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Observational Research in Childhood Respiratory Diseases

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ABSTRACT

Acute respiratory infections (ARIs) are common during the first two years of life, when infants and young children experience six to eight ARIs annually. In this age group, ARIs are the most commonly managed problems in general practice. Between 3 and 6% of infants are hospitalised in their first year of life with a severe ARI illness. Information about the epidemiology of ARI in children is based on historic community-based studies, cohorts of hospitalised patients, emergency department or primary healthcare presentations, and more recently from birth cohort studies principally involving children at high-risk of asthma. However, these studies do not completely identify the burden of mild-to-moderate ARIs in the community.

Recent community-based cohort studies have used sensitive polymerase chain reaction (PCR) assays. However, they have had one or more methodological limitations, including subject selection, length of study, non-representative sample populations, variable sampling frequency, and the lack of a control population. Furthermore, frequent detection of respiratory viruses in asymptomatic individuals questions their clinical and public health significance. Studies reporting the causal effect of individual respiratory viruses in ARI are needed to help address this question.

The Observational Research in Childhood Infectious Diseases (ORChID) study was a four year prospective, community-based, longitudinal birth cohort study of ARIs in 158 healthy children from birth to two years of age. ORChID sought to minimise some of the methodological limitations of previous studies. Parents completed a daily symptom diary and collected weekly nasal swabs, which were tested against 17 respiratory viruses. Healthcare-seeking behaviour was recorded in a separate ‘burden’ diary.

I found that young children experienced 0.56 (95% confidence interval (CI): 0.54, 0.59) ARIs per child-month. This equated to almost five cumulative months of respiratory symptoms during the first two years. Forty-eight percent of ARIs where a burden diary was completed initiated a visit to a family doctor. ARIs were associated with increasing age, the winter season, and childcare attendance.

Studies examining respiratory viruses in neonates have largely been from neonatal units or neonates presenting to hospital with respiratory symptoms. I was able to show

that respiratory virus infections were common (0.25 episodes per neonatal period, 95% CI: 0.18, 0.34), with diverse human rhinovirus (HRV) genotypes dominating (21/29; 72% of neonates with positive swabs). Almost 50% of respiratory virus infections in this period were asymptomatic. This subclinical shedding of all respiratory virus types complicates estimates of the true community burden of viral ARI in infants and young children.

To explore this further, I investigated the relative contribution of individual respiratory viruses to ARIs by calculating the virus-specific attributable fractions in exposed (AFE) children of ARIs and lower respiratory tract infections (LRTIs). The overall incidence of virus infections was 978 (95% CI: 930, 1029) per 100 child-years in the first two years of life. Viruses were detected in 75% of ARI episodes, while 23% of weekly swabs were positive for viruses during asymptomatic periods. RNA viruses, including HRV, influenza, parainfluenza, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), and human coronaviruses NL63 and OC43 were associated with a significantly increased risk of ARI symptoms. Support for causality was strongest for RSV (AFE 68%, 95% CI: 45%, 82%), and HMPV (AFE 69%, 95% CI: 43%, 83%) in children with LRTIs. In contrast, amongst the DNA viruses tested, only adenoviruses (AFE 29%, 95% CI; 12%, 42%) were significantly associated with an increased risk of ARI symptoms. Of HRV species, only HRV-C had a significant AFE result for LRTIs (AFE 22% (95% CI: 5%, 22%).

I went on to examine the timing of detection for each of the 17 respiratory viruses tested for in the ORChID infant cohort. Determining timing of first virus detection episodes (fVDEs) for different respiratory viruses in infants and young children identifies risk periods and informs preventive interventions, including vaccination. The median age for first HRV infections was 2.9 months (25^{th}–75^{th} centiles: 1.6, 5.1), while for all other respiratory viruses combined the median age was $\geq$13.9 months. Overall 52% of first HRV detections were symptomatic, compared with 57-83% with the other first virus detections. Thus, infants and young children do not always experience respiratory symptoms with their first viral detection episode, and for some viruses, such as RSV, these commonly occur when maternal vaccines may no longer offer protection.

Collectively, these findings highlight the important community-managed disease

burden caused by respiratory viruses in early childhood. They also provide a wealth of information about the relationship between respiratory virus infection and symptoms of respiratory illness. The ORChID study uses modern molecular-based techniques over four respiratory seasons to address questions about respiratory virus acquisition and infection.

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Sarna M, Ware RS, Sloots TP, Nissen MD, Grimwood K, Lambert SB. The burden of community-managed acute respiratory infections in the first 2-years of life. *Pediatric Pulmonology* 2016; 51(12):1336-46. DOI 10.1002/ppul.23480.

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List of Abbreviations

AFE	Attributable fraction in the exposed
AOM	Acute Otitis Media
ARI(s)	Acute respiratory infection(s)
AUD	Australian Dollars
BEACH	Bettering the Evaluation And Care of Health
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
ED	Emergency Department
EHV	Equine herpesvirus
ERV-3	Endogenous retrovirus-3
ETS	Environmental tobacco smoke
fVDE	First viral detection episode
FP	Family physician
GP	General Practitioner
HAdV	Human adenovirus
HBoV-1	Human bocavirus-1
HCoV	Human coronavirus
HMPV	Human metapneumovirus
HPyV	Human polyomavirus
HRV	Human rhinovirus
INFV	Influenza virus
ILI	Influenza-like illness
IR	Incidence rate
IRR	Incidence rate ratio
LRTI(s)	Lower respiratory tract infection(s)
NAAT	Nucleic acid amplification test
NPA	Nasopharyngeal aspirate
OR	Odds ratio
ORChID	Observational Research in Childhood Infectious Diseases study
QPID	Queensland Paediatric Infectious Diseases laboratory

PCR	Polymerase chain reaction
PIV	Parainfluenza virus
RNA	Ribonucleic acid
RR	Risk ratio
RSV	Respiratory syncytial virus
SD	Standard deviation
UI	Uncertainty Interval
UQ	The University of Queensland
URTI(s)	Upper respiratory tract infection(s)
VDE	Viral detection episode

Definitions

An **acute respiratory episode (ARI)** episode was defined by the presence of respiratory symptoms and/or signs of the upper and/or lower respiratory tracts listed below.

Upper respiratory tract infection (URTI): parent-reported runny nose/nasal congestion, dry cough, or doctor-diagnosed acute otitis media (AOM).

Lower respiratory tract infection (LRTI): parent-reported rattly breathing, moist cough, shortness of breath, wheeze, or doctor-diagnosed pneumonia.

If wheeze was a component of a child's LRTI, it was sub-categorised as a 'wheezy episode'.

ARIs were sub-categorised hierarchically as either a LRTI or URTI, respectively.

Fever as a symptom was accepted if the parent ticked the fever symptom box or if they recorded axillary temperatures >37.5°C. For the purposes of demarcating ARIs, a new episode was considered to have commenced if there were ≥3 symptom-free days since the last day with symptoms. Daily symptoms were recorded until the child's second birthday.

New virus detection episode (VDE): when a new virus (different virus or new species/subtype of the same genus or species respectively) was detected in a swab by PCR testing, or the same virus was detected after at least two negative intervening swabs, or the same virus was detected at least 30 days from the last positive swab.

Symptomatic new VDE: when respiratory symptoms were reported within seven days either side of detecting a new virus detection episode in the weekly nasal swab.

First viral detection episode (fVDE): when a respiratory virus not detected previously was first found in a child's weekly nasal swab specimen.

Symptomatic fVDE: when respiratory symptoms were reported within seven days either side of first detecting the virus of interest in the weekly nasal swab.

Symptomatic fVDEs were sub-categorized hierarchically as LRTI or URTI episodes respectively.

Thesis Introduction, Aims, and Structure

Introduction to the Thesis Topic

Acute respiratory infections (ARIs) are common in young children during the first two years of life, when infants and young children experience six to eight ARIs each year, and are the most frequently managed problems in general practice. Complications are common, and between 3 and 6% of young children are hospitalised with more severe disease. Much of what is known about the epidemiology of ARIs in young children is based on historic community-based studies, cohort studies of hospitalised patients, emergency department (ED) or primary healthcare presentations, and more recently from birth cohort studies that have involved mainly children with one or both parents being atopic and thus considered to be at high-risk of asthma. Findings from these studies may not identify the true community burden of mild-to-moderate disease in otherwise healthy children in the contemporary, modern community.

The discovery of novel viruses and the ability to detect them and other known viruses using sensitive molecular-based methods has led the way for several cohort studies being conducted using polymerase chain reaction (PCR) assay testing of specimens. However, these cohort studies have had several limitations, including subject selection, length of study, non-representative sample populations, variable sampling frequency, and the lack of a control population. Furthermore, with the detection of respiratory viruses in both asymptomatic individuals as well as children with ARI symptoms, the clinical and public health significance of respiratory viruses is often uncertain, as detection may represent persistent shedding from a recent illness or either a nascent or genuine subclinical infection. Studies reporting the causal effect of individual respiratory viruses in ARI are needed to help address this question.

Research Aims

The broad aims of this research are to:

- Describe the community-based epidemiology of respiratory viruses in the first two years of life, including the incidence, symptoms, management, and associated risk factors of ARIs;
- Determine the relative contributions of individual respiratory viruses to ARI in the first two years of life; and
- Determine the pathogenicity of newly discovered respiratory agents, including

the new HRV-C species, by comparing the timing and duration of detection in respiratory specimens with the presence of symptoms.

The specific hypotheses to be tested include that:

- Collectively, HRVs, and the six recently described respiratory viruses from four virus families (HMPV, HCoV-NL63, HCoV-HKU1, HBoV-1, HPyV-WU, HPyV-KI) are more frequently detected during ARIs in the first two years of life than the long-established viruses (RSV, PIVs, INFV, HAdVs) identified from hospital-based studies.
- Respiratory pathogens are more likely to be associated with symptoms when first acquired.
- Established respiratory viruses are individually more likely to be associated with symptoms than the recently described novel DNA viruses HPyV and HBoV-1.
- Most respiratory pathogen shedding is transient, ceasing within 2-4 weeks of initial detection.
- HRV-C is more likely to be associated with lower respiratory tract infections (LRTIs) than other HRV species.

The Observational Research in Childhood Infectious Diseases (ORChID) study was a four year prospective, community-based longitudinal, dynamic birth cohort study of ARI episodes in children from birth to two years of age. ORChID was designed to address some of the methodological limitations of previous studies. It employed prospective serial sampling, sensitive molecular testing, and intensive clinical follow-up in a community-based healthy infant birth cohort, all of whom were born at term. Data were collected on respiratory symptoms daily, and healthcare seeking behavior. Weekly nasal swabs were tested against 17 novel and established respiratory viruses.

Thesis Structure

This thesis is divided into seven chapters.

Chapter 1 provides a literature review of the impact of ARIs, risk factors associated with ARIs, the aetiology of common respiratory viruses, and a summary of the findings of community-based studies to date.

Chapter 2 outlines the basic methods employed in the study.

Chapters 3–6 report the findings of research studies based around specific questions. Each of these chapters presents a published manuscript.

Using data from the ORChID Study, in Chapter 3 I initially collated symptom data to report on the burden of community-based ARIs in the first two years of life. I demonstrate that ARIs are a common cause of early childhood morbidity in the community, where they are almost exclusively managed. While mainly self-limiting and of a viral nature, antibiotics are still commonly prescribed for ARIs with almost half the children receiving these agents after visiting their family doctor. The most common reason for prescribing antibiotics was for acute otitis media (AOM).

Combining symptom and nasal swab testing results, in Chapter 4 I first explored respiratory virus acquisition in neonates, and explored possible risk factors for ARI during this period. I found that respiratory viruses are detected frequently during infancy and are without symptoms in 45% of instances (13 of 29 neonates). Subclinical shedding by all respiratory virus types complicates estimates of the true community burden of viral ARI in infants.

I then investigated the relative contribution of individual respiratory viruses to ARIs by calculating the virus-specific attributable risk of ARIs and LRTIs in Chapter 5 over the two year study period. Asymptomatic detection of both RNA and DNA respiratory viruses occur commonly. The virus-specific attributable risk of ARIs and LRTIs, a neglected key metric for interpreting molecular diagnostic tests in ambulatory clinical settings, provides quantitative estimates of the relative proportion of virus-associated ARIs, including the more serious LRTIs, in the community, yielding important insights for prioritising public health interventions.

In Chapter 6 I looked at the timing of first infections of respiratory viruses, and the proportions that were symptomatic, in further detail. I asked whether specific factors were associated with symptomatic first infections. This analysis identified age, season and the number of children in the household as potential risk factors to symptomatic infection, although these varied by virus type.

In Chapter 7, I summarise the thesis findings as a whole, discuss the implications of the findings, and how they will inform future research and public health policy.

Chapter 1 – Introduction

The impact of viral acute respiratory infections

Acute respiratory infections (ARIs) are the most commonly experienced illnesses throughout life (1). Viruses are the most frequent causes of ARIs, either on their own or in synergy with bacterial pathogens. Previous research has estimated the childhood incidence of community-managed ARIs in Australia ranges from 3.2–5.8 cases of ARIs per child-year (2-4), with the highest incidence rates reported in the first two years of life (5, 6). While most ARIs result in mild, self-limited symptoms, complications such as acute otitis media (AOM) and sinusitis are common, especially in those younger than two years of age. Consequently, in countries such as the United Kingdom, ARIs account for 59% of general practitioner (GP) consultations in this age group (7). Furthermore, it is estimated that 3–6% of children younger than one year of age are hospitalised with lower respiratory infections (LRTI), including bronchiolitis, croup, pneumonia, or secondary bacterial pneumonia (7, 8), making up to 25% of all hospitalisations in this age group. These infections are a considerable burden on the health system (9). The majority of cases of ARIs are managed solely in the community (6) and these impose considerable costs upon families and society (10, 11).

In Australia, the disease burden in Aboriginal and/or Torres Strait Islander children is also greater than in non-Indigenous Australians. Hospitalisation rates of acute lower respiratory infection amongst Aboriginal and/or Torres Strait Islander children are nearly six times that of non-Indigenous children (12). Antecedent respiratory viral infection has been shown to increase nasopharyngeal bacterial density and progression to AOM with perforation in Indigenous children (13), who have some of the highest rates of AOM with perforation globally.

Early onset of ARIs accompanied by wheezing have been associated with subsequent recurrent wheezing illnesses in infants and young children, and a diagnosis of asthma later in childhood. Indeed, ARIs are probably the most frequent causes of asthma exacerbations in older children and adults. When ARIs involve the lower respiratory tract and are severe in infants and young children, they can be associated with impaired lung function, non-smoking-related chronic obstructive lung disease (COPD) in adulthood, and when severe, bronchiectasis (15-20).

Available information on the epidemiology of viral ARIs in children has largely been based on cohorts of hospitalised patients (21, 22), findings reflecting the most severe infections. These illnesses are not representative of mild-to-moderate disease managed in the community, and can under-estimate the underlying true community disease burden (23-25), an important consideration in planning future preventive strategies.

Respiratory illnesses are also the most commonly managed problems in general practice in Australia. An early epidemiological report on the nature of primary care consultations in general practice in Australia showed that nearly 50% of consultations in children younger than five years of age were for ARIs (26). A recent analysis of Australian GP activity described 26 of 100 encounters in this age group for common respiratory tract infections: upper respiratory tract infection (URTI), bronchitis/bronchiolitis, tonsillitis, and pneumonia (27). Others have shown that in this age group almost half of ARI episodes resulted in a medical consultation (10, 28) and their management had considerable impact upon healthcare services (29). Extrapolation of Australian data published in a Bettering the Evaluation And Care of Health (BEACH) survey suggest that 1.7 per 100 encounters in Australians of all ages attending primary care were for URTI alone and 6.2 encounters for cough (30). However, there are few published reports that provide detailed and up-to-date data regarding the rate and impact of respiratory events in the community, beyond the scope of those managed by GPs, particularly in young children.

Studies estimating the impact of respiratory illness have largely focused on direct hospitalisation and in-patient costs (31-34) or on evaluating the cost-effectiveness of introducing a vaccine (35, 36). They also vary in their method of analysis, including which cost drivers are used, making comparisons difficult. In general, the inclusion of societal and indirect costs such as productivity losses from lost work time caring for a sick child increases the overall cost of ARIs (10, 35, 37, 38). Children are also active transmitters of respiratory viruses to other family members (39-42). Palmer et al. (41) showed that employees with at least one child with an influenza-like illness (ILI) missed more days at work due to illness in another household member than employees without a child. A more recent study showed that parent work absenteeism was higher for parents with children in the 0 to 3 year age group compared to those with older children (43).

Risk factors for ARIs

Several risk factors associated with the development of ARIs have been identified by observational studies of natural infection, challenge inoculations in human volunteers, and *in vitro* experimental models.

Numerous studies have reported high rates of illness in children younger than five years of age. In addition to being active transmitters of infectious agents in households (39), children attending childcare are at higher risk of experiencing ARIs (44) and AOM (45). De Hoog et al. (45) also found that children who enter childcare in the first year of life have URTIs and AOM at an earlier age, leading to higher use of healthcare resources compared to non-attendees. However, an eight year study examining short- and long-term risk of infections as a function of group childcare attendance observed that although participation in large group childcare before 2.5 years of age was associated with increased infections at that time, it appeared to protect against infections during the elementary school years (46). Family household size and composition have also been identified as risk factors. Members of larger families (42), and children in families where younger children have older siblings (47), are at greater risk of developing an ARI.

Examination of pre- and post-natal factors has identified preterm birth on its own or in combination with other factors to be a significant risk factor for more severe disease from several viruses (48-50). This is presumably from a combination of reduced levels of protective maternal antibodies and small lower airway diameters that are easily obstructed. ARIs have also been reported to be more severe in children with underlying complex chronic comorbidities, or immunosuppression (49, 51, 52). The risk of developing severe disease from respiratory syncytial virus (RSV) infection was higher in preterm infants with or without chronic neonatal lung disease, congenital heart disease, immunodeficiency, chromosomal, neuromuscular or other underlying chronic disorders (53). The study by Simoes (48) also identified that for up to half of hospitalised patients, severe LRTI from RSV infection was associated with being male, being aged younger than six months, being born early in the RSV season, attending childcare, exposure to household crowding and older siblings, absence of breast-feeding, tobacco smoke exposure (48), and race/ethnicity (54). Race/ethnicity on its own has been shown to be an independent risk factor in Indigenous populations in

several countries (54-56). Being born in the six months before the annual respiratory virus season has also been identified as a risk factor in RSV and human metapneumovirus (HMPV) infections in other studies (49, 54).

Childhood wasting remains the leading risk factor for lower respiratory infection mortality in children younger than five years in underprivileged populations worldwide, estimated to be responsible for 61.4% of lower respiratory infection deaths in 2016 (95% UI: 45.7, 69.6) (57). The higher burden of disease in these populations is likely due to a combination of factors, including malnutrition and overcrowding.

Exposure to environmental tobacco smoke (ETS) is a well-recognised risk factor for both acute and chronic respiratory illness. Maternal smoking and ETS exposure influence lung development and are associated with both URTI and LRTI, wheezing, and asthma (58). There is evidence linking the effects of ETS exposure to impaired early-life immune function, resulting in an imbalance in Th1 and Th2 responses, which may increase susceptibility to allergic diseases and childhood ARIs (58). Exposure to ETS is also associated with more severe respiratory disease. ETS has been found to increase the risk of severe RSV disease as measured by hospitalisation and hypoxia in both infants and children (aOR 2.2, 3.8), and a 20% increased need for ICU admission and 12% increased risk of intubation in children with influenza compared to children without ETS exposure (58).

The effect of breastfeeding on mortality and morbidity from respiratory infections was assessed in a systematic review published by the World Health Organization (59). Breastfeeding reduced the risk of hospitalisation for respiratory infection by 57% [pooled relative risk (RR): 0.43 (95% CI: 0.33, 0.55)]. Studies that compared breastfed with non-breastfed children reported the highest protective effect [pooled RR: 0.33 (95% CI: 0.24; 0.46)] against hospitalisation for respiratory infection. Mortality from LRTIs was also reduced among breastfed children [pooled RR: 0.30 (95% CI: 0.16; 0.56)]. Furthermore, breastfeeding also reduced the prevalence or incidence of LRTIs [pooled RR: 0.68 (95% CI: 0.60; 0.77)] (59).

Importantly, this systematic review examined data from both low and high income countries. These results were robust, observed in high and low-income countries and across different respiratory infection-related outcomes.

Aetiology of respiratory viruses

Large epidemiological studies in the 1950s and 1960s demonstrated the importance of viruses in ARIs, despite employing relatively insensitive classical cell-based techniques available for viral detection at the time (60, 61). Historically, known respiratory viruses in childhood have included RSV, influenza viruses (INFV), parainfluenza viruses (PIV), human coronaviruses (HCoV) OC43 and 229E, adenoviruses (HAdV), and HRV-A and B species.

In the last two decades, several novel human respiratory viruses have been identified using either modern molecular techniques or improved culture methodologies. These viruses are now detected in routine diagnostic or research specimens using sensitive nucleic acid amplification tests (NAAT), such as PCR assays. These include HMPV (62), novel HCoVs NL63 and HKU1 (63, 64), human bocavirus-1 (HBoV-1) (65), and newly discovered human polyomavirus species (HPyV) (66, 67). A new species of HRV (HRV-C) has also been discovered, previously not detected as this new clade had been non-cultivable using traditional cell-culture techniques (68).

While there are other viruses responsible for respiratory symptoms in children, I have focused here on the respiratory viruses commonly associated with ARIs, and were therefore selected for study in the Observational Research in Childhood Infectious Diseases (ORChID) study cohort. Questions remain over their relative importance in disease managed within the community, and for some even their role as respiratory pathogens remains controversial.

RNA viruses

Respiratory Syncytial Virus (RSV)

RSV is an enveloped, non-segmented, negative strand RNA virus of the family *Paramyxoviridae* with two antigenic subtypes, A and B. RSV is well-recognised as an important viral respiratory pathogen in infancy and early childhood. It is the most frequent cause of acute LRTI in this age group (69), and the most common reason for infants to be hospitalised in the first year of life (70, 71).Globally in 2015, 33.1 million (95% UI: 21.6, 50.3) episodes of RSV-LRTI (28% of all LRTI) resulted in approximately 3.2 million (95% UI: 2.7, 3.8) hospital admissions, and 59, 600 (95% UI: 48,000, 74,500) in-hospital deaths in children aged younger than five years (72). The most recent estimates from 2016 place RSV as a leading contributor of LRTI deaths in this

age group (57), notwithstanding deaths from bacterial pneumonia may be underestimated given the insensitivity of blood cultures. Estimates of RSV hospitalisation rates for children younger than five years of age in Australia have used a variety of methods and have ranged from 2.2 (73) to 4.9 (31) per 1000 child-years, with an average annual cost (calculated between 2001 and 2010) of more than AUD$9 million in New South Wales alone (31).

Infection with RSV occurs early in life and it is a generally held belief that between 50% and 70% of infants are infected in the season they are first exposed to RSV (74). In the seminal Houston Family Study, 97% of children had serological evidence of infection by the end of their second season, 40% of them developed signs of LRTI (wheezing or croup), and 2–3% of these presented with severe symptoms and required hospitalisation (74). However, studies from Asia, Europe, and Latin America have observed lower RSV seroprevalence rates of 36–70% by age two years (75-78), while in Kenya a birth cohort project similar in design to the Houston study found 73% had RSV by two years of age (79).

RSV typically has a winter seasonal peak in temperate climates, but seasonality differs between temperate and tropical climates. In tropical climates, a seasonal peak is less well-defined (55, 80) and may even be bimodal (81). In subtropical climates, such as South-East Queensland, the peak is in mid-to-late autumn (March-April), tapering in late winter (June-August) (82). Incidence can be influenced by the severity of the annual epidemic, which varies from year to year, and opportunity for individual exposure, such as older siblings and childcare attendance (83).

Well-recognised risk factors for severe LRTI with RSV infection have been mentioned previously (see page 29/30 for further details), and include being born preterm, underlying chronic cardiopulmonary disorders, trisomy 21, and being infected in the first six weeks of life.

Nevertheless, most infants hospitalised with RSV infection are otherwise healthy and were born at term. It is thought that hospitalised infants have more severe disease because they have pre-existing smaller airways, immature immune systems, and/or lack protective maternal antibodies (84, 85). In a two year prospective study examining the viral aetiology of acute wheezing illnesses in hospitalised children in Finland, RSV was more prevalent in infants whereas picornaviruses dominated in older children

(86), presumably because as otherwise healthy infants become older, their airways grow, immune systems mature, and the symptoms of RSV disease lessen (74, 79). Findings from the Dutch WHISTLER birth cohort study showed decreased lung function at birth predisposed to severe RSV disease, and to post-RSV wheezing in term infants (84). RSV infection in young children is also more likely to be associated with respiratory symptoms (22) than asymptomatic detection.

Monoclonal antibodies such as palivizumab are currently offered to high-risk infants. Long-acting monoclonal antibodies and candidate antenatal vaccines undergoing trials in pregnant women to reduce the risk of RSV infection in young infants are also currently underway (87). A successful vaccine would go some way to determine whether RSV has an aetiological role in asthma.

Influenza virus (INFV)

INFVs are negative sense, enveloped, single-stranded, segmented RNA viruses of the family *Orthomyxoviridae.* Subtypes A, B, and C are known to infect humans. INFVs undergo frequent antigenic mutations that contribute to variability from year-to-year. INFV has a sharp seasonal pattern in temperate climates. Data from the Global Burden of Disease Study shows globally, influenza was the second most common aetiology among LRTI episodes (39.1 million episodes, 95% UI: 30.5, 48.4). In children under five years of age, the incidence of seasonal influenza was estimated to be 9.1 LRTIs per 1000 people (95% UI: 5.4, 14.8).

In Australia, influenza hospitalisation rates are highest in children aged 0-5 months (152 per 100,000 in 2013), followed by children 6-23 months of age (98 per 100,000 in 2013) (14).

Children have the highest attack rates of influenza. In otherwise healthy children, influenza is typically a mild to moderate illness and, in most children, resolves without complications (88). Although the vast majority of children are treated as outpatients (outpatient visits are 10–250 times more common than hospitalisations (88)), infants can experience a more severe clinical course and are frequently admitted to hospital for INFV-associated illnesses. The highest hospitalisation rates for seasonal INFV infections are in the first year of life (71, 89).

Influenza infections can be complicated by secondary infections, which add considerably to the burden of influenza. There is some evidence for the role of INFV in facilitating bacterial transmission and disease (90). Clinically, AOM is the most frequent INFV-associated complication (91). Another significant, though less common, complication of influenza is pneumonia (92). Such secondary infections are less frequent where protein-polysaccharide conjugate pneumococcal and *Haemophilus influenzae* type b vaccines are routinely administered to children (93). Influenza-related clinical deterioration is more likely in children with asthma (94).

Influenza infection in children has consequences beyond direct medical outcomes. Children shed influenza virus longer and in larger amounts than adults and thus play a major role in the transmission of influenza in families and society. Influenza also translates into a societal burden evident as a child's absenteeism from childcare or school, and parental work loss because of influenza in their children (95, 96). In a prospective study in Finland, the frequency and duration of parental work loss because of child influenza were highest among children younger than three years of age, among whom the mean duration of parental absenteeism in families in which a parent had to stay at home was 3.2 days (95).

Asymptomatic detection of INFV is not uncommon. Estimates of the asymptomatic fraction have ranged from 20–50% (97-99) and in a 2015 meta-analysis of 4–28% (100). Two studies have reported influenza viral RNA shedding in asymptomatic persons to be age and symptom related. In a household transmission study of INFV in Nicaragua, children younger than 6 years of age had a longer duration of pre-symptomatic INFV shedding than adults (101).The duration of post-symptomatic INFV shedding was longest in children 0–5 years old, followed by children 6–15 years of age, and then adults. In a community-based study in Hong Kong where healthy household contacts of symptomatic persons with INFV were followed up, viral RNA shedding occurred in pauci-symptomatic and asymptomatic cases, but was shorter and declined more rapidly than observed for symptomatic cases (102).

For children aged six months to <5years of age, influenza vaccination in Australia is recommended, but currently only funded as part of the National Immunisation Program for all Indigenous children (14), (103).

Parainfluenza virus (PIV)

PIVs are single-stranded, negative sense, enveloped RNA viruses of the *Paramyxoviridae* family containing four distinct antigenic types, PIV 1–4. PIVs are a common cause of ARIs in children, with clinical manifestations ranging from afebrile URTIs to severe LRTI symptoms (104). The most characteristic and clinically important syndrome associated with PIV-1 and PIV-2 is croup (laryngotracheobronchitis).

Morbidity data from prospective studies conducted in the latter half of the twentieth century in diverse populations of different ages and the types of illness surveyed (105-108) show the number of cases of croup and LRTI increase dramatically from the age of four months to six years. One study in the US found that PIVs accounted for 74% of isolates in cases of croup presenting to a paediatric practice over an 11 year period (109). The use of PCR detection in samples from children in the household and childcare settings (3, 110) has contributed to our understanding of PIV epidemiology by documenting the presence of prolonged shedding (up to 35 days), multiple infections and re-infections, and asymptomatic disease. PIVs have been studied specifically in children with asthma (111) and bronchiolitis (112). Data from the New Vaccine Surveillance Network show that 7% of all hospitalisations for fever and/or ARI in children younger than 5-years of age in the United States can be attributed to PIV (113). Half of these resulted from PIV-3 infection, with most of the remainder caused by PIV-1.

Human rhinoviruses (HRV)

HRV are non-enveloped, single, positive-stranded RNA viruses within the family *Picornaviridae* containing three species (A, B, C). HRVs are the most common cause of ARIs (114) and the most frequently detected of all respiratory viruses in all ages. HRV infections are associated with a wide range of clinical presentations, both URTIs (the ‘common cold’, AOM, rhinosinusitis) and LRTIs (2, 69, 115). HRV infections have been linked to bronchitis, bronchiolitis in infants, and childhood pneumonia, as well as to acute exacerbations of more chronic pulmonary disorders in older children and adults, such as asthma and COPD (116).

HRVs exhibit enormous diversity, with currently about 160 recognised subtypes. A large number of distinct types circulate each year with little cross-immunity, and while some predominate in a given season, they are replaced by others in subsequent years (117). HRVs are frequently transmitted from children to other family members and multiple virus types have been shown to circulate simultaneously in families (39) and in childcare (118). Cross-sectional PCR detection rate data do not comprehensively represent HRV circulation patterns because sequential infections by different strains occur and may appear as unbroken symptomatic episodes during a single observation period (117).

HRVs are second only to RSV for being detected in infants hospitalised with bronchiolitis. In a three year prospective study, Calvo and colleagues investigated the frequency of 16 respiratory viruses in 318 children hospitalised with bronchiolitis (69). RSV was found in 61% of cases of bronchiolitis and HRV in 17%. High-risk birth cohort studies have identified HRV, ahead of RSV and INFVs as the major upper and lower respiratory pathogens in the first year of life (2, 120, 121). For severe respiratory infections requiring hospitalisation, RSV dominates in infants and HRV in children older than 12 months of age (122).

Studies in hospitalised patients and atopic cohorts suggest HRV-associated wheeze in early childhood results in an increased risk for the later development of asthma, especially if already sensitised to aeroallergens (17, 123, 124). Children hospitalised for HRV-positive bronchiolitis used long-term asthma controller medication more often than those hospitalised for HRV-negative bronchiolitis during the first year after hospitalisation. HRV-associated wheezing illness was also associated with more courses of systemic corticosteroids during follow-up (125).

Results in healthy cohorts are scarcer, but one study on a healthy unselected birth cohort has shown that HRV and other viruses were associated with lower respiratory symptoms during infancy, as well as a high pre-symptomatic respiratory system resistance. Lung function was measured shortly after birth and before two months of age in 140 healthy infants. HRV presence during infancy was not associated with childhood wheezing, but wheeze during a HRV episode was an indicator of children at high risk for ongoing childhood wheeze, partly because of reduced neonatal lung function and airway compliance leading to airflow limitation (126).

The clinical significance of the different HRV species has been the subject of much debate. HRV-A and HRV-C species have been linked to more severe illness than HRV-B, especially in children (127-129), however this finding has not been consistent across studies (118, 128, 130, 131). Several studies have shown HRV-C viruses to be detected in infants with wheezing or persistent cough (132), or more often associated with severe exacerbations of asthma requiring intensive care admissions than other species (133-135). An observation in a West Australian cohort study was that infants who wheezed with infection by one HRV strain did not always wheeze with a second or third infection (2), suggesting that some strains may be more “wheezogenic” than others. HRV genotype-specific analysis of nasal lavages from high-risk infants enrolled in the COAST study has shown that HRV-A or -C clades of viruses cause more severe respiratory illnesses in infants (136). However, it is important to note that many of these studies were among children presenting for ambulatory or hospital-based medical care or in high-risk cohorts at risk of developing wheeze.

A further consequence of sensitive PCR testing has been the frequent detection of HRV in specimens from asymptomatic individuals, the role and clinical significance of which has been debated. HRV RNA has been detected before, during and after symptomatic infection, and in subclinical infection (137). Indeed, HRV has usually been the most frequent viral finding whether a person was symptomatic or not. Several studies have independently identified HRV RNA in 12% to 35% of asymptomatic subjects (138-140).

Considering their ubiquity, relatively low numbers of co-detections of other respiratory viruses occur with HRV strains (3, 117, 141). While up to 50% of HRV detections are found concurrently with another virus (117), higher rates of up to 80% co-detection can be found with some other viruses (142).

HRV shedding after an acute illness appears to be of short duration (143). More recent studies have shown HRV infections to disappear within 4–5 weeks (39, 144). A later study by the same authors showed that persistence of the same HRV type beyond 2 weeks was observed in only 5% of infections (122). However, prospective studies with repeated sampling combined with genotyping are few and essential to fully describe HRV epidemiology, to distinguish between shedding from a previous subclinical illness, and to define infection with different genotypes in an unbroken illness episode.

A limited number of longitudinal birth cohort studies have prospectively evaluated HRV infections in infants and young children but these have had limitations in size (145-147), frequency of sampling (144, 148), and limited asymptomatic sampling (118, 144, 149) and follow-up (144, 150). Furthermore, few studies sequenced serial HRV detections in the same child to distinguish between genotypes (118, 144, 145, 150-152). Studies that employed molecular genotyping to characterise strains did not identify persistent HRV shedding by a single strain and it seems instead the finding of generic “persistent” infections or prolonged shedding is from strain replacement and sequential infections caused by a succession of HRV strains (39, 137, 153).

Human metapneumovirus (HMPV)

HMPV is an enveloped, single-stranded, negative sense RNA virus in the family *Paramyxoviridae*. First identified in 2001 in RSV-negative children with bronchiolitis (154), HMPV is closely related to RSV and appears to have a similar seasonality and clinical spectrum associated with RSV infection, although HMPV infections occur in patients that are slightly older (155).

Overall annual rates of hospitalisation associated with HMPV infection were 1 per 1000 children less than five years of age, 3 per 1000 infants less than six months of age , and 2 per 1000 infants six to eleven months of age in a six year surveillance of three US counties (156). The estimated annual burden of outpatient visits was 55 clinic visits and 13 ED visits per 1000 children (156). A more recent meta-analysis estimated the prevalence of hospitalised ARI to be 6.4% (95% CI: 5.3-7.3) (157). Average annual incidence of HMPV in Queensland was 7.1% in respiratory samples from patients with acute LRTI collected for 4 years (158).

Infection with HMPV can range from a mild URTI (159), URTI with AOM (159), through to bronchiolitis, croup, or pneumonia (158, 160). Serological studies have shown that by age fiveyears, almost all children have been infected by HMPV (154), although children younger than two years of age are most at risk of severe disease (155, 158). Its association with wheeze and asthma has been highlighted in several studies (161-163). Clinical evidence suggests that HMPV is associated with acute exacerbations of asthma in both children and adults (164). Animal models have demonstrated that airway hyper-responsiveness and inflammation are triggered following HMPV infection, and HMPV is able to persist *in vivo* by inhibiting innate immune responses

and causing aberrant adaptive responses. Asymptomatic and subclinical infection appear to be uncommon (154, 156). Screening of 400 samples from infants without respiratory symptoms revealed no positive samples in one study (154) and 1.3% in another (156).

Human coronaviruses (HCoV)

HCoVs are enveloped, single-stranded, negative sense RNA viruses in the family *Coroniviridae* and were first described in the 1960s with the discovery of species 229E and OC43 (165-167). Together with HRVs, they are regarded as important 'common cold' agents. Increased interest in HCoVs followed the severe acute respiratory syndrome (SARS-HCoV) epidemic in 2003 and was rekindled with the emergence of the Middle East Respiratory Syndrome (MERS-HCoV) virus in 2012 (168). In the early part of this century, two other HCoVs, NL63 (63) and HKU1 (64) were also identified. Infection from these four non-SARS/MERS HCoV species occur in the first five years of life (169), and can cause URTIs (common cold) and LRTIs (ranging from croup (170), bronchiolitis (171), and pneumonia (172)). However, a systematic review found no association between HCoV infection and acute asthma exacerbations (173).

The newly discovered species appear to be present in higher rates than the two older species in both hospitalised and healthy children (174)**.** Most of the evidence of their involvement in severe disease comes from reports from hospital-based studies using PCR platforms (171, 175-177). Furthermore, HCoVs are frequently encountered with other viruses (178), which complicates determining the primary aetiology. It is thus difficult to say what proportion of ARIs that result in hospitalisation are in fact caused by these viruses. In a comparison of all HCoV types in a community cohort and a children's hospital in the same community over two years, children in the community generally had mild illness with few medically attended cases and were older. In hospitalised children, young age and chronic complex conditions were associated with severity of illness (179).

DNA viruses

Adenoviruses (HAdV)

HAdVs are non-enveloped, double-stranded DNA viruses within the family *Adenoviridae* and important pathogens in young children. They are most frequently

associated with febrile URTIs with pharyngitis, pharyngoconjunctivitis, or coryza, but can occasionally cause pneumonia. HAdVs have also been independently associated with acute otitis media with perforation in Indigenous children (13). They account for 5–10% of ARIs in infants and young children (180), and have been implicated in up to 10% of childhood pneumonia (181, 182). HAdVs are especially prevalent in childcare centres and households with young children. Longitudinal studies performed in families and paediatric facilities showed that species C HAdV infections occur early in life and are often endemic (183, 184).

These studies identified a large number of asymptomatic infections with species C and demonstrated a high frequency of faecal shedding of viruses (180). Infants and young children have the highest attack rates of endemic HAdV infections. Although most HAdV illnesses are acute and self-limited, infection may be prolonged, and asymptomatic infections are common. HAdVs have been isolated from at least 50% of surgically removed tonsils, from the kidney, and also from lymphocytes, suggesting that infection may remain latent for a very long time, possibly for life (185). Prolonged viral shedding following HAdV infection and ubiquitous environmental contamination have been implicated in disease transmission (186).

Human bocavirus (HBoV)

HBoV-1 was first identified in 2005 (65) in nasopharyngeal aspirates (NPAs) collected from children with ARI episodes. It is a small non-enveloped, single-stranded DNA virus in the family *Parvoviridae, genus Bocavirus*.

HBoV-1 is most commonly found in young children aged 6–24 months with ARIs and appears to be acquired early in life (51). HBoVs have also been detected in children diagnosed with community-acquired pneumonia in Brazilian children (187, 188).

Studies have shown that HBoV-1 may persist in the respiratory tract for a longer time than other respiratory agents, resulting in frequent detection of low load HBoV-1 carriage by PCR (189, 190). HBoV-1 DNA may be detectable in the nasopharynx of immunocompetent individuals for at least six months after infection (191, 192). Consequently, high rates of co-detection of HBoV-1 with other viruses (40–80%) and high prevalence rates (5-44%) in asymptomatic children (190, 191, 193-196) have been noted and its role as a pathogen has been questioned. Additional prospective studies are required to determine the actual pathogenic role of HBoV-1.

