Prevention of Acute Respiratory Infections among Indigenous Infants of the Northern Territory

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DECLARATION

I hereby declare that the work herein, submitted for the degree of Doctor of Philosophy at Charles Darwin University, is the result of my own investigations and that all references to the ideas and work of others have been specifically acknowledged. I certify that the work embodied in this thesis has not been accepted or submitted for any other degree.

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ABSTRACT

Indigenous children in the Northern Territory experience a disproportionately high burden of respiratory infectious diseases associated with conditions favouring microbial transmission and endemic carriage of respiratory pathogens. The lack of a substantive impact in the prevalence of respiratory diseases over the last two decades highlights the ongoing need for improvements in health equity for Indigenous Australians.

This thesis investigates the impact of maternal pneumococcal vaccination against, and the influence of maternal/infant vitamin D insufficiency on, the risk of infant respiratory infections. Novel vaccination and supplementation strategies have the potential to be translated into practice.

Principal findings:

1. In a randomised controlled trial of maternal pneumococcal vaccination for the prevention of infant middle ear disease, receiving the 23-valent pneumococcal polysaccharide vaccine in pregnancy:

   a. Stimulated a strong maternal vaccine serotype-specific antibody response in both blood and breast milk.

   b. Had a non-significant impact on overall infant ear disease and/or nasopharyngeal carriage of vaccine serotypes.
c. Reduced the prevalence of ear disease associated with concurrent carriage of vaccine serotypes (a more specific outcome).

d. Had a non-significant impact on the incidence of infant acute lower respiratory infection (ALRI) hospitalisations.

2. In a prospective cohort of Indigenous mothers and their infants, cord blood vitamin D insufficiency was common at birth and cord blood concentrations were lower among infants subsequently hospitalised with an ALRI compared to infants who were not.

3. In a cross-section of hospitalised Indigenous and non-Indigenous children, vitamin D insufficiency was unexpectedly less common among those with ALRI diagnoses compared to non-ALRI diagnoses (predominantly gastroenteritis).

While the data presented in this thesis need to be interpreted with caution due to small sample sizes and limited significance, the findings suggest that maternal pneumococcal vaccination or vitamin D supplementation may have potential as interventions for the prevention of infant respiratory infections. These data lay a strong foundation for the cohort studies or clinical trials necessary to allow informed decisions regarding the future use of these strategies in routine practice.
ACKNOWLEDGEMENTS

Like many others before me I began this journey deeply concerned about the unfairly high burden of respiratory disease endured by Indigenous children in this region. This thesis contributes a small amount of knowledge to the broad child health respiratory and immunisation research programs at Menzies School of Health Research. I am hopeful the contribution was worthy.

I would like to open by thanking my primary supervisor Ross Andrews who agreed to take me on as his student. Ross provided me with a broad and fresh public health perspective on research that I had perhaps lost after more a decade in the laboratory. I feel privileged to have been a part of the PneuMum trial, it was a fantastic learning experience and I walk away a much better researcher for it! Ross, thank you for your good humour, interesting ideas and valuable advice that always raised the quality of the research. I look forward to future collaborations and yes we will get those papers published someday!

A big thanks to Heidi Smith-Vaughan who supported me from the outset. Despite her busy schedule, Heidi’s door was always open and she provided me with sagely advice on countless topics both work related and beyond, often on the weekend or late into the evening. Heidi, thanks for your friendship, enthusiasm and down to earth advice. Robyn, with your own PhD still fresh in your mind your empathy and encouragement over the final months was invaluable. Your unwavering confidence in my ability to finish on time was infectious and I really appreciated the regular words of encouragement. “It’s a PhD, not a nobel prize” though hard to believe, will
always ring in my mind. I was also very fortunate to have the advice and support of Anne Chang. Anne, despite being based interstate, you provided amazingly prompt and candid feedback and your critical appraisal during the final write up phase was invaluable. Your “waffle detector” is second to none.

To the PneuMum chief investigators and senior scientists, Jonathan Carapetis, Amanda Leach, Peter Morris and Kim Mulholland: thanks for conceiving and supporting such a great project and providing valuable advice along the way. To the PneuMum clinical staff: Jane Nelson, Sarah Moberley, Melita McKinnon, Irene O’Meara, Marie Kirkwood, Christine Wigger, Sandy Nelson and Cate Wilson thanks for doing such a good job with the all the study visits, ear examinations, sample collection and data entry….all that hard work certainly made my job a lot easier. Thanks to Kim Hare and Vanya Hampton in the Menzies laboratory for processing the samples and doing the microbiology, and also to Anne Balloch at the Murdoch Children’s Research Institute in Melbourne for performing all the ELISA’s and for willingly chasing up my often difficult queries.

Many others have made the last three and a half years a great experience. Thanks to Amanda Leach, for excellent support and guidance on clinic trials and otitis media in the Northern Territory. Your passion for Indigenous health research is highly motivating. To my office buddy and fellow PhD student Susan Pizzutto, it was great to be able to share many of the common PhD highs and lows with you. Our often inane but ever hilarious conversations were a necessary distraction from reality and as for our tower of Eclipse mint containers……that was a sight to behold! Apparently it was very important to have fresh breath in such a small office. To my
fellow researcher and good friend Patiyan Andersson, in a division comprised almost exclusively of females (not that I’m complaining) the “bromance” was much appreciated, cheers mate! The longboarding, squash, rock climbing, and footy kept the waistline in check and the brain refreshed. Jemima Beissbarth, thanks for the database help and for chatting about all things STATA. Your willingness to help at the drop of a hat was much appreciated. For their amazing organisation, administrative skills and general ability to get things done, thanks also to Margaret Landrigan, Jennifer Wong and Caroline Sheridan. Also a little shout out to the workmen that slaved in the heat outside my window and whose colourful language initiated our regular use of the acronym “JFF”. The actual expansion will remain veiled but essentially it means get back to work!

Most importantly, I need to thank my family. Taking on a PhD with three young children, a busy wife and a half renovated house seemed like a good idea at the time! Paula Binks, I am eternally grateful for your enduring love and support over this journey. Though you must have felt like a single mum at times, you were there for me through the ups and downs and it was great to have someone to vent to at the end of the day. Promise we’ll go on a holiday next year! To my beautiful children, Zayd, Maiya and Marley Binks, thanks for your unwavering love and for taking my mind off things the second I walked in the door. Next time you ask “Dad can we play something?” the answer will definitely be yes. I would also like to thank my Mum and Dad for their interest in my studies, assistance around the house and for the 24 hour baby-sitting service they provided every dry season.
Lastly, I wish to thank all the children, families and carers that participated in these studies. I hope you all took something positive away from the experience. I would also like to acknowledge the funding support from the National Health and Medical Research Council, the Australian Academy of Science, the Channel 7 Children’s Research Foundation, and the Menzies School of Health Research that made this work possible.
STATEMENT OF RESEARCH CONTRIBUTION

This section lists my contribution to each of the research chapters contained in this thesis:

Chapter 3: PneuMum, Impact from an open label randomised controlled trial of maternal 23-valent pneumococcal polysaccharide vaccination on middle ear disease amongst Indigenous infants, Northern Territory, Australia.

My contribution to this work was primarily in the analysis and publication. I was responsible for scientific and statistical appraisal of the study data and prepared this chapter (and the numerous drafts of manuscript, which is to be submitted shortly) in consultation with Professor Ross Andrews (the chief investigator and my primary PhD supervisor) and Doctor Sarah Moberley (co-primary author). The senior co-investigators of this trial were Professors Jonathan Carapetis, Amanda Leach, Peter Morris, Mimi Tang, Paul Torzillo and Kim Mulholland. The trial was managed by many clinical, laboratory and administrative staff, guided by a Human Research Ethic Committee, an Indigenous reference group and a data safety monitoring board. Sarah Moberley has completed a PhD thesis based on other aspects of this trial.

Chapter 4: Impact of the 23-valent pneumococcal polysaccharide vaccination in pregnancy against infant acute lower respiratory infections in the Northern Territory of Australia.

This was a secondary analysis of the PneuMum randomised controlled trial. The idea was conceived by Professor Ross Andrews (my primary supervisor) and myself. I
performed all other aspects of this study including data collection, management and analysis.

Chapter 6: Cord blood vitamin D status and the risk of acute lower respiratory infection for Indigenous infants in the Northern Territory of Australia during their first year of life.

I conceived the idea for this chapter. The study design, funding applications, ethics approvals, sample handling and data analysis were performed by myself in consultation with Professor Andrews. Vitamin D assays were performed by Royal Melbourne Institute of Technology Drug Discovery Technologies Pty Ltd in Melbourne, Australia.

Chapter 7: Vitamin D insufficiency among hospitalised children in the Northern Territory

I conceived the idea for this chapter. The study design, ethics approvals, data collection and analysis were performed by myself in consultation with Professor Andrews. The published article was prepared by myself in consultation with the co-authors, Drs Heidi Smith-Vaughan and Naor Bar-Zeev and Professors Anne Chang and Ross Andrews. Vitamin D assays were performed by Royal Melbourne Institute of Technology Drug Discovery Technologies Pty Ltd, in Melbourne, Australia.
Publications arising from thesis


In preparation


Related publications during the course of the candidature


#I gave the oral presentation.


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### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALRI</td>
<td>Acute lower respiratory infections</td>
</tr>
<tr>
<td>7vPCV</td>
<td>7-valent pneumococcal conjugate vaccine</td>
</tr>
<tr>
<td>10vPHiD-CV</td>
<td>10-valent pneumococcal <em>Haemophilus influenzae</em> protein D conjugate vaccine</td>
</tr>
<tr>
<td>23vPPV</td>
<td>23-valent pneumococcal polysaccharide vaccine</td>
</tr>
<tr>
<td>NTHi</td>
<td>Nontypeable <em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td>25OHD2</td>
<td>25-hydroxy vitamin D2</td>
</tr>
<tr>
<td>25OHD3</td>
<td>25-hydroxy vitamin D3</td>
</tr>
<tr>
<td>25OHD</td>
<td>25-hydroxy vitamin D (vitamin D2 and D3 collectively)</td>
</tr>
<tr>
<td>1,25OHD2D</td>
<td>1, 25-dihydroxy vitamin D</td>
</tr>
<tr>
<td>OME</td>
<td>Otitis media with effusion</td>
</tr>
<tr>
<td>AOM</td>
<td>Acute otitis media</td>
</tr>
<tr>
<td>AOMwiP</td>
<td>Acute otitis media with perforation</td>
</tr>
<tr>
<td>CSOM</td>
<td>Chronic suppurative otitis media</td>
</tr>
<tr>
<td>VE</td>
<td>Vaccine efficacy/effectiveness</td>
</tr>
<tr>
<td>95% CI</td>
<td>95 percent confidence interval</td>
</tr>
<tr>
<td>GMC</td>
<td>Geometric mean concentration</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
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<tr>
<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>nmol/L</td>
<td>Nanomoles per litre</td>
</tr>
<tr>
<td>ng/ml</td>
<td>Nanograms per millilitre</td>
</tr>
<tr>
<td>TIV</td>
<td>Trivalent influenza vaccine</td>
</tr>
<tr>
<td>HIA</td>
<td>Haemagglutination inhibition assay</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immuno-sorbent assay</td>
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<tr>
<td>Th</td>
<td>T-helper lymphocytes</td>
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<td>T-cells</td>
<td>T lymphocytes</td>
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<td>B-cells</td>
<td>B lymphocytes</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>R²</td>
<td>Regression coefficient</td>
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CHAPTER 1

Introduction
Chapter 1: Introduction

1.1 Overview

This thesis focuses on two common conditions affecting Indigenous children of the Northern Territory: acute lower respiratory infections (ALRI) and middle ear infections (or otitis media). Most Indigenous children experience acute otitis media (AOM) in their first year of life which progresses to chronic suppurative otitis media (CSOM) in up to 15% of 6-30 month olds (Morris et al. 2005). A CSOM prevalence of over 4% is considered by the World Health Organization to be a substantial public health problem requiring immediate attention (World Health Organization 1996). The ALRI’s, bronchiolitis and pneumonia are the most common reason for hospitalisation and the leading cause of preventable mortality among Indigenous children in this region (Li et al. 2007; O'Grady et al. 2010c; Pink & Allbon 2008). In 2007, prevention of ALRI was listed as a performance target in the Australian Federal Government’s ‘Closing the Gap’ program (Australian Government 2007).

The ongoing high prevalence of both otitis media and ALRI reflect the socioeconomic and health disadvantages that persist among Indigenous populations in Northern Australia.

Chapter 1 outlines the burden of otitis media and ALRI among Indigenous infants in the Northern Territory over the past 20 years, highlighting the key pathogens and current immunisation strategies. The subsequent chapters form two distinct parts. The first part (Chapters 2, 3 and 4) focuses on maternal pneumococcal vaccination. Chapter 2 reviews the literature while Chapters 3 and 4 describe the findings from a randomised controlled trial for the prevention of infant otitis media and ALRI
respectively. The second part (Chapters 5, 6 and 7) focuses on vitamin D and ALRI. Chapter 5 reviews the literature around vitamin D as a risk factor for, and potential preventative strategy against, ALRI. Chapters 6 and 7 report data characterising the vitamin D status of Indigenous infants in the Northern Territory focusing on vitamin D insufficiency as a modifiable risk factor for ALRI. Chapter 8 is the final discussion, reiterating the main thesis findings and discussing the future implications of this research.

1.2 Otitis media

Otitis media or “inflammation of the middle ear” is the result of a bacterial infection in the middle ear cavity that manifests with varying degrees of clinical severity. Otitis media with effusion (OME) is a state of mild inflammation marked by fluid behind the ear drum without any acute symptoms; AOM indicates a proliferating infection with increased middle ear pressure and ear drum (tympanic membrane) bulging; acute otitis media with perforation (AOMwiP) occurs when middle ear pressure ruptures the tympanic membrane; and CSOM is a complicated perforated infection resulting in persistent ear discharge. Diagnostic criteria for otitis media differ around the world. For the purposes of this thesis, all diagnostic criteria and clinical management practices for otitis media studies are based on the standardised “Clinical care guidelines on the management of otitis media in Aboriginal and Torres Strait Islander populations” (Morris et al. 2001, 2010b).

1.2.1 Burden of otitis media

Indigenous children have some of the highest reported rates of acute and chronic ear infections in the world. Surveys conducted between 1977 and 1992 investigated ear
disease in over 27,000 Australian Indigenous children <15 years of age (Lewis et al. 1977; Moran et al. 1979; Sunderman & Dyer 1984; Watson & Clapin 1992). Across these studies the rates of ear infections with tympanic membrane perforation were 15-37% indicating a serious public health problem (Lewis et al. 1977; Moran et al. 1979; Sunderman & Dyer 1984; Watson & Clapin 1992). Persistent ear infections can result in permanent middle ear damage, hearing loss and educational disadvantage (O'Connor, Perry & Lannigan 2009).

A systematic literature search (Appendix A: Search 1) identified five articles that reported on the burden of otitis media (middle ear disease) among Indigenous children in the Northern Territory since 1990. A small prospective birth cohort study in 1994 found 75% (27/36) of remote Indigenous infants developed OME and 22% (8/36) developed AOM by 12 weeks of age (Leach et al. 1994). A subsequent small cross-sectional survey in 1995, found the prevalence of any otitis media was 95% (21/22) among 6-8 week old remote Indigenous children in this region compared to 30% (3/10) for their non-Indigenous child counterparts (Boswell & Nienhuys 1995). Two years later in a prospective cohort study, otitis media with effusion was diagnosed in 37% (739/2012) of ear examinations performed on 252 Indigenous and non-Indigenous children (<4 years of age) attending Darwin child care centres over a 24 week period (Skull et al. 1999). In a larger cross-sectional survey of 914 Indigenous children (<6 years of age) living in remote communities in 2001 (Morris et al. 2005), 91% of children had some form of otitis media and 15% of children had CSOM. A retrospective historical comparison (Mackenzie et al. 2009) across two time periods, 1996-2001 and 2001-2004, showed little change in the proportion of children identified as having OME (96%; 81/84 and 100%; 41/41) by 6 months of
age or AOM (89%; 75/84 and 88%; 36/41) and AOMwiP (34%; 29/84 and 35%; 17/41) by 12 months of age. Recent otitis media surveillance in this region (unpublished) suggests no significant decline in the rates of childhood (0-6 years of age) AOMwiP since 2001 (Figure 1.1) (Leach 2014).

Figure 1.1  Prevalence of tympanic membrane perforation among Indigenous children (0-6 years of age) living in remote communities of the Northern Territory.

Data were taken from (Leach 2014; Morris et al. 2005). Whiskers represent 95% confidence intervals (CI). Otitis media diagnoses were determined using tympanometry, pneumatic otoscopy and video-otoscopy according to standardised definitions (Morris et al. 2001, 2010b).
1.2.2 Otitis media in the era of pneumococcal conjugate vaccination

The pneumococcus is one of the major otitis media pathogens in the Northern Territory (Leach et al. 1994) (discussed further in Section 1.5.2). In the early 2000’s, two international trials demonstrated that the seven-valent pneumococcal conjugate vaccine (7vPCV) administered to infants as a 2, 4 and 6 months primary schedule reduced AOM episodes by 6-7% compared to control vaccinees (meningococcal C and hepatitis B respectively) (Black et al. 2000; Eskola et al. 2001). A subsequent study by O’Brien (O'Brien et al. 2008) among Apache infants showed PCV7 had no effect against AOM while Prymula (Prymula et al. 2006) found their prototype 11-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (11vPHiD-CV) reduced AOM by 34% (95% CI 21% to 44%). The trial conducted in Finland (Eskola et al. 2001) further demonstrated a 57% (95% CI 44% to 67%) reduction in the more specific outcome of AOM concurrent with detection of a vaccine serotype in the middle ear following tympanocentesis (9% among 7vPCV vaccinees versus 19% among controls).

The 7vPCV was introduced for Indigenous children of the Northern Territory in 2001 as a 2, 4 and 6 months primary schedule, with a 23-valent pneumococcal polysaccharide vaccine (23vPPV) booster at 18 months. The 7vPCV/23vPPV combination (June 2001 to Sept 2009) was replaced on the infant immunisation schedule by the 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine (10vPHiD-CV) in October 2009 and the 13-valent pneumococcal conjugate vaccine (13vPCV) in October 2011, for both primary and booster doses. A systematic search of the literature (Appendix A: Search 2) returned two relevant non-randomised studies investigating the impact of the 7vPCV against childhood (<3 years) otitis
media in the Northern Territory. One, the aforementioned study by Mackenzie et al. compared two birth cohorts of Indigenous children, one prior to (n=41; 1996-2001) and one in the period after (n=84; 2001-2004) the introduction of 7vPCV/23vPPV (Mackenzie et al. 2009). Clinical examinations including tympanometry and otoscopy were performed at regular intervals from birth until 24 months of age for both cohorts. By age 6 months, 69% of children had already experienced an episode of AOM with no difference between 7vPCV vaccinees and controls. At age 12 months, there was no difference in cumulative proportions of AOM (89% and 88%) or AOMwiP (34% and 35%) between groups; however, vaccinees had experienced significantly less recurrent perforation episodes (9%; 8/95 versus 22%; 11/51, p=0.037). The second study (Leach et al. 2014) compared data from cross-sectional otitis media surveillance of Indigenous children (<3 years of age) across 25 remote communities. Among children with at least one ear exam, those who received two or more doses of 7vPCV (n=432; 2008-2011) had less OME (41% versus 51%; p=0.002) and more AOM (35% versus 25%; p=0.003) compared to those that received two or more doses of 10vPHiD-CV (n=437; 2011-2012). The analysis included adjustment for risk factors such as the increased use of macrolide antibiotics and suggested a reduction in the severity of otitis media among 10vPHiD-CV vaccinees. No differences were seen in children less than 9 months of age where the prevalence of any suppurative otitis media (AOM, AOMwiP or CSOM) was already 60% (74/124). While these non-randomised comparisons from different eras were prone to temporal biases in the community and the research procedures, both studies suggest that infant pneumococcal conjugate vaccination had only a moderate impact against otitis media. Further, it was evident that there is a considerable burden of otitis media prior to expected protection afforded by the current schedule.
highlighting the need for earlier intervention strategies.

