Does Respiratory Syncytial Virus (RSV) infection in the first two years of life contribute to the development of asthma among children in Manitoba?

By

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ABSTRACT

Asthma is a 'multifactorial disease' with a large series of causative, inducing, triggering and aggravating factors. Respiratory syncytial virus (RSV) associated lower respiratory tract infection (LRTI) in infancy has been identified as a potential risk factor of childhood asthma in a number of studies. Conflicting evidence for the association between RSV-associated LRTI and subsequent development of childhood asthma was reported from some epidemiological and animal model studies. The current population based study was undertaken to determine whether RSV infection within first two years of life contributed to the subsequent development of asthma among the children in Manitoba.

The current study is limited to a total of 13980 children of the 1995 birth cohort, who were living in Manitoba by the end of December, 2006. A validated database definition of asthma was applied to this cohort to determine the asthma status of the children before the age of 3 years, at the age of 7 and 11 years. Asthma status at these points of life was used to define the asthma/wheezing phenotypes at the age of 11 years. Respiratory virus isolation report of Health Canada was used to define RSV-season(s) in Manitoba. Children with possible/potential RSV-associated LRTI were identified using the population based health care administrative data from the PHRDR. In addition to the children with confirmed diagnosis of RSV-associated LRTI after hospital admission, children who had lower respiratory tract infection in RSV-season(s) within the first two years of life were defined as subjects with clinically significant RSV-associated LRTI. Following the validity assessment of this definition of RSV-associated LRTI, the association between clinically significant RSV-associated LRTI within the first 2 years of life and risk of subsequent childhood asthma diagnosis was determined. The influences of

frequency and severity of clinically significant RSV-associated LRTI in the first 2 years of life and age-group at the time of first clinically significant RSV-associated LRTI on risk of childhood asthma diagnosis were determined as well.

Frequency and severity of clinically significant RSV-LRTI, and younger age at first occurrence of the infection were the most significant risk factors for asthma when the onset was within the first three years of life. The late onset of asthma phenotypes were not related to RSV-LRTI exposure. Clinically significant RSV-associated lower respiratory tract infection in the first two years of life was associated with higher risk of asthma at the age of 7 (OR=1.36, 95% CI=1.17-1.59) and at 11 (OR=1.26, 95% CI=1.08-1.47) years. RSV-LRTI within the first 2 years of life was also associated with higher risk of early persistent asthma (OR=2.91, 95% CI=2.20-3.85) and transient wheeze (OR=2.99, 95% CI=2.62-3.43) but not related to late transient asthma, late persistent asthma and late onset asthma.

Our study found that frequency of RSV-associated LRTI before age 2 years influenced the risk of asthma diagnosis at 7 (≥3 RSV-LRTI: OR=1.61, 95% CI=1.24-2.08) and 11 (≥3 RSV-LRTI: OR=1.42, 95% CI=1.09-1.86) years. Higher frequency of RSV-LRTI was related with greater risk of transient wheeze (≥3 RSV-LRTI: OR=8.21, 95% CI=6.84-9.85) and early persistent asthma (≥3 RSV-LRTI: OR=6.04, 95% CI=4.05-9.00) as well. Higher risk of asthma diagnosis at 7 (severe: OR=1.89, 95% CI=1.21-2.95) and at 11 (severe: OR=1.74, 95% CI=1.11-2.72) years was associated with more severe episode(s) of RSV-associated LRTI within the first 2 years of life. Severity was also associated with higher risk of early persistent asthma (severe: OR=8.98, 95% CI=4.86-16.61) and transient wheeze (severe: OR=9.36, 95% CI=7.01-12.49). First clinically significant RSV-LRTI between 6 and 12 months was associated with the highest risks of asthma diagnosis at 7 (OR=1.43, 95% CI=1.14-1.79) and at 11 (OR=1.36, 95% CI=1.08-1.71) years. But first RSV-associated LRTI within the first 6 months of life was associated with the highest risk early persistent asthma (OR=4.23, 95% CI=3.00-5.96) and transient wheeze (OR=4.55, 95% CI=3.87-5.35). The associations between severity and frequency of clinically significant RSV-LRTI and younger age at first occurrence and risk of subsequent childhood asthma were diminishing with increasing age of the children of the study cohort.

Only a few confounding variables were significantly associated with the risk of asthma diagnosis at 7 or at 11 years or asthma/wheezing phenotypes of the children of the current study cohort. Maternal asthma and male gender was associated with higher risk of asthma at 7 and at 11 years and all the asthma/wheezing phenotypes. With the exceptions of only transient wheeze and late onset asthma, having ≥2 sibling was associated with lower risk of asthma diagnosis at 7 and at 11 years and all other asthma/wheezing phenotypes. Children living in urban areas of Manitoba were at higher risk of asthma diagnosis at 7 and at 11 years and all asthma/wheezing phenotypes except for transient wheeze. With the exceptions of late onset asthma and late persistent asthma, receiving a higher number of antibiotics in their first year of life was associated with higher risk of asthma at 7 and at 11 years and all the asthma/wheezing phenotypes. Lower income and prematurity were associated with higher risk of transient wheeze only.

The current research revealed that a significant association existed between clinically significant RSV-associated lower respiratory tract infection within the first 2 years of life and risk of asthma diagnosis up to the age of 11 years among children in Manitoba.

Further research in this field would help to decrease asthma burden among the population of children in the future.

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LIST OF ABBREVIATIONS

AD	:	Anno Domini/Anno Domine
ALSPAC	:	Avon Longitudinal Study of Parents and Children
ATC	:	The Anatomical Therapeutic Chemical (ATC) Classification System
BCG	:	Bacillus Calmette-Guerin
CCHS	:	Canadian Community Health Survey
CDC	:	Centre for Disease Control
CI	:	Confidence interval
CIRID	:	The Centre for Immunization and Respiratory Infectious Diseases
DNA	:	Deoxyribonucleic acid
ECRHS	:	European Community Respiratory Health Survey
et al.	:	And others
FEF_{50}	:	Forced expiratory flow at 50%
FEV_1	:	Forced expiratory volume (FEV1)
FVC	:	Forced vital capacity
GP	:	General physician

HIPC	:	Health Information Privacy Committee
HMPV/hMPV	:	Human metapneumovirus
i.e.	:	That is
ICD9	:	International Classification of Diseases, version 9
ICD9CM	:	The International Classification of Diseases, 9th Revision, Clinical Modification
IFN	:	Interferon
IgE	:	Immunoglobulin E
IL	:	Interleukin
IRID	:	Immunization and Respiratory Infections Division
ISSAC	:	International Study on Asthma and Allergies in Childhood
KPNMDS	:	Kaiser Permanente Neonatal Minimum Data Set
LOS	:	Length of stay/Length of hospital stay
LRTI	:	lower respiratory tract infection
MAS	:	Multicentre Allergy Study
MCHP	:	Manitoba Centre for health Policy
mRNA	:	Messenger ribonucleic acid

No.	:	Number
NREVSS	:	National Respiratory and Enteric Virus Surveillance System
OR	:	Odds ratio
PEFR	:	Peak expiratory flow rate
PHIN	:	Personal identification number
PHRDR	:	Population Health Research Data Repository
PICNIC	:	Paediatric Investigators Collaborative Network on Infections in Canada
PIV	:	Para-influenza virus
RBEL	:	RSV Bronchiolitis in Early Life
REB	:	Research Ethics Board
ref.	:	Reference
RHA	:	Regional Health Authority
RR	:	Rate ratio
RR	:	Rate ratio
RSV	:	Respiratory Syncytial Virus (RSV)
RV	:	Rhinovirus
std	:	Standard deviation

TABS	:	Tennessee Asthma Bronchiolitis Study
UK	:	The United Kingdom
URTI	:	Upper respiratory infection
USA	:	The United States of America
V'_{maxFRC}	:	Maximal expiratory flow at functional residual capacity
vs.	:	Versus

Introduction & Background

1.1 Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (RSV) is considered as the single most important virus responsible for acute respiratory tract infections among infants. RSV was first isolated from a chimpanzee in 1956 and shortly afterwards from children with pneumonia and croup. In 60s and 70s, this virus was described as the most common cause of hospital acquired infection. The RSV epidemics generally occur at regular, predictable, yearly intervals. Human are the reservoir of this virus which transmits from one person to another by direct or close contact with contaminated respiratory secretions [1-3].

1.2 RSV-infection associated burden on heath care and population

In Canada, RSV infection is common among infants and represents a large public health burden. The Paediatric Investigators Collaborative Network on Infections in Canada study (PICNIC) reported that RSV accounted for almost 5800 annual hospitalizations for infants and children. The total estimated annual cost of RSV infection among children younger than 4 years of age in Canada was almost $18 million (in 1993 US dollars). Inpatient care for 0.7% of all infected children who required hospital admission accounted for most (62%) of the direct expenditures. Physician fees comprised only 4% of inpatient expenses and expenditures for ambulatory patients accounted for 38% of direct cost. An estimated 8611 workdays were lost for mothers of hospitalized children and 74,127 workdays were lost by mothers of outpatients. The economic value of this time was $1,505,813 [4].

Greenough found that infants with a confirmed RSV infection required more frequent and longer admissions to general paediatric wards and intensive care units, more outpatient attendance and GP consultations for respiratory disorders in the first 2 years after birth in the USA [5]. Shay and colleagues estimated that RSV accounted for up to 126,300 annual hospitalizations and as many as 510 annual deaths among children younger than 5 years in the USA over a period of 16 years [6, 7]. In 1985, the Institute of Medicine estimated that approximately 90,000 infants and children younger than five years of age were annually hospitalized in the USA for RSV bronchiolitis or pneumonia at a cost of $300 million per year [8]. Stang reported that a total of $365-$585 million per annum hospital charges (in 1998 US dollars) was attributable to RSV associated illness among US children younger than 5 years [9].

There were considerable differences between the Canadian and US estimates for RSV associated health care costs. Langley and colleagues suggested that the non-profit status of the Canadian health care system, along with regional practice variation, differential reimbursement by third-party payers, regional variation in charges and costs might account for the difference between the reported actual costs in Canada and charges billed in the United States [4]. In their attempt to estimate the cost of RSV infection in Canada, Langley and colleagues did not measure some direct cost including the out of pocket expenses and pharmacy prescriptions and the intangible costs. In addition, only the mother's lost wages were considered at the rate of a homemaker. The analysis did not consider the health care cost for conditions those were developed or exacerbated as a consequence of RSV infection. So the Canadian estimates appear to be an underestimate of actual RSV associated cost.

1.3 Epidemiology of RSV

Evidence of RSV infection is found globally. In temperate zones, RSV outbreaks usually occur in winter or spring. The spread of RSV in a community is easily recognized with a sharp rise in the number of bronchiolitis and pneumonia cases and a subsequent increase in the number of hospital admissions of young children with acute lower respiratory tract infections [3, 10]. RSV accounts for approximately 50% of all pneumonia in infancy and 50-90% of cases of bronchiolitis. In addition to that, RSV can be associated with 10-30% of cases of bronchitis among children. Other major respiratory pathogens are usually absent in the community during the peak of RSV activity [10]. Most of the children hospitalized in the USA with RSV infection between 1980 and 1996 were younger than 6 months and 81% were in the first year of their lives [6]. Virtually all children have at least one RSV-associated lower respiratory tract infection before the age of 2 years. RSV-associated reinfection of the lower respiratory tract is very common and usually mild. Reinfection is mostly limited to tracheobronchitis and reactive airway diseases [10, 11].

Introduction of RSV into the family is most likely to occur through a school-aged child and the infant becomes secondarily infected. Transmission of RSV from one individual to another is most likely to happen by close contact with infected persons or their infectious secretions. Spreading of the virus through small-particle aerosol is unlikely. Nose and eyes is the most likely route of inoculation of the virus with an incubation period of 2-8 days. Most of the infants show improvement of clinical conditions within 3-4 days after hospitalization with RSV-associated bronchiolitis and most are discharged from the hospital within 3 to 7 days of admission [10].

1.4 Risk factors associated with RSV infection

RSV infection within the first four weeks of life is relatively uncommon. The peak incidence of severe RSV-related illness occurs between 2 and 3 months of age. Male patients appear to experience more severe disease than their female counterparts. McNamara and Smyth reported that in industrialized countries, severe bronchiolitis is primarily seen in well-defined high-risk groups. Host-related risk factors for severe RSV disease reported from several studies include premature birth and low birth-weight, young age, infections before 6 months of age, chronic lung disease, congenital heart diseases, diabetes, bronchopulmonary dysplasia, cystic fibrosis and immunodeficiency [10, 12-15].

Young age, multiple gestation, malnutrition, family history of atopy, lack of parental education, lack of breast-feeding, household crowding, lower socio-economic conditions, older school-age siblings, day-care attendance, passive smoke exposure and discharge from a neonatal intensive care unit between September and December were reported as the environmental risk factors for more frequent and severe RSV infection [3, 10, 12, 13, 15-17].

In the USA, the highest prevalence of severe RSV infection was reported among infants of black mothers [12]. Wang and colleagues reported a significant association between complicated RSV-associated hospital admission and aboriginal race in Canada [18]. Study among the Alaska Native Children also found higher incidence of hospital admission among native children [19-21].

1.5 RSV-associated lower respiratory tract infections

Newborns lack the maturation of the immune system in comparison to older children and normal adults. RSV infection is most prevalent when the infant is least immunologically mature. During the first 2 months of life, passively acquired maternal immunoglobulins protect newborns against RSV infection. The presence of maternal antibodies decreases gradually during the first 6 months of life leaving most infants unprotected against RSV between 2 and 4 months of age [3, 13, 22, 23]. The majority of children infected with RSV under 1 year of age develop mild upper respiratory tract symptoms but up to 40% develop lower respiratory tract symptoms and 0.5%-2.0% of all infants require admission to hospital [13]. The clinical conditions, which can be attributable to RSV, are briefly described below.

1.5.1 Bronchiolitis

Acute bronchiolitis refers to a disease of infectious aetiology, mostly caused by viruses with especial affinity for the bronchiolar epithelium [24]. The primary sign and symptom of bronchiolitis is presented by obstruction of expiratory air flow, leading to wheezing with flaring of the nostrils and accessory muscles of respiration [25]. The first symptoms of RSV bronchiolitis are coryza and dry cough followed by breathlessness. Sometimes apnoeic spells are observed in the initial stage of the disease among infants younger than 2 months, especially among preterm babies. This stage is followed by hypoxemia, and carbon dioxide retention may lead to hypercarbia. Other clinical symptoms include sharp and dry cough, tachypnea and tachycardia. Chest radiograph shows over-inflated lungs. On chest auscultation, fine end-inspiratory crepitations are heard and the expiratory phase is prolonged. Wheezes may be audible with or without a stethoscope [26].

Acute bronchitis and bronchiolitis is coded as 466 in the International Classification of Diseases, version 9, clinical modification, (ICD9CM). This code has been divided into several subcategories: ICD9CM=466.0, 466.1, 466.11 and 466.19. Code 466.0 represents acute or subacute bronchitis; code 466.1 represents cases of acute bronchiolitis; acute bronchiolitis due to RSV is coded as 466.11 and code 466.19 represents the cases of acute bronchiolitis due to other organisms [27].

Respiratory syncytial virus (RSV) is the most important etiologic agent for bronchiolitis and has been reported as the only important etiologic consideration when bronchiolitis is epidemic in the community. Parainfluenza virus, influenza virus and rhinoviruses were also reported as the causative agents of bronchiolitis [25, 28]. The bronchiolitis cases in the summer and early fall are mostly associated with rhinovirus and parainfluenza virus [24]. Shah reported RSV as the most common (70%) causative agent of bronchiolitis and parainfluenza, influenza and adenovirus as less common. Human metapneumovirus, *Mycoplasma pneumoniae* and rhinoviruses were reported as rare etiologic agent of bronchiolitis [29].

In most of the cases, the subject is exposed to an older sibling or adult with the common cold and develops the illness in 5 to 7 days [28]. Bronchiolitis most commonly occurs during infancy and almost 80% of the cases within the first year of life. Children between 2 to 6 months of age are the most affected group with the peak age of hospitalization between 1 and 3 months. Premature children, children with family history of asthma and children with congenital hearth or lung disease are at high risk of bronchiolitis associated hospitalization [25]. Boys are more frequently affected by RSV-associated bronchiolitis than their female counterparts but the difference diminishes by the age of 9 years. Family history of asthma, lower socioeconomic conditions and the associated lifestyle including crowded living conditions, low level of maternal education, lack of breast feeding and exposure to tobacco smoke, have been found to be associated with higher risk of developing RSV-associated bronchiolitis [28].

1.5.2 Acute bronchitis

Acute bronchitis is a febrile illness with cough, rhonchi and referred breath sounds resulting from acute inflammatory disease and subsequent damage to the ciliated epithelium of the larger air passages including the trachea and the large and medium bronchi [30]. The initial signs and symptoms include low-grade fever, cough and rhinorrhea. Apnoea is not uncommon among children younger than 6 months. The initial signs and symptoms are followed by tachypnea, hypoxia, subcostal/intercostal retractions, rales, wheezing and rhonchi [29]. The signs and symptoms of acute bronchitis are very often accompanied by pharyngitis and rhinitis [30]. The cases of chronic bronchitis are coded as 491 and bronchitis which are not classified as either chronic or acute are coded as 490 in the ICD9CM [31, 32].

RSV, Adenovirus, influenza virus, parainfluenza virus and *Mycoplasma pneumoniae* have been found responsible for most cases of acute bronchitis among children [30, 33]. Chapman and Henderson found that RSV and parainfluenza type 3 were the most common etiologic agents of acute bronchitis in the first 6 years of life [33]. Despite the fact that different etiologic agents of acute bronchitis have different cytopathologic effects, all the infectious agents lead to similar symptoms as a result of airway obstruction [30].

Chronic bronchitis among children lacks a standardized definition. Recurrent episodes of acute bronchitis can often be misinterpreted as chronic bronchitis and virus induced exacerbation of asthma can be confused as chronic bronchitis. RSV, rhinoviruses, parainfluenza virus and influenza viruses, have been found to be associated with exacerbation of both asthmatic bronchitis and chronic bronchitis. The lack of a standardized definition of chronic bronchitis among children resulted in confusing data while appreciating the prevalence or aetiology of lower respiratory diseases [34].

1.5.3 Viral Pneumonia

Bronchiolitis and viral pneumonia among children often coexist and are not clearly distinguishable from one another. The clinical presentations of viral pneumonia among children include difficulty in breathing, non-productive cough accompanied by wheezing and increased breath sounds. Apnoeic episodes with fever have been reported among young infants with viral pneumonia [25]. Tachypnea, tachycardia, nasal flaring and retractions are the most often encountered physical findings during acute non-bacterial pneumonia [35]. Acute pneumonia is the most common among children younger than 2 years but the incidence rate decreases with increasing age [29, 35].

Bacteria are responsible for 30% to 40% of acute pneumonia cases, with *Streptococcus pneumoniae* as the most common etiologic agent. Respiratory viruses, including RSV, adenovirus, parainfluenza and influenza viruses are among the other common etiologic agent of acute pneumonia, especially among children between 1 month and less than 5 years of age. RSV is the most commonly associated etiologic agent of viral pneumonia among children [25, 29, 36]. *Mycoplasma pneumoniae* is the most common etiologic agent of non-bacterial pneumonia among children over 5 years of age. *Chlamydia* *pneumoniae* is now emerging as an important etiologic agent of pneumonia among older children and young adults [35].

Viral pneumonia is coded as 480 and has been divided into 480.0, 480.1, 480.2, 480.3, 480.8 and 480.9 in the ICD9CM. Code 480.1 represents viral pneumonia caused by RSV. Viral pneumonia caused by unspecified virus is coded as 480.9 [37]. Code 485 and 486 represent the cases of pneumonia where the causative organisms are not specified [38].

1.6 LRTI in RSV-season

Terletskaia-Ladwig and colleagues determined the onset, offset, peak and duration of RSV outbreaks for each year from 1996 to 2004 in Southern Germany. The investigators defined the onset week as the first of two consecutive weeks with at least 2 positive RSV isolations. The offset week of RSV outbreak was defined as the last of the final two consecutive weeks with at least two positive isolations of RSV [39]. The National Respiratory and Enteric Virus Surveillance System (NREVSS) in the USA is responsible for reporting a summary of testing data for several respiratory viruses including RSV and several enteric viruses. The NREVSS defined onset of RSV-season as the first of two consecutive weeks of ≥10% positive test results for RSV and at least two positive test results for both the weeks. The offset week was defined as the last of the final two weeks with at least 10% positive RSV isolation and at least two positive samples for both the weeks [40].

RSV infections follow distinctive seasonal patterns around the world. RSV epidemics have occurred primarily in late fall, winter or spring but never in summer in the temperate part of the world [41]. Several epidemiological studies compared the distribution of seasonal outbreaks caused by RSV and other respiratory viruses. From the Houston Family study, Glezen found that epidemics caused by different respiratory viruses did not occur simultaneously [42]. This was also supported by finding of Denny and colleagues from the Chapel Hill study. Denny and colleagues also found that PIV type 1 caused a large epidemic of croup in fall which was followed by a winter epidemic of RSV associated LRTI and a spring outbreak associated with influenza type B virus. RSV was rarely isolated in December and was most commonly isolated in January and February [43, 44]. Anderson and colleagues found that RSV and influenza A and B epidemics occurred every year in the winter, parainfluenza (PIV) 1 and 2 epidemics were detected every other year in the autumn and the PIV 3 were isolated through out the year with periodic increase isolation [45]. Knott and co-investigators and Hall reported very similar patterns for PIV outbreaks [46, 47]. Williams and colleagues found that though the seasonal activities of the respiratory viruses might have some overlaps, the numbers of influenza virus, adenovirus and hMPV isolated during the time of seasonal RSV activity were very small in comparison to number of RSV isolation. Very similar findings have been reported by several epidemiological studies conducted in different geographical settings in the world [48-52].

Essentially all the cases of bronchiolitis during the RSV epidemic season are caused by this virus [25, 53]. Other major respiratory pathogens are absent during peak RSV activity in the community. Despite the fact that RSV outbreaks and influenza A infection may overlap, their peaks hardly coincide with each other [10].

Major clinical conditions/symptoms caused by other respiratory virus infections are often different from those caused by RSV. von Linstow and co-investigators found that hMPV caused milder clinical consequences than RSV [49]. PIV infections are more likely to involve and limited in the upper respiratory tract [54]. PIV could be associated with bronchiolitis and pneumonia among young infants but the frequency is much less than that observed with RSV [25, 54]. Adenovirus infections most commonly occur in late winter, spring and early summer [55]. Though the seasonal activity of adenoviruses may overlap with that of RSV, adenoviruses cause febrile upper respiratory tract infections which are mostly subclinical, mild and self-limiting [56, 57]. Rhinovirus (RV) is the single most important causative agent of common cold. RV survives best in conditions of high relative humidity and has been reported as predominant virus in spring, summer and fall. RV activity has been reported throughout the year in the areas with humid climate [58-61]. Bosis and colleagues reported that most of the influenza viruses were isolated from subjects with upper respiratory tract infections including common cold, pharyngitis and acute otitis media [62]. Nicholson and colleagues also found that influenza caused significantly less lower-respiratory illness [48].

1.7 LRTI in the first two years of life

RSV infects almost all children in their first years of life and nearly all of the first infections are symptomatic. Several epidemiological studies found that reinfection by RSV was very common, but the first episode of RSV infection was the most severe and significant. In the Houston Family study, with the exception of only one child, all the children had at least one episode of RSV infection within the first two years of life.

Primary RSV infection after the age of 12 months had less severe consequences. The investigators found that reinfection was common, but reinfection associated illness was mild and lower respiratory disease was very uncommon as a consequence of reinfection with RSV [11, 63-65]. Henderson and colleagues and Denny and colleagues also found that severity of RSV-infection associated illness progressively diminished with reinfection [43, 66]. Iwane and colleagues found that 94% of the RSV isolates were detected among children younger than 2 years [67]. Rakes and co-investigators found that 68% of the wheezing infants younger than 2 years of age had positive RSV infection and only 6% of the wheezing children older than 2 years tested positive for RSV [68]. Hall reported that RSV-associated lower respiratory disease was almost entirely restricted among the children younger than 3 years of age [10, 63].

In The Chapel Hill study, similar rates of bronchiolitis in the first and the 2nd year of life were observed, but the rates declined sharply after the age of 2 years [43]. Chapman and colleagues reported that RSV was the single most commonly isolated agent of all lower respiratory tract illness before the age of 2 and 4 years, parainfluenza was the only other significant isolate within this age group. Influenza isolation for 104 months of the study was very low and almost negligible in the first four years of life [33]. Henderson and colleagues reported that the incidence of wheezing illness, which was more prominent among the children younger than 2 years of age, reached the peak simultaneously with the occurrences of RSV infection over the 11 years time period of the Chapel Hill study. The peak incidence of wheezing associated respiratory illness among children over 5 years of age coincided with the seasonal peak of *M. pneumoniae* and parainfluenza virus infection [69].

Influenza viruses are more likely to infect older children and have been reported as the most important cause of acute respiratory tract illness associated hospitalization of schoolchildren [70]. Anderson and colleagues found that the isolation of RSV strongly correlated with the winter peaks in LRTI-associated deaths among children between 1 and 11 months of age. The authors also found that the isolation of influenza had correlation with the winter peak in LRTI-associated deaths of children between 24 and 59 months of age [45]. Bosis and colleagues reported that mean age of the subjects with influenza virus (4.03 years, std ±3.66) and hMPV (3.31 years, std ±2.99 years) associated respiratory infections were significantly higher than that of subjects with RSV (1.13 years, std ±2.46 years) [62]. Several other epidemiological studies also reported that the mean age of subjects with RSV-associated LRTI was significantly less than the mean ages of subjects with respiratory illnesses caused by other respiratory viruses [48, 50, 71, 72].

1.8 Asthma

The word asthma was derived from the Greek word for panting or breathlessness, which was in fact a description of the primary symptom of this disease. Clinically asthma can be defined as recurrent airflow obstruction that causes intermittent wheezing, breathlessness, chest tightness and sometimes cough with sputum production. According to the National Asthma Education Panel, asthma can be defined as a clinical condition with: a) reversible airflow obstruction, b) airway inflammation and c) increased airway responsiveness to a variety of stimuli [73].

The clinical form and severity of asthma can be variable. In the case of childhood asthma, the acute asthma attack may last from hours to days with intervals. It may also occur as a chronic state in which the child has frequent minor and major exacerbations of the disease. Asthma may cause slight impact on lung function to serious handicap [74].

1.8.1 The epidemiological trend of asthma

International studies such as the European community Respiratory Health Survey (ECRHS) and the International Study on Asthma and Allergies in Childhood (ISSAC) revealed the emergence of a pattern of geographical distribution of asthma and provided comparable data on the prevalence of asthma in different parts of the world. Those studies reported that, with some exceptions, the highest prevalence of asthma was observed in westernized countries where English was the main language. Intermediate values were found in central and southern Europe and the lowest prevalence was found mostly in developing countries and in Eastern Europe [75, 76]. According to the ISSAC study report, the prevalence of wheezing among 13-14 year-old children was 11.2-19.7% in Finland and Sweden. The prevalence of asthma in this age group was 7.6-8.5% in Estonia, Latvia and Poland and 2.6-5.9% in Albania, Romania, Russia, Georgia and Uzbekistan. There was a significant increase in the prevalence of hay fever (2.3 % vs. 5.1%) in Leipzig, East Germany between 1991-'92 and 1995-'96. Atopic sensitization also increased significantly between the same time-period. After reviewing this evidence, von Mutius concluded that there was an apparent association between the dramatic changes towards western lifestyle in former East Germany and rise in the prevalence of hay fever and atopic sensitization in school-aged children [75].

Asthma is one of the most prevalent chronic conditions among Canadian children and a serious problem in adults as well. According to the 2000/01 Canadian Community Health

Survey (CCHS), asthma affected 2.2 million people or 8.5% of Canadians 12 years of age and over. Asthma imposes a heavy burden on the nation's health care expenditures and reduces the quality of life for individuals with asthma and their families. In 2000/01 alone, asthma accounted for 1.2% of overall hospitalization among Canadian males and females [77, 78].

1.8.2 Aetiology and risk factors of asthma

Kuzemko described childhood asthma as a heterogeneous disease involving both genetic and environmental factors [74]. Viral infections, weather changes, exercise, allergen exposure, irritant exposure and emotional upset can trigger acute asthma among children. House dust mite, cockroaches, pet-derived antigenic proteins, airborne moulds and pollens are most commonly implicated factors in chronic asthma. Occupational exposure to metal salts, wood dusts, vegetable dusts, industrial chemicals, pharmaceutical agents, biological enzymes and animal and insect materials are often associated with development of asthma. Exercise is a nonspecific stimulus to airflow obstruction and can be demonstrated in most patients with asthma. Dietary practice and factors associated with socio-economic status including higher rate of smoking among poorer parents, increased exposure of children to infections in day-care and poor quality housing may be contributing to the rise in asthma prevalence [73, 75, 79-81].

Considering the wide range of factors associated with the aetiology, asthma can be defined as a ‘multifactorial disease’ with a large series of causative, inducing, triggering and aggravating factors [76]. Health Canada reported asthma as the result of a complex interaction between three factors:

- Predisposing factors (such as atopy - a tendency to have an allergic reaction to foreign substances).
- Causal factors, which may sensitize the airways (such as cat and other animal dander, dust mites, cockroaches, or workplace contaminants).
- Contributing factors, which may include cigarette smoke during pregnancy and childhood, respiratory infections, and indoor and outdoor air quality [82].

Some of the mostly cited factors of asthma epidemic in the Westernized countries are very briefly presented below.

1.8.2.1 Pollution

Studies conducted in the UK, Sweden and Germany reported that preschool children admitted to hospital for asthma, wheeze and other respiratory conditions (including cough and Bronchiolitis) were more likely to live in areas with high traffic and increased outdoor NO_2 exposure resulting from traffic emission [75, 76]. Air pollution may trigger symptoms in individuals with asthma, but not-necessarily induces the development of new cases of atopy and asthma among individuals with previously healthy lungs [75, 83].

1.8.2.2 Nutrients

Westernization could be characterized by profound changes in dietary habits including increased salt intake, increased consumption of vegetable oils, decreased consumption of antioxidants and bottle feeding practice [76]. It has been hypothesized that the development of childhood asthma and allergies could have a relation with higher intake of certain polyunsaturated fatty acids, such as linoleic acid (predominantly from industrial sources) and low intake levels of vitamins [75].

1.8.2.3 Maternal smoking

A growing body of evidence suggested a relationship between in utero tobacco smoke exposure and the subsequent development of asthma and decreased lung function in childhood [84-89]. Children of smoking mothers have higher incidences of asthma and more frequent exacerbations, more severe symptoms and poorer lung function after adjustments for exposure to dust mite allergen, pet ownership, maternal asthma and child gender [86, 90]. Parental smoking has been associated with higher incidence of both upper and lower respirator tract illnesses including otitis media, asthma, bronchiolitis and pneumonia [89, 91, 92].

1.8.2.4 Indoor allergen and hygiene

Matricardi and Bonini suggested that exposure to indoor allergens had the strongest association with asthma in developed countries. The prevalence of asthma is very high in countries with highest mite allergen levels in the world [76].

The allergy epidemic has been attributed to changes in interactions between human and the microbes of their ecosystem as a consequence of Westernized lifestyle. This led to spreading of allergy among new cohorts according to gradients dictated by hygiene and by the individual degree of genetic predisposition to atopy. This hypothesis explains all the gradients observed in the distribution of atopy, as hygiene spread earlier with westernization in English-speaking countries and among the advantaged and infections are acquired less frequently and later in life in small families and among the first born [76].

1.8.2.5 Influences of other factors on asthma

Parental history of asthma, especially maternal asthma, has been reported as a strong predictor of childhood asthma in different parts of the world [93-95]. Studies conducted in Canada found that male gender, low birth-weight, parental atopy, maternal stress, living in urban areas, having one or no sibling and traffic related pollutions were significantly associated with higher risk of physician diagnosed asthma in preschool years [96-98]. Similar findings were reported from studies conducted in the USA and Europe and from several systematic review of published literature [99-106].