Nonetheless, there is some evidence to show that HBoV-1 is pathogenic and associated with ARI symptoms. The strongest evidence to date comes from studies where symptomatic ARIs have occurred in association with HBoV-1 as a single pathogen in respiratory secretions, associated with high viral loads, viraemia (195, 197), and seroconversion (198, 199). The study by Ricart et al. (197) also found that viral load was positively correlated with length of hospitalisation, but inversely correlated with days of respiratory effort before admission to hospital with acute bronchiolitis (suggesting a more rapid development of symptoms with higher viral loads). Other studies that have included asymptomatic controls have shown an association between the presence of the virus and symptomatic illness in PCR studies of children (196, 200, 201). HBoV-1 viral load was also associated with the presence of ARI symptoms, a metric along with HBoV-1 capsid messenger RNA that has been reported previously to be associated with disease severity (202). Capsid protein VP1 is thought to exert a direct pathogenic effect on human airway cells (203). Thus, a combination of tests involving serology, quantitative PCR of blood and respiratory secretions, and transcriptional assays will be needed to accurately diagnose HBoV-1 infection (204).

Human polyomaviruses (HPyV)

The *Polyomaviridae* constitute a family of small DNA viruses that infect a variety of hosts. In the last decade 11 new human polyomaviruses have been identified, including the phylogenetically related KI-HPyV and WU-HPyV (65, 67), the latter two identified in the respiratory tract specimens of children with acute respiratory symptoms.

Sero-epidemiological studies have shown that primary exposure occurs early in life (205), with seroprevalence in children under the age of two years for KI-HPyV at around 40% and WU-HPyV varying between 45-83%. Seroprevalence of KI-HPyV and WU-HPyV is high in the adult population (55-90% and 69-98%, respectively) (206, 207).

In healthy, non-immunocompromised children, KI-HPyV and WU-HPyV seem to have a minor aetiological role in ARIs, most often manifesting as mild respiratory illness or asymptomatic infections, and thus their pathogenic role has been questioned (208, 209). Attributing causality remains problematic. Studies that have included

asymptomatic controls have detected viral sequences at similar or higher frequencies in controls compared with ARI cases (11% vs. 7% WU/KI) (208), (4.2% vs 7% WU) (210) (0% vs 2.2% KI, 6% vs 7% WU) (211). KI-PyV and WU-PyV have also been co-detected with other viruses in specimens from symptomatic subjects (66, 212, 213), in some cases in the order of 70–80% (212). In a vaccine trial in Filipino children with lower respiratory disease, the prevalence and co-detection rates for KI-HPyV and WU-HPyV were 4.2% and 84%, and 5.3% and 74% respectively (214).

Epidemiological studies

In addition to the seminal, community-based epidemiological studies conducted in families during the 1950s and 1960s (see page 29), hospital-based studies have also contributed to our understanding of the epidemiology of more severe viral ARIs in infants. These studies have emphasised RSV and, to a lesser extent, seasonal INFV, as the most common reasons for infants to be hospitalised in the first year of life (70, 71), as well as more recently emphasised the roles of HRV, PIV, and HMPV (69, 215-218).

The advent of sensitive PCR testing combined with the collection of a less invasive clinical specimen such as an anterior nose swab and home-based self or parent collection provides the opportunity to conduct more intensive community-based studies. These studies' design features offer several potential advantages, including timely and easy collection of specimens during acute episodes of ARI, removing the need for trained staff to collect specimens. These characteristics combine to make large, community-based studies logistically and financially more achievable, and provide the means to collect previously unavailable detailed information about infections in the community.

In the last two decades, a number of prospective community-based birth cohort studies on respiratory viruses have been conducted (Table 1), in both infants at high-risk of asthma and healthy infants, although very few have employed the above methodologies, viz parent-collected symptom and swab information (rather than health care workers) (219). Birth cohort studies of high-risk infants have investigated the role of respiratory viruses in the development of wheezing illnesses, as well as provided valuable insights into asthma risk factors and the natural history of wheezing and asthma through childhood and beyond (2, 121, 220, 221). These studies have

identified HRV, ahead of RSV and INFVs, as the major upper and lower respiratory pathogens in the first year of life.

However, in many cases cohort studies of ARIs in healthy children have had one or more methodological limitations, including follow-up restricted to a single year or respiratory season, and in so doing failing to account for seasonal or year-to-year variability in respiratory virus activity (222-224). Other limitations include non-representative sample populations, variable sampling frequency (146, 225), testing confined to a single or limited number of pathogens (146, 224), or lacking a control population or control specimen sampling strategy (3, 42, 222, 223, 226). Several cohort studies have recruited through clinics or hospitals on presentation of an ARI (227), or looked at specific clinical outcomes (140, 160, 225, 227, 228).

Well-designed prospective community-based cohort studies of ARI in unselected, healthy children with longitudinal follow-up and intensive sampling have been few (148, 151, 229), and understandably so, as they are not without challenges. Cohort studies of long duration with intensive follow-up are biased towards smaller families of higher socioeconomic status (2, 230).

However, these longitudinal study designs can establish temporal associations and offer the ability to correctly classify infections as symptomatic or asymptomatic, account for background prevalence, and afford the opportunity to examine viral shedding. Clinical follow-up can be correlated with virological and demographic data, and other risk determinants.

Birth cohort studies extend this further and provide valuable insight into incipient and ‘first’ infections acquired from birth and thereafter, persistence, and true asymptomatic infections. As demonstrated in a birth cohort study on norovirus, for viruses that commonly infect and re-infect, sometimes sub-clinically, similar to respiratory viruses, longitudinal data are crucial for understanding the relationship between primary, secondary, and subsequent infections and the development of disease (231).

Table 1: Summary of prospective observational community-based birth cohort studies of respiratory viruses published this century

Reference	Study selection	Cohort size	Enrolment age/follow-up	Sampling/Symptom collection details	Main findings
High-risk cohorts					
Lemanske, 2005 (121) (US)	One allergic parent	285 infants	Birth–12 months	Nasal mucus specimens at scheduled visits (regardless of symptoms) and at onset of ARI; Daily symptom diaries	Symptomatic HRV illnesses during infancy biggest risk factor for development of preschool wheezing.
Legg, 2005 (220) (UK)	One atopic, asthmatic parent	88 infants	Birth–5 months	Nasal lavage at each ARI; daily diaries for ARI only	Picornaviruses most frequently detected; RSV infection associated with diagnosis of bronchiolitis.
Kusel, 2006 (2) (Australia)	One atopic parent	263 infants	Birth–12 months	NPA at each ARI; 2 control NPAs; daily symptom diaries	69% ARI virus-positive: HRV (49%) and RSV (11%) most common; RSV strongly associated with LRI requiring hospitalisation
Kusel, 2007 (28) (Australia)	Children at high atopic risk	198 children	Birth–5 years	Postnasal aspirates; all episodes of ARI	Viruses (69%) of aspirates; HRV (48%), RSV (11%). RSV/RV associated with wheezy LRI (OR, 4.1 (1.3-12.6).
Jartti, 2008 (137) (US)	One allergic parent	285 infants	Birth–12 months	Nasal mucus specimens at scheduled visits (regardless of symptoms) and at onset of ARI; Daily symptom diaries	HRV infections occur early, pervasively, and repetitively in high-risk infants. Infants with prolonged or recurrent respiratory illnesses have series of infections rather than persistent infection with one virus strain.
Jackson, 2008 (123) (US)	One allergic parent	259 children	Birth–6 years of age	Nasal mucus specimens at scheduled visits (regardless of symptoms) and at onset of ARI; Daily symptom diaries	Viral wheezing illnesses in infancy and early childhood caused by HRV infections are most significant predictors of subsequent development of asthma at age 6 years.
Bisgaard, 2010 (221) (Denmark)	Children of asthmatic mothers	411 children	4 weeks–3 years	NPA during wheezy episodes and at planned visits without LRI symptoms; Daily symptom diaries	Wheezy episodes associated with bacterial and viral infections
Healthy cohorts					
van Benten, 2003 (232) (The Netherlands)	Antenatal recruitment	126 infants	Birth–12 months	Nasal brush samples during routine visits every 6 months and during URTI	HRV most prevalent (40%) in URTI, rhinitis, followed by RSV (20%), HCoV (10%). HRV may affect maturation of immune system and development of allergic disease.
von Linstow, 2008 (190) (Denmark)	Antenatal recruitment	228 infants	Birth–12 months	Nasal swab sampling and symptom diaries at monthly visits	HBoV-1 detected 8% in swabs from ARTI, 9% from asymptomatic children.
von Linstow, 2008 (233) (Denmark)	Antenatal recruitment	228 infants	Birth–12 months	No sampling conducted; Daily symptom diaries, monthly visits	6 episodes/child/year; Determinants of ARI: age, winter, household size, childcare, presence of siblings

Reference	Study selection	Cohort size	Enrolment age/follow-up	Sampling/Symptom collection details	Main findings
Healthy cohorts					
Regamey, 2008 (226) (Switzerland)	Antenatal recruitment 2 hospitals	197 infants	Birth–12 months	Nasal swab on onset and 3 weeks later; Weekly standardised phone interviews	First ARI at 6 months; HRV, HCoV 55% <6 months; 25% ARI had wheeze; 97% acute rhinitis
Ede, 2009 (234) (US)	Nursery or primary care paediatric clinic	180 infants	Birth-12 months	Nasopharyngeal secretions during ARI and AOM	Increase in respiratory virus activity not due to pandemic virus, but spectrum of viruses did change during pandemic. HRV most common in URI (55%), RSV in LRTI (64%)
van der Zalm, 2009 (222) (The Netherlands)	Postnatally recruited through council register	305 infants	Birth–12 months	Nose and throat swabs on onset of illness; Daily questionnaires	High prevalence of respiratory pathogens in infants in first year of life; HRV most prevalent. RSV infections the most severe compared with HRV, but HRV highest overall burden of disease.
van der Zalm, 2009 (139) (The Netherlands)	Postnatally recruited through council register	18 children	Birth–7 years old	Nose swabs taken biweekly; Daily questionnaires	Respiratory pathogens frequently detected in asymptomatic children. Younger children more likely symptomatic, as are multiple detections.
Fairchok, 2010 (110) (US)	2 large childcare centres; children with LRTI	119 children	Birth–30 months	Nose swab 48 hours of onset of illness; Daily diary 10 days following onset of illness	Viruses identified in twice as many ARIs as previously reported in childcare cohort. HMPV, HCoV infections less frequent and severe than RSV, HAdV, HRV infections.
van der Zalm, 2011 (145) (The Netherlands)	Postnatally recruited through council register	18 children	Birth–7 years old	Nose swabs taken biweekly; testing HRV only; Daily questionnaires	Total lung resistance associated with HRV-associated wheeze. HRV-associated wheeze might be first sign to recognize infants with reduced neonatal lung function
Houben, 2011 (224) (The Netherlands)	Antenatal recruitment from 2 large hospitals	298 infants	Birth–12 months	Nose/throat swabs, daily symptom diaries (RSV LRTI only)	Predictors for RSV LRTI (14% of children): childcare, siblings, high education, birth weight >4 kg, birth Apr-Sep. First year wheezing in 62% of children with RSV LRTI
Mackay, 2013 (235) (Australia)	Antenatal recruitment	234 infants	Birth–12 months	Nose and throat swabs on onset of illness; Daily symptom diaries	HRV only. No clinical impact attributable to HRV species and genotypes.
Martin, 2013 (236) (US)	3 childcare centres on military base	225 children	5 weeks–30 months	Nasal swab at enrolment, then onset of illness; Daily symptom diary for 10 days following illness	High proportion (47%) attending childcare had multiple viruses; multiple viruses associated with less severe illness
van der Gugten, 2013 (237) (The Netherlands)	Postnatally recruited through council register	140 infants	Birth–12 months	Monthly samples; Daily symptom diaries; lung function measured <2 months of age	HRV during infancy not associated with wheezing, wheeze during HRV episode indicator of kids at high risk for wheeze because of reduced lung function

Reference	Study selection	Cohort size	Enrolment age/follow-up	Sampling/Symptom collection details	Main findings
Zomer-Kooijker, 2014 (15) (The Netherlands)	Postnatally recruited through council register	417 infants	Birth–12 months	Nose/throat swabs (RSV only), daily diaries	Median neonatal respiratory compliance lower, resistance higher in hospitalised RSV patients. Lower birth lung function predisposes to severe RSV disease and post-RSV wheeze.
Anders, 2015 (238)(Vietnam)	Antenatal recruitment from four hospitals	1,478 infants	Birth-12 months	NPS during ARI, clinic-based surveillance, hospital admissions, self-reports	HRV, RSV, INFV-A, HboV-1 most frequently detected viruses. ARI-associated hospitalisations associated with longer stays, frequent ICU admission.
Howard, 2015 (151) (Peru)	Household study	500 children	Children <3 years of age	Weekly nasal swabs, weekly questionnaires	INFV, HMPV, PIV and RSV detections in children with an ARI usually indicate causal relationship. RSV Ct lower during ARI.
Chonmaitree, 2015 (225) (US)	Nursery or primary care paediatric clinic	362 infants	Birth-12 months	Nasopharyngeal secretions during ARI and AOM	Viruses detected in 76% of URTI and 27% asymptomatic monthly specimens; latter associated with young age, male sex, low viral load, single detections, specific viruses.
Mack, 2016 (147)(Switzerland)	Antenatal recruitment from four hospitals	41 infants	Birth-12 months	Weekly telephone interviews and weekly nasal swabs	HRVs highly prevalent in the first year; 51% asymptomatic. Respiratory symptoms during HRV infection associated with age, maternal atopy, premorbid lung function
Toivonen, 2016 (149) (Finland)	Antenatal recruitment, public hospitals	923 children	Birth–24 months	Nasal swabs during ARI and at 2,13,24 months; Daily diaries and clinic visit data	HRV detected in 59% ARI, associated with 50% of AOM, 41% of wheezing illnesses, 49% of antibiotic treatments, and 48% of outpatient office visits for ARI. 9% of asymptomatic children were HRV-positive.
Kumar, 2017 (239) (India)	Enrolment through major hospital	310 infants	Birth-12 months	NPAs at each ARI.	63% ARI virus-positive, 18% viral coinfections. HRV most common virus, followed by RSV, PIV, HCoV.
Martin, 2018 (118) (US)	3 childcare centres on military base	225 children	5 weeks–30 months	Initial nasal swab, then onset of ARI; Daily symptom diary 10 days post ARI	Heterotypic co-circulation of many HRV genotypes.
Uddin, 2018 (240)(Nepal)	Pregnant women from 2 community-based, vaccine trials	3,505 infants	Birth-6 months	Weekly household visits, midnasal swabs during ARI	8% of infants had an HCoV-ARI in first six months. HCoV incidence higher in non-neonates. 46% coinfections with another virus.

Abbreviations: AOM: acute otitis media; ARI: acute respiratory infections; aRSV: asymptomatic RSV; Ct: cycle threshold; GP: general practice; HAdV: human adenoviruses; HCoV: human coronaviruses; HRV: human rhinovirus; ILI: influenza-like illness; INFV: influenza virus; LRTI: lower respiratory tract infection; NPA: nasopharyngeal aspirate; NPS: nasopharyngeal swab; NTS: nose/throat swab; OME: Otitis media with effusion; PIV: parainfluenza viruses; RSV: respiratory syncytial virus; URTI: upper respiratory tract infection

Determining pathogenicity in the molecular era: asymptomatic detections and causation

The use of PCR technology has become increasingly common in routine diagnostics and epidemiological studies of respiratory viruses. PCR is fast, sensitive, and successful at detecting viruses, including those that are fastidious or do not grow in standard cell culture. These contemporary detection methods identify nucleic acid rather than an infectious virus, which can prove problematic when interpreting positive results. The dogma of a positive result identifying the agent of a disease relates to traditional diagnostic methods of viral culture and serology, and may not necessarily hold true with a positive PCR result detecting target sequences of nucleic acids. Koch's postulates associate infection with disease, and may not be immediately relevant for newly identified viruses, many of which have not been cultured. Modified Koch's postulates were proposed by Fredricks and Relman (241) to help address this problem and include virus identification by molecular detection techniques (Appendix 2).

A positive PCR finding in a person who is deemed asymptomatic may reflect:

- mild, but unrecognised or falsely attributed symptoms to another infection,
- a nascent, incubating infection with testing prior to developing symptoms,
- past, resolved infection (i.e. residual viral genetic material following infection without active replication), or
- a genuine subclinical infection (acute, persistent, or reactivation of latent infection) without associated signs and symptoms.

All respiratory viruses have been detected in studies with asymptomatic subjects, or longitudinal study designs with asymptomatic periods, both individually or co-detected with other viruses, making the clinical significance of a positive result difficult to interpret. Understanding the aetiological role of viruses in illness requires not only appropriate study designs, but suitable approaches to the analysis and interpretation of virus detection data.

Cross-sectional designs cannot describe the causal chain of infection. Other approaches to assess the role of detected pathogens in cases have been to compare their infection status to control subjects without the disease. This case-control

analytical approach has limitations as it assumes 100% specificity among subjects who test positive for the pathogen.

One approach to estimating the aetiological role of a pathogen is to calculate an 'attributable fraction' in the exposed population (AFE). The AFE provides information about the percentage of cases amongst exposed individuals that can be attributed to the exposure. However, while the AFE approach addresses the limitations inherent with the case-control approach, it is a population level metric and cannot inform on the individual aetiology. The option for estimating aetiology for an individual case is to assign the group aetiological fraction for pathogens detected in that case, regardless of the findings for other pathogens detected (242).

Summary

Viral ARIs remain the most common illnesses experienced by children, particularly in the first two years of life. Information about the epidemiology of ARIs in children is based on historic community-based family studies, cohorts of hospitalised patients, ED, or primary healthcare presentations, and more recently mainly from birth cohort studies in children at high-risk of asthma. However, findings from these studies do not identify the contemporary burden of mild-to-moderate disease in otherwise healthy children that are overwhelmingly managed in the community, resulting in an overall under-estimate of disease burden. Contemporary published data on the nature and duration of ARIs in children in the community, and how these illnesses are managed, are limited. Intermittent wheeze triggered by ARIs in the first one to two years of life have also been implicated in the later development of asthma in susceptible individuals. Taken together, ARIs in children result in substantial costs to the families, society, and the healthcare system.

Since 2001, modern molecular-based techniques have discovered several new human viruses in patients with respiratory symptoms. The use of sensitive PCR assays has highlighted the detection of respiratory viruses in asymptomatic subjects, the co-detection of multiple viruses in respiratory secretions, and virus persistence following recovery from illness. The clinical significance of positive PCR findings therefore can be difficult to interpret because of few prospective, unselected healthy infant birth cohort studies where serial sampling and comprehensive molecular-based testing of respiratory secretions for pathogens is conducted before, during and after

respiratory episodes. Epidemiological studies conducted to date have had one or more limitations in study design preventing them from adequately addressing these questions. Furthermore, none of these cohort studies tested for all the newly discovered respiratory viruses. Few prospective studies examining the aetiological role of HRV in ARIs have conducted sequence-based typing of serial HRV detections to distinguish between species and genotypes.

The Observational Research in Childhood Infectious Diseases Study

The ORChID study was designed to address some of these methodological limitations.

The ORChID study was a four year prospective, community-based longitudinal birth cohort study of ARIs in children during the first two years of life (243), modelled on a previous cohort study conducted by Lambert et al. in Melbourne in 2003/2004 (3). Parents consented antenatally for their newborn infants to be recruited into the study shortly after birth at one of two metropolitan hospitals in Brisbane if they met the inclusion criteria (born healthy and at term (36–42 weeks) without congenital abnormalities or underlying chronic disorders). Brisbane is a subtropical city of more than 2 million inhabitants in Southeast Queensland, Australia.

Parents completed a daily symptom diary designed in a tick-box format that captured a set of pre-defined respiratory symptoms. Parents were also trained to take weekly nasal swabs from their child irrespective of symptoms, which were tested against a panel of 17 novel and established respiratory viruses. Illness episodes were documented further in a ‘burden diary’, which recorded healthcare-seeking behaviour, antibiotic prescriptions, and laboratory investigations. Full details of the methods employed in the study are outlined in Chapter 2.

The progressive two year recruitment and follow up until the second birthday means the study spanned multiple seasons, allowing for analysis of year-to-year variation in respiratory virus activity. Regular serial sampling in a healthy, unselected cohort allowed temporal relationships between respiratory virus detection and respiratory illness to be explored, improving the understanding of the role of respiratory viruses in illness. The longer follow-up period with this cohort and more frequent sampling increased the likelihood of sampling so-called ‘asymptomatic’ or genuine subclinical infections, as well as gave an indication of the duration of virus detection before,

during, and after symptomatic illness. As infants were swabbed from birth, the study design also allowed an examination of the prevalence, type, and timing of first infections with various viruses, including whether they were symptomatic, and the proportion of infants and their samples that were positive for viruses over the first two years of life.

Aims

The broad aims of this research are to:

- Describe the community-based epidemiology of respiratory viruses in the first two years of life, including the incidence, symptoms, management and associated risk factors of ARIs;
- Determine the relative contributions of individual respiratory viruses to ARI in the first two years of life; and
- Determine the pathogenicity of newly discovered respiratory agents, including the new HRV-C species, by comparing the timing and duration of detection in respiratory specimens with the presence of symptoms.

Hypotheses

The hypotheses to be tested are:

1. Collectively, HRVs, and the six recently described respiratory viruses from four virus families (HMPV, HCoV-NL63, HCoV-HKU1, HBoV-1, HPyV-WU, HPyV-KI) are more frequently detected during ARIs in the first two years of life than the long established viruses (RSV, PIVs, INFV, HAdVs) identified from hospital-based studies.
2. Respiratory pathogens are more likely to be associated with symptoms when first acquired.
3. Established respiratory viruses are more likely to be individually associated with symptoms than the recently described novel DNA viruses HPyV and HBoV-1.
4. Most respiratory pathogen shedding is transient, ceasing within 2-4 weeks of detection.
5. HRV-C is more likely to be associated with LRTIs than other HRV species.

Significance

At the commencement of this PhD, the role of some recently identified respiratory viruses was unclear. The prevalence of viruses in asymptomatic samples also posed a challenge in determining their role in infection. The clinical significance of HRV-C was a subject of ongoing debate. Findings from the ORChID study presented in this PhD have gone some way to address these information gaps in understanding respiratory virus epidemiology in early childhood.

Chapter 2 – Methods

Study population

The ORChID study (clinicaltrials.gov: NCT01304914) was conducted in the subtropical city of Brisbane, Australia (latitude 27° South, average monthly maximum temperature range 22-30°C, maximum rainfall in December-February, population 2.2 million) from September 2010 to October 2014. This was a dynamic cohort study, which enabled participants to leave the study temporarily (such as during family holidays) and re-join at a later date.

Recruitment was from antenatal clinics and was progressive over two years, which allowed infants born between September 2010 and October 2012 to be enrolled evenly throughout the study period to account for seasonal and year-to-year variation in respiratory virus activity. Parents provided written consent for their newborn infants to be recruited into the study shortly after their birth at one of two metropolitan hospitals (one private and one government-funded). Exclusion criteria for enrolment and ongoing participation included gestational age at birth of less than 36 weeks, major congenital abnormalities, chronic heart, respiratory (excluding asthma), gastrointestinal, neurological, or immunological disorders, parents unable to converse in English, living outside the Brisbane metropolitan region, or planning to move from the area within the next two years (243). Consequently, symptom data and nose swab specimen collection spanned more than four years from September 2010 to October 2014.

Demographic, social and clinical characteristics

Following the infant's recruitment into the study, parents provided their demographic, social and health characteristics as well as pregnancy and birth details. Telephone interviews were conducted every three months to update immunisation, feeding, and childcare attendance details. Childcare was categorised according to Australian Bureau of Statistics definitions as formal (regulated care outside the child's home) or informal (non-regulated care provided by family or friends) (244). The duration of exclusive breastfeeding was defined as the period of time from birth to introducing milk formula or solids, whichever was first.

Symptom data collection

We provided diary cards listed with pre-defined respiratory symptoms or diagnoses, to be completed daily, and returned by mail at the end of each month (Appendix 3). Parents were trained to recognise and record respiratory signs/symptoms, including runny nose/nasal congestion, distinguishing between a wet and dry cough and rattly breathing, and breathing difficulties, including wheezing, so as to minimise inaccuracy of reporting. The diary cards were designed in a tick-box format, modified from a prior study investigating influenza vaccine effectiveness (245) and used previously in a Melbourne cohort study (3). Parents were provided with a digital thermometer to measure temperature. Diagnoses of AOM and pneumonia were validated by a doctor's visit and where appropriate following review of the hospital ED or admission medical records (6).

Classification of acute respiratory infections

ARI episode was defined by respiratory symptoms and/or signs of the upper and/or lower respiratory tracts listed below (3, 6).
URTI: parent-reported runny nose/nasal congestion, dry cough, or doctor-diagnosed AOM (6).
LRTI: parent-reported rattly breathing, moist cough, shortness of breath, wheeze, or doctor-diagnosed pneumonia (6).

If wheeze was a component of a child's LRTI, it was sub-categorised as a 'wheezy episode'.

ARIs were sub-categorised hierarchically as either a LRTI or URTI, respectively.

Fever as a symptom was accepted if the parent ticked the fever symptom box or if they recorded axillary temperatures >37.5°C. For the purposes of demarcating ARIs, a new episode was considered to have commenced if there were $\geq$3 symptom-free days since the last day with symptoms (3). Daily symptoms were recorded until the child's second birthday.

ARI burden diary

Parents were asked to further document healthcare-seeking behaviour (visits to a family physician, an ED presentation, or a hospital admission) and antibiotic usage in

a separate ‘burden impact diary’ (Appendix 4) (3). Burden diaries were requested for all LRTIs, AOM and any URTI resulting in both nasal symptoms (nasal discharge or congestion) and cough. To minimise inconvenience we did not seek burden diaries for children with nasal symptoms alone as we reasoned under these circumstances impact would be minor and parents were unlikely to seek medical advice. Burden diaries were mailed back to study staff by parents with symptom diary cards at the end of each month. When reviewing hospital ED presentation or admission notes, the recorded principal diagnosis was accepted as the reason for consultation, regardless of whether the child had an ARI at the time.

Details of specimen collection, processing, PCR assays, quality control measures, and assay parameters used to identify respiratory viruses

Specimen collection

We supplied parents with swabs, a specimen collection instruction sheet, sealable bags, pre-addressed envelopes, and labelled stickers at the commencement of the study and at regular intervals (Appendices 5 and 6). All swabs were collected using a plastic-shaft, rayon-budded swab in a transport tube with a foam pad reservoir soaked with viral transport medium (Virocult MW950, Medical Wire & Equipment, Wiltshire, England). A single swab was used to sample both nostrils. Parents were taught by trained research staff to collect anterior nose swabs from their infant, beginning around the time of birth and then weekly thereafter. Parents were also reminded of correct swab technique when contacted by telephone for other information or if there were issues with the quality of swabs submitted. Immediately post-birth, parents also provided their own nasal swab specimens and reported respiratory symptoms within the previous two weeks.

The specimens were mailed at ambient temperature directly to the research laboratory by standard surface mail, taking a median of 3 (interquartile range 2-4) days and where they were processed and stored at -80°C until further analysis (243, 246). Swabs were batch-tested for HRV, RSV-A, RSV-B, INFV-A, INFV-B, PIV-1, PIV-2, PIV-3, HMPV, HCoV-OC43, NL63, 229E, HKU1, HAdV, WU-HPyV, KI-HPyV, and HBoV-1, using previously validated real-time PCR assays (243).

Quality Control measures

Prior to nucleic acid extraction, a known amount of whole equine herpesvirus (EHV) spiked into each sample assessed nucleic acid extraction quality and presence of PCR inhibitors (247). Any extract having a >3 cycle-threshold (Ct) difference to that of the expected value by EHV real-time PCR assays was considered to have failed quality control and the sample was re-extracted.

Specimen quality was assessed by testing for a marker of human genomic DNA, endogenous retrovirus-3 (ERV-3) (248). In addition, Ct values were used as semi-quantitative markers of viral load as in real-time PCR assays they are inversely proportional to the amount of specific virus nucleic acid present in the specimen (246). We have demonstrated previously that among specimens with an ERV-3 Ct value >38, respiratory virus detection declined significantly (odds ratio 0.35, 95% CI: 0.27, 0.44 when ERV-3 was undetectable) (249). Thus, virus-negative swabs with an ERV-3 Ct value >38 were deemed to be of poor quality and removed from parts of the analysis, such as incidence calculations.

Swab quality was monitored throughout the study and the number of ERV-3 negative specimens ranged from 19.6% to 44.6% in the first 8 months of the study (250). Analysis of ERV-3 positive and respiratory virus positive specimens showed that ERV-3 positive sample rates increased with age and varied by season (249), with ERV-3 positive rates in the first six months of age as a reference. Following a cluster of specimens that were negative for ERV-3, study nurses contacted parents and reminded them of the optimal swab technique they had been shown at enrolment. After this feedback, the number of ERV-3 specimens declined.

Nucleic Acid Extraction

Each swab was resuspended in 2mL of phosphate buffered saline from which 200µL was used for nucleic acid extraction. After being spiked with EHV, samples were extracted on the QIAxtractor automated high-throughput extraction platform using DX reagents according to the manufacturer's instructions (Qiagen, Australia). Total DNA and RNA were eluted into 150µL of elution buffer.

Detection of Viruses by Real-Time PCR

DNA viruses

All real-time PCR assays targeting DNA templates (EHV, ERV-3, HAdV, KI-HPyV, WU-HPyV, HBoV-1) used an identical set of master mix and cycling conditions. In brief, 8pmol of each primer, 3·2pmol of the respective probe(s), and 2µL of template were made in a 20µL final reaction volume using the Bioline Sensi Mix II Probe PCR mix kit (Bioline, Australia), and followed cycling conditions of: activation at 94°C for 2-minutes, followed by 45 cycles of 95°C for 15-seconds and 60°C for 60-seconds.

RNA viruses

For RNA viruses, real-time, one-step reverse-transcription PCR (RT-PCR) assays were used following a common protocol for all but INFV and HRV assays. In brief, 8pmol of each primer, 3·2pmol of the respective probe(s), and 2µL of template were made in a 20µL final reaction volume using the Bioline SensiFAST Probe One-Step RT-PCR kit (Bioline, Australia), and followed cycling conditions of: 45°C reverse transcriptase incubation for 20-minutes, 95°C activation for 2-minutes, followed by 45 cycles of 95°C for 15-seconds and 60°C for 60-seconds. The single deviation from the common RT-PCR protocol in the INFV A/B reaction was use of asymmetric amounts of INFV A and B probes (6·4pmol and 3·2pmol, respectively).

HRV typing

A concentration of 16pmol of each primer, as well as magnesium chloride additive of the same concentration, were used along with 2pmol of the respective probe in the HRV assay. The cycling conditions for the RT-PCR were as follows: 45°C reverse transcriptase incubation for 20-minutes, 94°C activation for 2-minutes, followed by 55 cycles of 95°C for 15-seconds to denature and 60°C for 60-seconds for the annealing step. Reactions were performed on ABI 7500 and Viia7 instruments (Life Technologies, Australia), as well as the Qiagen Rotorgene Q (Qiagen Australia).

Samples testing positive for HRV were genotyped by sequencing the variable region of VP4/VP2 genes using a nested PCR assay with two sets of primers (251-253). Samples that could not be amplified twice by the VP4/VP2 assay were further investigated by amplifying a 390 nucleotide fragment from the 5'UTR segment (254). PCR products were purified (QIAquick PCR purification kit, Qiagen, Australia) and

submitted for DNA sequencing to the Australian Equine Genetics Research Centre (The University of Queensland, Brisbane, Australia).

All detections with Ct values ≤40 were considered positive for the target respiratory virus. Details of probe and primer sequences for each respiratory virus tested are listed in Table 2.

Table 2: Details of primer and probe sequences by virus

Reaction Mix	Virus	Target Gene	Primer, Probe sequences (5'-3')	Source
1	Human rhinovirus[a]	5' UTR	CY+AGCC+TGCGTGGY GAAACACGGACACCCAAAGTA FAM-TCCTCCGGCCCCTGAATGYGGC-BHQ1	Lu, 2008. (251) Arden, 2010. (252)
2	Influenza A	Matrix	CTTCTAACCGAGGTCGAAACGTA GGTGACAGGATTGGTCTTGTCTTTA Q670-TCAGGCCCCCTCAAAGCCGAG-BHQ2	Whiley, 2005. (255)
	Influenza B	Matrix	GCATCTTTTGTTTTTTATCCATTCC CACAATTGCCTACCTGCTTTCA FAM-TGCTAGTTCTGCTTTGCCTTCTCCATCTTCT-BHQ1	Lambert, 2008. (256)
3	RSV-A	Nucleocapsid	AGATCAACTTCTGTCATCCAGCAA TTCTGCACATCATAATTAGGAGTATCAAT FAM-CACCATCCAACGGAGCACAGGAGAT-BHQ1	van Elden, 2003. (257)
	RSV-B[b]	Nucleocapsid	AAGATGCAAATCATAAATTCACAGGA TGATATCCAGCATCTTTAAGTATCTTTATAGTG YAK-TATGTCC+AGG+TTAGGAAG+G+G+AA-BBQ	van Elden, 2003. (257)

Reaction Mix	Virus	Target Gene	Primer, Probe sequences (5'-3')	Source
4	Parainfluenza-1	Hemagglutinin-neuraminidase	TTTAAACCCGGTAATTTCTCATACCT CCCCTTGTTCCTGCAGCTATT FAM-TGACATCAACGACAACAGGAAATCATGTTCTG-BHQ1	Lambert, 2008. (256)
	Parainfluenza-2	Nucleocapsid	AGAGTTCCAACATTCAATGAATCAGT CTCAAGAGAAATGTCATTCCCATCT YAK-CCTCTGTATTGCTCATGCATAGCACGGA-BBQ	Lambert, 2008. (256)
	Parainfluenza-3	Nucleocapsid	CGGTGACACAGTGGATCAGATT AGGTCATTTCTGCTAGTATTCATTGTTATT Q670-TCAATCATGCGGTCTCAACAGAGCTTG-BHQ2	Lambert, 2008. (256)
5	Human coronavirus-HKU1	Polymerase	CCTTGCGAATGAATGTGCT TTGCATCACCACTGCTAGTACCAC FAM-TGTGTGGCGGTTGCTATTATGTTAAGCCTG-BHQ1	Dare, 2007. (258)

Reaction Mix	Virus	Target Gene	Primer, Probe sequences (5'-3')	Source
6	Human coronavirus-OC43	Nucleocapsid	CGATGAGGCTATTCCGACTAGGT CCTTCCTGAGCCTTCAATATAGTAACC Q670-TCCGCCTGGCACGGTACTCCCT-BHQ2	van Elden, 2004. (259)
	Human coronavirus-NL63	Polyprotein 1a	ACGTACTTCTATTATGAAGCATGATATTAA AGCAGATCTAATGTTATACTTAAAACTACG YAK-ATTGCCAAGGCTCCTAAACGTACAGGTGTT-BBQ	Gunson, 2005. (260)
	Human coronavirus-229E	Nucleocapsid	CAGTCAAATGGGCTGATGCA AAAGGGCTATAAAGAGAATAAGGTATTCT FAM-CCCTGACGACCACGTTGTGGTTCA-BHQ1	van Elden, 2004. (259)
7	Human metapneumovirus	Nucleocapsid	CATATAAGCATGCTATATTAAAAGAGTCTC CCTATTTCTGCAGCATATTTGTAATCAG FAM-TGYAATGATGAGGGTGTCACTGCGGTTG-BHQ1	Maertzdorf, 2004. (261)
8	Adenovirus	Hexon	GCCACGGTGGGGTTTCTAAACTT GCCCCAGTGGTCTTACATGCACATC FAM-TGCACCAGACCCGGGCTCAGGTACTCCGA-BHQ1	Heim, 2003. (262)
9	Human bocavirus-1	VP1	GGCAGAATTCAGCCATACTCAAA TCTGGGTTAGTGCAAACCATGA FAM-AGAGTAGGACCACAGTCATCAGACACTGCTCC-BHQ1	Tozer, 2009. (263)

Reaction Mix	Virus	Target Gene	Primer, Probe sequences (5'-3')	Source
10	Human polyomavirus WU	NCCR	GCCGACAGCCGTTGGATATA TTTCAGGCACAGCAAGCAAT FAM-AGGGTCACCATTTTTATTTCAGATGGGCA-BHQ1	Antonsson, 2012. (264)
	Human polyomavirus KI	NCCR	GAACTTCTACTGTCCTTGACACAGGTA GGATTAGAACTTACAGTCTTAGCATTTCAG Q670-ACCCTTTGTAGGCCAAAGGAGAGTGAAGG-BHQ2	Antonsson, 2012. (264)
	Human polyomavirus KI	STAg	CACAGGTGGTTTTCTATAAATTTTGTACTT GAAGCAGTGGGATGTATGCATTC YAK-TGCATTGGCATTCGTGATTGTAGCCA-BBQ	Antonsson, 2012. (264)
11	Endogenous retrovirus-3	ENV gene	CATGGGAAGCAAGGGAACTAATG CCCAGCGAGCAATACAGAATTT FAM-TCTTCCCTCGAACCTGCACCATCAAGTCA-BHQ1	Yuan, 2001. (248)
	Equine Herpesvirus	Glycoprotein B gene	GATGACACTAGCGACTTCGA CAGGGCAGAAACCATAGACA Q670-TTTCGCGTGCCTCCTCCAG-BHQ2	Bialasiewicz, 2009. (247)

+ indicates Locked Nucleic Acid (LNA) base (eg: +A is a LNA Adenine analogue)

[a]Rhinovirus pan assay; [b]Modified probe from published version presented

Cohort characteristics

Of 891 potential participants approached, 165 (18.5%) eligible singleton infants from 163 families were enrolled (two families' enrolled two siblings) (Figure 2). Seven were excluded subsequently; one from ineligibility (born <36 weeks) and six for failing to provide any swabs. Of the remaining children, 158 provided 11,192 swabs. For consistency, we censored the end of the study period at the child's second birthday, at which point a total of 11,125 swabs (Figure 2; 68.3% of maximum expected; median 84.5, range 1-104) were submitted. Four families submitted swabs but no symptom diaries before withdrawing early from the study. In this analysis of clinical information, 154 children provided 87,547 child-days of symptom diaries (77.9% of maximum expected days; median 726 (range 1-730 days)) with 67% followed until at least 23 months (Figure 3). One or more respiratory symptoms were recorded on 16,877 (19.3%) days, and corresponded to 1,641 discrete ARI episodes. Demographic and social characteristics are reported in Table 3. Compared to the general population of Brisbane and the state of Queensland, study children were from smaller families of advantaged backgrounds (Table 4). Enrolled families were more likely to have children that attended childcare and less likely to smoke.

Figure 1: Recruitment of participants into the Observational Research in Childhood Diseases Study, September 2010-September 2012.