1.3 Acute lower respiratory infections (ALRI)

In this thesis, ALRI hospitalisations were defined by International Classification of Diseases coding (ICD-10-AM) (National Centre for Classification in Health 2009) while ALRI clinic presentations were identified via the electronic primary care information system (PCIS) using a standardised diagnostic algorithm described in detail in Chapter 4. ALRI broadly describes any acute infections of the lower respiratory mucosa from the trachea to the alveoli. Bronchiolitis and pneumonia are the most common ALRI’s but bronchitis, whooping cough (pertussis) and influenza infection are also important. The epidemiology, pathophysiology, aetiology and management of ALRI’s among Indigenous and non-Indigenous children are described in detail in the review by Chang et al. (Chang et al. 2009). Bronchiolitis is predominantly a viral infection characterised by airway inflammation, mucus production and necrosis of respiratory epithelial cells (American Academy of Pediatrics Subcommittee 2006). Clinical symptoms of bronchiolitis include cough, wheezing and increased work of breathing that is often evidenced by grunting, nasal flaring and intercostal recession (American Academy of Pediatrics Subcommittee 2006). Pneumonia is caused by a diverse range of microbes; however, establishing the specific aetiology of pneumonia is difficult due to the physical constraints of sampling the lung. Pneumonia symptoms include cough, difficulty breathing, tachypnoea and chest wall recession with radiographs providing definitive diagnosis (Cherian et al. 2005).

1.3.1 Burden of ALRI
Respiratory diseases kill 10 times more Australian Indigenous than non-Indigenous infants (81 versus 8.1 deaths per 100,000 population) (Australian Institute of Health and Welfare 2011). In the Northern Territory, one in five (22%; 2028/9295) Indigenous infants born between 1999 and 2004 were hospitalised with an ALRI before their first birthday at an incidence of 427 episodes per 1000 child-years of observation (O'Grady, Torzillo & Chang 2010). The frequency distribution was highest in the first four months of life prior to any expected immune protection from the 2, 4 and 6 month infant PCV schedule. The median age of admission was 4.6 months and the overall risk was twice as high among infants living in the Central Australian region of the Northern Territory.

1.3.2 ALRI in the era of pneumococcal vaccination

Across the globe pneumococcal conjugate vaccines have successfully reduced the burden of pneumonia (Loo et al. 2014). In the Northern Territory of Australia, there was no decline in all cause ALRI following the introduction of routine infant pneumococcal conjugate vaccination in 2001 (O'Grady et al. 2010b; O'Grady, Torzillo & Chang 2010) although the data relating to the more specific diagnosis of pneumonia are conflicting. Ecological studies have reported reductions in pneumonia hospitalisation rates among Northern Territory Indigenous infants in the years after implementation of the infant pneumococcal vaccination program (Jardine, Menzies & McIntyre 2012). In contrast, a retrospective cohort study utilising documented vaccination status, blinded radiological assessment of pneumonia (according to World Health Organization guidelines) and controlling for a number of confounding factors, showed no evidence of 7vPCV effectiveness against ALRI among Indigenous infants to 18 months of age (O'Grady et al. 2010a).
Whilst hospitalisation data identify serious illness, these data are generally a poor reflection of the magnitude and trends of disease in the community. A systematic review of ALRI among Northern Territory Indigenous children at a community level (Appendix A: Search 3) found that up to 75% of remote Indigenous infants presented to a health care clinic with at least one episode of ALRI before 12 months of age (Clucas et al. 2008; Kearns et al. 2013) (Table 1.1). There are no published ALRI burden data from remote Northern Territory communities after 2006.

<table>
<thead>
<tr>
<th>Author</th>
<th>Study type</th>
<th>Years conducted</th>
<th>Age (years)</th>
<th>Total Children</th>
<th>ALRI presenting children (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clucas et al.</td>
<td>Retrospective clinic record review</td>
<td>2001-2005</td>
<td>0-4.75</td>
<td>174</td>
<td>139 (80)</td>
</tr>
<tr>
<td>Kearns et al.</td>
<td>Retrospective clinic record review</td>
<td>2001-2006</td>
<td>0-1</td>
<td>320</td>
<td>239 (75)</td>
</tr>
</tbody>
</table>

### 1.4 Causal pathways and risk factors

A broad range of demographic, social, environmental, immunological and microbiologic risk factors are associated with the high burden of respiratory disease (otitis media and ALRI) among Indigenous children. These risk factors and the causal pathways leading to a vicious cycle of respiratory disease are well described in the literature (Figure 1.2) (Hare, Smith-Vaughan & Leach 2010; Lehmann et al. 2008; Mackenzie et al. 2010; Moore 2011; Moore et al. 2010; O'Grady & Chang 2010). In summary, the endemic respiratory disease among Indigenous children is associated with living conditions that (1) favour microbial transmission and (2) lead to impaired immunity. Common risk factors for transmission include overcrowded...
housing, seasonality, childcare attendance and poor hygiene whereas risk factors for impaired immunity include poor nutrition, young age, male gender, prematurity, low birth weight and exposure to tobacco or environmental smoke. Many of these risk factors are associated with remote dwelling, limited formal education, high unemployment, low socio-economic status, inadequate housing and inadequate access to under-resourced medical services.

![Diagram of the extended vicious cycle of infection hypothesis explaining the high rates of respiratory infection among Australian Indigenous children.](source)

Figure 1.2 Extended vicious cycle of infection hypothesis explaining the high rates of respiratory infection among Australian Indigenous children.

Sourced from Hare et al. (Hare, Smith-Vaughan & Leach 2010) following adaptation from Wiertsema and Leach (Wiertsema & Leach 2009).

1.5 Aetiology of ear and respiratory tract infections in Indigenous children.
The pneumococcus and nontypeable *Haemophilus influenzae* (NTHi) are considered the most important aetiological organisms of childhood respiratory disease and are frequently detected from the respiratory tract of Indigenous children during episodes of otitis media (Leach et al. 1994), pneumonia (Torzillo et al. 1999) and bronchiectasis (Hare et al. 2013).

### 1.5.1 Common respiratory bacteria in the nasopharynx

Indigenous infants living in remote communities of the Northern Territory experience dense and diverse nasopharyngeal bacterial colonisation within weeks of birth (Leach et al. 1994; Smith-Vaughan et al. 2008). Remote dwelling Indigenous children (aged 3-7 years) have almost ubiquitous carriage of pneumococcus (90%) and NTHi (80%), rates twice as high as their non-Indigenous counterparts (aged 0-4 years) attending urban child care centres (43% and 41% respectively) (Stubbs et al. 2005) and over three times higher than among non-Indigenous children (aged 0-2 years living in rural Western Australia (25% for both) (Watson et al. 2006).

It is likely that microbe laden nasopharyngeal secretions seed both middle ear and lower airway infections (Thach 2008). As such, nasopharyngeal microbiology is often used as a surrogate for both lower airway and middle ear microbiology where middle ear and lung aspirates are not routinely collected (Binks et al. 2011; Fairchok et al. 2010).

### 1.5.2 Bacteriology associated with otitis media

Leach et al., showed that Indigenous children living in remote communities had pneumococcal and NTHi nasopharyngeal colonisation within three weeks of birth,
directly correlated with the first evidence of otitis media, which occurred among 50% of these children by four weeks of age (Leach et al. 1994). As tympanocentesis is not routinely performed on Australian Indigenous children, the middle ear microbiology can only be assessed in discharge samples collected following spontaneous tympanic membrane perforation. Gibney et al. reported pneumococcus and NTHi in 29% and 32% of 38 ear discharge swabs collected from 13 children with AOMwiP (Gibney et al. 2005). Similarly, Morris et al. found pneumococcus and NTHi in 27% and 39% of 70 ear discharge swabs, also from children with AOMwiP (Morris et al. 2010a). More recently, a PCR based study showed NTHi was the dominant otopathogen, in terms of prevalence and density, in Indigenous children with AOMwiP (Smith-Vaughan et al. 2013). Numerous other bacteria such as Moraxella catarrhalis, Staphylococcus aureus and Alloiococcus otitidis contribute to ear disease but will not be discussed further in this review.

1.5.3 Bacteriology associated with ALRI

The pneumococcus is a major cause of pneumonia around the globe causing an estimated 11% of all childhood deaths in children aged <5 years (O’Brien et al. 2009). However, because collection of lung aspirations and bronchoalveolar lavages are invasive procedures and not generally justified during acute infections, only a few studies have directly characterised the lung microbiology of children with ALRI. Among 83 children hospitalised with pneumonia in Papua New Guinea, pneumococcus (34%) and Haemophilus influenzae (40%; over half of which were NTHi) were the most commonly isolated bacteria from lung aspirates and blood samples (Shann et al. 1984). In Malawi, the pneumococcus (32%; 31/95) was the leading bacterial pathogen detected by PCR of lung aspirates from children aged 2
months to 15 years with high rates of HIV (62%; 59/95) while adenovirus (16%; 15/95) was the most common virus (Carrol et al. 2011). In another African study, this one conducted in The Gambia (Howie et al. 2014), the pneumococcus (91%; 48/53) was the leading pathogen identified in lung or pleural aspirations of children with severe pneumonia using both culture and molecular detection assays; whilst *Haemophilus influenzae* (non-type b) was also frequent (23%; 12/53).

Few ALRI aetiology studies were identified among Indigenous children in Australia from a systematic literature search (Appendix A: Search 4). In the early 1990’s, Torzillo et al. found that 29% (92/322) of children hospitalised with ALRI in Central Australia had blood positive pneumolysin assays suggestive of a pneumococcal aetiology despite few positive blood cultures (Torzillo et al. 1999). Another study found high nasopharyngeal carriage rates of NTHi (44%; 21/48) and pneumococcus (23%; 11/48) among Northern Territory Indigenous children (aged 0.5-12 months) with bronchiolitis suggesting these bacteria may be aetiological (Hare, Smith-Vaughan & Leach 2010).

1.5.4 Virology associated with otitis media and ALRI

AOM is often a complication of a viral upper respiratory tract infection (Marom, Nokso-Koivisto & Chonmaitree 2012) and viruses have been detected in ear effusions (Nokso-Koivisto et al. 2004). A PCR-based study in the Northern Territory found respiratory viruses in 62% of 366 nasopharyngeal swabs taken from 114 otitis prone Indigenous children. Rhinovirus (38% of swabs) was identified as the most common and adenovirus (13% of swabs) was found to be independently associated with a 3-fold higher risk of AOM (Binks et al. 2011). During ALRI episodes,
respiratory syncytial virus (RSV) and rhinovirus are frequently detected in the nasopharynx of Indigenous children (McCallum et al. 2013; Torzillo et al. 1999) and although influenza is less common (approximately 5%) among young Indigenous children in the Northern Territory (Binks et al. 2011), it remains important because it potentiates invasive pneumococcal disease by over 100-fold (Edwards et al. 2011). Influenza has also been detected in infant (aged 2-24 months) middle ear fluid during acute otitis media episodes (Nokso-Koivisto et al. 2004) and there is evidence that influenza vaccination in infants is associated with a small reduction in AOM (4%) (Norhayati, Ho & Azman 2015).

A population based data linkage study of 8980 Western Australian children (aged 0-9 years) hospitalised for an ALRI with a respiratory sample and a laboratory record available, identified a respiratory virus in 55% of predominantly upper respiratory specimens, commonly RSV (36%) and influenza (4%) (Moore et al. 2011). RSV confirmed bronchiolitis is also common in Central Australia. Between 2000 and 2004 the annual incidence rate for RSV confirmed hospitalisation among Indigenous infants less than 2 years old was 30 per 1000, nearly 3-times higher than among non-Indigenous children (11 per 1000) (Dede et al. 2010).

1.5.5 Polymicrobial infections

For non-Indigenous children, otitis media is usually of uncomplicated aetiology resulting in a mild and self-resolving infection (Leach et al. 1994; Morris & Leach 2009). In contrast, Indigenous Australian children in the Northern Territory endure dense and repetitive polymicrobial acute middle ear infections that regularly progress to chronic infections. Among otitis-prone children in the Northern Territory, one
study concurrently detected pneumococcus, NTHi and *Moraxella catarrhalis* in 94% (48/51) of nasopharyngeal swabs from Indigenous children compared to 48% (25/52) of nasopharyngeal swabs from non-Indigenous children (Smith-Vaughan et al. 2006) while a second study simultaneously detected pneumococcus, NTHi and at least one respiratory virus in 56% (197/366) of nasopharyngeal swabs from 110 Indigenous children (Binks et al. 2011). In the latter study, bacterial density was higher with viral co-infection suggesting a synergistic relationship between bacteria and viruses. Mixed bacterial infections are also demonstrated in the middle ear of children in the Northern Territory. In a study of Indigenous children with spontaneous perforation, at least two bacterial pathogens (of pneumococcus, NTHi and *Moraxella catarrhalis*) were detected by PCR in half (51%; 28/55) of the middle ear fluids investigated; NTHi was dominant in terms of prevalence and density (Smith-Vaughan et al. 2013). In recent years, it has been demonstrated that biofilms play a pivotal role in the development of chronic respiratory disease. This has been well described in the literature (Hall-Stoodley et al. 2006; Tikhomirova & Kidd 2013). A detailed description of the polymicrobial infections and biofilm associated with respiratory disease is beyond the scope of this review.

1.6 The impact of pneumococcal conjugate vaccines (PCV) on nasopharyngeal pneumococcal carriage among Indigenous children

Clinical trials conducted in The Americas, Europe, Asia and Africa, employing two or more infant doses of a PCV (7vPCV, 10vPHiD-CV or 13vPCV) have found reduced nasopharyngeal carriage of vaccine serotypes (Fleming-Dutra et al. 2014). The impact of PCVs on pneumococcal carriage among Indigenous children of the
Northern Territory is poorly captured in the literature. A systematic literature search (Appendix A: Search 5) returned 34 results of which only one compared pneumococcal carriage among Indigenous children with respect to pneumococcal vaccination. This study surveyed Indigenous children (0-6 years of age) living in remote Northern Territory communities in 2003 (n=902) and 2005 (n=818) (Leach et al. 2009), two and four years after the 7vPCV/23vPPV schedule was introduced for Indigenous infants in this region. Coverage of the 7vPCV improved from one survey to the next with 76% (2003) and 84% (2005) of children completing the 2, 4 and 6 month primary 7vPCV schedule. Pneumococcal carriage was compared between surveys. A small reduction in overall pneumococcal carriage was evident among 12 to 24 month old children between 2003 and 2005 (82% versus 76%; p=0.002), while across all ages between 2003 and 2005, there were reductions in 7vPCV serotype carriage (11% versus 8%; p=0.011), and increases in non-vaccine serotypes (57% versus 67%; p<0.001). Some care should be taken with interpretation of these non-randomised studies that are prone to temporal biases such as improvements in housing, antibiotic therapy and education.

A combination of published and unpublished surveillance data (Leach, Morris & Mathews 2008) (Leach et al. 2009) (Hare 2014; Leach 2014) suggests there has been no significant change in overall pneumococcal carriage since the introduction of the pneumococcal conjugate vaccination program.
Figure 1.3 Temporal nasopharyngeal carriage prevalence of pneumococcus and NTHi among Indigenous children living in remote communities of the Northern Territory.

Early data (1996-2000) were taken from a trial of amoxicillin for the prevention of otitis media (Leach, Morris & Mathews 2008) while the subsequent data (2003, 2005, 2007, 2009, 2010) were obtained from ongoing surveillance (Leach et al. 2009) (Hare 2014; Leach 2014)
1.7 Moving forward

Otitis media and ALRI are largely considered to be diseases of poverty. Contemporary prevention and intervention strategies including the advent and implementation of pneumococcal conjugate vaccines, advances in antibiotic therapy (Morris et al. 2010a) and efforts to improve housing (Bailie et al. 2011; Bailie et al. 2005), hygiene (McDonald & Bailie 2010), employment (Carson & McConnel 2011), education (Nutton, Bell & Fraser 2013), access to fresh food (Colles, Maypilama & Brimblecombe 2014) and medical services(Si et al. 2007) are crucial but as yet have not managed to overcome the endemicity of mucosal microbial infections among Indigenous children living in either urban or remote communities. The pneumococcus and NTHi are important pathogens to overcome but others must be considered, particularly respiratory viruses such as RSV. Novel and multifaceted approaches are required to treat existing disease, modify key risk factors for pathogen transmission and to build overall resilience against the perpetual cycles of infection existing in this region.

1.8 Hypotheses and aims

The research in this thesis was based on the potential future use of maternal pneumococcal vaccination and/or vitamin D supplementation as strategies for the enhanced prevention against respiratory infections among Indigenous infants of the Northern Territory of Australia. Investigative chapters had the following hypotheses and aims:
**Hypothesis 1**

The presence of serotype-specific pneumococcal antibodies at birth and during early infancy will prevent or delay nasopharyngeal colonisation, proliferation and duration of serotype-specific pneumococci, reducing the subsequent burden respiratory disease.

*Aim (Chapter 3):*

To determine if maternal immunisation with the 23-valent pneumococcal polysaccharide vaccine (23vPPV), given antepartum or immediately postpartum, is immunogenic and can reduce infant pneumococcal carriage and middle ear disease between birth and age 7 months.

*Aim (Chapter 4):*

To determine whether receipt of the 23vPPV during pregnancy can reduce infant ALRI hospitalisations or clinic presentations in the first year of life.

**Hypothesis 2**

Indigenous mothers and infants of the Northern Territory have high rates of vitamin D insufficiency despite the tropical climate, increasing the risk of infant ALRI.

*Aim (Chapter 6):*

To describe the vitamin D status of Indigenous mothers and infants living in the Northern Territory and determine if cord blood vitamin D insufficiency is associated with the risk of ALRI hospitalisation during the first year of life.

*Aim (Chapter 7):*

To determine the prevalence of vitamin D insufficiency amongst Indigenous and non-Indigenous children hospitalised with ALRI in the Northern Territory.
CHAPTER 2

Maternal Pneumococcal vaccination: Literature Review
Chapter 2: Maternal pneumococcal vaccination: Literature review.