Phase III of the ISAAC study found that use of antibiotic in the first year of life was associated with higher risk of asthma among 6 and 7 years old children. This was supported by several epidemiological studies and meta-analysis of published literature [107-109]. Association of BCG vaccination with lower prevalence of asthma among children has been reported from studies conducted in different parts of the world [110-113]. As reported in several review articles, a number of studies in last decade revealed the higher prevalence of asthma in urban areas than in rural areas [114-116]. Studies conducted in Asia, Europe and South America reported that children living in urban environment for a long time were at higher risk of reduced lung function and children living in rural environments were at lower risk of development of asthma and aeroallergen sensitization [117-120]. In addition to respiratory virus infection(s), involvement of bacterial, fungal and parasitic infections were also reported in the pathogenesis and expression of asthma [73, 121]

Considering the evidences on the associations between the above mentioned factors and childhood asthma, Matricardi and Bonini hypothesized that hygiene would be the major primary cause of the atopy and epidemic of atopic diseases including allergic asthma. It would facilitate type-2 responses against environmental non-microbial antigens among the genetically predisposed individuals. Westernized diets would contribute to this phenomenon as it deprives the individual of daily and diversified microbial stimulation. Pre-existing high concentration of allergens in indoor environment would contribute to steeper increase in asthma prevalence in certain countries, particularly the English-speaking ones. At any stage, pollution would either aggravate or trigger asthma attacks among already affected patients. The respiratory infections, on the other hand, might facilitate bronchial inflammation in subjects with atopic sensitization [76].

1.9 RSV infection and asthma

A number of population based epidemiological studies as well as some animal model studies were conducted around the world to reveal/establish the association between RSV-associated respiratory tract infection during infancy and subsequent development of childhood asthma. Some of the studies reported that a significant association existed between RSV-infection in infancy and subsequent development of asthma. On the other hand, some studies did not find any significant association.

After reviewing a number of publications on asthma and possible aetiology, Sigurs indicated that though no single specific cause of asthma was found, lower respiratory tract infection (LRTI) caused by RSV in infancy was identified as a potential risk factor [122]. In a review of literatures on the association between RSV infection in first 3 years of life and the subsequent development of asthma or bronchial hyper-reactivity, Perez-Yarza and colleagues concluded that there was an association between RSV infection in infancy and the emergence of different asthma phenotypes with a clear gradient effect and progressive disappearance of this effect with increasing age [123]. Several other reviews of currently available literature also concluded in favour of the positive association between RSV-associated respiratory tract infection during infancy and subsequent development of asthma [124-127]. Reviews of currently available literature also reported that RSV infection in early childhood was not an independent risk factor for childhood asthma [128, 129]. After consulting few animal studies, Mejias and colleagues concluded that RSV-infected mice with persistent airway hyperresponsiveness exhibited the presence of abnormal chronic inflammatory changes and mucus over-production that could contribute to long term airway disease [130].

1.9.1 Human studies on association between RSV infection and asthma

Several studies which were conducted to reveal the association between RSV infection among human in early life and subsequent development of asthma are very briefly presented below.

1.9.1.1 The Tucson children's respiratory study: This prospective study was conducted in Tucson, Arizona to study the interrelations of a variety of factors with development of acute lower respiratory tract illness and/or chronic airways diseases. A total of 1246 newborns were enrolled in the study between May 1980 and January 1984. The study revealed that, RSV-LRTI before 3 years of age was independently associated with a significant increase in risk of subsequent wheezing during the first ten years of life but the relationship rapidly subsided with age and was no longer significant at 13 years [131, 132].

1.9.1.2 The Oslo birth cohort study: In 1992-1993, a birth cohort was established to study the environmental determinant of respiratory health among children. Children who were born during a period of 15 months in the city of Oslo, Norway, were selected for the study. A total of 3754 children out of 4973 eligible children were enrolled in the study. The investigators reported that respiratory infection during infancy increased the risk of developing bronchial obstruction during the first 2 years of life and of having asthma at 4 years of age [133, 134].

1.9.1.3 A Swedish prospective cohort study: Sigurs and colleagues conducted a prospective cohort study in Sweden to study the occurrence of bronchial obstructive symptoms and immunoglobulin E (IgE) after RSV bronchiolitis in infancy. A total of 47 children who were admitted to hospital with laboratory-verified RSV bronchiolitis under the age of 1 year and 93 age and sex matched controls were enrolled in the study. After follow-ups at the mean age of 1 year, 3 years, 7.5 years and 13.4 years, the investigators concluded that RSV bronchiolitis in infancy, sever enough to cause hospitalization, was highly associated with the development of asthma and allergic sensitization in early adolescence [135-137].

1.9.1.4 A German multicentre longitudinal study: Illi and colleagues conducted a nested longitudinal cohort study to investigate the association between early childhood infections and subsequent development of asthma. A total of 1314 children, who were born in 1990 in five different German cities, were recruited for their study. These children were initially recruited for the longitudinal Multicentre Allergy Study (MAS). The findings of the study suggested that lower respiratory tract infections in early life were positively associated with subsequent development of asthma, wheeze and bronchial hyperreactivity. Repeated viral infections, other than lower respiratory tract infections, in early life might reduce the risk of developing asthma up to school age [138].

1.9.1.5 The ALSPAC study in Avon, UK: The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited 14541 pregnant women living in Avon, UK with expected dates of delivery between April 1st, 1991 and December 31st, 1992. The children were assessed for doctor diagnosed asthma at 91 months. They were also assessed for the presence and frequency of wheezing between 30 and 42 months and between 69 and 81 months. From the study, Henderson and colleagues found that severe RSV bronchiolitis in infancy, which required hospital admission, was associated with a significantly increased risk of doctor diagnosed asthma in later childhood [139].

1.9.1.6 Tennessee Asthma Bronchiolitis Study (TABS): This population-based retrospective birth-cohort study was carried out among more than 90,000 term healthy infants with normal birth-weight, who were born between 1995 and 2002. Medicaid and linked vital records files were used to identify health care visits, asthma-specific medications and demographics. The study found that children who had bronchiolitis in their infancy were more likely to develop childhood asthma (between 4 and 5.5 years) than children who did not have bronchiolitis as an infant. The study also found that more severe bronchiolitis was associated with higher risk of childhood asthma [140]. Another report from the same study revealed that clinically significant bronchiolitis at any age during infancy was associated with increased risk of childhood asthma. Children who were born four months prior to the seasonal virus peak were more likely to have childhood asthma [141].

1.9.1.7 RSV bronchiolitis in early life (RBEL) cohort study: In this prospective cohort study, a total of 206 previously healthy children who were admitted to St. Louis Children's hospital with RSV infection from 1998 to 2001 were enrolled. The children were assessed for immunological responses after RSV infection and after 2, 4 and 6 years. The authors reported that sever RSV infection in early life was associated with a high incidence of asthma and eczema [142].

1.9.1.8 The Canadian Asthma Primary Prevention study: The Canadian Asthma Primary Prevention study was a prospective, randomized, controlled study with 278 subjects in intervention and 267 in control group. The study found a clear temporal relationship between viral exposures during the first year of life and the occurrence of possible asthma and atopy in the second year. The children who were exposed to either parainfluenza virus or RSV during first 12 months had significantly higher odds of having new onset of possible asthma in the second year of life [143].

1.9.1.9 A follow-up study among the Alaska Native Children: Karron and colleagues conducted a case control study in 1993-'96 to determine the risk factors for hospitalization with RSV infection among Alaska Native children. After follow-up of this cohort during 1999-2001, Singleton and colleagues concluded that hospitalization with RSV infection among Alaska Native children before the age of 2 years was associated with a significant increase in wheezing, LRTI and asthma diagnosis during the first 4 years of life. But the association was declining with increasing age and was no longer significant by the age of 5 years [19, 144].

1.9.1.10 A 10 years prospective cohort study in Newcastle upon Tyne, UK: Pullan and Hey reviewed all the children who were admitted to hospital with RSV associated lower respiratory tract infections in Newcastle upon Tyne during the winters of 1968-69 and 1969-70. A total of 130 children were interviewed for follow-up after 10 years and 107 of them completed a full pulmonary reassessment. A total of 111 age, sex and social class matched children were selected as the control for the study. The authors found that wheezing was significantly higher among the index cases than in the controls. The difference was most significant in the first four years of life and the difference declined with increasing age. Overall, more index cases reported recurrent upper respiratory tract symptoms than children in the control group [145].

1.9.1.11 A 10 years prospective follow-up study in Nottingham, UK: Noble and colleagues conducted this prospective study to evaluate the relationship between acute bronchiolitis in infancy and subsequent respiratory problems. A total of 101 infants (54 boys and 47 girls) who were admitted to the children's wards of the University Hospital, Nottingham during winter bronchiolitis epidemics in 1979-'81, were enrolled in the study. Age, sex, ethnicity, and maternal smoking history matched controls were also recruited. The authors found that there was an excess of respiratory symptoms and lung function abnormalities, compatible with an increased prevalence of asthma, persisting for at least nine years after episodes of well documented bronchiolitis [146].

1.9.1.12 Study in Greece: Cassimos and colleagues evaluated a total of 189 children who were hospitalized with RSV-associated acute bronchiolitis in infancy. The investigators compared these children with a total of sixty non-asthmatic matched controls with no history of bronchiolitis in infancy. Follow-up at a median of 7 years after their hospitalization for acute bronchiolitis revealed that the PEFR, FEV_1 and FEF_{50} of the cases were significantly lower than children in the control group. The investigators concluded that a history of RSV-associated acute bronchiolitis in infancy increased the risk of developing asthma during childhood [147].

1.9.1.13 A clinic-based cohort study in The Gambia: Weber and colleagues conducted a passive, clinic-based cohort study in The Gambia to determine the frequency of later respiratory tract morbidity after RSV infection in infancy. A total of 105 children were enrolled in the index group, 105 in the first control group and 102 in the second control group in early 1994 and in early 1995. The investigators found that the prevalence of pneumonia and wheezing were more common in the index group than in the both control groups and the incidence rates were higher for younger children. The incidence declined rapidly with increasing age and no significant difference was detected among the groups by the age 3 years [148].

1.9.2.14 Prospective study on phenotypes of RSV-LRTI and outcome: Elphick and colleagues investigated the long-term outcome of different phenotypes of acute RSV associated lower respiratory tract infections. A total of 56 children who were admitted to The Sheffield Children's Hospital with confirmed RSV-associated LRTI before the age of 1 year were enrolled in the study. After a follow-up period of 3 years, the authors concluded that RSV associated LRTIs in infancy did not induce allergic asthma [149].

1.9.1.15 A 20 years prospective cohort study in Kuopio, Finland: Korppi and colleagues prospectively followed a cohort of 127 children since 1981-82 until the year 2000 who were hospitalized for either viral bronchiolitis or pneumonia before the age of 2 years. The authors reported that RSV infection was not a significant risk factor for asthma or for bronchial reactivity, but RSV infection that required hospitalization in infancy was an independent risk factor for abnormal lung function [150]. In 2005, the investigators reported that no association could be found between early RSV infection and teenage asthma [151]. In 2007, the study group reported that infants with non-RSV bronchiolitis, requiring treatment in hospital, were at increased risk for subsequent asthma in adulthood [152].

1.9.1.16 A randomized, controlled follow-up study in Kuopio, Finland: Reijonen and colleagues investigated the effects of allergic sensitization and RSV infection on the development of asthma. A total of 100 children were enrolled in the study. These children were treated for infection associated bronchial obstruction before the age of 2 years between January 1, 1992 and November 2, 1993 in the Department of Paediatrics, Kuopio University Hospital. The investigators found that identification of RSV at entry was associated with decreased risk of asthma (RR 0.3, CI: 0.08-0.80). The investigators also reported that wheezing patients with RSV infection had much better prognosis than those without RSV infection [153]. In a follow up study in 2004, the investigators found that asthma was relatively rare among children who had RSV infection than children who had other viral infection or no viral infection at all [154]. Though the FVC values were lower in RSV-positive than in RSV-negative cases, the values were within normal limits and asthma was more common after RSV-negative than after RSV-positive bronchiolitis [155, 156].

1.9.1.17 A follow-up study in Turku, Finland: Valkonen and co-investigators conducted a follow-up study in Turku, Finland among 416 children younger than 2 years who were hospitalized with bronchiolitis between August and December during 1988 and 2001. The children were assessed after 1 year, 2 years and 3 years after hospitalization for bronchiolitis. The investigators found that children with non-RSV associated bronchiolitis were more likely to develop recurrent wheezing than children with RSV-associated bronchiolitis within the first year after hospitalization and at all time points of a 3-year follow-up study [157].

1.9.1.18 Population based study among Danish twins: Stensballe and colleagues investigated the causal direction of the associations between RSV-hospitalization and asthma in Denmark. The prospective population based cohort study enrolled 18,614 twins born between 1994 and 2003 in Denmark. The investigators found that severe RSV infection was associated with a short term increase in the risk of subsequent asthma and asthma was associated with a long-term increased susceptibility for severe RSV disease. They concluded that severe RSV infection and asthma could share a common genetic predisposition or environmental exposure [158]. In another report the authors concluded that severe RSV-associated hospitalization was not responsible for causing asthma but was an indicator of the genetic predisposition to asthma [159]. Another epidemiological study in Oxford, UK also reported that primary RSV associated bronchiolitis and atopy shared a genetic determinant [160].

1.9.1.19 An 8 years follow-up study in Newcastle, UK: Sims and colleagues prospectively followed a cohort of children for 8 years who had RSV associated bronchiolitis in infancy in Newcastle. A total of 35 children who had RSV associated bronchiolitis in the winter epidemic of 1967-1968 and 35 age, sex and social class matched children were selected for the study. Though more children in the bronchiolitis in infancy group had subsequent episodes of wheezing, in most of the cases that was neither severe nor frequent enough for a hospital inpatient or outpatient referral. The wheezing episodes disappeared in most of the cases by the age 8 years. The investigators concluded that clinical prognosis of infants with RSV associated bronchiolitis was good at the age of 8 years and there was no strong relationship between RSV bronchiolitis and childhood asthma [161].

1.9.1.20 Study in Norway: Fjaerli and colleague investigated the effect of virus associated acute bronchiolitis in infancy on lung function and other respiratory symptoms up to seven years. A total of 57 children who were hospitalized with acute respiratory diseases were enrolled in the follow-up study. The authors found that children hospitalized for viral bronchiolitis during infancy had decreased lung function, more often wheezing episodes and current medication and follow-up for asthma at seven years of age than the age matched controls. The authors did not find any significant difference between the RSV-positive and RSV-negative groups [162].

1.9.1.21 A seven years follow-up study in Edinburgh, UK: A total of 376 children under 1 year of age were admitted to the Royal Hospital for Sick Children in Edinburgh during the winter of 1971-72, 1972-73 and 1973-74 with bronchitis, bronchiolitis or pneumonia. Mok and Simpson conducted a retrospective study with these children and age, sex and height matched controls to assess the prevalence and severity of subsequent respiratory symptoms and established respiratory disorders, lung function and the possible influence of social and environmental factors. The study found that children with acute LRTI in infancy, severe enough to require hospital admission, had increased respiratory symptoms, bronchitis and asthma, impairment of ventilatory function and increased bronchial hyperreactivity than the controls seven years after the infection [163].

1.9.1.22 A prospective follow-up study in Bochum, Germany: Schauer and colleagues recruited 42 children who were hospitalized with laboratory verified RSV-bronchiolitis from December 1999 to April 2001. Two age and sex matched controls were also enrolled in each subject. The children were followed-up at a mean age of 1 year. The investigators found that severe RSV bronchiolitis during the first year of life was an important risk factor for the development of recurrent wheezing and sensitization to common allergens during the subsequent years [164].

1.9.1.23 Study on HMPV and RSV as risk factor for childhood asthma in Spain: The study was conducted among a total of 85 children of more than 2 years of age who had a history of viral infection associated hospitalization before the age of 2 years. The study found that hospital admission with RSV-bronchiolitis was associated with increased risk of recurrent wheezing and asthma. The author also found that human metapneumovirus infection in infancy was one of the most significant independent risk factors for the development of pre-school asthma. The association was as strong as the association between RSV-bronchiolitis and subsequent development of asthma (OR=5.2 for hMPV and OR=4.68 for RSV) [165].

1.9.1.24 Study on hMPV, RSV and RV in France: Manoha and colleagues conducted an epidemiological study to compare the clinical features of hMPV, RSV and rhinovirus infections among 931 children less than 3 years of age who required emergency medical care with acute respiratory illness. The investigators found that hMPV and RV were more associated with asthma than RSV. But the study did not provide information on the impact of viral infection on subsequent development of asthma [51].

1.9.1.25 An epidemiological survey in Australia: A total of 73 children less than 5 years of age were enrolled in the study from the emergency department at Royal Children's hospital in Melbourne between June and September, 1998. The investigators reported that children with positive RSV infection were more likely to have more days of cough and wheeze before their hospital attendance than children who were negative. Parents of the RSV-positive group graded the severity of the asthma episodes as significantly worse compared with the RSV-negative group. High rates of RSV infections were reported in exacerbations of asthma or recurrent wheeze in young children during peak RSV season [166].

1.9.1.26 A retrospective cohort study in Qatar: In this retrospective cohort study, a total of 70 infants were selected as index cases who were admitted to Hamad General Hospital in Qatar between January 1988 and June 1989 with RSV associated bronchiolitis. Seventy more children who were admitted to the same hospital unit at the same time but did not have any respiratory illness were selected as the controls. The investigators reported that, 44% subjects in the index groups and 13% in the control group developed wheezing 2-years after hospitalization. Nearly half of the infants hospitalized with RSV associated bronchiolitis developed recurrent episodes of wheezing in 2 years following the hospitalization. This finding was independent of positive family history of atopy [167].

1.9.1.27 A prospective follow-up study in Oakland, California: Adler and colleagues conducted this study to determine the effects of passive exposure to tobacco smoke and RSV-LRTI during infancy on the subsequent evolution of bronchial hyperresponsiveness. A total of 86 cases and 78 controls were recruited during the RSV seasons from November, 1995 through March, 1998. The investigator reported that having RSV associated LRTI during the first 6 months of life did not have any effect on either V'_{maxFRC} or on bronchial responsiveness, assessed by methacholine challenge over the subsequent 12-18 months [168].

1.9.1.28 Population based survey in Oulu, Finland: Dunder and co-investigators conducted a population based survey in Finland to evaluate whether being 0-6 months of age during RSV epidemic had an impact on the use of asthma medication later in the childhood when the subjects were over 3 years of age. The authors did not find any significant difference in consumption of asthma medication between the unexposed and the exposed cohorts [169]. The same group of investigators conducted a prospective cohort study with the blood samples collected from 1084 newborn children during autumn 2001 in Oulu, Finland. The findings suggested that natural differences in innate immunity were responsible for predisposing children to severe RSV infection rather than the infection modifying immune response in childhood [170].

1.9.2 Animal model studies on association between RSV infection and asthma

Several studies which were conducted to reveal the association between RSV infection and subsequent development of asthma or abnormal respiratory functions, using animal models, are very briefly presented below.

1.9.2.1 Persistence of RSV and airway responsiveness in guinea-pigs: Bramley and colleagues found that the guinea-pigs inoculated with RSV had significantly elevated airway hyperresponsiveness and airway eosinophils than the uninfected control animals. The authors concluded that long-term persistence of RSV in guinea-pig lung was associated with airway hyperresponsiveness and airway eosinophilia and these changes might be pertinent to the pathogenesis of post-bronchiolitis wheezing and asthma among children [171].

1.9.2.2 RSV infection and airway hyperresponsiveness in mice: From a study using murine model, Schwarze and colleagues found that previous infection with RSV resulted in enhanced airway responsiveness to methacholine after ovalbumin sensitization. These effects were observed even if the exposure to ovalbumin was initiated after the effects of RSV infection subsided. The investigators concluded that RSV infection resulted in airway hyperresponsiveness in the acute phase and led to changes in immune function that could enhance the effects of airway to antigen after infection [172].

1.9.2.3 Mechanism of RSV associated allergic airway responses in mice: Lukacs and colleagues investigated the ability of an initial RSV infection to exacerbate and promote a more severe asthmatic-type response by using a murine model. The investigators found that RSV infection of the airways could induce an IL-13 dependent change in the airway function and promoted an environment that contributed to the development of severe allergic asthmatic responses [173].

1.9.2.4 Recurrent RSV infection in allergen-sensitized mice: From their study with BALB/c mice, Matususe and colleagues reported that allergen sensitization and RSV infection together enhanced lung inflammation. Recurrent RSV infection was responsible for enhanced chemokine and Th-2 type cytokine response as well as increased production of total serum IgE in allergen sensitized mice. The authors concluded that these observed effects were characteristics of asthma [174].

1.9.2.5 RSV infection in neonates: Seven days old mice were infected with RSV and were allowed to mature to adulthood. These mice were sensitized and challenged with ovalbumin and were subjected to tests for lung function, histopathology, cytokine production and cellularity in bronchoalveolar lavage. The investigators found that RSV infection in neonates alone led to inflammatory airway disease. The pulmonary phenotype was exacerbated if the early RSV infection was followed by allergen exposure. You and colleagues concluded that neonatal RSV exposure resulted in long term pulmonary inflammation and exacerbated allergic airway disease [175].

1.9.2.6 Immune interaction between RSV infection and allergen sensitization: Peebles and colleagues conducted this study to reveal immune response to RSV infection in allergically sensitized and non-sensitized mice. The investigators found that prior RSV infection decreased allergen induced airway hyperresponsiveness, allergen-induced lung eosinophilia and allergen induced production of IL-13 in lung. On the other hand, allergen sensitization before RSV infection increased RSV-induced airway hyperresponsiveness and resulted in increased numbers of lung lymphocytes. Prior sensitization to allergen also decreased the level of RSV induced IFN-γ. The investigators concluded that RSV infection before allergic sensitization protected against development of allergen induced airway hyperresponsiveness and RSV did not directly induce the allergic phenotype [176].

1.9.2.7 Prior immunization with RSV and RSV-enhanced allergic inflammation: Barends and colleagues investigated whether prior infection with RSV enhanced or protected against RSV-enhanced ovalbumin allergy. Increased pulmonary expression of IL-4, IL-5 and IL-13 mRNAs aggravated alveolitis and hypertrophy of mucus producing cells were observed only when the ovalbumin sensitized mice were infected with RSV shortly before or during ovalbumin challenge. Prior inoculation with RSV could not result RSV-enhanced, ovalbumin-induced expression of Th-2 type cytokines in the lung. The investigators concluded that, inoculation with RSV enhanced allergic disease only when the immune system was already primed to Th-2 type response by allergen and RSV-enhanced allergy was not completely abrogated by prior inoculation with RSV [177].

1.10 Study objectives & hypotheses

The purpose of the current research is to determine whether respiratory syncytial virus (RSV) infection within first two years after birth contributes to subsequent development of the asthma among the children in Manitoba. With this objective, the research hypotheses are mentioned as follows:

1. Respiratory syncytial virus (RSV) associated lower respiratory tract infection in first two years of life increases the risk of development of asthma in later stage of life,
2. The risk of development of asthma, as a consequence of RSV-associated LRTI in the first 2 years of life, decreases with increase in age of the infants at the time of RSV-associated lower respiratory tract infection,
3. The risk of development of asthma, as a consequence of RSV-associated LRTI in the first 2 years of life, increases with increase in severity of RSV-associated lower respiratory tract infection,
4. The risk of development of asthma, as a consequence of RSV-associated LRTI in the first 2 years of life, increases with the increase in frequency of RSV-associated lower respiratory tract infection(s),
5. The risk of development of asthma, as a consequence of RSV-associated lower respiratory tract infection in the first 2 years of life, is independent of factors including maternal asthma, gender of the subject, gestational age, number of antibiotic

prescriptions in 1^{st} year, parents' income quintile (child health income quintile) and area of residence (urban/rural).

Methods

2.1 Study population

About 16000 children were born in Manitoba in 1995. The current study was limited to the children of 1995 birth cohort, who were living in Manitoba by December, 2006. The children of this cohort who left Manitoba or who died by this time were excluded from the study. The necessary information about the subjects was retrieved from the Population Health Research Data Repository (PHRDR), maintained by the Manitoba Centre for health Policy (MCHP).

2.2 Sample size

The whole population of the 1995 birth-cohort residing Manitoba by the end of December 2006 was included for this study. So the calculation of sample size was not required. By the end of December 2006, a total of 13,980 children who were born in Manitoba in the year 1995 were still living in Manitoba. Health care information on all of these children was available from the PHRDR.

2.3 Data sources

2.3.1 The Population Health Research Data Repository (PHRDR)

The MCHP provides accurate information in timely manner to health care decision-makers, analysts and health care providers to ensure effective and efficient services to improve the health of Manitobans. The unique PHRDR is the key to describe and explain patterns of care and profiles of illness as well as to explore other factors which influence health, including income, education, employment and social status [178].

Since the 1970s, Manitoba Health has provided copies of computerized health care utilization files to the University of Manitoba. All records deposited in the Repository have been processed by Manitoba Health to remove names and addresses while preserving the capacity to link records together to form individual histories of health care use. Confidentiality is maintained in the Repository through a number of procedures and security measures. No patient names or addresses and no physician names or addresses are contained in the data base. The file layouts are altered so that the University of Manitoba files bear little resemblance to the original data files. In addition, physical access is highly controlled and monitored. The integrity of MCHP's security procedures is regularly verified through security audits [178].

The Population Health Research Data Repository contains anonymized encounter-based records of individuals' interactions with the provincial health care system. It is derived from information contained in the Manitoba Health Services Insurance Plan registry, from health insurance claims routinely filed by physicians and health care facilities with Manitoba Health. Manitoba Health provides MCHP with copies of several files which have been identified as necessary to carry out MCHP deliverables, including the hospital file, medical claims file, long-term care file and the registry. In addition, in recognition of the unique research opportunity that the Repository offers and stringent protocols for protecting data confidentiality, other agencies including the Office of Vital Statistics, and Cancer Care Manitoba also deposit data with MCHP [178].

The entire population of Manitoba is covered in the PHRDR. Individuals covered by the Manitoba Health Services Insurance Plan, migration into and out of the province, and mortality can be traced from 1970 onward. Thus the longitudinal data, which covers multiple years in the same database, allow researchers to study change over time for numerous variables and building histories of individuals is possible. The data repository enhances the ability to study the health of the entire population as the physician claims contains diagnostic information and 90% of the population have physician contacts over a two-year history with more than four visits per year [178].

2.3.2 Public Health Agency of Canada (PHAC)

The Respiratory Virus Detection Surveillance System at the PHAC reports on respiratory viruses in Canada. Each week, selected laboratories report numbers of tests performed and numbers positive for Influenza, Respiratory Syncytial Virus, Parainfluenza, and Adenovirus to the Immunization and Respiratory Infections Division (IRID), Public Health Agency of Canada [179].

A personal communication was made with the PHAC, requesting the respiratory virus isolation reports in Canada. Complete reports on the isolation of the above mentioned respiratory viruses since 1994 to 2006 were sent from Health Canada upon that request. The reports contained the numbers of influenza virus, RSV, parainfluenza viruses and adenoviruses isolated weekly in all of the Canadian provinces over the years mentioned above.

2.4 Defining subjects with asthma and asthma/wheezing phenotypes

2.4.1 Defining subjects with asthma

According to the International Classification of Diseases, version 9, clinical modification (ICD9CM), asthma is coded as 493 [180]. The PHRDR database has the information on prescription, medical claim and hospitalization for specific disease conditions including asthma and respiratory tract infections for the 1995 birth cohort since January 01, 1995. The children of the current study cohort were defined asthmatic if all/any of the following criteria are applicable to them within a time-span of one year since birth until December 2006:

1. At least one diagnosis of asthma (ICD9CM=493) at hospital or
2. At least two diagnoses of asthma (ICD9CM=493) in the medical claim or
3. At least two prescriptions for any asthma drugs including inhaled, injectable or oral bronchodilators; inhaled, oral or injectable steroids, inhaled non-steroid prophylaxis drugs; oral non-steroid prophylaxis drugs.

The above health care database definition of asthma based on prescription and health care utilization has been developed and validated by Kozyrskyj and colleagues [181]. This definition was applied to the study cohort to identify children with asthma at the following points of their lives up to the age of 11 years (December, 2006):

1) Within the first three years of life,
2) At 7 years and (December, 2002)
3) At 11 years (December, 2006).

2.4.2 Defining asthma/wheezing phenotypes

Martinez and colleagues classified wheezing phenotypes as 1) transient early wheezing 2) late-onset wheezing and 3) persistent wheezing. Rusconi and colleagues [182], de Sario and colleagues [183] and Kurukulaaratchy and colleagues [184-186] also used the same classifications for asthma and wheezing phenotypes. The ALSPAC study group has classified wheezing phenotypes as 1) early-onset transient wheezing, 2) Intermediate-onset transient wheezing, 3) early-onset persistent wheezing, 4) intermediate-onset persistent wheezing and 5) late onset-wheezing [187]. Considering these classifications and the age(s) of assessment of asthma status, the following asthma/wheezing phenotypes were defined for the current study cohort:

1) Transient wheeze: Positive diagnosis of asthma-like symptoms within the first 3 years of life but no asthma was detected at 11 years,
2) Late-onset asthma: No positive asthma-like diagnosis within the first 3 years of life and at 7 years but positive diagnosis of asthma at 11 years,
3) Late-transient asthma: No positive asthma-like diagnosis within the first 3 years of life and at 11 years, but positive asthma diagnosis at 7 years,
4) Early persistent asthma: Positive diagnosis of asthma-like symptoms within the first 3 years of life and also at 11 years,
5) Late persistent asthma: No asthma-like diagnosis within the first 3 years of life but positive diagnosis of asthma at 7 years and at 11 years.

In addition to the above mentioned phenotypes at the age of 11 years, there was another group of children who were never asthmatic up to the age of 11 years.

2.5 Defining subjects with RSV associated LRTI

The health care records of the children of the study cohort with lower respiratory tract infection(s) within the first two years of life were extracted from the PHRDR. Health care record(s) for any of the following clinical conditions/diagnoses in the hospital abstract or medical claim were identified as episodes of lower respiratory tract infection(s):

1) Acute bronchitis and bronchiolitis (ICD9CM=466),
2) Viral pneumonia (ICD9CM=480),
3) Pneumococcal pneumonia (ICD9CM=481),
4) Other bacterial pneumonia (ICD9CM=482),
5) Pneumonia due to other specified organisms (ICD9CM=483),
6) Pneumonia in infectious diseases classified elsewhere (ICD9CM=484),
7) Bronchopneumonia organism unspecified (ICD9CM=485),
8) Pneumonia organism unspecified (ICD9CM=486),
9) Influenza (ICD9CM=487),
10) Influenza due to identified avian influenza virus (ICD9CM=488),
11) Bronchitis not specified as acute or chronic (ICD9CM=490) and
12) Chronic bronchitis (ICD9CM=491).

The ICD9CM codes used for the extraction of the health care records (ICD9CM=466, 480-488, 490 and 491) of lower respiratory tract infection included all the confirmed and potential/possible cases of RSV-associated LRTI and all the non-RSV-associated LRTI. A child was identified positive for acute RSV associated bronchiolitis if s/he had at least one diagnosis of ICD9CM=466.11 in the medical claim or hospital abstract. Very similarly, a child was identified positive for RSV associated pneumonia if s/he had at least one diagnosis of ICD9CM=480.1 in the medical claim or hospital abstract. But specific aetiologies (i.e., recorded up to the 5-digits, ICD9CM=466.11) of the clinical conditions were not always recorded or were not always available from the database (i.e., recorded only as ICD9CM=466 or ICD9CM=466.1). A definition was required to identify RSV associated episodes of LRTI from the episodes of lower respiratory tract infections. A total of fourteen definitions were considered to identify subjects with RSV associated lower respiratory tract infections (Appendix B). After careful review of the literature, the following definition was selected to identify subjects with RSV-associated LRTI:

"Any physician visit and/or hospitalization with confirmed (ICD9CM=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated lower respiratory tract infection (ICD9CM=466, 466.0, 466.1, 480, 485, 486, 490 and 491) in RSV season before the age of 2 years."

The definition was expected to identify children with RSV-associated LRTIs, only when the clinical conditions were severe enough to require utilization of health care (i.e., physician visit and/or hospital admission). Considering this aspect of the definition, the episodes which were identified by this definition as RSV-associated LRTIs, were designated as 'clinically significant RSV-associated LRTI' for the current study.

2.6 Defining RSV season

Health Canada publishes respiratory virus isolation report on weekly basis for all the provinces of Canada. From that report it was possible to identify the period of RSV isolation. This report was used to identify the weeks when isolation of RSV started to rise, reached the seasonal peak and started to decline followed by a period when there was almost no isolation of RSV. Using this report and following the definition of RSV season proposed by NREVSS [40] and Terletskaia-Ladwig and co-investigators [39], the onset and offset of RSV seasons for the current study were identified for the years 1995,1996 and 1997. The following definitions of the onset and offset of RSV-seasons were used for the current study:

2.6.1 Onset of RSV-season: The first of two consecutive weeks with at least two samples tested for RSV were positive for the virus in Manitoba and were reported to The Centre for Immunization and Respiratory Infectious Diseases (CIRID), Public Health Agency of Canada.