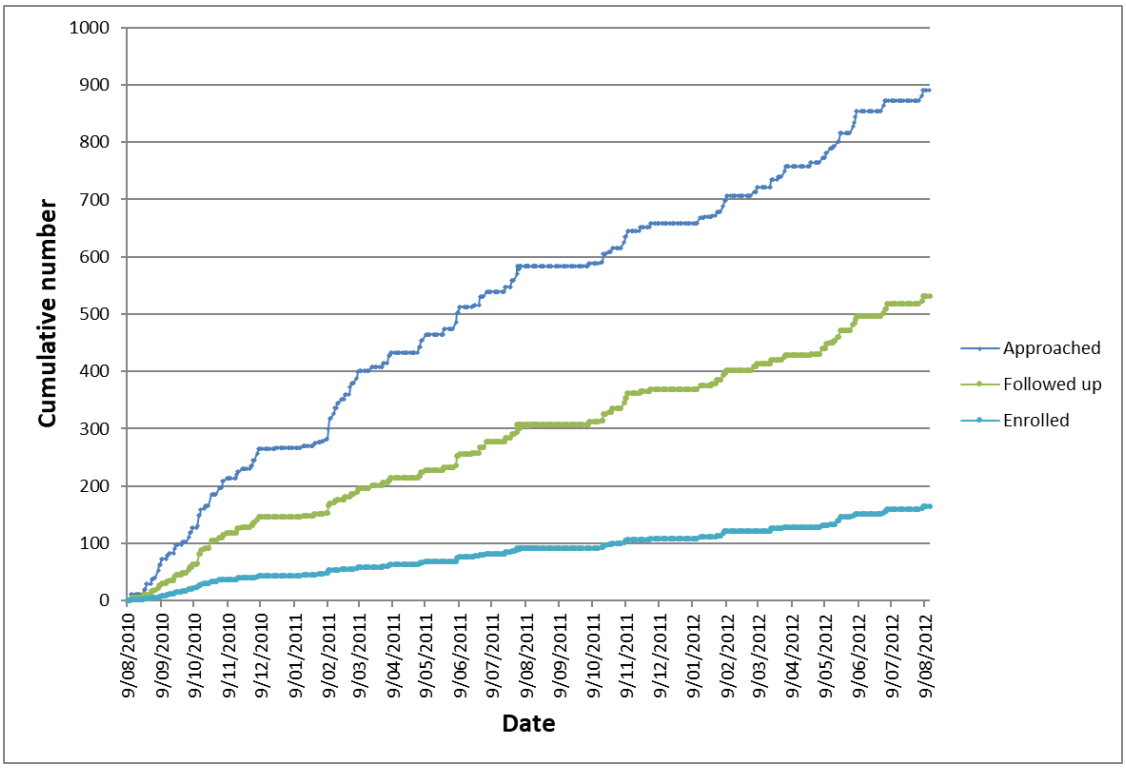

Figure 2: Submission of nasal swabs and symptom diaries from participants in the Observational Research in Childhood Infectious Diseases Study

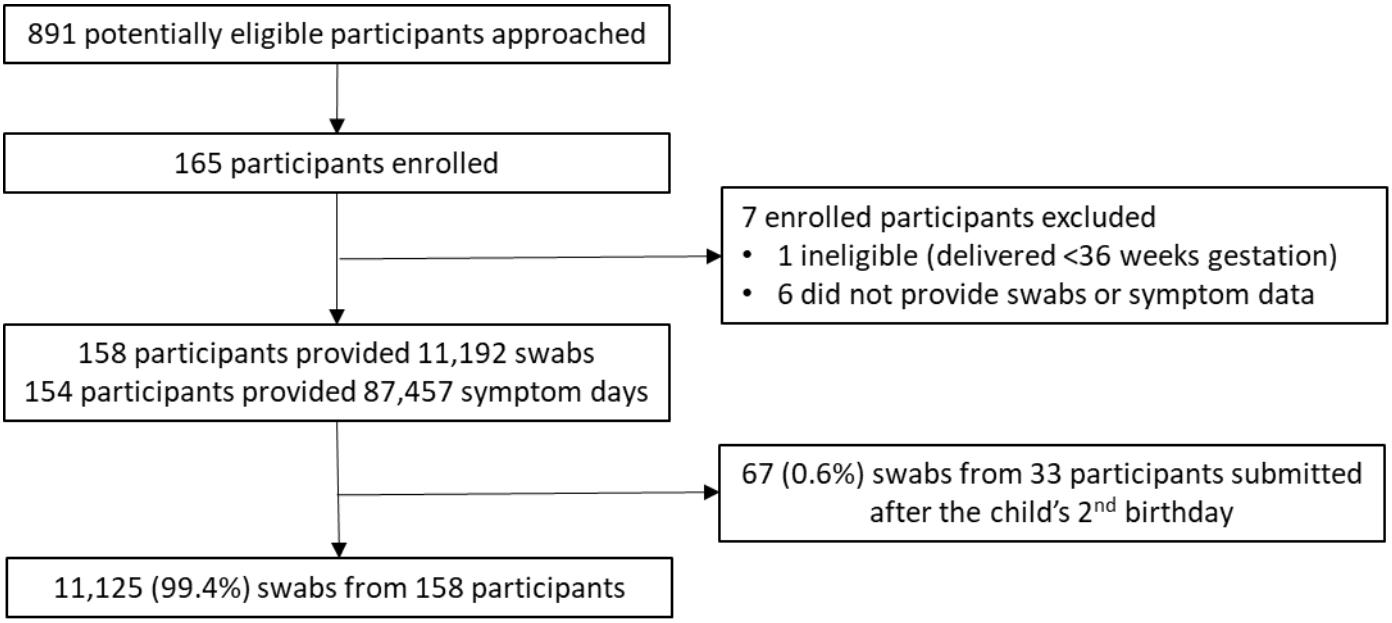

Figure 3: Proportion of swab and/or diary return for 158 participants in the Observational Research in Childhood Infectious Diseases Study

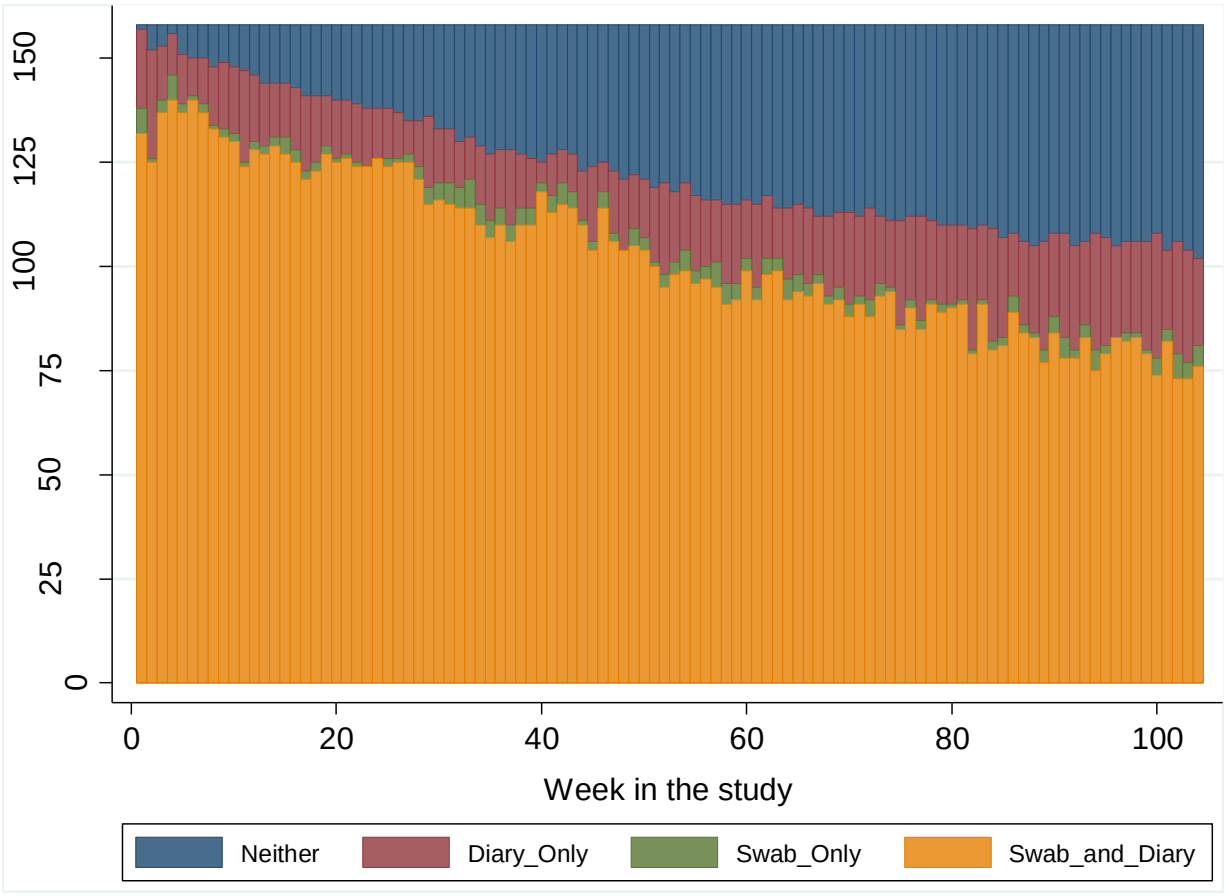

Table 3: Characteristics of children enrolled in the Observational Research in Childhood Infectious Diseases Study.

Characteristic (n=158 unless stated)	Frequency (%) or Mean (±SD)
Sex (male)	75 (47.5%)
Gestational age (weeks)	39.8 (±1.3)
Birth weight (g)	3530.8 (± 430.4)
Delivery	
Vaginal	107 (67.7%)
Birth order (maternal)	
First born	106 (67.1%)
Parental mean age	
Mother (years) (n=157)	31.8 (± 4.6)
Family history of asthma or eczema	
Mother	50 (31.6%)
Father	44 (27.8%)
Sibling	15 (9.5%)
First degree relative with asthma or eczema	83 (52.5%)
Smoke exposure at birth	
Mother (n=156)	5 (3.2%)
Other householder (n=155)[a]	17 (11.0%)
Maternal educational status (n=157)	
Primary school	5 (3.2%)
High school	15 (9.6%)
Diploma/Certificate	38 (24.2%)
University or higher university degree	99 (63.1%)
Household income (n=155)[b]	
Lowest quarter	0
Lower quarter	17 (11.0%)
Higher quarter	52 (33.5%)
Highest quarter	86 (55.5%)
Child Immunisation status to 18 months (n=154)[c]	
Fully immunised	141 (91.6%)
Exclusive breastfeeding	
At birth (n=149)	142 (95.3%)
At 3 months (n=142)	97 (68.3%)
At 6 months (n=133)	5 (3.8%)
Childcare	
At 6 months (n=133)	33 (24.8%)
At 9 months (n=123)	48 (39.0%)
At 12 months (n=115)	72 (62.6%)
At 15 months (n=110)	87 (79.1%)

[a]Includes 2 single parent households; [b]Income categories based on Australian Bureau of Statistics income quartiles (Quartile 1: <$26,000; Quartile 2: $26,000-$67,499; Quartile 3: $67,500-$114,999; Quartile 4: ≥$115,000); [c]11/154 (7.1%) infants did not receive a full course of rotavirus vaccines because they presented for immunisation outside of required age limits; [c]2 children had chickenpox diagnosed before the varicella vaccine could be administered at the scheduled age of 18 months, but were up-to-date for all other vaccines; another 2 children withdrew early and information was unavailable on their later immunisations. Influenza vaccines are not part of the national Australian infant immunisation schedule. Only 7 children received the influenza vaccine in the first two years of life. Abbreviations: SD: standard deviation.

Table 4: Comparison of socio-demographic characteristics of the ORChID Study cohort and the general population of Brisbane or the State of Queensland, 2011.

	ORChID cohort	Brisbane/Queensland
Sex (% male)	75 (47.5%)	51.3%[a]
Aboriginal/Torres Strait Islander status	2 (1.3%)	3.4%[b]
Caesarean delivery	51 (32.3%)	34.2%[c]
Maternal age (years)	31.8 (±4.6)	29.3[c]
No. of children in the household		
Average children per family	1.5 (±0.8)	1.9[a]
Household income		
<$26,000	0%	14.3%[d]
$26,000-$67,499	10.5%	31.2%
$67,500-$114,999	35.8%	23.7%
≥$115,000	53.7%	30.8%
Smoking exposure		
Mother smokes	5 (3.2%)	14.8%[e]
Other householder smokes	17 (11.1%)	
Immunisation		
Fully immunised to 18 months	141 (91.6%)	92.8%[f]
Childcare		
At 12 months	72 (62.6%)	47.6%[g]

[a]Australian Bureau of Statistics 2011 Census of Population and Housing Basic Community Profile (Catalogue number 2001.0) - Brisbane (UCL301001). Table B04. Data presented are an average for children 0-2 years of age. http://www.censusdata.abs.gov.au/census_services/getproduct/census/2011/quickstat/UCL301001?opendocument&navpos=220. Accessed 28 May 2017.

[b]Australian Bureau of Statistics 2011 Census of Population and Housing Basic Community Profile (Catalogue number 2001.0) - Brisbane (UCL301001). Table B07. Data presented are an average for children 0-4 years of age. http://www.censusdata.abs.gov.au/census_services/getproduct/census/2011/quickstat/UCL301001?opendocument&navpos=220. Accessed 24 October 2017.

[c]Source: 'Australian mothers and babies 2011' report. Australian Institute of Health and Welfare. http://www.aihw.gov.au/WorkArea/DownloadAsset.aspx?id=60129545698. Data presented are for deliveries in Queensland. Accessed 28 May 2017.

[d]Australian Bureau of Statistics report 6523.0 Household Income and Income Distribution, Australia, 2011–12. Table 2; http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/6523.02011-12?OpenDocument. Accessed 28 May 2017.

[e]Australian Bureau of Statistics report 43640DO001: Australian Health Survey 2011-2012. Tables 1-17 – Queensland, Table 7.2 – Smoker status. Data presented are for current female smokers aged 25-34 years in Queensland; http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0012011-12?OpenDocument. Accessed 28 May 2017.

[f]Australian Immunisation Register. Data presented are for 2011 Queensland State summary of percentage of fully immunised children 24-<27 months; http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/acir-ann-cov-hist-data.htm. Accessed 28 May 2017.

[g]Australian Bureau of Statistics report 44020DO001_201106 Childhood Education and Care, Australia, June 2011, Table 1 and 6; http://www.abs.gov.au/ausstats/abs@.nsf/mf/4402.0. Accessed 28 May 2017

Chapter 3 – The burden of community-managed acute respiratory infections in the first two years of life

This chapter is presented as a published original article:

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Keywords: respiratory tract infections; children; respiratory symptoms; management

ABSTRACT

Introduction:

Contemporary information on acute respiratory infections (ARIs) in children is based on hospital cohorts, primary healthcare presentations, and high-risk birth cohort studies. We describe the burden and determinants of symptomatic episodes of ARIs in unselected healthy children in the first two years of life.

Methods:

One hundred and fifty-four children from subtropical Brisbane, Australia participated in a longitudinal, community-based birth cohort study. A daily tick-box diary captured pre-defined respiratory symptoms. Parents also completed a burden diary, recording family physician and hospital visits, and antibiotic use.

Results:

Participants contributed 88,032 child-days (78.2% of expected), of which 17,316 (19.7%) days were symptomatic during 1,651 ARI episodes: incidence rate 0.56 ARIs per child-month (95% CI: 0.54, 0.59). Runny nose (14,220 days; 6.0 days median duration) and dry cough (6,880 days; 4.0 days median duration) were reported most frequently. Overall, 955 burden diaries recorded 455 family physician visits (1-8 visits per ARI) and 48 hospital presentations, including six hospital admissions. Antibiotics were prescribed on 209 occasions (21.9% of ARI episodes where burden diary submitted). Increasing age, non-summer seasons, and attendance at childcare were associated with an increased risk of ARI.

Conclusions:

ARIs are a common cause of morbidity in the first two years of life, with children experiencing 13 discrete ARI episodes and almost five months of respiratory symptoms. Most ARIs are managed in the community by parents and family physicians. Antibiotic prescribing remains common for ARIs in young children. Secular societal changes, including greater use of childcare in early childhood, may have maintained the high ARI incidence in this age group.

INTRODUCTION

Respiratory symptoms are exceedingly common, occurring at every age, and often caused by infections. ARIs occur throughout life, but are most frequent during the first two years (114). Most early childhood ARIs are caused by viruses and are self-limiting in nature, involving mainly the upper respiratory tract where symptoms of nasal discharge or congestion predominate. Nevertheless, secondary bacterial infections from pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* can often occur in this age group and may lead to AOM and sinusitis complicating the original illness (265, 266). Although less common than URTIs, infections involving the lower respiratory tract may affect as many as one-third of infants in the first year of life (267). As with URTIs, the majority of LRTIs are caused primarily by viruses (268), but 3-5% of infants are hospitalised because of bronchiolitis, croup, virus-associated wheezing, or secondary bacterial pneumonia (269). Information about the epidemiology of ARIs in children is based on historic community-based studies (60, 61), cohorts of hospitalised patients (119, 270), Emergency Department (23) or primary healthcare presentations (271), and more recently from birth cohort studies in children at high-risk of asthma (2, 221). However, findings from these latter groups may not identify the burden of mild-to-moderate disease in healthy children managed in the community, while hospital-derived data can under-estimate the disease burden from illnesses, such as influenza and pertussis within the community (88, 272).

An early Australian study reported ARIs were the most commonly managed problems in primary care, making up nearly 50% of consultations in children aged <5 years (26). A recent analysis of Australian family physician activity described 26 per 100 encounters in this age group were for common respiratory tract infections (URTI, bronchitis/bronchiolitis, tonsillitis, and pneumonia) (27). Others have shown that in this age group almost half the ARI episodes resulted in a medical consultation (3, 28) and their management had considerable impact upon healthcare services (29).

Several risk factors have been associated with the development of ARIs in various settings, including well-established determinants such as age (114), household size (114), exposure to other children, especially siblings (114) (28) or group child care attendance (28), environmental exposures, particularly tobacco smoke (273), a family history of atopy (273), and more recently gestational age at birth (274). Contemporary

published data on the nature and duration of symptoms in children with ARIs in the community are limited (3, 222, 233). We describe the incidence, symptoms, management and determinants of ARIs in a community-based birth cohort of healthy Australian children in their first two years of life, information that will guide strategies to minimise disease transmission and over-use of antibiotics in ARI, and help predict the future impact of novel respiratory vaccines.

MATERIALS AND METHODS

Details of the study population, demographic and symptom data collection, the ARI burden diary, and classification of ARIs have been outlined in Chapter 2.

Analysis

Summary statistics are presented as mean (standard deviation, SD) or median (interquartile range, IQR) for continuous variables and frequency (percentage) for categorical variables. ARI mean incidence rates (IR), averaged over the two year period, and associations between participant characteristics and new episodes of ARI, were calculated using Poisson regression. The association between potential risk factors, including age, sex, type of delivery, gestational age at birth, season, family history of asthma or eczema, tobacco smoke exposure, household size, maternal education status, mode of feeding, and type of childcare attendance were examined using both univariable and multivariable analyses. Breastfeeding and childcare attendance were analysed as time-varying variables using mixed-effects Poisson regression with child entered as a random effect. Multivariable models were adjusted for all of the above risk factors and were calculated using mixed effects regression. All models included the natural logarithm of time-at-risk-of-ARI as an offset. Time-at-risk began with the first recorded symptom diary day completed and ended at age 24 months or when the child was lost to follow-up or left the study. This was a dynamic study and families could also leave the study for a short duration (e.g. for a holiday) and re-enter at a later date. Days forming part of an ARI and the next three days were excluded from the calculation of time-at-risk. Effect estimates are presented as incidence rate ratios (IRRs) with 95% CI. Data were analysed using Stata v12 for Windows (StataCorp, Texas, USA).

RESULTS

Of 891 potential participants approached, 165 (18.5%) infants from 163 families were enrolled between September 2010 and November 2012 (Figure 2). Eleven participants were excluded from this analysis due to pre-term birth (n=1) or because they did not supply symptom data (n=10). Symptom diary information was received for 154 children from 152 families. The number of children for whom symptom information was received at 6, 12, 18, and 24 months was 139 (90.3%), 122 (79.2%), 110 (71.4%), and 100 (64.9%) respectively. In total 88,032 child-days of follow-up were recorded (78.2% of total expected days; median 731; range 7-759).

Cohort characteristics

Two-thirds of the children were first-born and more than half came from advantaged families (Table 5). Immunisation coverage was high. The median duration of exclusive breast-feeding was 19 (range 0-30) weeks. In 19 (12.3%) households, 23 adults (five mothers) were active smokers at birth. Two children (1.2%) were Aboriginal, consistent with the proportion of the Indigenous population in Brisbane. Eighty-one (52.6%) families had a first-degree relative (mother, father or sibling) with a history of asthma or eczema. By age 15 months, 79% of children were in some form of childcare.

Overall, 1,651 ARIs were recorded, providing a mean IR of 0.56 ARI per child-month (95% CI: 0.54, 0.59); 1,370 (83.0%) as URTIs (mean IR=0.47 URTI per month, 95% CI: 0.44, 0.49) and 281 (17.0%) as LRTIs (mean IR=0.10 LRTI per month, 95% CI: 0.09, 0.11) (Figure 4). Fever was reported in 320 (19.4%) ARIs, a dry cough in 584 (42.6%) URTIs, while a moist cough was present in 234 (83.3%) and wheezing in 93 (33.1%) LRTI episodes (Table 7). AOM was reported in 56 children (36.4%, mean IR=0.04 ARI with AOM per child-month (95% CI: 0.03, 0.05). These episodes of AOM complicated 76 (5.5%) URTIs and 40 (14.2%) LRTIs respectively. The longest duration of continuous respiratory symptoms in any individual child was 192 days. We did not observe any clustering of events in the different years.

Table 5: Characteristics of children enrolled in the Observational Research in Childhood Infectious Diseases Study who returned symptom diaries

Characteristic (n=154 unless stated)	frequency (%) or mean (±SD)
Gender (male)	74 (48.1%)
Gestational age (weeks)	39.8 (±1.3)
Birth weight (g)	3514.5 (± 511)
Delivery	
Vaginal	105 (68.2%)
Caesarean	49 (31.8%)
Birth order (maternal)	
First born	103 (66.9%)
Parental mean age	
Mother (years) (n=153)	31.7 (± 4.6)
Father (years) (n=152)	34.2 (± 6.2)
Family history of asthma or eczema	
Mother	49 (31.8%)
Father	42 (27.3%)
Sibling	15 (9.7%)
First degree relative with asthma or eczema	81 (52.6%)
Smoke exposure at birth	
Mother (n=152)	5 (3.3%)
Other householder (n=149)	18 (12.1%)
Maternal educational status (n=153)	
High school	14 (9.2%)
Diploma/Certificate	38 (24.8%)
University or higher university degree	96 (62.7%)
Household income (n=151)[a]	
Lowest	0
Lower	17 (11.1%)
Higher	51 (33.8%)
Highest	83 (55.0%)
Child Immunisation status (n=154)[b]	
Fully immunised	137 (89.0%)
Exclusive breastfeeding	
At birth (n=149)	142 (95.3%)
At 3 months (n=142)	97 (68.3%)
At 6 months (n=133)	5 (3.8%)
Childcare	
At 6 months (n=133)	31 (23.3%)
At 9 months (n=123)	48 (39.0%)
At 12 months (n=115)	72 (62.6%)
At 15 months (n=110)	87 (79.1%)

[a]Income categories based on Australian Bureau of Statistics income quartiles (Quartile 1: <$26,000; Quartile 2: $26,000-$67,499; Quartile 3: $67,500-$114,999; Quartile 4: >$115,000). [b]10/154 (6%) subjects did not receive a full course of rotavirus vaccines because they presented for immunisation beyond the required age constraints; three subjects withdrew early and information was unavailable on their later immunisations. Influenza vaccines are not part of the national Australian infant immunisation schedule. Thus, only seven children received the influenza vaccine in the first two years of life. Abbreviations: SD: standard deviation

Frequency, type and duration of symptoms and ARI

Respiratory symptoms were recorded on 17,316 (19.7%) days, equivalent to 4.7 months over the first two years of life. The most common and persistent symptoms were nasal discharge/congestion and dry cough (Table 6 and Table 7). Overall, 826 days (0.9%) of fever associated with an ARI were reported in 107 (69%) children, with temperatures ranging from 37.5-41.0°C.

Table 6: Frequency of symptoms. Total number of child-days on record = 88,032.

Characteristic	No. child-days	%
Days acute respiratory infections	17,316	19.7
Days upper respiratory tract infection	12,324	14.0
Days lower respiratory tract infection	4,992	5.7
Symptom		
Fever associated with acute respiratory infection	826	0.9
Nasal discharge/congestion	14,220	16.2
Dry cough	6,880	7.8
Moist cough/pulmonary congestion	2,091	2.4
Shortness of breath	109	0.1
Wheeze	409	0.5
Diagnoses (doctor-diagnosed)		
Otitis media	712	0.8
Pneumonia	13	<0.1

Table 7: Duration of respiratory symptoms and acute respiratory infection episodes

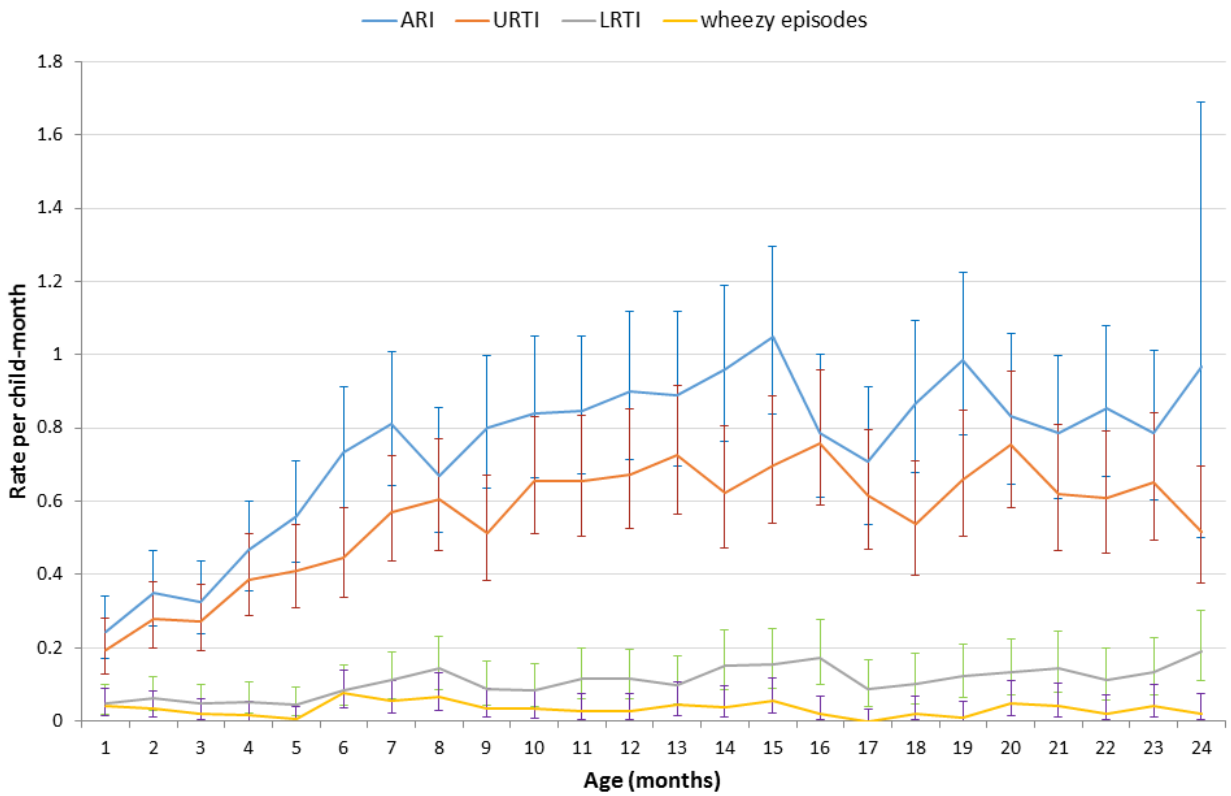
Duration of illness	1-3 days		4-7 days		8-14 days		15-21 days		22-28 days		>28 days	
	n	%	n	%	n	%	N	%	n	%	n	%
ARI[a] (n=1,651)	427	25.9	451	27.3	456	27.6	151	9.1	72	4.4	94	5.7
URTI (n=1,370)	414	30.2	410	29.9	347	25.3	101	7.4	45	3.3	53	3.9
LRTI (n=281)	13	4.6	41	14.6	109	38.8	50	17.8	27	9.6	41	14.6
Number of days with symptom/diagnosis for URTIs[b]												
Fever (n=215)	174	80.9	37	17.2	4	1.9	0	0	0	0	0	0
Nasal discharge (n=1,233)	386	31.3	374	30.3	321	26.0	77	6.2	35	2.8	40	3.2
Dry cough (n=584)	223	38.2	166	28.4	121	20.7	36	6.2	18	3.1	20	3.4
Otitis media (n=76)	19	25	40	52.6	15	19.7	2	2.6	0	0	0	0
Number of days with symptom/diagnosis for LRTIs												
Fever (n=105)	74	70.5	28	26.7	2	1.9	1	1.0	0	0	0	0
Moist cough (n=234)	50	21.4	84	35.9	70	29.9	13	5.6	8	3.4	9	3.8
Shortness of breath (n=31)	18	58.1	10	32.3	3	9.7	0	0	0	0	0	0
Wheeze (n=93)	53	57.0	22	23.7	16	17.2	2	2.2	0	0	0	0
Pneumonia (n=3)	2	66.7	0	0	1	33.3	0	0	0	0	0	0
Otitis media[c] (n=40)	13	32.5	15	37.5	8	20	2	5	2	5	0	0

[a]Abbreviations: ARI: acute respiratory infection; LRTI: lower respiratory tract infection; URTI: upper respiratory tract infection; [b] within an ARI, symptoms may overlap and/or be sequential, thus duration of symptoms does not correspond to duration of ARI ;[c]complicating the LRTI episode

Management

Overall, 955 completed burden diaries were submitted for 1,007 ARIs (94.8%) that met the threshold for burden diary completion. Noting that a burden diary was not required for all URTIs, diaries captured 688 (50.2%) URTIs and 267 (95.0%) LRTIs. Episodes of URTI where a burden diary was not required (isolated nasal congestion or cough) had a median duration of 4 (IQR 2-7) days compared with 10 (IQR 5-16) days for those submitting a diary. For ARIs covered by burden diaries, a family physician visit occurred with 455 (47.6%) episodes, 300 for URTIs and 155 for LRTIs (Table 8). Additional family physician visits (range 1-7) were required in 169 (17.7%) ARI episodes. A further 34 consultations were made with other healthcare professionals, most commonly paediatricians, n=10 (Table 8). Antibiotics were prescribed at least once for 209 (21.9%) ARI episodes (median 1.0, range 1-4 courses), which represented 45.9% of ARIs prompting a family physician visit, most commonly for AOM (98, 46.9%). Two children with pneumonia were administered antibiotics and managed by their family physician. Children with a LRTI were more likely to be prescribed antibiotics than children with an URTI (unadjusted odds ratio (OR) = 2.0 (95% CI: 1.5, 2.8).

Figure 4: Incidence rate of acute respiratory infection episodes per child-month by age and type of respiratory infection



The 955 episodes included 48 hospital presentations resulting in 6 (0.6%) hospitalisations (IR 0.002 admissions per month, mean length of stay 1.3 (range 0-3) days) (Table 8). All 6 hospitalisations were for LRTI: croup (2), pneumonia (1), acute wheezy episode treated with albuterol and prednisolone (1), bronchiolitis (1), and bronchiolitis with bilateral AOM (1). Two children also underwent elective grommet insertions for recurrent AOM. There were significant differences in seeking healthcare for LRTIs vs URTIs, including family physician visits (unadjusted OR=1.6 (95% CI: 1.2, 2.1)), and hospital presentations (unadjusted OR=4.3 (95% CI: 2.3, 8.2)).

Table 8: Healthcare-seeking behaviour by type of acute respiratory infection

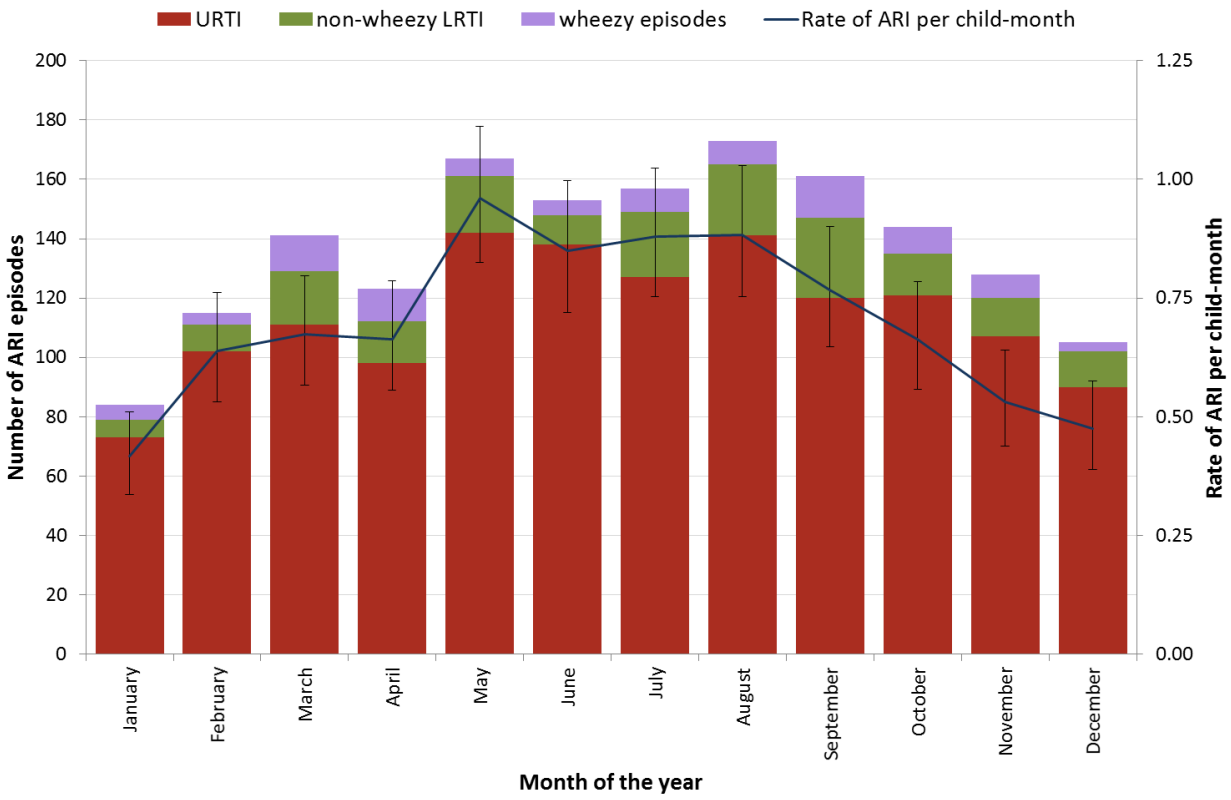
	Type of respiratory infection		
Healthcare-seeking behaviour	**URTI[a] (n=688)**	**LRTI (n=267)**	**Total[b] (n=955)**
Family physician visits	300 (43.6%)	155 (58.1%)	455 (47.6%)
Other healthcare professional	22 (3.2%)	12 (4.5%)	34 (3.6%)
Hospital			
Presentations	19 (2.8%)	29 (10.9%)	48 (5.0%)
Admissions	0 (0.0%)	6 (2.2%)	6 (0.6%)
Antibiotics	126 (18.3%)	83 (31.1%)	209 (21.9%)

[a]Abbreviations: URTI: upper respiratory tract infection, LRTI: lower respiratory tract infection; [b]symptom information not available for four burden diaries

Risk factors

Increasing age, non-summer seasons, and childcare attendance were independent risk factors for ARI (Table 9). We did not observe an association between family history of atopy and LRTI or wheezing episodes. We also did not see an association between household size, tobacco smoke exposure, gestational age at birth, and ARI. The rate of ARI increased progressively during the first six months of life, then stabilised for the rest of the study period (Figure 3). The mean age at first ARI was 6.7 months (95% CI: 6.5, 7.0); children aged 15 months had the highest rates of ARI at 1.05 ARI per child-month (Figure 3). A seasonal peak in the rate of ARI per child-month was observed between the months of May to September, which in Australia encompass late fall, winter, and early spring seasons (Figure 5).

Figure 5: Number of acute respiratory infection episodes and rate of acute respiratory infection per child-month by type of infection and month of the year[a], September 2010 – October 2014.



[a]This combines all months of the study. All numerator ARIs and denominator child-days were summed for each month of the year over four years

Table 9: Number of children, child-months, acute respiratory illness episodes, days exposed, and association between participant characteristics and acute respiratory infections

Explanatory variables	No. of children	No. of child-months	No. of ARI	Incident rate per child-month (95% CI)	Unadjusted Incidence rate ratio (95% CI)	Adjusted Incidence rate ratio[a] (95% CI)
Age (months)						
0-<3	154	406.7	129	0.3 (0.3, 0.4)	Referent rate	Referent rate
3-<6	141	353.1	183	0.5 (0.4, 0.6)	**1.6 (1.3, 2.0)**	**1.8 (1.4, 2.3)**
6-<12	135	541.9	465	0.9 (0.8, 0.9)	**2.7 (2.2, 3.3)**	**2.9 (2.2, 4.0)**
12-<24	117	905.9	865	1.0 (0.9, 1.0)	**3.0 (2.5, 3.6)**	**2.6 (1.9, 3.6)**
Sex						
Male	74	1017.9	720	0.7 (0.7, 0.8)	Referent rate	Referent rate
Female	80	1189.6	922	0.8 (0.7, 0.8)	1.1 (1.0, 1.2)	1.1 (0.9, 1.4)
Type of delivery						
Vaginal	105	1465.0	1141	0.8 (0.7, 0.8)	Referent rate	Referent rate
Caesarean	49	742.5	501	0.7 (0.6, 0.7)	0.9 (0.8, 1.0)	0.8 (0.6, 1.0)
Gestational age						
Term (39w0d-41w6d)	119	1746.5	1327	0.8 (0.7, 0.8)	Referent rate	Referent rate
Late pre-term/early term (36w0d-38w6d)	35	461.0	315	0.7 (0.6, 0.8)	0.9 (0.8, 1.0)	1.0 (0.8, 1.4)
Season						
Summer	142	600.9	319	0.5 (0.5, 0.6)	Referent rate	Referent rate
Autumn	138	549.6	452	0.8 (0.7, 0.9)	**1.6 (1.4, 1.9)**	**1.6 (1.4, 1.8)**
Winter	138	497.3	478	1.0 (0.9, 1.0)	**2.0 (1.7, 2.3)**	**1.9 (1.6, 2.2)**
Spring	143	559.7	393	0.7 (0.6, 0.8)	**1.4 (1.2, 1.6)**	**1.3 (1.1, 1.5)**
Family history						
No family history	73	1021.1	741	0.7 (0.7, 0.8)	Referent rate	Referent rate
Either parent asthma/eczema	78	1142.5	870	0.7 (0.7, 0.8)	1.0 (0.9, 1.1)	1.0 (0.8, 1.3)
First degree relative asthma/eczema[b]	81	1186.4	901	0.8 (0.7, 0.8)	1.0 (0.9, 1.2)	1.0 (0.8, 1.3)

Explanatory variables	No. of children	No. of child-months	No. of ARI	Incident rate per child-month (95% CI)	Unadjusted Incidence rate ratio (95% CI)	Adjusted Incidence rate ratio[a] (95% CI)
Tobacco smoke exposure						
No exposure	130	1885.5	1406	0.7 (0.7, 0.8)	Referent rate	Referent rate
Either parent smokes	19	244.9	171	0.7 (0.6, 0.8)	0.9 (0.8, 1.1)	0.8 (0.6, 1.2)
Household size						
No other children in household	100	1495.6	1052	0.7 (0.7, 0.7)	Referent rate	Referent rate
More than one child in household	54	712.0	595	0.8 (0.8, 0.9)	1.2 (1.1, 1.3)	1.2 (0.9, 1.5)
Maternal education status						
High school	14	186.2	141	0.8 (0.6, 0.9)	1.0 (0.8, 1.2)	0.9 (0.6, 1.4)
Diploma/Certificate	38	526.7	353	0.7 (0.6, 0.7)	0.9 (0.8, 1.0)	0.8 (0.6, 1.1)
University/higher university degree	96	1443.3	1099	0.8 (0.7, 0.8)	Referent rate	Referent rate
Mode of feeding						
Formula	142	1739.4	1447	0.8 (0.8, 0.9)	Referent rate	Referent rate
Breast milk	146	467.6	195	0.4 (0.4, 0.5)	**0.5 (0.4, 0.5)**	1.2 (0.9, 1.6)
Childcare attendance						
No childcare	153	1238.4	679	0.5 (0.5, 0.6)	Referent rate	Referent rate
Informal childcare only	48	343.2	197	0.6 (0.5, 0.7)	**1.3 (1.0, 1.5)**	0.9 (0.7, 1.1)
Formal and/or informal childcare[c]	90	625.5	766	1.2 (1.1, 1.3)	**2.6 (2.3, 3.0)**	**1.9 (1.6, 2.3)**

[a]adjusted for age, sex, type of delivery, gestational age, season, family history, tobacco smoke exposure, household size, maternal education status, mode of feeding, and childcare attendance; [b]mother, father or sibling; [c]formal and informal childcare attendance were combined because of the small number of children attending informal childcare alone; abbreviations: ARI: acute respiratory infection. Discrepancies in the numbers of children in Table 1 and Table 4 are either because of no ARIs before leaving the cohort and/or missing data.