2.1 Rationale

Neonates have an immature and naïve immune system which limits their capacity to protect against many viral, bacterial and fungal pathogens (Prendergast, Klenerman & Goulder 2012). Hence, neonates are prone to severe infections. This is largely because neonatal cellular immune responses deviate toward regulatory and T-helper (Th) type 2 responses that have limited effectiveness against intracellular pathogens and encapsulated bacteria, such as the pneumococcus (Adkins, Leclerc & Marshall-Clarke 2004). The aim of maternal vaccination is to stimulate production of maternal antibodies against a pathogen of interest, such that they are systemically transferred to the foetus via the placenta and the baby is born with protective antibodies against this pathogen. With this strategy, neonates also receive vaccine-specific antibodies via ingestion of breast milk.

2.2 Basic immunological principles

Vaccine induced systemic immunoglobin (Ig) G generated in the mother are transferred to the foetus via a selective active transport mechanism in the placenta that confers protective immunity to the neonate. Briefly, maternal IgG binds to neonatal antibody (Fc) receptors on the placental synctiotrophoblast (the interface between the maternal and foetal circulatory systems) where they are endocytosed, transported across the interface and then released into the foetal blood stream. Preferential transport of human Ig subtypes (IgG1>IgG3>IgG4>IgG2) is directly
related to their affinity to bind the neonatal Fc receptor (Niewiesk 2014; Simister 2003). The longevity of these antibodies in the infant is vaccine antigen specific and related to the concentration of maternal antibody generated (Esposito et al. 2012; Faucette et al. 2014). Vaccine specific IgA, IgG, IgM and IgD are also secreted into breast milk and are ingested by the neonate upon breastfeeding with the potential to bind pathogens and stimulate an infant immune response (Faucette et al. 2014). While this strategy has proven clinically effective some caution needs to be taken as circulating maternal IgG antibodies in the neonate/infant can suppress their vaccine-induced immune responses (Jones et al. 2014). Two recent reviews concluded that a deeper understanding of the immunological mechanisms across a broader range of vaccine formulations is required in the future (Esposito et al. 2012; Faucette et al. 2014).

2.3 History and current use of maternal vaccination

Proof of the principle of the maternal vaccination strategy was first demonstrated by two studies that showed tetanus vaccination among women of childbearing age successfully reduced neonatal tetanus cases (Black, Huber & Curlin 1980; Demicheli, Barale & Rivetti 2013; Newell et al. 1966). Maternal vaccination has been subsequently used against infant pertussis and influenza. In a randomised controlled trial, the inactivated influenza vaccine in pregnancy reduced proven influenza illness by 63% in infants up to 6 months of age compared to a control group that received the 23vPPV (Zaman et al. 2008). For maternal pertussis vaccination, infants receive only short lasting antibodies (6 weeks) (Gall, Myers & Pichichero 2011; Leuridan et al. 2011) yet recent United Kingdom surveillance data, before (2011 and 2012) and after (2013) maternal DTPa introduction, indicated a
vaccine effectiveness of 91% against confirmed pertussis cases in children aged <3 months (Amirthalingam et al. 2014). To date, there has been no evidence of a risk to the foetus from maternal vaccinations using inactivated vaccines or toxoids (Centers for Disease Control and Prevention 2011). However, live or live attenuated vaccines are generally contraindicated in pregnancy due to the theoretical risk to the developing foetus. The inactivated influenza vaccine and the diphtheria, tetanus and acellular pertussis vaccine are routinely recommended for use in pregnancy in the United States of America (Kim et al. 2015), the United Kingdom (Salisbury & Ramsay 2013), Canada (Canadian Government 2014) and Australia (Australian Government 2013).

2.4 Introduction to maternal pneumococcal vaccination

Administering the pneumococcal polysaccharide vaccine (PPV) in pregnancy generates an immune response that provides infant offspring with antibodies against vaccine serotype pneumococci from birth. The first use of a PPV in pregnancy occurred in a large randomised controlled trial for the prevention of adult pneumonia in Papua New Guinea in 1973, where 187 pregnant mothers inadvertently received a 14-valent PPV and 167 received a saline placebo because local custom prevented inquiries about pregnancy and women without obvious signs of pregnancy were included (Riley et al. 1977). Morbidity surveillance showed 14vPPV vaccination in pregnancy had an encouraging 14% vaccine efficacy among children in utero at the time of vaccination (p=0.100) and a 17% vaccine efficacy (p=0.020) among children aged 1-17 months of age at time of vaccination, against episodes of ALRI until 3 years of age. This was the first suggestion that maternal pneumococcal vaccination may protect infants against pneumococcal disease. Trials involving pneumococcal
polysaccharide vaccination in pregnancy have subsequently been conducted in Asia (Lehmann et al. 2002; Quiambao et al. 2003; Shahid et al. 1995; Zaman et al. 2008), Africa (O'Dempsey et al. 1996; Obaro et al. 2004), North America (Munoz et al. 2001) and South America (Lopes et al. 2009).

2.5 Significance of maternal pneumococcal vaccination

Pneumococcal infections are estimated to cause 11% (up to 1 million) of all deaths in children under 5 years of age (O'Brien et al. 2009) and are a prominent cause of morbidity and mortality among Indigenous infants in the Northern Territory (Krause & Cook 2012; Leach et al. 1994; Mackenzie et al. 2010; Torzillo et al. 1999). Since the introduction of PCV’s for Indigenous children in 2001, invasive pneumococcal disease rates have halved yet remain a concern (146 cases/100,000; 2002-2011), excessive ALRI hospitalisations episodes rates persist (O'Grady, Torzillo & Chang 2010) (427 episodes per 1000 child-years; 1999-2004) with little evidence of recent improvement (Jardine, Menzies & McIntyre 2012) and up to 50% of infants continue to experience suppurative otitis media by 6 months of age (Leach et al. 2014). Maternal pneumococcal vaccination offers potential immune protection from birth, when infants are at their most vulnerable, prior to protection afforded by routine PCV schedules.

2.6 The 23-valent Pneumococcal Polysaccharide Vaccine

The 23-valent pneumococcal polysaccharide vaccine (23vPPV, PNEUMOVAX® 23, Merck, United States of America) contains 25µg of polysaccharide antigens derived from the common disease causing pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F.
In Australia, the 23vPPV is approved for use in persons over 2 years of age at high risk of pneumococcal disease and is routinely recommended for all Aboriginal and Torres Islander persons aged ≥15 years within the study catchment area (Australian Government 2013). Further, until 2009 the 23vPPV was used as a booster subsequent to the routine infant 7vPCV schedule at 2, 4 and 6 months of age.

### 2.7 General efficacy of the 23vPPV

Vaccination of children with polysaccharide antigens has been controversial due to weak immunogenicity, poor induction of memory responses and only transitory protection (Lesinski & Westerink 2001). Polysaccharide antigens are generally considered T-cell independent, inducing systemic but not mucosal antibodies limiting their effect on bacterial carriage (Herva et al. 1980). Despite issues with immunogenicity in infants, pneumococcal polysaccharide vaccines have proven to be clinically useful in adults. In a large Swedish study, over 100,000 adults aged over 65 years were vaccinated with either the 23vPPV or the trivalent inactivated influenza vaccine. The total mortality was 57% lower among the vaccinated cohort in the 6 months after vaccination (15.1 vs 34.7 deaths per 1000 inhabitants) (Christenson et al. 2001) and there were fewer hospitalisation admissions for influenza (risk ratio 0.68), pneumonia(risk ratio 0.78) and invasive pneumococcal disease (risk ratio 0.46) (Hedlund et al. 2003) in the 12 months after vaccination.

### 2.8 Studies of maternal 23vPPV in pregnancy

Despite the demonstrated success of maternal vaccination against tetanus and influenza, the efficacy of maternal 23vPPV against infant pneumococcal disease outcomes requires further validation. A PubMed literature search (Appendix A:
Search 6) identified 12 relevant studies of pneumococcal vaccination in pregnancy. Here I review the outcomes of these studies in terms of safety, immunogenicity, prevention of clinical disease and reductions in pneumococcal carriage.

2.8.1 Safety of the 23vPPV in pregnancy

Nearly 1000 women have received the 23vPPV in pregnancy without evidence of adverse effects (Lehmann et al. 2002; Lopes et al. 2009; Munoz et al. 2001; O'Dempsey et al. 1996; Obaro et al. 2004; Quiambao et al. 2003; Quiambao et al. 2007; Riley et al. 1977; Shahid et al. 1995; Zaman et al. 2008), however, there remains some concern about the possibility of rare serious adverse events.

2.8.2 Immunogenicity of 23vPPV in pregnancy

Nine studies, mostly randomised controlled trials, investigated immunogenicity outcomes of 23vPPV in pregnancy with varying results (Almeida Vde et al. 2009; Lehmann et al. 2002; Lehmann et al. 2003; Munoz et al. 2001; O'Dempsey et al. 1996; Obaro et al. 2004; Quiambao et al. 2003; Shahid et al. 1995; Steinhoff et al. 2010). Across these studies, 23vPPV was administered late in the 2nd trimester or during the 3rd trimester (range: 24-38 weeks) and timeliness in this range appeared to have little correlation on the magnitude of the mean serotype-specific antibody concentration at birth (Lehmann et al. 2002; Shahid et al. 1995). No studies have measured antibodies to all 23vPPV serotypes.

2.8.2.1 Maternal immunogenicity

In general, mothers mounted a good immune response to most serotypes tested (including serotypes 1, 3, 5, 14, 19F, 23F) with weaker responses seen for serotypes
and stronger responses seen to serotype 14 (Lehmann et al. 2002; O'Dempsey et al. 1996; Quiambao et al. 2003). Generally, the vaccine specific cord blood IgG concentration was 50-60% of the maternal IgG concentration (Lehmann et al. 2002; Munoz et al. 2001; O'Dempsey et al. 1996; Shahid et al. 1995), but ranged from as low as 24% in the Gambian study where placental IgG transfer was poor (O'Dempsey et al. 1996) to 72% among infants of HIV-infected mothers vaccinated in pregnancy with 23vPPV (Almeida Vde et al. 2009). Further to this, Munoz et al. showed that the IgG1 subclass was preferentially transported across the placenta compared to IgG2 subclass antibodies which have a weaker affinity for the neonatal antibody receptor at the maternal-foetal interface of the placenta (Munoz et al. 2001).

2.8.2.2 Passive infant immunogenicity

Vaccine specific IgG persisted in the infant for 1 to 5 months after birth. Similar to the mother, IgG against serotypes 6B and 7F were the shortest lasting (1 to 2 months), antibodies to serotype 1, 3, 5, 23F were variable (up to 4 months) and antibodies to serotype 14 and 19F were consistently the longest lasting (up to 5 months) (Lehmann et al. 2002; Munoz et al. 2001; O'Dempsey et al. 1996; Shahid et al. 1995) most likely reflecting the magnitude of antibody received by the infant. Quiambao et al. considered antibody decay rates and infant growth levels and calculated that cord blood serotype-specific IgG concentrations of 4.4µg/ml are required to enable protective antibodies (>0.35µg/ml) to persist in the infant for 4 months (Quiambao et al. 2007). In their 2003 maternal 23vPPV trial in the Philippines, 60% of the vaccinees had serotype-specific (1, 5, 6B, 14, 18C and 19F)
cord blood antibody concentrations exceeding 4.4 µg/ml compared to only 10% in controls (Quiambao et al. 2003).

Only one study performed functional antibody assays. Munoz et al. showed that cord blood antibodies of 23vPPV vaccinees were highly opsonic against the pneumococcus (serotypes 6B, 14, 19F were tested) compared to controls (Munoz et al. 2001). More recent work showed that the 23vPPV in pregnancy is immunogenic even among HIV-infected mothers (Almeida Vde et al. 2009), while Holmlund et al. demonstrated that the serotype-specific immune responses to childhood 23vPPV at either 7 weeks or 3 years of age were similar regardless of whether their mother had received the 23vPPV in pregnancy (Holmlund et al. 2011) suggesting that interference by pre-existing vaccine antibodies was not an issue for the infant.

2.8.2.3 Breast milk antibodies

The potential protection afforded to infants following maternal vaccination is not confined to systemic antibody production. Breast milk is a rich source of immunomodulatory compounds including maternal antibodies, that when ingested, can protect the infant respiratory mucosa against bacterial colonisation (Deubzer et al. 2004). In the Northern Territory of Australia breast feeding rates are high amongst both urban (62% at 3 months, 51% at 6 months) and rural (97% at 3 months, 92% at 6 months)(Li et al. 2007).

Breast milk immunogenicity following 23vPPV in pregnancy was measured in four studies, again with considerable heterogeneity in the outcomes. In Bangladesh, Shahid et al. showed moderately higher IgA concentrations in the breast milk of
23vPPV vaccinees compared to controls for serotype 19F (7-times higher) and 6B (3-times higher) that waned rapidly at 7 and 2 weeks respectively (Shahid et al. 1995). Women in Papua New Guinea who received the 23vPPV showed only weakly elevated breast milk IgA (1.1 to 1.8-times at 6 months) compared to population controls (Lehmann et al. 2003) while in the United States of America, Munoz et al. demonstrated that women who received the 23vPPV in the third trimester had higher IgA titres to serotypes 6B, 14, 19F and 23F at 2 months post-partum (and to 7 months for serotype 19F), compared to \textit{Haemophilus influenzae} type b vaccinated controls (Munoz et al. 2001) as well as higher breast milk IgG levels against serotypes 6B and 14 at 2 months post-partum which may be important in the presence of pneumococcal IgA protease. In 2004, a trial in The Gambia (Obaro et al. 2004) showed that following 23vPPV in pregnancy, breast milk antibodies against serotype 4, 6B and 14 were higher than controls at 6 months post-partum although antibodies to serotype 19F (4 months) and 23F (birth) were weaker. Importantly, the avidity of the breast milk antibodies for 23vPPV antigens was significantly higher among 23vPPV vaccinees compared to controls at all time points suggesting function in the absence of magnitude. In a follow up to this trial, Deubzer et al. showed that colostrum of 23vPPV vaccinated mothers inhibited the colonisation of pneumococcal serotypes 6B and 14 to pharyngeal cells \textit{in vitro} (Deubzer et al. 2004).

2.8.3 Prevention of clinical disease

Two trials have investigated and published clinical outcomes in infants following 23vPPV in pregnancy. In The Gambia, infants of vaccinated mothers underwent one year of surveillance following maternal vaccination. Respectively among 23vPPV compared to meningococcal vaccinees there were: 4 versus 2 infant mortalities; 4
versus 8 cases of pneumonia; and 0 versus 3 episodes of otitis media, none of which were statistically significant (O'Dempsey et al. 1996). In a Brazilian trial, infants were examined and mothers were questioned monthly to identify the occurrence of acute respiratory infections during the first 6 months of life. There was no difference in the prevalence of acute respiratory infections between infants of vaccinated and unvaccinated mothers at 3 (8.5% versus 8.5%) or 6 (25.5% versus 29.8%) months of age (Lopes et al. 2009). A Cochrane review (first published in 2006 and updated in 2012) collectively examined the clinical outcomes from these two studies (n=241) and concluded that there was insufficient evidence to support the use of pneumococcal vaccination (23vPPV) in pregnancy for reducing infant infections (Chaithongwongwatthana et al. 2012).

2.8.4 Reduction in pneumococcal carriage

Two studies have reported infant pneumococcal carriage as an outcome of maternal 23vPPV vaccination. The trial conducted in the United States of America in 1995-1996 by Munoz et al. reported an infant pneumococcal carriage rate of 11% (2/18) in the 23vPPV group compared with 34% (13/38) in the control group at seven months of age (p=0.120) and of 17% (3/18) compared with 50% (19/38) at 16 months of age (p=0.021) (Munoz et al. 2001). In the most recently published trial however, Lopes et al. found no difference in the rate of pneumococcal carriage among infants at age 6 months if the mother was vaccinated during the third trimester (17%; 7/42), at delivery (18%; 8/45), or not at all (15%;7/46). In a combined meta-analysis of the 146 pregnancies that investigated carriage, there was insufficient evidence to indicate maternal 23vPPV in pregnancy had an effect on pneumococcal carriage at 3
or 6 months of age but there was evidence of a significant decrease in pneumococcal carriage at 16 months of age (Chaithongwongwatthana et al. 2012).

2.9 Summary

In summary, 23vPPV vaccination in pregnancy generates a maternal immune response that is passed onto the infant systemically via the placenta (IgG) and mucosally via breast milk (IgA and IgG). The magnitude of the immune response is serotype-specific and persistence in the infant correlates with the concentration of antibody received. Opsonophagocytosis and antibody avidity assays suggest the antibodies received by the infant are functional. Of note, many of 23vPPV serotypes important in carriage and disease among Indigenous infants of the Northern Territory (19A, 10A, 11A, 15B, 33F (Leach et al. 2009)) were not investigated in the aforementioned immunogenicity studies limiting the predictability of maternal 23vPPV in this region. While there was limited evidence to suggest a benefit against acute pneumococcal infections in the infant, none of the maternal vaccine trials were adequately powered to demonstrate statistically significant differences in nasopharyngeal carriage and/or respiratory disease during infancy. In a setting where pathogens colonise the respiratory tract directly after birth, the potential for maternal pneumococcal vaccination strategies to improve infant respiratory outcomes is significant and will be investigated in Chapters 3 and 4.
CHAPTER 3

PneuMum: Impact from an open label randomised controlled trial of maternal 23-valent pneumococcal polysaccharide vaccination on middle ear disease amongst Indigenous infants, Northern Territory, Australia
Chapter 3: PneuMum: Impact from an open label randomised controlled trial of maternal 23-valent pneumococcal polysaccharide vaccination on middle ear disease amongst Indigenous infants, Northern Territory, Australia

3.1 Context

In 2009, the Northern Territory had an estimated resident population of about 227,900 people dispersed across 1,346,200 km² which is about five times the size of the United Kingdom (Australian Institute of Health and Welfare 2011). Thirty percent of the population (approximately 70,000 people) identify as Indigenous (Aboriginal or Torres Strait Islander) for which socioeconomic disadvantage and shorter life expectancy (10 years less than non-Indigenous Australians) is well documented (Australian Institute of Health and Welfare 2014). Respiratory diseases (predominantly infectious) are the leading cause of preventable morbidity and mortality among infants in this region (Li et al. 2007).

3.2 Abstract

Introduction

We assessed maternal 23-valent pneumococcal polysaccharide (23vPPV) vaccine efficacy (VE) against infant middle ear disease and pneumococcal carriage among a group of high risk Australian Indigenous children.

Methods
Pregnant Indigenous women (n=227) were randomised 1:1:1 to receive the 23vPPV at: 30-36 weeks gestation, birth, or to serve as unvaccinated controls. Independent co-primary outcomes were infant middle ear disease and nasopharyngeal carriage of a 23vPPV serotype (23v carriage) at age 7 months. Ear disease associated with concurrent carriage of a 23vPPV-type or the related serotype 6A (23v6A ear disease) was assessed in a post-hoc analysis.

Results
The consent rate was 50% (313/632) with 227 eligible participants subsequently randomised: 77 controls, 75 pregnancy vaccinees; 75 birth vaccinees; (Figure 3.1). The retention rate was 86% (n=66), 89% (n=67) and 88% (n=66) respectively. The 23vPPV was immunogenic against all 23v serotypes with no evidence of adverse outcomes related to pregnancy vaccination. At 7 months of age, the prevalence of infant middle ear disease was: 71% (47/66) controls, 63% (42/67) pregnancy vaccinees, 76% (50/66) birth vaccinees; and the prevalence of infant 23v carriage was: 26% (17/66) controls, 18% (12/67) pregnancy vaccinees, 18% (12/66) birth vaccinees. For pregnancy vaccinees, VE was 12% (95% CI -12% to 31%) against infant ear disease and 30% (95% CI -34% to 64%) against 23v carriage. Post hoc analysis showed that the prevalence of 23v6A ear disease was 27% (18/66) among control infants and 13% (9/67) among infants of pregnancy vaccinees; VE 51% (95% CI -2% to 76%).