2.6.2 Offset of RSV-season: The last of the final two consecutive weeks with at least two samples tested for RSV were positive for the virus in Manitoba and were reported to The Centre for Immunization and Respiratory Infectious Diseases (CIRID), Public Health Agency of Canada.

2.6.3 RSV-season: The time period(s) from the onset of the RSV-season(s) to the offset of the RSV-season(s) was defined as the RSV-season(s) for the corresponding years (1995, 1996 and 1997) of the current study.

In the definition of RSV-associated LRTI, the first two years of life was used as the cut-off point. Children of the current study cohort who were born by the end of the year 1995 turned 2 years old by the end of the year 1997. So, the data on the isolation of respiratory viruses from Health Canada was used to identify the RSV seasons for 1995, 1996 and 1997 only.

2.7 Severity categories for RSV-associated LRTI

The majority of children infected with RSV under 1 year of age develop mild upper respiratory tract symptoms but only up to 40% develop lower respiratory tract symptoms and 0.5%-2.0% of all infants require admission to hospital [13]. So the cases of RSV associated LRTIs which require physician visits and result in hospital admissions are expected to be severe. For the current study, the RSV-LRTIs which resulted in utilization of health care system were defined as clinically significant RSV-LRTIs. Particular length of hospital stay can not be used to predict a particular degree of severity of the clinical condition(s), but higher degree of severity is associated with longer hospital stay [188-191]. Considering that, the information on length of hospital stay (LOS) and the type of health care required for RSV-associated LRTI were used to determine the severity categories of the LRTI episodes. The RSV-associated LRTI episodes were classified into the following severity categories for the current study:

1) No RSV-LRTI: No health care for RSV-associated LRTI within the first 2 years of life was required,

2) Mild: Only physician visit was required for RSV-associated LRTI within the first 2 years of life,

3) Moderate: Hospital admission was required with three days or less hospital stay (LOS ≤3 days) for RSV-associated LRTI within the first 2 years of life,

4) Severe: Hospital admission was required with more than three days hospital stay (LOS >3 days) for RSV-associated LRTI within the first 2 years of life.

2.8 Extraction of data from PHRDR

A data extraction request was made to the MCHP to identify the subjects of the current study cohort with asthma from birth until the end of December 2006 and lower respiratory tract infection(s) within the first two years of life. For the extraction, the following criteria were followed:

2.8.1 Subjects with asthma

History of asthma associated physician visit, prescription history of asthma medication and history of asthma associated hospital admission were identified from the MCHP database for children of the study cohort. The database definition of asthma, which was mentioned earlier, was applied to the study cohort and the subjects who were positive for asthma at the following stages of their lives were identified:

1) Before the third birthday,

2) At the age of 7 years (December, 2002) and

3) At the age of 11 years (December, 2006).

2.8.2 Subjects with LRTI

To identify subjects with lower respiratory tract infections, which obviously include RSV-associated lower respiratory tract infections, health care records of children of the study cohort for the following ICD9 codes within the first two years of life were identified from the PHRDR:

1) Acute bronchitis and bronchiolitis (ICD9CM=466)
2) Viral pneumonia (ICD9CM=480)
3) Pneumococcal pneumonia (ICD9CM=481)
4) Other bacterial pneumonia (ICD9CM=482)
5) Pneumonia due to other specified organism (ICD9CM=483)
6) Pneumonia in infectious diseases classified elsewhere (ICD9CM=484)
7) Bronchopneumonia organism unspecified (ICD9CM=485)
8) Pneumonia organism unspecified (ICD9CM=486)
9) Influenza (ICD9CM=487)
10) Influenza due to identified avian influenza virus (ICD9CM=488)
11) Bronchitis not specified as acute or chronic (ICD9CM=490 and)
12) Chronic bronchitis (ICD9CM=491).

2.9 Parental asthma

Parental history of asthma associated utilization of health care was determined from the MCHP database. The parent(s) of the subject was defined asthmatic if he/she required at least one hospitalization or at least one physician visit for asthma (ICD9CM=493) between 1990 and 1995 or at least one asthma drug prescription (excluding oral steroids) in 1995.

2.10 Maternal stress

The mother of the child was defined as stressed if she received at least one anxiolytic (ATC=N05B) or hypnotic/sedative (ATC=N05C) or antidepressant (ATC=N06A) or had at least one diagnosis of episodic mood disorder (ICD9CM=296) or anxiety, dissociative & somatoform disorder (ICD9CM=300) or adjustment reaction (ICD9CM=309) or depressive disorder (not classified elsewhere) (ICD9CM=311). This condition was assessed between January 01, 1995 and child's first birthday, between child's 1st and 4th birthday and between child's 5th and 8th birthday.

2.11 Urban/rural residence

Urban and rural areas were defined on the basis of population concentration and population density based on the previous census. The areas with a minimum population concentration of 1000 and a population density of at least 400/square kilometre were considered as urban. The territories outside the urban areas were considered as rural.

Winnipeg and Brandon were defined as urban areas and all other regional health authorities in Manitoba were classified as rural.

2.12 Northern RHAs

All the regional health authorities of Burntwood, Norman and Churchill were designated as Northern Regional Health Authorities (RHAs).

2.13 Parent's income (child health income quintile)

The income quintiles were created separately among the urban (Winnipeg and Brandon, U1-U5) and rural (other areas in Manitoba, R1-R5) population for individual year based on public use census files developed by Statistics Canada. Instead of reflecting an individual income, the 'income' represents an area-level income measure since each person living in an area is attributed the average household income of the area [192]. The mean income of U1 and R1 groups in 1995 were below 30,000/year which was used as the first cut-off point by Statistics Canada in its attempt to define low-income groups [193, 194]. The lowest income quintiles in the urban areas and rural areas were combined together and designated as 'low' income group and the rest of the income quintiles (U2 to U5 for urban and R2 to R5 for rural areas) were combined together to form 'high' income group for this current study.

2.14 Execution of the study

This study was conducted as a historical-cohort study. The subjects with asthma and lower respiratory tract infection (including clinically significant RSV-associated LRTI) were identified according to the criteria mentioned for the data extraction from the PHRDR. The database provided information associated with the episodes of health care utilization including the date of the physician visit or hospitalization, date of discharge from the hospital and requirement of emergency hospital admission. The database also provided the demographic information of the subjects including the number of siblings, the location of residence and socio-economic condition of parents. Information on antibiotic prescription history, BCG immunization and paternal history of asthma was also available from the PHRDR.

New variables were created from the existing information to address the research questions. The association between RSV infection in early childhood and subsequent development of asthma was analyzed considering the possible influences of confounding variables mentioned in Table 2.1.

The date of first onset of the asthma was compared to the history of clinically significant LRTI (all aetiology) and clinically significant RSV-associated LRTI (exclusively RSV aetiology) to determine the temporal relationship between the infections and onset of asthma. The children who had asthma (outcome) onset before having clinically significant RSV-associated LRTI (exposure) were not included in the final analyses of the study.

Table 2.1: Explanatory variables.

Measure	**Operational definition**
RSV infection in 2 years	If the child had RSV-LRTI associated utilization of health care before the age of 2 years
Frequency of RSV infection in 2 years	Number of RSV-LRTI associated health care in first 2 years of life
Types of RSV care (severity categories)	The type of health care required for RSV-LRTI, the care required was used as a measure of severity [i.e.: hospital admission with long (>3 days, which was median LOS) stay represented most severe and only physician visit represented the least severe RSV-LRTI]
Recurrent RSV infection in 2 years	If the child had more than one clinically significant RSV-LRTI within the first 2 years of life
Recurrent hospitalization in 2 years	If the child required to be admitted at the hospital with RSV-LRTI for more than once within the first 2 years of life
Age-groups at 1st RSV-associated care	Age-group of the children at the time of first RSV-LRTI associated utilization of health care
Age-groups at 1st RSV-associated hospitalization	Age-group of the children at the time of first RSV-LRTI associated hospital admission
BCG vaccination in 2 years	Completion of 2 year BCG vaccination schedule
Urban/rural residence	Residence of the infant in urban or rural region

Measure	Operational definition
Living north	If the child is living in either of the 3 northern RHAs
Gender	Gender of the infant
Parental asthma history in 1995	Asthma status of the parents in 1995 (the year of children's birth)
Maternal stress	Maternal stress from January, 1995
Having ≥2 sibling in 2002	Whether the child had more than one sibling by the end of 2002
Income (child health income quintile)	Income level of the parent ('low' or 'high' as defined for the study)
Prematurity	Gestational age ≤36 weeks
Low birth-weight	Birth-weight ≤2499 grams
Antibiotic use in 1st year	Receiving antibiotic(s) (# of antibiotics received) in the first year of life

To address the first hypothesis, lower respiratory tract infection by respiratory syncytial virus (RSV) in first two years of life increases the risk of developing asthma in later stage of life, asthma status of the children with clinically significant RSV-associated LRTI in the first two years of life was compared with asthma status of those who did not have any RSV-associated LRTI within first 2 years of life. The likelihood of asthma at the age of 7 years (end of December 2002) and 11 years (end of December 2006) among the children in the study following the RSV infection in the first two years of life was determined as odds-ratio after adjusting for the possible confounders. Multivariate logistic regression was conducted to adjust for the effects of the confounding variables on the asthma status of the subjects. The influences of clinically significant RSV-associated LRTI within the first 2 years of life on asthma/wheezing phenotypes were also determined following a very similar procedure.

To address the second hypothesis, the risk of developing childhood asthma decreases with increase in age of the infants at the time of first RSV-associated LRTI within the first two years of life, subjects were divided into several age-groups on the basis of their age at the time of first clinically significant RSV-associated LRTI. Asthma status of children in different age-groups at the age of 7 and 11 years were compared with children who did not have clinically significant RSV-LRTI within first 2 years. Multivariate logistic regression analysis was conducted to determine the effect of age at the time of first RSV-associated LRTI on the development of asthma later in childhood after adjusting for confounders. The influences of age-group at the time of first clinically significant RSV-associated LRTI within the first 2 years of life on asthma/wheezing phenotypes were also determined following a very similar procedure.

To address the third hypothesis, the risk of developing asthma increases with the increase in severity of lower respiratory tract infection by RSV, the subjects were divided into several severity categories. Asthma status of the children in different severity categories at the age of 7 and 11 years were compared with children who did not have clinically significant RSV-LRTI within first 2 years of life. Multivariate logistic regression analysis was conducted to determine the effect of severity of RSV-associated LRTI on development of childhood asthma after adjustment for confounders. The influences of severity of clinically significant RSV-associated LRTI within the first 2 years of life on asthma/wheezing phenotypes were also determined following a very similar procedure.

To address the fourth hypothesis, the risk of developing asthma increases with increase in the frequency of lower respiratory tract infection(s) by RSV in the first two years of life, the subjects were divided into three categories on the basis of number of RSV infections in the first 2 years of life. Asthma status of children in these categories at the age of 7 and 11 years were compared to those who did not have clinically significant RSV-LRTI before the age of 2 years. The effect of number (frequency) of RSV-associated LRTI episodes on the development of childhood asthma was determined after adjustment for confounding factors in multivariate logistic regression analysis. The influences of frequency of clinically significant RSV-associated LRTI within the first 2 years of life on asthma/wheezing phenotypes were also determined following a very similar procedure.

The fifth hypothesis of the study stated that the risk of developing asthma as a consequence of RSV-associated LRTI was independent of confounding factors including maternal asthma, gender of the subject, gestational age, number of antibiotic prescriptions, parents' income quintile (child health income quintile) and area of residence (urban/rural). To address the first four hypotheses, multivariate logistic regression analyses were run to determine the effects of main explanatory variables on the outcome. Along with the main explanatory variables, all of these multivariate logistic regression models also included the above mentioned confounding factors to adjust for their influences. So, the associations between the outcome and the main explanatory variables revealed from these multivariate models were independent of the influences of these confounding factors.

Multivariate logistic regression analysis was proposed for most of the analyses. This approach weighted the impact of the confounding factors against the exposure variable of concern and identified if the exposure (clinically significant RSV-associated LRTI) had any significant influence on the outcome (development of childhood asthma and asthma/wheezing phenotypes). Odds ratio and adjusted odds ratio provided the likelihood of developing childhood asthma among subjects with RSV-associated LRTI against those without the infection.

Initially univariate logistic regression analyses were run with all the eligible children of the study to determine the relation between the factors under consideration and the asthma status of the children at 7 and 11 years (as well as the asthma/wheezing phenotypes). The factors which were not significantly associated with asthma and asthma/wheezing phenotypes at these ages were excluded from the multivariate logistic regression equations. Multivariate logistic regression equations with backward selection method were run to determine the effects of the significant factors and the effects were reported as odds ratio with 95% confidence intervals.

Maternal asthma has been cited as very important risk factor for the development of childhood asthma. The children in the current study were stratified into children with and without maternal history of asthma. Univariate logistic regression analyses were run with both the strata to determine the relation between the confounding factors and the asthma status at 7 years and 11 years for children with and without maternal history of asthma. Univariate logistic regressions were also run to determine the relation between the confounding factors and asthma/wheezing phenotypes for children with and without maternal history of asthma.

Instead of laboratory based diagnosis, a database definition of clinically significant RSV-associated LRTI was used to identify the cases of the current study. Content validity and criterion related validity were considered appropriate approaches for the validation of the definition of RSV-associated LRTI for the current study and construct validity was not within the scope of the current study. To determine the content/face validity of the definition, different aspects of the definition were discussed under the light of current knowledge from earlier studies to justify the inclusion of those aspects in the definition. To determine the criterion related validity of the definition, descriptive analyses were run on several parameters (including prematurity, socio-demographic characteristics and residence in northern RHAs as a proxy measure of ethnicity). Results from those analyses were compared to results on those parameters reported from studies conducted earlier. The similarities and/or discrepancies were discussed to justify the concurrent validity of the definition. Similar analyses were also conducted for overall LRTI (all aetiology) in addition to RSV-associated LRTI only.

2.15 Data analysis

The data was analyzed by SAS software (version 9.1) at MCHP. The likelihoods of asthma at 7 years and at 11 years (as well as asthma/wheezing phenotypes) among children of the study cohort following the RSV-associated LRTI in the first two years of life were determined as odds-ratio after adjusting for the possible confounders. The confidence level was set at 95%. Multivariate logistic regression analyses were conducted to adjust for the effects of the confounding variables. The closely related variables were tested for collinearity. To compare the asthma status of subjects to address the research hypotheses, some categorical variables were created from continuous variables (for example, age-groups from age of subjects in days). As mentioned earlier, only the significant variables in the univariate logistic regression analyses were included for the multivariate logistic regression. If a variable was not statistically significant ($p \geq 0.05$), it was not included in the multivariate model.

2.16 Ethical considerations

The study has been approved by Bannatyne Campus Research Ethics Board (REB), University of Manitoba (Protocol Reference Number: H2006:016) and Health Information Privacy Committee (HIPC), Manitoba Health, (reference file number: HIPC #: 2005/2006 -30).

The PHRDR databases use unique scrambled personal identification number (PHIN) that can not be traced back to the subjects. The datasets did not contain any identifiable personal information, for example, name and address of individual user of health care system or their physicians. No personal contact was made with any subject and the study did not involve any invasive procedure.

The analyses were conducted under the secure computing environment at MCHP. Strict security measures including multiple passwords, encryption of information when transmitted and firewalls were in place to protect the data files. Destruction and/or storage of the study data was in accordance with MCHP's policies.

Results

Asthma and asthma/wheezing phenotypes

3.1 Study cohort at the end of 2006

As mentioned in the methods, the asthma/wheezing status of the subjects was assessed using the hospitalization records, medical claim and prescription history within the first three years of life, at the end of 2002 when the subjects were 7 years old and at the end of 2006 when the subjects were 11 years old. Depending on the asthma/wheezing status at these points, the phenotypes of asthma/wheezing for the subjects were identified as well. According to the definitions, a total of 917 (6.56% of 13980) children of the current study cohort had current asthma when they were 7 years of age, a total of 910 children (6.51%) had current asthma when they were 11 years of age and a total of 2643 children (18.91%) ever had asthma/wheezing at some point(s) of their lives up to the age of 11 years. Table 5 (Appendix) presents the characteristics of the children of the study cohort and their asthma/wheezing status up to the age of 11 years.

Out of 13980 children of the study cohort, a total of 4117 had clinically significant RSV-associated lower respiratory tract infection before the age of 2 years which required utilization of health care. Three hundred and seventy eight children of the study cohort were diagnosed with asthma before they had clinically significant RSV-associated LRTI. Since these children had outcome of interest (asthma) before the exposure (clinically significant RSV-associated LRTI), they were excluded from the final analyses. A total of 13602 children were considered eligible for the final analyses of the study.

3.2 Asthma at 7 years (December, 2002)

A total of 13602 children of the current study cohort were considered eligible for the final analyses, 3739 of these children required RSV-LRTI associated utilization of health care before the age of 2 years and 852 (6.26%) children were diagnosed with current asthma when they were 7 years of age. Univariate logistic regression analyses were run to determine the associations between asthma at 7 years and potential contributing factors. The associations between these potential contributing factors and the primary exposure variable (RSV-LRTI) were also determined. The results of the associations are presented in Table 6 (Appendix).

Univariate logistic regression showed that children with RSV-associated lower respiratory tract infection within the first two years of their lives were more likely to be asthmatic at the age of 7 years than children who did not have that infection in the first 2 years of life (OR= 1.58, 95% CI=1.37-1.83). The association was significant for children without a maternal history of asthma (OR= 1.58, 95% CI=1.35-1.85) but not significant for children with a maternal history of asthma (OR= 1.36, 95% CI=0.964-1.91). After adjustment for other factors in multivariate logistic regression, having RSV-associated LRTI within the first two years of life remained as a significant risk factor of asthma at the age of 7 years (Table 3.1).

Table 3.1: Association between risk of asthma diagnosis at 7 years and explanatory variables (multivariate logistic regression), with clinically significant RSV-LRTI.

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
RSV-LRTI in 2 years					
• Yes	3717	311	8.37	1.36 (1.17-1.59)	<.0001
• No RSV-LRTI (ref.)	9803	535	5.46		
Residence					
• Urban	7764	599	7.72	1.61 (1.37-1.90)	<.0001
• Rural (ref.)	5756	247	4.29		
Living north					
• Yes	1376	41	2.98	0.68 (0.48-0.96)	0.0270
• No (ref.)	12144	805	6.63		
Gender					
• Male	6829	546	8.00	1.79 (1.55-2.07)	<.0001
• Female (ref.)	6691	300	4.48		
Asthmatic mother					
• Yes	1374	156	11.35	1.86 (1.54-2.25)	<.0001
• No (ref.)	12146	690	5.68		
Maternal stress					
• Stressed	2536	202	7.97	1.20 (1.01-1.42)	0.0337
• Not stressed (ref.)	10984	644	5.86		

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
No. of sibling in 2002					
• ≥2 sibling	6752	339	5.02	0.69 (0.60-0.80)	<.0001
• 0/1 sibling (ref.)	6768	507	7.49		
Low birth-weight					
• Yes	657	54	8.22	1.36 (1.01-1.82)	0.0403
• No (ref.)	12863	792	6.16		
Antibiotic received in 1st year					
• 1-2 antibiotics	5056	316	6.25	1.31 (1.09-1.57)	0.0038
• 3-4 antibiotics	2177	163	7.49	1.50 (1.20-1.86)	0.0003
• >4 antibiotics	1645	160	9.73	1.97 (1.57-2.47)	<.0001
• No antibiotic (ref.)	4642	207	4.46		

*82 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.2.1 Frequency of RSV-LRTI and asthma at 7 years

From univariate logistic regression it appeared that frequency of clinically significant RSV-LRTI was associated with risk of asthma diagnosis at 7 years as higher number of infection was associated with higher risk. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.2). Having 1-2 clinically significant RSV-LRTI within first two years of life was not associated with higher risk of asthma at 7 years for children with a maternal history of asthma, but having three or more clinically significant RSV-LRTIs was a significant risk factor for this group of children. Though having 1-2 RSV-LRTI or more than 2 RSV-LRTI within first 2 years of life was associated with higher risk of asthma diagnosis at 7 years, the risks of asthma diagnosis at 7 years between these two categories (1-2 RSV-LRTI vs. ≥ 3 RSV-LRTI) were not significantly different.

Table 3.2: Association between risk of asthma diagnosis at 7 years and explanatory variables (multivariate logistic regression), with frequency of RSV-LRTI.

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
Frequency of RSV infection in 2 years					
• 1-2 infection(s)	2863	229	8.00	1.30 (1.10-1.54)	0.0021
• ≥ 3 infections	854	82	9.60	1.61 (1.24-2.08)	0.0003
• No RSV-LRTI (ref.)	9803	535	5.46		
Residence					
• Urban	7764	599	7.72	1.62 (1.37-1.91)	<.0001
• Rural (ref.)	5756	247	4.29		
Living north					
• Yes	1376	41	2.98	0.68 (0.48-0.95)	0.0245
• No (ref.)	12144	805	6.63		
Gender					
• Male	6829	546	8.00	1.79 (1.54-2.07)	<.0001
• Female (ref.)	6691	300	4.48		
Asthmatic mother					
• Yes	1374	156	11.35	1.86 (1.54-2.24)	<.0001
• No (ref.)	12146	690	5.68		
Maternal stress					
• Stressed	2536	202	7.97	1.19 (1.01-1.41)	0.0384
• Not stressed (ref.)	10984	644	5.86		

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
No. of sibling in 2002					
• ≥2 sibling	6752	339	5.02	0.69 (0.59-0.80)	<.0001
• 0/1 sibling (ref.)	6768	507	7.49		
Low birth-weight					
• Yes	657	54	8.22	1.35 (1.01-1.82)	0.0425
• No (ref.)	12863	792	6.16		
Antibiotic received in 1st year					
• 1-2 antibiotics	5056	316	6.25	1.31 (1.09-1.58)	0.0036
• 3-4 antibiotics	2177	163	7.49	1.49 (1.20-1.85)	0.0003
• >4 antibiotics	1645	160	9.73	1.94 (1.55-2.44)	<.0001
• No antibiotic (ref.)	4642	207	4.46		

*82 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.2.2 Age at 1st RSV-LRTI care and asthma at 7 years

The mean age of having first clinically significant RSV-associated LRTI for subjects who were not asthmatic at 7 years was 270 days (std ±186 days, median 229 days) and for the subjects who were asthmatic at 7 years was 264 days (std ±173 days, median 232 days). Difference in age at the time of first clinically significant RSV-associated LRTI between children who were asthmatic and who were not asthmatic at 7 years was not statistically significant. Univariate logistic regression suggested that having first clinically significant RSV-associated LRTI before the age of 6 months was not associated with risk of asthma diagnosis at 7 years. But when adjusted for other factors in multivariate logistic regression, first clinically significant RSV-LRTI in any age-group appeared to be associated with higher risk of asthma diagnosis at 7 years in comparison to children who did not have clinically significant RSV-LRTI within the first 2 years of life (Table 3.3).

The risk of asthma diagnosis at 7 years was not significantly different between age-groups at the time of first clinically significant RSV-associated LRTI (i.e., between ≤6 months, >6-12 months and >12-24 months). First clinically significant RSV-associated LRTI between 6 and 12 months appeared to be associated with the highest risk of asthma at 7 years. The following graph (Figure 3.1) shows the distributions of asthmatic children in different age-groups at the time of first clinically significant RSV-LRTI.

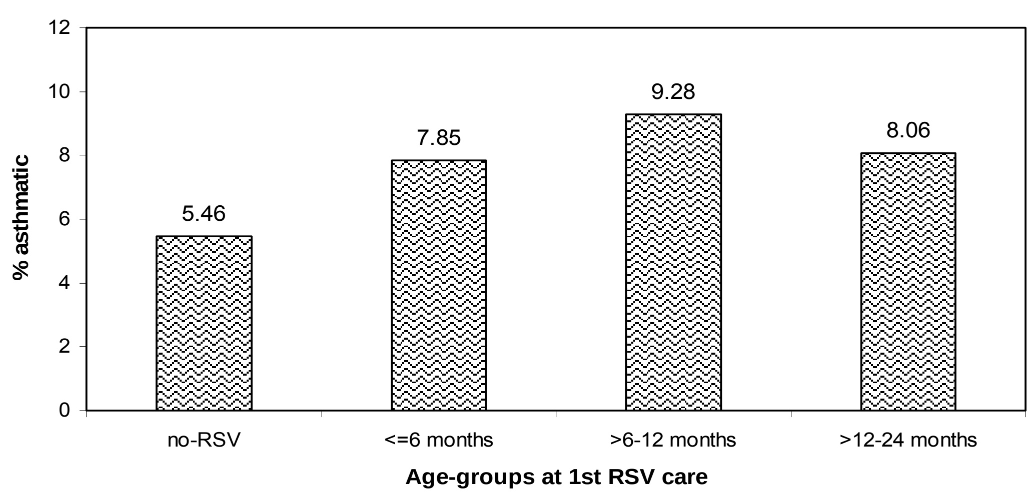

Figure 3.1: Proportion of asthmatic children (at 7 years) in different age-groups at 1st clinically significant RSV-LRTI.

Table 3.3: Association between risk of asthma diagnosis at 7 years and explanatory variables (multivariate logistic regression), with age-group at 1st RSV-LRTI.

Features/variables	**Asthma at 7 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Age-groups at 1st RSV-associated care					
• ≤6 months	1491	117	7.85	1.32 (1.06-1.65)	0.0120
• >6 to 12 months	1196	111	9.28	1.43 (1.14-1.79)	0.0018
• >12 to 24 months	1030	83	8.06	1.34 (1.05-1.71)	0.0181
• No RSV-care (ref.)	9803	535	5.46		
Residence					
• Urban	7764	599	7.72	1.61 (1.37-1.90)	<.0001
• Rural (ref.)	5756	247	4.29		
Living north					
• Yes	1376	41	2.98	0.68 (0.48-0.96)	0.0284
• No (ref.)	12144	805	6.63		
Gender					
• Male	6829	546	8.00	1.79 (1.55-2.07)	<.0001
• Female (ref.)	6691	300	4.48		
Asthmatic mother					
• Yes	1374	156	11.35	1.86 (1.54-2.24)	<.0001
• No (ref.)	12146	690	5.68		
Maternal stress					
• Stressed	2536	202	7.97	1.20 (1.02-1.42)	0.0323
• Not stressed (ref.)	10984	644	5.86		

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
No. of sibling in 2002					
• ≥2 sibling	6752	339	5.02	0.69 (0.60-0.80)	<.0001
• 0/1 sibling (ref.)	6768	507	7.49		
Low birth-weight					
• Yes	657	54	8.22	1.36 (1.01-1.82)	0.0406
• No (ref.)	12863	792	6.16		
Antibiotic received in 1st year					
• 1-2 antibiotics	5056	316	6.25	1.31 (1.09-1.57)	0.0041
• 3-4 antibiotics	2177	163	7.49	1.49 (1.20-1.86)	0.0003
• >4 antibiotics	1645	160	9.73	1.96 (1.56-2.46)	<.0001
• No antibiotic (ref.)	4642	207	4.46		

*82 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.2.3 Severity of RSV-LRTI and asthma at 7 years

The subjects were divided into four severity categories depending on the required health care for RSV-associated lower respiratory tract infection: 1) no RSV-LRTI, 2) mild RSV-LRTI, 3) moderate RSV-LRTI and 4) severe RSV-LRTI in the first 2 years of life. Univariate logistic regression suggested that all the severity categories were associated with higher risk of asthma at 7 years than children who did not have clinically significant

RSV-LRTI within first 2 years of life. Children who had moderate clinically significant RSV-LRTI before the age of 2 years were at the highest risk of asthma at 7 years. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.4).

The risk of asthma at 7 years was significantly higher for children who had moderate RSV-associated LRTI than children who had mild RSV-associated LRTI within first 2 years of life. No other significant difference in the risk of asthma at 7 years between the severity categories (i.e., severe vs. mild and severe vs. moderate) was detected for the study cohort.

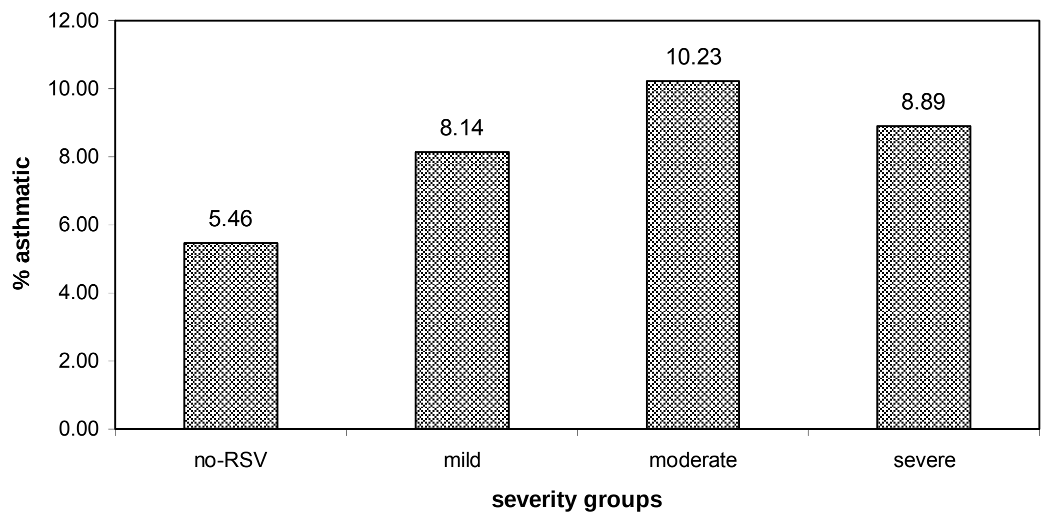

Figure 3.2: Proportions of asthmatic children at 7 years in different severity groups.

Table 3.4: Association between risk of asthma diagnosis at 7 years and explanatory variables (multivariate logistic regression), with severity of RSV-LRTI.

Features/variables	**Asthma at 7 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Severity of RSV-LRTI in 2 years					
• Mild	3144	256	8.14	1.29 (1.09-1.51)	0.0023
• Moderate	303	31	10.23	2.08 (1.40-3.10)	0.0003
• Severe	270	24	8.89	1.89 (1.21-2.95)	0.0049
• No RSV in 2 years (ref.)	9803	535	5.46		
Residence					
• Urban	7764	599	7.72	1.65 (1.39-1.95)	<.0001
• Rural (ref.)	5756	247	4.29		
Living north					
• Yes	1376	41	2.98	0.66 (0.47-0.93)	0.0184
• No (ref.)	12144	805	6.63		
Gender					
• Male	6829	546	8.00	1.78 (1.54-2.07)	<.0001
• Female (ref.)	6691	300	4.48		
Asthmatic mother					
• Yes	1374	156	11.35	1.87 (1.55-2.25)	<.0001
• No (ref.)	12146	690	5.68		
Maternal stress					
• Stressed	2536	202	7.97	1.20 (1.01-1.42)	0.0354
• Not stressed (ref.)	10984	644	5.86		

Features/variables	Asthma at 7 years				
	No.	n	%	OR (95% CI)	P-value
No. of sibling in 2002					
• ≥2 sibling	6752	339	5.02	0.68 (0.59-0.79)	<.0001
• 0/1 sibling (ref.)	6768	507	7.49		
Antibiotic received in 1st year					
• 1-2 antibiotics	5056	316	6.25	1.31 (1.09-1.57)	0.0038
• 3-4 antibiotics	2177	163	7.49	1.49 (1.20-1.85)	0.0004
• >4 antibiotics	1645	160	9.73	1.94 (1.55-2.44)	<.0001
• No antibiotic (ref.)	4642	207	4.46		

*82 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.2.4 Confounding factors and asthma at 7 years

The associations between several confounding factors and childhood asthma are well documented in currently available literature. The associations between these factors (Appendix B) and risk of asthma diagnosis at the age of 7 years were analyzed for the current study cohort as well. The results are briefly mentioned in the following section.

3.2.4.1 Gender and family history

Univariate logistic regression suggested that male children were significantly at higher risk of asthma at the age of 7 years, which was equally applicable for children with or without maternal history of asthma. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.2).

Maternal asthma and maternal stress were associated with higher risk of asthma at 7 years (Table 3.2). In multivariate logistic regression, a total of 4933 observations were excluded because of missing information on paternal history of asthma. With the available information it appeared that the highest risk of asthma diagnosis at 7 years was associated with both parents being asthmatic, followed by only mother being asthmatic and only father being asthmatic (Figure 3.3).