DISCUSSION

In this community-based cohort we found that during their first two years of life, healthy Australian children living in a subtropical city had on average 13 discrete symptomatic episodes of ARI. Further, these combined episodes amounted to a cumulative total of almost five months of any respiratory symptoms in the first two years of life. Just 17% of all episodes were LRTIs, including 6% with recorded symptoms of wheezing. Parents consulted their family physician for almost half (48%) of the ARIs captured by a burden diary and an antibiotic was prescribed in 46% of these episodes. Increasing age, non-summer seasons, and attendance at childcare were independent risk factors for ARI.

Our incidence estimate is consistent with other reports of an annual mean 5.0-6.3 ARI episodes per child during their first 1-2 years of life (222, 233, 266, 275). However, this contrasts with a lower annual mean incidence of 4.2 ARI episodes in a community-based, high-risk birth cohort in Perth, Western Australia (28). The lower IR in that study probably reflects study design, which required parents to contact research staff within 24-hours of ARI onset and staff to make visits within 48-hours of notification. Lower rates have also been reported in similar study designs involving high-risk infant cohorts where mild infections went under-reported (275). Furthermore, a smaller proportion of Western Australian cohort participants attended childcare (32%), which could also explain lower ARI incidence (28). Other studies have focused primarily on LRTIs requiring medical attention and hospitalisation (21) or extrapolated from other data sources such as Emergency Department presentations (276).

Nasal discharge/congestion and cough were the major ARI symptoms in both frequency and duration, whereas fever and wheeze were less common and resolved quickly. A recent systematic review of ARI in children attending primary care (277) estimated that 90% of healthy children with nasal symptoms from ARI will recover within two weeks, longer than the 7-10 days cited by current guidelines (278, 279). Our observations on symptom duration are similar for nasal discharge/congestion, although in our cohort 90% of cough also resolved by two weeks rather than the estimated 25 days from the systematic review (277). This information is relevant to both parents and clinicians to help set more realistic expectations for ARI episodes and for parents to decide when to re-consult for persistent symptoms.

Many children in our study were first-born and from well-educated, affluent families; factors thought to decrease the rate of infection in a household. Secular changes in Australia have led to an increase in the proportion of households where both parents are employed outside the home, and their children are more likely to attend childcare at an earlier age than other sectors of the population (244). A slightly higher proportion (80-85%) of our cohort attended some form of care by the age of 15-24 months, compared to 74% of children in the Australian population (244), with childcare attendance an independent risk factor for ARI (11, 29). We did not observe a relationship between ARI and either tobacco smoke exposure or household size, however our power to detect such associations was small because of the low number of household smokers in our cohort and two-thirds of participants were first-born. In contrast to recent studies reporting a continuum of increasing risk of adverse respiratory outcomes with increasing prematurity (274), we did not see an association between gestational age at birth and ARI, although our eligibility criteria excluded early pre-term children (<36 weeks gestation) and only two children in our cohort were born late pre-term/early term (36w0d-37w6d). Consistent with other paediatric studies, age, greater respiratory virus activity outside summer months, and childcare attendance were independently associated with an increased risk of ARI in our cohort (2, 3, 60, 114, 233, 269).

Of ARI episodes captured in a burden diary, nearly half required a medical consultation, with significantly higher likelihood of consultation for LRTIs. Thus, studies estimating ARI from primary care visits alone could underestimate disease burden by 50% or more. Studies with parent-completed diaries are able to capture mild-to-moderate disease in the community that may not generate a doctor's visit (3). When an ARI-related medical consultation occurred in our cohort, almost half were prescribed an antibiotic, more likely for AOM than for other URTIs, so that by the end of the study more than half the cohort had been prescribed an antibiotic on at least one occasion. This degree of antibiotic exposure is comparable with other Australian studies (3, 28, 280) and within the broad range of prescribing found within Europe (222, 233, 281), the United Kingdom (282), and in the United States of America (283). A European study observed that while the frequency of infection episodes did not differ between countries, antibiotic prescribing habits were determined by other factors, such as physician attitudes and socio-economic factors (281). This was highlighted by a

recent cross-sectional survey of common ARI management in young children in Australian family practice (27). It found highest antibiotic prescribing rates for URTI were from older male doctors who were also less likely to educate parents about these infections. While antibiotic prescribing in our cohort was higher for LRTI than for URTI, few cases of pneumonia were diagnosed and thus educating parents and physicians on the self-limiting and viral nature of most ARIs remains a priority for promoting good antimicrobial stewardship.

Strengths and limitations

Our study had a good retention rate for an intensive two year study with daily data collection, providing 78% of total expected person-days of follow-up recorded and 95% of expected burden diaries submitted. We captured burden diary information for 50% of URTIs. The remaining URTIs where data were not collected were mild events involving either nasal symptoms or dry cough in isolation, and unlikely to impact upon resources at the family level. The prospective study design with parent-reported diary cards gives a comprehensive account of ARIs, including minor symptoms. Although we did not validate symptom information captured in the daily diaries (other than doctor-diagnosed AOM and pneumonia), we minimised inaccuracy of reporting by training parents how to recognize symptoms and complete the diary before they commenced the study. Furthermore, this study design has been used before successfully (3) and this and other studies have shown that parents can be trained to recognize and document symptoms of interest (3), often as reliably as health professionals (284, 285). While visits by trained healthcare workers to validate all symptoms would have been ideal, a study of this magnitude and scale would have been logistically challenging and resource intensive to conduct without parent participation. We used relatively specific case definitions for both URTI and LRTI without the inclusion of non-specific constitutional symptoms, which may be unreliable in this age group. It is possible therefore that some minor respiratory symptoms may have been missed when more non-specific symptoms, such as fever, irritability, and poor feeding dominated the clinical picture. Our cohort were from families of more advantaged backgrounds, which is common in longitudinal cohort studies (28, 230) given the long-term, intensive nature of the study. However, our findings of the exposures within the cohort on the outcomes of interest remain valid and provide

estimates on ARI burden and management in an Australian subtropical urban community.

Conclusions

Our study adds to current knowledge about ARIs in the first two years of life as a common cause of early childhood morbidity in the community. Most are URTIs and their symptom duration, particularly nasal symptoms of the common cold, can be much longer than commonly published estimates. When healthcare assistance is sought, family physicians are consulted most often, with very few children requiring hospitalisation. Nevertheless, antibiotics are still commonly prescribed for ARIs. Secular societal changes and the increasing use of childcare in early childhood may have impacted on the incidence of ARIs in this age group. Future studies examining the aetiologies of these ARIs are underway and will help guide prevention strategies.

Chapter 4 – Respiratory viruses in neonates

This chapter is presented as a published original article:

Sarna M, Alsaleh A, Lambert SB, Ware RS, Mhango LP, Mackay IM, Whiley DM, Sloots TP, Grimwood K. Respiratory viruses in neonates: a prospective, community-based birth cohort study**.** *Pediatric Infectious Diseases Journal* 2016; 35(12):1355-57. DOI: 10.1097/INF.1316.

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Key words: respiratory viruses, neonate, rhinovirus

ABSTRACT

Introduction:

Acute viral respiratory infections are important causes of morbidity and mortality in infants worldwide. However, little is known about these infections in the first four weeks of life (the neonatal period). The few studies describing respiratory viruses in neonates are predominantly from neonatal units or neonates presenting to hospital with respiratory symptoms, and are unlikely to be widely generalisable. Respiratory virus infection in this age group, even if asymptomatic, may influence future respiratory health. Hence we describe the respiratory viruses detected in neonates in a community-based birth cohort.

Methods:

One hundred and fifty-seven children from subtropical Brisbane, Australia, participated in a longitudinal, community-based birth cohort study. Parents completed a daily tick-box diary capturing pre-defined respiratory symptoms, and collected weekly nasal swabs, which were tested for 17 respiratory viruses by polymerase chain reaction assays. The association between potential risk factors and new virus infections was assessed using Poisson regression.

Results:

An average of 0.25 (95% CI: 0.18, 0.34) respiratory virus infections per neonatal period were detected in 157 full-term infants. Human rhinoviruses of diverse subtypes dominated and almost 50% were asymptomatic. Virus shedding was transient (5-14 days) and serial HRV detections were as likely to result from new genotype acquisition as continued detection of the original infection. Being the first-born child was associated with a reduced incidence of infection (IRR 0.5; 95% CI: 0.2, 0.9, p=0.02). There was no evidence of transmission between parents harbouring respiratory viruses around the time of delivery and their newborn infants.

Conclusions:

Respiratory viruses are common and often unrecognised in healthy neonates. HRV predominates. This early exposure to HRV means preventative strategies against HRV need to begin early.

INTRODUCTION

Despite acute viral respiratory infections being important causes of morbidity and mortality in infants worldwide, little is known about these infections in the first four weeks of life (referred henceforth as the neonatal period). The few studies describing respiratory viruses in neonates are unlikely to be widely generalizable as they are predominantly from neonatal units (286, 287) or neonates presenting to hospital with respiratory symptoms (288, 289). While several community-based birth cohort studies tested for respiratory viruses (144, 146), few collected samples before four weeks of age and then only tested for a limited number of viruses (290). It is important to understand respiratory virus exposure in this age group as early infection, even if asymptomatic, may influence future respiratory health (291). Hence we describe the respiratory viruses detected in neonates in a community-based birth cohort.

MATERIALS AND METHODS

Details of the study population, demographic and symptom data collection, classification of ARIs, anterior nose swab sample collection, transport, processing, and testing, have been outlined in Chapter 2.

Analysis

A new virus detection occurred when a new virus species or another HRV genotype was detected. We defined each swab as representing seven days of study-time, with a maximum of 28 days per neonate. The association between potential risk factors (sex, mode of delivery – vaginal vs Caesarean, first-born status, season, parental history of asthma or eczema, household tobacco smoke exposure, and breastfeeding) and new virus infections was assessed using Poisson regression. As categories of season and breastfeeding were not mutually exclusive for individual neonates, these two variables were analysed using mixed-effects Poisson regression with neonate included as a random effect. All models were offset using the natural logarithm of study-time. Effect estimates are expressed as incidence rate ratios with 95% CI. A failure curve of first virus detections was constructed from life tables. Data were analysed using Stata v12.0 (StataCorp, Texas, USA).

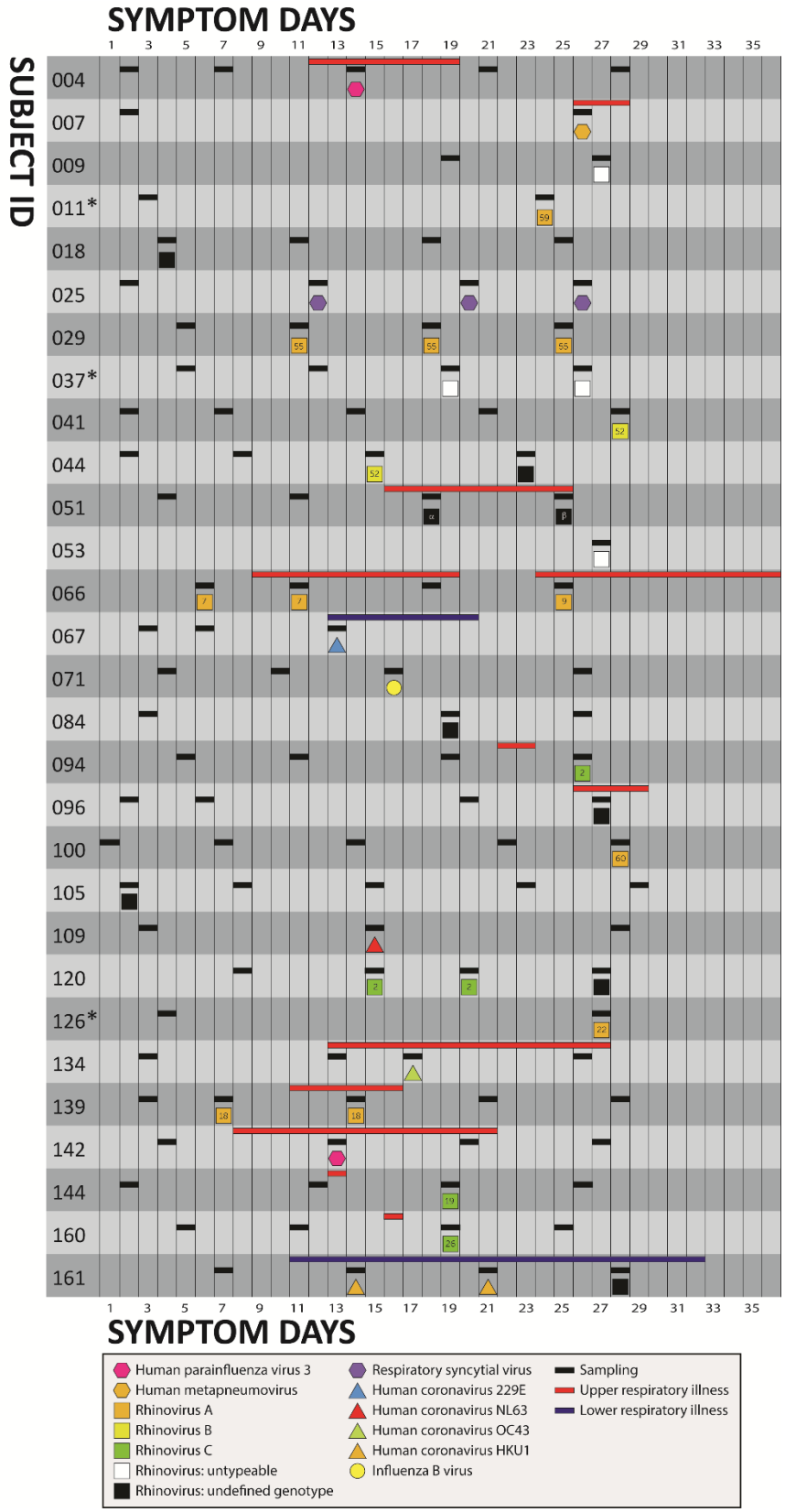
RESULTS

Viruses detected and symptoms in neonates

Overall, 157/164 enrolled and eligible (74 males, 47.1%) neonates provided 574 nasal swab specimens (range 1-5 per neonate), yielding 552 weeks of data (87.9% of maximum expected). One infant did not provide any swabs in the neonatal period. Of the 43 virus-positive swabs from 29 (10 male) neonates (Figure 6), new infections were detected in 34 swabs (incidence rate 0.25 virus infections per neonatal period; 95% CI: 0.18, 0.34). The earliest virus detection was at two days of age and the age at first detected infection was consistent throughout the neonatal period (Figure 7).

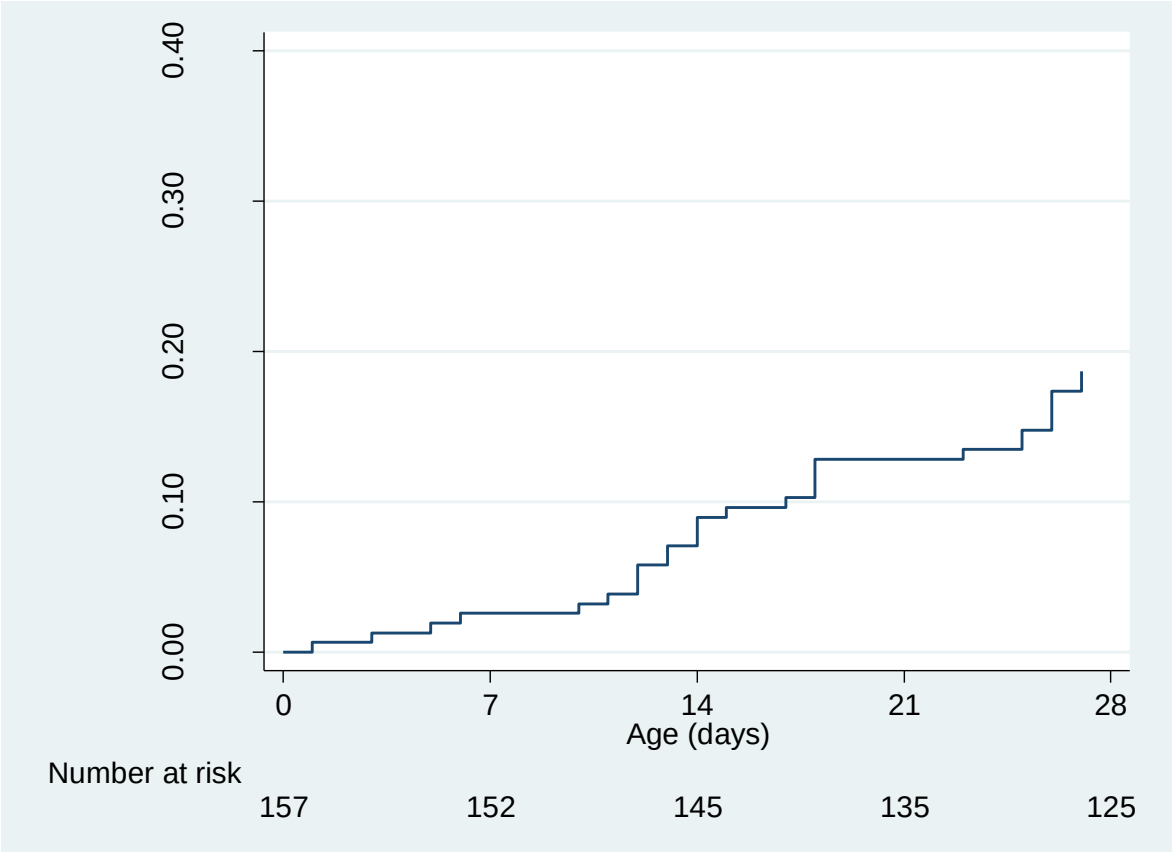
HRV was observed in 21/29 (72.4%) neonates with positive detections. Overall, HRV was found in 31 weekly swabs, including from all 4 neonates with viruses detected in the first-week of life. HCoV was detected in 4 neonates (5 swabs) and PIV-3 virus in 2 neonates (2 swabs), with 1 neonate each having RSV-A (3 swabs), INFV-B virus (1 swab), and HMPV (1 swab). No co-detections were identified.

Figure 6: Nature, timing and symptoms associated with respiratory viruses detected in 29 healthy, full-term neonates during the neonatal period



*No symptom diary was completed for subjects 11, 37 and 126

Figure 7: Time to detection of first respiratory virus (n=157)*



*one infant did not provide any swabs during the neonatal period

Thirteen neonates had 14 symptomatic episodes associated with virus detection (Figure 6). The earliest symptomatic virus detections were in the first week of life, on days 6 and 7 (both HRV-A), and associated symptoms commenced on days 9 and 11, respectively. Eleven showed upper respiratory symptoms of nasal discharge/congestion (four also had a dry cough), including subject 66 who experienced two discrete respiratory episodes (corresponding to two different strains of HRV-A). The remaining two infants exhibited lower respiratory symptoms: subject 67 had wheezing (HCoV-229E detected on day-13), while subject 161 had wheezing accompanied by fever and a wet cough (HCoV-HKU1 detected on days-14 and 21, and HRV on day-28). Subjects 4, 51, 67, 134, 142, 161 sought medical advice, with subject 51 diagnosed with otitis media at 22 days of age. None were hospitalised. In contrast, 13 neonates had asymptomatic viral detections. This included subject 71 with

influenza-B on day-16, and subject 25 shedding RSV-A between days 9-26. Symptom diary data were unavailable for three neonates with single HRV detections.

Being the first-born child was associated with a reduced incidence of infection (IRR 0.5; 95% CI: 0.2, 0.9, p=0.02) (Table 10). In contrast, no associations were observed between other risk factors (sex, mode of delivery, season, parental history of asthma/eczema, household tobacco exposure, or exclusive breastfeeding) and virus infection as a neonate.

HRV typing

Of the 31 HRV-positive samples (21 neonates), nine (29.0%) were sequenced, but could not be assigned a specific HRV genotype (unclassified novel VP4/VP2 sequences) based on a GenBank database search (http:www.ncbi.nlm.nih.gov), while 4 (12.9%) HRV detections could not be sequenced, usually because of insufficient viral RNA template loads. Of the 18 samples able to be typed, 11 (61.1%) were HRV-A, 2 (11.1%) were HRV-B, and 5 (27.8%) were HRV-C. Overall, there were 17 different subtypes amongst the 27 HRVs able to be sequenced. Apart from HRV-B52 and HRV-C02 (each detected in 2 unrelated neonates), clustering of HRV genotypes was confined to individuals. Repeat detection of homologous genotypes for 1-2 weeks was observed in 4 neonates (subjects 29, 66, 120, 139). In contrast, 4 subjects (44, 51, 66, 120) had either different HRV genotypes or different unclassified HRV sequences in subsequent swabs.

Viruses detected and symptoms in parents

Two-hundred-and-thirty-nine (127 mothers) parents submitted swabs. Nine (three mothers) parents (3.8%, 95% CI: 1.9%, 7.5%) of eight neonates had respiratory viruses detected, 8 with HRV and 1 with influenza-A virus, with no co-detections. Five parents with HRV reported nasal discharge/congestion, while one other complained of fever, myalgia, and lassitude. Of the eight neonates with infected parents, subject 51 had different HRV genotypes detected on days 18 and 25, subject 100 had a different HRV genotype detected on day-28, and subject 71, whose parents had HRV, became infected with influenza-B. The remaining 5 neonates remained asymptomatic and virus-negative. No transmission events between parents and infants were detected.

Table 10: Number of children and swabs, and association between participant characteristics and new virus detections of the ORChID community-based birth cohort in the neonatal period.

Characteristics	Total no. of children N=157	No. of positive children N=29	Total no. of Swabs N=574	New virus detections, N=34 n (%)	Unadjusted incidence rate ratio (95% CI), p value
Sex					
Males	74	10	273 (47.6%)	11 (32.4%)	reference
Females	83	19	301 (52.4%)	23 (67.6%)	1.8 (0.9, 3.8), 0.09
Mode of delivery					
Vaginal	106	20	385 (67.1%)	24 (70.6%)	reference
Caesarean	51	9	189 (32.9%)	10 (29.4%)	0.8 (0.4, 1.7), 0.60
Birth order					
Not first-born	105	14	185 (32.2%)	17 (50.0%)	reference
First-born	52	15	389 (67.8%)	17 (50.0%)	0.5 (0.2, 0.9), 0.02
Season[a]					
Summer	52	10	171 (29.8%)	10 (29.4%)	reference
Autumn	35	5	102 (17.8%)	7 (20.6%)	0.9 (0.3, 2.9), 0.92
Winter	47	6	147 (25.6%)	9 (26.5%)	0.9 (0.3, 2.5), 0.81
Spring	34	8	154 (26.8%)	8 (23.5%)	0.8 (0.3, 2.2), 0.64
Parent with asthma/eczema					
No	79	16	284 (49.5%)	15 (44.1%)	reference
Yes	138	25	290 (50.5%)	19 (55.9%)	1.2 (0.6, 2.3), 0.63
Household tobacco exposure					
No	21	2	509 (88.7%)	29 (85.3%)	reference
Yes	140	25	65 (11.3%)	5 (14.7%)	1.4 (0.5, 3.5), 0.53
Breastfeeding[a,b]					
Received milk formula	21	2	66 (11.8%)	2 (6.5%)	reference
Received breastmilk	140	25	493 (88.2%)	29 (93.5%)	1.5 (0.4, 6.1), 0.55

[a]Total number of children does not add up to 157 because an infant could be in more than one category

[b]Data from 559 swabs from 152 neonates; one neonate had new infection in both categories

DISCUSSION

In a community-based, urban Australian birth cohort of 157 healthy, full-term infants with high exclusive breastfeeding rates and little household tobacco exposure, we detected a mean of 0.25 (95% CI: 0.18, 0.34) new respiratory virus infections per neonatal period. These infections occurred consistently throughout the neonatal period. Being an only child, and presumably less exposed to other children, was associated with a reduced risk of virus detection. HRVs representing diverse genotypes predominated and almost half of all episodes associated with respiratory virus detections were asymptomatic. Virus shedding was transient (5-14 days) and serial HRV detections were as likely to result from new genotype acquisition as continued detection of the original infection. Finally, although numbers were small, there was no evidence of transmission between parents harbouring respiratory viruses around the time of delivery and their newborn infants.

It is possible that the frequent subclinical viral detections we observed were due to some protection against symptomatic illnesses afforded by maternal trans-placental and breastmilk antibodies (292). Indeed, neonates who appear healthy may be important sources of virus transmission. Early exposure to HRV could also have implications for infants with a family history of atopy or asthma. High-risk birth cohort studies have found a close relationship between HRV infections in infancy and subsequent asthma development, particularly with HRV-A and C species (124). Recently, a cross-sectional study of the Danish asthma birth cohort ($COPSAC_{2010}$) detected viruses (predominantly HRV) in 12% of neonates at four weeks of age (291). Some of the infected neonates exhibited an exaggerated type-2 mucosal immune response, suggesting early HRV exposure may trigger aberrant immune programing in susceptible individuals and promote subsequent development of asthma and allergic sensitization (124, 291). Any preventive intervention will therefore need to begin early.

HRV genotype diversity occurs in neonates as well as in infants and young children (150). Moreover, even during single acute respiratory infection episodes, continued HRV presence in neonates is most likely from sequential infections by different HRV genotypes rather than continued shedding of the original genotype. In HRV-positive infants with sequential detections, we observed a change of genotype 50% of the time. These findings, and those from other reports (144, 150), emphasise the importance of nucleotide sequencing when studying HRV infections.

Our study's strengths include the prospective collection of swabs at birth and then weekly thereafter, allowing respiratory viruses to be detected during the neonatal period. Parent swabs and symptom history also enabled us to examine possible transmission from parent to newborn, which appeared limited, although this observation was constrained by the small number of positive swabs. It is also possible that parents may have missed identifying mild respiratory and non-specific symptoms in their newborn infants. Finally, and similar to other community-based HRV studies (150), 41.9% of sequences from HRV detections either failed to align with HRV reference prototype sequences or could not be genotyped, usually because of low viral RNA template loads. Nevertheless, the ORChID study design, with its weekly sampling and HRV-sequencing, was able to demonstrate sequential detection by the same and different HRV genotypes.

In conclusion, respiratory virus infections were common in neonates and many were asymptomatic. HRV predominated and our future studies will assess the association between early HRV exposure and ensuing frequency and severity of respiratory infections, as well as with later respiratory health, mucosal immunity, and lung function.

Chapter 5 – Viruses causing lower respiratory symptoms in children younger than two years of age: findings from the ORChID birth cohort

This chapter is presented as a published original article:

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Keywords: acute respiratory infections; respiratory viruses; children; attributable risk

ABSTRACT

Introduction:

Viral acute respiratory infections (ARIs) cause substantial child morbidity. Sensitive molecular-based assays aid virus detection, but the clinical significance of positive tests remains uncertain as some viruses may be found in both acutely ill and healthy children. We describe disease-pathogen associations of respiratory viruses and quantify virus-specific attributable risk of ARIs in healthy children during the first two years of life.

Methods:

One hundred fifty-eight term newborn babies in Brisbane, Australia, were recruited progressively into a longitudinal, community-based, birth cohort study conducted between September 2010 and October 2014. A daily tick-box diary captured predefined respiratory symptoms from birth until their second birthday. Weekly parent-collected nasal swabs were batch-tested for 17 respiratory viruses by PCR assays, allowing calculation of virus-specific attributable fractions in the exposed (AFE) to determine the proportion of virus-positive children whose ARI symptoms could be attributed to that particular virus.

Results:

Of 8100 nasal swabs analysed, 2646 (32.7%) were virus-positive (275 virus co-detections, 3.4%), with human rhinoviruses accounting for 2058/2646 (77.8%) positive swabs. Viruses were detected in 1154/1530 (75.4%) ARI episodes and in 984/4308 (22.8%) swabs from asymptomatic periods. Respiratory syncytial virus (AFE: 68% (95% CI: 45%, 82%)) and human metapneumovirus (AFE: 69% (95% CI: 43%, 83%)) were strongly associated with higher risk of lower respiratory symptoms.

Conclusions:

The strong association of respiratory syncytial virus and human metapneumovirus with ARIs and lower respiratory symptoms in young children managed within the community indicates successful development of vaccines against these two viruses should provide substantial health benefits.

INTRODUCTION

Viral ARIs are a common cause of morbidity in children, either on their own or in synergy with bacterial pathogens (293). Virus-positive ARIs early in life are associated with developing wheeze and asthma during childhood and adolescence (114). Community-based studies from the 1950-1960s examining the aetiology of ARIs by traditional laboratory detection methods remain the template for understanding childhood respiratory virus epidemiology (60). Advances in molecular diagnostics, including polymerase chain reaction (PCR) testing, have improved virus detection in those with ARIs (294). However, this increased test sensitivity has also resulted in viruses being detected in asymptomatic individuals (151, 294). The clinical significance of finding respiratory viruses is not always straightforward, and a better understanding of the proportion of infections that are symptomatic for individual viruses is needed.

The AFE provides information about the percentage of cases amongst exposed individuals that can be attributed to the exposure. In our study, the AFE measures the percentage of ARIs in virus-positive children attributable to that particular virus. The AFE does not inform on the aetiology at an individual level, but is a key population level metric for interpreting positive specimen results. It assists with estimation of the true burden of disease, thus guiding preventative strategies for respiratory infections, such as immunisation. Virus-specific AFE estimates for ARI in children are reported infrequently, with most studies reporting AFEs having important methodological limitations. Except for one community study (151), these investigations recruited either highly-selected hospitalised children or outpatients as study cases (182, 295, 296), or used controls from outpatient clinics (182, 295). A meta-analysis of 23 studies that calculated virus-specific AFEs included 22 studies employing hospital-based case ascertainment and only three using community-based controls (22). Other studies comparing symptomatic and asymptomatic groups calculated odds ratios (ORs) (147, 225, 297), or relative risks (298), without calculating AFEs. Moreover, several studies focussed solely either on specific viruses (147, 297) or outcomes (182, 225, 297, 298).

Longitudinal, community-based studies employing sensitive molecular-based assays with regular and frequent sampling, irrespective of illness, are best suited to explore the relationship between respiratory virus detections and ARI symptoms, especially as they represent most ARI episodes. The aim of our study was to examine the disease-pathogen

associations of respiratory viruses in young children participating in a community-based birth cohort project (243) and to calculate their virus-specific AFE values.

MATERIALS AND METHODS

Details of the study population, demographic and symptom data collection, classification of ARIs, anterior nasal swab sample collection, transport, processing, and testing, have been outlined in Chapter 2.

Definitions

New virus detection episode (VDE): when a new virus (different virus or new species/subtype of the same genus or species respectively) was detected in a swab by PCR testing, or the same virus was detected after at least two negative intervening swabs, or the same virus was detected at least 30 days from the last positive swab.

Symptomatic new VDE: when respiratory symptoms were reported within seven days either side of first detecting the virus of interest in the weekly nasal swab.

Analysis

The incidence rate of new single VDEs per 100 child-years was calculated using Poisson regression. To account analytically for the differing lengths of time children remained in the study, the natural logarithm of the number of swabs returned was included in the model as an offset. Swabs were removed from incidence rate calculations for individual viruses when the participant was not at risk of a new VDE (a positive consecutive swab following the initial positive swab, and two swabs after the last positive swab, as per our definition of a new VDE), or the swabs were of lesser quality (to avoid under-estimating incidence rates) (249). When calculating incidence rates for ‘any virus’ we assumed children were at risk of being positive for new viruses in every swab.

The virus-specific risk and AFE of single VDEs in cases of ARI and LRTI were estimated using a generalised linear model with binomial family and log link. Robust variance estimates were calculated using sandwich estimators to account for repeated measures within children. We analysed LRTIs separately as children with these infections are more likely to visit family physicians, present to hospital and receive antibiotics (6). The AFE

was calculated as 100% *(1-RR^{-1}), where RR (risk ratio) is the proportion of children positive for a virus with ARI symptoms, divided by the proportion of children negative for the virus who have ARI symptoms (Table 11).

Table 11: Calculation of attributable fraction in the exposed

The AFE calculates the proportion of symptomatic acute respiratory infections (ARI) in virus-positive individuals that can be attributed to the virus.

Swab status	Acute respiratory infection	No symptoms	Total population
Virus-positive	a	b	a+b
Virus-negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

The risk of having symptoms given that a child is virus-positive = a/(a+b)

The risk of having symptoms given that a child is virus negative = c/(c+d)

The risk ratio of having symptoms if a child is virus-positive compared to if they are virus-negative is a/(a+b)/c/(c+d)

Attributable fraction in the exposed is $\frac{100\% * (a/a+b)-(c/c+d)}{(a/a+b)}$

The AFE can also be written in terms of the risk ratio (RR): AFE = 100% * (1-RR^{-1}), where RR is the proportion of children positive for a virus with ARI symptoms, divided by the proportion of children negative for the virus who have ARI symptoms.
For example,

- AFE = 0% - the risk of having symptoms when virus-positive is equal to the risk of having symptoms when virus-negative – that is, the presence of the virus is not associated with the presence of symptoms;
- AFE = 50% - 50% of symptomatic episodes when virus-positive were attributable to the presence of the virus;
- AFE = 100% - the risk of having symptoms when virus-negative = 0.

Only virus-negative swabs of lesser quality (Ct >38) were excluded in these analyses of association, as swab quality of positive swabs did not modify the association between VDEs and ARI symptoms (Table 12).

The association between potential risk factors (age, sex, type of delivery, gestational age at birth, season, family history of asthma or eczema, tobacco smoke exposure, household size, maternal education status, mode of feeding, category of childcare attendance), and symptomatic and asymptomatic virus detection was examined using mixed-effects logistic regression with the child entered as a random effect to account for repeated measurements within children. Both univariable and multivariable analyses were conducted. Breastfeeding and childcare attendance were analysed as time-varying variables. Multivariable models included all the above risk factors with no forward or backward selection. Only virus-negative lesser quality swabs were excluded in these analyses. As it was not possible to determine individual virus contributions to ARI symptoms during virus co-detections, samples with more than one virus detected were analysed descriptively (Table 17). Data were analysed using Stata v12.1 (StataCorp, Texas, USA).

Table 12: Acute respiratory infections, lower respiratory tract infections and asymptomatic single new virus detections, risk ratios, and attributable fractions by respiratory virus in children from the ORChID birth cohort with virus positive detections with cycle-threshold values >38 excluded (n=7,856).

	Acute respiratory infections		Lower respiratory tract infections	
Virus	**Risk ratio[a] (95% CI)**	**Attributable fraction in the exposed (%)**	**Risk ratio[a] (95% CI)**	**Attributable fraction in the exposed (%)**
Single new detections				
All HRV	**1.5 (1.4, 1.7)**	**33 (29, 41)**	1.1 (1.0, 1.2)	9 (0, 17)
HRV-A	**1.6 (1.5, 1.7)**	**37 (32, 42)**	1.2 (1.0, 1.6)	19 (-3, 36)
HRV-B	1.2 (1.0, 1.5)	18 (-1, 33)	0.8 (0.4, 1.6)	-19, -130, 39)
HRV-C	**1.6 (1.4, 1.7)**	**38 (30, 40)**	**1.4 (1.1, 1.7)**	**28 (12, 41)**
INFV-A	**1.7 (1.2, 2.3)**	**41 (17, 57)**	1.6 (0.4, 5.7)	38 (-150, 82)
INFV-B	1.0 (0.5, 2.8)	0 (-1, 64)	0	0
PIV-1	**1.8 (1.3, 2.5)**	**44 (23, 60)**	2.5 (0.8, 7.8)	**60 (25, 87)**
PIV-2	n/c	n/c	n/c	n/c
PIV-3	**1.6 (1.3, 2.0)**	**38 (23, 50)**	**2.0 (1.2, 3.3)**	**50 (17, 70)**
RSV-A	**1.4 (1.1, 1.7)**	**29 (9, 41)**	**3.0 (2.0, 4.5)**	**67 (50, 78)**
RSV-B	**1.8 (1.4, 2.2)**	**44 (29, 55)**	**3.1 (1.8, 5.4)**	**68 (44, 81)**
HCoV-OC43	**1.6 (1.3, 2.0)**	**38 (23, 50)**	**2.3 (1.4, 3.7)**	**57 (29, 73)**
HCoV-NL63	**1.7 (1.4, 2.1)**	**41 (29, 52)**	1.7 (0.9, 3.3)	**41 (11, 70)**
HCoV-229E	0.9 (0.3, 2.5)	-11 (-233, 60)	1.2 (0.2, 7.7)	17 (-4, 87)
HCoV-HKU1	1.1 (0.7, 1.6)	9 (-43, 38)	0.8 (0.2, 2.9)	25 (-4, 66)
HMPV	**1.6 (1.2, 2.1)**	**38 (17, 52)**	**3.2 (1.7, 6.0)**	**69 (41, 83)**
HAdV	**1.5 (1.2, 1.8)**	**33 (17, 44)**	1.0 (0.4, 2.4)	0 (-15, 58)
WU-PyV	1.3 (0.9, 1.8)	23 (-11, 44)	1.4 (0.6, 3.5)	29 (-67, 71)
KI-PyV	0.8 (0.6, 1.2)	25 (-67, 17)	1.2 (0.6, 2.2)	17 (-67, 55)
HBoV-1	1.3 (1.0, 1.6)	23 (0, 38)	1.3 (0.8, 2.2)	23 (-25, 55)
Any virus	**1.6 (1.5, 1.7)**	**38 (33, 41)**	**1.4 (1.2, 1.5)**	**29 (17, 33)**

[a]adjusted for clustering using sandwich estimators to account for within-infant correlation between observations; [b]Individual rhinovirus species do not add up to 'All rhinovirus' detections because of a combination of more than one rhinovirus species detected within the same ARI episode, or single new virus detection episodes where HRV positive specimens could not be sequenced. Abbreviations: n/c: not calculable as there were no cases in the asymptomatic group. HRV: human rhinovirus, INFV: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus, HCoV; human coronavirus, HMPV: human metapneumovirus, HAdV: human adenovirus, WU-PyV: WU polyomavirus, KI-PyV: KI polyomavirus, HBoV-1: human bocavirus-1.