Conclusions
This was the first study designed to assess the ability of maternal pneumococcal vaccination to prevent infant ear disease. While the 23vPPV during pregnancy or at
birth was safe, immunogenic and well tolerated among Indigenous mothers and infants, we were unable to demonstrate an impact against co-primary outcomes of all-cause ear disease or 23v carriage at infant age 7 months. The post-hoc analysis suggests a revised outcome of ear disease with concurrent carriage of a vaccine-related serotype might be a more specific outcome of interest in future studies conducted in the absence of tympanocentesis. Additional analysis suggests the impact was limited to infant ear disease concurrent with carriage of the most common 23vPPV serotypes (19A, 10A, 6A, 15B and 33F) (ClinicalTrials.gov number, NCT00714064).

3.3 Background

3.3.1 Otitis media in the Northern Territory

Indigenous Australian children experience the highest published rates of acute and chronic ear infections in the world (Morris et al. 2005). This may result in permanent middle ear damage, hearing loss and educational disadvantage. These infections are mainly bacterial and the pneumococcus and NTHi are the predominant pathogens. Pneumococcal colonisation begins within days of birth (Leach et al. 1994), months before any direct immunological protection from infant pneumococcal conjugate vaccine may be expected. The inflammation and mucosal damage caused by early recurrent infections (commonly pneumococcal) predisposes to chronic suppurative otitis media, chronic bronchitis and bronchiecstasy. All of these conditions are common among Indigenous populations (Chang et al. 2014; Chang et al. 2002; Morris et al. 2005). Prevention or delay of the early pneumococcal colonisation (described in more detail in Chapter 1) is likely to be an important approach to reducing the burden of infant middle ear disease in this region.
3.3.2 Maternal Immunisation

Maternal immunisation with the 23vPPV is one strategy that may protect infants from birth. Several randomised controlled trials of 23vPPV in pregnancy have been conducted in Asia (Lehmann et al. 2002; Quiambao et al. 2003; Shahid et al. 1995; Zaman et al. 2008), Africa (O’Dempsey et al. 1996; Obaro et al. 2004) and the Americas (Lopes et al. 2009; Munoz et al. 2001), as described in detail in Chapter 2. Briefly, higher vaccine specific antibody levels have been demonstrated in maternal, cord and infant blood, and in breast milk samples following 23vPPV in pregnancy compared to controls (Lehmann et al. 2002; Munoz et al. 2001; O’Dempsey et al. 1996; Obaro et al. 2004; Quiambao et al. 2003; Shahid et al. 1995) but only a small number of serotypes have been investigated. Few studies have assessed infant pneumococcal carriage as an outcome of interest. Munoz et al. reported an infant carriage rate of 11% (2/18) in the 23vPPV group compared with 34% (13/38) in the control group at seven months of age while Lopes et al. showed no difference in pneumococcal carriage among infants at age 6 months following maternal 23vPPV in pregnancy (17%:7/42), at birth (18%:8/45), or not at all (15%:7/46). In the only study to investigate otitis media, O’Dempsey et al. found no cases of otitis media by one year of age among infants of vaccinated mothers (n=75) in the Gambia compared to three cases among the control group (n=75) (O’Dempsey et al. 1996).

3.3.3 Aims

The aim of our randomised controlled trial, called “PneuMum”, was to determine whether serotype-specific antibodies generated by maternal 23vPPV (in pregnancy or at birth) could reduce infant ear disease and 23vPPV serotype-specific
nasopharyngeal pneumococcal colonisation among infants at 7 months of age in a setting where both are endemic.

3.4 Methods

3.4.1 Study Design

We conducted a three arm (Figure 4.1), outcome-assessor blinded, prospective, open label, parallel group, randomised controlled trial of maternal 23vPPV (PNEUMOVAX® 23, Merck, United States of America). The complete PneuMum study protocol is available at: ClinicalTrials.gov number, NCT00714064.

3.4.2 Outcomes

The outcomes for this study were:

Co-primary outcomes

- Infant ear disease at age 7 months
- Nasopharyngeal carriage of a 23vPPV pneumococcal serotype (23v carriage) at age 7 months

Secondary outcomes

- Safety of maternal 23vPPV, study attrition rates and participant characteristics
- Nasopharyngeal pneumococcal carriage serotypes among infants at age 7 months
- Nasopharyngeal pneumococcal carriage serotypes among mothers at birth
- Infant ear disease and nasopharyngeal pneumococcal carriage at ages 1 and 2 months
• Comparison of ear diagnosis by research nurse and blinded assessor at age 7 months
• Vaccine specific IgG (blood) and IgA (breast milk) concentrations in mothers and infants across the visits from 30-36 weeks gestation until 7 months post-partum

Post hoc outcomes (potentially vaccine preventable disease)

In post hoc analysis, serotype 6A was included based on demonstrated cross-reactivity with serotype 6B, a 23vPPV serotype (Park et al. 2008) (MacIntyre et al. 2014).
• Nasopharyngeal carriage of a 23vPPV serotype or the related serotype 6A (23v6A carriage) at age 7 months
• Ear disease concurrent with nasopharyngeal 23v6A carriage (23v6A ear disease) at age 7 months
• Most common 23v6A ear disease serotypes at age 7 months

3.4.3 Participants

Pregnant Indigenous women were recruited in Darwin, Alice Springs and remote communities of the Northern Territory of Australia from August 2006 to January 2011. At 30-36 weeks gestation (inclusive), eligible participants were randomised in blocks of six using a computerised random number generator to one of three groups:
• Pregnancy vaccinees – maternal 23vPPV at the randomisation visit
• Birth vaccinees – maternal 23vPPV within 72 hours of infant birth
• Controls – maternal 23vPPV offered at study exit (7 months post-partum)
Randomisation was stratified by community of residence with allocation concealment maintained using sealed, opaque envelopes. Eligibility criteria, confirmed prior to randomisation but no earlier than 28 weeks gestation, required each participant to: be an Indigenous Australian aged 17-39 years with a singleton uncomplicated pregnancy (no existing or pre-existing condition judged by the clinical investigator to make pregnancy high-risk); be a resident of the catchment area; be intending to give birth at a participating hospital; have no human immunodeficiency virus, history of severe allergy, uncontrolled asthma or splenectomy; and to have no history of 23vPPV vaccination within the previous three years. Study infants received the routine pneumococcal conjugate vaccination via primary health care providers as per the recommended schedule (2, 4 and 6 months of age): 7vPCV from June 2001, and 10vPHiD-CV from October 2009.

3.4.4 Sample collection and testing

Maternal nasopharyngeal swabs were collected at birth and infant nasopharyngeal swabs at ages 1, 2 and 7 months. Pneumococci were isolated by experienced laboratory staff, blinded to the treatment allocation and sample identification, according to World Health Organization guidelines (O'Brien & Nohynek 2003). Briefly, pneumococci were identified by α-haemolysis, colony morphology and susceptibility to optochin. To maximise serotype detection, where identified, four colonies per swab were serotyped by Quellung reaction using serotype-specific antisera (Statens Serum Institute, Copenhagen, Denmark). Nontypeable pneumococci and optochin non-susceptible isolates were not considered further. *Haemophilus influenzae* and *Moraxella catarrhalis* were cultured using standard microbiological methods (Smith-Vaughan et al. 2013).
Only pregnancy vaccinees had maternal blood collected immediately prior to vaccination. In all randomisation groups, maternal venous and cord blood was collected at birth and infant blood was collected at age 7 months. Expressed breast milk was collected at birth, 1, 2 and 7 months post-partum. Vaccine-specific antibodies in serum and breast milk were assessed by trained staff, blinded to the randomised allocation, at the Pneumococcal Laboratory, Murdoch Children’s Research Institute, Melbourne, Australia. Serotype-specific serum IgG for all 23vPPV serotypes and serotype 6A were measured by a validated 3rd generation World Health Organization enzyme-linked immuno-sorbent assay (ELISA) (Baloch et al. 2010; Wernette et al. 2003).

3.4.5 Breast milk sample processing and ELISA

Breast milk samples were collected by maternal manual expression into a specimen collection jar, cooled to 4°C for transport to the laboratory, then stored at -80°C until testing. Prior to testing, samples were brought to room temperature, mixed well and centrifuged at 2000 rpm for 10 minutes. The lipid layer was discarded and the milk was again centrifuged at 4000 rpm for 30 minutes. This soluble interphase was used in a novel breast milk ELISA assay to determine concentrations of serotype-specific breast milk IgA.

Microtitre wells were coated with 15 of the 23v pneumococcal polysaccharides (serotypes 1, 10A, 11A, 12F, 14, 15B, 19A, 19F, 22F, 23F, 3, 33F, 6B, 7F and 9V) at 5µg/ml diluted in phosphate buffered saline. Plates were incubated at room temperature overnight. To neutralise non-specific cell wall polysaccharide
antibodies, samples, controls and standard (reference serum 89SF, Food and Drug Administration, Bethesda MD) were diluted 1:50 in 10% foetal bovine serum in phosphate buffered saline containing cell wall polysaccharide at 20 µg/ml and also incubated overnight at 4°C. On the day of testing, coated plates were washed with PBS containing 0.05% Tween 20 then blocked for 1 hour with 10% foetal bovine serum in PBS (sample diluent). All plate incubations were performed at room temperature on a horizontal shaker at 500 rpm. At 1 hour the blocking solution was discarded and doubling dilutions of standard 89SF, patient sample dilutions of 1:50, 1:150, 1:450 and 1:1250 and the three controls (high, medium and low) were applied to the plate and incubated for 2 hours. At 2 hours the plates were washed with 0.05% Tween PBS and then 50µl of a 1:5000 dilution of sheep anti-human IgA (α-chain specific), affinity-isolated, horseradish peroxidase-conjugated antiserum (Chemicon, Australia) was added to each well and incubated for a further 2 hours. Finally the plates were washed with 0.05% Tween PBS, followed by distilled water and 50µl of the peroxidase substrate (TMB microwell peroxidase substrate system; KPL, Maryland, USA) was added to each well. The reaction was stopped at 12 minutes with 50µl of 2M H₃PO₄ and the optical density was measured at 450nm, reference filter 620nm on an ELX808 ultramicroplate reader (Biotek Instruments, Vermont, USA). The concentration of specific IgA was proportional to the colour development of substrate. Using the software KCJunior, Biotek Autoreader, Version 1.40.3 (Biotek Instruments, Vermont, USA), an 8-point standard curve was derived using the 4-parameter log curve-fitting algorithm. Control and unknown sample concentrations were read from the standard curve.
3.4.6 Ear assessments

3.4.6.1 Research nurse ear assessments

Clinical ear assessments for each child (both ears) were undertaken at the 1, 2 and 7 month visits by trained research nurses who used tympanometry and otoscopy and recorded all assessment outcomes on a standard proforma. The research nurses were not blinded to the randomisation allocation (unblinded ear diagnosis) and tympanocentesis was not performed.

3.4.6.2 Blinded assessment of primary ear disease outcome at 7 months

For the primary outcome at 7 months, recordings of the tympanometry and pneumatic video-otoscopy collected by the research nurses were reviewed by independent assessors blinded to the randomisation allocation (blinded ear diagnosis). Where the unblinded and blinded ear diagnoses (ear disease present/absent) were concordant these were accepted as final. Where discordant, the tympanometry and pneumatic video-otoscopy recordings were reviewed by a second independent assessor blinded to all previous diagnoses and the randomisation allocation. The second assessor’s diagnosis was accepted as final and completed the blinded primary diagnosis. The presence or absence of ear disease was based on the infant’s worst ear and was determined according to recommended guidelines for clinical practice in this population (Morris et al. 2001, 2010b) (Table 3.1). Outcomes for sub-categories of ear disease are not presented here.
Table 3.1  Ear disease diagnosis algorithm utilised by the blinded independent assessors based on pneumatic video-otoscopy and tympanometry recording


<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sub-category</th>
<th>Observation (based on pneumatic video-otoscopy and/or tympanogram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any ear disease</td>
<td>Otitis Media with effusion (OME)</td>
<td>Intact and non-bulging TM* AND/OR Type B tympanogram</td>
</tr>
<tr>
<td>Acute Otitis Media without perforation (AOMwoP)</td>
<td></td>
<td>Any bulging of the TM*</td>
</tr>
<tr>
<td>Acute Otitis Media with perforation (AOMwiP)</td>
<td></td>
<td>Discharge from the middle ear AND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) intact TM (with or without bulging) OR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) &lt;2% TM* pars tensa perforation</td>
</tr>
<tr>
<td>Chronic Suppurative Otitis Media (CSOM)</td>
<td></td>
<td>Discharge from the middle ear AND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥2% TM* pars tensa perforation</td>
</tr>
</tbody>
</table>

3.4.7 Analyses

Based on local data (Mackenzie et al. 2009; Morris et al. 2005), at age 7 months the expected prevalence of infant middle ear disease was 90% and of nasopharyngeal carriage of 23vPPV serotypes was 55%. We aimed to recruit 70 subjects per group (210 total), sufficient for 80% power to demonstrate a 23% (90 to 69%) reduction in ear disease and a 45% (55 to 30%) reduction in vaccine-serotype pneumococcal carriage.
Mother-infant pairs were considered to have successfully completed the study follow-up if the infant had both a nasopharyngeal swab cultured and a valid independent ear assessment performed (tympanometry and/or video-otoscopy) at the 7 month visit. All analyses were performed on an intention-to-treat basis. Withdrawals, loss to follow up, or those without assessment data for both primary outcomes at age 7 months were excluded from analysis of study outcomes.

Primary analyses were independent comparisons between the risks at age 7 months of ear disease and vaccine-serotype pneumococcal carriage amongst infants of the control group compared with those of pregnancy vaccinees and birth vaccinees respectively. VE was calculated (1 minus the risk ratio) with 95% CI. Confidence intervals were not adjusted for multiple comparisons, those excluding zero were considered statistically significant. Analyses of ear disease and carriage prevalence at other time points were assessed using Fisher’s exact test.

Antibody titres were log normalised and the geometric mean concentrations (GMC) of the serotype-specific antibody concentrations were compared between vaccinees and controls using a two-tailed Student’s t-test. Differences were considered statistically significant when p<0.05. GMC’s of IgG in maternal, cord and infant blood, and IgA in breast milk, were assessed at all visits where the relevant samples were collected. The presented GMC’s and 95%CI’s are exponents of the log normal titre summary data.

As a post-hoc analysis, we included serotype 6A on the basis of demonstrated cross-reactivity with serotype 6B (Park et al. 2008) and investigated the prevalence of
23v6A carriage and 23v6A ear disease among vaccinees compared to controls. This strategy was designed to maximise sensitivity for ascertainment of potentially vaccine-preventable ear disease.

3.4.8 Ethics/clinical trial registration

The Human Research Ethics Committee of the Northern Territory Department of Health and Community Services and Menzies School of Health Research approved the study (05/52), which was registered at clinicaltrials.gov (NCT00714064, formerly NCT00310349).
Figure 3.1 PneuMum participant flow diagram.
3.5 Results

3.5.1 Participants

The consent rate was 50% (313/632) with 227 eligible participants subsequently randomised: 75 pregnancy vaccinees; 75 birth vaccinees; 77 controls (Figure 3.1). The retention rate was 89% (n=67), 88% (n=66) and 86% (n=66) respectively. Neither the withdrawals (n=13) nor those lost to follow up at 7 months (n=15) were differentially clustered by randomisation group. Reasons for withdrawal were: moved outside study catchment area (n=3); personal (n=6); not specified (n=4). Reasons for loss to follow-up were: moved outside study catchment area (n=6); unable to be located (n=8); refusal of 7 month ear exam (n=1) (Figure 3.1). Median time between receipt of the 23vPPV in pregnancy and birth was 6 weeks (range 1-10 weeks). The 23vPPV was received as intended by all mothers of infants that completed the study.

Participant characteristics were similar among the allocated groups (Table 3.2). Stratification of randomisation was reflected in equal proportions of remote dwelling participants. Median maternal age at enrolment was 23-25 years with self-reported smoking rates high, both during pregnancy (44-48%) and at infant age 7 months (56-69%). Few women (12-21%) received seasonal influenza vaccine during pregnancy, whilst most infants (84-89%) had received ≥2 PCV (7vPCV or PHID-10CV) doses at least 14 days prior to the 7 month visit.
Table 3.2  Participant characteristics.

<table>
<thead>
<tr>
<th>Maternal characteristics at enrolment</th>
<th>Control group n=77</th>
<th>Pregnancy vaccinees n=75</th>
<th>Birth vaccinees n=75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years</td>
<td>24 (17-38)</td>
<td>23 (17-39)</td>
<td>25 (17-37)</td>
</tr>
<tr>
<td>Household occupancy</td>
<td>5 (2-11)</td>
<td>4 (1-12)</td>
<td>4 (2-15)</td>
</tr>
<tr>
<td>Remote community residence</td>
<td>24 (31%)</td>
<td>23 (31%)</td>
<td>24 (32%)</td>
</tr>
<tr>
<td>Primigravida</td>
<td>29 (38%)</td>
<td>29 (39%)</td>
<td>22 (29%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>34 (44%)</td>
<td>36 (48%)</td>
<td>36 (48%)</td>
</tr>
<tr>
<td>Influenza vaccine in pregnancy</td>
<td>16 (21%)</td>
<td>9 (12%)</td>
<td>14 (19%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infant characteristics at birth n=76</th>
<th>n=76</th>
<th>n=75</th>
<th>n=74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight in grams</td>
<td>3381 (2132-4620)</td>
<td>3293 (2060-4425)</td>
<td>3334 (2080-4330)</td>
</tr>
<tr>
<td>Male</td>
<td>42 (55%)</td>
<td>36 (48%)</td>
<td>42 (57%)</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500 grams)</td>
<td>4 (5%)</td>
<td>7 (9%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>2 (3%)</td>
<td>7 (9%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Special or intensive care admission</td>
<td>16 (21%)</td>
<td>14 (19%)</td>
<td>15 (20%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infant characteristics at 7 months n=66</th>
<th>n=66</th>
<th>n=67</th>
<th>n=66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit age in months</td>
<td>7.2 (6.7-8.1)</td>
<td>7.2 (6.6-9.2)</td>
<td>7.2 (6.6-8.3)</td>
</tr>
<tr>
<td>Breast fed</td>
<td>38 (58%)</td>
<td>43 (64%)</td>
<td>45 (68%)</td>
</tr>
<tr>
<td>Breast fed exclusive</td>
<td>28 (42%)</td>
<td>30 (45%)</td>
<td>35 (53%)</td>
</tr>
<tr>
<td>Mother smoking</td>
<td>37 (56%)</td>
<td>49 (69%)</td>
<td>37 (56%)</td>
</tr>
<tr>
<td>≥2 doses of any PCV</td>
<td>57 (86%)</td>
<td>56 (84%)</td>
<td>59 (89%)</td>
</tr>
</tbody>
</table>

Maternal characteristics are described at time of enrolment (prior to withdrawals/loss to follow-up). Infant characteristics at birth exclude 2 pre-birth withdrawals. Infant characteristics at 7 month visit exclude 13 withdrawals and 15 lost to follow-up. PCV coverage, calculated at 14 days prior to the 7 month visit, includes doses of 7vPCV or 10vPHID-CV.
3.5.2 Safety

Four pregnancy vaccinees (5%) reported local pain, swelling or mild nausea post-
23vPPV. Two pregnancy vaccinees had preterm births (35 weeks, 37 weeks) of
otherwise healthy babies. Prevalence of preterm birth, low birth weight, or neonatal
special or intensive care admission were similar for pregnancy vaccinees compared
with those not in the pregnancy vaccine group. Two study participants (non-
pregnancy vaccinees) withdrew prior to birth.