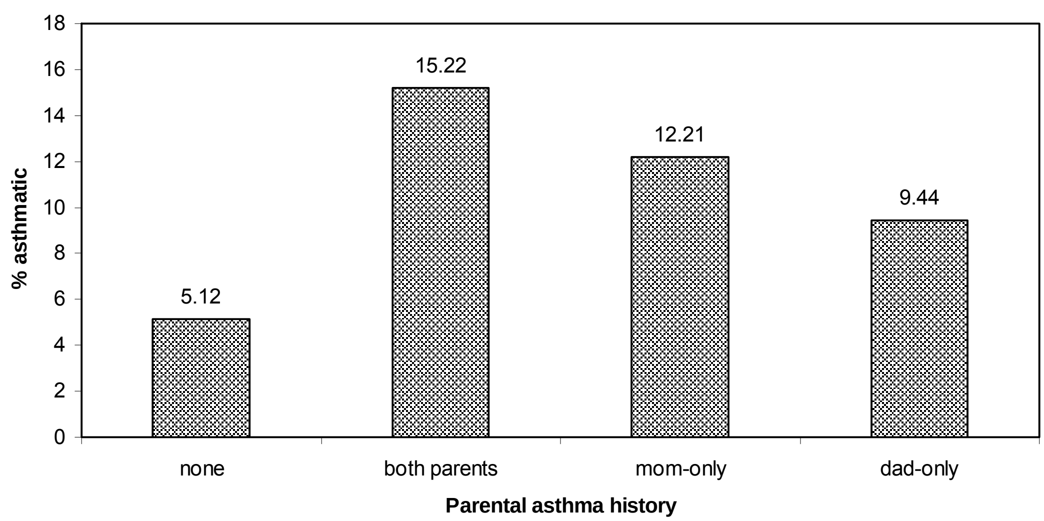

Figure 3.3: Proportions of asthmatic children at 7 years with parental history of asthma.

Analyses were carried out to find the sibling effect on the asthma status of children of the current study cohort. Univariate logistic regression suggested that children who had two or more siblings at the end of 2002 were less likely to be asthmatic at the age of 7 years than children who had either one or no sibling. In univariate logistic regression, this association was similar for children with and without maternal history of asthma. Having two or more siblings in 2002 remained significantly associated with less risk of having asthma at the age of 7 years after adjustment for other factors in multivariate logistic regression (Table 3.2).

3.2.4.2 Income

Univariate logistic regression suggested that parents' income level did not influence the asthma status of the children at the age of 7 years (Table 6, Appendix). Same association was found for children with and without maternal history of asthma. For further investigating the influence of income on the asthma status of the children of the study cohort, asthma status at 7 years of the children from the lowest income quintile was compared with children of each of the higher income quintiles (quintile-2, quintile-3, quintile-4 and quintile-5). Univariate logistic regression suggested that the asthma status of the children of parents in the lowest income quintile was not significantly different from asthma status of the children from the parents in the higher income quintiles at the age of 7 years.

3.2.4.3 Prematurity

Information on the gestational age was available for 13593 of the children who were eligible for the final analyses of the study. The median gestational age of the children who were asthmatic at 7 years was 39 weeks (mean=38.97 weeks, std±2.31 weeks) and the median gestational age of the children who were not asthmatic was 40 weeks (mean=39.19 weeks, std±1.98 weeks). Univariate logistic regression suggested that the gestational age of the asthmatic children was significantly less than the gestational age of non-asthmatic children. Univariate logistic regression also indicated that premature children were more likely to be asthmatic at 7 years. But multivariate logistic regression revealed that gestational age (prematurity) was not a significant risk factor of asthma at 7 years when adjusted for other variables.

Birth weight information was available for 13598 of the children who were eligible for the final analyses of the study. Median birth-weight of the children who were asthmatic at the age of 7 yeas was 3428 gm (mean=3395.26 gm, std±596.73 gm) and the median birth-weight of the children who were not asthmatic at the age of 7 years was 3479 gm (mean 3452.37 gm, std±574.91 gm). Univariate logistic regression suggested that asthmatic children at 7 years had significantly less birth-weight than non-asthmatic children and low birth-weight was a significant risk factor for asthma diagnosis at 7 years. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.2).

When the children were stratified according to maternal history of asthma, neither prematurity nor low birth-weight was associated with risk of asthma diagnosis at 7 years for children with or without maternal history of asthma.

3.2.4.4 BCG vaccination history

Univariate logistic regression suggested that completion of BCG vaccination within the first two years of life did not influence the asthma status of the children at 7 years (Table 6, Appendix). This finding was essentially the same for children with or without maternal history of asthma.

3.2.4.5 Antibiotic use in the first year of life

Univariate logistic regression revealed that children who received at least one antibiotic in the first year of life were significantly at higher risk of being asthmatic at the age of 7 years (Table 6, Appendix). When the number of antibiotic prescriptions in the first year and risk of asthma diagnosis at the age of 7 years were considered, it was found that higher number of antibiotic prescriptions in the first year of life was associated with higher risk of asthma diagnosis at 7 years. Children who received more than four antibiotics in the first year of life were as much as twice more likely to be diagnosed with asthma at 7 years than children who did not receive any antibiotic in their first year. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.2).

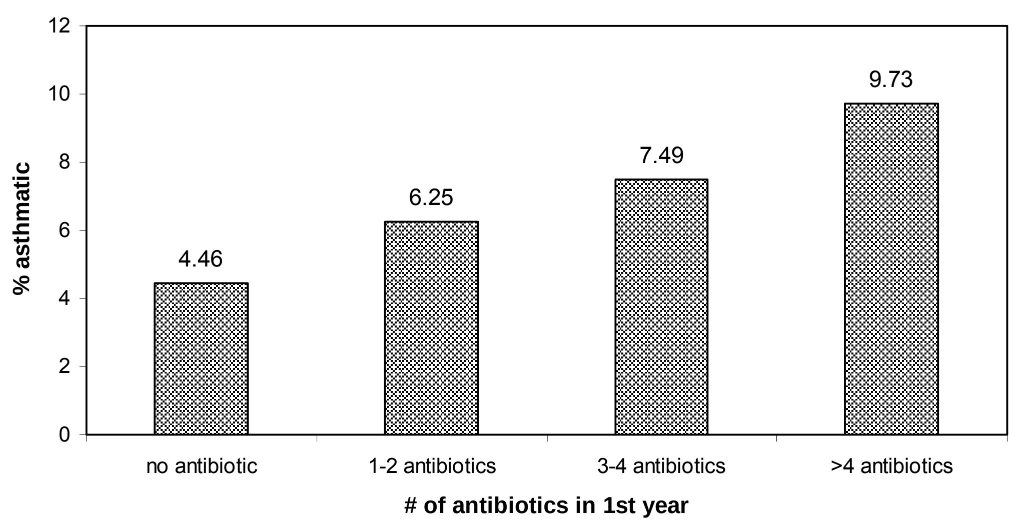

Figure 3.4: Overall antibiotic use in 1st years of life and asthma at 7 years.

Children who received four or more antibiotics in the first year of life were 47% more likely than children who received 1-2 antibiotics and 30% more likely than children who received 3-4 antibiotics to have asthma diagnosis at 7 years. In stratified univariate logistic regression, higher number of antibiotics in the first year of life was associated with higher risk of asthma at 7 years for children with and without maternal history of asthma.

3.2.4.6 Urban/rural residence

Children living in rural areas were less likely to be diagnosed with asthma at 7 years than children living in urban areas of Manitoba (Table 6, Appendix). This was applicable for children without maternal history of asthma but not for children with maternal history of asthma. Children living in three northern RHAs were less likely to be asthmatic at 7 years in comparison to the children who were not living in those RHAs (Table 6, Appendix). This association was very similar for children with and without maternal history of asthma. Living in rural areas or living in three northern regional health authorities remained significantly associated with less risk of asthma diagnosis at the age of 7 years after adjustment for other factors in multivariate logistic regression analysis (Table 3.2).

3.3 Asthma at 11 years (December, 2006)

Out of total 13980 children of the study cohort, a total of 910 children (6.51%) were asthmatic at the age of 11. After the exclusion of the 378 children who had asthma onset before having clinically significant RSV-associated LRTI, a total of 13602 children were considered for the final analyses of the study. Among these children, a total of 3739 children had clinically significant RSV-associated LRTI before the age of 2 years and 856 children (6.29%) were asthmatic at the age of 11 years. Univariate logistic regression analyses were run to determine the associations between asthma at 11 years and potential contributing factors. The associations between these potential contributing factors and the primary exposure variable (clinically significant RSV-LRTI) were also analysed. The results of these associations are presented in Table 7 (Appendix).

Univariate logistic regression suggested that children who had clinically significant RSV-associated lower respiratory tract infection within the first two years of their lives were more likely to have asthma at the age of 11 years. But this factor was not significant for children with maternal history of asthma. When controlled for other factors in multivariate logistic regression, having clinically significant RSV-LRTI before the age of 2 years remained significantly associated with risk of asthma diagnosis at 11 years (Table 3.5).

Table 3.5: Association between risk of asthma diagnosis at 11 years and explanatory variables (multivariate logistic regression), with clinically significant RSV-LRTI.

Features/variables	**Asthma at 11 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
RSV-LRTI in 2 years					
• Yes	3721	288	7.74	1.26 (1.08-1.47)	0.0038
• No RSV-LRTI (ref.)	9811	567	5.78		
Residence					
• Urban	7769	591	7.61	1.59 (1.36-1.85)	<.0001
• Rural (ref.)	5763	264	4.58		
Gender					
• Male	6837	520	7.61	1.54 (1.33-1.77)	<.0001
• Female (ref.)	6695	335	5.00		
Asthmatic mother					
• Yes	1375	160	11.64	2.02 (1.68-2.42)	<.0001
• No (ref.)	12157	695	5.72		
No. of sibling in 2002					
• ≥2 sibling	6760	344	5.09	0.70 (0.60-0.81)	<.0001
• 0/1 sibling (ref.)	6772	511	7.55		

Features/variables	Asthma at 11 years				
	No.	n	%	OR (95% CI)	P-value
Antibiotic received in 1st year					
• 1-2 antibiotics	5062	336	6.64	1.26 (1.06-1.50)	0.0105
• 3-4 antibiotics	2179	147	6.75	1.22 (0.98-1.51)	0.0795
• >4 antibiotics	1646	137	8.32	1.51 (1.20-1.90)	0.0004
• No antibiotic (ref.)	4645	235	5.06		

*70 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.3.1 Frequency of RSV-LRTI and asthma at 11 years

Univariate logistic regression suggested that higher number of clinically significant RSV-associated LRTI within the first 2 years of life was associated with higher risk of asthma diagnosis at 11 years. The association remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.6). The difference in risk of asthma diagnosis at 11 years between children who had 1-2 clinically significant RSV-LRTI and children who had $\geq$ 3 clinically significant RSV-associated LRTI before the age of 2 years was not statistically significant. When the children were stratified according to the maternal history of asthma, frequency of clinically significant RSV-associated LRTI was not significantly associated with risk of asthma at 11 years for children with maternal asthma but significant for children without maternal asthma.

Table 3.6: Association between risk of asthma diagnosis at 11 years and explanatory variables (multivariate logistic regression), with frequency of RSV-LRTI.

Features/variables	Asthma at 11 years				
	No.	n	%	OR (95% CI)	P-value
Frequency of RSV infection in 2 years					
• 1-2 infection(s)	2866	216	7.54	1.21(1.03-1.43)	0.0248
• ≥ 3 infections	855	72	8.42	1.42 (1.09-1.86)	0.0096
• No RSV-LRTI (ref.)	9811	567	5.78		
Residence					
• Urban	7769	591	7.61	1.59 (1.37-1.86)	<.0001
• Rural (ref.)	5763	264	4.58		
Gender					
• Male	6837	520	7.61	1.53 (1.33-1.77)	<.0001
• Female (ref.)	6695	335	5.00		
Asthmatic mother					
• Yes	1375	160	11.64	2.01 (1.67-2.42)	<.0001
• No (ref.)	12157	695	5.72		
No. of sibling in 2002					
• ≥ 2 sibling	6760	344	5.09	0.69 (0.60-0.80)	<.0001
• 0/1 sibling (ref.)	6772	511	7.55		

Features/variables	Asthma at 11 years				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Antibiotic received in 1st year					
• 1-2 antibiotics	5062	336	6.64	1.26 (1.06-1.50)	0.0101
• 3-4 antibiotics	2179	147	6.75	1.21 (0.97-1.51)	0.0850
• >4 antibiotics	1646	137	8.32	1.50 (1.19-1.88)	0.0006
• No antibiotic (ref.)	4645	235	5.06		

*70 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.3.2 Age at 1st RSV-LRTI care and asthma at 11 years

The mean age of first RSV-associated utilization of health care for children who were asthmatic at the age of 11 was 262 days (std±171 days, median=234 days) and 270 days (std±186 days, median=229 days) for the children who were not asthmatic at the age of 11 years. Univariate logistic regression suggested that the difference in age at the time of first clinically significant RSV-associated LRTI was not statistically significant between children who were asthmatic at 11 years and who were not asthmatic at that point of life.

Univariate logistic regression suggested that children who had first clinically significant RSV-associated LRTI within the first 12 months of life were at higher risk of asthma at 11 years. Children who had first clinically significant RSV-LRTI between 6 and 12 months were at the highest risk of asthma diagnosis at 11 years. The association between age-group at the time of first health care for RSV-associated LRTI and risk of asthma diagnosis at 11 years remained very similar after adjustment for other factors in multivariate logistic regression (Table 3.7).

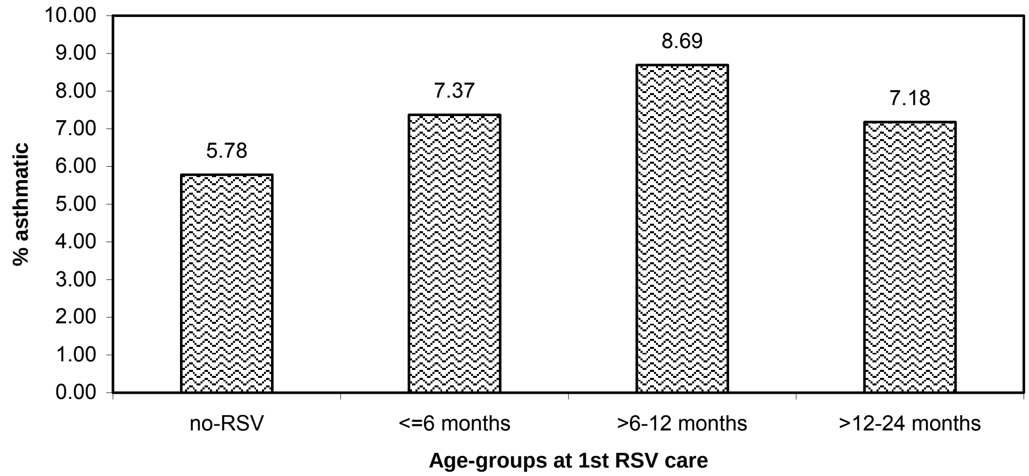

Figure 3.5: Proportion of asthmatic children (at 11 years) in different age-groups at 1st clinically significant RSV-LRTI.

No significant difference in risk of asthma diagnosis at 11 years was detected between the age-groups at the time of first clinically significant RSV-associated LRTI (i.e., between ≤6 months, >6-12 months and >12-24 months). The above graph (Figure 3.5) represents the distributions of asthmatic children (at 11 years) in different age-groups at the time of first clinically significant RSV-associated LRTI.

Table 3.7: Association between risk of asthma diagnosis at 11 years and explanatory variables (multivariate logistic regression), with age-group at 1st RSV-LRTI.

Features/variables	**Asthma at 11 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Age-groups at 1st RSV-associated care					
• ≤6 months	1493	110	7.37	1.25 (1.001-1.56)	0.0486
• >6 to 12 months	1197	104	8.69	1.36 (1.08-1.71)	0.0078
• >12 to 24 months	1031	74	7.18	1.15 (0.90-1.49)	0.2700
• No RSV-care (ref.)	9811	567	5.78		
Residence					
• Urban	7769	591	7.61	1.59 (1.36-1.85)	<.0001
• Rural (ref.)	5763	264	4.58		
Gender					
• Male	6837	520	7.61	1.53 (1.33-1.77)	<.0001
• Female (ref.)	6695	335	5.00		
Asthmatic mother					
• Yes	1375	160	11.64	2.01 (1.68-2.42)	<.0001
• No (ref.)	12157	695	5.72		
No. of sibling in 2002					
• ≥2 sibling	6760	344	5.09	0.70 (0.60-0.81)	<.0001
• 0/1 sibling (ref.)	6772	511	7.55		

Features/variables	Asthma at 11 years				
	No.	n	%	OR (95% CI)	P-value
Antibiotic received in 1st year					
• 1-2 antibiotics	5062	336	6.64	1.25 (1.05-1.49)	0.0125
• 3-4 antibiotics	2179	147	6.75	1.21 (0.97-1.50)	0.0930
• >4 antibiotics	1646	137	8.32	1.49 (1.19-1.88)	0.0007
• No antibiotic (ref.)	4645	235	5.06		

*70 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

3.3.3 Severity of RSV-LRTI and asthma at the age of 11 years

Univariate logistic regression suggested that only the children who had mild clinically significant RSV-LRTI before the age of 2 years were at higher risk of asthma diagnosis at 11 years than children who did not have that infection within the first 2 years of life. Moderate and severe RSV-associated LRTI before the age of 2 years were not associated with risk of asthma diagnosis at 11 years. After adjustment for other factors in multivariate logistic regression, all of the mild and severe clinically significant RSV-LRTI before the age of 2 years appeared to be associated with higher risk of asthma diagnosis at 11 years (Table 3.8).

Table 3.8: Association between risk of asthma diagnosis at 11 years and explanatory variables (multivariate logistic regression), with severity of RSV-LRTI.

Features/variables	Asthma at 11 years				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Severity of RSV-LRTI in 2 years					
• Mild	3147	242	7.69	1.21 (1.03-1.43)	0.0211
• Moderate	303	23	7.59	1.44 (0.92-2.24)	0.1077
• Severe	271	23	8.49	1.74 (1.11-2.72)	0.0151
• No RSV in 2 years (ref.)	9811	567	5.78		
Residence					
• Urban	7769	591	7.61	1.61 (1.38-1.87)	<.0001
• Rural (ref.)	5763	264	4.58		
Gender					
• Male	6837	520	7.61	1.53 (1.33-1.77)	<.0001
• Female (ref.)	6695	335	5.00		
Asthmatic mother					
• Yes	1375	160	11.64	2.02 (1.68-2.42)	<.0001
• No (ref.)	12157	695	5.72		
No. of sibling in 2002					
• ≥2 sibling	6760	344	5.09	0.69 (0.60-0.80)	<.0001
• 0/1 sibling (ref.)	6772	511	7.55		

Features/variables	Asthma at 11 years				
	No.	n	%	OR (95% CI)	P-value
Antibiotic received in 1st year					
• 1-2 antibiotics	5062	336	6.64	1.26 (1.06-1.50)	0.0098
• 3-4 antibiotics	2179	147	6.75	1.22 (0.98-1.51)	0.0807
• >4 antibiotics	1646	137	8.32	1.51 (1.20-1.90)	0.0004
• No antibiotic (ref.)	4645	235	5.06		

*70 observations were deleted from multivariate logistic regression model due to missing values for explanatory variable(s).

The risk of asthma diagnosis at 11 years did not differ significantly between the severity categories (i.e., between severe vs. moderate, severe vs. mild and mild vs. moderate).

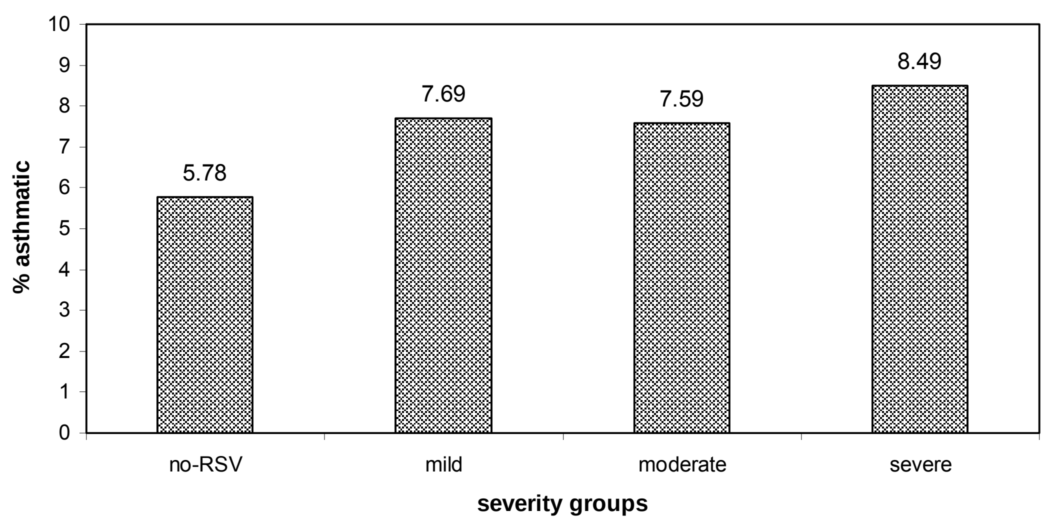

Figure 3.6: Proportion of asthmatic children at 11 years in different severity groups.

3.3.4 Confounding factors and asthma at 11 years

As mentioned earlier, the associations between several confounding factors and childhood asthma are well documented in currently available literature. The associations between these factors (Appendix B) and risk of asthma diagnosis at the age of 11 years were analysed for the current study and are briefly discussed in the following sections.

3.3.4.1 Gender and family history

Both univariate and multivariate logistic regression analyses suggested that male children were more likely to have asthma at 11 years in comparison to the female children of the study cohort (Table 7, Appendix and Table 3.6). Male gender was associated with higher risk of asthma at 11 years for children with and without maternal history of asthma.

Both univariate and multivariate logistic regression analyses suggested that maternal asthma was significantly associated with higher risk of asthma at 11 years (Table 7, Appendix and Table 3.6). When the paternal history of asthma was also considered for multivariate logistic regression, a total of 4939 observations were excluded because of missing information on paternal history of asthma. With the available information, it appeared that the highest risk of asthma diagnosis at 11 years was associated with both parents being asthmatic (OR=3.89, 95% CI=2.26-6.71). Only mother (OR=2.20, 95% CI=1.68-2.87) or only father (OR= 2.21, 95% CI=1.64-2.99) being asthmatic was associated with almost similar risk of childhood asthma at 11 years.

Though univariate logistic regression suggested that children of the stressed mothers were more likely to be diagnosed with asthma at 11 years, the association was no longer significant when adjusted for other factors under consideration in the current study (Table 7, Appendix and Table 3.6).

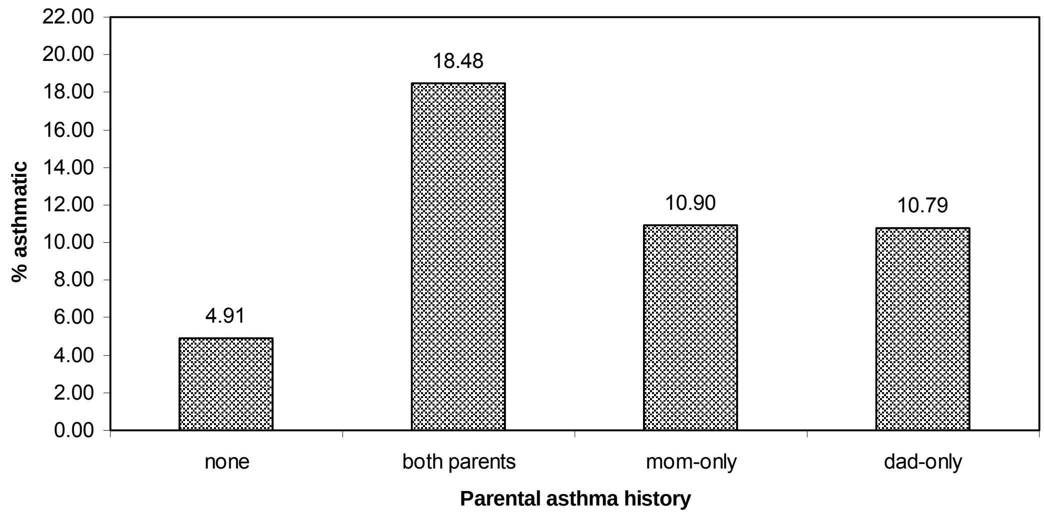

Figure 3.7: Proportions of asthmatic children at 11 years with parental history of asthma.

Univariate logistic regression suggested that children who had two or more sibling at the end of 2002 were less likely to have asthma at 11 years than children who had one or no sibling (Table 7, Appendix). This association was not significant for children with maternal history of asthma, but significant for children without maternal asthma. Having at least two or more sibling remained significantly associated with lower risk of asthma diagnosis at 11 years after adjustment for other factors in multivariate logistic regression (Table 3.6).

3.3.4.2 Income

Univariate logistic regression suggested that parental income was not associated with risk of asthma diagnosis at the age of 11 years (Table 7, Appendix). The influence of parental income was further investigated by comparing asthma status of children at 11 years from the lowest income quintile (quintile-1) with children from each of the higher income quintiles (quintiles 2-5). Univariate logistic regression suggested that, at the age of 11 years, asthma status of the children from the lowest income quintile was not significantly different from children from the higher income quintiles.

3.3.4.3 Prematurity

The median gestational age of children who were asthmatic at 11 years was 39 weeks (mean 39.06 weeks, std±2.25 weeks) and 40 weeks (mean 39.19 weeks, std±1.99 weeks) for children who were not asthmatic at 11 years. There was no statistically significant difference between the gestation age of children who were asthmatic and who were not asthmatic at 11 years. Univariate logistic regression suggested that prematurity was not associated with risk of asthma diagnosis at 11 years (Table 7, Appendix).

The median birth-weight of children who were asthmatic at 11 years was 3460.50 gm (mean 3448.36 gm, std±580.58 gm) and 3475.50 gm (mean 3448.82 gm, std± 576.19 gm) for children who were not asthmatic at 11 years. Like gestational age, there was no significant difference between the birth-weight of children who were asthmatic at 11 years and who were not asthmatic at that point of life. Birth weight was not associated with risk of asthma diagnosis at the age of 11 years, as revealed by univariate logistic regression analysis (Table 7, Appendix).

3.3.4.4 BCG vaccination history

Univariate logistic regression suggested that completion of BCG vaccination within 2 years of life did not significantly influence the asthma status of the children at 11 years

(Table 7, Appendix). This finding was very similar for children with and without maternal history of asthma.

3.3.4.5 Antibiotic use in the first year of life

Univariate logistic regression suggested that children who received at least one antibiotic in the first year of life were more likely to be asthmatic at 11 years than children who did not receive any antibiotic in the first year (Table 7, Appendix). Receiving antibiotic in the first year was associated with higher risk of asthma at 11 years for children without maternal history of asthma, but not for children with maternal history of asthma.

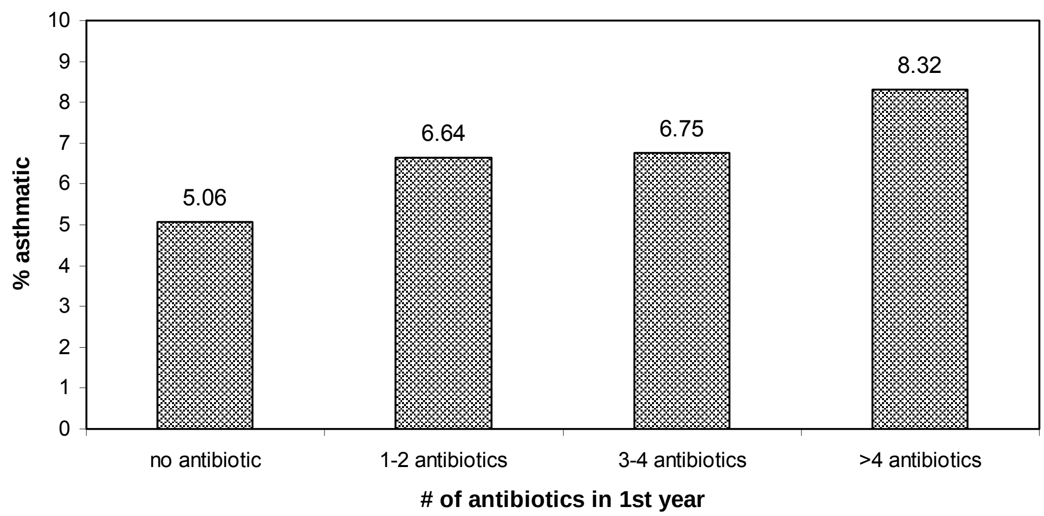

Figure 3.8: Overall antibiotic use in 1st years of life and asthma at 11 years.

Higher risk of asthma diagnosis at 11 years appeared to be associated with higher number of antibiotics in the first year of life. Multivariate logistic regression suggested that receiving 3-4 antibiotics in the first year of life was not associated with risk of asthma diagnosis at 11 years, though it was significant in univariate logistic regression (Table 7, Appendix and Table 3.6). Risk of asthma diagnosis at 11 years was not significantly different between children who received different numbers of antibiotics (i.e., 1-2, 3-4 and >4 antibiotics) in the first year of life.

3.3.4.6 Urban/rural residence

Univariate logistic regression suggested that living in urban areas was significantly associated with higher risk of asthma at the age of 11 years. This association was also supported by multivariate logistic regression analysis after adjustment for other variables (Table 7, Appendix and Table 3.6). Living in urban area was associated with higher risk of asthma diagnosis at 11 years for children with and without maternal history of asthma.

Children living in three northern regional health authorities were less likely to be asthmatic at 11 years than children who were not living in north (Table 7, Appendix). This factor was not significant for children with maternal history of asthma. Multivariate logistic regression suggested that living in north was not significantly associated with risk of asthma diagnosis at 11 years when adjusted for the influences of other variables.

3.4 Asthma/wheezing phenotypes at 11 years (December, 2006)

By the end of the year 2006, when children of the current study cohort were 11 years of age, a total of 2265 children (out of 13602) were ever diagnosed with asthma/wheezing at some point of their lives up to the age of 11 years. As defined in the methods, 1096 children were diagnosed with transient wheeze, 372 were diagnosed with late onset asthma, 263 were diagnosed with late persistent asthma and 221 children were diagnosed with early persistent asthma. If only these three asthma/wheezing phenotypes were considered some of the children who had the asthma onset later in their lives and were not asthmatic at 11 years would be missing. This asthma/wheezing phenotype was defined as late transient asthma and a total of 313 were identified with this asthma/wheezing phenotype at the end of 2006 when the children were 11 years old. Univariate logistic regression analyses were run to determine the associations between the asthma/wheezing phenotypes and potential contributing factors. The associations between these potential contributing factors and clinically significant RSV-LRTI (primary exposure) were determined as well. The results of the associations are presented in Table 8 - Table 12 (Appendix).

3.4.1 RSV-associated LRTI, frequency of RSV-LRTI and asthma/wheezing phenotypes

Both univariate and multivariate logistic regression analyses suggested that clinically significant RSV-LRTI within first 2 years of life was associated with higher risk of early persistent asthma (Table 8 and 13, Appendix). Clinically significant RSV-LRTI before 2 years was a significant risk factor for children with and without maternal history of asthma for this asthma phenotype. Univariate logistic regression revealed that children who had 1-2 clinically significant RSV-LRTI before 2 years were almost 3 times more likely to have early persistent asthma, but the risk was increased to 8 times for children who had more than 2 clinically significant RSV-LRTI before 2 years. When adjusted for other variables under consideration of the current study in multivariate logistic regression, more than 2 clinically significant RSV-LRTI before 2 years remained associated with almost 6 times higher risk of early persistent asthma (Table 3.9).

Univariate and multivariate logistic regressions suggested that children who had clinically significant RSV-associated lower respiratory tract infection before the age of 2 years were more likely to have transient wheeze than children who did not have clinically significant RSV-LRTI within the first 2 years of life (Table 14, Appendix). Clinically significant RSV-LRTI before 2 years was significant risk factor for transient wheeze for children with and without maternal history of asthma. When the number of clinically significant RSV-LRTI was considered, univariate logistic regression suggested that children who had 1-2 episodes of clinically significant RSV-LRTI before 2 years were as much as twice more likely to have transient wheeze, but who had 3 or more RSV-LRTI were almost 13 times more likely to have this wheezing phenotype. After adjustment for other factors in multivariate logistic regression, having 3 or more episodes of clinically significant RSV-LRTI was still associated with 8 times higher risk of transient wheeze (Table 3.10).

Table 3.9: Associations between risk of early persistent asthma and explanatory variables (multivariate logistic regressions), with frequency and severity of RSV-LRTI and age at 1st occurrence.