RESULTS

Cohort characteristics

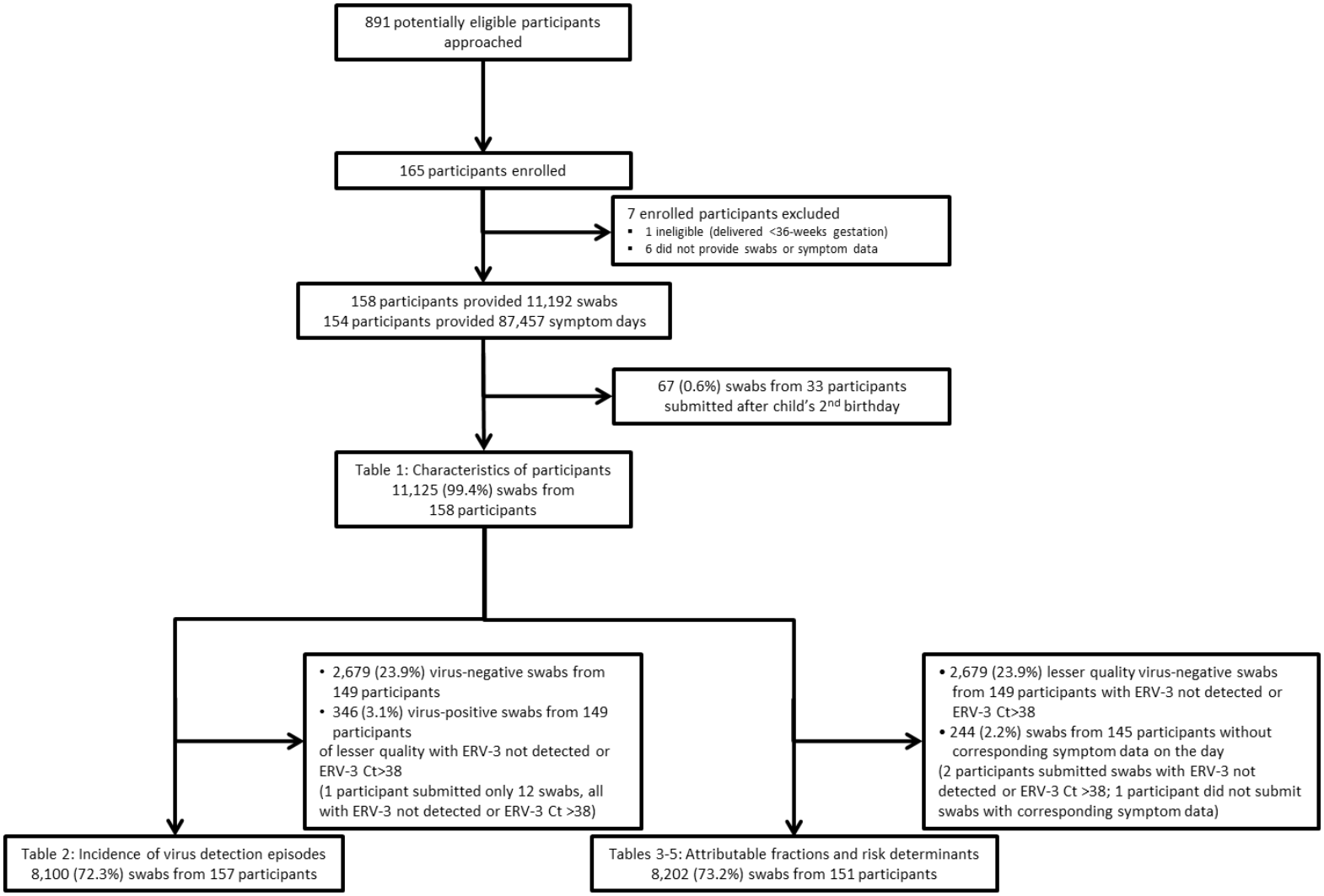
Of 891 potential participants approached, 165 (18.5%) eligible singleton infants from 163 families were enrolled (two families enrolled two siblings) (Figure 8). Seven were excluded subsequently; one from ineligibility (born <36 weeks) and six for failing to provide any swabs. Of the remaining children, 158 provided 11,125 swabs (Figure 8; 68.3% of maximum expected; median 84.5, range 1-104) from birth until their second birthday, and 154 children provided 87,547 child-days of symptom diaries (77.9% of maximum expected days; median 726 (range 1-730 days)) with 67% followed until at least 23 months (Figure 3). One or more respiratory symptoms were recorded on 16,877 (19.3%) days, and corresponded to 1,641 discrete ARI episodes. Demographic and social characteristics are reported in Table 3).

Virus detections

When calculating incidence rates, of the 11,192 total swabs submitted by all children, 3,025 (27.2%) lesser-quality swabs (346 virus-positive and 2,679 virus-negative) were removed from the analyses, as were 67 swabs submitted after the child's 2nd birthday, leaving 8,100 swabs from 157 children (Figure 8). Overall, 2,646/8,100 (32.7%) swabs were positive for respiratory viruses (Table 13), of which 1,520 were new VDEs.

The incidence of new VDEs increased in the first 9 months of life, with symptomatic episodes dominating after six months of age (Figure 8). There was an overall incidence rate of 978 new VDEs per 100 child-years (95% CI: 923, 1,029, Table 13). More than one virus was detected in 275/8,100 (3·4%) specimens. HRV was the most commonly detected virus (2,058/2,646 detections, 77·8% of all detections, mean rate of 1,002 new VDEs per 100 child-years, 95% CI: 942, 1,066), followed by HBoV-1 (60 new VDEs per 100 child-years, 95% CI: 49, 73), and KI polyomavirus (56 new VDEs per 100 child-years, 95% CI: 45, 69) (Table 13).

Figure 8: Submission of nasal swabs and symptom diaries from participants in the ORChID Study.



Ct = cycle-threshold; ERV-3=endogenous retrovirus-3

Figure 9: Incidence rate of new virus detection episodes per child-month by age and type of infection (N=151).

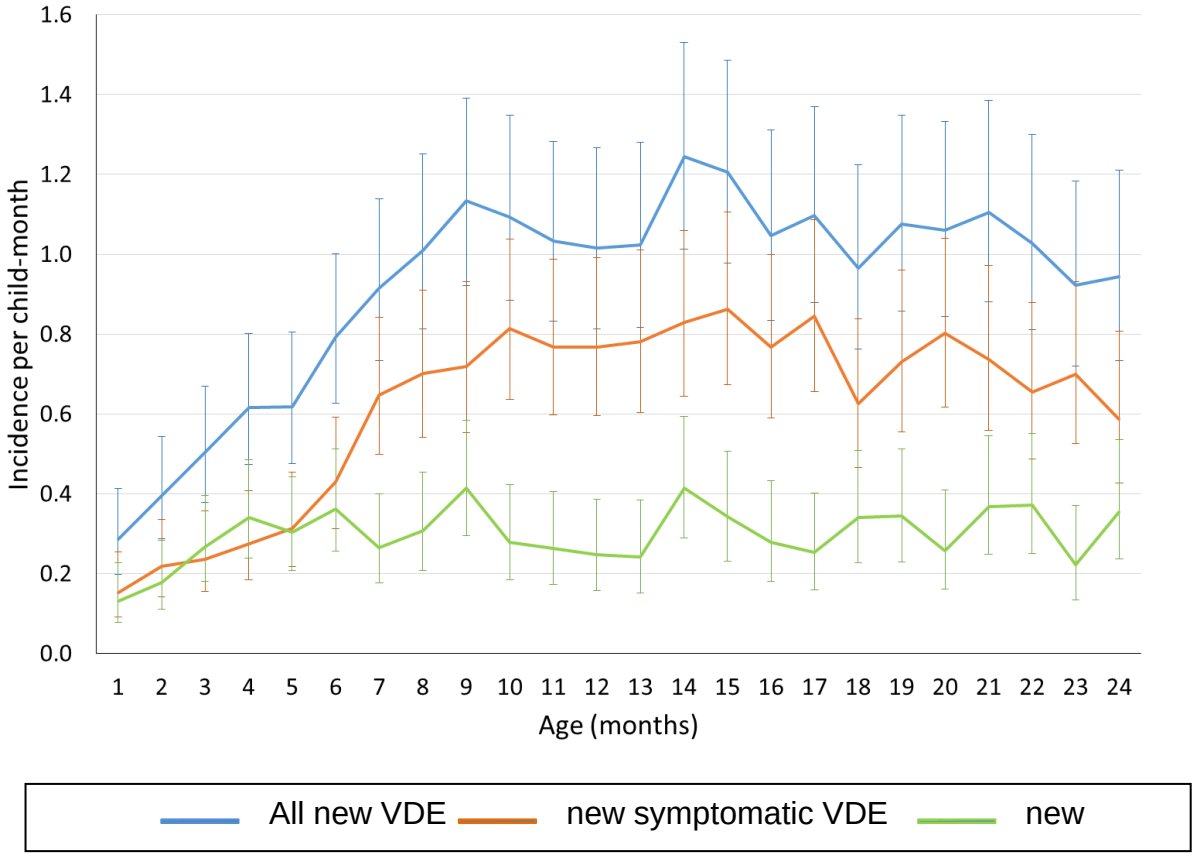


Table 13: Respiratory viruses detected in 8,100 high-quality nasal swabs collected from 157 children from the ORChID birth cohort in the first two years of life. Incidence rates/100 child-years presented overall, and for the first and second years of life separately.

Virus	Virus positive swabs	New virus detections N=1,520[a]	New VDE/100 child-years (Overall year mean)	New VDE/100 child-years (1st-year)	New VDE/100 child-years (2nd-year)
	n (%)	n (%)	Mean (95%CI)	Mean (95% CI)	Mean (95% CI)
All HRV[b]	2,058 (25.4)	1,012 (66.6)	1,002 (942, 1066)	770 (703, 842)	1354[c] (1245, 1472)
HRV-A	702 (8.7)	440 (28.9)	323 (295, 355)	258 (224, 297)	409 (361, 464)
HRV-B	154 (1.9)	97 (6.4)	64 (53, 78)	60 (45, 79)	69 (52, 92)
HRV-C	635 (7.8)	495 (32.6)	363 (332, 396)	295 (259, 336)	448 (398, 505)
INFV-A	12 (0.1)	12 (0.8)	7.7 (4.4, 13.6)	4.7 (1.8, 12.5)	11.5 (5.7, 22.9)
INFV-B	2 (0.0)	2 (0.1)	1.3 (0.3, 5.2)	1.2 (0.2, 8.3)	1.4 (0.2, 10.1)
PIV-1	7 (0.1)	7 (0.5)	4.5 (2.2, 9.5)	2.3 (0.6, 9.3)	7.2 (3.0, 17.2)
PIV-2	4 (0.0)	4 (0.3)	2.6 (1.0, 6.9)	1.2 (0.2, 8.3)	4.3 (1.4, 13.3)
PIV-3	59 (0.7)	56 (3.7)	36.5 (28.1, 47.4)	27.1 (18.0, 40.8)	47.8 (34.0, 67.3)
RSV-A	69 (0.9)	58 (3.8)	37.9 (29.3, 49.0)	30.7 (20.9, 45.1)	46.4 (32.8, 65.6)
RSV-B	22 (0.3)	20 (1.3)	12.9 (8.3, 20.1)	8.2 (3.9, 17.2)	18.7 (10.9, 32.2)
HCoV- OC43	51 (0.6)	42 (2.8)	27.3 (20.2, 37.0)	18.8 (11.5, 30.7)	39.1 (26.8, 57.0)
HCoV-NL63	56 (0.7)	40 (2.6)	26.0 (19.1, 35.5)	18.8 (11.5, 30.7)	37.7 (25.6, 55.3)
HCoV-229E	14 (0.2)	9 (0.6)	5.8 (3.0, 11.2)	4.7 (1.8, 12.5)	7.2 (3.0, 17.2)
HCoV-HKU1	34 (0.4)	28 (1.8)	18.2 (12.5, 26.3)	10.5 (5.5, 20.3)	27.4 (17.5, 43.0)
HMPV	27 (0.3)	23 (1.5)	14.9 (9.9, 22.4)	12.9 (7.1, 23.3)	17.2 (9.8, 30.4)
HAdV	104 (1.3)	80 (5.3)	52.6 (42.2, 65.4)	47.6 (34.9, 64.9)	58.3 (42.8, 79.5)
WU-PyV	101 (1.2)	60 (3.9)	39.3 (30.5, 50.6)	16.5 (9.8, 27.8)	67.6 (50.6, 90.2)
KI-PyV	168 (2.1)	84 (5.5)	55.7 (45.0, 68.9)	51.6 (38.3, 69.5)	60.4 (44.5, 82.1)
HBoV-1	137 (1.7)	90 (5.9)	59.6 (48.5, 73.3)	47.8 (35.1, 65.2)	73.8 (55.9, 97.4)
Any virus[d]	2,646 (32.7)	1,520 (18.8)	978 (930, 1029)	794 (736, 856)	1206 (1127, 1290)
Co-detections[d]	275 (3.4)	237 (15.6)	147 (130, 168)	117 (96, 142)	184 (155, 219)

[a]includes co-detections; [b]666 HRV (28.5%) positive samples could not be sequenced primarily because of low viral loads; [c]The rate of HRV new VDEs and the mean incidence of HRV new VDEs may equal or exceed the total rate of all new VDEs depending on the denominator after all swabs with cycle-threshold values >38 are excluded; [d]days at risk were not removed from the denominator as an infant could be at risk of being positive for other viruses in every swab. Abbreviations: HRV: human rhinovirus, INFV: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus, HCoV; human coronavirus, HMPV: human metapneumovirus, HAdV: human adenovirus, WU-PyV: WU polyomavirus, KI-PyV: KI polyomavirus, HBoV-1: human bocavirus-1; VDE: virus detection episode

Virus-positive ARIs and asymptomatic episodes

When considering virus-positive ARIs and asymptomatic episodes, we retained 346 virus-positive swabs with ERV Ct values >38, but removed a further 244 swabs with missing symptom data, to give a final dataset of 8,202 swabs from 151 participants (3 participants submitted swabs, but no symptom data before withdrawing early in the study) (Table 14, Table 15, Table 16).

Of 8,202 swabs, 3,894 (47·5%) were taken during symptomatic periods and 4,308 (52·5%) from asymptomatic periods. Of all swabs taken anytime during symptomatic periods, 1,903/3,894 (48·9%) were positive for a virus, and 1,120/3,894 (28·8%) were new VDEs. Overall, 984/4,308 (22·8%) swabs taken during asymptomatic periods were positive for a virus, of which 562 (57·1%) were new VDEs and 491/562 (87.4%) were new single virus detections (Table 14 and Table 15).

Table 14 shows that for new VDEs involving only single virus detections, HRV-A, HRV-C, INFV-A, PIV-1, PIV-3, RSV-A, RSV-B, HCoV-OC43, HCoV-NL63, HMPV, and HAdV had statistically significant, positive AFE values, indicating they were significantly more often associated with ARI episodes than asymptomatic periods.

In contrast, HRV-B, HCoV-229E, HCoV-HKU1, WU-PyV and KI-PyV, and HBoV-1 were frequently detected in asymptomatic periods and had low point estimates for AFE, indicating they were less likely to have caused the ARI.

Of the total 1,641 ARIs, 111 (6·8%) did not have swabs submitted during an ARI episode, including swabs taken seven days before or seven days after the last day of the illness. Of the remaining 1,530 ARIs, 1,154 (75·4%) ARIs (935 (81%) URTI, 219 (19.0%) LRTI) had ≥1 swabs with a virus detected during this period.

RSV and HMPV were more strongly associated with LRTI than asymptomatic detections (Table 15), with higher risk ratios than other viruses. While significant, the association between LRTI and HRV-C, HCoV-OC43 and PIV-3 detection was weaker. Interestingly, co-detected specimens had amongst the strongest AFE values for both ARIs and LRTIs (Table 14, Table 15), with HRV the most frequently detected virus, usually in combination with DNA viruses (Table 17).

Table 14: Acute respiratory infections and asymptomatic single new virus detections, adjusted risk ratios and attributable fractions by respiratory virus in 151 children from the ORChID birth cohort (n=8,202).

Virus	ARI-associated single new virus detections N=3,894 swabs	Asymptomatic single new virus detections N=4,308 swabs	Adjusted risk ratio[a] (95% CI); p value	Attributable fraction in the exposed (%, 95% CI)
Single new detections	**n (%)**	**n (%)**		
All HRV	679 (17.4)[c]	328 (7.6)	**1.5 (1.4, 1.6); <0.001**	**34 (29, 38)**
HRV-A	289 (7.4)	122 (2.8)	**1.5 (1.4, 1.6); <0.001**	**34 (28, 39)**
HRV-B	49 (1.3)	41(1.0)	1.2 (0.9, 1.4); 0.17	14 (-6, 30)
HRV-C	328 (8.4)	137 (3.2)	**1.5 (1.4, 1.7); <0.001**	**35 (30, 40)**
INFV-A	9 (0.2)	2 (0.0)	**1.7 (1.3, 2.3); <0.001**	**42 (23, 57)**
INFV-B	2 (0.1)	2 (0.0)	1.1 (0.4, 2.8); 0.90	6 (-150, 64)
PIV-1	6 (0.2)	1 (0.0)	**1.8 (1.3, 2.5**); **<0.001**	**45 (26, 59)**
PIV-2	3 (0.1)	0 (0.0)	n/c	n/c
PIV-3	32 (0.8)	11 (0.3)	**1.5 (1.3, 1.9); <0.001**	**35 (21, 46)**
RSV-A	29 (0.7)	14 (0.3)	**1.4 (1.2, 1.7); <0.001**	**30 (16, 42)**
RSV-B	19 (0.5)	3 (0.1)	**1.8 (1.5, 2.2); <0.001**	**46 (35, 54)**
HCoV-OC43	26 (0.7)	8 (0.2)	**1.6 (1.3, 2.0); <0.001**	**38 (25, 50)**
HCoV-NL63	27 (0.7)	8 (0.2)	**1.6 (1.3, 2.0); <0.001**	**37 (21, 49)**
HCoV-229E	3 (0.1)	4 (0.1)	0.9 (0.4, 2.2); 0.83	-10 (-161, 54)
HCoV-HKU1	12 (0.3)	10 (0.2)	1.2 (0.8, 1.7); 0.42	14 (-24, 40)
HMPV	12 (0.3)	4 (0.1)	**1.6 (1.2, 2.1); 0.001**	**37 (16, 53)**
HAdV	28 (0.7)	16 (0.4)	**1.4 (1.1, 1.7); 0.008**	**29 (12, 42)**
WU-PyV	16 (0.7)	15 (0.3)	1.1 (0.8, 1.6); 0.62	9 (-31, 36)
KI-PyV	23 (0.6)	34 (0.8)	0.9 (0.6, 1.2); 0.37	-17 (-65, 17)
HBoV-1	35 (0.9)	31 (0.7)	1.1 (0.9, 1.4); 0.30	11 (-12, 29)
Any virus	961 (24.6)	491 (11.4)	**1.5 (1.4, 1.6); <0.001**	**35 (31, 38)**
New co-detections[d]				
≥ 2 viruses	168 (4.3)	62 (1.4)	**1.6 (1.4, 1.7); <0.001**	**37 (30, 42)**

[a]adjusted for repeated measures within children using sandwich estimators; [b]confidence intervals for attributable fractions were calculated from confidence intervals for corresponding risk ratios; [c]individual HRV species do not add up to 'All HRV' detections because of a combination of more than one HRV species detected within the same ARI episode, or single new virus detection episodes where HRV positive specimens could not be sequenced; [d]includes 219 (160 symptomatic) new co-detections and another 11 co-detections with two and three viruses co-detected respectively where at least one co-detected virus was a new detection. Abbreviations: ARI: acute respiratory infection; n/c: not calculable, no cases in the asymptomatic group. HRV: human rhinovirus, INFV: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus, HCoV; human coronavirus, HMPV: human metapneumovirus, HAdV: human adenovirus, WU-PyV: WU polyomavirus, KI-PyV: KI polyomavirus, HBoV-1: human bocavirus-1.

Table 15: Lower respiratory tract infections and asymptomatic single new virus detections, adjusted risk ratios and attributable fractions by respiratory virus in 151 children from the ORChID birth cohort (n=5,267 swabs).

Virus	LRTI-associated single new virus detections N=959 swabs	Asymptomatic single new virus detections N=4,308 swabs	Risk ratio[a] (95% CI); p-value	Attributable fraction in the exposed (%, 95% CI)[b]
Single detections	**n (%)**	**n (%)**		
All HRV	126 (13.1)[c]	328 (7.6)	1.1 (1.0, 1.2); 0.22	8 (-5, 20)
HRV-A	57 (6.0)	122 (2.8)	1.2 (1.0, 1.5); 0.11	17 (-4, 34)
HRV-B	8 (0.8)	41(1.0)	0.8 (0.4, 1.5); 0.43	-31 (-157, 33)
HRV-C	68 (7.1)	137 (3.2)	**1.3 (1.1, 1.6); 0.01**	**22 (5, 36)**
INFV-A	2 (0.2)	2 (0.0)	1.6 (0.4, 5.7); 0.50	36 (-131, 82)
INFV-B	0 (0.0)	2 (0.0)	0.0 (0.0, 0.0); <0.001	0 (0, 0)
PIV-1	2 (0.2)	1 (0.0)	2.5 (0.8, 7.8); 0.13	59 (-28, 87)
PIV-2	1 (0.1)	0 (0.0)	n/c	n/c
PIV-3	10 (1.0)	11 (0.3)	**2.0 (1.2, 3.3); 0.006**	**50 (18, 70)**
RSV-A	15 (1.6)	14 (0.3)	**3.0 (2.1, 4.5); <0.001**	**67 (51, 78)**
RSV-B	8 (0.8)	3 (0.1)	**3.2 (1.8, 5.5); <0.001**	**68 (45, 82)**
HCoV-OC43	9 (0.9)	8 (0.2)	**2.3 (1.4, 3.8); 0.001**	**56 (29, 73)**
HCoV-NL63	7 (0.7)	8 (0.2)	1.7 (0.9, 3.3); 0.10	42 (-10, 70)
HCoV-229E	1 (0.1)	4 (0.1)	1.2 (0.2, 7.7); 0.83	19 (-407, 87)
HCoV-HKU1	2 (0.2)	10 (0.2)	0.8 (0.2, 2.9); 0.71	-28 (-376, 66)
HMPV	6 (0.6)	4 (0.1)	**3.2 (1.7, 6.1); <0.001**	**69 (43, 83)**
HAdV	5 (0.5)	16 (0.4)	1.0 (0.4, 2.5); 0.96	-2 (-156, 59)
WU-PyV	5 (0.5)	15 (0.3)	1.4 (0.6, 3.5); 0.48	28 (-80, 71)
KI-PyV	8 (0.8)	34 (0.8)	1.2 (0.7, 2.2); 0.55	17 (-53, 56)
HBoV-1	10 (1.0)	31 (0.7)	1.3 (0.8, 2.2); 0.33	24 (-30, 55)
Any virus	217 (22.6)	491 (11.4)	**1.4 (1.2, 1.5); <0.001**	**27 (18, 35)**
New co-detections[d]				
≥ 2 viruses	58 (6.0)	62 (1.4)	**2.2 (1.7, 3.0); <0.001**	**55 (41, 66)**

[a]adjusted for repeated measures within children using sandwich estimators; [b]confidence intervals for attributable fractions were calculated from confidence intervals for corresponding risk ratios; [c]individual HRV species do not add up to 'All HRV' detections because of a combination of more than one HRV species detected within the same LRTI episode, or single new virus detection episodes where HRV positive specimens could not be sequenced; [d]includes 110 (51 symptomatic) new co-detections and another 10 co-detections with 2 and 3 viruses co-detected respectively where at least one co-detected virus was a new detection. Abbreviations: LRTI: lower respiratory tract infection; n/c: not calculable as there were no cases in the asymptomatic group. HRV: human rhinovirus, INFV: influenza virus, PIV: parainfluenza virus, RSV: respiratory syncytial virus, HCoV; human coronavirus, HMPV: human metapneumovirus, HAdV: human adenovirus, WU-PyV: WU polyomavirus, KI-PyV: KI polyomavirus, HBoV-1: human bocavirus-1.

Viruses and association with risk factors

Independent risk factors associated with symptomatic rather than asymptomatic VDEs, included increasing age, particularly after 6 months, and virus infections during non-summer seasons (Table 16). The regression model revealed childcare attendance was also independently associated with higher rates of VDEs, both symptomatic and asymptomatic, but no other risk factors were found to be associated with asymptomatic VDEs.

Table 16: Number of children, child-months, virus-associated symptomatic and asymptomatic episodes and association between participant characteristics and acute respiratory infections in 151 children.

Risk factor	Children (N)	Child-months (N)	Symptomatic new VDE Odds Ratio (95% CI); p-value			Asymptomatic new VDE Odds Ratio (95% CI); p-value		
			n	Unadjusted	Adjusted[a]	n	Unadjusted	Adjusted
Age (months)								
0-<3	151	423.3	60	Referent rate	Referent rate	58	Referent rate	Referent rate
3-<6	142	398.0	92	**1.6 (1.2, 2.3); 0.003**	**1.7 (1.2, 2.4); 0.003**	90	**1.7 (1.2, 2.3); 0.003**	1.5 (1.0, 2.2); 0.03
6-<12	135	715.4	354	**3.5 (2.6, 4.6); <0.001**	**3.6 (2.4, 5.4); <0.001**	146	**1.5 (1.1, 2.0); 0.01**	1.2 (0.8, 1.8); 0.46
12-<24	117	1250.6	627	**3.5 (2.7, 4.6); <0.001**	**3.1 (2.0, 4.7); <0.001**	270	**1.6 (1.2, 2.1); 0.001**	1.2 (0.7, 1.8); 0.54
Sex								
Male	74	1255.1	481	Referent rate	Referent rate	262	Referent rate	Referent rate
Female	77	1532.2	652	1.1 (1.0, 1.4), 0.17	1.1 (0.9, 1.3); 0.33	302	1.0 (0.8, 1.2); 0.79	0.9 (0.7, 1.2); 0.53
Type of delivery								
Vaginal	103	1876.3	802	Referent rate	Referent rate	387	Referent rate	Referent rate
Caesarean	48	910.9	331	0.8 (0.7, 1.0); 0.12	0.8 (0.7, 1.0); 0.09	177	0.9 (0.7, 1.2); 0.64	1.0 (0.8, 1.3); 0.94
Gestational age at birth								
Term (39w0d-41w6d)	117	2219.1	937	Referent rate	Referent rate	446	Referent rate	Referent rate
Late pre-term/early term (36w0d-38w6d)	34	568.1	196	0.8 (0.7, 1.1); 0.17	0.9 (0.7, 1.2); 0.48	118	1.1 (0.8, 1.5); 0.58	1.1 (0.8, 1.5); 0.40
Season								
Summer	140	678.8	196	Referent rate	Referent rate	138	Referent rate	Referent rate
Autumn	138	702.0	290	**1.4 (1.2, 1.7); <0.001**	**1.4 (1.2, 1.7); <0.001**	135	0.9 (0.7, 1.2); 0.64	1.0 (0.8, 1.2); 0.75
Winter	138	706.6	350	**1.7 (1.4, 2.1); <0.001**	**1.6 (1.3, 1.9); <0.001**	142	1.0 (0.8, 1.3); 0.93	0.9 (0.7, 1.2); 0.67
Spring	142	699.9	297	**1.5 (1.2, 1.8); <0.001**	**1.4 (1.2, 1.7); <0.001**	149	1.1 (0.8, 1.3); 0.68	1.1 (0.8, 1.4); 0.56

Risk factor	Children (N)	Child-months (N)	Symptomatic new VDE Odds Ratio (95% CI); p-value			Asymptomatic new VDE Odds Ratio (95% CI); p-value		
			n	Unadjusted	Adjusted[a]	n	Unadjusted	Adjusted
Family history								
None present for asthma/eczema	73	1289.6	533	Referent rate	Referent rate	286	Referent rate	Referent rate
Either parent asthma/eczema	75	1447.3	582	1.0 (0.8, 1.2); 0.93	1.0 (0.8, 1.2); 0.85	264	0.8 (0.7, 1.1); 0.16	0.9 (0.7, 1.1); 0.22
1st degree relative asthma/eczema[b]	78	1497.7	600	1.0 (0.8, 1.2); 0.80	1.0 (0.8, 1.2); 0.71	278	0.9 (0.7, 1.1); 0.22	0.9 (0.7, 1.1); 0.36
Tobacco smoke exposure								
No exposure	129	2383.4	973	Referent rate	Referent rate	472	Referent rate	Referent rate
Either parent smokes	17	307.4	127	1.0 (0.7, 1.4); 0.86	0.9 (0.6, 1.1); 0.29	75	1.2 (0.8, 1.7); 0.42	1.1 (0.8, 1.7); 0.51
Household size								
No other children in household	99	1864.9	723	Referent rate	Referent rate	350	Referent rate	Referent rate
>1 child in household	52	922.4	410	1.1 (0.9, 1.4); 0.28	1.1 (0.9, 1.4); 0.17	214	1.3 (1.0, 1.7); 0.05	1.4 (1.0, 1.7); 0.04
Maternal education status								
High school	14	246.7	113	1.1 (0.8, 1.6); 0.56	1.0 (0.8, 1.4); 0.81	62	1.4 (0.9, 2.1); 0.11	1.4 (0.9, 2.1); 0.17
Diploma/Certificate	36	650.2	245	0.9 (0.7, 1.1); 0.37	0.9 (0.7, 1.1); 0.25	149	1.2 (0.9, 1.6); 0.29	1.2 (0.9, 1.6); 0.15
University/higher university degree	95	1828.3	750	Referent rate	Referent rate	346	Referent rate	Referent rate
Mode of feeding								
Formula	142	2281.5	1039	Referent rate	Referent rate	484	Referent rate	Referent rate
Breastmilk	145	505.5	94	**0.4 (0.3, 0.5); <0.001**	1.2 (0.9, 1.7); 0.28	80	0.7 (0.6, 0.9); **0.01**	0.9 (0.6, 1.3); 0.47
Childcare attendance								
No childcare	150	1423.6	423	Referent rate	Referent rate	242	Referent rate	Referent rate
Informal childcare only	47	389.5	117	1.2 (0.9, 1.5); 0.13	0.8 (0.6, 1.1); 0.16	93	**1.3 (1.0, 1.7); 0.10**	1.3 (0.9, 1.7); 0.16
Formal and/or informal childcare	90	973.8	593	**2.2 (1.9, 2.5); <0.001**	**1.5 (1.3, 1.9); <0.001**	229	**1.4 (1.1, 1.7); 0.002**	**1.4 (1.0, 1.8); 0.03**

VDE: virus detection episode. [a]adjusted for age, sex, type of delivery, gestational age, season, family history, tobacco smoke exposure, household size, maternal education status, mode of feeding, and childcare attendance; [b]mother, father or sibling.

Table 17: Number of symptomatic and asymptomatic new virus co-detection episodes[a] (n=219).

		HRV	INFV- A	INFV- B	PIV-1	PIV-2	PIV-3	RSV A	RSV B	HCoV-OC43	HCoV-NL63	HCoV-229E	HCoV-HKU1	HMPV	HAdV	WU-HPyV	KI-HPyV	HBoV-1	Total asym	
SYMPTOMATIC DETECTIONS (n=160)	HRV		0	0	0	0	3	3	0	1	0	1	1	0	6	9	13	3	40	ASYMPTOMATIC DETECTIONS (n=59)
	INFV-A	1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	INFV-B	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	PIV-1	1	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	PIV-2	0	0	0	0		0	0	0	0	1	0	0	0	0	0	0	0	1	
	PIV-3	8	0	0	0	0		0	0	0	0	0	0	0	0	2	0	1	3	
	RSV A	6	0	0	0	0	0		0	0	0	0	0	0	0	0	0	2	2	
	RSV B	2	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	
	HCoV-OC43	1	0	0	0	0	0	0	1		0	0	0	0	1	0	0	0	1	
	HCoV-NL63	5	0	0	0	0	0	0	0	0		1	0	0	0	0	0	0	1	
	HCoV-229E	1	0	0	0	0	0	0	0	0	0		0	1	1	0	0	0	2	
	HCoV-HKU1	0	0	0	0	0	2	0	0	0	0	0		0	0	2	1	0	3	
	HMPV	1	0	0	0	0	0	1	0	0	0	0	0		0	0	0	0	0	
	HAdV	29	0	0	0	0	0	0	0	0	0	0	0	0		1	0	1	2	
	WU-HPyV	17	0	0	0	0	0	3	0	0	1	0	0	0	2		1	1	2	
	KI-HPyV	24	1	0	0	0	1	1	0	4	1	0	1	0	0	4		2	2	
	HBoV-1	32	0	0	0	0	0	1	0	0	0	0	2	0	2	2	1		59	
	Total sym	128	1	0	0	0	3	7	1	4	2	0	3	0	4	6	1	160	219	

[a] does not include 11 co-detections with 3 viruses each specimen; HRV: human rhinovirus; INFV: influenza virus; PIV: parainfluenza virus; RSV: respiratory syncytial virus; HCoV: human coronavirus; HMPV: human metapneumovirus; HAdV: human adenovirus; HPyV: human polyomavirus; HBoV-1: human bocavirus-1.

DISCUSSION

Healthy children in the ORChID community-based birth cohort experienced an incidence rate of 978 new VDEs per 100 child-years during the first two years of life, with HRV playing a dominant role. This high rate of viral events equated to almost 10 new viral detection episodes per year and includes both ARI episodes and episodes of asymptomatic virus shedding. Overall, respiratory viruses were detected by PCR in regular weekly swabs at least once during 75% of ARIs, while in asymptomatic periods 23% of weekly nasal swabs were positive by PCR for respiratory viruses, accounting for 33% of all new VDEs. We demonstrated evidence of attribution for ARIs by RNA viruses, which included HRV-A, HRV-C, INFV-A, PIV-1, PIV-3, RSV-A, RSV-B, HCoV-OC43, HCoV-NL63, and HMPV; and HAdV as the single DNA virus representative. All these viruses were detected significantly more often in children with an ARI than during asymptomatic periods and had statistically significant positive AFEs. The strongest associations with LRTI were observed for RSV and HMPV. Increasing age ≥6 months, non-summer months, and childcare attendance were independent risk factors for symptomatic VDEs. Age as a risk factor for symptomatic infection independent of childcare attendance is also noted in other studies (229), and is presumably due to protection afforded by maternal transplacental and breastmilk antibodies in the very young (292). Environmental risk factors of season and childcare attendance for ARIs may be related to the intensity and opportunity for exposure in these settings experienced by young children (147, 299).

The virus-specific attribution of ARIs was quantified by identifying VDEs during asymptomatic periods and calculating the virus-specific AFE, an infrequently reported metric for helping to determine the relative causal roles of different viruses at a population level. A limitation of the AFE approach is that it cannot inform on the aetiology at an individual level: in individual cases it does not differentiate between those with a true positive test identifying the virus responsible for the ARI and those with a false positive result from asymptomatic virus shedding. Nevertheless, knowing the AFE for respiratory viruses can help inform clinicians on the likely clinical significance of detecting one or more viruses in nasal swab specimens taken from infants or young children during an ARI episode, by assigning the group aetiological fraction for viruses detected in that child. Importantly, AFE estimates can be applied to studies of ARIs in different settings to estimate the true burden of disease in the

community, thus informing which viruses should be prioritised in future public health policies and interventions.

Most previous studies have overestimated AFE values by using odds ratios, rather than risk ratios, in calculations (22, 151, 182, 296). Odds ratios overestimate the effect size when the event is not rare, as occurs with ARIs in children. These studies are principally cross-sectional, hospital-based, and often have poorly matched controls. Consequently our estimates of AFE being lower than reported elsewhere is not surprising, and, we believe, are likely to provide more accurate estimates of the ARI and LRTI burden attributable to these viruses in the broader paediatric population.

The proportion of asymptomatic infections in our study highlights the risks of extrapolating hospital-based data from sick children to the community setting. Previously, the significance of detecting viruses by PCR was tempered by concerns that this may be from an incubating or unrelated subclinical infection or represent continued virus nucleic acid shedding from a recent ARI that has resolved. The intensive longitudinal specimen and symptom sampling from our cohort, as well as the definitions of VDEs and symptomatic VDEs used, helped identify nascent and subclinical infections. In this context, our observations emphasise the frequency of subclinical infections by respiratory viruses in young children, who when otherwise well, may act as important ‘silent’ reservoirs of infection. Similar observations and conclusions were reported recently in a household study involving both child and adult contacts of symptomatic cases of laboratory-confirmed influenza (102). Nevertheless, in addition to influenza, established viruses, such as HRV, PIV-3, RSV, and HMPV are also important community respiratory pathogens, especially the latter two viruses and potential candidates for vaccine and other therapeutic interventions (300). This contrasts with the novel DNA viruses, HPyVs and HBoV-1, which have low AFEs calculated for both ARIs and LRTIs, despite their higher detection rates (202, 301). However, as suggested by this cohort, synergy may exist between multiple viruses causing ARIs. This involved mostly HRV and DNA viruses and warrants further study.

Strengths and limitations

The 78% of expected child-days and 68% of expected swab returns was a very good retention rate given the intensive and prolonged nature of the study for participating families. Analysis of incidence rates of ARI in participants that withdrew from the study

at different periods showed no clear association between time of drop-out and ARI incidence. Regular parent-collected nasal swabs avoids the need for home visits by research staff or clinic attendance during an ARI, minimising possible biased estimates of ARI events and specimen availability from losing families failing to seek healthcare. Moreover, regular weekly nasal swab collections increase the likelihood of virus detections during an ARI episode rather than relying simply upon results from a single specimen. Our previous work found that with sensitive PCR techniques, parent-collected nose or nose-throat swabs sent to the laboratory by standard mail had comparable sensitivity to nasopharyngeal swabs obtained by healthcare workers (246, 256, 302). Longitudinal data collection also allows assessment of asymptomatic status by considering past and future illness history, as some viruses are detectable days before and after ARI symptoms develop (303), and are therefore less likely to yield false positive findings.

There are also some important limitations to consider. To reduce the chances of false negative test results (249), we excluded from analysis 2,679 (23.9%) swabs where the internal control for human DNA, ERV-3, was either undetected or present at very low levels. We excluded both virus-positive and -negative swabs to avoid over-estimating our incidence and prevalence rates. In addition, some viruses were detected rarely, in particular influenza, a finding reflected in other community studies (3), but nevertheless limiting our ability to provide precise AFE estimates for these agents. We also excluded co-detections in individual virus assessments as multiple aetiological agents make individual contributions of each agent difficult to ascertain, but analysed them separately to look at the association patterns of individual viruses. Symptom information, other than doctor-diagnosed otitis media and pneumonia, captured by daily diaries was not validated. To maximise accuracy, parents were trained to recognise symptoms before commencing the study. While healthcare worker-validated symptoms would be ideal, a study of this scale is logistically challenging without parent participation. This study design has been used previously (302) and with others have shown that parents can be trained to recognise symptoms of interest (302), often as reliably as health professionals (284, 285). Our rates of ARI, including those associated with wheeze, are comparable with other community studies (233, 237).

Finally, as is common for these types of studies, families in our cohort were from more advantaged backgrounds and ARI episodes were predominantly of a mild-moderate nature (6). While many children in our study were first-born and from advantaged families, factors thought to decrease the rate of ARIs, secular changes in Australia have led to an increased proportion of children from these families attending childcare at an earlier age than other sectors of the population. A slightly higher proportion (80–85%) of our cohort attended some form of care by the age of 15–24 months, compared to 74% of children in the Australian population, with childcare attendance an independent risk factor for ARIs (299). Moreover, the incidence of ARIs in the ORChID cohort (6) is comparable to other reports in this age group (28, 230, 233) and RSV and HRV-associated ARIs approximate other community-based studies conducted in more temperate climates of Australia (Perth) and Europe (2, 220, 222). Our findings for this cohort remain valid and provide important estimates on community respiratory virus exposures and ARIs in Australian children in a subtropical, urban setting.