3.5.3 Co-primary outcomes

For the primary outcomes at age 7 months (Table 3.3), the prevalence of middle ear
disease was 71% (47/66) among infants in the control group compared with 63%
(42/67) for infants of pregnancy vaccinees (VE 12%, 95% CI -12% to 31%) and
76% (50/66) for infants of birth vaccinees (VE -6%, 95% CI -15% to 23%). At the
same age, 26% (17/66) of infants in the control group had carriage of a 23vPPV
serotype compared with 18% (12/67) for infants of pregnancy vaccinees (VE 30%,
95% CI -34% to 64%) and 18% (12/66) for infants of birth vaccinees (VE 29%, 95%
CI -36% to 63%).
Table 3.3 Co-primary ear disease and carriage outcomes at infant age 7 months.

<table>
<thead>
<tr>
<th>Outcomes at infant age 7 months</th>
<th>Control group n=66</th>
<th>Pregnancy vaccinees n=67</th>
<th>Birth vaccinees n=66</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>VE(^1) % (95%CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Ear disease</td>
<td>47 (71)</td>
<td>12 (-12,31)</td>
<td>50 (76)</td>
</tr>
<tr>
<td>23v carriage</td>
<td>17 (26)</td>
<td>12 (18)</td>
<td>12 (18)</td>
</tr>
</tbody>
</table>

23v carriage: nasopharyngeal carriage of a pneumococcal serotype contained in the 23vPPV. VE\(^1\): vaccine efficacy of pregnancy vaccinees versus control group. VE\(^2\): vaccine efficacy of birth vaccinees versus control group. VE = 1 minus the risk ratio.

3.5.4 Secondary outcomes

3.5.4.1 Pneumococcal carriage and serotypes at age 7 months

At age 7 months, carriage of any pneumococcal serotype was 62% (41/66) for control infants compared with 48% (32/67) for infants of pregnancy vaccinees (p=0.118) and 59% (39/66) for infants of birth vaccinees (p=0.859). The most common serotypes at age 7 months (Figure 3.2, Table 3.4) were non-vaccine serotypes 6C and 16F (n=23 and n=18 infants respectively). Of the 23vPPV carriage serotypes, 19A, 10A, 15B and 33F predominated (n=28 infants in total). Three of the four predominant serotypes (19A, 15B and 33F) were lower among infants of vaccinees compared to controls (Figure 3.2, Table 3.4) while serotype 10A was most common among birth vaccinees. Five infants had the vaccine-related serotype 6A.
Figure 3.2 Pneumococcal nasopharyngeal carriage isolates detected at age 7 months by serotype for infants of controls, pregnancy and birth vaccinees.

Individual (bars; left axis) and cumulative (lines; right axis) serotypes detected in the nasopharynx of infants at 7 months of age are shown for each randomisation group. Multiple carriage of 23v serotypes occurred in two infants (infant 1: serotypes 15B and 3; infant 2: serotypes 19A and 19F), both of whom were in the control group. 23v types: pneumococcal serotypes contained in the 23vPPV.
Table 3.4 Ear disease and nasopharyngeal pneumococcal carriage at the 1, 2 and 7 month study visits among infants of controls, pregnancy and birth vaccinees.

<table>
<thead>
<tr>
<th>Outcomes per infant</th>
<th>Infant age 1 month</th>
<th>Infant age 2 months</th>
<th>Infant age 7 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls n=66</td>
<td>Pregnancy vaccinees n=67</td>
<td>Birth vaccinees n=66</td>
</tr>
<tr>
<td>Blinded assessor diagnosed infant ear disease, n (%)</td>
<td>13 (25%)</td>
<td>14 (25%)</td>
<td>12 (28%)</td>
</tr>
<tr>
<td>p&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.000</td>
<td>p&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.817</td>
</tr>
<tr>
<td>23vPPV carriage serotypes, (n)</td>
<td>2 (3%)</td>
<td>4 (7%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Serotype 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 6B&lt;sup&gt;PCV&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 8</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 9N</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serotype 9V&lt;sup&gt;PCV&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 10A</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 11A</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Serotype 15B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 19A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 19F&lt;sup&gt;PCV&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Serotype 22F</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 23F&lt;sup&gt;PCV&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Serotype 33F</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overall 23vPPV carriage, n (%)</td>
<td>2 (3%)</td>
<td>4 (7%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Non-23vPPV carriage serotypes, (n)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Serotype 6A</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Other serotypes</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Any Pneumococcal carriage, n (%)</td>
<td>8 (14%)</td>
<td>9 (15%)</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>p&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1.000</td>
<td>0.793</td>
<td>1.000</td>
</tr>
<tr>
<td>No NP swab, (n)</td>
<td>8</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>No blinded tympanometry, (n)</td>
<td>14</td>
<td>12</td>
<td>23</td>
</tr>
</tbody>
</table>

The primary infant ear disease outcome at age 7 months was determined by expert independent blinded assessors using tympanometry and video-otoscopy recorded by research nurses in the field. Infants were only included in the table if they completed the 7 month study follow-up: both a nasopharyngeal swab cultured and a valid independent ear assessment performed (tympanometry and/or video-otoscopy). Difficulty obtaining high quality video-otoscopy images at one or two month visits precluded their use for blinded diagnosis at these visits. Ear disease outcomes at age 1 and 2 months were determined solely by blinded assessment of the tympanometry. **Multiple serotype carriage:** (m<sub>1</sub>) 3 and 15B; (m<sub>2</sub>) 19F and 19A; (m<sub>3</sub>) 15C and 15B; (m<sub>4</sub>) 19A and 35F; (m<sub>5</sub>) 10A and 22A; (m<sub>6</sub>) 6C and 10A; (m<sub>7</sub>) 6C and 19A. **PCV serotypes** (PCV): pneumococcal serotypes contained in the 7vPCV or 10vPHID-CV. **23vPPV carriage:** nasopharyngeal carriage of pneumococcal serotypes contained in the 23vPPV. **p**<sub>1</sub>: 23vPPV in pregnancy versus controls and **p**<sub>2</sub>: 23vPPV at birth versus controls; significance determined using Fisher’s exact test. Missing data are described at the bottom of the table. NP swabs were not available for 28 infants at 1 month and 22 infants at 2 months. No blinded assessment of the tympanometry was available for 49 infants at 1 month, 45 infants at 2 months and 16 infants at 7 months.
3.5.4.2 Maternal pneumococcal carriage at birth

Maternal nasopharyngeal pneumococcal carriage rates at birth were similar among pregnancy vaccinees (7%; 5/67) and non-pregnancy vaccinees (7%; 9/132) with serotypes 10A, 11A, 16F and 34 most common (n=2 for each).

3.5.4.3 Infant ear disease and pneumococcal carriage at 1 and 2 months

At the one and two month visits, neither the prevalence of tympanometry diagnosed infant middle ear disease nor nasopharyngeal carriage of 23vPPV serotypes were significantly different between controls and respective maternal vaccination groups (Table 3.4). Collectively, PCV serotypes were infrequent at 1 (n=3), 2 (n=8) and 7 months (n=5) of age.

3.5.4.4 Comparison of ear diagnosis between the independent blinded assessor and research nurse at infant age 7 months - a analysis

At age 7 months, the ear disease prevalence ascertained by the research nurses was lower than that determined by the independent blinded assessor (co-primary outcome) for controls (62% versus 71%), pregnancy (54% versus 63%) and birth vaccinees (62% versus 76%) but followed a similar trend. Overall, there were 157 concordant and 32 discordant ear disease diagnoses (presence/absence) between the research nurses and blinded assessors at the 7 month assessment. Where the blinded assessor diagnosed ear disease and the research nurse did not (n=25) the discordant diagnoses comprised: 11 where the blinded assessor interpreted the tympanometry as type B and the research nurse as normal; 8 where tympanometry was interpreted as type B by both blinded assessor and research nurse; 6 where tympanometry was missing or normal and video-otoscopy determined diagnosis. The blinded ear
diagnoses were all according to protocol. Where the research nurse diagnosed ear disease and the blind assessor did not (n=7) the discordant diagnoses comprised: 6 with normal or missing tympanometry where otoscopy determined diagnosis; 1 where tympanometry was interpreted as type B by both blinded assessor and research nurse.

In a sensitivity analysis of the primary ear disease outcome at age 7 months including the additional 7 cases identified by the research nurse, the prevalence of middle ear disease was 76% (50/66) among infants for the control group compared with 64% (43/67) for infants of pregnancy vaccinees (VE 15%, 95% CI -6% to 32%) and 80% (53/66) for infants of birth vaccinees (VE -6%, 95% CI -27% to 12%).

3.5.5 Post hoc outcomes

3.5.5.1 Analysis of “23v6A carriage” and “23v6A ear disease”

To reiterate, 23v6A carriage refers to: nasopharyngeal carriage of a 23vPPV serotype or the vaccine-related serotype 6A; and 23v6A ear disease: refers to ear disease concurrent with nasopharyngeal 23v6A carriage.

At 7 months of age, the 23v6A carriage prevalence was 32% (21/66) for control infants compared with 18% (12/67) for infants of pregnancy vaccinees (VE 44%, 95% CI -5% to 70%) and 20% (13/66) for infants of birth vaccinees (VE 38%, 95% CI -13% to 66%). At the same time, the prevalence of 23v6A ear disease (Figure 3.3, Table 3.5) was 27% (18/66) among infants in the control group compared with 13% (9/67) for infants of pregnancy vaccinees (VE 51%, 95% CI -2% to 76%) and 17% (11/66) for infants of birth vaccinees (VE 39%, 95% CI -19% to 69%). In contrast,
the prevalence of 7 month ear disease associated with non-23v6A serotypes, nontypeable *Haemophilus influenzae* and/or *Moraxella catarrhalis* in the absence of pneumococcus, or where no detectable pathogen was found, were similar between vaccinees and controls (Figure 3.3).

Table 3.5  Frequency of 23v6A serotypes detected in the nasopharynx of infants with ear disease at age 7 months.

<table>
<thead>
<tr>
<th>Infant 23v6A ear disease at 7 months: by serotype</th>
<th>Control group</th>
<th>Pregnancy vaccinees</th>
<th>Birth vaccinees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype 3&lt;sup&gt; (m2) &lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Serotype 6A</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Serotype 6B&lt;sup&gt; (PCV) &lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Serotype 8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serotype 9N</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serotype 10A</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Serotype 11A</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Serotype 15B&lt;sup&gt; (m2) &lt;/sup&gt;</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Serotype 19A&lt;sup&gt; (m1) &lt;/sup&gt;</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Serotype 19F&lt;sup&gt; (m1) (PCV) &lt;/sup&gt;</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serotype 22F</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Serotype 33F</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total 23v6A ear disease</strong></td>
<td>18 (27%)</td>
<td>9 (13%)</td>
<td>11 (17%)</td>
<td>38 (19%)</td>
</tr>
</tbody>
</table>

Multiple serotype carriage: (<sup>m1</sup>) 19A and 19F; (<sup>m2</sup>) 15B and 3. PCV serotypes (<sup>PCV</sup>): pneumococcal serotypes contained in the 7vPCV or 10vPHID-CV. 23v6A ear disease: ear disease associated with concurrent nasopharyngeal carriage of a 23v6A serotype. <sup>p1</sup>: 23vPPV in pregnancy versus controls and <sup>p2</sup>: 23vPPV at birth versus controls; significance determined using Fisher’s exact test.
3.5.5.2 Predominant serotypes causing “23v6A ear disease” at 7 months

The predominant serotypes present in children with 23v6A ear disease (Table 3.5) were 19A, 10A, 15B, 33F and 6A (76%; 29/38); these were also the most common 23v6A carriage serotypes overall as reported in Table 3.4 (72%; 33/46). Compared to controls, the prevalence of ear disease associated with each of the individual serotypes 19A, 15B, 33F and 6A was lower among infants of vaccinees compared to controls while serotype 10A was most commonly associated with ear disease among infants of birth vaccinees (Table 3.5). Seven other 23vPPV serotypes (3, 6B, 8, 9N, 11A, 19F, 22F) were less frequently associated with 23v6A ear disease at age 7 months (≤1 case per group) and the remaining 12 vaccine serotypes (1, 2, 4, 5, 7F, 9V, 12F, 14, 17F, 18C, 20 and 23F) were not seen at all among infants with ear disease. Only three infants (one in each group) had ear disease associated with a PCV serotype (7vPCV or 10vPHiD-CV) at age 7 months (Table 3.5).
Figure 3.3 Nasopharyngeal carriage concurrent with ear disease at age 7 months among infants of controls, pregnancy and birth vaccinees
The proportion of infants with Any ear disease (bold lines) are sub-grouped (dotted lines) as follows: Any pneumococcus: concurrent NP carriage of any pneumococcal serotype; 23v6A ear disease: ear disease with concurrent nasopharyngeal carriage of a 23v6A serotype (black wedge, exploded). Note: Two infants, both in the control group, had ear disease with concurrent multiple 23v6A serotypes (serotypes 15B and 3; 19A and 19F). Infants with multiple serotype carriage of a 23v6A and a non23v6A serotype are shown exclusively within the 23v6A wedge: one infant of pregnancy vaccinees (serotypes 10A and 22A), two infants of birth vaccinees (serotypes 6C and 10A; 6C and 19A) and two control infants (serotypes 15B and 15C; 19A and 35F). NTHi: Nontypeable Haemophilus influenzae. Mcat: Moraxella catarrhalis. Pnc: Pneumococcus.
3.5.6 Vaccine-specific antibody responses

3.5.6.1 Maternal sera

In maternal blood (Appendix B) compared to corresponding controls, pregnancy vaccinated mothers had higher post-vaccination IgG to all 23v serotypes at both birth (venous and cord) and 1 month post-partum, while birth vaccinated mothers had higher IgG against all 23v serotypes 1 month post-partum (the only post-vaccination maternal sample). Maternal IgG to the vaccine-related serotype 6A was also elevated in the venous blood of pregnancy and birth vaccinated mothers. This did not translate into significantly higher serotype 6A IgG in the cord blood of the pregnancy vaccinees.

3.5.6.2 Breast milk

In maternal breast milk (Appendix B) compared to corresponding controls, pregnancy vaccinees had higher IgA for 14/15 serotypes tested at 1 month (excluding serotype 23F), 15/15 at 2 months and 13/15 serotypes at 7 months (excluding serotypes 19A and 23F). Birth vaccinees had higher IgA for 12/15 serotypes at 1 month (excluding serotypes 3, 19A, 23F), 13/15 serotypes at 2 months (excluding serotypes 19A and 23F) and 6/15 serotypes at 7 months (excluding serotypes 3, 6B, 10A, 11A, 12F, 14, 19A, 19F and 23F). Serotype-specific breast milk IgA was generally higher among pregnancy compared to delivery vaccinees at 1 month (0.9 to 1.61 fold), 2 month (1.2 to 1.5 fold) and 7 months (1.1 to 1.7 fold). IgA concentrations were all <0.4ug/ml.
3.5.6.3 *Infant sera*

In infant blood at age 7 months (Appendix B), excluding PCV serotypes there were no immunologically relevant differences between serotype specific IgG among infants in the vaccinated groups and controls. Although IgG concentration of serotypes 5, 7F, 8, 20 and 17F were higher among infants of pregnancy vaccinated mothers compared to controls at 7 months, the concentrations were low and none exceeded 0.35µg/ml. Of the 70 infants with available serum that received ≥2 routine 7vPCV doses at least 14 days prior to the 7 month visit, the IgG GMC’s were consistently >1.0 µg/ml for all 7vPCV serotypes regardless of study group. Of the 11 infants with available serum that received ≥2 doses of 10vPHiD-CV 14 days prior to the 7 month visit, the IgG GMC’s were >1.0 µg/ml for 8 of the 10vPHiD-CV serotypes, while serotypes 1 (0.86ug/ml) and 5 (0.65ug/ml) were lower. 10vPHiD-CV antibody concentrations were consistent across study groups. Notably, across all children at 7 months, the IgG GMC’s against the PCV-related serotypes 6A and 19A were both >0.35ug/ml (Appendix B).

The immune response to the commonly carried serotypes, 19A, 10A, 15B and 33F, were typical of most 23vPPV serotypes (Figure 3.4).
Figure 3.4  Immunogenicity of 23vPPV in venous blood, cord blood and breast milk by randomisation group.

Temporal antibody concentrations to four commonly carried serotypes (19A, 10A, 15B and 33F) as measured in mothers (venous blood, cord blood and breast milk) and their infants (blood) at scheduled study visits for each randomisation group.
3.6 Discussion

This is the first maternal pneumococcal vaccine trial designed to assess infant middle ear and nasopharyngeal carriage as primary outcomes. Vaccination with the 23vPPV during pregnancy elicited a strong maternal antibody response in blood and breast milk but evidence of an impact against infant ear disease (VE 12%, 95% CI -12% to 31%) and vaccine serotype nasopharyngeal carriage (VE 30%, 95% CI -34% to 64%) at 7 months of age was non-significant compared to controls. There was no evidence to suggest that 23vPPV at birth was effective against infant ear disease (VE -6%, 95% CI -15% to 23%) or vaccine serotype nasopharyngeal carriage (VE 29%, 95% CI -36% to 63%). Breast milk antibody responses were weaker among birth vaccinees than among pregnancy vaccinees. A lower than predicted prevalence of the a priori outcomes, ear disease (control infants 71% rather than predicted 90%) and NP carriage of 23vPPV serotypes (control infants 26% rather than predicted 55%) at infant age 7 months limited the ability to assess small effects.

3.6.1 Impact against potentially vaccine preventable ear disease

Nasopharyngeal carriage is a pre-requisite for ear disease with high serotype concordance between the nasopharynx and middle ear (Simell et al. 2012; Syrjanen et al. 2005). In the absence of tympanocentesis, the post hoc analyses of ear disease concurrent with nasopharyngeal carriage of 23v6A serotypes, 23v6A ear disease, provided a potential proxy indicator of ear disease that was vaccine-preventable within the study population. In our study, 27% of control infants had 23v6A ear disease at age 7 months compared to 13% for infants of pregnancy vaccinees (Figure 3.3). This was equivalent to a 51% relative reduction (95% CI -2% to 76%).
The reduction in 23v6A ear disease between infants of control (27%) and pregnancy vaccinees (13%) approached significance (VE 51%, 95% CI -2% to 76%) suggesting a vaccine impact. Interestingly, the impact was largely confined to the five predominant 23v6A ear disease serotypes (19A, 10A, 6A, 15B or 33F) where prevalence was 24% (16/66) among control infants and 4% (3/67) among infants of pregnancy vaccinees (Table 3.5). There was no difference in the prevalence of ear disease associated with the other 23vPPV serotypes (3, 6B, 11A, 22F, 8, 9N, 19F), non-23v6A serotypes, nontypeable *Haemophilus influenzae* and/or *Moraxella catarrhalis* in the absence of pneumococcus, or among those where no NP carriage was detected.