Features/variables	No.	n	%	Frequency of RSV-LRTI		Severity of RSV-LRTI		Age-group at 1st RSV-LRTI	
				OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Frequency of RSV infection in 2 years									
• 1-2 infection(s)	2328	78	3.35	2.34 (1.72-3.18)	<.0001	Not applicable for this multivariate equation		Not applicable for this multivariate equation	
• ≥ 3 infections	491	42	8.55	6.04 (4.05-9.00)	<.0001				
• No RSV-LRTI (ref.)	8640	100	1.16						
Severity of RSV-LRTI in 2 years									
• Mild	2466	95	3.85	Not applicable for this multivariate equation		2.61 (1.94-3.50)	<.0001	Not applicable for this multivariate equation	
• Moderate	200	11	5.50			4.62 (2.37-9.00)	<.0001		
• Severe	153	14	9.15			8.98 (4.86-16.61)	<.0001		
• No RSV-LRTI (ref.)	8640	100	1.16						
Age-groups at 1st RSV-associated care									
• ≤6 months	1050	60	5.71	Not applicable for this multivariate equation		Not applicable for this multivariate equation		4.23 (3.00-5.96)	<.0001
• >6 to 12 months	893	41	4.59					2.85 (1.93-4.19)	<.0001

Features/variables	No.	n	%	Frequency of RSV-LRTI		Severity of RSV-LRTI		Age-group at 1st RSV-LRTI	
				OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
• >12 to 24 months	876	19	2.17					1.58 (0.96-2.60)	0.0743
• No RSV-care (ref.)	8640	100	1.16						
BCG vaccination in 2 years									
• Complete	322	12	3.73	2.00 (1.05-3.82)	0.0358	NS		2.17 (1.14-4.10)	0.0178
• Not complete (ref.)	11137	208	1.87						
Residence									
• Urban	6450	148	2.29	1.66 (1.22-2.25)	0.0012	1.59 (1.18-2.14)	0.0023	1.64 (1.21-2.22)	0.0015
• Rural (ref.)	5009	72	1.44						
Gender									
• Male	5603	156	2.78	2.41 (1.79-3.25)	<.0001	2.40 (1.79-3.23)	<.0001	2.42 (1.80-3.25)	<.0001
• Female (ref.)	5856	64	1.09						
Asthmatic mother									
• Yes	1036	50	4.83	2.48 (1.78-3.46)	<.0001	2.53 (1.82-3.53)	<.0001	2.52 (1.81-3.52)	<.0001
• No (ref.)	10423	170	1.63						

Features/variables	No.	n	%	Frequency of RSV-LRTI		Severity of RSV-LRTI		Age-group at 1st RSV-LRTI	
				OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
No. of sibling in 2002									
• ≥2 sibling	5757	89	1.55	0.60 (0.45-0.80)	0.0004	0.60 (0.45-0.80)	0.0004	0.59 (0.44-0.79)	0.0003
• 0/1 sibling (ref.)	5702	131	2.30						
Premature birth									
• Yes	675	21	3.11	NS		NS		1.63 (1.02-2.61)	0.0421
• No (ref.)	10784	199	1.85						
Antibiotic received in 1st year									
• 1-2 antibiotics	4316	81	1.88	1.89 (1.26-2.83)	0.0021	1.89 (1.26-2.83)	0.0021	1.83 (1.22-2.74)	0.0035
• 3-4 antibiotics	1756	52	2.96	2.56 (1.64-3.99)	<.0001	2.62 (1.68-4.09)	<.0001	2.48 (1.58-3.87)	<.0001
• >4 antibiotics	1182	52	4.40	3.27 (2.08-5.14)	<.0001	3.59 (2.29-5.63)	<.0001	3.27 (2.08-5.16)	<.0001
• No antibiotic (ref.)	4205	35	0.83						

*Total 11459 observations were included in multivariate logistic regression models for early persistent asthma.

Univariate logistic regression suggested that clinically significant RSV-LRTI before the age of 2 years was associated with higher risk of late transient asthma. Clinically significant RSV-LRTI was not significant risk factor for children with maternal history of asthma for this asthma phenotype. Clinically significant RSV-associated LRTI was no longer a significant risk factor for late transient asthma when adjusted for other variables in multivariate logistic regression. When the numbers of clinically significant RSV-LRTI before the age of 2 years was considered, univariate logistic regression suggested that having 1-2 RSV-LRTI before 2 years was associated with higher risk of late transient asthma but more than 2 RSV-LRTI before 2 years was not a significant risk factor for this asthma phenotype. The number of clinically significant RSV-LRTI was not significantly associated with the risk of late transient asthma when adjusted for other factors in multivariate logistic regression (Table 3.11).

Univariate logistic regression suggested that clinically significant RSV-LRTI before the age of 2 years was not associated with risk of late onset asthma and late persistent asthma. Obviously the number of clinically significant RSV-LRTI was not significant for these asthma phenotypes as well (Table 3.11).

3.4.2 Age at 1st RSV-LRTI and asthma/wheezing phenotypes

Children who had early persistent asthma were significantly younger at the time of first clinically significant RSV-LRTI than children who did not have this asthma phenotype. Univariate logistic regression suggested that children in the younger age-groups at the time of first clinically significant RSV-LRTI were at higher risk of early persistent asthma. The trend remained very similar after adjustment for other factors in multivariate logistic regression, with the highest risk was associated with first clinically significant RSV-LRTI before 6 months. First clinically significant RSV-LRTI after 12 months was no longer associated with risk of early persistent asthma in multivariate logistic regression (Table 3.9).

At the time of first RSV-LRTI associated utilization of health care, children who had transient wheeze were significantly younger than children who did not have this wheezing phenotype. Both univariate and multivariate logistic regression analyses suggested that younger age at the time of first clinically significant RSV-LRTI was associated with higher risk of transient wheeze. Multivariate logistic regression revealed that children who had first RSV-LRTI before 6 months were over four times more likely to have transient wheeze. First RSV-LRTI after 12 months of age did not put the children at risk of transient wheeze (Table 3.10).

Table 3.10: Associations between risk of transient wheeze and explanatory variables (multivariate logistic regressions), with frequency and severity of RSV-LRTI and age at 1st occurrence.

Features/variables	**No.**	**n**	**%**	**Frequency of RSV-LRTI**		**Severity of RSV-LRTI**		**Age-group at 1st RSV-LRTI**	
				OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Frequency of RSV infection in 2 years									
• 1-2 infection(s)	2557	308	12.05	1.89 (1.61-2.21)	<.0001	Not applicable for this multivariate equation		Not applicable for this multivariate equation	
• >2 infections	764	315	41.23	8.21 (6.84-9.85)	<.0001				
• No RSV-LRTI (ref.)	9000	462	5.13						
Severity of RSV-LRTI in 2 years									
• Mild	2805	435	15.51	Not applicable for this multivariate equation		2.43 (2.10-2.80)	<.0001	Not applicable for this multivariate equation	
• Moderate	272	83	30.51			5.36 (4.01-7.17)	<.0001		
• Severe	244	105	43.03			9.36 (7.01-12.49)	<.0001		
• No RSV-LRTI (ref.)	9000	462	5.13						
Age-groups at 1st RSV-associated care									
• ≤6 months	1346	357	26.52	Not applicable for this multivariate equation		Not applicable for this multivariate equation		4.55 (3.87-5.35)	<.0001
• >6 to 12 months	1053	201	19.09					2.86 (2.37-3.45)	<.0001

Features/variables	No.	n	%	Frequency of RSV-LRTI		Severity of RSV-LRTI		Age-group at 1st RSV-LRTI	
				OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
• >12 to 24 months	922	65	7.05					1.19 (0.90-1.56)	0.2248
• No RSV-care (ref.)	9000	462	5.13						
Gender									
• Male	6106	662	10.84	1.48 (1.29-1.70)	<.0001	1.49 (1.30-1.70)	<.0001	1.50 (1.31-1.72)	<.0001
• Female (ref.)	6215	423	6.81						
Asthmatic mother									
• Yes	1164	178	15.29	1.67 (1.38-2.02)	<.0001	1.69 (1.40-2.04)	<.0001	1.69 (1.40-2.03)	<.0001
• No (ref.)	11157	907	8.13						
Maternal stress									
• Stressed	2273	276	12.14	1.24 (1.06-1.46)	0.0071	1.29 (1.10-1.50)	0.0016	1.27 (1.09-1.49)	0.0024
• Not stressed (ref.)	10048	809	8.05						
Child health income quintile									
• Low	3046	385	12.64	1.39 (1.20-1.61)	<.0001	1.38 (1.19-1.59)	<.0001	1.45 (1.26-1.67)	<.0001
• High (ref.)	9275	700	7.55						

Features/variables	No.	n	%	Frequency of RSV-LRTI		Severity of RSV-LRTI		Age-group at 1st RSV-LRTI	
				OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Premature birth									
• Yes	755	102	13.51	1.57 (1.23-1.99)	0.0003	1.52 (1.19-1.93)	0.0007	1.62 (1.28-2.06)	<.0001
• No (ref.)	11566	983	8.50						
Antibiotic received in 1st year									
• 1-2 antibiotics	4579	346	7.56	2.03 (1.66-2.49)	<.0001	2.02 (1.65-2.48)	<.0001	1.93 (1.58-2.37)	<.0001
• 3-4 antibiotics	1965	262	13.33	3.19 (2.56-3.98)	<.0001	3.27 (2.63-4.07)	<.0001	3.07 (2.46-3.82)	<.0001
• >4 antibiotics	1462	332	22.71	4.97 (3.99-6.20)	<.0001	5.44 (4.38-6.76)	<.0001	4.96 (3.99-6.17)	<.0001
• No antibiotic (ref.)	4315	145	3.36						

*Total 12321 observations were included in multivariate logistic regression models for transient wheeze.

At the time of first clinically significant RSV-LRTI, there was no significant difference between the age of children who had late onset asthma or late persistent asthma or late transient asthma and who did not have these asthma phenotypes. Univariate logistic regression suggested that age-group at the time of first clinically significant RSV-LRTI was not associated with risk of either late onset asthma, late persistent asthma or late transient asthma.

3.4.3 Severity of RSV-LRTI and asthma/wheezing phenotypes

In univariate logistic regression, all the severity categories were significantly associated with higher risk of transient wheeze and early persistent asthma. The highest risks were associated with the most severe infections for these asthma/wheezing phenotypes. This trend was also observed for transient wheeze and early persistent asthma for children with and without maternal asthma history. The association between severity and transient wheeze remained similar in multivariate logistic regression. The most severe RSV-LRTI was associated with more than nine times higher risk of transient wheeze (Table 3.10). More severe RSV-LRTI was associated with higher risk of early persistent asthma as well. The most severe cases of RSV-LRTI were associated with almost nine times higher risk of early persistent asthma (Table 3.9).

Univariate logistic regression suggested that only mild clinically significant RSV-LRTI was associated with higher risk of late transient asthma but none of the severity categories was significantly associated with higher risk of late transient asthma when adjusted for other factors in multivariate logistic regression. Univariate logistic regression suggested that none of the severity categories was associated with risk of late onset asthma or late persistent asthma.

3.4.4 Confounding factors and asthma/wheezing phenotypes

The associations between well cited confounding factors (Appendix B) and different asthma/wheezing phenotypes are briefly discussed in the following sections.

3.4.4.1 Gender and family history

Both univariate and multivariate logistic regressions suggested that male children were more likely to have transient wheeze (Table 9, Appendix A and Table 3.10), early persistent asthma (Table 8, Appendix A and Table 3.9), late persistent asthma (Table 10, Appendix A and Table 3.11) and late transient asthma (Table 11, Appendix A and Table 3.11). Male gender was associated with higher risk of early persistent asthma and transient wheeze for children with and without maternal history of asthma. Gender was not a significant factor for late persistent asthma and late transient asthma for the children with maternal history of asthma.

As suggested by univariate logistic regression, male children were more likely to have late onset asthma (Table 12, Appendix A). This factor was not significant for children with maternal history of asthma. Gender of the children was no longer significant for late onset asthma after adjustment for other factors in multivariate logistic regression.

Univariate and multivariate logistic regression analyses suggested that maternal asthma was associated with higher risk of all the asthma/wheezing phenotypes defined for the current study (Table 8-12, Appendix and Table 3.9-3.11). Over 5000 observations had missing information on paternal asthma. When the available information on paternal asthma was considered, the highest risks of transient wheeze and late persistent asthma were associated with both of the parents being asthmatic, followed by only mother being asthmatic and only father being asthmatic. Only maternal history of asthma was significantly associated with higher risk of early persistent asthma. Both of the parents or only the father being asthmatic was not associated with risk of early persistent asthma. Only maternal asthma or only paternal asthma was associated with higher risk of late transient asthma, but both of the parents being asthmatic was not associated with risk of this asthma phenotype. Both parents being asthmatic was associated with higher risk of late onset asthma, followed by only the father and only the mother being asthmatic.

Univariate logistic regression suggested that maternal stress was associated with higher risk of early persistent asthma (Table 8, Appendix A), transient wheeze (Table 9, Appendix) and late onset asthma (Table 12, Appendix A) but was not significantly associated with risk of late persistent asthma (Table 10, Appendix A) and late transient asthma (Table 11, Appendix A). When adjusted for other variables under consideration of the current study in multivariate logistic regression, it appeared that maternal stress was a significant risk factor only for transient wheeze (Table 3.10) and not significantly associated with risk of early persistent asthma and late onset asthma.

Table 3.11: Associations between risk of late persistent asthma, late transient asthma and late onset asthma and explanatory variables (multivariate logistic regressions), with frequency of RSV-LRTI.

Features/variables	**Late persistent asthma**					**Late transient asthma**					**Late onset asthma**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**
Frequency of RSV infection in 2 years															
• 1-2 infection(s)	2322	61	2.63	1.18 (0.88-1.59)	0.2706	2338	77	3.29	1.22 (0.93-1.60)	0.1539	2337	77	3.29	1.02 (0.79-1.32)	0.8797
• > 2 infections	462	12	2.60	1.26 (0.69-2.29)	0.4515	466	16	3.43	1.38 (0.82-2.35)	0.2300	467	17	3.64	1.20 (0.73-1.99)	0.4693
• No RSV-LRTI (ref.)	8756	190	2.17			8781	215	2.45			8841	277	3.13		
Residence															
• Urban	6515	188	2.89	1.76 (1.33-2.31)	<.0001	6543	216	3.30	1.47 (1.13-1.90)	0.0040	6579	254	3.86	1.69 (1.35-2.11)	<.0001
• Rural (ref.)	5025	75	1.49			5042	92	1.82			5066	117	2.31		
Living north															
• Yes	Factor not included for this multivariate equation					1208	9	0.75	0.37 (0.19-0.74)	0.0047	Factor not included for this multivariate equation				
• No (ref.)						10377	299	2.88							
Gender															
• Male	5631	164	2.91	1.80 (1.40-2.32)	<.0001	5650	183	3.24	1.53 (1.22-1.93)	0.0003	5663	199	3.51	1.24 (1.01-1.53)	0.0403
• Female (ref.)	5909	99	1.68			5935	125	2.11			5982	172	2.88		
Asthmatic mother															
• Yes	1045	52	4.98	2.47 (1.81-3.38)	<.0001	1036	43	4.15	1.53 (1.10-2.13)	0.0126	1051	58	5.52	1.87 (1.40-2.49)	<.0001

Features/variables	Late persistent asthma					Late transient asthma					Late onset asthma				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**
• No (ref.)	10495	211	2.01			10549	265	2.51			10594	313	2.95		
No. of sibling in 2002															
• ≥2 sibling	5787	96	1.66	0.62 (0.48-0.81)	0.0003	5810	119	2.05	0.67 (0.53-0.85)	0.0011	Factor not included for this multivariate equation				
• 0/1 sibling (ref.)	5753	167	2.90			5775	189	3.27							
Low birth-weight															
• Yes	Factor not included for this multivariate equation					Factor not included for this multivariate equation					536	8	1.49	0.43 (0.21-0.88)	0.0204
• No (ref.)											11109	363	3.27		
Antibiotic received in 1st year															
• 1-2 antibiotics	Factor not included for this multivariate equation					4376	124	2.83	1.38 (1.04-1.83)	0.0262	Factor not included for this multivariate equation				
• 3-4 antibiotics						1768	59	3.34	1.59 (1.12-2.24)	0.0087					
• >4 antibiotics						1176	42	3.57	1.72 (1.16-2.53)	0.0065					
• No antibiotic (ref.)						4265	83	1.95							

* Total 11540 observations were included in multivariate logistic regression models for late persistent asthma.

** Total 11585 observations were included in multivariate logistic regression models for late transient asthma.

*** Total 11645 observations were included in multivariate logistic regression models for late onset asthma.

Univariate logistic regression suggested that children with two or more siblings in 2002 were more likely to have transient wheeze than children who had one or no sibling in 2002 (Table 9, Appendix A). This association was very similar for children without maternal history of asthma but not significant for children with maternal asthma. Having more than one sibling became insignificant risk factor for transient wheeze after adjustment for other factors in multivariate logistic regression.

Children who had more than one sibling in 2002 were less likely to have early persistent asthma (Table 8, Appendix A), late onset asthma (Table 12, Appendix A), late persistent asthma (Table 10, Appendix A) and late transient asthma (Table 11, Appendix A) than children who had only one or no sibling. Having one or no sibling was not significantly associated with risk of any of these four asthma phenotypes for children with maternal history of asthma. Having two or more siblings remained significantly associated with lower risk of early persistent asthma (Table 3.9), late persistent asthma and late transient asthma (Table 3.11) but was no longer significant for late onset asthma when adjusted for other factors in multivariate logistic regression analyses.

3.4.4.2 Income

Univariate logistic regression suggested that children of the low income parents were more likely to develop early persistent asthma (Table 8, Appendix A) and transient wheeze (Table 9, Appendix A) in comparison to children of high income parents. The association of lower income and transient wheeze was very similar for children with and without maternal history of asthma. After adjustment for other factors in multivariate logistic regression, lower income remained significantly associated with higher risk of transient wheeze (Table 3.10) but was no longer associated with risk of early persistent asthma. As univariate logistic regression revealed, income level of the parents was not associated with the risk of late persistent asthma (Table 10, Appendix A), late transient asthma (Table 11, Appendix A) and late onset asthma (Table 12, Appendix A).

3.4.4.3 Prematurity

In univariate logistic regression, prematurity appeared to be associated with higher risk of early persistent asthma (Table 8, Appendix A) and transient wheeze (Table 9, Appendix A). Prematurity was not a significant risk factor for transient wheeze for children with maternal history of asthma. On the other hand, prematurity was associated with higher risk of early persistent asthma for children with maternal history of asthma but not for children without maternal history of asthma. Prematurity was no longer significantly associated with risk of early persistent asthma but remained associated with higher risk of transient wheeze after adjustment for other factors in multivariate logistic regression (Table 3.10). As suggested by univariate logistic regression, prematurity was not associated with risk of late persistent asthma (Table 10, Appendix A), late transient asthma (Table 11, Appendix A) and late onset asthma (Table 12, Appendix A).

Univariate logistic regression showed that low birth weight was associated with higher risk of transient wheeze (Table 9, Appendix A) and lower risk of late onset asthma (Table 12, Appendix A) and was not associated with risk of any other asthma phenotype (Table 8, 10 and 11, Appendix A). Low birth-weight was not a significant risk factor for transient wheeze and late onset asthma for children with maternal history of asthma. After adjustment for other factors under consideration of the current study in multivariate logistic regression analyses, low birth-weight was no longer significantly associated with risk of transient wheeze but still remained significantly associated with lower risk of late onset asthma (Table 3.11).

3.4.4.4 BCG vaccination history

Univariate logistic regression suggested that children who completed BCG vaccination within first two years of life were more likely to have early persistent asthma (Table 8, Appendix A) and transient wheeze (Table 9, Appendix A) than children who did not complete BCG vaccination in first 2 years. Completion of BCG vaccination was not significant for children with maternal asthma history for transient wheeze. Completion of BCG vaccination was still significantly associated with higher risk of early persistent asthma (Table 3.9) but no longer a significant factor for transient wheeze after adjustment for other factors in multivariate logistic regression.

Completion of BCG vaccination in first 2 years of life was not associated with the risk of late persistent asthma (Table 10, Appendix A), late transient asthma (Table 11, Appendix A) or late onset asthma (Table 12, Appendix A), as suggested by univariate logistic regression.

3.4.4.5 Antibiotic use in the first year of life

Univariate logistic regression analyses suggested that receiving at least one antibiotic in the first year of life was associated with higher risk of early persistent asthma (Table 8, Appendix A), transient wheeze (Table 9, Appendix A), late transient asthma (Table 11, Appendix A) and late onset asthma (Table 12, Appendix A). Receiving at least one antibiotic was associated with higher risk of early persistent asthma and transient wheeze for children with and without maternal history of asthma. Receiving antibiotic(s) in the first year of life was not associated with risk of late transient asthma and late onset asthma for children with maternal history of asthma. Univariate logistic regression suggested that receiving at least one antibiotic in the first year of life was not significantly associated with risk of late persistent asthma (Table 10, Appendix A).

Multivariate logistic regression suggested that receiving higher number of antibiotics was associated with higher risk of early persistent asthma and transient wheeze. Receiving more than four antibiotics in the first year of life was associated with more than 3 times higher risk of early persistent asthma (Table 3.9) and almost five times higher risk of transient wheeze (Table 3.10) than children who did not receive any antibiotic in the first year of life. Receiving antibiotic in the first year of life remained significantly associated with higher risk of late transient asthma but the magnitude of risk did not change much with number of antibiotics received (Table 3.11). Receiving antibiotic(s) in the first year of life was no longer significantly associated with risk of late onset asthma after adjustment for other factors in multivariate logistic regression.

3.4.4.6 Urban/rural residence

Univariate logistic regression suggested that children living in urban Manitoba were more likely to have early persistent asthma (Table 8, Appendix A), late persistent asthma (Table 10, Appendix A) late transient asthma (Table 11, Appendix A) and late onset asthma (Table 12, Appendix A). But living in urban/rural area was not associated with risk of transient wheeze (Table 9, Appendix A). Living in urban area was not associated with risk of late onset asthma, late transient asthma, early persistent asthma and late persistent asthma for children with maternal history of asthma. Living in urban Manitoba remained significantly associated with higher risk of late onset asthma, late transient asthma, late persistent asthma (Table 3.11) and early persistent asthma (Table 3.9) after adjustment for other factors under consideration of the current study in multivariate logistic regression analyses.

Living in three northern RHAs was not associated with risk of transient wheeze (Table 9, Appendix A), late persistent asthma (Table 10, Appendix A) and late onset asthma (Table 12, Appendix A). But children living in those RHAs were less likely to have late transient asthma (Table 11, Appendix A) and early persistent asthma (Table 8, Appendix A). Living in northern RHAs was not associated with risk of early persistent asthma for children with maternal history of asthma. No children who were living in northern RHAs and also had maternal history of asthma were diagnosed with late transient asthma. Living in northern RHAs remained significantly associated with lower risk of late transient asthma (Table 3.11) but was no longer associated with risk of early persistent asthma after adjustment for other factors in multivariate logistic regression.

Validation of RSV definition

4.1 Defining RSV-season

On weekly basis, selected laboratories across Canada report on the number of sample tested for respiratory syncytial virus (RSV), influenza, parainfluenza and adenovirus and number of virus isolation to the Centre for Immunization and Respiratory Infectious Diseases (CIRID), Public Health Agency of Canada [179]. A personal communication was made with Health Canada for the isolation report on these respiratory viruses in the year 1995, 1996 and 1997. From that report, the onset and offset of RSV seasons for the current study were identified for the corresponding years following the definition of RSV season mentioned in the methods. The RSV-seasons for the years 1995, 1996 and 1997, as identified following the definition, are mentioned below:

4.1.1 RSV season for 1995: Onset: February 19th, 1995 and offset June 24th, 1995 with the duration of 18 weeks. In addition to the main epidemic period, as evident from the graph, there was another small outbreak period that met the criteria for RSV-season (onset and offset) starting on November 05, 1995 and ending on December 02, 1995 with duration of 4 weeks.

4.1.2 RSV season for 1996: Onset: January 21st, 1996 and offset May 18th, 1996 with the duration of 17 weeks.

4.1.3 RSV season for 1997: Onset: January 01st, 1997 and offset April 05, 1997 with the duration of 14 weeks.

The following graphs (Figure 4.1) represent the weekly isolation of RSV in Manitoba for the year 1995, 1996 and 1997.

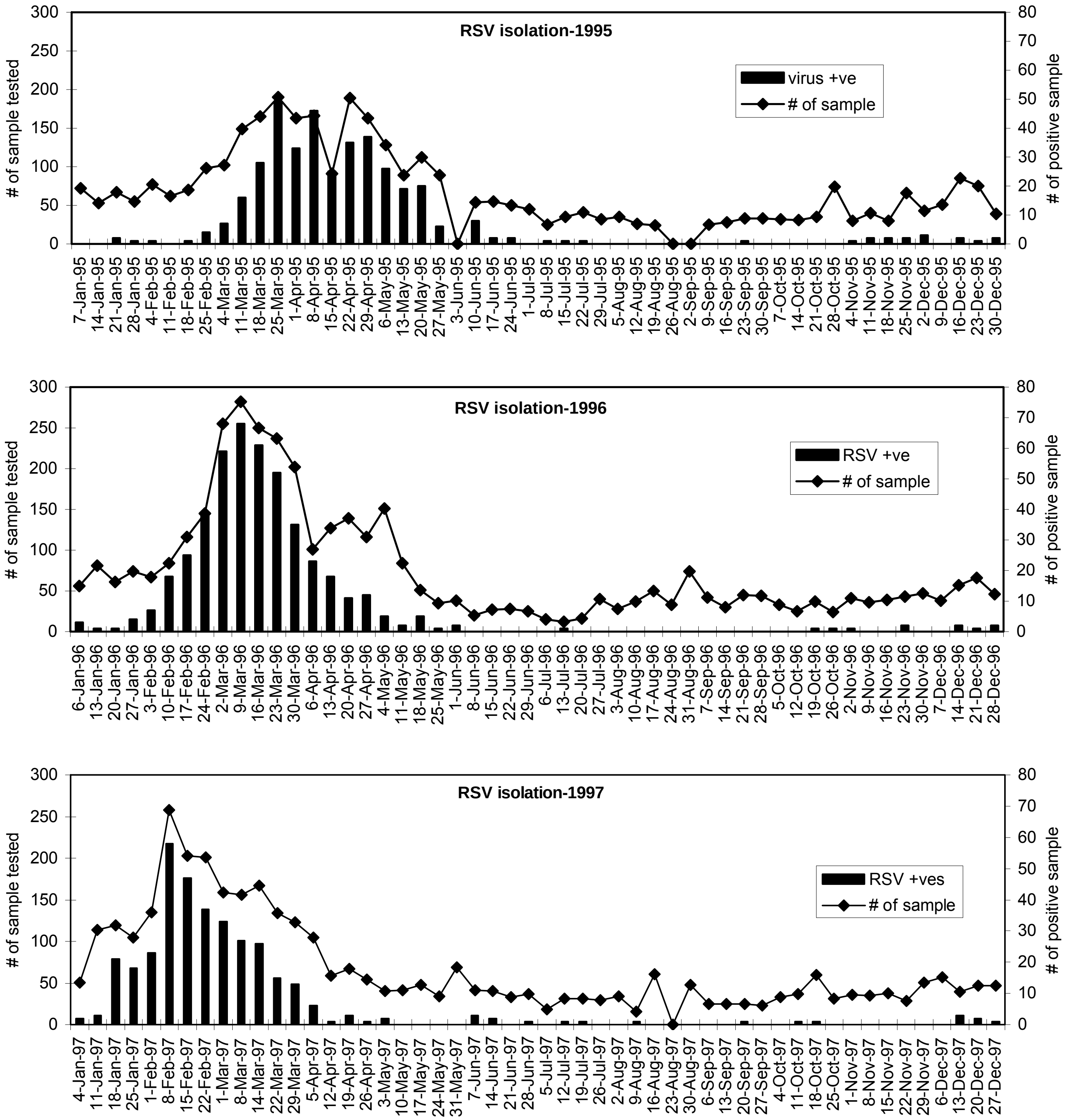

Figure 4.1: Weekly isolation of RSV in Manitoba, 1995-1997.

4.2 Proportion of children required health care for RSV-LRTI

The current definition identified that 4117 (29.45% of total 13980) children of the study cohort had clinically significant RSV-associated lower respiratory tract infection within the first two years of life. Among these children, 1888 (45.86% of 4117 RSV-positive and 13.51% of total 13980) had more than one RSV-associated LRTI within the first two years of life. A total of 2954 children (71.75% of 4117 RSV-positive) required utilization of health care for RSV-associated LRTI in the first year of life and 43.40% of these children had the infection(s) more than once. In the second year of life 1838 children of the study cohort (44.64% of 4117) had clinically significant RSV-associated LRTI and 29.65% of them had the infection(s) more than once.

A total of 6138 children (43.91%) of the current study cohort had lower respiratory tract infections (overall) before the age of 2 years, 3630 of them (59.14%) had the infection(s) more than once. In the first year of life 4351 (70.89% of 6138 LRTI positive and 31.12% of total 13980) children required LRTI-associated utilization of health care, 2358 of them (54.19% of 4351) required it more than once. In the second year of life, 3461 children (56.39% of 6138) had overall LRTI and 42.96% (1487 of 3461) of these children had the infection(s) more than once.

A total of 665 children (16.15% of 4117 RSV-positive and 4.76% of the total 13980) of the study cohort were hospitalized with RSV-associated LRTI within the first 2 years of life. One hundred and sixty nine children (25.41% of 665) were hospitalized with RSV-associated LRTI more than once before the age of 2 years. Five hundred and thirty nine children were hospitalized with RSV-LRTI in the first year of life. The recurrence rate of hospitalization with RSV-associated LRTI in the first year was 22.26% (120 of 539) and in the second year was 16.96% (29 of 171).

One thousand and thirty one children (16.8% of 6138 LRTI positive and 7.37% of total 13980) of the current study cohort required hospitalization for all lower respiratory tract infections within the first two years of life. Three hundred and fifty eight children (34.72% of 1031) were hospitalized with LRTI more than once within the first two years. Eight hundred and thirty children (80.5% of 1031) were hospitalized with LRTI in the first year. The reoccurrence rate of hospitalization with LRTI in the first year was 31.2% (259 of 830) and in the second year was 26.07% (85 out of 326).

4.3 Severity of lower respiratory tract infection

Length of hospital stay (LOS) was used as an indicator of severity of the episodes of lower respiratory tract infections. The median length of hospital stay for overall lower respiratory tract infection was 3 days (mean=3.92 days, std ±4.05 days). The median length of hospital stay for RSV-associated lower respiratory tract infection was 3 days (mean=3.78 days, std ±3.03 days) and median length of hospital stay for non-RSV-associated lower respiratory tract infection was also 3 days (mean=4.12 days, std ±5.08 days). There was no statistically significant difference between the length of hospital stay for children with RSV-associated LRTI and children with non-RSV-associated LRTI. The mean LOS for the lower respiratory tract infections are compared in the following graph (Figure 4.2).

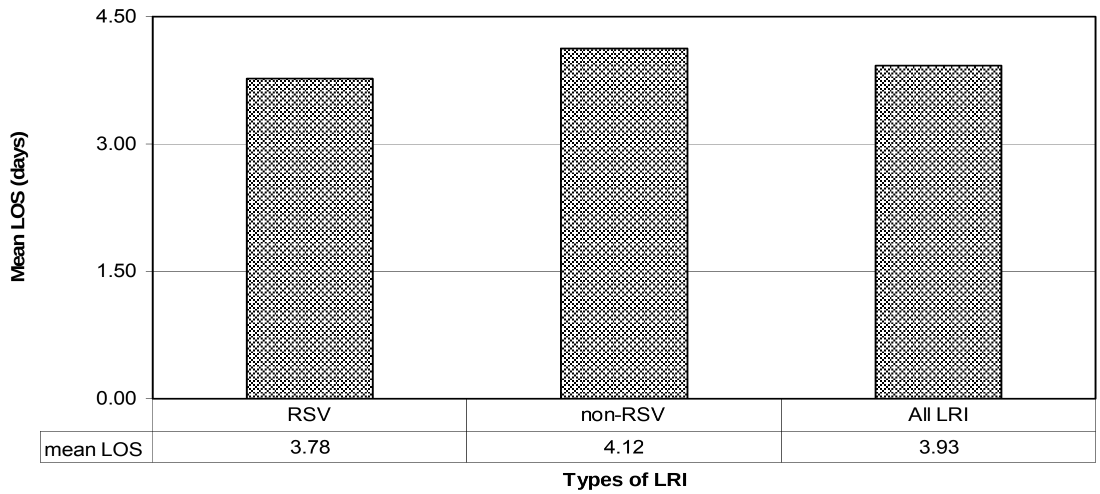

Figure 4.2: Comparing mean length of hospital stay (LOS) for RSV-associated LRTI, non-RSV-associated LRTI and overall LRTI.