Conclusions

Respiratory viruses, particularly HRV, were detected with remarkable frequency in healthy Australian children in the first two years of life, with at least one-third of new VDEs unaccompanied by symptoms. With molecular methods used increasingly to detect respiratory viruses in young children, our study provides insights into supporting attribution for virus-specific ARIs in this age group managed within the community. The established RNA respiratory viruses and HAdV were more strongly associated with symptomatic than asymptomatic infections, while no such associations were found for the newly described DNA viruses, HPyVs and HBoV-1. These data emphasise the high community levels of HRV circulating amongst young children. Importantly, they also provide evidence to suggest that the current clinical trials of candidate RSV and HMPV vaccines seeking to protect infants against severe disease and hospitalisation might also deliver substantial health benefits for all young children within the community (300).

Chapter 6: Timing of first respiratory virus detections in infants: a community-based birth cohort study

This chapter is presented as a published original article.

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Keywords: respiratory viruses; infant; primary infection; human rhinovirus; respiratory syncytial virus; human metapneumovirus; cohort study.

ABSTRACT

Introduction:

Determining timing of first virus detection episodes (fVDEs) for different respiratory viruses in infants identifies risk periods and informs preventive interventions, including vaccination. We describe the ages and nature of fVDEs in an infant birth cohort and explore factors associated with increased odds of symptomatic fVDEs.

Methods:

The Observational Research in Childhood Infectious Diseases (ORChID) study is a community-based birth cohort describing acute respiratory infections in young children until their second birthday. Parents recorded daily symptoms and collected nose swabs weekly, which were batch-tested using polymerase chain reaction assays for 17 respiratory viruses.

Results:

One hundred fifty-eight children participated in ORChID. The median age for fVDEs was 2.9 months for human rhinovirus (HRV) but was ≥13.9 months for other respiratory viruses. Overall, 52% of HRV fVDEs were symptomatic, compared with 57%-83% of other fVDEs. Respiratory syncytial virus and human metapneumovirus fVDEs were more severe than HRV fVDEs. Older age and the winter season were associated with symptomatic episodes.

Conclusions:

Children do not always experience respiratory symptoms with their fVDE. Predominance of early HRV detections highlights the need for timing any intervention early in life. fVDEs from other respiratory viruses most commonly occur when maternal vaccines may no longer provide protection.

INTRODUCTION

Despite better living conditions, improved nutrition, and greater access to healthcare and vaccine programs, ARIs remain the most common illness experienced by people of all ages (1). Viruses are the most frequent cause of ARIs and the highest incidence rates occur during the first two years of life where on average young children experience 6-8 episodes per annum (5, 6). While only 3-5% of all children are hospitalised for viral ARIs (269), they are responsible for 25% of all hospitalisations in this age group imposing a major burden and cost to the health system for children (9). With the overwhelming majority of cases managed solely in the community (6), ARIs in young children also levy considerable additional costs upon families and society (10, 11).

Much of our recent understanding of the epidemiology of viral ARIs in young children relies upon hospital-based studies (21, 22). These emphasize RSV as the predominant cause of bronchiolitis and the most common reason for infants to be hospitalised in the first year of life (70, 71), and seasonal INFVs, which also have high hospitalisation rates in this age group (71, 89). Importantly, the highest hospitalisation rates for ARIs associated with both viruses are during the first six months of life (89, 304-306). However, hospital-based surveillance systems capture only the most severe illnesses and under-estimate the substantial burden of disease within the community caused by these and other respiratory viruses in older infants and young children (23-25). Indeed, community-based birth cohort studies of infants at high-risk of asthma have instead identified HRVs, ahead of RSV and INFVs as the major upper and lower respiratory pathogens in the first year of life (2). Moreover, those with HRV-induced wheezing from an early age are also at increased risk of developing asthma, especially if already sensitised to aeroallergens (123, 124, 307).

Although evidence is limited, first infections with respiratory viruses, particularly those caused by RSV, are thought to be almost always symptomatic (308) and by implication more likely to be brought to medical attention. As a large disease burden may be missed by hospital-based studies, understanding community epidemiology is essential when planning effective control measures. Consequently, in light of the aforementioned studies it is important to determine the timing different respiratory viruses are first detected in otherwise healthy young infants. This will help identify

when risk periods commence and to inform future public health interventions, including maternal and infant immunisation strategies for this age group.

Recently, we reported that healthy, term-born infants participating in a community-based birth cohort study had an average 0.25 (95% CI 0.18, 0.34) respiratory virus detection episodes in the first 28 days of life, of which 72% were HRV, with 45% of these being asymptomatic (309). In view of this early exposure to respiratory viruses, we now report findings for virus detection during the first two years of life by describing the ages at which each of nine respiratory virus groups, comprising 17 different species and subtypes, were first detected in this birth cohort and in a setting where influenza vaccines are not part of the national infant immunisation program. For each of the nine viruses, we determined: the nature of the respiratory symptoms associated with their first detection, including virus shedding characteristics and healthcare use; and whether sociodemographic and seasonal factors were associated with increased odds of developing respiratory symptoms at the time of the first detection episode.

MATERIALS AND METHODS

Details of the study population, demographic and symptom data collection, the ARI burden diary, classification of ARIs, anterior nasal swab sample collection, transport, processing, and testing have been outlined in Chapter 2.

Definitions

fVDE: when a respiratory virus not detected previously was first found in an infant's weekly nasal swab specimen.

Symptomatic fVDE: when respiratory symptoms were reported within seven days either side of first detecting the virus of interest in the weekly nasal swab.

Symptomatic fVDEs were sub-categorized hierarchically as LRTI or URTI episodes respectively.

Definition of symptom duration for an ARI episode associated with the first detection of a respiratory virus.

If symptoms began within seven days of first detecting the virus, the commencement of the ARI episode was marked from the day of symptom onset and its duration counted until the day the ARI symptoms ceased and the infant remained symptom free for at least the next three days.

If ARI symptoms began more than seven days before detecting a new positive virus swab, the beginning of the episode associated with the virus was judged to be either seven days before its detection or up to the time of the previous swab collection irrespective of its result (i.e. negative or positive for a different virus or virus species), whichever time interval was the shorter. Alternatively, if no prior swabs had been taken since the symptomatic ARI episode began, the timing of the episode was from the day of symptom onset and continued until symptoms ceased. Similarly, if following a positive swab, subsequent specimens were not taken during the same ARI episode, the duration of the episode was determined by when symptoms ceased. However, if during a single continuous symptomatic ARI episode additional viruses were co-detected sequentially in successive weekly samples, the symptoms were attributed equally to each virus until seven days after the virus was no longer detected or the symptoms settled, whichever came sooner. This was done to avoid over-estimating the episode length attributed to the virus that was detected initially.

Analysis

Summary statistics are presented as medians (25^{th} – 75^{th} percentile) for continuous variables and frequency (percentage) for categorical variables. First analysed separately, HRV, RSV, PIV, INFV, HCoV and HPyV subtypes and species were then grouped for additional analyses. The time-to-fVDE and symptomatic fVDE for each virus were calculated using life tables with the day of birth as study entry time. Children were censored at either the date of the last swab submitted if the next swab was not returned for more than 30 days, or at 730 days, whichever came sooner. The association between symptomatic/asymptomatic fVDEs and both the Ct value and length of shedding were compared using Mann-Whitney tests. For clinical characteristics of fVDEs, associations between virus types and symptoms were

compared using absolute risk differences for binary outcomes, and median regression for continuous outcomes. In all cases HRV was the reference virus. Thus, the risk differences were the proportion of children with first viral detection episodes with, for e.g., RSV who had symptoms of ARI divided by the proportion of children with first viral detection episodes with HRV who had symptoms of ARI. The association between potential risk factors (age, sex, season, breastfeeding, childcare attendance, number of children in the household) and symptomatic fVDEs was examined for the nine respiratory virus groups using logistic regression. Risk factors were categorised as: age (0-<3 months; 3-<6 months; 6-<12 months, and 12-24 months); sex (male, female); season (winter (June to August); spring (September to November); summer (December to February); fall (March to May)); breastfeeding (exclusively breastfed for ≥4 months, exclusively breastfed for <4 months or never breastfed); childcare attendance (no childcare, any form of childcare); number of children in the household (no other children, at least one other child). Both univariable and multivariable models were constructed. Multivariable models were adjusted for all variables listed above. Data were analysed using Stata v12·1 (StataCorp, TX, USA).

RESULTS

Cohort characteristics

Of 891 potential participants approached, 165 (18·5%) eligible infants from 163 families were enrolled, with seven subsequently excluded for protocol breaches (Figure 10). The remaining 158 children provided 11,192 swabs (68.3% of maximum expected; median 84·5, range 1-104). Subsequently, 1,327 swabs were censored due to a gap of >30 days between successive swabs, as were another 67 swabs submitted after the child's second birthday, leaving 9,798 swabs to be included in the full analysis (Figure 10, Figure 11). A further 204 swabs without corresponding symptom data were excluded from the analysis leaving 9,594 swabs describing the association between symptoms and fVDEs. Compared to the general population, study children were from smaller families of more advantaged backgrounds (Table 18).

Figure 10: Number of nasal swabs submitted by participants of the Observational Research in Childhood Infectious Diseases Study.

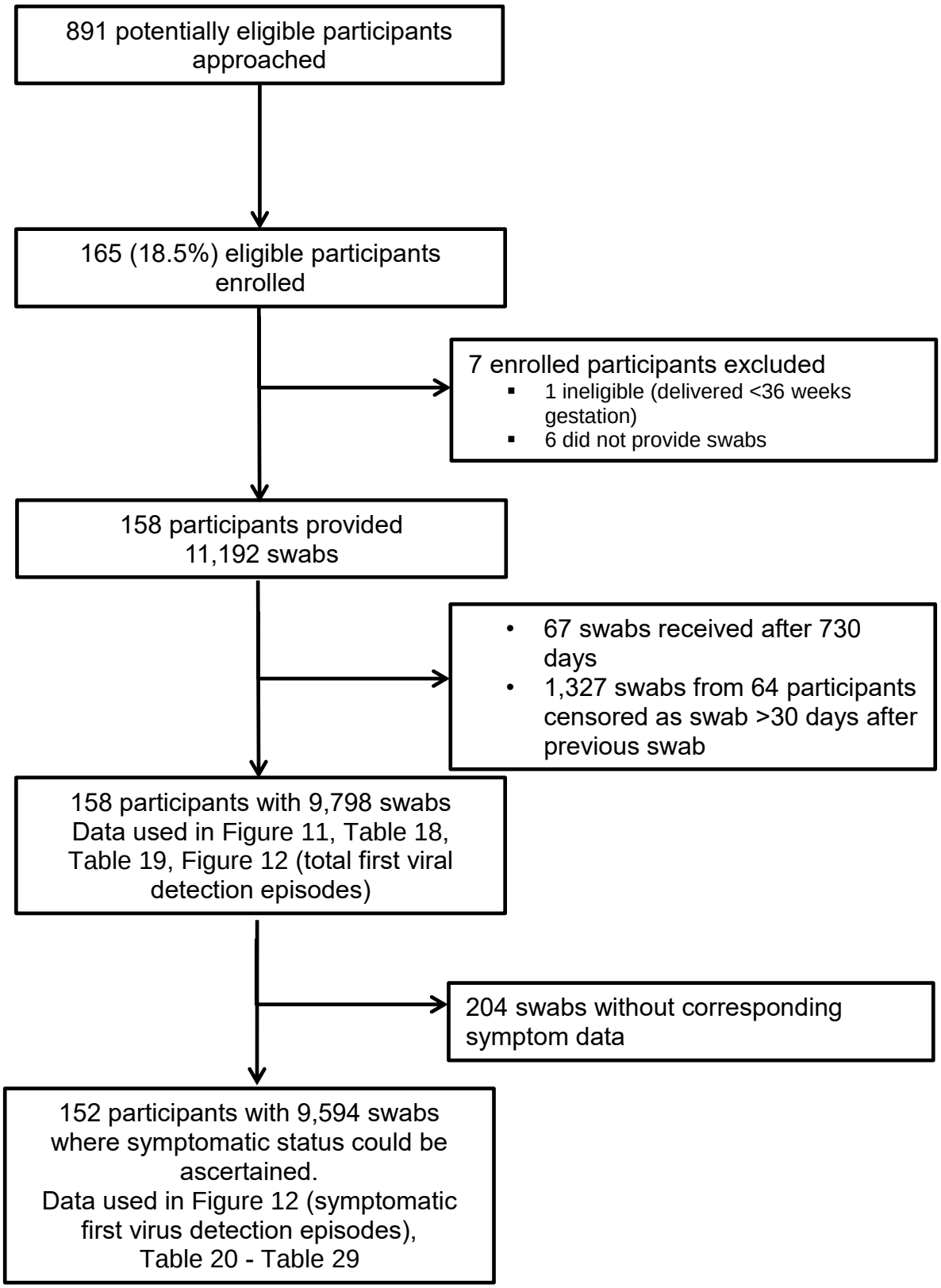
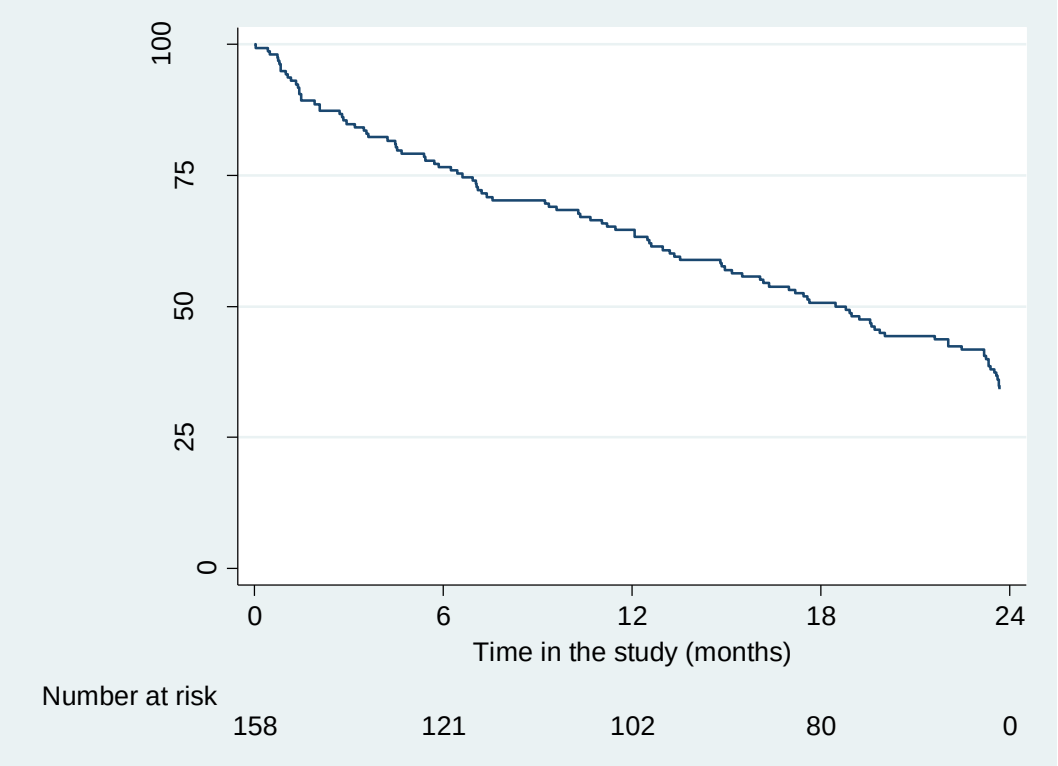


Figure 11: Kaplan-Meier curve of proportion of children contributing swabs to time to first virus detection episode analysis in the Observational Research in Childhood Infectious Diseases Study (N=158).



Footnote: Children left the study when they did not return a swab for more than 30 days, or at age two years, whichever was sooner.

Table 18: Characteristics of children enrolled in the Observational Research in Childhood Infectious Diseases Study.

Characteristic	No. of children (%) N=158	No. of swabs (%)[a] N=9,798
Sex (male)	75 (47·5%)	4,366 (44.6%)
First-born child in household	106 (67·1%)	6,596 (67.3%)
Smoke exposure at birth		
Mother (n=156)	5 (3·2%)	184 (1.9%)
Other householder (n=155)	17 (11·0%)	971 (9.9%)
Maternal educational status (n=157)		
Primary school	5 (3.2%)	150 (1.5%)
High school	15 (9·6%)	822 (8.4%)
Diploma/Certificate	38 (24·2%)	2,244 (22.9%)
University degree or higher	99 (63.1%)	6,582 (67.3%)
Exclusive breastfeeding		
At 4 months (n=143)[b]	84 (58.7%)	5,924 (60.8%)
Childcare[c]		
At 12 months (n=115)	72 (62.6%)	6,180 (65.1%)

[a]children were censored at either the date of the last swab submitted if the next swab was not returned for more than 30 days, or at 730 days, whichever came sooner; [b]15 participants withdrew from the study before four months; [c]formal and/or informal childcare.

First virus detection episodes

At least one virus was detected in 2,542/9,798 (25.9%) swabs, while co-detections of two or more viruses were observed in 241 (2.5%) specimens. HRV was the earliest and most commonly detected virus (present in 1,964/2,542 (77.3%) positive swabs). Table 19 and Figure 12 show that HRV-C (found in 98% of children) and HRV-A (94%) were more frequently detected than HRV-B (56%) by age two years and at a younger age (median age of fVDEs was 6.7, 6.1, and 19.6 months for each HRV species, respectively). HRV was detected as early as two days of life and by age three months 50% of the cohort had HRV detected at least once.

Other respiratory viruses were not detected as often in the first six months of life (Table 19 and Figure 12). However, by their second birthday, 50%-60% of the cohort had experienced their fVDEs by RSV, PIV-3 and HAdV species, and 70-77% of cohort subjects had HCoV, HPyV KI/WU, and HBoV-1 virus species detected on at least one occasion. Influenza virus, PIV-1, and PIV-2 were detected in $\leq$11% of the cohort.

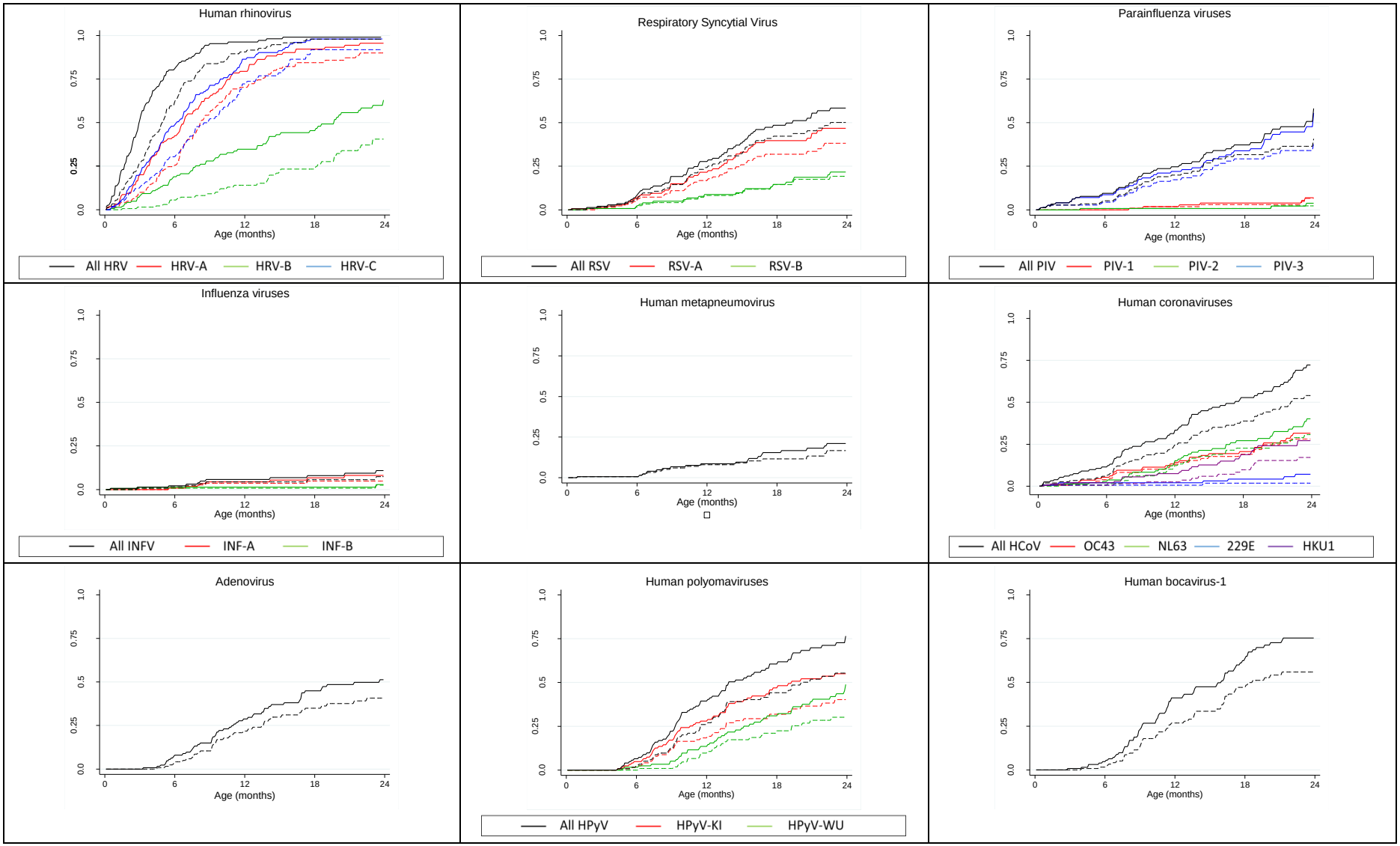
Table 19: Time to first detection of respiratory viruses collected from 158 children in the first two years of life in the Observational Research in Childhood Infectious Diseases (ORChID) birth cohort (n=9,798 swabs).

Virus	Virus positive swabs[a] N=9,798	Age at first virus detection (months)[b]	First virus detection proportions			
	n (%)	Median (25th, 75th centile)	(cumulative % by age in months)			
			6	12	18	24
HRV combined[c]	**1,964 (20.0)**	**2·9 (1·6, 5·1)**	**81·1**	**96·3**	**99·1**	**99·1**
HRV-A	652 (6.7)	6.7 (3.7, 10.7)	42.4	79.5	92.2	94.4
HRV-B	150 (1.5)	19.6 (7.7, -)	18.9	34.7	45.4	63.0
HRV-C	599 (6.1)	6.1 (3.5, 10.1)	48.8	86.4	98.1	98.1
RSV combined	**88[d] (0.9)**	**19.4 (11.2, -)**	**8.5**	**27.7**	**48.6**	**58.4**
RSV-A	67 (0·7)	- (13·3, -)	7·0	21·6	39·7	46·8
RSV-B	22 (0·2)	- (-, -)	2·4	8·7	14·6	21·7
PIV combined	**71 (0.7)**	**23.2 (12.4, -)**	**9.4**	**24.7**	**37.3**	**58.2**
PIV-1	6 (0·1)	-	0·0	1·8	3·9	6·9
PIV-2	3 (0·0)	-	0·8	0·8	0·8	3·7
PIV-3	62 (0·6)	23·9 (14·2, -,)	8·7	22·1	33·9	55.7
INFV combined[d]	**11 (0.1)**	**-**	**2.2**	**5.8**	**8.0**	**10.9**
INFV-A	8 (0·1)	-	0·8	4·4	6·7	8·0
INFV-B	3 (0·0)	-	1·4	1·4	1·4	2·9
HMPV	**24 (0·2)**	**-**	**0·7**	**8·6**	**15·4**	**21·1**

Virus	Virus positive swabs[a] N=9,798	Age at first virus detection (months)[b]	First virus detection proportions			
	n (%)	Median (25th, 75th centile)	(cumulative % by age in months)			
			6	12	18	24
HCoV combined	**138 (1.4)**	**17.2 (9.1, -)**	**11.5**	**33.4**	**52.9**	**72.2**
HCoV-OC43	43 (0·4)	- (19·7, -)	5·3	14·2	20·6	31·6
HCoV-NL63	52 (0·5)	- (16·8, -)	2·9	15·1	27·1	40·2
HCoV- 229E	10 (0·1)	-	2·0	2·0	4·3	7·2
HCoV-HKU1	33 (0·3)	- (22·7, -)	2·0	7·4	18·8	27·2
HAdV	**95 (1·0)**	**23·5 (11·0, -)**	**8·0**	**28·7**	**44·9**	**51·3**
HPyV combined	**250[e] (2.6)**	**13.9 (9.3, 23.9)**	**6.4**	**39.4**	**60.6**	**76.6**
HPyV-KI	157 (1.6)	19·2 (10·6, -)	4·8	28·0	46·9	55·0
HPyV-WU	98 (1·0)	- (15·1, -)	1·6	13·5	30·9	48.9
HBoV-1	**138 (1·4)**	**16·0 (9·2, 21·3)**	**4·7**	**41·2**	**63·7**	**75·4**

[a]includes co-detections; children were censored if a swab was not returned for more than 30 days;
[b]age at which 50% (25%, 75%) of the cohort have had their first detection with the virus; a dash in the median (25th centile, 75th centile) space reflects fewer than 50% (25%, 75%) of children were infected with this virus;
[c]562 isolates were not able to be typed, 1 swab extract missing and not sequenced; [d] includes 1 RSV-A/RSV-B co-detection; [e] includes 5 HPyV-KI/HPyV-WU co-detections.
[d]Influenza vaccines are not part of the Australian National Immunisation Program; 28 (17.7%) mothers of children in the ORChID cohort had the influenza vaccine during their pregnancy, and just seven children received the influenza vaccine in the first two years of life [3].
Abbreviations: HAdV, human adenovirus; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HMPV, human metapneumovirus; HPyV, human polyomavirus; HRV, human rhinovirus; INFV, influenza virus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Figure 12: Time to first respiratory virus detection episode by virus and subtype/species.



Solid line represents all censored first detections for that respiratory virus or virus sub-type; dashed lines represent all censored symptomatic first detections; Abbreviations: HCoV: human coronavirus; HPyV: human polyomavirus; HRV: human rhinovirus; INFV: influenza virus; PIV: parainfluenza virus; RSV, respiratory syncytial virus.

Nature of first virus detection episodes

While fVDEs for HRV were symptomatic 52% of the time, infections with the other RNA viruses and HAdV had symptoms for 69-83% of these episodes (Table 20, Table 21). fVDEs with other DNA viruses, HBoV-1, and HPyV WU and KI, were symptomatic 57-66% of the time. Symptomatic fVDEs with RSV and HBoV-1 had significantly lower Ct values (higher viral loads) in their nasal swabs than corresponding asymptomatic fVDEs (Table 20). No such associations were observed with Ct values for the other viruses and no differences in virus shedding duration was seen between symptomatic and asymptomatic fVDE groups.

Table 21 shows that children with RSV and HMPV fVDEs had the highest proportions of LRTIs (46.3% and 50.0% respectively). Compared with HRV, RSV, PIV, HMPV, HCoV, and HAdV fVDEs were significantly more likely to be symptomatic and for HAdV and HPyV symptomatic fVDEs to last longer. Overall, 128 of 527 (24.3%) fVDEs resulted in medical visits and antibiotics were prescribed in 63 (49.2%) of these illness episodes. Compared with HRV, those with RSV, PIV, HMPV, HCoV, HAdV and HBoV-1 symptomatic fVDEs were significantly more likely to seek medical advice and for those with RSV, INFV, HAdV, HPyV and HBoV-1 to receive antibiotics. While family physician consultations were common, children were infrequently taken to the ED or hospitalised, and there were no deaths in the cohort.

Table 20: Number of symptomatic and asymptomatic first respiratory virus detection episodes, virus-specific median cycle threshold values, and median duration of virus shedding, in the first two years of life in 152 children participating in the ORChID birth cohort (n=9,594 swabs).

	First respiratory virus detection episodes								
		Symptomatic episodes			Asymptomatic episodes				
Virus (No.)	**Children with single positive swab, %[a]**	**Children (No.)**	**Median (range) Ct value**	**Median (range) virus shedding (weeks)[a]**	**Children (No.)**	**Median (range) Ct value**	**Median (range) virus shedding (weeks)[a]**	**p-value[b]**	**p-value[c]**
HRV(130)	47.7	68	29.2 (18.8, 39.8)	1.5 (1, 9)	62	29.2 (20.6, 39.1)	2 (1, 11)	0.5	0.4
RSV (54)	83.3	42	29.3 (22.3, 37.3)	1 (1, 3)	12	36.0 (27.4, 39.6)	1 (1, 3)	**0.003**	0.5
PIV (52)	94.2	37	31.0 (22.0, 38.5)	1 (1, 2)	15	31.4 (26.0, 38.3)	1 (1, 1)	0.3	0.3
INFV (11)	100.0	8	31.9 (29.5, 38.2)	1 (1, 1)	3	30.2 (29.6, 30.3)	1 (1, 1)	0.2	n/c
HMPV (18)	83.3	15	33.7 (23.8, 39.2)	1 (1, 2)	3	34.4 (34.0, 35.5)	1 (1, 1)	0.5	0.4
HCoV (67)	74.6	47	29.3 (21.5, 38.9)	1 (1, 3)	20	28.8 (21.4, 38.5)	1 (1, 2)	0.8	0.06
HAdV (52)	76.9	36	31.3 (18.6, 39.4)	1 (1, 7)	16	33.6 (24.4, 39.1)	1 (1, 2)	0.7	0.05
HPyV (70)	37.1	40	27.5 (19.0, 39.1)	2.5 (1, 7)	30	32.2 (18.4, 39.3)	2 (1, 5)	0.1	0.2
HBoV-1 (73)	56.2	48	28.0 (16.9, 38.4)	2 (1, 4)	25	31.6 (18.1, 38.9)	1 (1, 4)	**0.001**	0.3

[a]Nasal swabs collected weekly by parents. [b] Mann-Whitney test comparing median Ct values for the two groups.[c] Mann-Whitney test comparing median shedding duration for the two groups. Abbreviations: HAdV, human adenovirus; Ct, cycle threshold; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HMPV, human metapneumovirus; HPyV, human polyomavirus; HRV, human rhinovirus; INFV, influenza virus; N/c, not calculable; No., number; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Table 21: Clinical characteristics of the first respiratory virus detection episodes in the first two years of life in 152 children participating in the ORChID birth cohort (n=9,594 swabs).

Virus[a]	No. of children	ARI episodes URTI/ LRTI,[b] %	Risk difference[c] for ARI episodes, % (95%CI)	Median ARI symptom duration, days (IQR)	Median difference of ARI episode duration, days (95%CI)[d]	Medical visits FP/ED/ Hospitalised[e] %	Risk difference for medical visits, % (95%CI)	Antibiotics after any medical visit[f] (%)	Risk difference for antibiotics %, (95%CI)
HRV	130	46.2/ 6.2	0.0 (ref)	5.5 (3, 10)	0.0 (ref)	13.1/ 0.0/ 0.8	0.0 (ref)	23.5	0.0 (ref)
RSV	54	31.5/ 46.3	**25.5 (11.4, 39.5)**	9 (7, 11)	1.8 (-0.2, 6.2)	37.0/ 5.6/ 1.9	**24.0 (9.8, 38.1)**	55.0	**34.4 (4.4, 64.4)**
PIV	52	50.0/ 21.2	**18.8 (3.8, 33.9)**	9 (4, 14)	1.8 (-0.4, 6.4)	26.9/ 1.0/ 0.0	**15.8 (2.2, 29.4)**	35.7	12.2 (-20.0, 44.4)
INFV	11	54.5/ 18.2	20.4 (-7.3, 48.1)	11 (7, 16.5)	1.9 (-0.2, 12.2)	36.4/ 0.0/ 0.0	23.3 (-5.7, 52.3)	75.0	**51.5 (4.5, 98.5)**
HMPV	18	33.3/ 50.0	**31.0 (11.8, 50.2)**	9 (6, 11)	1.3 (-1.7, 7.7)	33.3/ 5.6/ 0.0	**20.3 (1.7, 25.9)**	33.3	9.8 (-33.0, 52.6)
HCoV	67	46.3/ 23.9	**17.8 (3.9, 31.8)**	9 (3, 13)	1.9 (-0.1, 6.1)	25.4/ 4.4/ 0.0	**13.8 (1.7, 25.9)**	50.0	26.5 (-4.2, 57.1)
HAdV	52	44.2/ 25.0	**16.9 (1.7, 32.1)**	10 (5, 13.5)	**2.9 (1.6, 8.4)**	32.7/ 0.0/ 0.0	**19.6 (5.6, 33.6)**	58.8	**35.3 (4.4, 66.2)**
HPyV	70	41.4/ 15.7	4.8 (-9.7, 19.3)	11 (4, 17.5)	**3.0 (1.7, 8.3)**	15.7/ 0.0/ 0.0	4.1 (-6.5, 14.6)	66.6	**43.1 (9.7, 76.6)**
HBoV-1	73	50.7/ 15.1	13.4 (-0.4,27.3)	9 (5, 14)	1.9 (-0.1, 8.0)	26.0/ 2.7/ 0.0	**13.0 (1.3, 24.6)**	57.9	**34.4 (4.4, 64.4)**

[a]Detected in nasal swabs collected weekly by parents. [b]Mutually exclusive hierarchical classification of ARI episodes (LRTI>URTI). [c] Risk difference is the proportion of children with first viral detection episodes with a virus who had symptoms of ARI divide by the proportion of children with first viral detection episodes with HRV (reference) who had symptoms of ARI. [d]Median difference of ARI episode duration calculated by quantile regression technique. [e]Medical visits were not mutually exclusive categories as an infant may have more than one medical encounter in different settings during a single ARI episode. [f]Medical visits and antibiotic information were derived from the ARI burden diary triggered in 223/233 (95.7%) eligible recorded ARI episodes. Abbreviations: HAdV, human adenovirus; ARI, acute respiratory infection; asym, asymptomatic; ED, Emergency Department; FP, family physician; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HMPV, human metapneumovirus; HPyV, human polyomavirus; HRV, human rhinovirus; INFV, influenza virus; IQR, interquartile range; LRTI, lower respiratory tract infection; No., number; PIV, parainfluenza virus; ref, reference; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection.

Because virus co-detections may confound individual virus contributions to ARI symptoms, analyses of Ct values, shedding duration and clinical characteristics were repeated for single only fVDEs (Table 22 and Table 23). Overall, these gave similar results, although DNA rather than RNA virus fVDEs were significantly more likely to have virus co-detections (risk ratio 2.15 (95%CI 1.62, 2.78). A separate analysis found that single fVDEs with DNA viruses were consistently less likely to be symptomatic than corresponding fVDEs when other viruses were also present, reaching statistical significance for HPyV-KI and HBoV-1 (Table 24).

Table 22: Number of symptomatic and asymptomatic single first respiratory virus detection episodes, virus-specific median cycle threshold values, and median duration of virus shedding, in the first two years of life in 152 children participating in the ORChID birth cohort (n=9,594 swabs).

	Single first respiratory virus detection episodes								
Virus (No.)	**Symptomatic episodes**				**Asymptomatic episodes**				
	Children with single positive swab, %[a]	**Children (No.)**	**Median (range) Ct value**	**Median (range) virus shedding (weeks)[a]**	**Children (No.)**	**Median (range) Ct value**	**Median (range) virus shedding (weeks)[a]**	**p-value[b]**	**p-value[c]**
HRV(128)	47.7	67	29.2 (18.8, 39.8)	2 (1, 9)	61	29.2 (20.6, 39.1)	2 (1, 11)	0.5	0.4
RSV (43)	83.7	34	29.3 (22.3, 37.3)	1 (1, 3)	9	36.3 (29.4, 39.6)	1 (1, 3)	**0.003**	0.8
PIV (35)	97.1	26	31.1 (22.0, 37.5)	1 (1, 2)	9	31.4 (30.1, 38.3)	1 (1, 1)	0.3	0.6
INFV (10)	100.0	7	31.4 (29.5, 38.2)	1 (1, 1)	3	30.2 (29.6, 30.3)	1 (1, 1)	0.4	N/c
HMPV (13)	76.9	11	33.7 (23.8, 36.4)	1 (1, 2)	2	34.2 (34.0, 34.4)	1 (1, 1)	0.9	0.4
HCoV (55)	76.4	39	29.2 (21.5, 38.9)	1 (1, 3)	16	28.0 (21.4, 38.5)	1 (1, 2)	0.8	0.05
HAdV (29)	75.9	17	31.5 (18.6, 39.4)	1 (1, 7)	12	31.8 (24.4, 38.9)	1 (1, 2)	0.7	0.09
HPyV (39)	46.2	18	27.5 (22.0, 34.9)	2 (1, 7)	21	31.3 (18.4, 37.9)	1 (1, 4)	0.1	0.2
HBoV-1 (46)	63.0	25	28.7 (16.9, 38.1)	1 (1, 4)	21	32.7 (26.6, 38.9)	1 (1, 4)	**0.004**	0.2

[a]Nasal swabs collected weekly by parents. [b] Mann-Whitney test comparing median Ct values for the two groups. [c] Mann-Whitney comparing median shedding duration for the two groups. Abbreviations: HAdV, human adenovirus; Ct, cycle threshold; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HMPV, human metapneumovirus; HPyV, human polyomavirus; HRV, human rhinovirus; INFV, influenza virus; N/c, not calculable, No., number; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Table 23: Clinical characteristics of the single first respiratory virus detection episodes in the first two years of life in 152 children participating in the ORChID birth cohort (n=9,594 swabs).

Virus	No. of children	ARI episodes URTI/ LRTI,[b] %	Risk difference for ARI episodes, % (95%CI)	Median ARI symptom duration, days (IQR)[c]	Median difference for ARI episode duration, days (95%CI)	Medical visits FP/ED/ hospitalised[d, e] %	Risk difference for medical visits, % (95%CI)	Antibiotics after any medical visit[e] (%)	Risk difference for antibiotics %, (95%CI)
HRV	128	46.9 / 5.5	0.0 (ref)	5 (3, 10)	0.0 (ref)	12.5 / 0.0 / 0.8	0.0 (ref)	18.8	0.0 (ref)
RSV	43	37.2 / 41.9	**26.7 (11.8, 41.6)**	9 (7, 11)	**2.3 (0.5, 7.5)**	34.9 / 7.0 / 2.3	**20.1 (4.9, 35.2)**	42.9	24.1 (-8.1, 56.3)
PIV	35	54.3 / 20.0	**21.9 (5.1, 38.8)**	9 (4, 13)	**2.6 (1.2, 8.8)**	28.6 / 2.9 / 0.0	**18.9 (2.5, 35.3)**	18.2	1.3 (-30.1, 32.6)
INFV	10	50.0 / 25.0	17.7 (-12.0, 47.3)	10 (5, 14)	1.5 (-1.6, 11.6)	40.0 / 0.0 / 0.0	27.5 (-3.4, 58.4)	75.0	**56.3 (9.7, 102.8)**
HMPV	13	38.5 / 46.2	**32.3 (10.8, 53.7)**	9 (6, 11)	1.5 (-1.4, 9.4)	38.5 / 7.7 / 0.0	26.0 (-1.1, 53.0)	20.0	1.3 (-38.7, 41.2)
HCoV	55	49.1 / 21.8	**18.6 (3.8, 33.4)**	7 (3, 14)	1.2 (-1.3, 5.3)	21.8 / 3.6 / 0.0	11.1 (-1.4, 23.7)	61.5	**42.8 (10.2, 75.4)**
HAdV	29	48.3 / 10.3	6.3 (-13.6, 26.2)	7 (5, 15)	0.9 (-2.5, 6.5)	31.0 / 0.0 / 0.0	**18.5 (0.7, 36.3**)	77.8	**59.0 (25.8, 92.2)**
HPyV	39	35.9 / 10.3	-6.2 (-24.1, 11.7)	9 (3, 17)	**2.3 (0.6, 9.4)**	7.7 / 0.0 / 2.6	-2.2 (-13.4, 8.9)	100.0	**81.3 (62.1, 100.4)**
HBoV-1	46	39.1 / 15.2	2.0 (-14.8,18.8)	9 (5, 14	**2.0 (0.1, 7.9)**	26.1 / 2.2 / 0.0	13.6 (-0.3, 27.5)	58.3	**39.6 (5.8, 76.4)**

[a]Detected in nasal swabs collected weekly by parents. [b]Mutually exclusive hierarchical classification of ARI episodes (LRTI>URTI). [c]Median difference of ARI episode duration calculated by quantile regression technique. [d]Medical visits were not mutually exclusive categories as an infant may have more than one medical encounter in different settings during a single ARI episode. [e]Medical visits and antibiotic information were derived from the ARI burden diary triggered in 223/233 (95.7%) eligible recorded ARI episodes.