### 3.6.2 23vPPV in pregnancy and infant pneumococcal carriage

Two previous maternal 23vPPV studies have investigated pneumococcal carriage outcomes. In Texas, USA (Munoz et al. 2001) investigators showed that 23vPPV in pregnancy induced protective antibodies that were associated with a non-significant reduction in pneumococcal carriage at 7 months of age (largely due to low numbers) reaching significance by 16 months of age. Sero-groups 6 and 23 were common to infants of both 23vPPV and control vaccinated mothers, but there was a lower diversity of 23vPPV serotypes among infants born to 23vPPV vaccines and no increase in non-vaccine serotypes was observed. The Brazilian study by Lopes et al. (Lopes et al. 2009) showed that 23vPPV in pregnancy had no effect on the risk of infant pneumococcal carriage or acute respiratory infections at 6 months of age but fewer infants of pregnancy vaccinees carried serotype 6A/6B compared to controls. Neither of these studies were able to demonstrate early carriage reductions due to small sample sizes and low pneumococcal carriage rates (17% at 6 months (Munoz et
al. 2001) and 27% at 7 months (Lopes et al. 2009)) compared to that in our current study in the Northern Territory of Australia (56% at 7 months). Further, neither had a childhood PCV program in place nor investigated the impact of carriage in the context of ear disease. Despite this, both reported subtle serotype shifts among infants of vaccinees compared to controls.

3.6.3 Immunogenicity of 23vPPV in pregnancy

Of the nine studies that have investigated serotype specific immunogenicity following 23vPPV in the second or third trimester of pregnancy (Almeida Vde et al. 2009; Lehmann et al. 2002; Lehmann et al. 2003; Munoz et al. 2001; O'Dempsey et al. 1996; Obaro et al. 2004; Quiambao et al. 2003; Shahid et al. 1995; Steinhoff et al. 2010) ours has included the largest number of serotypes tested in both blood and breast milk (Appendix B). Pre-vaccination antibody concentrations to serotypes 19A, 19F, 22F, 15B, 6A and 33F were high at baseline among mothers randomised to receive the 23vPPV in pregnancy, suggesting previous maternal exposure to these serotypes. Pregnancy vaccinees had robust serotype specific antibody responses for all 23 serotypes in maternal and cord blood yet, consistent with previous studies (Lehmann et al. 2002; Munoz et al. 2001; O'Dempsey et al. 1996; Shahid et al. 1995), there was little evidence of persistence in the infant to 7 months.

3.6.3.1 Maternal antibody response

At 1 month post-vaccination, the magnitude of maternal blood antibody concentrations to the common 23v6A ear disease serotypes (19A, 15B, 33F) were among the highest for both pregnancy and birth vaccinees (>7µg/ml) and were the highest of the 23 serotypes tested in the cord blood (>4µg/ml) of pregnancy
vaccinees. Previous exposure to serotypes 19A, 15B, 33F, as indicated by high pre-vaccination antibody concentrations, may have primed the vaccine immune response to these types. Response to the other common 23v6A carriage serotypes 10A and 6A were weaker (<2 µg/ml) 1 month post vaccination for both pregnancy and birth vaccinees yet both were significantly greater than controls.

3.6.3.2 Maternal 23vPPV antibodies in the infant

Previous studies suggest that passively acquired 23vPPV specific IgG can persist in the infant for up to 5 months after birth, depending on the serotype (Lehmann et al. 2003; Munoz et al. 2001; Obaro et al. 2004; Quiambao et al. 2003; Shahid et al. 1995) with persistence shown to correlate with the magnitude of the maternal serotype-specific antibody concentration (Quiambao et al. 2007). Quiambao et al. considered antibody decay rates and infant growth levels and estimated that cord blood serotype specific IgG concentrations of 4.4µg/ml are required to enable persistence in the infant for 4 months (Quiambao et al. 2007). In our study, cord blood IgG GMC’s to serotypes 19A, 33F, and 15B were greater than 4.4ug/ml suggesting the potential persistence to 4 months of age despite their falling to background concentrations by 7 months of age. This may explain the small reductions in carriage of these serotypes. Cord blood IgG GMC’s to serotype 10A and the vaccine-related serotype 6A were below 4.4ug/ml as were IgG GMC’s to most of the other 23vPPV serotypes except serotypes 14 and 19F. There was no evidence to suggest that maternal 23vPPV in pregnancy inhibited infant antibody responses to PCV at age 7 months.
3.6.3.3 Breast milk antibodies

Breast milk antibody concentrations were also elevated among vaccinees for most of the 23vPPV serotypes at the 1, 2 and 7 month visits, particularly among pregnancy vaccinees. The higher breast milk antibody production seen among pregnancy compared to birth vaccinated mothers (up to 1.7 fold higher) has not been reported previously. For pregnancy vaccinees, concentrations of breast milk IgA specific to serotypes 19A and 15B were consistently the highest across all visits. While there are no defined protective levels of breast milk IgA the strong response suggests a potential supporting role for mucosal IgA in reducing in ear disease associated with carriage of the 23v6A serotypes. In a 2004 trial in The Gambia, maternal 23vPPV vaccination elicited a strong breast milk IgA (including some IgA2) response with high avidity against the 23vPPV antigens (Obaro et al. 2004) and a follow up study showed that colostrum of 23vPPV vaccinated mothers inhibited the colonisation of pneumococcal serotypes 6B and 14 to pharyngeal cells in vitro (Deubzer et al. 2004). However, the ability of IgA to prevent colonisation is largely circumvented by pneumococcal IgA1 protease which has the ability to cleave and inactivate IgA1 but not IgA2. The effect of IgA against infection is more complex because the host subsequently produces antibodies capable of neutralising the proteolytic activity of IgA1 protease (Janoff et al. 2014).

3.6.4 Hypotheses for impact against common vaccine and related serotypes

Given the sample size of our study it was difficult to draw a conclusion with respect to vaccine impact against individual serotypes. However, several of the strongest serotype-specific antibody responses (19A, 15B and 33F) were associated with reductions in ear disease concurrent with carriage of those serotypes suggesting this
effect maybe real. Interestingly, four of the common 23v6A ear disease serotypes
(19A, 10A, 6A and 33F) at age 7 months are prominent causes of invasive
pneumococcal disease among Indigenous children aged <5 years in the Northern
Territory (Krause & Cook 2012). It is plausible that inflammation caused by these
virulent serotypes might have exposed them to circulating vaccine specific IgG
resulting in local phagocytosis in the mucosa of the nasopharynx and/or middle ear.
Another thing to consider is that we may have only seen effects against infant ear
disease associated with serotypes 19A, 15B, 33F, 10A and 6A simply because they
were common enough that we could actually discern a difference between the
groups.

3.6.5 Conclusions
Contemporary prevention and intervention strategies have largely failed to overcome
the endemicity of microbial infections among Indigenous children living in both
urban and remote communities in the Northern Territory of Australia. As such, ear
disease continues to be a lived experience for Indigenous families. Undertaking a
maternal vaccination trial in this setting was challenging; however, the consent rate
(51%) and achievement of the intended sample size, demonstrate that such studies
are possible. Engagement with participants, local communities (Dunbar et al. 2007),
an Indigenous Reference Group (Andrews et al. 2012) and the Independent Data
Safety Monitoring Board that included community representation were integral to the
success of this trial.

Use of the 23vPPV during pregnancy or at birth was safe, immunogenic and well
tolerated. Despite the smaller than expected effect size and lower than predicted
prevalence of the co-primary outcomes, our data are suggestive of a reduction in infant ear disease associated with concurrent carriage of common 23v6A serotypes (19A, 10A, 6A, 15B and 33F). Unfortunately, we found no impact against the co-primary outcomes, collective carriage of 23vPPV serotypes or all-cause ear disease. Given the observed absence of infant middle ear disease associated with serotypes contained within the 7vPCV, for which uptake of at least two doses in infancy was >80% within our study population, the future of maternal 23vPPV in pregnancy as a strategy to prevent infant ear disease may be to target serotypes not contained in current PCV formulations (example 10A, 15B and 33F). Whilst promising, the potential impact against overall ear disease seems likely to be modest without substantial improvements in health equality for Indigenous Australians. The ongoing ear disease burden remains unacceptably high and alternatives, including increased uptake of influenza vaccination in pregnancy or the use of alternative pneumococcal vaccines (such as the Haemophilus influenzae protein D containing 10vPHiD-CV) in pregnancy warrant further investigation.
CHAPTER 4

Impact of the 23-valent pneumococcal polysaccharide vaccination in pregnancy against infant acute lower respiratory infections in the Northern Territory of Australia.
Chapter 4: Impact of the 23-valent pneumococcal polysaccharide vaccination in pregnancy against infant acute lower respiratory infections in the Northern Territory of Australia.

4.1 Context
The data in Chapter 3 showed that vaccination with the 23vPPV in pregnancy elicited a strong maternal immune response in both blood and breast milk. While the effect against overall infant ear disease among high risk Indigenous children was not significant, reductions in ear disease associated with commonly carried serotypes suggested that the vaccine may prove to be effective with a larger sample size. As described in Chapter 1.3, ALRI’s are prominent among Indigenous children of the Northern Territory with rates of radiologically confirmed pneumonia some of the highest reported in the world (O’Grady et al. 2010c). Although the aetiology of ALRI is poorly characterised, it was considered that maternal pneumococcal vaccination may be effective against infant ALRI in this setting of high pneumococcal carriage. In this chapter we investigated ALRI as a secondary outcome of the PneuMum trial.

4.2 Abstract

Introduction
Globally, the pneumococcus is one of the most common causes of pneumonia. Indigenous children are colonised with the pneumococcus early in life antecedent to
a high prevalence of acute lower respiratory infections and otitis media. Early immune protection may attenuate pneumococcal disease.

Methods
This was an open label randomised controlled trial of maternal 23vPPV (PNEUMOVAX® 23, Merck, USA) in pregnancy, called PneuMum. The primary ear disease outcomes were reported in Chapter 3. As a secondary outcome of this trial, we investigated the efficacy of pregnancy vaccination against the incidence of ALRI hospitalisations and clinic presentations (birth to 12 months of age) compared to controls.

Results
The incidence of first ALRI hospitalisation events (Table 4.2) was 8 per 100 child-years among infants of pregnancy vaccinees compared to 14 per 100 child-years among control infants (VE 39%, 95% CI -58 to 80). The incidence of first ALRI clinic presentation (Table 4.2) was 132 per 100 child-years among infants of pregnancy vaccinees compared to 127 per 100 child-years among control infants (VE -4%, 95% CI -90 to 45). For remote dwelling infants, the incidence of ALRI hospitalisation was 9 per 100 child-years among vaccinees compared to 33 per 100 child-years among controls (VE 72%, 95% CI -22 to 97). There was no difference in the incidence of ALRI hospitalisation between vaccinees and controls among urban dwelling infants.
Conclusions

Pneumococcal vaccination in pregnancy marginally reduced the incidence of ALRI hospitalisations compared to controls, and this reduction was more pronounced among remote dwelling participants, but we could not exclude no effect. There was no impact against ALRI clinic presentations. Larger trials involving 23vPPV or pneumococcal conjugate vaccines with active monitoring of respiratory outcomes are logical next steps.

4.3 Background:

Nasopharyngeal pneumococcal colonisation of Indigenous Australian infants, is not only concurrent with the onset of otitis media (Leach et al. 1994), but also with a high frequency of early hospital admissions and clinic presentations for acute lower respiratory infection (ALRI) (Kearns et al. 2013; O'Grady, Torzillo & Chang 2010). In the Northern Territory, childhood pneumococcal vaccination programs have had only a limited impact against ALRI (O'Grady, Torzillo & Chang 2010). Akin to our strategy against otitis media described in Chapter 3, we hypothesised that earlier and broader immunity against common pneumococcal serotypes might also reduce the burden of infant ALRI.

As described in Chapter 2.8, administering the 23-valent pneumococcal polysaccharide vaccine (23vPPV) in pregnancy generates antibodies that are transferred to the infant in utero via the placenta (serum IgG) and during early infancy via breast feeding (breast milk IgA) (Almeida Vde et al. 2009; Lehmann et al. 2002; Lehmann et al. 2003; Munoz et al. 2001; O'Dempsey et al. 1996; Obaro et al. 2004; Quiambao et al. 2003; Shahid et al. 1995; Steinhoff et al. 2010). The
immunogenicity outcomes of our own maternal 23vPPV trial, reported in Chapter 3, were consistent with these previous studies, though the breast milk response was more robust among pregnancy vaccinees than previously shown, persisting to 7 months for most serotypes. The presence of these systemic and mucosal pneumococcal antibodies from birth has the potential to protect against pneumococcal colonisation and disease prior to immunity generated by the infant PCV schedule (2, 4 and 6 months in Australia). To date, no maternal 23vPPV studies have adequately investigated infant ALRI outcomes (Lopes et al. 2009; O'Dempsey et al. 1996).

As a secondary outcome of our randomised controlled maternal pneumococcal vaccine trial, “PneuMum” reported in Chapter 3, we investigated the impact of 23vPPV in pregnancy against ALRI hospitalisations and/or clinic presentations among a high risk cohort of Indigenous infants between birth and 12 months of age.

4.4 Methods

4.4.1 Study Design

Open label randomised controlled trial of maternal 23vPPV (PNEUMOVAX® 23, Merck, USA) in pregnancy, called PneuMum. As a secondary outcome, we compared the incidence of ALRI hospitalisations and clinic presentations (<12 months of age) between infants of pregnancy vaccinees and non-pregnancy vaccinees.
4.4.2 Participants

PneuMum randomised 227 healthy pregnant Indigenous women (aged 17-39 years) in Darwin or remote Northern Territory communities of Australia to receive the 23vPPV during pregnancy (n=77), at birth (n=75), or 7 months post-partum (n=75; offered only). Trial design (Chapter 3.4.1), eligibility (Chapter 3.4.3), randomisation procedures (Chapter 3.4.3) and primary outcomes (Chapter 3.5) are described previously. Given that maternal 23vPPV at birth had no impact against the PneuMum primary outcomes of infant pneumococcal carriage and/or ear disease as described in Chapter 3.5, for this investigation we decided *a priori* to assess participants in two groups:

- **Pregnancy vaccinees** - 23vPPV during pregnancy (30-36 weeks gestation inclusive)
- **Controls** - no 23vPPV during pregnancy (birth and 7 month vaccinees combined)

After two pre-birth withdrawals, 75 infants of pregnancy vaccinees and 150 control infants were eligible for follow-up of ALRI outcomes (Figure 4.1). All mothers assigned as pregnancy vaccinees received the 23vPPV in pregnancy as intended and all participants indicated their intent to remain in the study catchment area for the duration of the study.

4.4.3 Outcomes

Infants underwent 12 months of clinical observation for hospitalisations and clinic presentations of any cause. The primary study outcomes were ALRI hospitalisations and ALRI clinic presentations. To avoid episode bias by individual participants, the
incidence (per 100 child-years) and time to event outcomes were based on the first event only.

ALRI hospitalisation episodes were identified by International Classification of Diseases coding (ICD-10-AM: J09-J22; A37-A37.9) recorded in the Northern Territory-wide government hospital discharge dataset (National Centre for Classification in Health 2009). Data were extracted by the Acute Care Information Unit at Royal Darwin Hospital. ALRI clinic presentations (remote participants only) were identified by applying a standardised diagnostic algorithm to medical records accessed via the electronic Primary Care Information System (PCIS). ALRI clinic presentations were defined by at least two primary indicators: ALRI diagnosis in clinic notes, chest recession, tachypnoea (<2mo: ≥60 breaths per minute; 2-12mo: ≥50 breaths per minute) or cough (any); or one of these primary indicators and at least two secondary indicators including: wheeze, crackles, fever (≥38°C) or specific treatment with at least 2 days of enteral antibiotics. Any hospitalisations (identified by any ICD-10-AM hospital coding) and any clinic presentation (identified by any non-scheduled presentation recorded in PCIS) were also documented for comparison. An episode was defined as unique with at least 1 day of separation following hospital discharge or leaving the clinic. Hospitalisations and clinic presentations within 7 days of birth were excluded from analysis to exclude vertically transmitted infections yet maximise case ascertainment.

4.4.4 Sample size

In a post hoc determination of sample size, we assumed 22% ALRI hospitalisations (O’Grady, Torzillo & Chang 2010) and 75% ALRI clinic presentations (Kearns et al.
2013) among Northern Territory Indigenous infants before 12 months. Given 75 available subjects in the intervention group and 150 in the control group, the study was adequately powered (80%; \( \alpha = 0.05 \)) to detect a 3-fold reduction in the rate of infant ALRI hospitalisations. Given 23 remote dwelling subjects in the intervention group and 47 in the control group (Figure 4.1) we are adequately powered (80%; \( \alpha = 0.05 \)) to detect a 2-fold reduction in the rate of infant ALRI clinic presentations between the groups.

4.4.5 Analysis

We compared the incidence rate and time to first event for both infant ALRI hospitalisations (all eligible infants) and ALRI clinic presentations (remote infants only) between infants of vaccinated and unvaccinated mothers during the period from birth and 12 months of age. The incidence rate and time to first hospitalisations of any cause were presented for comparison. Fisher’s exact test was used to compare proportions and incidence rates and the logrank test was used to evaluate the survivor functions of time event between groups. All data analysis was performed using STATA, version 13 (StataCorp, USA).

4.4.6 Ethics

The study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC 05/52). Written consent was obtained for access to each child’s medical records to complete all of the described outcomes of the study.
Hospital records were investigated for all 225 infants born during the study. Clinic presentations were investigated among the 70 infants from remote communities for whom electronic medical records were available.
4.5 Results:

4.5.1 Participant characteristics

All 225 study infants births were accounted for; 217 at Royal Darwin Hospital, six at Alice Springs Hospital and two at Darwin Private Hospital. Mild local pain, fever or nausea was reported by 4 (5%) women, all of whom were pregnancy vaccinees. Two (3%) preterm births at 35 and 37 weeks were deemed as possibly related to pregnancy vaccination by the data safety monitoring board; both resulted in healthy babies. Participant characteristics were similar between groups (Table 4.1). Uptake of the influenza vaccine during pregnancy was low (12%-21%), yet the completion of the primary infant PCV schedule (2, 4 and 6 months) was timely (median 194-199 days) with 85%-86% of infants fully PCV vaccinated by 12 months of age. Median time from 23vPPV in pregnancy to birth was 6 weeks (range 1-10 weeks).