Though there was no significant difference in LOS between LRTI, RSV-associated LRTI and non-RSV-associated LRTI, more episodes of severe lower respiratory tract infections were associated with RSV than with non-RSV. Almost 56% of the severe cases and 55% of the moderate episodes of lower respiratory tract infection before the age of 2 years were caused by RSV (Figure 4.3).

When the episodes of RSV-associated and non-RSV-associated lower respiratory tract infections were classified and compared by the severity categories, 7.65% of the total RSV-associated LRTI were severe and 8.50% were moderate infections. On the other hand, only 3.56% of the lower respiratory tract infections of non-RSV aetiology were severe and 5.05% were moderate. Since the moderate and severe lower respiratory tract infections involved hospital admission, it was clearly evident that 16.15% of the children who had RSV-associated lower respiratory tract infections required hospital admission while only 8.61% of the children who had non-RSV-associated lower respiratory tract infections required hospital admission.

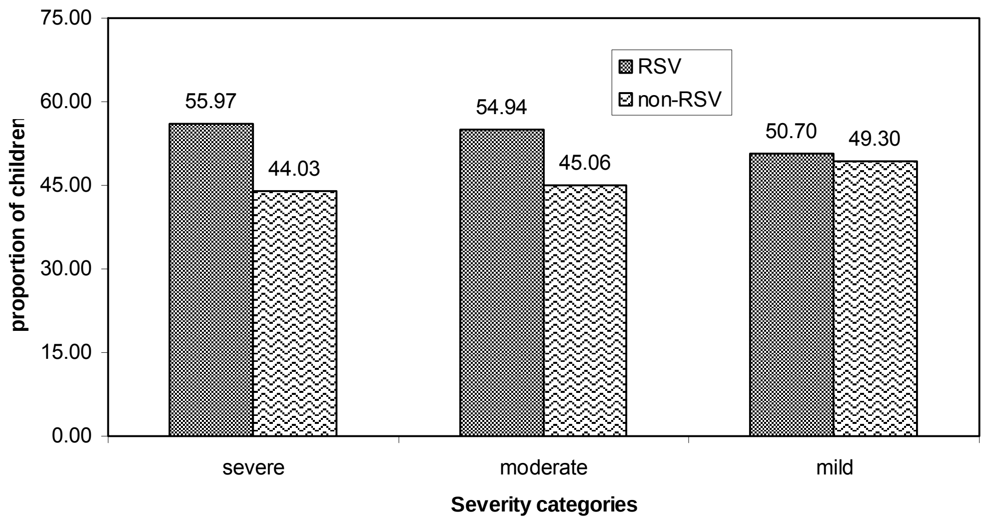

Figure 4.3: Proportions of children with RSV-associated and non-RSV-associated LRTI in different severity categories.

4.4 Age at health care for RSV-LRTI

Within the first two years of life, 6138 children of the current study cohort accounted for 17614 episodes of clinically significant lower respiratory tract infections which required utilization of health care system. According to the definition of RSV-associated lower respiratory tract infection, 9232 of these episodes were caused by RSV and the rest 8382 were attributable to non-RSV. RSV was the predominant organism to cause lower respiratory tract infection(s) before the age of 6 months. Non-RSV organisms were the major causes of lower respiratory tract infection(s) after the first 12 months of life. As presented in the following graph, RSV was associated with almost 70% of the total cases of lower respiratory tract infections before the age of 3 months and over 60% of total cases of lower respiratory tract infections between 3 and 6 months. Mean age of clinically significant RSV-associated LRTI were significantly less (mean age= 288 days, std ±188 days) than mean age of non-RSV-associated LRTI (mean age= 353 days, std ±194 days).

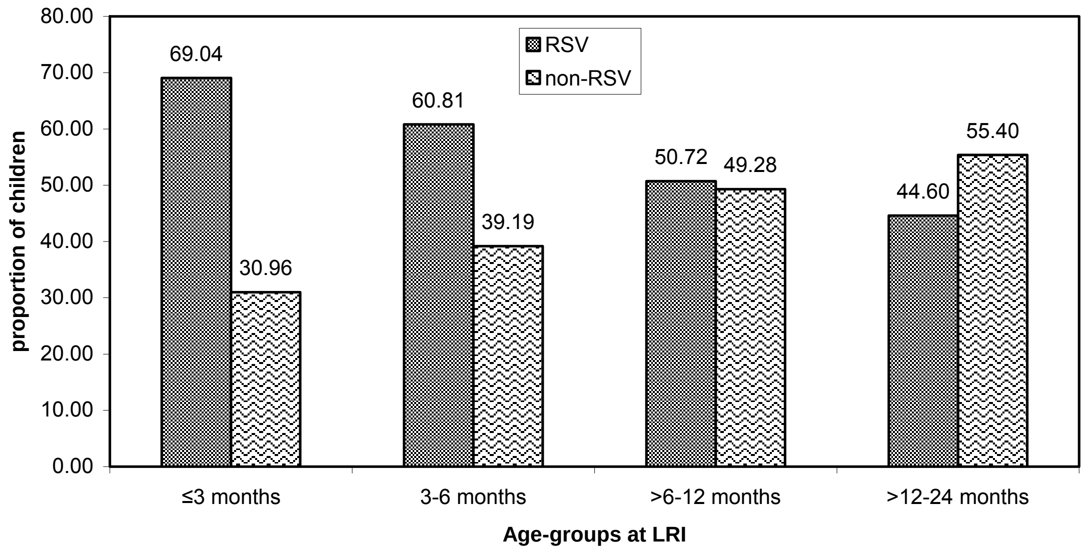

Figure 4.4: Proportions of RSV-associated and non-RSV-associated LRTI in different age-groups.

The mean age at the time of first RSV infection was 275 days (std ±184 days, median= 238 days). The highest proportion of children (33.54%) had first RSV-LRTI associated utilization of health care between 6 and 12 months of life and the highest proportion of children (32.48%) required RSV-LRTI associated hospital admission for the first time within first 3 months of life. RSV-LRTI associated hospital admission was the lowest (18.95) among the children of the oldest age-group (>12-24 months). The mean age at the time of first overall LRTI was 275 days (std ±187 days, median= 233 days). The highest proportion of children (32.14%) required LRTI associated utilization of health care for the first time between 6 and 12 months of life. Very similar to the RSV-associated LRTI, the highest proportion of children (34.14%) required LRTI associated hospitalization for the first time within the first 3 months of life. LRTI-associated hospitalization was also the lowest (19.5%) among children in the oldest age-group (>12-24 months).

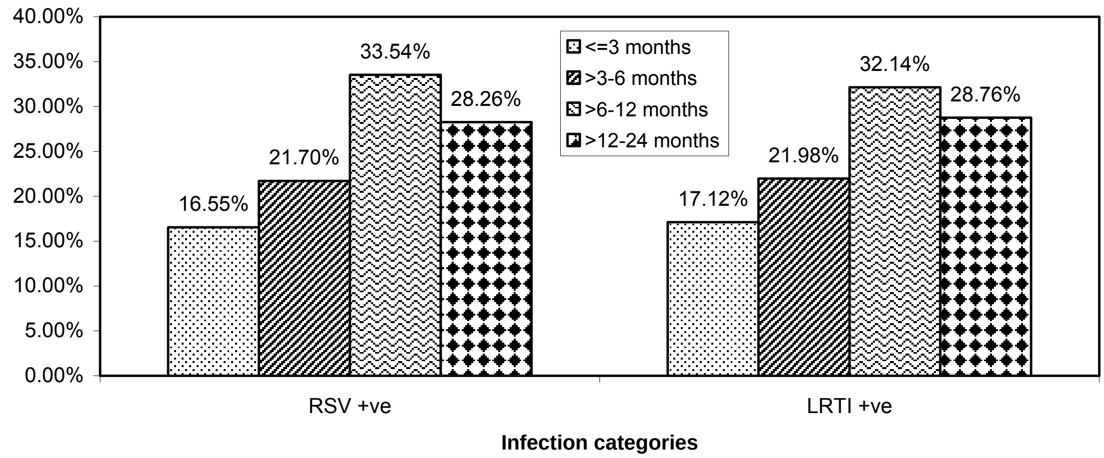

Figure 4.5: Proportions of children in different age-groups at first overall LRTI and first RSV-associated LRTI.

4.5 Gender and RSV-LRTI

The number of male and female children in the study cohort was 7116 and 6864, respectively. As presented in Table 4.1, male children were at significantly higher risk of having RSV-associated LRTI as well as overall LRTI before the age of 2 years than their female counterparts. Male children were also at higher risk of hospitalization with both RSV-associated LRTI and overall LRTI. Three hundred and ninety eight of the children who required hospital admission for RSV-associated LRTI within the first 2 years of life were male (59.85% of 665) and 267 (40.15% of 665) were female. Most of the children (59.26% of 1031) who required hospital admission with overall LRTI were also male.

Table 4.1: Comparing proportions of children with overall LRTIs and RSV-associated LRTIs within the first 2 years of life.

Feature	LRTI (N=6138)				RSV-LRTI (N=4117)			
	n	%	OR (95% CI)	P-value	n	%	OR (95% CI)	P-value
Prematurity								
• Yes	433	48.54	1.22 (1.07-1.40)	0.0039	307	34.42	1.28 (1.11-1.48)	0.0008
• No	5701	43.59			3806	29.10		
Living North								
• Yes	676	47.47	1.17 (1.05-1.31)	0.0042	455	31.95	1.14 (1.01-1.28)	0.0288
• No	5462	43.50			3662	29.17		
Low birth-weight								
• Yes	329	47.47	1.16 (1.00-1.36)	0.0513	233	33.62	1.23 (1.04-1.44)	0.0132
• No	5805	43.71			3881	29.22		
Urban/rural residence								
• Rural	2681	44.86	1.07 (1.00-1.14)	0.0505	1817	30.40	1.08 (1.01-1.17)	0.0331
• Urban	3457	43.20			2300	28.74		

Feature	LRTI (N=6138)				RSV-LRTI (N=4117)			
	n	%	OR (95% CI)	P-value	n	%	OR (95% CI)	P-value
No. of sibling in 2002								
• ≥ 2 siblings	3338	47.59	1.35 (1.26-1.44)	<.0001	2323	33.12	1.43 (1.33-1.54)	<.0001
• 0/1 sibling	2800	40.20			1794	25.75		
Older sibling								
• Yes	3869	46.61	1.31 (1.23-1.40)	<.0001	2673	32.20	1.39 (1.29-1.50)	<.0001
• no	2269	39.95			1444	25.43		
Gender								
• Male	3369	47.34	1.33 (1.24-1.42)	<.0001	2329	32.73	1.38 (1.28-1.49)	<.0001
• Female	2769	40.34			1788	26.05		
Childhood income quintile								
• Low	1866	53.48	1.68 (1.55-1.81)	<.0001	1318	37.78	1.67 (1.54-1.81)	<.0001
• High	4248	40.65			2783	26.63		

4.6 Prematurity

The median gestational age of the children of current study cohort was 40 weeks. A total of 892 children of the study cohort were identified as premature. Very similar to the whole birth-cohort, the median gestational age of children who required RSV-associated utilization of health care was 40 weeks. The median gestational age of children with overall lower respiratory infection before the age of 2 years was 40 weeks as well. As presented in Table 4.1, premature children were more likely to have both RSV-associated LRTI and overall LRTI within the first 2 years of life than their normal counterparts. Premature children were also at higher risk of hospital admission with RSV-associated LRTI (OR=2.21, CI=1.74-2.81) and overall LRTI (OR=2.58, CI=2.13-3.12).

A total of 693 children of the study cohort were identified with low birth weight. Children with low-birth weight were more likely to have RSV-associated LRTI in first two years of life than children with normal birth-weight. But no association between low birth-weight and overall LRTI was observed (Table 4.1). Children with low birth-weight were more likely to be hospitalized with RSV-associated LRTI (OR=1.84, CI=1.38-2.44) and overall LRTI (OR=2.24, CI=1.80-2.80) than children with normal birth-weight.

4.7 Region of residence

In the current study a total of 5977 children of the 1995 birth-cohort were living in rural Manitoba and 8003 children were living in urban areas of Manitoba. Children living in the rural areas were more likely to have clinically significant RSV-associate LRTI than children living in urban areas, though the association was barely significant. Living in urban/rural areas was not a significant risk factor for overall LRTI for the current study cohort (Table 4.1). But children living in rural Manitoba were over three times more likely to be hospitalized with RSV-associated LRTI (OR=3.19, CI=2.70-3.78) and overall LRTI (OR=3.42, CI=2.98-3.93) than children living in urban area.

A total of 1424 children of the study cohort were living in three northern regional health authorities. Children living in those northern RHAs were more likely to have RSV-associated LRTI than children not living in north. The risk of overall LRTI was barely associated with living in these three northern RHAs (Table 4.1). Children living in north were over three times more likely to be hospitalized with RSV-associated LRTI (OR=3.36, CI=2.8-4.04) and almost four times more likely to be hospitalized with overall LRTI (OR=3.91, CI=3.37-4.55) than children who were not living in north.

4.8 Socioeconomic status

The information about the income-quintile of parents' was available for 13939 children of the current study cohort. According to the definition used in the current study, families of 10450 children were defined as 'high' income and families of 3489 children were defined as 'low' income families. In the current study, children from low income families were at higher risk of having clinically significant RSV-associated LRTI and overall LRTI than children from the higher income families (Table 4.1). Children from lower-income families were over three times (OR=3.10, CI=2.65-3.63) more likely to be hospitalized with RSV-associated LRTI than children from the higher income families. Risk of hospital admission with overall LRTI was also higher for the children from lower income families than children from higher income families (OR=3.02, CI=2.65-3.43).

As presented in the following graph (Figure 4.6), 37.78% children of quintile-1 had RSV-associated LRTI within first 2 years of life while only 23.70% children of quintile-5 had RSV-associated LRTI. Similarly, 53.48% children of quintile-1 had lower respiratory tract infection (all aetiology) within the first 2 years of life while 36.85% children of quintile-5 had overall lower respiratory tract infections.

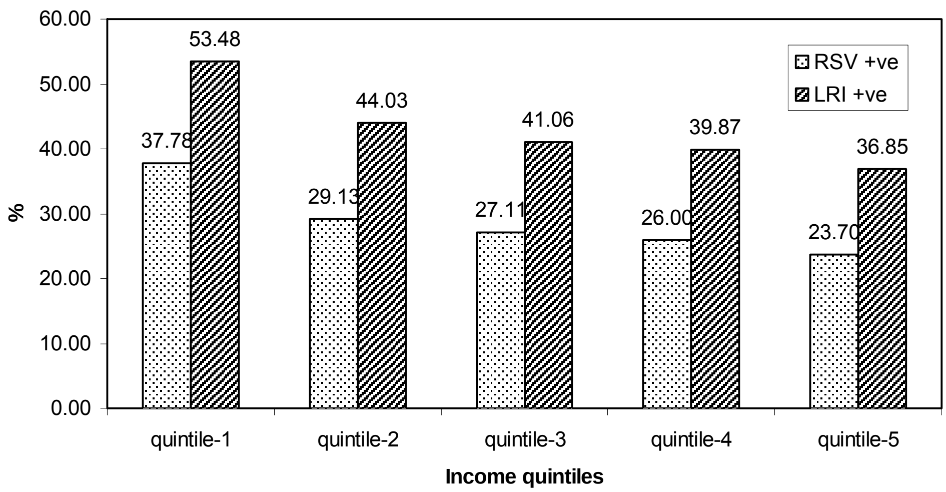

Figure 4.6: RSV-associated LRTI and overall LRTI in different income quintile.

4.9 Number of siblings

A total of 7014 children of the study cohort had two or more siblings by the end of 2002. As evident from Table 4.1, children with two or more siblings were more likely to have clinically significant RSV-LRTI and overall LRTI before the age of 2 years than children who had either one or no sibling in 2002. Children with two or more siblings were over three times (OR= 3.27, 95% CI=2.73-3.92) more likely to require hospital admission with RSV-LRTI within first two years of life than children with one or no sibling in 2002.

Children with two or more siblings were also more likely (OR=2.72, CI=2.36-3.13) to be hospitalized with overall LRTI than children with one or no sibling in 2002.

In the current study, 8301 children had at least one older sibling in 1995. Children with at least one older sibling at their birth were at higher risk of having RSV-associated LRTI and overall LRTI in the first two years of their lives than children who did not have any older sibling (Table 4.1). Risk of RSV-associated hospitalization was almost double among children with at least one older sibling at birth than children who did not have any older sibling (OR=2.14, CI=1.79-2.56). Children with at least one older sibling were also at higher risk of overall LRTI-associated hospital admission than children without any older sibling (OR=1.93, CI=1.67-2.22).

Discussion

5.1 Summary of research findings

Frequency and severity of clinically significant RSV-LRTI, and younger age at first occurrence were significant risk factors for asthma when the onset was within the first three years of life (i.e., early persistent asthma, Table 3.9 and transient wheeze, Table 3.10). The late onset of asthma phenotypes were not related to RSV-LRTI exposure. Clinically significant RSV-associated lower respiratory tract infection in the first two years of life was associated with higher risk of asthma at the age of 7 (Table 3.1) and at 11 years (Table 3.5). However, the association diminished with increasing age of children. Specifically, our study found that frequency of RSV-associated LRTI before age 2 years influenced the risk of asthma diagnosis at 7 (Table 3.2) and 11 (Table 3.6) years. Higher risk of asthma diagnosis was associated with more severe episode(s) of RSV-associated LRTI (Table 3.4 and Table 3.8). Children of the current study cohort who's first RSV-associated LRTI episode was between 6 and 12 months of life were at the highest risk of asthma at both the ages (Table 3.3 and Table 3.7). These findings were independent of children's maternal history of asthma, gender, number of siblings, premature birth, area of residence, socio-economic status and antibiotic treatment in the first year of life. Each will be discussed in more detail in the sections that follow.

5.1.1 RSV-associated LRTI and asthma

Children of the study cohort with clinically significant RSV-associated LRTI before the age of 2 years were at significantly higher risk of having asthma at the age of 7 and 11 years than children who did not have RSV-associated LRTI. The risk of asthma gradually diminished with increasing age. Children who had clinically significant RSV-associated LRTI before 2 years of age were almost 3 times more likely (OR=2.94, 95% CI=2.60-

3.32) to have asthma-like diagnoses within 3 years of life than children who did not have RSV infection (Appendix A-Table 1). This likelihood of asthma from RSV-LRTI dropped considerably when children were 7 years old (OR=1.36, 95% CI=1.17-1.59) (Table 3.1). The odds ratio for asthma at age 11 years fell further to 1.26 (95% CI=1.08-1.47, Table 3.5).

The association observed between RSV-LRTI and subsequent development of asthma, and the changing trend of the association with increased age followed findings from earlier studies. A number of epidemiological studies in different populations and settings reported that RSV-associated lower respiratory tract infection during infancy was a significant risk factor for the subsequent development of asthma later in life. The diminishing trend of the RSV-asthma association was also found in a few other studies [19, 131, 132, 144-146, 148]; in some the association was no longer apparent as early as at age 3 years [131-139, 144-146, 148, 163-165, 167, 195]. A recent review by Perez-Yarza and colleagues concluded that RSV infection in early life was an important risk factor in the long-term for the development of asthma or wheezing in childhood but the effect of RSV progressively disappeared with increasing age [123]. On the other hand, studies have reported that RSV-associated LRTI in infancy continued to be associated with asthma and abnormal lung function up to the age of 13 years [137, 146]. Very similar to our study, the Tucson Children's Respiratory Study, the Swedish prospective cohort study and study conducted by Noble and colleagues found that RSV-associated LRTI before the age of 2 yeas remained a significant risk factor of asthma at age 11 years.

5.1.2 Frequency of RSV-associated LRTI and asthma

Our study found that recurrence of clinically significant RSV-associated LRTI within the first two years of life was associated with greater risk of asthma at age 7 (Table 3.2) and age 11 (Table 3.6) years. It was clearly evident that the association between the number of clinically significant RSV-LRTI episodes and risk of asthma gradually declined with increase in age of the children. For example, the odds ratio (OR) of asthma-like diagnoses at age 3 years for children who had three or more RSV-associated LRTI was 7.70 (95% CI=6.50-9.13, Appendix A-Table 2). At the age of 7 years, the OR declined to 1.61 (95% CI=1.24-2.08) and at 11 years the OR was 1.42 (95% CI=1.09-1.86). A similar trend was observed for children who had 1-2 clinically significant RSV-LRTI within first 2 years of life.

An association between recurrent infections in early life and the development of childhood asthma is well documented in the literature. Von Mutius and colleagues found that repeated episodes of fever in early life were strongly associated with asthma and current wheeze at school-age [196]. From the MAS study group in Germany, a strong dose-response relationship between the number of lower respiratory tract infections in the first three years of life and physician diagnosed asthma, current wheeze and bronchial hyperreactivity at age 7 years was reported [138]. Arshad and colleagues, and at least 2 other studies, found that recurrent chest infections in the first year of life was an important factor for the occurrence of wheeze and asthma at 10 years [145, 197, 198]. Unlike our study, it is unknown whether RSV infection was involved in these studies, except the study conducted by Pullan and Hay.

5.1.3 Severity of RSV-associated LRTI and asthma

More severe RSV-associated LRTI before age 2 was a greater risk factor for asthma at 7 (Table 3.4) and at 11 (Table 3.8) years of age. At age 7 years, the odds ratio for asthma was 1.89 for severe RSV infection versus 1.29 for mild infection. As with recurrence of RSV infection, the association between RSV severity and asthma gradually declined with increasing child age. Children who had the most severe RSV-associated LRTI within the first 2 years of life were over nine times more likely (OR=9.15, 95% CI=6.95-12.04) to be diagnosed with asthma-like conditions before the age of 3 years (Appendix A-Table 3). The likelihood of asthma at the age of 7 years from severe early life RSV-LRTI was 1.89 (95% CI=1.21-2.95). The OR declined to 1.74 (95% CI=1.11-2.72) at the age of 11 years. Very similar trends were observed for children who had mild and moderate episodes of RSV-associated LRTI within the first two years of life.

Longer hospital stay has been used to predict disease severity or complications in children with asthma, pneumonia and bronchiolitis [188-191]. Silber and colleagues indicated that an LOS >5 days can be used as a tool to identify subjects with complications in pneumonia [191]. Willson and colleagues reported that average LOS for children who were hospitalized for uncomplicated bronchiolitis or pneumonia was 3 days [190]. We found the median LOS for lower respiratory tract infections (all aetiology) and for RSV-associated lower respiratory tract infections to be 3 days. On this basis, 3 days LOS was used as the cut off point to define moderate and severe episodes of RSV-associated LRTI. Thus, data on length of hospital stay (LOS) and the type of required health care were used to determine the severity of RSV-associated LRTI episodes in this study. RSV severity was defined as: 1) no RSV-LRTI care, 2) mild (physician visit only), 3) moderate (hospital admission with LOS ≤3 days) and 4) severe (hospital admission with LOS >3 days).

Caroll and colleagues were the first to report on the dose response relationship between severity of bronchiolitis among infants and subsequent risk of childhood asthma, using the following severity classification: 1) no health care visit for RSV, 2) outpatient physician visit, 3) emergency department visit and 4) hospitalization for bronchiolitis [140]. Similar to our study, they also found that greater asthma risk from bronchiolitis during early childhood was associated with more severe infection. Their study was not restricted to bronchiolitis caused by RSV only, but also included bronchiolitis caused by other respiratory viruses as well. The current study identified all lower respiratory tract infections (including but not only limited to bronchiolitis) caused exclusively by RSV within the first 2 years of life. So, to the best of our knowledge, ours is the first study to investigate the dose response relationship between the severity of clinically significant RSV-LRTI and risk of asthma and asthma phenotypes in childhood.

5.1.4 Age during RSV-associated LRTI and asthma

First clinically significant RSV-associated LRTI within the first 12 months of life was associated with higher risk of asthma diagnosis later in childhood. The highest risks of asthma diagnosis at 7 (Table 3.3 and Figure 3.1) and at 11 years (Table 3.7 and Figure 3.5) were associated with first clinically significant RSV-associated LRTI between 6 and 12 months of life. But the highest risk of asthma-like diagnosis within the first 3 years of life was related to first clinically significant RSV-LRTI within the first 6 months of life (Table 4, Appendix A). First RSV-associated LRTI after the first 12 months of life was not associated with risk of asthma-like diagnosis within the first 3 years of life (Table 4, Appendix A) and asthma diagnosis at the age of 11 years (Table 3.7).

Very similar to severity and recurrence of RSV infection, the association between age-group at the time of first clinically significant RSV-LRTI and asthma also declined with increasing age. Children who had first clinically significant RSV-LRTI within the first 6 months of life were over four times more likely to have asthma-like symptoms before the age of 3 years (OR=4.47, 95% CI=3.85-5.19, Table 4, Appendix A). The OR for asthma diagnosis of these children at 7 years declined to 1.32 (95% CI=1.06-1.65, Table 3.3) and was barely significant at the age of 11 years (OR=1.25, 95% CI=1.001-1.56, Table 3.7).

The association between younger age at the time of RSV-associated LRTI and higher risk of subsequent asthma is well documented in literature [51, 199-202]. Animal studies also reported that younger age at the time of RSV infection predisposed the subject to respiratory morbidity in adulthood [203, 204]. Wu and colleagues found that children who were 4 months old at the time of winter virus peak were at the highest risk of developing childhood asthma [141]. RSV infection is most prevalent when the infant is least immunologically mature. The presence of maternal antibodies decreases gradually during the first 6 months of life leaving most infants unprotected against RSV between 2 and 4 months of age and severe RSV-related illness occurs between 2 and 3 months [3, 13]. Findings from our study supported the current knowledge on the association between RSV infection at younger age and subsequent development of childhood asthma. But the highest risk of our study cohort was associated with first clinically significant infection between 6 and 12 months, which was slightly higher than those reported from studies conducted before.

5.1.5 Asthma phenotypes and RSV-associated LRTI

Clinically significant RSV-LRTI was a significant risk factor for subsequent childhood asthma when the onset of asthma occurred within the first 3 years of life. RSV-LRTI before the age of 2 years was associated with higher risk of early persistent asthma (OR=2.91, 95% CI=2.20-3.85) (Table 13, Appendix A) and transient wheeze (OR=2.99, 95% CI=2.62-3.43) (Table 14, Appendix A). But RSV-LRTI was not associated with risk of late transient asthma, late persistent asthma and late onset asthma. Obviously frequency and severity of the infection(s) and age at the time of first infection were insignificant for these three asthma phenotypes as well.

Very similar to the risk of asthma diagnosis at 7 and at 11 years, higher number(s) of clinically significant RSV-associated LRTI was significantly associated with greater risk of early persistent asthma (Table 3.9) and transient wheeze (Table 3.10). Children of the study cohort who had 1-2 clinically significant RSV-LRTI within the first 2 years of life were over twice more likely to have early persistent asthma (Table 3.9) and almost twice more likely to have transient wheeze (Table 3.10). But having three or more RSV-LRTI was associated with over six times more likelihood of early persistent asthma (Table 3.9) and over eight times more likelihood of transient wheeze (Table 3.10).

The current study found that more severe RSV-LRTI was associated with greater risk of both early persistent and transient wheeze, the trend was evident from Table 3.9 and Table 3.10. Children who had mild RSV-LRTI before the age of 2 years were over twice more likely to have early persistent asthma (Table 3.9) and transient wheeze (Table 3.10). But children who had severe episode(s) of clinically significant RSV-LRTI were almost nine times more likely to have early persistent asthma (Table 3.9) and transient wheeze (Table 3.10).

First clinically significant RSV-LRTI within the first 12 months of life was significantly associated with higher risk of early persistent asthma (Table 3.9) and transient wheeze (Table 3.10) than children who did not have the infection within first 2 years of life. Having first clinically significant RSV-LRTI after the first 12 months of life was not associated with any significant risks of these asthma phenotypes. Unlike the risks of asthma diagnosis at 7 and at 11 years, the highest risks of both early persistent asthma (Table 3.9) and transient wheeze (Table 3.10) were associated with having first clinically significant RSV-LRTI within the first 6 months of life.

5.1.6 Confounding factors and asthma

The current study determined the associations between clinically significant RSV-associated LRTI, frequencies and severity the infection and younger age at the time of first infection on the development of childhood asthma. These associations were determined after the adjustments for the influences of the most commonly cited confounding factors (Appendix B) in multivariate logistic regression analyses.

Children of the current study cohort with maternal history of asthma were at higher risk of asthma at 7 (Table 3.2) and at 11 (Table 3.6) years than children without maternal history of asthma. Maternal asthma was also associated with higher risk of all the asthma/wheezing phenotypes as well (Table 3.9, 3.10 and 3.11). Maternal stress appeared to be significantly associated with higher risk of asthma at 7 years (Table 3.2) and transient wheeze (Table 3.10) only.

Lower income of the parents was not associated with risk of asthma diagnosis at 7 or 11 years for children of the current study cohort. Lower income was only associated with higher risk of transient wheeze (Table 3.10) but not with any other asthma/wheezing phenotype.

Children of the current study cohort who had 2 or more siblings were less likely to have asthma diagnosis at 7 (Table 3.2) and 11 (Table 3.6) years than children who had either one or no sibling. With the exceptions of only transient wheeze and late onset asthma, having two or more sibling was associated with lower risks of all other asthma/wheezing phenotypes (Table 3.9 and 3.11). Male children were at higher risk of being diagnosed with asthma at 7 years (Table 3.2), at 11 years (Table 3.6) and all of the asthma/wheezing phenotypes (Table 3.9, 3.10 and 3.11) than their female counterparts.

Children living in urban areas of Manitoba were at greater risk of asthma diagnosis at 7 (Table 3.2) and at 11 (Table 3.6) years than children living in rural areas. With the exception of only transient wheeze, living in urban area was related with higher risks of all other asthma phenotypes (Table 3.9 and 3.11). Children living in three northern RHAs of Manitoba were less likely to have asthma diagnosis at 7 years (Table 3.2) than children who were not living in north. Living in northern RHAs found to be associated with lower risk of late transient asthma (Table 3.11) as well. But this factor was not associated with risk of asthma at 11 years and any other asthma/wheezing phenotype.

Prematurity was not a significant risk factor of asthma at 7 or 11 years for the current study cohort. Premature children were at higher risk of transient wheeze than full-term children (Table 3.10) of the study. Prematurity was not associated with risk of any other asthma phenotype. Low birth-weight was significantly associated with higher risk of asthma at 7 years (Table 3.2) and lower risk of late onset asthma at 11 years (OR=0.44, 95% CI=0.21-0.88) (Table 3.11). The number of low birth-weight children who had late onset asthma was very small (Table 3.11). With such a small number (and very small proportion of the study cohort), it was difficult to draw any conclusion on this association. Low birth-weight was not related to risk of asthma at 11 years or any other asthma/wheezing phenotype.

Receiving at least one antibiotic in the first year of life was not associated with risk of late onset asthma and late persistent asthma for the children of the current study cohort. With these two exceptions, higher number of antibiotics in the first year of life was associated with greater risk of asthma at age 7 (Table 3.2) and 11 years (Table 3.6). Children who received more antibiotics in the first year of life were also at higher risk of early persistent asthma, transient wheeze and late transient asthma (Table 3.9, 3.10 and 3.11). Completion of BCG vaccination was associated with higher risk of early persistent asthma (Table 3.9) only.

The associations of above mentioned factors with childhood asthma have been well documented in current literature. The Canadian Early Childhood Development Study revealed that male sex, low birth-weight, parental atopy and low socioeconomic condition put the children at significantly higher risk of physician diagnosed asthma in their preschool years. Having more than one sibling at birth and living in rural areas were related with lower risk of childhood asthma [96]. Findings of Shankardass and colleagues in Toronto supported this study [97]. Similar association between socioeconomic status and risk of asthma among European children was reported by several epidemiological studies [99-101]. A number of studies in different geographic areas across the world reported that children living in rural environments were at lower risk of development of asthma and aeroallergen sensitization [114-120].

A number of studies reported parental history of asthma as the strongest predictor of childhood asthma [93-95]. Few studies also found that maternal stress in early life was related to childhood asthma [98, 102, 103]. A number of epidemiological studies and reviews of published literature reported that wheezing and asthma were more prevalent among male than female children, but the trend was reversed in adolescence [95, 104, 105, 205]. In a systematic review Karmaus and Botezan indicated that children with higher numbers of siblings were less likely to have asthma [106].