Abbreviations: HAdV, human adenovirus; ARI, acute respiratory infection; asym, asymptomatic; ED, Emergency Department; FP, family physician; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HMPV, human metapneumovirus; HPyV, human polyomavirus; HRV, human rhinovirus; INFV, influenza virus; IQR, interquartile range; LRTI, lower respiratory tract infection; No., number; PIV, parainfluenza virus; ref, reference; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection.

Table 24: Association between (i) single first detection of the DNA viruses, adenoviruses, human polyomaviruses KI and WU, and human bocavirus-1, and (ii) when other respiratory viruses were present at the time these DNA viruses were first detected; and respiratory symptoms.

Virus	Single first virus detection episode				First virus detection episode with other respiratory viruses present[a]				Risk ratio[b] (95%CI)
	Symptoms		Asymptomatic		Symptoms		Asymptomatic		
	n	%	n	%	n	%	n	%	
HAdV (n=52)	17	58.6	12	41.4	19	82.6	4	17.4	0.71 (0.50, 1.02)
HPyV-KI (n=55)	17	53.1	15	46.9	19	82.6	4	17.4	**0.63 (0.43, 0.91)**
HPyV-WU (n=41)	9	42.9	12	57.1	12	60.0	8	40.0	0.77 (0.43, 1.43)
HBoV-1 (n=73)	25	54.3	21	45.7	23	85.2	4	14.8	**0.63 (0.48, 0.83)**

[a] HAdV: 20 first detections had one other virus present (16 HRV; 2 HCoV; 2 HPyV-WU) and three first detections had two other viruses present (HRV and HPyV-KI; 2 RSV and HPyV-KI).

HPyV-KI: 21 first detections had one other virus present (14 HRV; 1 RSV; 3 HCoV; 2 HPyV-WU; 1 HBoV-1) and two first detections had two other co-detected viruses (HRV and HAdV; HRV and HCoV).

HPyV-WU: 19 first detections had one other virus present (10 HRV, 1 RSV, 1 PIV; 2 HCoV; 4 HPyV-KI) and one first detection had two other co-detected viruses (HRV and RSV).

HBoV-1: 27 first detections had one other virus present (22 HRV, 1 RSV, 1 HCoV, 1 HPyV-KI, 2 HPyV-WU).

[b] Risk ratio represents the relative risk of an infant developing ARI symptoms associated with a single fVDE by a DNA virus compared to an fVDE by the same DNA virus when other viruses are co-detected in the nasal swab specimen.

Abbreviations: HAdV, human adenovirus; ARI, acute respiratory infection; fVDE, first virus detection episode; HBoV-1, human bocavirus-1; HCoV, human coronavirus; HPyV, human polyomavirus; HRV, human rhinovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

Factors associated with symptomatic first virus detection episodes

After adjusting for potentially confounding variables, characteristics associated with symptomatic fVDEs included the winter season, as opposed to summer for HRV and spring and summer for HPyV, while age between 6-<12 months was identified for PIV-3. No sociodemographic or seasonal factors were identified for symptomatic fVDEs for the other respiratory viruses (Table 25–Table 29).

Table 25: Association between selected participant characteristics and symptomatic first virus detection episodes for human rhinoviruses and respiratory syncytial viruses (n=9,594 swabs).

Risk factor	Human rhinoviruses				Respiratory Syncytial Viruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	130	68 (52.3%)			54	42 (77.8%)		
Age (months)								
0-<3	70	36 (51.4%)	Reference	Reference	3	2 (66.7%)	0.7 (0.1, 8.7)	1.4 (0.0, 64.3)
3-<6	40	18 (45.0%)	0.8 (0.4, 1.7)	0.7 (0.3, 1.7)	8	6 (75.0%)	1.0 (0.2, 6.3)	0.3 (0.0, 2.9)
6-<12	17	11 (64.7%)	1.7 (0.6, 5.2)	1.6 (0.5, 5.7)	19	16 (84.2%)	1.8 (0.4, 8.3)	2.2 (0.3, 17.2)
12-<24	3	3 (100.0%)	n/c	n/c	24	18 (75.0%)	Reference	Reference
Sex								
Male	62	29 (46.8%)	Reference	Reference	27	19 (70.4%)	Reference	Reference
Female	68	39 (57.4%)	1.5 (0.8, 3.1)	1.8 (0.8, 3.9)	27	23 (85.2%)	2.4 (0.6, 9.3)	2.5 (0.4, 13.7)
Season								
Winter	25	17 (68.0%)	Reference	Reference	13	10 (76.9%)	Reference	Reference
Spring	25	12 (48.0%)	0.4 (0.1, 1.4)	**0.2 (0.1, 0.9)**	4	3 (75%)	0.9 (0.1, 12.2)	1.9 (0.1, 42.7)
Summer	33	12 (36.4%)	**0.3 (0.1, 0.8)**	**0.2 (0.1, 0.7)**	10	8 (80.0%)	1.2 (0.2, 9.0)	0.4 (0.1, 6.1)
Fall	47	27 (57.4%)	0.6 (0.2, 1.76)	0.4 (0.1, 1.2)	27	21 (77.8%)	1.1 (0.2, 5.1)	0.6 (0.1, 4.5)

Risk factor	Human rhinoviruses				Respiratory Syncytial Viruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	130	68 (52.3%)			54	42 (77.8%)		
Breastfeeding								
Exclusively breastfed <4 months/never breastfed	53	28 (52.8%)	Reference	Reference	22	14 (63.6%)	Reference	Reference
Exclusively breastfed ≥4 months	76	39 (51.3%)	0.9 (0.5, 1.9)	1.0 (0.4, 2.3)	32	28 (87.5%)	4.0 (1.0, 15.6)	5.8 (1.0, 33.1)
Childcare attendance[b]								
No childcare	40	22 (55.0%)	Reference	Reference	16	11 (68.8%)	Reference	Reference
Formal and/or informal childcare	80	41 (51.3%)	0.9 (0.4, 1.8)	1.1 (0.5, 2.4)	37	30 (81.1%)	2.0 (0.5, 7.4)	2.4 (0.4, 13.8)
No. children in household								
No other children	84	49 (58.3%)	Reference	Reference	32	22 (68.8%)	Reference	Reference
One/more children	46	19 (41.3%)	0.5 (0.2, 1.0)	0.7 (0.3, 1.5)	22	20 (87.0%)	4.5 (0.9, 23.3)	4.7 (0.7, 32.9)

[a]adjusted for age, sex, season, breastfeeding, childcare and number of siblings in household;
[b]childcare attendance at 12 months;
Abbreviations: n/c: not calculable; OR: odds ratio; CI: confidence interval

Table 26: Association between selected participant characteristics and symptomatic first virus detection episodes for parainfluenza and influenza viruses.

Risk factor	Parainfluenza viruses				Influenza viruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	52	37 (71.2%)			11	8 (72.7%)		
Age (months)								
0-<3	7	4 (57.1%)	0.8 (0.1, 4.3)	0.5 (0.1, 4.7)	2	1 (50.0%)	n/c	n/c
3-<6	6	3 (50.0%)	0.6 (0.1, 3.5)	0.5 (0.1, 4.0)	1	1 (100%)	n/c	n/c
6-<12	17	16 (94.1%)	**9.1 (1.1, 82.4)**	**14.0 (1.1, 181)**	4	3 (75.0%)	0.3 (0.0, 11.9)	n/c
12-<24	22	14 (63.6%)	Reference	Reference	4	3 (75.0%)	Reference	Reference
Sex								
Male	28	20 (71.4%)	Reference	Reference	4	3 (75.0%)	Reference	Reference
Female	24	17 (70.8%)	1.0 (0.3, 3.2)	1.7 (0.3, 8.8)	7	5 (71.4%)	0.8 (0.1, 13.6)	n/c
Season								
Winter	8	7 (87.5%)	Reference	Reference	6	6 (100%)	Reference	Reference
Spring	29	19 (65.5%)	0.3 (0.0, 2.5)	0.2 (0.0, 4.3)	4	2 (50.0%)	n/c	n/c
Summer	12	8 (66.7%)	0.3 (0.0, 56.9)	0.5 (0.0, 12.0)	1	0 (0.0%)	n/c	n/c
Fall	3	3 (100.0%)	n/c	n/c	0	0 (0.0%)	n/c	n/c

Risk factor	Parainfluenza viruses				Influenza viruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	52	37 (71.2%)			11	8 (72.7%)		
Breastfeeding								
Exclusively breastfed <4 months /never breastfed	21	16 (76.2%)	Reference	Reference	4	2 (50.0%)	Reference	Reference
Exclusively breastfed ≥4 months	31	21 (67.7%)	1.1 (0.3, 3.8)	0.5 (0.1, 2.6)	7	6 (85.7%)	6.0 (0.3, 107.4)	n/c
Childcare attendance[b]								
No childcare	15	8 (53.3%)	Reference	Reference	3	2 (66.7%)	Reference	Reference
Formal and/or informal childcare	36	28 (77.8%)	2.6 (0.7, 9.2)	4.3 (0.8, 24.1)	8	6 (75.0%)	1.5 (0.1, 26.9)	n/c
No. children in household								
No other children	34	24 (70.6%)	Reference	Reference	6	4 (66.7%)	Reference	Reference
One/more children	18	13 (72.2%)	1.1 (0.3, 3.9)	0.6 (0.1, 3.3)	5	4 (80.0%)	2.0 (0.1, 32.0)	n/c

[a]adjusted for age, sex, season, breastfeeding, childcare and number of siblings in household;
[b]childcare attendance at 12 months;
Abbreviations: n/c: not calculable due to small number of detections; OR: odds ratio; CI: confidence interval

Table 27: Association between selected participant characteristics and symptomatic first virus detection episodes for human metapneumoviruses and human coronaviruses.

Risk factor	Human metapneumovirus				Human coronaviruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	18	15 (83.3%)			67	47 (70.1%)		
Age (months)								
0-<3	1	1 (100.0%)	n/c	n/c	10	5 (50.0%)	0.4 (0.1, 1.8)	0.2 (0.0, 1.3)
3-<6	0	0 (0.0%)	n/c	n/c	6	3 (50.0%)	0.4 (0.1, 2.4)	0.4 (0.0, 3.2)
6-<12	9	8 (88.9%)	2.7 (0.2, 36.8)	n/c	23	19 (82.6%)	1.9 (0.5, 7.4)	1.5 (0.3, 6.8)
12-<24	8	6 (75.0%)	Reference	Reference	28	20 (71.4%)	Reference	Reference
Sex								
Male	7	6 (85.7%)	Reference	Reference	28	18 (64.3%)	Reference	Reference
Female	11	9 (81.8%)	0.8 (0.1, 10.2)	n/c	39	29 (74.4%)	1.6 (0.6, 4.6)	2.4 (0.6, 8.7)
Season								
Winter	9	7 (71.4%)	Reference	Reference	32	26 (81.3%)	Reference	Reference
Spring	6	5 (83.3%)	1.4 (0.1, 20.4)	n/c	17	10 (58.8%)	0.3 (0.1, 1.2)	0.4 (0.1, 1.6)
Summer	0	0 (0.0%)	n/c	n/c	9	5 (55.6%)	0.3 (0.1, 1.4)	0.2 (0.0, 1.3)
Fall	3	3 (100.0%)	n/c	n/c	9	6 (66.7%)	0.5 (0.3, 2.4)	0.5 (0.1, 4.3)

Risk factor	Human metapneumovirus				Human coronaviruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	18	15 (83.3%)			67	47 (70.1%)		
Breastfeeding								
Exclusively breastfed <4 months /never breastfed	4	2 (50.0%)	Reference	Reference	30	20 (66.7%)	Reference	Reference
Exclusively breastfed ≥4 months	13	12 (92.3%)	12 (0.7, 203.1)	n/c	37	27 (73.0%)	1.3 (0.5, 3.8)	0.9 (0.3, 3.2)
Childcare attendance[b]								
No childcare	3	2 (66.7%)	Reference	Reference	40	25 (62.5%)	Reference	Reference
Formal and/or informal childcare	14	12 (85.7%)	3.0 (0.2, 50.8)	n/c	27	22 (81.5%)	1.7 (0.6, 5.2)	3.0 (0.7, 12.2)
No. children in household								
No other children	10	9 (90.0%)	Reference	Reference	40	26 (65.0%)	Reference	Reference
One/more children	8	6 (75.0%)	0.3 (0.0, 4.5)	n/c	27	21 (77.8%)	1.9 (0.6, 5.8)	2.1 (0.3, 7.3)

[a]adjusted for age, sex, season, breastfeeding, childcare and number of siblings in household;
[b]childcare attendance at 12 months;
Abbreviations: n/c: not calculable due to small number of detections; OR: odds ratio; CI: confidence interval

Table 28: Association between selected participant characteristics and symptomatic first virus detection episodes for human adenovirus and human polyomaviruses.

Risk factor	Human adenoviruses				Human polyomaviruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	52	36 (69.2%)			70	40 (57.1%)		
Age (months)								
0-<3	0	0	n/c	n/c	0	0 (0.0%)	n/c	n/c
3-<6	9	4 (44.4%)	0.5 (0.1, 2.3)	n/c	8	2 (25.0%)	0.2 (0.0, 1.0)	**0.1 (0.0, 0.9)**
6-<12	24	20 (83.3%)	2.9 (0.7, 12.1)	n/c	35	20 (57.1%)	0.7 (0.2, 1.9)	0.4 (0.1, 1.3)
12-<24	19	12 (63.2%)	Reference	Reference	27	18 (66.7%)	Reference	Reference
Sex								
Male	24	15 (62.5%)	Reference	Reference	32	19 (59.4%)	Reference	Reference
Female	28	21 (75.0%)	1.8 (0.5, 5.9)	n/c	38	21 (55.3%)	0.8 (0.3, 2.2)	1.5 (0.4, 3.9)
Season								
Winter	23	14 (60.9%)	Reference	Reference	17	14 (82.4%)	Reference	Reference
Spring	10	7 (70.0%)	1.5 (0.3, 7.4)	n/c	28	13 (46.4%)	**0.2 (0.0, 0.8)**	**0.1 (0.0, 0.6)**
Summer	6	4 (66.7%)	1.3 (0.2, 8.5)	n/c	15	6 (40.0%)	**0.1 (0.0, 0.7)**	**0.1 (0.0, 0.7)**
Fall	13	11 (84.6%)	3.5 (0.6, 19.8)	n/c	10	7 (70.0%)	0.5 (0.1, 3.1)	0.4 (0.1, 3.1)

Risk factor	Human adenoviruses				Human polyomaviruses			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	52	36 (69.2%)			70	40 (57.1%)		
Breastfeeding								
Exclusively breastfed <4 months /never breastfed	24	16 (66.7%)	Reference	Reference	31	19 (61.3%)	Reference	Reference
Exclusively breastfed ≥4 months	28	20 (71.4%)	1.3 (0.4, 4.1)	n/c	39	21 (53.8%)	0.7 (0.3, 1.9)	0.4 (0.1, 1.4)
Childcare attendance[b]								
No childcare	17	10 (61.9%)	Reference	Reference	21	10 (47.6%)	Reference	Reference
Formal and/or informal childcare	34	25 (74.2%)	1.9 (0.6, 6.7)	n/c	48	30 (62.5%)	1.8 (0.7, 5.2)	2.5 (0.7, 8.7)
No. children in household								
No other children	30	23 (76.7%)	Reference	Reference	52	28 (53.8%)	Reference	Reference
One/more children	22	13 (59.1%)	0.4 (0.1, 1.5)	n/c	18	12 (66.7%)	1.7 (0.6, 5.3)	2.3 (0.6, 8.7)

[a]adjusted for age, sex, season, breastfeeding, childcare and number of siblings in household;
[b]childcare attendance at 12 months;
Abbreviations: n/c: not calculable due to small number of detections; OR: odds ratio; CI: confidence interval

Table 29: Association between participant characteristics and symptomatic first virus detection episodes for human bocavirus-1.

Risk factor	Human bocavirus-1			
	N	Symptomatic n (%)	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
Total cases	73	48 (65.8%)		
Age (months)				
0-<3	1	0 (0.0%)	n/c	n/c
3-<6	5	2 (40.0%)	0.2 (0.0, 1.6)	0.1 (0.0, 1.4)
6-<12	39	25 (64.1%)	0.6 (0.2, 1.7)	0.5 (0.2, 1.6)
12-<24	28	21 (75.0%)	Reference	Reference
Sex				
Male	31	22 (71.0%)	Reference	Reference
Female	42	26 (61.9%)	0.7 (0.2, 1.8)	0.7 (0.3, 2.2)
Season				
Winter	37	26 (70.3%)	Reference	Reference
Spring	11	8 (72.7%)	1.1 (0.3, 5.1)	1.5 (0.3, 7.8)
Summer	8	4 (50.0%)	0.4 (0.1, 2.0)	0.9 (0.1, 5.6)
Fall	17	10 (58.8%)	0.6 (0.2, 2.0)	0.7 (0.2, 2.6)
Breastfeeding				
Exclusively breastfed <4 months /never breastfed	30	18 (60.0%)	Reference	Reference
Exclusively breastfed ≥4 months	43	30 (69.8%)	1.5 (0.6, 4.1)	1.3 (0.5, 3.9)
Childcare attendance[b]				
No childcare	22	13 (59.1%)	Reference	Reference
Formal and/or informal childcare	51	35 (68.6%)	1.5 (0.5, 4.3)	1.9 (0.6, 6.2)
No. children in household				
No other children	52	36 (69.2%)	Reference	Reference
One/more other children	21	12 (57.1%)	0.6 (0.2, 1.7)	0.6 (0.2, 2.1)

[a]adjusted for age, sex, season, breastfeeding, childcare and number of siblings in household; [b]childcare attendance at 12 months;
Abbreviations: n/c: not calculable due to small number of detections; OR: odds ratio; CI: confidence interval.

DISCUSSION

In this community cohort of healthy Australian children, we detected respiratory viruses early in life, with HRV playing a dominant role from birth, and other viruses detected generally after six months of age. Although only about half of the HRV fVDEs were associated with respiratory symptoms, 70-83% of fVDEs with other respiratory RNA viruses were symptomatic. While 57-69% of fVDEs with HAdV, HPyV KI/WU and HBoV-1 DNA viruses had symptoms, this decreased to 43-59% when these were the sole detected agents. High viral loads for RSV and HBoV-1 were associated with symptoms, but shedding duration was independent of symptom status. Overall, RSV and HMPV fVDEs were most likely to be associated with LRTIs and to result in medical visits where antibiotics were commonly prescribed irrespective of the virus or viruses detected around the time of presentation. The winter season was an independent risk factor for symptomatic HRV and HPyV fVDEs, and age >6 months was a risk factor for symptomatic PIV fVDEs. No risk factors were identified for other common viruses.

First respiratory virus infections are believed to be almost invariably symptomatic and, in the case of RSV, to result in more severe disease because of the infant's immature immune system and small airways, with re-infections thought to have a lower risk of illness (74, 308). Our data show that during their fVDEs children do not always experience respiratory symptoms. The cumulative proportion of children in our cohort with fVDEs from viruses other than HRV increased steadily after age six months. The delayed appearance of these other viruses may be from the protective effects of maternal antibodies and reduced exposure, as attendance at childcare centres is less common in this age group (6, 310).

Our findings differ from the Houston Family study, which relying heavily upon serology, reported 68% of infants had RSV by age 12 months and virtually all were infected by their second birthday. However, studies from Asia, Europe and Latin America observed RSV seroprevalence rates of 36-70% by age two years (75-77, 311, 312), while in Kenya a birth cohort project similar in design to the Houston study found 73% had RSV by two years of age (79). These results are more in keeping with our own and possible explanations for our lower incidence of RSV fVDEs during the first two years of life than in the Houston study include variations in intensity and duration of seasonal exposure (313), fewer subjects with older siblings to introduce RSV into the household (79, 314), and relying upon PCR rather than serology to detect the virus. The Houston study also enrolled families that lived

throughout the metropolitan area and were representative of low income groups (315), in contrast to the ORChID cohort.

Hospital-based studies are skewed towards those with the most severe infections and represent <5% of the infant population (269). While RSV predominates in hospitalised infants aged <6 months with LRTIs, HRV is the most common virus detected in older infants and young children admitted with wheezing illnesses, which are often recurrent and associated with a subsequent increased risk of asthma (123, 124, 307, 316). In contrast, community-based studies like our own find symptomatic fVDEs with HRV occurring by age 4-6 months, and with RSV and other viruses appearing on average beyond this age (2, 79, 226). Viruses such as RSV, PIV, INFV and HMPV are more likely than other viruses to be associated with respiratory symptoms (22), and consequently, although less commonly exposed to these viruses than HRV at a young age, it is thought that young infants hospitalised with more severe disease have pre-existing smaller airways, immature immune systems and/or are lacking protective maternal antibodies (15, 317). As these infants become older and their airways grow and immune systems mature, the symptoms of infections by these other viruses lessen (74, 79).

While wheezing illnesses following early exposure to HRV are associated with a greater likelihood of developing asthma in high-risk cohorts (123, 124, 307), the role of early asymptomatic HRV infections has received less attention. A recent report suggested that HRV detection in the first four weeks of life, even if asymptomatic, may help program immune memory with an exaggerated Th2 (rather than Th1) mucosal immune response and impaired anti-viral responses (291). This suggests a potential pathway for the later development of asthma and allergic sensitization in susceptible individuals. If true, interventions seeking to reduce HRV infections in high-risk infants will need to be introduced early. Although maternal vaccines to protect infants from early respiratory virus infections is an active area of research, especially for RSV and influenza viruses (310), our study emphasises the predominance of early HRV detections, the long-term effects of which remain uncertain in healthy infants. It also highlights that for many in our study population, their first infection from non-HRV viruses occurs at an age when maternal vaccines may no longer be protective (310).

The fVDEs by DNA viruses were significantly more likely than RNA viruses to have additional viruses co-detected during this episode. In part, this might be explained by most HRV fVDEs occurring during the first six months of life where other viruses were detected uncommonly.

When sole fVDEs by these DNA viruses were examined they were found to be similar to HRV fVDEs in that about 50% of episodes were associated with symptoms, although symptomatic episodes with HPyV and HBoV-1 were significantly more prolonged than those associated with HRV. These findings indicate that while common, these viruses often cause mild or no ARI symptoms, even after a fVDE (22, 202, 301). Interestingly, HBoV-1 viral load was associated with the presence of ARI symptoms, a metric along with HBoV-1 capsid mRNA that has been reported previously to be associated with disease severity (202).

The ORChID cohort spanned multiple seasons and its strengths include the comprehensive surveillance provided by regular weekly nasal swab collections and daily symptom diary recordings by parents. The return rate of 68% of expected swab is noteworthy given the project's intensive and prolonged nature. Potential limitations include parents missing very mild symptoms. However, we minimised reporting inaccuracy by training parents on symptom recognition and diary completion before they commenced the study and included only doctor-diagnosed episodes of otitis media and pneumonia (6). This study design has been used successfully before where parents were trained to recognize respiratory symptoms (256). Another limitation is parents having a suboptimal nasal swabbing technique (249). However, sensitive molecular detection methods combined with mailed parent-collected nasal swabs provide comparable results with nasopharyngeal swabs collected by health professionals (246, 256, 318). For logistical reasons we did not include other respiratory viruses, such as INFV-C or PIV-4, in our assays, although 17 viruses still provide a comprehensive survey of early life exposures. Additionally, 29% of HRV-positive specimens were unable to be typed, mainly due to low viral loads, a finding comparable with other community-based cohort studies (150). We may have under-estimated virus shedding duration because of missing swabs or swabs being collected only weekly. Similarly, ascribing symptoms to viruses when swab collection was not always done weekly or if other viruses were detected during the fVDE could compromise accuracy, although sub-analyses of sole fVDEs were reassuring. It is also likely we were unable to identify risk factors associated with symptomatic non-HRV fVDEs because of a lack of power, as numbers for some sub-categories were small. As is common with cohort studies, our families were from more advantaged backgrounds, but our findings for infections rates, such as RSV, approximate recent studies (79, 308, 312) and provide an insight into respiratory viruses affecting children from a developed country in a subtropical, urban setting.

In conclusion, respiratory viruses are detected early in life, with HRV playing a dominant role from birth. The onset of fVDEs for other respiratory viruses increased after age six months, with RSV and HMPV having the more severe symptoms, but identifiable sociodemographic and seasonal factors were not consistently associated with symptomatic fVDEs in our cohort. Whether early fVDEs, particularly from HRV, are important or preventable and have adverse long-term effects on some individuals warrants further study.

Chapter 7 – General Discussion and Conclusions

Summary of findings

For my PhD I undertook analysis of data collected from the ORChID cohort study. The findings presented in Chapters 3–6 offer insights into respiratory virus epidemiology and respiratory illness in a community cohort of healthy Australian infants and young children less than two years of age from the subtropical urban environment of the city of Brisbane.

My work has resulted in four chapters in the form of published papers. The key findings, as they relate to the hypotheses outlined in Chapter 1, are:

a) ARIs are a frequent and common cause of early childhood morbidity in the community. They are, for the most part, mild illnesses with few hospitalisations. However, almost 50% of ARI episodes lead to primary care consultations when they involve more than simple nasal discharge or congestion, or an isolated dry cough.

b) Diverse respiratory virus species are detected from a very early age. HRV detections dominate throughout, but especially in the first six months of life. Despite their dominance, only 34% of HRV detections were associated with symptoms. While my typing studies were limited to the neonatal period, diverse HRV genotypes were identified, with serial detections often reflecting sequential infections with new HRV strains.

c) Several RNA viruses, including various HRV, INFV, PIV, RSV, HMPV, and HCoV species and subtypes, were associated with an increased risk of ARI symptoms when found in respiratory secretions of an infant. Support for causality was strongest for RSV and HMPV in children with LRTIs. Of the three HRV species, HRV-C was associated with an increased risk of LRTIs.

d) Contrary to the view that first infections with viruses are almost always symptomatic, particularly with RSV (74), and more likely to result in more severe disease and therefore to require medical care, first detections of respiratory viruses did not always result in illness. HRV fVDEs were detected early (median of 2.9 months of age), in contrast with all other respiratory viruses, which were first detected later (median >13.9 months).

What this research adds

In the last two decades, several prospective studies have been conducted in cohorts of both healthy infants and young children, and in infants deemed to be at high risk of asthma and/or atopy. These studies have sought to examine the contribution of individual respiratory viruses to respiratory illness using molecular testing techniques. While several new human viruses have been discovered in patients with respiratory illness, they and other respiratory viruses have also been detected in asymptomatic subjects. The lack of well-designed longitudinal epidemiological studies in healthy community cohorts has meant the clinical significance of these detections remains unclear. The frequent detection of HRV in respiratory specimens without subsequent sequence-based typing to distinguish between species and genotypes has made the aetiology of HRV difficult to ascertain. The role of HRV-C species in LRTI and wheezing illness has also been the subject of ongoing debate.

The ORChID study findings provide a wealth of information about the relationship between respiratory virus infection and symptoms of respiratory illness. The study builds on historic community-based studies of respiratory virus epidemiology, which established the conceptual framework for the community epidemiology of respiratory virus infection. However, the ORChID study uses modern molecular-based techniques over four respiratory seasons to address questions about respiratory virus acquisition and infection, the only study reported to date to sample intensively over this length of time in a healthy cohort.

The ORChID data highlight the differences in epidemiological profiles displayed by hospital and community cohorts, and the risk of extrapolating hospital-based data to the community setting. Hospital-based studies are skewed towards those with the most severe disease and represent a minority of infants and infections. In contrast to hospitalised cohorts, HRVs are the predominant viruses circulating in the community and infants are exposed to the virus in the first few weeks of life. Fortunately, most illnesses are mild, and although HRVs do not play a large attributable role for ARIs, they are nevertheless major contributors to respiratory illness burden because of their greater prevalence.

In contrast, studies have reported that RSV, and to a much lesser extent, seasonal INFV, are the most common reasons for infants to be hospitalised in the first year of life (70, 71, 89). RSV is considered to be more virulent than HRV as detection of RSV in young children is more likely to be associated with respiratory symptoms, as observed in other studies (22), and from our own observations (319). Although less commonly exposed to RSV than HRV,

it is thought that otherwise well infants hospitalised with RSV have more severe disease and represent a subset with pre-existing smaller airways, immature immune systems, and/or lack protective maternal antibodies (15, 317). As infants and young children become older and their airways grow and immune systems mature the symptoms of initial RSV infection lessen.

Studies in community-based, high-risk cohorts, however, have shown that HRV is the predominant virus in the first year of life associated with wheezing illness (2, 120, 121). In hospital-based studies, infants hospitalised with HRV-associated wheezing or LRTIs are older and are more likely to have a prior history of wheezing (320). HRV is the most common virus detected in older infants and young children admitted with wheezing illnesses. These infections are often recurrent and associated with a subsequent increased risk of asthma (122), (123, 124, 307, 316).

The ORChID data analysis also underlines the early and frequent interaction of diverse HRVs (symptomatic and asymptomatic) at a time when the immune system is not fully developed. HRVs are known to alter innate immune responses, and have been suggested as contributing to more severe disease in asthmatics and those genetically predisposed to allergic phenotypes (321, 322). These impaired innate immune responses to HRV may increase susceptibility to HRV-induced wheezing and asthma (321). A recent study of the Danish asthma birth cohort ($COPSAC_{2010}$) showed that HRV infection in the first four weeks of life, even if asymptomatic, induced an exaggerated type-2 mucosal immune response in some infected neonates (291). Asymptomatic presence of HRVs in the neonatal airway was a potent activator of the local mucosal immune response. Thus, early HRV exposure may trigger aberrant immune programming in susceptible individuals and promote subsequent development of asthma and allergic sensitisation (124, 291). It has been suggested HRVs play a role as early life ‘educators’ of the immune system in healthy children through normal virus-host interactions, which in atopic children take on the role of a ‘miseducator’ (323).

Finally, the ORChID study findings show that, while relatively common (319), the frequency of RSV infection in early life is also less than identified in some previous studies. The seminal Houston study showed 97% of children had culture or serological evidence of at least one RSV infection by the same age (74). Subsequently, most serologically-based studies have not been able to replicate such high levels of RSV infection in the first two years of life. While most show high seroprevalence by the age of three years, many have had methodological limitations (small numbers, cross-sectional design), differences in age categories, and

seasonal variations in the incidence of RSV infection. The most robust of these studies and similar in design to the Houston study was the community study conducted in Kenya, which recorded 73% of children with serological evidence of prior RSV infection by the age of two years (79).

The ORChID study findings are more in keeping with other recent studies (75-77, 79, 311, 312) showing a lesser likelihood of RSV infection early in childhood. There are several reasons for this finding, including population, geographic, temporal, sociodemographic, and technical factors. The use of weekly PCR instead of serology may have missed some infections. However, the median age at first detection in our cohort was at 19.4 months (25^{th} centile 11.2 months), and by 24 months of age, only 58% of the cohort had experienced a first RSV infection. Variations in intensity and duration of seasonal exposure (313) may also have resulted in a lower incidence of RSV fVDEs than observed in the Houston study. RSV activity varies in severity from year to year. In cities like Houston with high annual rainfall, activity can be prolonged (324). Queensland Health data show in the two years involving 2012 and 2013, the mid-point period of the study, RSV activity in South-east Queensland was below average (https://www.health.qld.gov.au/clinical-practice/guidelines-procedures/diseases-infection/surveillance/reports/flu).

In addition, 67% of children in our cohort were first born and thus were less likely to have older siblings to transmit RSV into the household in the first few months before attending childcare (79, 325). Cohort subjects also had low exposure to environmental tobacco smoke, including maternal smoking during pregnancy and while breastfeeding and less crowded households, factors known to be associated with hospitalisation (308). Further community-based studies in cohorts of low socioeconomic status and high disadvantage, in high-risk groups such as Indigenous populations, are warranted to examine if symptomatology in these groups is more frequent or severe than urban infants from small families.

However, if these findings are confirmed in other studies, the ‘delayed’ appearance of first RSV infections in our study has important implications for immunisation strategies such as antenatal maternal immunisation. Less than 10% of the ORChID cohort experienced an RSV first infection by the age of six months, an age after whichpassive maternal antibodies may no longer be protective. Thus, while an antenatal RSV vaccine may reduce the hospitalisation rate of RSV infections in infants in the first few months of life (of which only ~2% are hospitalised in the first year of life), it may not have as great an impact upon the

community burden of RSV infection. .

It is also pertinent to place the ORChID study in a broader context with other longitudinal studies conducted in non-atopic/allergic populations at high risk of ARIs. Similar to the ORChID study, a study in healthy Aboriginal and non-Aboriginal children (326), as well as a study conducted among children with LRTI of similar age in the PNG highlands (327), both employing sensitive molecular techniques, identified a diversity of respiratory viruses in the first two years of life, with early dominance by HRV. The Kalgoorlie study also showed sequential infections were a result of HRV serotype replacement, similar to what we have noted with sequential HRV positive specimens during the neonatal period.

However, in contrast to the ORChID study, where HRV-C was associated with LRTI symptoms, the Kalgoorlie study found HRV-C was associated with upper respiratory symptoms in all children. HRV of any individual species was also not associated with LRTI in the PNG study (327). Instead, LRTI was associated with detection of the DNA viruses adenovirus B and C, influenza A, and RSV. HRV-A and HRV-C were also associated with carriage of respiratory bacteria (326). Respiratory viruses, including HRV, have been shown to exacerbate nasopharyngeal bacterial load in another birth cohort study in Aboriginal children in the Northern Territory (13).

As well as differences in viral aetiology, there were also differences in the seasonality of virus circulation, and other environmental and social risk factors. HRV-A was found to be more common in summer in Aboriginal children, thought to be due to increased social interaction (326). In non-Aboriginal children, childcare attendance and exclusive breastfeeding at age 6-8 weeks were associated with the detection of HRV-A. Gestational smoking was positively associated with HRV-C detection among non-Aboriginal but not Aboriginal children. A decreased risk of HRV detection was associated with larger non-Aboriginal households. These findings suggest that further community studies are warranted in diverse settings to gain a greater understanding of the mechanistic role of respiratory viruses in early childhood, including modulation of viral load and frequency from seasonal and environmental factors, and their interaction with respiratory bacteria in these environments.

Strengths

Notable features of the ORChID study design included the relatively large size, being community based, enrolment from birth, and prospective intensive specimen sampling and

symptom data collection irrespective of illness. This last feature allowed assessment of asymptomatic status with consideration of past and future illness history. This has in turn enabled the calculation of a population-level attributable fraction for individual respiratory viruses in healthy children (Chapter 5).

Recruitment over two years and data collection over four years accounted for seasonality and year-to-year variability in respiratory virus circulation within the community. An extensive panel of real-time PCR assays was used, with sequencing to species level able to be performed on 73% of HRV-positive swabs, a proportion comparable with other community-based studies (118, 145, 150).

Parental participation in obtaining regular nasal swabs avoided the need for home visits by research staff or clinic attendance during an ARI. The requirement for home visits by research staff in other studies may have led to a loss of families or incomplete specimen collection during ARIs, potentially resulting in biased estimates of ARI events and specimen availability. The sensitivity of this method of collection was compared in some of our previous work, which found that parent-collected nose or nose-throat swabs sent to the laboratory by regular mail had comparable sensitivity to nasopharyngeal swabs obtained by healthcare workers (256).

These attributes of the ORChID study design have several advantages that cross-sectional study designs cannot address. The community-based design has captured the non-hospitalised disease burden in young children and allowed for more accurate estimates of the burden of community ARI. Recruitment through GP clinics alone would have underestimated disease burden by more than a factor of three.

Limitations

The ORChID cohort study design also presented some challenges. Given the size of the study, the decision to involve parents in the data collection process was made for methodological, logistical, and cost considerations. A study of this magnitude would have been cost intensive had we employed research staff to collect information on every ARI and undertake visits on a weekly basis. Specimen quality resulting from poor swabbing technique and accuracy in recognising respiratory symptoms were large considerations in this study. We tried to minimise these limitations by training parents at the beginning of the study, which has been shown to be successful in other settings (284, 285).

The symptom card used in the ORChID study was one modified from a previous study on seasonal INFV vaccine effectiveness and used in another cohort study successfully in Melbourne (3). It included both respiratory and systemic signs and symptoms, intended to capture INFV-like symptoms in older children. We restricted the ARI definition to include only respiratory symptoms in these analyses, given the age of the children, to increase reliability. Symptom information, other than doctor-diagnosed AOM and pneumonia, captured by daily diaries was not validated. We tried to minimise inaccuracy of reporting by training parents how to recognise symptoms and complete the diary before they commenced the study. A parental questionnaire such as the Canadian Acute Respiratory Flu Scale has been available since the beginning of this century, but they are not suitable for home-based parent collection on a recurring basis (328). There is a need for a simple, recurrent, validated tool for the collection of respiratory illness symptoms in children.

Besides controlling for specimen quality at the beginning of the study, we also incorporated internal controls for the amount of human DNA present on nasal swabs. Low or absent levels indicated poor quality samples taken with a sub-optimal swabbing technique containing few host epithelial cells, resulting in a decreased odds of detecting viruses (249). Parents were also contacted when specimens of poor quality were observed. We reinforced good swabbing techniques with additional instructions on these occasions. We may also have underestimated virus shedding duration because of missing swabs or swabs being collected only weekly.

Analysis of ERV-3 positive and respiratory virus positive specimens showed that ERV-3 positive sample rates increased with age and varied by season (249), with ERV-3 positive rates in the first six months of age as a reference. It is possible, therefore, that swab quality was not as good as when children were first recruited compared to later, and this might bias identification of virus versus another.

As mentioned earlier, 27% of sequences from HRV detections either failed to align with HRV reference prototype sequences or could not be typed to species level. These combined limitations would result in a ‘minimum estimate’ and an under-estimation of species-specific incidence rates. Furthermore, some viruses, such as INFV, HMPV, and some PIVs, were detected in small numbers in this cohort limiting the ability to draw conclusions about these viruses in the community, and so, interpretation of results has been much more circumspect given relatively small numbers.

My analysis also excluded co-detections in individual virus assessments as multiple aetiological agents make individual contributions of each agent difficult to ascertain. Instead, I analysed viruses separately to look at the association patterns of individual viruses. However, multiple respiratory viruses do co-circulate at any one time and a comprehensive metagenomics study in the future that includes the respiratory virome will need to take into consideration multiple concurrent virus-virus, virus-bacteria, and virus-host interactions. We also observed arbitrary rules when assigning illness duration with mixed detections.