4.5.2 Episodes of care

By 12 months of age, 25% (60/225) of infants were admitted to hospital for 88 episodes of care and 11% (25/225) with an ALRI for 34 episodes of care. The respective median ages of first hospitalisation (any cause) and ALRI hospitalisation, were 98 days (range 9 to 347) and 155 days (range 13 to 346). Over the 12 month observation period, hospitalisations were more frequent among remote compared to urban dwelling infants: any cause (43%; 30/70 versus 23%; 35/155) and ALRI (21%; 15/70 versus 6%; 10/155). By 12 months of age, all 70 remote dwelling infants had presented to their medical clinic for 759 episodes of care and 74% (52/70) with an ALRI for 140 episodes of care. The respective median ages of first clinic presentation (any cause) and ALRI clinic presentation, were 46 days (range 7 to 317) and 148 days (range 36 to 364).
Table 4.1 Maternal and infant characteristics by randomisation group.

<table>
<thead>
<tr>
<th>Maternal characteristics at randomisation</th>
<th>23vPPV in Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=75)</td>
</tr>
<tr>
<td>Age in years median(range)</td>
<td>23 (17-39)</td>
</tr>
<tr>
<td>Household occupancy median(range)</td>
<td>4 (1-12)</td>
</tr>
<tr>
<td>Household occupancy (&lt;5yrs)median(range)</td>
<td>1 (0-4)</td>
</tr>
<tr>
<td>Completed year 10 n(%)</td>
<td>61 (82)</td>
</tr>
<tr>
<td>Remote dwelling n(%)</td>
<td>23 (31)</td>
</tr>
<tr>
<td>Smoker n(%)</td>
<td>36 (48)</td>
</tr>
<tr>
<td>Influenza vaccine in pregnancy n(%)</td>
<td>9 (12)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n(%)</td>
<td>36 (48)</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500g) n(%)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Premature birth (&lt;37weeks) n(%)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Intensive Care Admission n(%)</td>
<td>14 (19)</td>
</tr>
<tr>
<td>Breast fed 1 month visit n(%)</td>
<td>61 (81)</td>
</tr>
<tr>
<td>2 month visit n(%)</td>
<td>55 (73)</td>
</tr>
<tr>
<td>7 month visit n(%)</td>
<td>43 (57)</td>
</tr>
<tr>
<td>Mother smoking 1 month visit n(%)</td>
<td>35 (53)</td>
</tr>
<tr>
<td>2 month visit n(%)</td>
<td>34 (57)</td>
</tr>
<tr>
<td>7 month visit n(%)</td>
<td>46 (69)</td>
</tr>
<tr>
<td>3 doses of PCV &lt;12 months n(%)</td>
<td>64 (85)</td>
</tr>
<tr>
<td>7vPCV n(%)</td>
<td>38 (51)</td>
</tr>
<tr>
<td>10vPHiD-CV n(%)</td>
<td>15 (20)</td>
</tr>
<tr>
<td>Mixed schedule (7v/10v) n(%)</td>
<td>11 (15)</td>
</tr>
<tr>
<td>Age at PCV1 median(range)</td>
<td>63 (41-240)</td>
</tr>
<tr>
<td>Age at PCV2 median(range)</td>
<td>129 (103-311)</td>
</tr>
<tr>
<td>Age at PCV3 median(range)</td>
<td>199 (150-249)</td>
</tr>
</tbody>
</table>

Study vaccinations, sample collection and clinical examinations occurred over 5 visits: pregnancy (30-36 weeks), birth (<72 hours) and infant age 1, 2 and 7 months. Complete data were not available for some characteristics. For pregnancy vaccinated, and non-pregnancy vaccinated groups respectively the denominators for household occupancy(<5yrs) were: 75 and 149; for completed year 10 were: 74 and 149; and for maternal smoking were: 66 and 129 at 1 month; 60 and 127 at 2 months; and 67 and 135 at 7 months. PCV: pneumococcal conjugate vaccine; includes both the 7vPCV and 10vPHiD-CV.
4.5.3 Primary outcomes

The incidence of ALRI hospitalisation events (Table 4.2) was 8 per 100 child-years among infants of pregnancy vaccinees compared to 14 per 100 child-years among control infants (VE 39%, 95% CI -58 to 80). The incidence of ALRI clinic presentation (Table 4.2) was 132 per 100 child-years among infants of pregnancy vaccinees compared to 127 per 100 child-years among control infants (VE -4%, 95% CI -90 to 45). Incidence of any cause hospitalisation (30 versus 33 per 100 child-years) and any clinic presentation (584 versus 424 per 100 child-years) were consistent among infants of vaccinees and controls.

Table 4.2 Primary outcomes: incidence of ALRI hospitalisation episodes and ALRI clinic presentations among infants of pregnancy vaccinees versus controls.

<table>
<thead>
<tr>
<th>23vPPV in Pregnancy</th>
<th>Yes (n=75)</th>
<th>No (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First episodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALRI Hospitalisation</td>
<td>n</td>
<td>child-years</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>72.2</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>13.6</td>
</tr>
</tbody>
</table>

IR: incidence rate per 100 child-years. IRR: incidence rate ratio. VE: vaccine efficacy. #ALRI clinic presentation data were only available for the 70 infants from remote communities (23vPPV n=23; no 23vPPV n=47).
4.5.4 ALRI episodes

Consistent with the primary incidence outcomes, the proportion of children hospitalised with an ALRI during the first 12 months was 8% (6/75) among infants of pregnancy vaccinees compared to 13% (19/150) among controls (Table 4.3), while ALRI clinic presentations were similar between infants of pregnancy vaccinees (78%) and controls (72%). Multiple ALRI hospitalisation episodes occurred among one infant in the 23vPPV group (n=4) and two infants in the control group (n=2 and n=6). There were no significant differences in the median number of episodes per child, number of children with multiple episodes, length of hospital stay, or time between episodes, for either ALRI hospitalisations or clinic presentations, between infants of pregnancy vaccinees and controls.

Table 4.3 Characterisation of all infant ALRI episodes (<12 months)
among pregnancy vaccinees and controls.

<table>
<thead>
<tr>
<th></th>
<th>23vPPV in Pregnancy</th>
<th>ALRI hospitalisations</th>
<th>23vPPV in Pregnancy</th>
<th>ALRI clinic presentations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=75)</td>
<td>No (n=150)</td>
<td>Yes (n=23)</td>
<td>No (n=47)</td>
</tr>
<tr>
<td>Children, n (%)</td>
<td>6 (8)</td>
<td>19 (13)</td>
<td>18 (78)</td>
<td>34 (72)</td>
</tr>
<tr>
<td>Episodes, n</td>
<td>9</td>
<td>25</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>Episodes per child, median (range)</td>
<td>1 (1-4)</td>
<td>1 (1-6)</td>
<td>3 (1-6)</td>
<td>4 (1-8)</td>
</tr>
<tr>
<td>Length of stay in days, median (range)</td>
<td>3 (1-7)</td>
<td>3 (1-39)</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Children with multiple episodes, n (%)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>14 (61)</td>
<td>21 (45)</td>
</tr>
<tr>
<td>Days between episodes median (range)#</td>
<td>23 (21-211)</td>
<td>27 (2-85)</td>
<td>51 (7-182)</td>
<td>39 (2-202)</td>
</tr>
</tbody>
</table>

#Days between episodes calculated for children with multiple episodes.
4.5.5 ALRI hospitalisation outcomes by dwelling

ALRI hospitalisations were more frequent among remote compared to urban dwelling infants. To investigate any differential effect, we repeated the primary analysis of ALRI hospitalisation stratified by dwelling (Table 4.4). For remote dwelling infants, the incidence of ALRI hospitalisation was lower among infants of pregnancy vaccinees (9 per 100 child-years) compared to controls (33 per 100 child-years) but not statistically significant (p=0.119) while the incidence rates of any cause hospitalisation were similar between groups. For urban dwelling infants, there was no difference in the incidence of any cause hospitalisation or ALRI hospitalisations among infants of pregnancy vaccinees compared to controls.

Table 4.4 Incidence of hospitalisation episodes among infants of controls versus pregnancy vaccinees, stratified by dwelling.

<table>
<thead>
<tr>
<th>23vPPV in Pregnancy</th>
<th>Yes (n=75)</th>
<th>No (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Hospitalisation episodes</td>
<td>n (child-years)</td>
</tr>
<tr>
<td>Any cause</td>
<td>Urban</td>
<td>12 44.7</td>
</tr>
<tr>
<td></td>
<td>Remote</td>
<td>7 17.6</td>
</tr>
<tr>
<td>ALRI</td>
<td>Urban</td>
<td>4 50.4</td>
</tr>
<tr>
<td></td>
<td>Remote</td>
<td>2 21.9</td>
</tr>
</tbody>
</table>

IR: incidence rate per 100 child-years. IRR: incidence rate ratio. VE: vaccine efficacy. Clinic medical records were only available for remote dwelling infants (n=70).
4.5.6 Time to ALRI (equality of Kaplan-Meier failure functions)

Time to event analysis was used to further elucidate the trend in time to first ALRI events (Figure 4.2). There was a delay in time to first ALRI hospitalisation among infants of pregnancy vaccinees compared to controls but the inequality of the Kaplan-Meier failure functions was not statistically significant (p=0.284). Stratification by dwelling showed that any potential vaccine effect against time to first ALRI hospitalisation occurred predominantly among remote participants where the difference in failure functions approached statistical significance (p=0.071). There were no differences in the time to hospitalisations of any cause, nor in the time to clinic presentations for any cause or ALRI. Further, there was no evidence of a hazard bias due to PCV dosing or timeliness with respect to ALRI hospitalisation outcomes in either 23vPPV randomisation group (Table 4.5).
Pneumococcal conjugate vaccination timeliness (2, 4 and 6 month schedule) was investigated between birth and 12 months of age for infants of pregnancy vaccinees compared to controls, by ALRI hospitalisation status. \( p \): Fisher’s exact and Wilcoxon ranksum tests were used to compare proportions and non-parametric continuous data respectively.
Cumulative proportion of first hospitalisations between birth and 12 months of age for infants of pregnancy vaccinees (dashes) compared to controls (solid lines). Any and ALRI hospitalisation (A1; overall) (A2; by dwelling). Any and ALRI clinic presentations (B; data available for the 70 remote participants only). The logrank test was used to compare the equality of the Kaplan-Meier failure functions.
4.6 Discussion

Vaccinating Indigenous mothers with the 23vPPV during pregnancy had a non-significant impact on the incidence of first infant ALRI hospitalisation episodes (VE 39%, 95% CI -58% to 80%) and no effect on the incidence first infant clinic presentations (VE -4%, 95% CI -90% to 45%) during the first year of life (Table 4.2). The non-significant difference in the incidence of first ALRI hospitalisation episodes between infants of vaccinees and controls occurred predominantly during the first 6 months of age and was largely confined to remote dwelling participants (Figure 4.2). Overall, only half the expected proportion of infants were hospitalised with an ALRI before age 12 months (expected 22%, actual 11%) reducing our statistical power to proving differences greater than 4-fold in magnitude. The observed risk difference in ALRI hospitalisation between groups was 1.6-fold.

4.6.1 Consistency with previous studies

Like preceding maternal 23vPPV trials (Lopes et al. 2009; O'Dempsey et al. 1996) these data did not show a significant impact against infant ALRI’s. In 1973, a large randomised controlled trial of a 14-valent PPV (14vPPV) was carried out in Papua New Guinea (Riley et al. 1977) which inadvertently enrolled over 300 women in the early stages of pregnancy. Among infants followed for 3 years post-partum, there were encouragingly fewer ALRI episodes among infants of 14vPPV versus placebo vaccinees (68%, 57/84 versus 79%, 73/93; p=0.100). Twenty years later O’Dempsey et al. showed that infants of 23vPPV vaccinees had marginally fewer pneumonia episodes than infants receiving a control vaccine (7 versus 4; not-significant) (O'Dempsey et al. 1996). In the most recent study, Lopes et al. found no difference in the period prevalence of acute respiratory infections among infants followed from
birth to 6 months of age following 23vPPV during the third trimester (17%:7/42), at delivery (18%:8/45), or not at all (17%:8/46) (Lopes et al. 2009).

4.6.2 Considerations for future trials
Future maternal pneumococcal vaccination studies for the prevention of respiratory infection need to carefully consider study power and sample size. For Indigenous children of the Northern Territory, the pneumococcus is just one of a diverse range of common respiratory pathogens. Chapter 3 demonstrated that at 7 months of age, nasopharyngeal carriage prevalence of pneumococcus was 62% (41/66), nontypeable *Haemophilus influenzae* was 53% (35/66) and *Moraxella catarrhalis* was 38% (25/66). All of these bacteria are capable of causing disease. Additionally, respiratory viruses are historically detected in the nasopharynx of up to 90% of Northern Territory Indigenous children under 6 months to 2 years of age (Binks et al. 2011). In Chapter 3 we also highlighted that only 26% (17/66) of control infants in the PneuMum trial had nasopharyngeal carriage of a 23vPPV serotype (representative of community prevalence) and that only 24% (16/66) had ear disease concurrent with carriage of a 23vPPV serotype. Assuming hypothetically that approximately one quarter of the ALRI hospitalisations in the community (prevalence 13%; 19/150 among non-maternally vaccinated controls) were related to a pneumococcal infection, as was the case for ear disease in Chapter 3, then less than 5% of these Northern Territory Indigenous infants would have had an ALRI hospitalisation associated with a 23vPPV serotype. This illustrates that such a targeted strategy may have a limited potential and that much larger, potentially non-feasible, sample sizes are required.
To prove whether the 1.6-fold risk reduction in ALRI hospitalisations seen during this study was real (13% among controls, 8% among vaccinees), we would have required a sample size of almost 1200 mother-infant pairs. Based on the recruitment rate of the Pneumum trial this would take over three decades to complete. While these calculations are speculative they may not be unrealistic. To enhance the power of existing studies of maternal 23vPPV against infant clinical outcomes, there is scope for pooled data meta-analysis; however, in order to prove a local effect against ALRI among Indigenous children, large multi-site trials across northern Australia are required.

4.6.3 Lack of effect against ALRI clinic presentations

For the 70 remote participants, the clinic presentation algorithm was designed to detect all potential acute infections related to the lower airways, from minor to severe. We expected the frequency of ALRI events would improve our power to detect a vaccine effect; however, while the pneumococcus is the leading aetiological pathogen of pneumonia (Wardlaw et al. 2006), its role in less severe ALRI’s, such as those seen during clinic presentations, is not well understood. The absence of an effect against ALRI clinic presentations among remote Indigenous infants (74%; 52/70) may simply reflect a high proportion of non-pneumococcal ALRI’s in the community. By this logic, the small effect against ALRI hospitalisations, should it be real, may be an indication that severe ALRI’s are more commonly of pneumococcal aetiology. The microbiology component of the PneuMum trial was confined to 1, 2 and 7 month visits in conjunction with the infant ear exams (co-primary outcomes) and was not able to be meaningfully linked with the ALRI presentations or hospitalisations.
4.6.4 Conclusions

Maternal 23vPPV vaccination in pregnancy generated a robust maternal antibody response as described in Chapter 3.5.6, yet had only a small non-significant impact on the incidence of ALRI hospitalisations and no impact at all on the incidence of ALRI clinic presentations in remote communities. It is unclear whether the limited efficacy of 23vPPV in pregnancy against ALRI was due to the low burden of ALRI caused by vaccine serotypes, or whether the polysaccharide antibody response is inadequate to protect against mucosal disease. In Chapter 3 we demonstrated potential efficacy against carriage of several common serotypes: 19A, 15B, 33F and 6A (23vPPV related). Future trials of 23vPPV in pregnancy need to show serotype specific effectiveness and must consider: (a) whether or not a pneumococcal conjugate vaccine schedule is in place; and (b) whether or not there is sufficient disease in the target population attributed to 23vPPV serotypes that are not contained in any currently employed conjugate vaccine formulation. In the Northern Territory, vaccination with the 23vPPV in pregnancy remains attractive due to high carriage of several 23vPPV non-conjugate vaccine serotypes such as 15B, 33F and 10A. While maternal vaccine trials involving the more immunogenic pneumococcal conjugate vaccines may be an option for the future, herd immunity generated by high infant PCV coverage rates, may mean this strategy is of little added benefit.
CHAPTER 5

Vitamin D and Acute Lower Respiratory Infection: Literature Review
Chapter 5: Vitamin D and acute lower respiratory infection: literature review.

5.1 Significance

Vitamin D insufficiency has been associated with the risk of childhood ALRI in several international studies (Belderbos et al. 2011; Berry et al. 2011; Camargo et al. 2012; Camargo et al. 2011; Roth et al. 2010; Wayse et al. 2004) yet trials of vitamin D supplementation for the prevention of ALRI have been inconsistent (Bergman et al. 2013; Mao & Huang 2013). Despite the sunny climate of the Northern Territory, Indigenous populations have several risk factors for vitamin D insufficiency, such as pigmented skin. Vitamin D insufficiency may thus be related to the high burden of ALRI occurring among Indigenous children in the Northern Territory; however, to date very few data are available. Vitamin D insufficiency is modifiable by simple supplementation strategies.

5.2 What is role of vitamin D in the human body?

Approximately 80% of human vitamin D is generated endogenously following exposure of the skin to the ultraviolet radiation of sunlight (Figure 5.1). UVB radiation catalyses the production of vitamin D3 from 7-dehydrocholesterol which is subsequently circulated to and converted by the liver into the major circulating metabolite, 25-hydroxy vitamin D3 (25OHD3) (Hughes & Norton 2009; Lips 2006). 25-hydroxy vitamin D2 (25OHD2) is the plant-based vitamin D epimer used in commercial supplements and is only found in humans following consumption of these supplements. While 25OHD3 predominates, often 25OHD2 and 25OHD3 are
measured collectively and simply referred to as 25OHD, which reflects total body stores of vitamin D and is considered the best measure of vitamin D status (Vieth et al. 2007). The active hormonal form of vitamin D, 1, 25-dihydroxy vitamin D (1,25OH2D), is produced from the circulating 25OHD by the kidney, or locally when required, by cells such as those of the immune system (Hughes & Norton 2009). Active vitamin D is a gene regulator. When 1,25OH2D enters a target cell, it binds to the vitamin D receptor and forms a heterodimer complex with the retinoid receptor before binding to the response element of a vitamin D responsive gene to stimulate its expression (White 2008). Conventionally, 1,25OH2D is known for the up-regulation of calcium binding protein, enhancing calcium absorption in the gut, making it important for maintaining bone density (Lips 2006). However, vitamin D has numerous other functional/regulatory roles, some of which will be discussed in more detail.

5.3 What are the optimal levels of vitamin D?

The optimal level of circulating vitamin D is contentious. Previously vitamin D was considered sufficient at levels that prevented rickets; however, discovery of the requirement of vitamin D for prevention of diseases such as cancer, cardiovascular and infectious diseases has resulted in re-evaluation of these levels (Melamed et al. 2008). Several studies have highlighted an increased risk of respiratory infection for 25OHD3 levels <75 nmol/L (Belderbos et al. 2011; Bergman et al. 2013; Camargo et al. 2012) while a large survey of 13,000 adults in the USA suggested that vitamin D levels between 62.5 nmol/L and 120 nmol/L were associated with the lowest all-cause mortality rates (Melamed et al. 2008). The most widely accepted definitions of vitamin D status are:
- Vitamin D deficiency is a serum 25OHD concentration <50 nmol/L
- Vitamin D insufficiency is 50-74 nmol/L; and
- Vitamin D sufficiency is ≥75 nmol/L (Holick et al. 2011).