Phase III of The ISAAC study found that use of antibiotics in the first year of life was associated with higher risk of current asthma among 6 and 7 years old children [109]. This finding was supported by several epidemiological studies as well [107, 108, 206]. Conflicting evidence on the protective effect of BCG vaccination in early life was reported by several epidemiological studies and systemic review of the published literature [110-113, 207-209].

The associations observed in the current study between the above mentioned factors and childhood asthma were fairly similar to observations reported from studies conducted earlier. But few discrepancies with current knowledge were identified as well. Most of the current literature reported that children from lower income families were more likely to be diagnosed with asthma. But the current study found that income level of the parents was significantly associated with risk of transient wheeze only. The current study also found that completion of BCG vaccination was associated with risk of early persistent asthma and living in the northern RHAs was associated with risk of late transient asthma only.

Studies conducted earlier reported that in Manitoba childhood asthma was not more likely among children from lower income quintiles or among premature children or among children living in northern Manitoba [210-213]. The findings of the current study on these issues were very similar to these reports. The association between BCG vaccination and childhood asthma had conflicting evidence. Our study supported the finding of no association between asthma and BCG vaccination in early life.

The current study observed that only few factors including living in urban areas, child's gender, maternal asthma and number of sibling(s) (Table 3.11) were related with risk of asthma/wheezing phenotypes when the onset of asthma symptoms occurred after the age of 3 years (i.e., late onset asthma, late persistent asthma and late transient asthma).

5.2 Validity of the current definition of RSV-associated LRTI

Validity refers to whether a measurement instrument or procedure accurately measures the characteristics or attributes it is intended to measure and a valid instrument truly reflects the concept it is supposed to measure. Depending on the purpose of the measurement, one or more validity approaches could be of interest [214-218]. Validity can be broadly divided into three basic types, which are: 1) content validity, 2) criterion related validity and 3) construct validity [216, 219]. The content/face validity and criterion related validity issues of the definition of RSV-associated LRTI are discussed below. Construct validity was not within the scope of the current study.

5.2.1 Content validity for the current definition of RSV-associated LRTI

Content validity, which has been described as the most common approach to establish validity of a measurement scale, addresses the question "how representative of the construct are the items that comprise the measure" [216, 219]? A content validity approach addresses the issue of adequacy of coverage of the content area being measured and compares the contents of the measurement technique to the known literature on the topic [215, 218, 220].

As mentioned earlier, RSV can be associated with more than one clinical condition of the lower respiratory tract including acute bronchitis, bronchiolitis and viral pneumonia. In addition to RSV, some other organisms including parainfluenza virus, influenza virus, adenovirus and rhinovirus have been reported as the causative agents of one or more of these clinical conditions [10, 24, 25, 28-30, 33-36]. After identifying all the possible/potential cases of RSV-associated lower respiratory tract infections, the next step of defining RSV-associated LRTI was differentiating RSV-associated infections from non-RSV-associated infections. Epidemiologic features including age, season and clinical presentation can be useful for estimating the aetiology of LRTI [54]. The current literature shows that there are differences in the ages of the subjects infected by different respiratory viruses. Seasonal patterns of different respiratory virus infection among children have also been reported [25, 58, 221-223]. The following features of the definition of RSV-associated LRTI will be discussed separately for content validity:

1) Inclusion of relevant ICD9CM codes in the definition,

2) Defining the RSV season and limiting the LRTIs to the RSV season only

3) Choosing 2 years as the cut-off age to define RSV-associated LRTI.

a) Inclusion of relevant ICD9CM codes in the definition: Respiratory syncytial virus (RSV), described as the most important respiratory pathogen in the infancy and early childhood, can be associated with more than one clinical condition of the lower respiratory tract including acute bronchitis, bronchiolitis and viral pneumonia [10, 25, 28, 30, 33, 35]. To identify the RSV-associated lower respiratory tract infections it is imperative that all the clinical conditions with a possible RSV aetiology are considered.

As noted earlier, acute bronchitis and bronchiolitis is coded as 466, chronic bronchitis is coded as 491, bronchitis which is not classified as either chronic or acute is coded as 490, viral pneumonia is coded as 480, bronchopneumonia where the causative organism is not specified is coded as 485, and the ICD-9CM code 486 represents the cases of pneumonia where the causative organism is not specified [27, 31, 32, 37, 38]. Chronic bronchitis (ICD9CM=491) lacks a standardized definition among children and the clinical entity or pathology of this condition is not well defined. Recurrent episodes of acute bronchitis can be interpreted as chronic bronchitis [34]. In lieu of the confusion over the diagnosis of chronic bronchitis, and possible overlap with the diagnosis of acute bronchitis, the ICD9CM code 491 was included in the criteria for identifying RSV-associated LRTI.

Unless the etiologic agent of LRTI was specified as an organism other than RSV, there was always the possibility that the etiologic agent for a particular episode of LRTI was actually RSV. Thus, it was crucial to include the unspecified cases of LRTI as possible/potential cases of RSV-associated LRTI to maximize the chance to identify all actual cases of RSV-associated LRTI. In summary, the inclusion of ICD9CM codes 466, 480-488, 490 and 491 and associated subcategories appeared to represent all the possible health care episodes where RSV-associated LRTI was responsible for the clinical condition(s).

b) Defining RSV season and limiting LRTIs to the RSV season only: The beginning and end of the RSV-season has been defined in several reports. Terletskaia-Ladwig and colleagues defined the onset week of RSV-season as the first of two consecutive weeks with at least 2 positive RSV isolations. The offset week of RSV outbreak was defined as the last of the final two consecutive weeks with at least two positive isolations of RSV [39]. The NREVSS in the USA defined onset of RSV-season as the first of two consecutive weeks of ≥10% positive isolation of RSV with at least two positive test results for both the weeks. The offset week was defined as the last of the final two weeks with at least 10% positive RSV isolation with at least two positive samples for both the weeks [40]. The definition of RSV-associated LRTI used for the current study followed these criteria.

Data on the isolation of respiratory viruses from Health Canada was used to define the onset, offset and duration of RSV-seasons for 1995, 1996 and 1997 only. The onset and offset dates were not the same for 1995, 1996 and 1997, which was very reasonable as Mullins and colleagues reported that the onset weeks and durations of RSV season vary substantially by year and within ± 4 weeks of the median value of that region [40]. Similar findings about the yearly variation was also reported by Gilchrist and co-investigators [224]. However, the Health Canada clearly showed the beginnings and ends of RSV outbreaks in Manitoba in 1995, 1996 and 1997. Outside those periods almost no RSV was isolated but other viruses were isolated throughout the year.

c) Choosing 2 years as the cut-off age to define RSV-associated LRTI: Another criterion used to differentiate RSV-associated LRTI from non-RSV-associated LRTI was choosing LRTI within the first two years of life only. Currently available literature suggests that RSV is essentially the most prevalent virus in lower respiratory tract infection up to the age of 2 years. Almost all children are infected with RSV within the first two years of life. The number of other respiratory viruses associated with the lower respiratory tract illness before 2 years were negligible. Nearly all of the first RSV infections are symptomatic. Though reinfection with RSV is very common, reinfection-associated illness are mild and lower respiratory disease is uncommon as a consequence of reinfection. Studies also found that the mean age of RSV-associated LRTI was significantly lower than the mean ages of LRTI caused by other respiratory viruses including influenza and hMPV [10, 11, 33, 43, 45, 48, 50, 62-72].

d) Overall content/face validity of the definition: In the current study, we identified all children who required utilization of health care with a RSV-associated LRTI. In other words, these episodes of RSV-associated LRTIs were expected to be severe enough to require utilization of health care. Considering the features of the definition of RSV-associated LRTI and the rationale for their selection in the published literature, it was reasonable to conclude that lower respiratory tract infections, coded as ICD9CM=466, 480-488, 490 and 491 in RSV season within the first two years of life were most likely to represent RSV-associated lower respiratory tract infections. This definition of clinically significant RSV-associated LRTI satisfied the requirement for content/face validity for the exposure variable of RSV in the current study.

5.2.2 Criterion related validity for the current definition of RSV-associated LRTI

In the concurrent criterion validity approach assessment, the measure being tested for validity and the measurement of related criterion are compared to determine current performance in respect to that criterion. A measurement has concurrent validity if the measurement generates results which are comparable and highly correlated with an established measurement [214, 217, 218, 220].

a) Proportion of children requiring LRTI care: Over 43% of cohort children required utilization of health care for lower respiratory tract infection before the age of 2 years. Almost 30% had clinically significant RSV-associated LRTI before the age of 2 years. Over 7% children of the cohort were admitted to the hospital with lower respiratory tract infection and less than 5% required hospital admission with RSV-associated LRTI. According to their health care utilization patterns, about 46% of children with RSV (13.5% of total study cohort) were reinfected with RSV in the first 2 years of life, while almost 60% of the LRTI-positive (all LRTI) children of the study cohort had recurrent lower respiratory tract infection.

These patterns of utilization are similar to those documented by others. It has been reported that up to 40% of children infected with RSV develop lower respiratory tract symptoms and 0.5%-2.0% of all infants require admission to hospital [10, 13]. Virtually all children in the Houston Family Study were infected at least once by 24 months of age, and about one half had experienced two infections [11]. Over 20% of the study population in the Chapel Hill study were reinfected naturally with RSV [43].

Health care records were used in the current study to identify children with RSV-associated LRTI and overall LRTI. RSV infections not severe enough to result in a physician visit or hospitalization would not be identified by the current definition. Re-infection with RSV would also infrequently be captured with the definition since RSV-associated re-infections are usually milder and rarely involve the lower respiratory tract. Thus, the prevalence of RSV-associated LRTI in the current study would be expected to be less than that reported in epidemiological studies where cell culture or immunological techniques were applied to confirm RSV infection. Considering the similarity with RSV health care patterns in the literature, the proposed definition of RSV-associated LRTI provided a reasonable representation of RSV-associated lower respiratory tract infection before the age of two.

b) Severity of LRTI: The type of required health care and the length of hospital stay (LOS) were used to define severity of the infection. No difference in LOS was detected between children with RSV-LRTI, non-RSV-LRTI and overall-LRTI for the current study cohort (Figure 4.2). But more children with RSV-associated than non-RSV-associated LRTI required hospital admission. As evident from Figure 4.3, RSV caused more severe cases of LRTI than non-RSV etiologic agent(s). Epidemiological studies suggested that RSV is responsible for more severe episodes of lower respiratory tract infections than other respiratory viruses [21, 49, 52, 62, 225, 226]. Similar to this study, Iwane and colleagues did not find a difference in LOS between children with RSV, PIV and influenza viruses, but RSV was associated with more severe infections than other viruses [67].

c) Age and gender of subject: In the current cohort, the mean age at the time of clinically significant RSV-associated LRTI was significantly less than the mean age at the time of non-RSV-associated LRTI (288 days vs. 353 days). Most of the RSV-associated lower respiratory tract infections occurred before the age of 6 months (Figure 4.4). Male children were more likely to have RSV-associated lower respiratory tract infections than their female counter parts (Table 4.1).

A number of epidemiological studies have reported similar age and gender distributions of RSV-associated LRTI. In his review, Welliver noted the peak incidence of severe RSV-associated illness to occur between 2 and 3 months of age, consistent with data from reports by Hall, Bosis and co-investigators, von Linstow and colleagues and by Manoha and colleagues. Several studies found the mean age of RSV-associated lower respiratory tract infection to be greater than the age mentioned in the review by Welliver, but well within the first year of life. All of the studies reported that the risk of RSV-associated LRTI was higher for boys than their girl counterparts [3, 10, 33, 36, 48, 49, 51, 52, 61, 62, 67, 69, 71, 72, 223, 227].

d) Prematurity: Premature children and children born low birth-weight in the current study cohort were more likely to have clinically significant RSV-associated lower respiratory tract infection than full-term children and children with normal birth-weight (Table 4.1). As noted by Welliver, premature children are at increased risk of acquiring more severe RSV infections. Similar findings regarding the influence of prematurity and low birth-weight on the prevalence and severity of RSV-associated lower respiratory tract infection have been reported by other study groups as well [3, 17, 61, 223, 228].

e) Residence: Children living in rural areas of Manitoba and the three northern regional health authorities (RHAs) were more likely to have clinically significant RSV-associated LRTI than children who were living in urban areas or not in northern RHAs. Similarly, Kozyrskyj et al found that all Manitoba children living in the urban area are less likely to be hospitalized [212]. Most of the children hospitalized for lower respiratory tract infection in the Chapel Hill study were residents of a rural area [227]. The rate of RSV-LRTI associated hospital admission has been reported to be higher among the native population in Canada and the United States [18-21]. The majority of the residents in the northern RHAs in Manitoba are of aboriginal background.

f) Socio-economic class: Children from the lower income quintiles were more likely to have clinically significant RSV-associated LRTI than children in the higher income quintiles (Figure 4.6 and Table 4.1). Lower household socioeconomic status and associated exposures such as crowded living conditions, low maternal education, lack of breast feeding and exposure to tobacco smoke have been associated with a higher risk of developing RSV-associated lower respiratory tract infections and greater severity of the infection as well [3, 10, 17, 28, 84, 229, 230]. The higher prevalence of RSV-associated LRTI among lower income children in this study cohort mirrors this literature.

g) Sibling(s) of subject: The current study found that having two or more siblings, or at least one older sibling was associated with higher likelihood of having clinically significant RSV-associated lower respiratory tract infection before the age of 2 years (Table 4.1). Introduction of RSV into the family is most likely to occur through a school-aged child and the infant becomes secondarily infected. Several epidemiological studies have reported an association between having siblings and increased risk of respiratory infections [10, 28, 223, 227, 231].

h) Overall criterion-related validity of the definition: The associations found between RSV-associated LRTI and "criteria" such as age, sex, ethnicity, history of premature birth, older sibling(s), socio-economic status, and region of residence among children in the current study cohort were consistent with those reported in the currently available literature. Thus, the study definition of RSV-associated LRTI met the requirements for concurrent criterion related validity since it was correlated with established criteria for RSV infection [220].

5.2.3 Choosing current definition of RSV

It was obvious that overall lower respiratory tract infection before age 2, as well as RSV-associated lower respiratory tract infection, followed very similar patterns when tested against the selected criteria in this study (Table 4.1). So one could argue to include all the cases of lower respiratory tract infections in the definition of RSV-associated LRTI.

According to the current RSV definition, a total of 4117 children of study children had clinically significant RSV-associated LRTI. So, 67.1% of children with at least one lower respiratory tract infection (total 6138, RSV associated LRTI and non-RSV associated LRTI combined) before 2 years of age had RSV-associated LRTI. In the validity assessments, the associations of overall LRTI and RSV-associated LRTI with different criteria (for example: prematurity, birth-weight and other socio-demographic characters) was compared. It was very likely that the results for the overall LRTI group were largely influenced by the children with RSV, which were nested in this group. This would explain the similar associations for overall LRTI and RSV-associated LRTI presented in the Table 4.1.

5.3 Internal validity of the current research

Woodward defined bias as uncontrolled features in the data that could lead to distorted results and thereby to misleading conclusions [232]. According to Greenberg and colleagues, bias is a systemic error in a study that leads to distortion of the results. Bias is particularly important for the observational studies because of the lack of randomization increases the chance that study groups will differ with respect to important characteristics [233]. Bias may arise from 1) poor measurement, leading to measurement or information bias; 2) sample being unrepresentative of the target population, leading to selection bias and 3) the differential effects of other determinants on the association of interest, leading to confounding bias [234].

5.3.1 Selection bias

In most of the cases, it is not possible to include all individuals with a particular health condition or exposure in a study and selection of sample of subjects is required. An unrepresentative sample of the target population leads to the selection bias [234]. Though the method for inclusion of subjects leads to a valid comparison group under optimal circumstances, the selection process itself may increase or decrease the chance that a relationship between the exposure and the outcome of interest will be detected which leads to selection bias [233]. According to Rothman and Greenland, selection biases are distortions that result from procedures used to select subjects and from factors that influence study participation [235]. Selection bias occurs when the relation between exposure and disease is different for those who participate and those who should be theoretically eligible for the study, including those who do not participate. As a consequence of this type of bias, the associations observed in the study represent a mix of forces determining participation and the forces determining disease as well [235]. Loss to follow-up can also lead to selection bias in a clinical trial or cohort study. The subjects may discontinue their participation after enrolment in the study. Greenberg and colleagues indicated that certain types of subjects are more likely than others to drop out of a study. Subjects may also die from causes other than the outcome of interest during the study. Biased estimates of risk may be obtained from the analyses of such studies if the lost subjects differ in their risk of the outcome of interest [233].

5.3.2 Measurement bias

Bias in evaluating an effect can occur from errors in obtaining the required information and whenever there are errors in the measurements. When the misclassification depends on the values of other variables, it is designated as differential misclassification. When a particular group of subjects seek more medical attention than others because of a pre-existing condition, it is likely that they could be diagnosed with some other clinical condition(s) that is unusually higher than the normal population. This could lead to a differential misclassification bias as the under-diagnosis of that particular clinical condition occurs more frequently in the otherwise normal population groups [235]. The classification error that is not dependent on the values of the other variables is designated as nondifferential misclassification. This type of error occurs when the proportion of subjects misclassified on exposure does not depend on disease status or when the proportion of subjects misclassified on disease does not depend on exposure. For example, subjects may answer a question about the exposure with a socially acceptable but inaccurate response regardless of whether they have the disease of interest [233, 235].

5.3.3 Confounding bias

According to Greenberg and colleagues, confounding refers to the mixing of effect of an extraneous variable with the effects of the exposure and disease of interest [233]. Rothman and Greenland indicated confounding as a confusion of effects when the apparent effect of the exposure of interest is distorted because of the effect of an extraneous factor is mistaken for or mixed with actual exposure effect. They defined confounders as the extraneous factors responsible for difference in disease frequency between the exposed and unexposed. A confounder must be associated with both the exposure and outcome of interest of the study. Confounding can lead to over- or underestimation of an effect and even can change the apparent direction of an effect. The exposed and the unexposed group of the study could differ on many factors other than the exposure of interest. In these situations, the comparison of the exposed and unexposed is confounded because the disease frequency in the exposed and unexposed groups can result from a mixture of several factors, including any exposure effect [235].

5.3.4 How the current study addressed biases

5.3.4.1 Addressing selection bias

Since the whole population of the 1995 birth-cohort (living in Manitoba at the end of the study period) was included, the study sample represented the population of interest. This eliminates the possibility of introducing selection bias. Further, the data PHRDR data repository has updated health information on any subject who requires medical attention in Manitoba, including all episodes of physician visits and hospital admission[178].

Unless the subject left the province or died, s/he was in the database which minimized/eliminated the adverse effect of drop out on the study.

5.3.4.2 Addressing measurement bias

Poor measurement leads to the introduction to information or measurement bias [234]. For the current study, exposure (clinically significant RSV-associated LRTI) was identified on the basis of a lower respiratory tract infection in RSV season before the age of 2 years. As discussed earlier, the definition of clinically significant RSV-LRTI was chosen carefully on the basis of published research and assessments were conducted for face/content validity and criterion related validity. The asthma cases, the subjects with the outcome of interest of the study, were identified from this data repository on the basis of physician visit(s) for asthma, hospital admission(s) for asthma and prescription for drugs to treat asthma using a database definition of asthma, validated by Kozyrskyj and colleagues [236]. This minimized the possibility of introducing measurement or information bias.

5.3.4.3 Addressing confounding bias

The differential effects of other determinants on the association of interest can result in confounding bias [234]. As mentioned earlier, the PHRDR contains anonymized encounter-based records of individuals' interactions with the provincial health care system, derived from the Manitoba Health Services Insurance Plan registry, from health insurance claims routinely filed by physicians and from health care facilities in Manitoba. Manitoba Health annually provides MCHP with copies of several files including the hospital file, medical claims file, long-term care file and the registry. In addition, other agencies including the Office of Vital Statistics, and Cancer Care Manitoba deposited data to MCHP [178]. As a consequence, a wide range of information was available on the subjects. It included information on subject's gender, maternal asthma status, use of antibiotics, BCG vaccination, history of physician visits, history of hospitalization, residence in urban or rural area, income quintile of the parent(s), age at infection and season of infection. Thus, the chance of confounding bias was minimized for this study as well.

5.3.4.4 Overall internal validity of the study

The current research was an observational study of the association between clinically significant RSV-associated LRTI and subsequent development of asthma. The cohort study design ensured temporality between the exposure and the outcome. To confirm temporality, analyses were run after the exclusion of children with asthma diagnoses before the RSV exposure. Validity assessments were conduced for the RSV exposure measure. An already-validated definition was used to identify children with the outcome of interest (asthma). Since the possibility of selection bias, measurement bias and confounding bias were minimal, it can be concluded that the current study had good internal validity.

As mentioned earlier, a total of 13,980 children of the 1995 birth cohort were included for the current study. Some of the children of the study cohort had the onset of asthma before they had clinically significant RSV-associated LRTI within the first 2 years of life. If these children were included in the final analyses of the study, the multivariate logistic regression models would have individuals who had outcome before the exposure. The association(s) from these models would not represent the actual influence of exposure (clinically significant RSV-LRTI) on the subsequent development of outcome (asthma and asthma/wheezing phenotypes).

To ensure that this issue was addressed in the current study, the associations between clinically significant RSV-associated LRTI and risk of asthma (and asthma/wheezing phenotypes) were determined in two different sets of multivariate logistic regression models with the same variables. In the first set of models, all the children of the study cohort were included (n=13980). In the second set, children who had asthma onset before having clinically significant RSV-LRTI were not included (n=13602).

The results of these analyses (table 17, Appendix A) showed that when the children who had asthma onset before clinically significant RSV-LRTI were excluded from the analyses, the magnitude of the associations always changed/decreased. All the associations reported in the current study (Table 3.1-3.11 and table 1-16, Appendix A) were obtained from multivariate logistic regression models where children having asthma before clinically significant RSV-LRTI were excluded. The effects of the confounding factors were also adjusted for. Excluding children who had asthma onset before having RSV-LRTI ensured that the temporal relationship between exposure and subsequent outcome was taken into account for current study. So, the findings truly reflected the influences of clinically significant RSV-associated LRTI, frequency and severity of clinically significant RSV-associated LRTI and younger age at the time of first clinically significant RSV-associated LRTI on subsequent development of childhood asthma and asthma/wheezing phenotypes.

5.4 External validity of the current research

External validity assesses the extent to which study findings can be generalized to a wider population. The current study was conducted involving all the children of the 1995 birth cohort who were still living in Manitoba at the end of the study period. Considering the validity and sensitivity of study definitions and the reliability of the PHRDR database, in general, it can be concluded that the study had good external validity. Findings can be generalized to children of other birth cohorts in Manitoba in the 1990s. The study findings can also be generalized to children over the same time period in other Canadian provinces with similar geographic and demographic characteristics.

5.5 Implication of the current study

The current study was the first population-based birth cohort study in Manitoba to determine the association between RSV-associated LRTI and subsequent development of childhood asthma. It found that clinically significant RSV-LRTI was an important risk factor for asthma up to the age of 11 years when asthma onset occurred within the first 3 years of life. More severe infections and more frequent infections were very highly associated with risk of asthma and asthma phenotypes. Since study findings are applicable to children born in other regions of North America and the birth cohort was large (n=13980), the current study will provide strong evidence for the debate regarding the association between RSV-associated LRTI and childhood asthma. They will provide invaluable information for strategies regarding prevention of RSV-associated LRTI. Finally, several measures were developed in this study which could be used in other research on the outcome of clinically significant RSV-associated LRTI.

5.5.1 Case definition of RSV

A database definition was developed to identify children with clinically significant RSV-associated lower respiratory tract infection from children who required utilization of health care system with lower respiratory tract infection(s). Content/face validity and criterion related validity approaches were used to justify the validity of the definition and the definition appeared to meet the validity criteria. In addition to that this definition identified 93.46% of confirmed RSV-positive cases of LRTI in hospital admission as RSV-associated LRTI. So, the definition was expected to be valid measure to identify RSV-associated LRTI when laboratory based diagnosis/identification of RSV was not available.

5.5.2 Severity measure for RSV-associated LRTI

The current study developed and used a measure to determine the severity of the clinically significant RSV-associated LRTI using the type of required health care utilization and the length of hospital stay associated with the episode of LRTI. It found a clear trend of increasing risk of asthma with increasing severity of RSV-associated LRTI.

Caroll and colleagues reported that, their study on the dose response relationship between severity of bronchiolitis among infants and subsequent risk of childhood asthma was the first study of that kind [140]. The investigators found that increased severity of bronchiolitis among infants was associated with increased asthma specific morbidity during early childhood. Current study findings, using the severity measurements, were similar to their study. However, our study identified all lower respiratory tract infections (including but not limited to bronchiolitis) caused exclusively by RSV (not all respiratory viruses). To our knowledge, out study appears to be the first to investigate the dose response relationship between the severity of clinically significant RSV-LRTI and risk of asthma and asthma/wheezing phenotypes later in childhood.

5.5.3 Effect of age-group at 1st clinically significant RSV-LRTI

Considering the fact that RSV is the most prominent respiratory virus before the age of 2 years and currently available information on peak age of RSV-associated LRTI, several age-groups at the time of first clinically significant RSV-associated LRTI were proposed for the current study. When the age-group classifications were applied, the study found that first clinically significant RSV-LRTI before the age of 1 year was associated with higher risk of asthma and most significant risk was associated with first clinically significant RSV-LRTI between 6 and 12 months. This was very similar to the current literature. The age-group classifications can be used to determine the effect of first clinically significant RSV-LRTI at different ages up to the age of 2 years.

5.5.4 RSV-LRTI and asthma/wheezing phenotypes

The current study determined the influences of major explanatory variables and confounding factors on asthma/wheezing phenotypes (i. e.; early persistent asthma, transient wheeze), in addition to determining the influences of these factors/variables on asthma/wheezing status before 3 years, at 7 years and at 11 years. Currently available literature, reporting the association between RSV-associated LRTI and asthma, did not always report the association between the infection and asthma/wheezing phenotype(s).

5.6 Justification of pursuing the current study

If a relationship is causal, it is expected to exist consistently in different studies in different settings. To meet this criterion, numerous experiments have to be done before meaningful statements can be made about the causal relationship between two or more items [237, 238]. Several epidemiological studies were reviewed earlier which provided evidence in favour of a positive association between RSV infection in infancy and subsequent development of childhood asthma [19, 131-148, 163-167, 195]. These studies were conducted by different groups of investigators in various geographical locations and involved different study populations. Similarly, the studies that found evidence against the association or found no association between RSV infection in infancy and subsequent development of childhood asthma were conducted by different group of investigators with different groups of study population [51, 149-162, 168-170]. Though few of these studies were conducted in the Scandinavian countries, the groups of investigators, study populations, size of the study populations and duration of the follow-up time were all different. So, currently available literature provided consistent evidence both in favour and against the association between the RSV infection in early life and subsequent development of asthma. Very similar to epidemiological studies involving human subjects, animal model studies also provided evidence both in favour [171-175] and against [176, 177] the association. This suggested that the association between RSV infection in infancy and subsequent development of asthma was still inconclusive and proved the worthiness of pursuing the current study.

5.7 Limitations of the current study

RSV exposure was identified from the patients who developed lower respiratory tract illness as a consequence of RSV infection(s) and required utilization of the health care system. If the infection was not severe enough to require utilization of health care, it could not be identified by our study definition.

The possible cases of RSV infection were identified by a definition that was mentioned earlier. Though RSV is strictly a seasonal virus and the parameters which were considered to define the RSV cases should identify the RSV cases, without a definite laboratory diagnosis/identification, it was not possible to conclude that every single case of lower respiratory tract infections during the RSV season(s) within the first 2 years of life was caused by RSV only. Human metapneumovirus (hMPV) is a fairly new virus and the Health Canada database did not have information on the isolation of this virus. HMPV was often isolated from subjects with RSV and their epidemiological characteristics often overlapped. It was possible that the case definition of RSV would identify some subjects as RSV cases while the actual etiological agent was hMPV or other overlapping respiratory virus(es). Health Canada data also showed that there could be very few cases of clinically significant RSV-associated lower respiratory tract infection before or after the defined RSV-season. Choosing the current definition of clinically significant RSV-associated LRTI would miss those cases.

The database definition of asthma was validated at the age of 7 years and was assumed to be a valid measure of asthma at the age of 11 years. In addition to that, the definition for asthma-like diagnoses at age 3 includes ICD9CM codes for bronchiolitis, bronchitis and asthma (466/490/491/493). These codes were used in the definition of RSV-LRTI, which may explain the high association between RSV-associated LRTI and transient wheeze. This could be a source of measurement bias for the current study.

Over 16,000 children were born in Manitoba in 1995. More than 2000 children of the 1995 birth cohort were not living in Manitoba at the end of 2006, hence were excluded from the current study. This could introduce selection bias to the current study.

In addition to that the huge population size of the administrative database could raise risk of statistical significance with little clinical or no policy significance at all.

5.8 Recommendations and future directions

Our study found that clinically significant RSV-associated lower respiratory tract infection before the age of 2 years was a significant risk factor for childhood asthma and asthma/wheezing phenotypes. The study also found that severe and recurrent clinically significant RSV-LRTI and the infection(s) before the age of 12 months were associated with higher risks of asthma and asthma/wheezing phenotypes.

5.8.1 Prevention of clinically significant RSV-LRTI

When asthma care is placed in the context of prevention, the intervention(s) can be introduced at several stages of the progression of the disease. As Murphy indicated, the primary prevention of asthma involved preventing the disease in healthy individuals by removing the risk factors [239]. Findings from the primary prevention of asthma studies revealed that decrease in allergen exposure in very early stage of life had some protective effects against development of allergic symptoms/disorders later in life [240-252]. So, prevention of severe and recurrent RSV-associated LRTI in early life could lead to a lessning of the childhood asthma burden. Instead of strictly following a particular strategy, a combination of following strategies should be practiced for an effective reduction of RSV infection and RSV associated hospitalization in Manitoba:

a) RSV infection control practice in hospital: Nosocomial transmission is one of the major risks for contracting the virus [253]. Decontaminating hands after contact with patient or respiratory specimen and wearing protective gears including masks, gown and disposable gloves are highly recommended to prevent transmission of the virus from one individual to another. CDC also recommended limiting visitors and cohorting RSV patients in a separate hospital ward or room, if possible [254].

b) Use of palivizumab for prophylaxis against RSV infection: Strategies are required to prevent non-hospital acquired RSV infection as well. Palivizumab prophylaxis can produce substantial cost savings if the therapy is restricted to infants who were at high-risk for developing severe RSV infections [255]. Prophylaxis is recommended for premature infants with a gestational age of ≤32 weeks who are <6 months of age at the start of or during the RSV season and children with chronic lung or congenital heart disease. The family history of asthma, socio-economic status and number of family member should also be considered to define high-risk children. The PHAC also recommended palivizumab prophylaxis for children who are living in remote northern communities of Manitoba [256].

5.8.2 Future directions

A data linkage study with the RSV-data from the CadHam provincial Laboratory would provide further information on the validity of the current definition of clinically significant RSV-associated LRTI, hence the association between RSV-LRTI and childhood asthma and asthma/wheezing phenotypes.

Studies on association between RSV-associated lower respiratory tract infection and subsequent development of childhood asthma reported that the association could be significant up to the age of 11 years but became insignificant after that age. In the current study, children of the study cohort were followed up to the age of 11 years. The association between clinically significant RSV-LRTI and risk of asthma was still significant at that point, though a declining trend with increasing age was observed. Further follow up study could provide important information on the persistence of the association beyond that point.

Currently available findings from few studies indicate that severe RSV-associated LRTI and asthma share a common genetic predisposition and that severe RSV-LRTI is not responsible for causing asthma but an indicator of the genetic predisposition to asthma. Further study on the current cohort with asthma as exposure and clinically significant RSV-LRTI as outcome variable could provide some important information on the causal direction of the association between RSV-associated LRTI and childhood asthma.

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Appendices

Appendix A

Table 1: Association between risk of asthma/transient wheeze before 3 years and explanatory variables (multivariate logistic regression), with clinically significant RSV-LRTI.