Finally, as is common for these types of studies, families in the ORChID cohort were from more advantaged backgrounds. Longitudinal cohort studies of this intensity and duration are often over-represented with households from higher income brackets (2, 230). Recruitment of families with lower than average mean household income presents a challenge for researchers with this study design. Targeting recruitment efforts in the Melbourne study to lower socioeconomic areas (3) did not significantly improve representation from low income households. However, the effect of families with higher socioeconomic status can be complex. While these factors are thought to decrease the rate of ARIs, secular changes in Australia have led to an increased proportion of children from these families attending childcare at an earlier age than other sectors of the population. The majority of children in the ORChID study were first born (67%) compared to 41% of families with one child in the Brisbane urban area recorded in the 2011 Census (329), and this would impact on the rate of ARI in children in the household. Like other studies, we have shown the presence of siblings to be a risk determinant for ARIs (47).

Clinical implications

The development of more sensitive, specific, and rapid assays of respiratory specimens is increasingly being used in the diagnosis and clinical management of patients. Methods such as MassTag PCR have been developed for differential diagnosis of respiratory infections (330), where multiplex primer sets were designed to identify up to 22 respiratory pathogens in a single reaction. Assay kits will allow for rapid screening and are more likely to be used in the future as antiviral therapies become available. While vaccines have only been developed against INFV so far, there are good prospects of an imminent vaccine against RSV, currently entering Phase II trials (331), and vaccines in development against several other respiratory viruses (300). Rapid identification of the aetiological cause of an ARI could improve patient management and antibiotic stewardship. Timely detection of viral pathogens

by PCR would reduce the risk of unnecessary antibiotic use in those with a compatible clinical illness.

However, the use of PCR to test swabs taken during symptomatic and asymptomatic periods in the ORChID study has increased the appreciation of a wide clinical spectrum of respiratory virus impact, ranging from subclinical infections to mild to severe infections requiring hospitalisation. Calculations of the attributable fraction have highlighted the relative importance of RSV, HMPV, and PIV, in LRTIs in children in the community. While recent molecular detection assays using multiplex quantitative RT-PCR have good sensitivity, negative predictive value, and have been optimised to detect a panel of respiratory viruses, findings from the ORChID study challenge the notion of specificity and positive predictive value for some viruses with high rates of asymptomatic detection, such as HRV and HAdV. Attempts to link viruses with clinical disease are confronted by the high rates of recovery from asymptomatic individuals for some viruses, as well as concurrent viral infections in some specimens. Distinguishing incidental and unrelated asymptomatic viral shedding from symptomatic disease due to the virus will require other methods of analysis such as querying the host response (see ‘Future research’, page 162).

Public health implications

Quantitative estimates of the relative proportion of virus-associated ARIs in the community yield important insights for prioritising public health interventions. The strong association of RSV and HMPV with LRTIs in young children managed within the community indicates successful development of vaccines against these two viruses should provide substantial health benefits. Findings on the timing of first VDEs of individual viruses are important in identifying at-risk groups and determining the optimal timing of maternal and infant immunisation strategies. Evidence from this thesis shows that, along with other data on burden and healthcare costs, virus-specific data are required to populate models for the cost effectiveness of any potential therapeutic treatment or vaccine to be considered.

The high prevalence of HRV compared with other respiratory viruses, and its possible role in immunological programming and asthma in susceptible individuals, presents a challenge when developing an HRV vaccine, particularly with multiple genotypes and little evidence of cross-protective immunity between genotypes.

Future research

Emerging evidence indicates infections early in life, along with other environmental insults to developing airways in susceptible children, may contribute to later development of chronic respiratory conditions such as asthma in childhood and COPD in adults (18, 19, 332-334). Children who participated in the ORChID study have been enrolled in a follow-on study (the Early Life Lung Function Study) exploring the impact of respiratory viral infections in the first two years of life and respiratory health in later childhood (3–7 years of age) as measured by lung function measurements. The relative impact and role of each of the viral pathogens tested, the frequency of infection, and severity of illness will also be recorded. Additional studies will also examine the role of common respiratory bacterial pathogens (*Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*) and their interactions with the 17 respiratory viruses, including the effect of these interactions on airway epithelial cells *ex vivo* and upon their immune recognition *in vivo*. Outcomes from this research could inform interventions made at a critical time in lung development to attenuate or even prevent loss of lung function.

Whether early fVDEs are important or preventable and have adverse long-term effects on some individuals warrants further study. The ORChID study findings have highlighted the extremely frequent interaction between HRV and the immature immune system. The concept of HRV as an early 'educator' of the immune system fits well with an emerging view that one of the key functions of the infant's microbiome is education of the immune system. This is a growing area of research (335, 336). Greater understanding of the mechanistic role of viruses in early childhood in both healthy children and in those at risk of atopy/asthma would help inform public health strategies such as immunisation and identify at risk groups.

Future studies need to examine not only the composition, but also the functioning, of complex microbial communities in the upper airways and their relationship to health and disease. Emerging evidence indicates that pathobiological processes such as bronchiolitis involve a complex interplay among viruses, airway microbiome, and host immunity (337-339), with the suggestion that microbiota form the mediator between early-life environmental risk factors for and susceptibility to LRTI over the first year of life (340). The ORChID study was not designed to specifically address these important questions, focusing as it did on viruses.

Many aspects of HRV epidemiology, immunobiology, strain characterisation, and clinical impact remained poorly addressed. Few data have tracked strains from year-to-year,

reported if strains recur each year, if homotypic strains re-infect and if so, how long protective immunity lasts. There are no data that convincingly identify a distinct clinical outcome for any single HRV subtype, and whether some subtypes are more virulent than others (117). Many studies have detected some strains more or less frequently, but they do not seek all likely microbial causes or even span sufficient time to encompass commonly circulating HRV strains (117). The heterogeneity of HRVs hinders the significance of detecting the virus on clinical respiratory PCR panels, with the result that repeated detections cannot distinguish between reinfection with a new subtype versus prolonged shedding.

There is also a current need for a validated, parent-collection tool for respiratory symptoms. The swabs used in this study, with the VTM-soaked sponge, are no longer manufactured. Flocked swabs for anterior nasal swabbing would be useful, but at present, no system free of frank liquid is available that would allow their transport through surface mail in Australia.

Given the high background rate of PCR positivity in asymptomatic individuals, approaches to better define ‘infection’ or more appropriate analytical approaches have been proposed. One option to better define infection is to query the host response. Several groups have investigated host gene expression in peripheral blood leukocytes and shown differences in response to viral versus bacterial infection or in response to symptomatic versus asymptomatic infection (341, 342). Proteomics and metabolomics provide alternative paths for evaluating host response and interactions with local microbial communities. Evidence of active replication and transcription (188), or host gene expression assays and biomarkers of anti-viral responses to identify respiratory virus infections have also been explored (343). An approach that combines pathogen detection with characterisation of host response may yield a test or tests that could inform clinicians of the likelihood of a bacterial or mixed viral-bacterial infection and the need to start antibiotic therapy. Clinicians could withhold antibiotics when bacterial infection was unlikely, lessening the overuse of antibiotics to treat viral respiratory infections (344).

Finally, the ORChID study also captured information on time off work, missed childcare and missed activities. A future cost analysis will evaluate the economic and social impact of ARIs in these Australian families.

Conclusions

Through analysis of the ORChID study data and by compilation of these publications and this thesis, I have made important, original contributions to understanding respiratory virus epidemiology in healthy children in the community. My work complements other research on respiratory illness in hospital and vulnerable populations, such as children with underlying chronic respiratory disorders. Future metagenomics studies linked with high density sampling of an ORChID-type study to determine the biological mechanisms of health and respiratory disease, and to identify diagnostic, therapeutic and vaccine targets, would be beneficial not only to the child and their family, but for future adult respiratory health and society.

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APPENDICES

Appendix 1: Ethics approvals from the Royal Brisbane and Women's Hospital, the Royal Children's Hospital, and the University of Queensland

Appendix 2: Koch's modified postulates

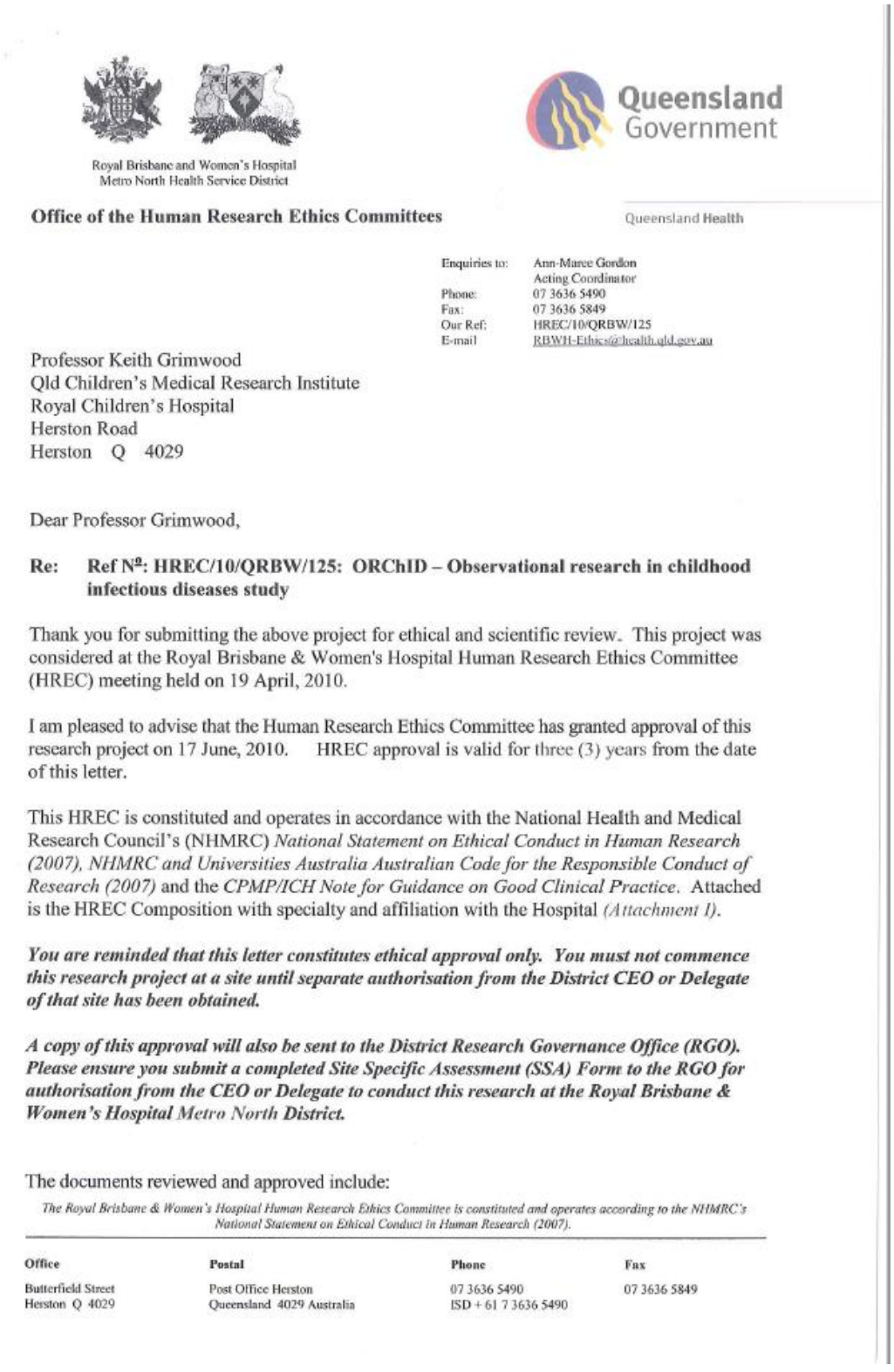
Appendix 3: The Observational Research in Childhood Infectious Diseases study daily diary template

Appendix 4: The Observational Research in Childhood Infectious Diseases study burden diary template

Appendix 5: The Observational Research in Childhood Infectious Diseases study specimen collection instruction sheet

Appendix 6: The Observational Research in Childhood Infectious Diseases study swab and symptom collection pack

Appendix 1: Ethics approvals from the Royal Brisbane and Women's Hospital, the Royal Children's Hospital, and the University of Queensland Human Research Ethics Committees



Royal Brisbane and Women's Hospital
Metro North Health Service District

Queensland Government

Office of the Human Research Ethics Committees

Queensland Health

Enquiries to: Ann-Maree Gordon
Acting Coordinator
Phone: 07 3636 5490
Fax: 07 3636 5849
Our Ref: HREC/10/QRBW/125
E-mail RBWH-Ethics@health.qld.gov.au

Professor Keith Grimwood
Qld Children's Medical Research Institute
Royal Children's Hospital
Herston Road
Herston Q 4029

Dear Professor Grimwood,

Re: Ref Nº: HREC/10/QRBW/125: ORChID – Observational research in childhood infectious diseases study

Thank you for submitting the above project for ethical and scientific review. This project was considered at the Royal Brisbane & Women's Hospital Human Research Ethics Committee (HREC) meeting held on 19 April, 2010.

I am pleased to advise that the Human Research Ethics Committee has granted approval of this research project on 17 June, 2010. HREC approval is valid for three (3) years from the date of this letter.

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. Attached is the HREC Composition with specialty and affiliation with the Hospital *(Attachment I)*.

You are reminded that this letter constitutes ethical approval only. You must not commence this research project at a site until separate authorisation from the District CEO or Delegate of that site has been obtained.

A copy of this approval will also be sent to the District Research Governance Office (RGO). Please ensure you submit a completed Site Specific Assessment (SSA) Form to the RGO for authorisation from the CEO or Delegate to conduct this research at the Royal Brisbane & Women's Hospital Metro North District.

The documents reviewed and approved include:

The Royal Brisbane & Women's Hospital Human Research Ethics Committee is constituted and operates according to the NHMRC's National Statement on Ethical Conduct in Human Research (2007).

Office	Postal	Phone	Fax
Butterfield Street Herston Q 4029	Post Office Herston Queensland 4029 Australia	07 3636 5490 ISD + 61 7 3636 5490	07 3636 5849

Document	Version	Date
Covering Letter		26 March 2010
Application: NEAF	2.0	23 March 2010
Infection Impact Diary	2	04 March 2010
Daily Symptom Diary	1	01 March 2010
Parent General Instructions	1	01 March 2010
Information Flyer	1	01 March 2010
Advertising Poster	1	01 March 2010
Letter of approval from Qld Children's Health Services District (RCH) Ethics Committee		16 March 2010
Curriculum Vitae of Keith Grimwood		
Curriculum Vitae of Dr Stephen Bernard Lambert		
Curriculum Vitae of David Wang		
Curriculum Vitae of A/Prof Michael Desmond Nissen		
Curriculum Vitae of Theodorus Pieter Sloots		
Response to Request for Further Information *(Received on 10.06.2010)*		22 April 2010
Protocol	3	22 April 2010
Participant Information Sheet & Consent Form	3	22 April 2010
Specimen Collection Instructions	2	22 April 2010

Please note the following conditions of approval:

1. The Principal Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:
    - Unforeseen events that might affect continued ethical acceptability of the project. Serious Adverse Events must be notified to the Committee as soon as possible. In addition, the Investigator must provide a summary of the adverse events, in the specified format, including a comment as to suspected causality and whether changes are required to the Patient Information and Consent Form. In the case of Serious Adverse Events occurring at the local site, a full report is required from the Principal Investigator, including duration of treatment and outcome of event.

2. Amendments which do not affect either the ethical acceptability or site acceptability of the project (e.g. typographical errors) should be submitted in hard copy to the HREC Coordinator. These should include a covering letter from the Principal Investigator providing a brief description of the changes and the rationale for the changes, and

accompanied by all relevant updated documents with tracked changes.

3. Proposed amendments to the research project which may affect both the ethical acceptability and site suitability of the project must be submitted firstly to the HREC for review and, once HREC approval has been granted, then submitted to the Research Governance Office.

4. Amendments to the research project which only affect the ongoing site acceptability of the project are not required to be submitted to the HREC for review. These amendment requests should be submitted directly to the Research Governance Office (by-passing the HREC).

5. Amendments to the research project which may affect the ongoing ethical acceptability of a project must be submitted to the HREC for review. Major amendments should be reflected in a revised online NEAF (accompanied by all relevant updated documentation and a covering letter from the Principal Investigator, providing a brief description of the changes, the rationale for the changes, and their implications for the ongoing conduct of the study). Hard copies of the revised NEAF, the cover letter and all relevant updated documents with tracked changes must also be submitted to the HREC Coordinator as per standard HREC SOP. Further advice on submitting amendments is available from http://www.health.qld.gov.au/ohmr/documents/researcher_userguide.pdf

6. The HREC will be notified, giving reasons, if the project is discontinued at a site before the expected date of completion.

7. The HREC will be notified, giving reasons, on any sponsor reports or other information which might affect the ongoing ethical acceptability in line with the requirements of the ICH GCP guidelines as annotated by the TGA: http://www.tga.gov.au/docs/pdf/euguide/ich/ich13595.pdf

8. The Principal Investigator will provide an Annual Report to the HREC and at completion of the study in the specified format.

9. The District Administration and the Human Research Ethics Committee may inquire into the conduct of any research or purported research, whether approved or not and regardless of the source of funding, being conducted on Hospital premises or claiming any association with the Hospital, or which the Committee has approved if conducted outside Royal Brisbane & Women's Hospital Metro North Health Service District.

Should you have any queries about the HREC's consideration of your project please contact the HREC Coordinator on 07 3636 5490. The HREC terms of Reference, Standard Operating Procedures, membership and standard forms are available from http://www.health.qld.gov.au/ohmr/html/regu/regu_home.asp

Once authorisation to conduct the research has been granted, please complete the Commencement Form *(Attachment II)* and return to the office of the Human Research Ethics Committee.

The HREC wishes you every success in your research.

Yours sincerely,

Dr Conor Brophy
Chairperson RBWH Human Research Ethics Committee
Metro North **District**
18.06.2010

CHILDREN'S HEALTH QUEENSLAND
HOSPITAL AND HEALTH SERVICE
HUMAN RESEARCH ETHICS COMMITTEE

Professor Alan Isles AM (Chair) 3069 7002
Mrs Amanda Smith (Co-ordinator) 3069 7002

Queensland
Government

Level 7, Centre for Children's Health Research
Lady Cilento Children's Hospital Precinct
62 Graham Street, South Brisbane QLD 4101
Telephone (07) 3069 7002

28th August 2017

Professor Keith Grimwood
Professor of Infectious Diseases
Griffith University & Gold Coast University Hospital
Gold Coast Campus QLD 4222

Dear Professor Grimwood,

HREC Reference number: HREC/10/QRCH/16
Project title: ORChID – Observational research in childhood infectious diseases study

Many thanks for the Annual Report dated 22nd August for the above project. This has now been reviewed and the Committee is happy to give approval for the continuation of this important work.

The Children's Health Queensland Hospital and Health Service Human Research Ethics Committee (HREC) is constituted and operates in accordance with the National Health and Medical Research Council's *"National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the "*CPMP/ICH Note for Guidance on Good Clinical Practice"*.

Please note that all conditions of original approval still apply.

Yours sincerely,

Professor Alan Isles AM
Chair
Children's Health Queensland
Hospital and Health Service
Human Research Ethics Committee

Cc: Ethics Committee Files

annual report 280817

THE UNIVERSITY OF QUEENSLAND

Institutional Human Research Ethics Approval

Project Title:	ORChID - Observational Research In Childhood Infectious Diseases Study - 19/09/2016 - AMENDMENT
Chief Investigator:	Prof Keith Grimwood
Supervisor:	None
Co-Investigator(s):	Dr Stephen Lambert, A/Prof Michael Nissen, A/Prof Theo Sloots, A/Prof David Wang, Dr Rebecca Kimble, Ms Minda Sarna
School(s):	Queensland Children's Medical Research Institute
Approval Number:	2010000820
Granting Agency/Degree:	NHMRC
Duration:	18th June 2019

Comments/Conditions:

Amendment reviewed on the basis of approval by the Metro North HHS HREC dated 15/08/2016 (HREC/10/QRBW/125)

- Correspondence, 18/07/2016
- Annual Report, 20/07/20126
- Response to Request for Further Information, 05/08/2016

Note: If this approval is for amendments to an already approved protocol for which a UQ Clinical Trials Protection/Insurance Form was originally submitted, then the researchers must directly notify the UQ Insurance Office of any changes to that Form and Participant Information Sheets & Consent Forms as a result of the amendments, before action.

Name of responsible Committee:
University of Queensland Human Research Ethics Committee A
This project complies with the provisions contained in the *National Statement on Ethical Conduct in Human Research* and complies with the regulations governing experimentation on humans.

Name of Ethics Committee representative:
Professor Emerita Gina Geffen
Chairperson
University of Queensland Human Research Ethics Committee A
Registration: EC00456

Signature G. Geffen Date 20/12/2016

Appendix 2: Koch's modified postulates

(i) A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased (i.e., with anatomic, histologic, chemical, or clinical evidence of pathology) and not in those organs that lack pathology.

(ii) Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.

(iii) With resolution of disease (for example, with clinically effective treatment), the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.

(iv) When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.

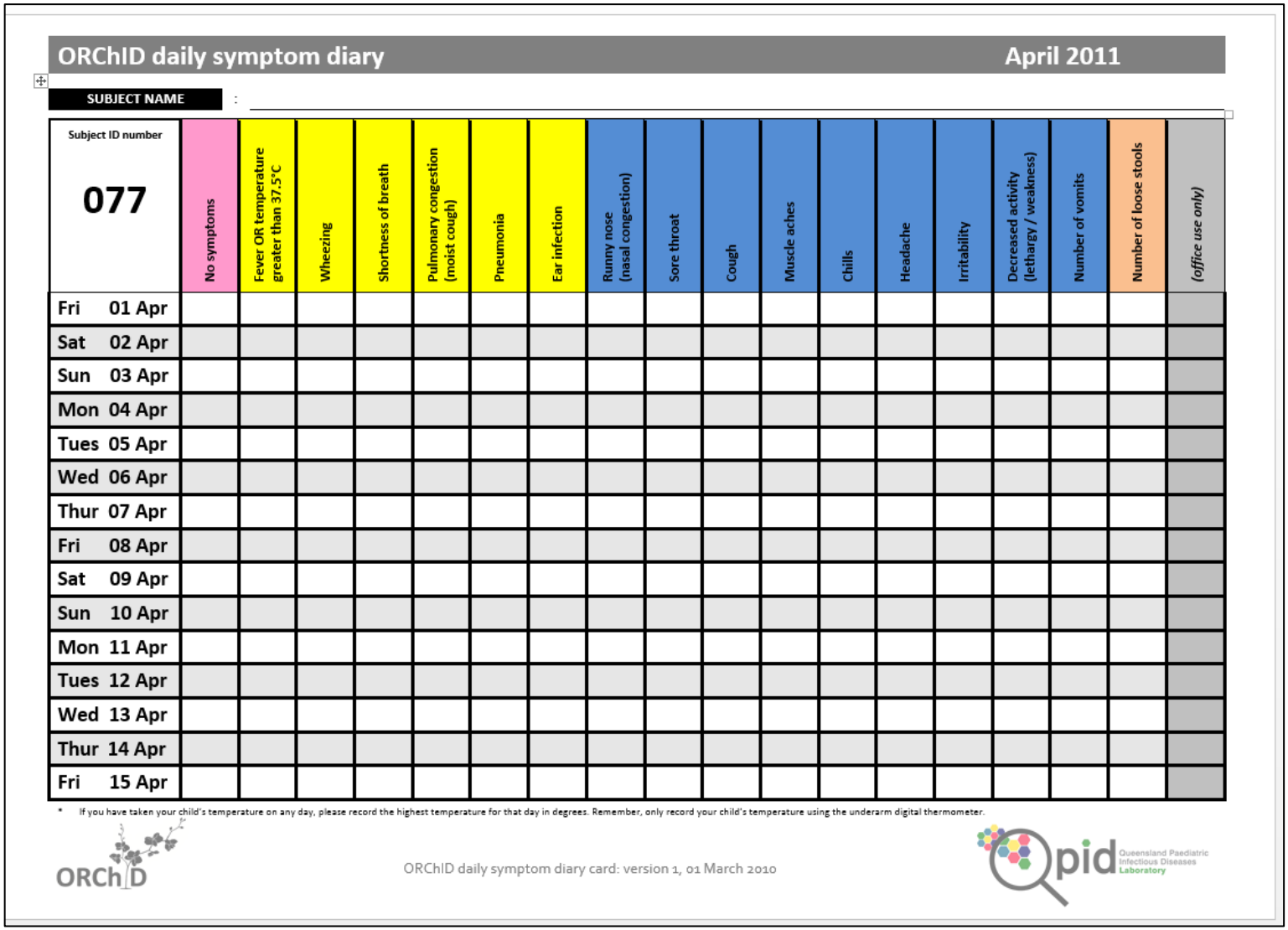
(v) The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms. When phenotypes (e.g., pathology, microbial morphology, and clinical features) are predicted by sequence-based phylogenetic relationships, the meaningfulness of the sequence is enhanced.

(vi) Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequences to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.

(vii) These sequence-based forms of evidence for microbial causation should be reproducible."

Reference: Fredricks DN and Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clinical Microbiology Reviews* 1996;9:18-33

Appendix 3: The Observational Research in Childhood Infectious Diseases study daily diary template



ORChID daily symptom diary **April 2011**

SUBJECT NAME :

Subject ID number 077	No symptoms	Fever OR temperature greater than 37.5°C	Wheezing	Shortness of breath	Pulmonary congestion (moist cough)	Pneumonia	Ear infection	Runny nose (nasal congestion)	Sore throat	Cough	Muscle aches	Chills	Headache	Irritability	Decreased activity (lethargy / weakness)	Number of vomits	Number of loose stools	*(office use only)*
Fri 01 Apr																		
Sat 02 Apr																		
Sun 03 Apr																		
Mon 04 Apr																		
Tues 05 Apr																		
Wed 06 Apr																		
Thur 07 Apr																		
Fri 08 Apr																		
Sat 09 Apr																		
Sun 10 Apr																		
Mon 11 Apr																		
Tues 12 Apr																		
Wed 13 Apr																		
Thur 14 Apr																		
Fri 15 Apr																		

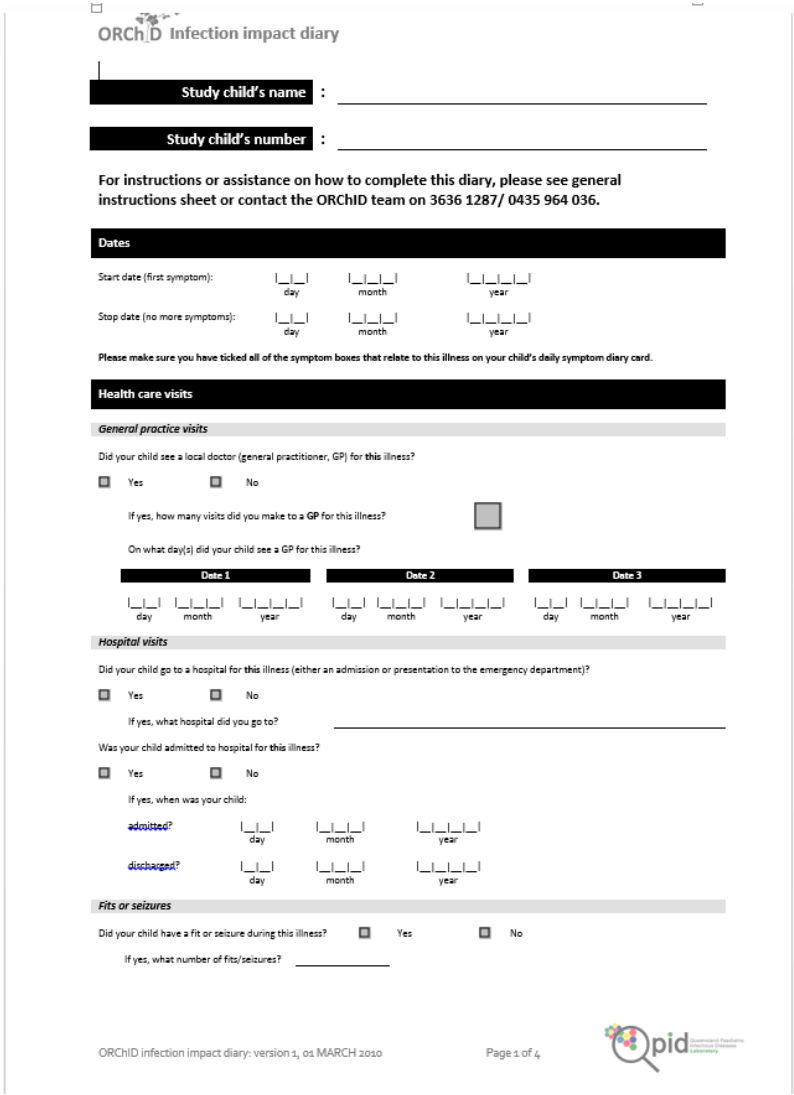
* If you have taken your child's temperature on any day, please record the highest temperature for that day in degrees. Remember, only record your child's temperature using the underarm digital thermometer.

ORChID

ORChID daily symptom diary card: version 1, 01 March 2010

pid Queensland Paediatric Infectious Diseases Laboratory

Appendix 4: The Observational Research in Childhood Infectious Diseases study burden diary template



ORChID Infection impact diary

Study child's name : _____

Study child's number : _____

For instructions or assistance on how to complete this diary, please see general instructions sheet or contact the ORChID team on 3636 1287/ 0435 964 036.

Dates

Start date (first symptom): |__|__| day |__|__| month |__|__|__|__| year

Stop date (no more symptoms): |__|__| day |__|__| month |__|__|__|__| year

Please make sure you have ticked all of the symptom boxes that relate to this illness on your child's daily symptom diary card.

Health care visits

General practice visits

Did your child see a local doctor (general practitioner, GP) for **this** illness?

☐ Yes ☐ No

If yes, how many visits did you make to a GP for this illness? ☐

On what day(s) did your child see a GP for this illness?

Date 1	Date 2	Date 3
|__|__| day |__|__| month |__|__|__|__| year	|__|__| day |__|__| month |__|__|__|__| year	|__|__| day |__|__| month |__|__|__|__| year

Hospital visits

Did your child go to a hospital for **this** illness (either an admission or presentation to the emergency department)?

☐ Yes ☐ No

If yes, what hospital did you go to? _____

Was your child admitted to hospital for **this** illness?

☐ Yes ☐ No

If yes, when was your child:

~~admitted~~? |__|__| day |__|__| month |__|__|__|__| year

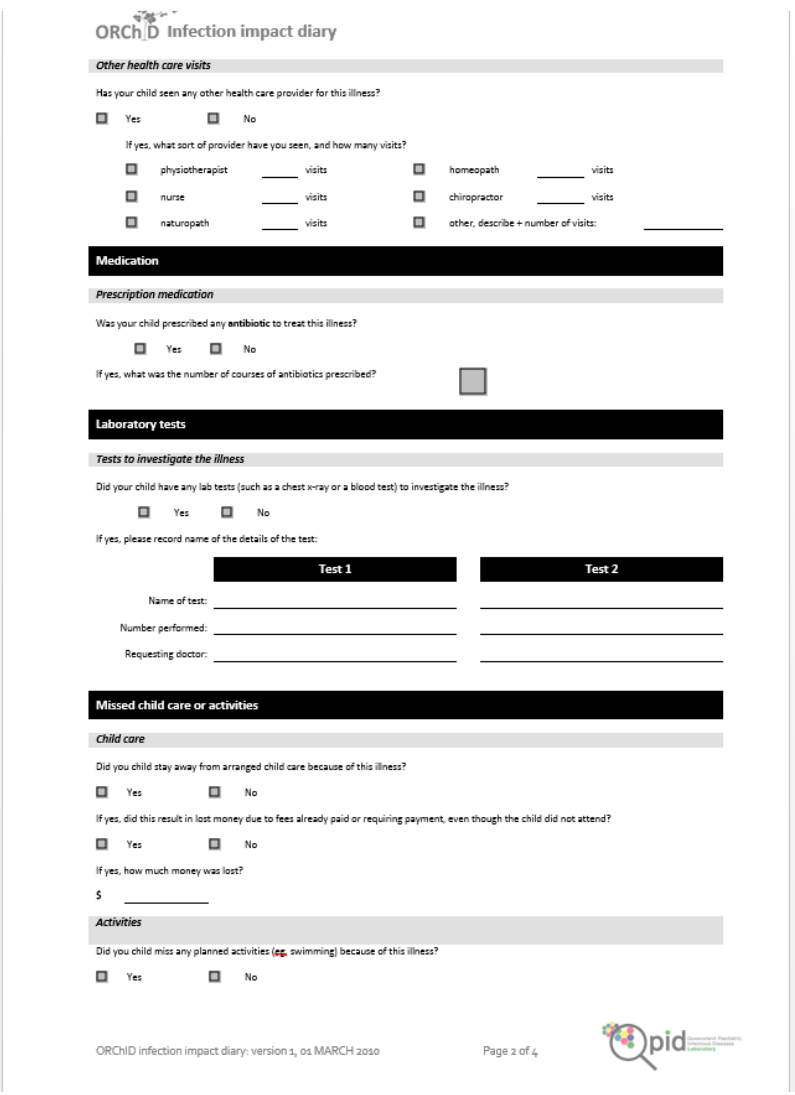
~~discharged~~? |__|__| day |__|__| month |__|__|__|__| year

Fits or seizures

Did your child have a fit or seizure during this illness? ☐ Yes ☐ No

If yes, what number of fits/seizures? _____

ORChID infection impact diary: version 1, 01 MARCH 2010 Page 1 of 4



ORChID Infection impact diary

Other health care visits

Has your child seen any other health care provider for this illness?

☐ Yes ☐ No

If yes, what sort of provider have you seen, and how many visits?

☐ physiotherapist _____ visits ☐ homeopath _____ visits

☐ nurse _____ visits ☐ chiropractor _____ visits

☐ naturopath _____ visits ☐ other, describe + number of visits: _____

Medication

Prescription medication

Was your child prescribed any **antibiotic** to treat this illness?

☐ Yes ☐ No

If yes, what was the number of courses of antibiotics prescribed? ☐

Laboratory tests

Tests to investigate the illness

Did your child have any lab tests (such as a chest x-ray or a blood test) to investigate the illness?

☐ Yes ☐ No

If yes, please record name of the details of the test:

	Test 1	Test 2
Name of test:		
Number performed:		
Requesting doctor:		

Missed child care or activities

Child care

Did you child stay away from arranged child care because of this illness?

☐ Yes ☐ No

If yes, did this result in lost money due to fees already paid or requiring payment, even though the child did not attend?

☐ Yes ☐ No

If yes, how much money was lost?

$ _____

Activities

Did you child miss any planned activities (**eg.** swimming) because of this illness?

☐ Yes ☐ No

ORChID infection impact diary: version 1, 01 MARCH 2010 Page 2 of 4

If yes, did this result in lost money due to fees already paid or requiring payment, even though the child did not attend?

☐ Yes ☐ No

If yes, how much money was lost?

$ _____

Other people's time during this illness

Time seeking health care only

Was any time spent seeking healthcare for this illness?

☐ Yes ☐ No

If yes, who was the adult who went with the child for the health care visit?

Name: _____

Sex: ☐ Male ☐ Female Relationship to child: _____

Work / occupation: _____

What was the **total time** spent by this **care giver seeking health care?**

_____ hours _____ minutes

From this **total time**, how much of the time **seeking health care** was:

Time off work with pay lost? _____ hours _____ minutes

Time off work with no pay lost? _____ hours _____ minutes

Time off usual activity (not work)? _____ hours _____ minutes

All other time caring for ill child

Who was the main adult (primary care giver) who cared for the child during this illness?

Sex: ☐ Male ☐ Female Relationship to child: _____

Work / occupation: _____

What was the **total extra time** spent by **the primary care giver caring for the child** during this illness (**excluding** time for health care visits counted above)?

Please note, this time should be the **extra time** in excess of **time normally spent caring for the child**. For example, if you normally spend 6 hours a day caring for the child, but you need to spend 7 hours and 30 minutes caring for the child because of the illness, the extra time should be recorded is 1 hour and 30 minutes.

_____ hours _____ minutes

From this **total time**, how much of the time **caring for the child** was (these 3 should add to the value above):

Time off work with pay lost? _____ hours _____ minutes

Time off work with no pay lost? _____ hours _____ minutes

Time off usual activity (not work)? _____ hours _____ minutes

In the time when the primary care giver was not caring for the ill child, were other adult care givers involved in caring for the ill child?

☐ Yes ☐ No

Name: _____

Sex: ☐ Male ☐ Female Relationship to child: _____

Work / occupation: _____

What was the **total time** spent by the **other care giver caring for the child** during this illness (**excluding** time for health care visits counted above)?

_____ hours _____ minutes

From this **total time**, how much of the time **caring for the child** was (these 2 should add to the value above):

Time off work with pay lost? _____ hours _____ minutes

Time off work with no pay lost? _____ hours _____ minutes

Time off usual activity (not work)? _____ hours _____ minutes

Comments and other details

If you have any questions about completing this diary, please contact the ORChID study team on 3636 1287/ 0435 964 036.

Appendix 5: The Observational Research in Childhood Infectious Diseases study specimen collection instruction sheet

ORChID: specimen collection instructions

What will I need to take the anterior nose swabs?

- Provided children's nose swab.
- **Sponge** transport tube.
- Packing material: specimen bag, mailing bag.
- Specimen collection form.

What will I need to take the dirty nappy swab?

- Provided children's nappy swab.
- A nappy with baby's poo.
- **Sponge** transport tube.
- Packing material: specimen bag, mailing bag.
- Specimen collection form.

What should I do?

Every week, on the same day, we would like you to collect a simple swab from both nostrils, and a swab from a dirty nappy.

Before starting

- Prepare well by being in an area with good lighting and have all equipment available.
- Try to ensure that your child does not blow his/her nose before taking the nostril swabs.
- Label the sponge transport tubes before starting with your child's name, date of birth, study number, and the date on which the specimen is collected.
- Get assistance to hold your child's head if required for the nostril swabs.
- Wash your hands before taking the specimens.

Nostril swabs

- Tilt your child's head back gently, with one hand to steady the chin.
- With the other hand, insert the end of a new swab into the front part of one of the nostrils.
- Rub swab gently against the inner wall of the nostril, whilst getting a good sample of mucous if your child has a runny nose at the time.
- Remove the swab from the nostril, and using the same swab collect a swab of the other nostril.
- Remove the cap from the sponge transport tube.
- Place the swab in the tube and push the cap down to seal the lid securely.
- Squeeze the sponge so the end of the swab gets moist.

Dirty nappy swab

- Wait until you have a dirty nappy with baby's poo (stool) available.
- Use the swab to collect a specimen of stool, enough to cover one side of the swab.
- Remove the cap from the sponge transport tube.
- Place the swab in the tube and push the cap down to seal the lid securely.
- Squeeze the sponge so the end of the swab gets moist.

Now what?

- Place the specimen sponge transport tubes in the zip lock bag as you have been shown.
- Wash your hands thoroughly using soap and warm water.
- Complete the specimen collection form and place it in the sleeve of one of the zip lock bags.
- Place the zip lock bag with the specimen collection form into the pre-addressed pre-paid mailing bag/envelope.
- IF YOU HAVE ANY OTHER COMPLETED PAPERWORK, SUCH AS COMPLETED MONTHLY DIARY OR INFECTION IMPACT DIARY, PLEASE INCLUDE IT WITH THIS SHIPMENT.
- As soon as possible, place the pre-addressed, pre-paid mailing bag in a red Australia Post mailing post box.
- Let the ORChID team know you have collected the specimen by calling on **3636 1567.**

Remember, if you have any questions or problems with collecting the specimen, call your research assistant or the ORChID team on:

3636 1567

Thank you!

ORChID

Appendix 6: The Observational Research in Childhood Infectious Diseases study swab and symptom collection pack