Features/variables	Asthma/transient wheeze before 3 years				
	No.	n	%	OR (95% CI)	P-value
RSV-LRTI in 2 years					
• Yes	3701	743	20.08	2.94 (2.60-3.32)	<.0001
• No RSV-LRTI (ref.)	9780	562	5.75		
Gender					
• Male	6807	818	12.02	1.57 (1.39-1.77)	<.0001
• Female (ref.)	6674	487	7.30		
Asthmatic mother					
• Yes	1366	228	16.69	1.69 (1.44-2.00)	<.0001
• No (ref.)	12115	1077	8.89		
Maternal stress					
• Stressed	2529	329	13.01	1.23 (1.07-1.42)	0.0037
• Not stressed (ref.)	10952	976	8.91		
Child health income quintile					
• Low	3322	451	13.58	1.47 (1.29-1.67)	<.0001
• High (ref.)	10159	854	8.41		

Features/variables	Asthma/transient wheeze before 3 years				
	No.	n	%	OR (95% CI)	P-value
Premature birth					
• Yes	834	123	14.75	1.59 (1.29-1.97)	<.0001
• No (ref.)	12647	1182	9.35		
Antibiotic received in 1st year					
• 1-2 antibiotics	5039	427	8.47	1.91 (1.59-2.29)	<.0001
• 3-4 antibiotics	2170	314	14.47	3.03 (2.49-3.69)	<.0001
• >4 antibiotics	1641	384	23.40	4.74 (3.90-5.77)	<.0001
• No antibiotic (ref.)	4631	180	3.89		

Table 2: Association between risk of asthma/transient wheeze before 3 years and explanatory variables (multivariate logistic regression), with frequency of RSV-LRTI.

Features/variables	**Asthma/transient wheeze before 3 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Frequency of RSV infection in 2 years					
• 1-2 infection(s)	2850	386	13.54	1.93 (1.68-2.23)	<.0001
• ≥3 infections	851	357	41.95	7.70 (6.50-9.13)	<.0001
• No RSV-LRTI (ref.)	9780	562	5.75		
Gender					
• Male	6807	818	12.02	1.56 (1.37-1.77)	<.0001
• Female (ref.)	6674	487	7.30		
Asthmatic mother					
• Yes	1366	228	16.69	1.71 (1.44-2.02)	<.0001
• No (ref.)	12115	1077	8.89		
Maternal stress					
• Stressed	2529	329	13.01	1.21 (1.04-1.39)	0.0120
• Not stressed (ref.)	10952	976	8.91		
Child health income quintile					
• Low	3322	451	13.58	1.36 (1.19-1.55)	<.0001
• High (ref.)	10159	854	8.41		
Premature birth					
• Yes	834	123	14.75	1.54 (1.24-1.92)	0.0001
• No (ref.)	12647	1182	9.35		

Features/variables	Asthma/transient wheeze before 3 years				
	No.	n	%	OR (95% CI)	P-value
Antibiotic received in 1st year					
• 1-2 antibiotics	5039	427	8.47	1.95 (1.63-2.35)	<.0001
• 3-4 antibiotics	2170	314	14.47	3.01 (2.46-3.67)	<.0001
• >4 antibiotics	1641	384	23.40	4.40 (3.60-5.37)	<.0001
• No antibiotic (ref.)	4631	180	3.89		

Table 3: Association between risk of asthma/transient wheeze before 3 years and explanatory variables (multivariate logistic regression), with severity of RSV-LRTI.

Features/variables	**Asthma/transient wheeze before 3 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Severity of RSV-LRTI in 2 years					
• Mild	3129	530	16.94	2.44 (2.14-2.79)	<.0001
• Moderate	303	94	31.02	5.18 (3.94-6.81)	<.0001
• Severe	269	119	44.24	9.15 (6.95-12.04)	<.0001
• No RSV in 2 years (ref.)	9780	562	5.75		
Gender					
• Male	6807	818	12.02	1.56 (1.38-1.77)	<.0001
• Female (ref.)	6674	487	7.30		
Asthmatic mother					
• Yes	1366	228	16.69	1.72 (1.46-2.04)	<.0001
• No (ref.)	12115	1077	8.89		
Maternal stress					
• Stressed	2529	329	13.01	1.23 (1.07-1.42)	0.0043
• Not stressed (ref.)	10952	976	8.91		
No. of sibling in 2002					
• ≥2 sibling	6732	693	10.29	0.88 (0.78-0.99)	0.0418
• 0/1 sibling (ref.)	6749	612	9.07		

Features/variables	Asthma/transient wheeze before 3 years				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Child health income quintile					
• Low	3322	451	13.58	1.36 (1.19-1.56)	<.0001
• High (ref.)	10159	854	8.41		
Premature birth					
• Yes	834	123	14.75	1.51 (1.21-1.88)	0.0002
• No (ref.)	12647	1182	9.35		
Antibiotic received in 1st year					
• 1-2 antibiotics	5039	427	8.47	1.96 (1.63-2.35)	<.0001
• 3-4 antibiotics	2170	314	14.47	3.09 (2.53-3.77)	<.0001
• >4 antibiotics	1641	384	23.40	4.82 (3.95-5.88)	<.0001
• No antibiotic (ref.)	4631	180	3.89		

Table 4: Association between risk of asthma/transient wheeze before 3 years and explanatory variables (multivariate logistic regression), with age-group at 1st RSV-LRTI.

Features/variables	**Asthma/transient wheeze before 3 years**				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**
Age-groups at 1st RSV-associated care					
• ≤6 months	1487	417	28.04	4.47 (3.85-5.19)	<.0001
• >6 to 12 months	1187	242	20.39	2.80 (2.35-3.33)	<.0001
• >12 to 24 months	1027	84	8.18	1.23 (0.97-1.57)	0.0934
• No RSV-care (ref.)	9780	562	5.75		
Gender					
• Male	6807	818	12.02	1.57 (1.39-1.78)	<.0001
• Female (ref.)	6674	487	7.30		
Asthmatic mother					
• Yes	1366	228	16.69	1.73 (1.46-2.04)	<.0001
• No (ref.)	12115	1077	8.89		
Maternal stress					
• Stressed	2529	329	13.01	1.23 (1.06-1.42)	0.0051
• Not stressed (ref.)	10952	976	8.91		
Child health income quintile					
• Low	3322	451	13.58	1.41 (1.24-1.61)	<.0001
• High (ref.)	10159	854	8.41		

Features/variables	Asthma/transient wheeze before 3 years				
	No.	n	%	OR (95% CI)	P-value
Premature birth					
• Yes	834	123	14.75	1.58 (1.27-1.96)	<.0001
• No (ref.)	12647	1182	9.35		
Antibiotic received in 1st year					
• 1-2 antibiotics	5039	427	8.47	1.87 (1.56-2.25)	<.0001
• 3-4 antibiotics	2170	314	14.47	2.88 (2.36-3.52)	<.0001
• >4 antibiotics	1641	384	23.40	4.40 (3.61-5.37)	<.0001
• No antibiotic (ref.)	4631	180	3.89		

Table 5: Current study cohort at the age of 11 years (December, 2006).

Variables/Factors	Yes N (%)	No N (%)
Asthma and asthma/wheezing phenotypes		
• Current asthma at age 7 years	917 (6.56)	13063 (93.44)
• Current asthma at age 11 years	910 (6.51)	13070 (93.49)
• Ever asthma up to 11 years	2643 (18.91)	11337 (81.09)
• Transient wheeze	1420 (10.16)	
• Early persistent asthma at 11 years	275 (1.97)	
• Late onset asthma at 11 years	372 (2.66)	
• Late persistent asthma at 11 years	263 (1.88)	
• Late transient asthma at 11 years	313 (2.24)	
Gender (male)	7116 (50.90)	6864 (49.10)
Prematurity (<36 weeks)	892 (6.39)	13078 (93.61)
Low birth-weight (<2500 gms)	693 (4.96)	13282 (95.04)
Antibiotic receive in 1st year of life		
• Overall antibiotic use in 1st year of life	9289 (66.44)	4691 (33.56)
• Antibiotic for LRTI in 1st year	2857 (20.44)	11123 (79.56)
• Antibiotic for URI in 1st year	7455 (53.33)	6525 (46.67)
• Antibiotic for NRI in 1st year	769 (5.50)	13211 (94.50)
Complete BCG vaccination (2 years)	414 (2.96)	13566 (97.04)

Variables/Factors	**Yes** **N (%)**	**No** **N (%)**
Older sibling at birth	8301 (59.38)	5679 (40.62)
Younger sibling in 2002	7275 (52.04)	6705 (47.96)
Urban residence	8003 (57.25)	5977 (42.75)
Living North	1424 (10.19)	12556 (89.81)
Child health income quintile (low)	3489 (25.03)	10450 (74.97)
Maternal stress	2625 (18.78)	11355 (81.22)
Parental asthma		
• Asthmatic mother	1442 (10.37)	12465 (89.63)
• Both parents asthmatic	98 (0.70)	13882 (99.30)

Table 6: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and asthma status at 7 years.

Features/variables	RSV-LRTI in first 2 years of life				Asthma at 7 yrs		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	6862	2075	30.24	<.0001	547	7.97	<.0001
• Female (ref.)	6740	1664	24.69		305	4.53	
Asthmatic mother							
• Yes	1375	469	34.11	<.0001	156	11.35	<.0001
• No (ref.)	12157	3252	26.75		690	5.68	
Maternal stress							
• Stressed	2536	807	31.82	<.0001	202	7.97	<.0001
• Not stressed (ref.)	11066	2932	26.50		650	5.87	
Child health income quintile							
• Low	3341	1170	35.02	<.0001	224	6.70	0.2386
• High (ref.)	10220	2553	24.98		627	6.14	
No. of sibling in 2002							
• ≥ 2 siblings	6793	2102	30.94	<.0001	341	5.02	<.0001
• 0/1 sibling (ref.)	6809	1637	24.04		511	7.50	
Urban/rural residence							
• Urban	7782	2079	26.72	0.0195	600	7.71	<.0001
• Rural (ref.)	5820	1660	28.52		252	4.33	

Features/variables	RSV-LRTI in first 2 years of life				Asthma at 7 yrs		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1390	421	30.29	0.0136	41	2.95	<.0001
• No (ref.)	12212	3318	27.17		811	6.64	
BCG vaccination in 2 years							
• Complete	397	190	47.86	<.0001	18	4.53	0.1489
• Not complete (ref.)	13205	3549	26.88		834	6.32	
Premature birth							
• Yes	856	271	31.66	0.0047	69	8.06	0.0254
• No (ref.)	12737	3465	27.20		783	6.15	
Low birth-weight							
• Yes	668	208	31.14	0.0300	54	8.08	0.0468
• No (ref.)	12930	3529	27.29		798	6.17	
Antibiotic use in 1st year of life							
• Yes	8937	3050	34.13	<.0001	645	7.22	<.0001
• No (ref.)	4665	689	14.77		207	4.44	

Table 7: Percentage distribution of confounding factors in relation to RSV-LRTI within the first 2 years of life and asthma status at 11 years.

Features/variables	**RSV-LRTI in first 2 years of life**				**Asthma at 11 yrs**		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	6862	2075	30.24	<.0001	521	7.59	<.0001
• Female (ref.)	6740	1664	24.69		335	4.97	
Asthmatic mother							
• Yes	1375	469	34.11	<.0001	160	11.64	<.0001
• No (ref.)	12157	3252	26.75		695	5.72	
Maternal stress							
• Stressed	2536	807	31.82	<.0001	192	7.57	0.0033
• Not stressed (ref.)	11066	2932	26.50		664	6.00	
Child health income quintile							
• Low	3341	1170	35.02	<.0001	198	5.93	0.3185
• High (ref.)	10220	2553	24.98		655	6.41	
No. of sibling in at age 7 years							
• ≥2 sibling	6793	2102	30.94	<.0001	344	5.06	<.0001
• 0/1 sibling (ref.)	6809	1637	24.04		512	7.52	
Urban/rural residence							
• Urban	7782	2079	26.72	0.0195	591	7.59	<.0001
• Rural (ref.)	5820	1660	28.52		265	4.55	

Features/variables	RSV-LRTI in first 2 years of life				Asthma at 11 yrs		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1390	421	30.29	0.0136	59	4.24	0.0009
• No (ref.)	12212	3318	27.17		797	6.53	
BCG vaccination in 2 years							
• Complete	397	190	47.86	<.0001	23	5.79	0.6773
• Not complete (ref.)	13205	3549	26.88		833	6.31	
Premature birth							
• Yes	856	271	31.66	0.0047	59	6.89	0.4590
• No (ref.)	12737	3465	27.20		797	6.26	
Low birth-weight							
• Yes	668	208	31.14	0.0300	42	6.29	0.9934
• No (ref.)	12930	3529	27.29		814	6.30	
Antibiotic use in 1st year of life							
• Yes	8937	3050	34.13	<.0001	621	6.95	<.0001
• No (ref.)	4665	689	14.77		235	5.04	

Table 8: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and early persistent asthma.

Features/variables		**RSV-LRTI in first 2 years of life**			**Early persistent asthma**		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	5645	1513	26.80	<.0001	157	2.78	<.0001
• Female (ref.)	5913	1333	22.54		64	1.08	
Asthmatic mother							
• Yes	1043	330	31.64	<.0001	50	4.79	<.0001
• No (ref.)	10455	2502	23.93		171	1.64	
Maternal stress							
• Stressed	2056	585	28.45	<.0001	54	2.63	0.0091
• Not stressed (ref.)	9502	2261	23.79		167	1.76	
Child health income quintile							
• Low	2743	857	31.24	<.0001	66	2.41	0.0293
• High (ref.)	8782	1978	22.52		154	1.75	
No. of sibling at the end of 2002							
• ≥2 sibling	5809	1587	27.32	<.0001	89	1.53	0.0027
• 0/1 sibling (ref.)	5749	1259	21.90		132	2.30	
Urban/rural residence							
• Urban	6487	1556	23.99	0.0721	149	2.30	0.0006
• Rural (ref.)	5071	1290	25.44		72	1.42	

Features/variables	RSV-LRTI in first 2 years of life				Early persistent asthma		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1222	322	26.35	0.1385	12	0.98	0.0121
• No (ref.)	10336	2524	24.42		209	2.02	
BCG vaccination in 2 years							
• Complete	327	144	44.04	<.0001	12	3.67	0.0186
• Not complete (ref.)	11231	2702	24.06		209	1.86	
Premature birth							
• Yes	694	183	26.37	0.2709	21	3.03	0.0273
• No (ref.)	10856	2661	24.51		200	1.84	
Low birth-weight							
• Yes	555	147	26.49	0.2959	16	2.88	0.0872
• No (ref.)	11000	2698	24.53		205	1.86	
Antibiotic use in 1st year of life							
• Yes	7322	2257	30.82	<.0001	186	2.54	<.0001
• No (ref.)	4236	589	13.90		35	0.83	

Table 9: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and transient wheeze.

Features/variables	**RSV-LRTI in first 2 years of life**				**Transient wheeze**		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	6158	1830	29.72	<.0001	670	10.88	<.0001
• Female (ref.)	6275	1526	24.32		426	6.79	
Asthmatic mother							
• Yes	1172	394	33.62	<.0001	179	15.27	<.0001
• No (ref.)	11197	2946	26.31		913	8.15	
Maternal stress							
• Stressed	2278	701	30.77	<.0001	276	12.12	<.0001
• Not stressed (ref.)	10155	2655	26.14		820	8.07	
Child health income quintile							
• Low	3064	1055	34.43	<.0001	387	12.63	<.0001
• High (ref.)	9331	2286	24.50		703	7.53	
No. of sibling at the end of 2002							
• ≥2 sibling	6329	1915	30.26	<.0001	609	9.62	0.0012
• 0/1 sibling (ref.)	6104	1441	23.61		487	7.98	
Urban/rural residence							
• Urban	6974	1817	26.05	0.0077	636	9.12	0.1761
• Rural (ref.)	5459	1539	28.19		460	8.43	

Features/variables	RSV-LRTI in first 2 years of life				Transient wheeze		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1322	400	30.26	0.0047	112	8.47	0.6415
• No (ref.)	11111	2956	26.60		984	8.86	
BCG vaccination in 2 years							
• Complete	370	173	46.76	<.0001	55	14.86	<.0001
• Not complete (ref.)	12063	3183	26.39		1041	8.63	
Premature birth							
• Yes	776	237	30.54	0.0213	103	13.27	<.0001
• No (ref.)	11648	3116	26.75		992	8.52	
Low birth-weight							
• Yes	613	183	29.85	0.1009	74	12.07	0.0035
• No (ref.)	11816	3171	26.84		1021	8.64	
Antibiotic use in 1st year of life							
• Yes	8086	2722	33.66	<.0001	950	11.75	<.0001
• No (ref.)	4347	634	14.58		146	3.36	

Table 10: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and late persistent asthma.

Features/variables		**RSV-LRTI in first 2 years of life**			**Late persistent asthma**		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	5652	1476	26.11	<.0001	164	2.90	<.0001
• Female (ref.)	5948	1322	22.23		99	1.66	
Asthmatic mother							
• Yes	1045	312	29.86	<.0001	52	4.98	<.0001
• No (ref.)	10495	2472	23.55		211	2.01	
Maternal stress							
• Stressed	2058	573	27.84	<.0001	56	2.72	0.1273
• Not stressed (ref.)	9542	2225	23.32		207	2.17	
Child health income quintile							
• Low	2730	831	30.44	<.0001	53	1.94	0.1829
• High (ref.)	8838	1957	22.14		210	2.38	
No. of sibling at the end of 2002							
• ≥2 sibling	5816	1567	26.94	<.0001	96	1.65	<.0001
• 0/1 sibling (ref.)	5784	1231	21.28		167	2.89	
Urban/rural residence							
• Urban	6526	1533	23.49	0.0721	188	2.88	<.0001
• Rural (ref.)	5074	1265	24.93		75	1.48	

Features/variables	RSV-LRTI in first 2 years of life				Late persistent asthma		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1229	319	25.96	0.1117	19	1.55	0.0724
• No (ref.)	10371	2479	23.90		244	2.35	
BCG vaccination in 2 years							
• Complete	319	136	42.63	<.0001	*	*	0.2176
• Not complete (ref.)	11281	2662	23.60		259	2.30	
Premature birth							
• Yes	691	176	25.47	0.3923	18	2.60	0.5406
• No (ref.)	10901	2620	24.03		245	2.25	
Low birth-weight							
• Yes	557	143	25.67	0.3793	18	3.23	0.1174
• No (ref.)	11040	2654	24.04		245	2.22	
Antibiotic use in 1st year of life							
• Yes	7315	2207	30.17	<.0001	179	2.45	0.0892
• No (ref.)	4285	591	13.79		84	1.96	

Table 11: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and late transient asthma.

Features/variables	**RSV-LRTI in first 2 years of life**				**Late transient asthma**		
	No.	**n**	**%**	**P-value**	**n**	**%**	**P-value**
Child's gender							
• Male	5671	1494	26.34	<.0001	183	3.23	0.0004
• Female (ref.)	5979	1325	22.16		130	2.17	
Asthmatic mother							
• Yes	1036	313	30.21	<.0001	43	4.15	0.0018
• No (ref.)	10549	2491	23.61		265	2.51	
Maternal stress							
• Stressed	2068	567	27.42	0.0002	66	3.19	0.1175
• Not stressed (ref.)	9582	2252	23.50		247	2.58	
Child health income quintile							
• Low	2756	843	30.59	<.0001	79	2.87	0.5222
• High (ref.)	8862	1966	22.18		234	2.64	
No. of sibling at the end of 2002							
• ≥2 sibling	5840	1571	26.90	<.0001	120	2.05	<.0001
• 0/1 sibling (ref.)	5810	1248	21.48		193	3.32	
Urban/rural residence							
• Urban	6555	1539	23.48	0.0398	217	3.31	<.0001
• Rural (ref.)	5095	1280	25.12		96	1.88	

Features/variables	RSV-LRTI in first 2 years of life				Late transient asthma		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1219	318	26.09	0.1036	9	0.74	<.0001
• No (ref.)	10431	2501	23.98		304	2.91	
BCG vaccination in 2 years							
• Complete	319	134	42.01	<.0001	*	*	0.1086
• Not complete (ref.)	11331	2685	23.70		309	2.73	
Premature birth							
• Yes	694	182	26.22	0.1983	21	3.03	0.5710
• No (ref.)	10948	2635	24.07		292	2.67	
Low birth-weight							
• Yes	552	145	26.27	0.2439	13	2.36	0.6209
• No (ref.)	11095	2673	24.09		300	2.70	
Antibiotic use in 1st year of life							
• Yes	7366	2225	30.21	<.0001	230	3.12	0.0001
• No (ref.)	4284	594	13.87		83	1.94	

Table 12: Percentage distributions of confounding factors in relation to RSV-LRTI within the first 2 years of life and late onset asthma.

Features/variables	RSV-LRTI in first 2 years of life				Late onset asthma		
	No.	n	%	P-value	n	%	P-value
Child's gender							
• Male	5688	1490	26.20	<.0001	200	3.52	0.0420
• Female (ref.)	6021	1330	22.09		172	2.86	
Asthmatic mother							
• Yes	1051	312	29.69	<.0001	58	5.52	<.0001
• No (ref.)	10597	2493	23.53		313	2.95	
Maternal stress							
• Stressed	2084	573	27.50	<.0001	82	3.93	0.0296
• Not stressed (ref.)	9625	2247	23.35		290	3.01	
Child health income quintile							
• Low	2756	836	30.33	<.0001	79	2.87	0.2994
• High (ref.)	8919	1974	22.13		291	3.26	
No. of sibling at the end of 2002							
• ≥2 sibling	5879	1578	26.84	<.0001	159	2.70	0.0034
• 0/1 sibling (ref.)	5830	1242	21.30		213	3.65	
Urban/rural residence							
• Urban	6592	1542	23.39	0.0468	254	3.85	<.0001
• Rural (ref.)	5117	1278	24.98		118	2.31	

Features/variables	RSV-LRTI in first 2 years of life				Late onset asthma		
	No.	n	%	P-value	n	%	P-value
Living in northern RHAs							
• Yes	1238	326	26.33	0.0504	28	2.26	0.0522
• No (ref.)	10471	2494	23.82		344	3.29	
BCG vaccination in 2 years							
• Complete	322	135	41.93	<.0001	7	2.17	0.2980
• Not complete (ref.)	11387	2685	23.58		365	3.21	
Premature birth							
• Yes	693	177	25.54	0.3548	20	2.89	0.6501
• No (ref.)	11008	2641	23.99		352	3.20	
Low birth-weight							
• Yes	547	142	25.96	0.2927	8	1.46	0.0192
• No (ref.)	11159	2677	23.99		364	3.26	
Antibiotic use in 1st year of life							
• Yes	7392	2223	30.07	<.0001	256	3.46	0.0209
• No (ref.)	4317	597	13.83		116	2.69	

Table 13: Association between risk of early persistent asthma and explanatory variables (multivariate logistic regression), with clinically significant RSV-LRTI.

Features/variables	Early persistent asthma				
	No.	n	%	OR (95% CI)	P-value
RSV-LRTI in 2 years					
• Yes	2819	120	4.26	2.91 (2.20-3.85)	<.0001
• No RSV-LRTI (ref.)	8640	100	1.16		
BCG vaccination in 2 years					
• Complete	322	12	3.73	2.29 (1.21-4.33)	0.0108
• Not complete (ref.)	11137	208	1.87		
Residence					
• Urban	6450	148	2.29	1.60 (1.18-2.17)	0.0023
• Rural (ref.)	5009	72	1.44		
Gender					
• Male	5603	156	2.78	2.42 (1.80-3.25)	<.0001
• Female (ref.)	5856	64	1.09		
Asthmatic mother					
• Yes	1036	50	4.83	2.52 (1.81-3.51)	<.0001
• No (ref.)	10423	170	1.63		
No. of sibling in 2002					
• ≥2 sibling	5757	89	1.55	0.61 (0.46-0.81)	0.0006
• 0/1 sibling (ref.)	5702	131	2.30		

Features/variables	Early persistent asthma				
	No.	n	%	OR (95% CI)	P-value
Premature birth					
• Yes	675	21	3.11	1.64 (1.03-2.62)	0.0390
• No (ref.)	10784	199	1.85		
Antibiotic received in 1st year					
• 1-2 antibiotics	4316	81	1.88	1.87 (1.25-2.79)	0.0025
• 3-4 antibiotics	1756	52	2.96	2.60 (1.67-4.05)	<.0001
• >4 antibiotics	1182	52	4.40	3.48 (2.22-5.47)	<.0001
• No antibiotic (ref.)	4205	35	0.83		

Table 14: Association between risk of transient wheeze and explanatory variables (multivariate logistic regression), with clinically significant RSV-LRTI.

Features/variables	Transient wheeze				
	No.	n	%	OR (95% CI)	P-value
RSV-LRTI in 2 years					
• Yes	3321	623	18.76	2.99 (2.62-3.43)	<.0001
• No RSV-LRTI (ref.)	9000	462	5.13		
Gender					
• Male	6106	662	10.84	1.50 (1.31-1.71)	<.0001
• Female (ref.)	6215	423	6.81		
Asthmatic mother					
• Yes	1164	178	15.29	1.67 (1.38-2.01)	<.0001
• No (ref.)	11157	907	8.13		
Maternal stress					
• Stressed	2273	276	12.14	1.28 (1.10-1.49)	0.0017
• Not stressed (ref.)	10048	809	8.05		
Child health income quintile					
• Low	3046	385	12.64	1.51 (1.32-1.74)	<.0001
• High (ref.)	9275	700	7.55		
Premature birth					
• Yes	755	102	13.51	1.64 (1.30-2.08)	<.0001
• No (ref.)	11566	983	8.50		

Features/variables	Transient wheeze				
	No.	n	%	OR (95% CI)	P-value
Antibiotic received in 1st year					
• 1-2 antibiotics	4579	346	7.56	1.96 (1.60-2.40)	<.0001
• 3-4 antibiotics	1965	262	13.33	3.22 (2.59-4.00)	<.0001
• >4 antibiotics	1462	332	22.71	5.34 (4.31-6.63)	<.0001
• No antibiotic (ref.)	4315	145	3.36		

Table 15: Associations between risk of late persistent asthma, late transient asthma and late onset asthma and explanatory variables (multivariate logistic regressions).

Features/variables	Late persistent asthma					Late transient asthma					Late onset asthma				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**
Residence															
• Urban	6515	188	2.89	1.75 (1.33-2.31)	<.0001	6543	216	3.30	1.46 (1.13-1.90)	0.0041	6579	254	3.86	1.68 (1.35-2.10)	<.0001
• Rural (ref.)	5025	75	1.49			5042	92	1.82			5066	117	2.31		
Living north															
• Yes	Factor not included for this multivariate equation					1208	9	0.75	0.37 (0.19-0.74)	0.0049	Factor not included for this multivariate equation				
• No (ref.)						10377	299	2.88							
Gender															
• Male	5631	164	2.91	1.81 (1.41-2.34)	<.0001	5650	183	3.24	1.55 (1.23-1.95)	0.0002	5663	199	3.51	1.25 (1.01-1.54)	0.0374
• Female (ref.)	5909	99	1.68			5935	125	2.11			5982	172	2.88		
Asthmatic mother															
• Yes	1045	52	4.98	2.50 (1.83-3.42)	<.0001	1036	43	4.15	1.55 (1.11-2.15)	0.0101	1051	58	5.52	1.87 (1.40-2.50)	<.0001
• No (ref.)	10495	211	2.01			10549	265	2.51			10594	313	2.95		

Features/variables	Late persistent asthma					Late transient asthma					Late onset asthma				
	No.	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**	**No.**	**n**	**%**	**OR (95% CI)**	**P-value**
No. of sibling in 2002															
• ≥2 sibling	5787	96	1.66	0.63 (0.49-0.82)	0.0004	5810	119	2.05	0.68 (0.54-0.87)	0.0016	Factor not included for this multivariate equation				
• 0/1 sibling (ref.)	5753	167	2.90			5775	189	3.27							
Low birth-weight															
• Yes	Factor not included for this multivariate equation					Factor not included for this multivariate equation					536	8	1.49	0.44 (0.21-0.88)	0.0209
• No (ref.)											11109	363	3.27		
Antibiotic received in 1st year															
• 1-2 antibiotics	Factor not included for this multivariate equation					4376	124	2.83	1.42 (1.07-1.88)	0.0157	Factor not included for this multivariate equation				
• 3-4 antibiotics						1768	59	3.34	1.67 (1.19-2.34)	0.0033					
• >4 antibiotics						1176	42	3.57	1.84 (1.26-2.69)	0.0017					
• No antibiotic (ref.)						4265	83	1.95							

Table 16: Comparing associations between different aspects of clinically significant RSV-associated LRTI and risk of asthma/wheezing phenotypes.

	Asthma/asthma-like symptoms in 3 yr	**Asthma at 7 yr**	**Asthma at 11 yr**	**Early persistent asthma**	**Transient wheeze**
RSV-associated LRTI (ref. no RSV-LRTI in 2 yrs)					
• Yes	2.94 (2.60-3.32)	1.36 (1.17-1.59)	1.26 (1.08-1.47)	2.91 (2.20-3.85)	2.99 (2.62-3.43)
Frequency of RSV-associated LRTI (ref. no RSV-LRTI in 2 yrs)					
• 1-2	1.93 (1.68-2.23)	1.30 (1.10-1.54)	1.21(1.03-1.43)	2.34 (1.72-3.18)	1.89 (1.61-2.21)
• ≥ 3	7.70 (6.50-9.13)	1.61 (1.24-2.08)	1.42 (1.09-1.86)	6.04 (4.05-9.00)	8.21 (6.84-9.85)
Severity of RSV-associated LRTI (ref. no RSV-LRTI in 2 yrs)					
• mild	2.44 (2.14-2.79)	1.29 (1.09-1.51)	1.21 (1.03-1.43)	2.61 (1.94-3.50)	2.43 (2.10-2.80)
• moderate	5.18 (3.94-6.81)	2.08 (1.40-3.10)	1.44 (0.92-2.24)	4.62 (2.37-9.00)	5.36 (4.01-7.17)
• severe	9.15 (6.95-12.04)	1.89 (1.21-2.95)	1.74 (1.11-2.72)	8.98 (4.86-16.61)	9.36 (7.01-12.49)
Age-group at RSV-associated LRTI (ref. no RSV-LRTI in 2 yrs)					
• ≤ 6 months	4.47 (3.85-5.19)	1.32 (1.06-1.65)	1.25 (1.001-1.56)	4.23 (3.00-5.96)	4.55 (3.87-5.35)
• >6-12 months	2.80 (2.35-3.33)	1.43 (1.14-1.79)	1.36 (1.08-1.71)	2.85 (1.93-4.19)	2.86 (2.37-3.45)
• >12-24 months	1.23 (0.97-1.57)	1.34 (1.05-1.71)	1.15 (0.90-1.49)	1.58 (0.96-2.60)	1.19 (0.90-1.56)

Table 17: Comparing association between RSV-LRTI and asthma/wheezing phenotype(s) for all children and children who did not have asthma onset before RSV-LRTI.

Asthma/asthma phenotype	**All children of the study cohort (n=13980)**	**Excluding subjects with asthma onset before RSV-LRTI (n=13602)**
Asthma/transient wheeze before 3 years	4.35 (3.88-4.88)	2.94 (2.60-3.32)
Asthma at 7 years	1.48 (1.28-1.72)	1.36 (1.17-1.59)
Asthma at 11 years	1.35 (1.16-1.56)	1.26 (1.08-1.47)
Ever asthma up to 11 years	2.69 (2.45-2.95)	2.04 (1.85-2.25)
Early persistent asthma	4.15 (3.20-5.39)	2.91 (2.20-3.85)
Transient wheeze	4.47 (3.95-5.07)	2.99 (2.62-3.43)

Appendix B

a) Definitions considered to identify RSV-associated LRTI

1) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) within winter (between December 21st to March 19th),

2) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) within winter and in late fall and early spring (between November 30 to April 09),

3) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) within RSV season,

4) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 2 years,

5) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) in winter (between December 21st to March 19th) before the age of 2 years,

6) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) within winter and in late-fall and early-spring (between November 30th to April 09th before the age of 2 years,

7) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) in RSV season before the age of 2 years,

8) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 2 years and within RSV season and in winter,

9) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 2 years and within RSV season and in winter and late-fall and early-spring,

10) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) within RSV season and in winter and late-fall and early-spring,

11) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI

(ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 5 years and within RSV season and in winter and late-fall and early spring,

12) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 3 years and within RSV season and in winter and late-fall and early-spring,

13) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 3 years within RSV season,

14) Any physician visit and/or hospitalization with confirmed (ICD9=466.11, 480.1) diagnosis of RSV in any time of the year and/or possible RSV-associated LRTI (ICD9=466, 466.0, 466.1, 480, 485, 486, 490 and 491) before the age of 5 years within RSV season.

b) Confounding factors

1) Parental history of asthma,

2) Maternal stress,

3) Socio-economic status,

4) Number of sibling,

5) Gender,

6) Urban/rural residence,

7) Prematurity,

8) Antibiotic use in early life and

9) BCG vaccination.