**Figure 5.1** Simplified representation of vitamin D synthesis and classical role in calcium regulation.

“Reproduced with permission from (Holick 2007), Copyright Massachusetts Medical Society”. 1 ng/ml = 2.5 nmol/L
In Australia the vitamin D insufficiency definition (<75 nmol/L) is not commonly utilised, there is no routine food fortification and no infant vitamin D screening or supplementation, unlike the United States of America, Canada and the United Kingdom. The Australian National Health and Medical Research Council guidelines advocate that pregnant women and young children receive reasonable sunlight exposure. To prevent deficiency in adults, recommendations are for direct sun exposure to the face or upper limbs on most days for 5-50 minutes, depending on skin tone and season and taking care to avoid sunburn (Paxton et al. 2013). Safe exposure levels for children are unknown and the general recommendation is simply to encourage outside physical activity (Paxton et al. 2013). Australian guidelines recommend infants and pregnant mothers with 25OHD between 30-50 nmol/L be supplemented with 400 international units (IU; 1 IU=25 ng)/day and that infants with 25OHD <30 nmol/L be supplemented with 1000 IU/day, for 3 months (Paxton et al. 2013). Some maternity hospitals in Australia are providing infants of vitamin D deficient mothers with a single 50,000 IU dose at delivery, followed by subsequent daily supplementation and re-checking of vitamin D status at 6 weeks; however, compliance with ongoing daily supplementation in these infants is unknown (Danchin 2014).

5.4 Risk factors for vitamin D insufficiency

As described in section 5.2, sunlight is the most important determinant of vitamin D levels. Modern lifestyle patterns such as extended periods watching television or working on computers, as well as the avoidance of sun exposure due to skin cancer concerns, increase the risk of vitamin D insufficiency (Kumar et al. 2009). As a result, infant vitamin D insufficiency is increasingly common and has become a
major public health concern. For pregnant women, the demand for vitamin D further increases to enhance calcium absorption. Population studies in Victoria, Australia, found that 66% of pregnant women were vitamin D insufficient (Teale & Cunningham 2010). Further, infant vitamin D status directly reflects that of their mother and breast fed infants are almost 6 times more likely to be vitamin D insufficient (risk ratio, 5.7; 95% CI, 2.7–10.2) than formula fed infants (Grant et al. 2009). Other risk factors for vitamin D insufficiency include wearing covering clothing (often for cultural reasons), obesity, southerly latitude and season, dark skin, premature birth, poor diet, socioeconomic status, female sex and medical conditions affecting vitamin D metabolism (Black et al. 2014; Paxton et al. 2013).

5.5 Vitamin D as an anti-infective agent

Prior to the discovery of antibiotics, infectious diseases were the most common cause of death around the world. During this time sunshine exposure and cod liver oil were recognized as effective therapies against tuberculosis infections (Grad 2004). These two therapies had one thing in common: vitamin D. Subsequently, vitamin D supplementation has been used successfully as therapy for tuberculosis (Khajavi & Amirhakimi 1977; Martineau et al. 2007; Salahuddin et al. 2013) and a strong association has been demonstrated between seasonal variations in vitamin D levels and the incidence of respiratory infections (Cannell et al. 2006; Grant 2008).

5.6 Vitamin D as an immune regulator

It was demonstrated in the 1980s that vitamin D stimulated the bactericidal activity of macrophages against *Mycobacterium tuberculosis* (Rook et al. 1986). Current literature suggests that vitamin D has a strong immunomodulatory role, affecting
both innate and adaptive immunity (Figure 5.2) (Mora, Iwata & von Andrian 2008). Pathogen activation of toll like receptors (TLRs) on innate immune cells such as monocytes, dendritic cells, macrophages, peripheral blood mononuclear cells and respiratory epithelial cells, in the presence of adequate circulating vitamin D, induces expression of the anti-microbial peptides cathelicidin and human β-defensin 2 (Liu et al. 2006). More specifically, TLR stimulation results in transcriptional induction of 1α-hydroxylase and the vitamin D receptor. 1α-hydroxylase subsequently converts circulating 25OHD into 1,25OH2D which couples with the vitamin D receptor to transcriptionally induce cathelicidin and human β-defensin 2 expression. 1,25OH2D also stimulates monocyte differentiation into macrophages (Krutzik et al. 2008), and promotes mechanisms associated with intracellular lysosomal encapsulation and degradation of pathogens, or autophagy (Gutierrez et al. 2004; Shin et al. 2010; Yuk et al. 2009). The antimicrobial activity of cathelicidin in combination with autophagy enhances intracellular killing of both bacteria and viruses (Liu & Modlin 2008; Liu et al. 2006). In 2009, Adams et al. demonstrated that cathelicidin expression in TLR induced monocyte cultures, was significantly enhanced by serum from patients supplemented with two doses of 50,000 IU 25OHD2 (plant-derived vitamin D) per week for five weeks (Adams et al. 2009). Additionally, monocytes cultured in vitamin D-deficient cord blood plasma (<30 nmol/L) exhibited decreased TLR-induced cathelicidin expression that was recovered with the addition of exogenous 25OHD (Walker et al. 2011). Interestingly, Leow et al. did not find an association between serum 25OHD levels and serum levels of cathelicidin or human β-defensin 2, suggesting a localised anti-microbial peptide response in vivo (Leow et al. 2011).
Figure 5.2 Immune and non-skeletal functions of vitamin D.

Vitamin D regulates innate and adaptive immune responses as well as several homeostatic processes including blood pressure and blood sugar control. “Reproduced with permission from (Holick 2007), Copyright Massachusetts Medical Society”.

Adaptive immune responses are broadly regulated by vitamin D (Figure 5.2). In vitro, 1,25OH2D inhibits the production of Th1 cytokines, particularly interferon gamma, suppressing T-cell proliferation and cytotoxicity while promoting Th2 activity via enhanced IL-4 production (and the suppression of interferon gamma). Further, 1,25OH2D is shown to inhibit in vitro Th17 cellular responses, early B-cell
proliferation and plasma cell differentiation while inducing the expansion of regulatory T-cells (Mora, Iwata & von Andrian 2008). In general 1,25OH2D switches a Th1 response towards a Th2 response potentially reducing inflammatory tissue damage that can occur with persistent infections or autoimmune diseases. The influence of vitamin D on the immune system is complex with few of the findings validated in human studies.

5.7 Vitamin D and vaccine responses

The strongest evidence regarding the influence of vitamin D on mucosal and systemic immune responses to vaccination comes from in vitro and animal studies. The addition of 1,25OH2D to vaccine preparations in animal studies induced increased immunity to influenza, herpes simplex virus, diphtheria toxoid, tetanus toxoid, hepatitis B surface antigen, poliovirus and HIVgp160 (Daynes et al. 1996; Kriesel et al. 1995; Mitchell, Ding & Baird 1996). While 1,25OH2D has been shown to blunt B-cell functions, despite the up regulation of a Th2 response, a study by Enioutina et al. demonstrated that vaccination with lipo-polysaccharide derived adjuvants induced local production of 1,25OH2D from circulating 25OHD, altering mature dendritic cell migration to both draining and non-draining lymphoid organs. This created a situation where B- and T-cells were able to mount both a systemic and mucosal immune response akin to that of an infection in peripheral or mucosal tissues (Enioutina, Bareyan & Daynes 2009). Furthermore, co-administration of 1,25OH2D with a trivalent influenza vaccine (TIV) in mice enhanced both mucosal and systemic antibody responses, and the animals’ ability to neutralize live influenza virus instilled in the nose (Daynes et al. 1996). While these data are supportive, human studies have been conflicting.
Several studies have investigated the influence of vitamin D and vaccine immune response in humans. In these studies, vitamin D status or supplementation has been associated with improved vaccine immune responses to influenza, hepatitis B and tetanus antigen (Chadha et al. 2011; Heine et al. 2011; Zitt et al. 2012); however, two studies were carried out among high-risk medical patients (Chadha et al. 2011; Zitt et al. 2012) and not all were supportive of an association (Principi et al. 2013; Sundaram et al. 2013). In a placebo-controlled randomised controlled trial of young adults, Heine et al. demonstrated that 10 weeks of vitamin D supplementation (2000 IU/day) prior to vaccination with tetanus toxoid significantly improved both vitamin D levels (80.3 nmol/L versus 29.1 nmol/L) and the tetanus toxoid-specific IgG response (Heine et al. 2011). Conversely, two studies have shown no effect of vitamin D on vaccine immune responses. Firstly, among 1100 healthy adults over 60 years of age, Sundaram et al. showed that baseline vitamin D levels had no consistent effect on sero-protective rates to a single dose of TIV (Sundaram et al. 2013). Secondly, in a recent randomised controlled trial, 3-year-old children were randomised to receive daily vitamin D (1000 IU) or placebo for four months. All of the children received 2 doses of TIV, 1 month apart, with supplementation beginning at the first TIV dose (Principi et al. 2013). Post-vaccine immunogenicity assays showed no difference in the sero-protective rate or haemagglutination inhibition assay (HIA) titres between the vitamin D and placebo groups. However, this study had several limitations: the trial was not specifically designed to evaluate the impact of vitamin D on TIV immune response with the primary outcome being recurrent otitis media; vitamin D was given simultaneously with the first TIV dose (biologically meaningful increases in vitamin D levels would have taken several
weeks); the study was only powered to detect a $\geq 25\%$ increase in sero-conversion in the vitamin D group compared to placebo due to small sample size; 1000 IU per day of vitamin D may have been inadequate for those who were deficient; and HIA titres were measured 3 months after the second dose rather than 1 month. Thus, there is considerable heterogeneity in the study findings. To date, studies have not targeted high-risk groups for vitamin D insufficiency, such as breast fed infants.

5.8 Vitamin D epidemiology

Vitamin D deficiency appears to be a worsening global issue, perhaps due to increasingly sedentary lifestyles. A large national survey in the United States of America showed declining vitamin D levels over a 16 year time period, and noted that African Americans returned the poorest levels throughout, suggesting that skin pigmentation is also a contributing factor (Ginde, Liu & Camargo 2009).

In 2011, a large national survey found that 73% of Australian adults over 25 years of age (n=11,247) had vitamin D levels $<75$ nmol/L (Daly et al. 2012). While the northern regions of Australia have sunshine hours and UV index well above the national average, population data on vitamin D status in this region are lacking. In one study from far North Queensland, only 7% of 116 women presenting for antenatal care were vitamin D insufficient ($<75$ nmol/L) (Bendall et al. 2012) whereas Daly et al. showed that during the summer and autumn months in the northern regions of Australia (latitude of $<30^\circ$S) over one third of males and almost two thirds of females had vitamin D levels $<75$ nmol/L (Daly et al. 2012). In southeast Queensland ($27^\circ$S), 41% and 15% of women were deficient ($<50$ nmol/L) in winter-spring and summer respectively (van der Mei et al. 2007). Based on
comparisons with more southerly regions, the authors demonstrated a 1 nmol/L decrease in serum vitamin D levels for every degree increase in latitude; however, season and latitude combined accounted for less than 20% of the variation in vitamin D highlighting the importance of the other common risk factors (van der Mei et al. 2007). These studies show that vitamin D insufficiency is common across Australia although perhaps less common in the sunnier northern regions as expected. Inconsistencies in the data suggest that region specific or population differences, testing bias or study design might also influence reported 25OHD3 levels.

5.9 Vitamin D levels among Indigenous Australians

Few studies have investigated vitamin D among Indigenous Australians (Appendix A: Search 7). A small case control study conducted in South Australia in 2006 showed that Aboriginal patients living with muscle pain had lower serum 25OHD levels than those without muscle pain (41 nmol/L versus 58 nmol/L; p=0.017) (Benson et al. 2006). More recently, Vanlint et al. demonstrated a mean serum 25OHD level of 57 nmol/L among another cross-section of urban Indigenous South Australians. In this study, sampling was evenly distributed across the seasons with lower vitamin D levels evident in winter. Overall, 62% (36/58) of participants had 25OHD levels below 60 nmol/L (Vanlint et al. 2011). Among 116 women (20% Indigenous) presenting for antenatal care in north Queensland, most had vitamin D levels above 100 nmol/L. Of the women with vitamin D levels below 100 nmol/L, Indigenous women (78 nmol/L; n=9) had lower median vitamin D levels than non-Indigenous women (89 nmol/L; n=27) (Bendall et al. 2012). A recent publication reported vitamin D data from a cohort of 592 Indigenous participants (aged 16 years and above) followed from 2007 to 2011 across the Northern Territory, north
Queensland and Western Australia. In this cohort, 31% had 25OHD <50 nmol/L and this was associated with a worse cardio-metabolic profile and an increased risk of diabetes compared to participants with 25OHD ≥50 nmol/L (Maple-Brown et al. 2014).

5.10 Vitamin D levels in Australian infants

Vitamin D levels below 75 nmol/L (insufficiency) have been shown in up to 76% of Australian neonates in winter and spring (Jones et al. 2012) and in Australia and New Zealand, independent of season, between 40-57% of neonates were shown to have 25OHD levels <50 nmol/L (Bowyer et al. 2009; Camargo et al. 2011; Grant et al. 2009). The search strategy for Indigenous Australians used in section 5.9 (Appendix A: Search 6) captured one childhood study. In the tropical climate of Darwin, a study by Dyson et al. showed that 22% (21/98) of children (43% Indigenous, median age 5 years) presenting to hospital, mostly as outpatients, had 25OHD <75 mol/L (Dyson et al. 2014)

5.11 Vitamin D and ALRI

5.11.1 Adults

A recent cohort study of 6789 British adults demonstrated significant seasonal variations in vitamin D levels with both inter- and intra-seasonal variations in vitamin D levels correlated with the prevalence of respiratory infections (Figure 5.3) (Berry et al. 2011; Hypponen & Power 2007). Berry et al. modelled that for each 10 nmol/L increase in 25OHD, the risk of respiratory infection was reduced by 7% (95%CI 3-11%) (Berry et al. 2011). In the USA, adults with 25OHD ≥95 nmol/L had a two-fold reduction in the risk of developing ALRI (p<0.001) compared to those
<95 nmol/L (Sabetta et al. 2010) while among adult patients hospitalised with community acquired pneumonia in New Zealand, those with severe 25OHD3 deficiency (<30 nmol/L) had a significantly higher 30 day mortality compared to those with 25OHD3 levels >50 nmol/L (OR 12.7, 95%CI 2.2-73.3, p=0.004) (Leow et al. 2011). These studies suggest that the protective effect of vitamin D against the risk of ALRI occurs across a broad range of vitamin D levels.

Figure 5.3 Prevalence of respiratory infections in the 3 weeks prior to measurement of serum 25OHD among 6789 British adults aged ≥45 years

Sourced from Berry et al. (Berry et al. 2011). Data are presented by season with 25OHD subcategories from darkest grey (<25 nmol/L) to lightest grey (>100 nmol/L). Respiratory infections were most common in winter when vitamin D levels were lowest; however, decreasing vitamin D levels were associated with increased risk of respiratory infection regardless of season (Berry et al. 2011).
5.11.2 Infants

The relationship between vitamin D insufficiency and ALRI is similarly shown among infants. Case-control studies from India, Turkey and Bangladesh each show that hospitalisation for ALRI is associated with low serum vitamin D metabolite levels (Karatekin et al. 2009; Roth et al. 2010; Wayse et al. 2004). In the Bangladesh study by Roth et al., conditional logistic regression showed that the odds of ALRI halved for each 10 nmol/L increase in circulating 25OHD3 (Roth et al. 2010). Five prospective birth cohort studies (Appendix A: Search 8) have shown that lower cord blood vitamin D levels are associated with the risk of respiratory infection (Belderbos et al. 2011; Camargo et al. 2011; Mohamed & Al-Shehri 2012; Shin et al. 2013). In the Netherlands, cord blood 25OHD3 levels were demonstrated to be lower in infants who developed RSV diagnosed ALRI within 12 months compared to controls (65 nmol/L versus 84 nmol/L; p=0.009) (Belderbos et al. 2011) and in New Zealand, lower cord blood 25OHD3 levels were associated with an increased risk of any respiratory infection by 3 months of age (odds ratio: 1.00 [reference] for ≥75 nmol/L, 1.39 for 25-74 nmol/L, and 2.16 for <25 nmol/L) (Camargo et al. 2011). Further, a study in Korea found that 90% (472/525) of cord bloods tested had vitamin D levels below 75 nmol/L, with descending cord blood vitamin D levels strongly associated with the risk of acute nasopharyngitis within the first 6 months of life (Shin et al. 2013). In this study, more cases of both otitis media and bronchiolitis were also identified with lower cord vitamin D levels suggestive of an association (Shin et al. 2013). Most recently, a German study (Luczynska et al. 2014) demonstrated that low cord blood vitamin D levels, <25 nmol/L compared to >50 nmol/L, were associated with an increased risk of ALRI within the first 12 months of life (Risk ratio 1.32; 95% CI 1.00-1.77). While there is increasing evidence to
suggest that neonatal vitamin D insufficiency (or deficiency) is associated with an increased risk of respiratory infection, not all studies support this association. Two Canadian studies found no difference in serum vitamin D levels between ALRI cases and controls (McNally et al. 2009; Roth et al. 2009).

Several international studies have demonstrated an inverse association between vitamin D levels and acute lower respiratory infection (ALRI) in children even in regions considered low risk due to abundant sunshine (Grant et al. 2009; Mohamed & Al-Shehri 2012; Roth et al. 2010; Wayse et al. 2004). The tropical climate in the Top End of the Northern Territory may not insulate Indigenous populations from vitamin D deficiency, and vitamin D levels among young children in this region are yet to be adequately characterised.

5.12 Vitamin D supplementation for the prevention of respiratory infection

In 2013, two independent systematic reviews scrutinised randomised controlled trials of vitamin D supplementation for the prevention of respiratory tract infections (Bergman et al. 2013; Mao & Huang 2013). These reviews came to opposing conclusions.

The systemic review by Mao and Huang included 7 trials and 4827 participants. Baseline age ranged from 1 month to 63 years, study dosage from 300 to 6800 IU/day, and the study duration from 1.75 to 18 months. The pooled relative risk of respiratory tract infection in subjects that received vitamin D supplementation compared to controls was 0.98 (95%CI 0.93-1.03) and the authors concluded that
routine vitamin D supplementation was not effective against respiratory tract infections (Mao & Huang 2013) (Figure 5.4). Conversely, Bergman et al. included eleven placebo-controlled studies in their meta-analysis of 5660 patients. The average age was 16 years, average dose of vitamin D was 1600 IU/day and average interval between doses was one day to three months. Vitamin D had a protective effect against respiratory tract infections with a pooled odds ratio of 0.64 (95%CI 0.49-0.84) (Bergman et al. 2013) (Figure 5.5).

Both trials were conducted using appropriate expertise and statistical rigour but subtle differences in methodology led to the conflicting outcomes. The review by Mao et al. used stricter inclusion criteria encompassing studies with healthy participants at baseline and with a Jadad score of >3 (Jadad et al. 1996) indicating the study methodology was of high quality. Bergman et al. on the other hand did not exclude pre-existing respiratory conditions, including asthma (Bergman et al. 2012; Majak et al. 2011) and pneumonia (Manaseki-Holland et al. 2010) and included two trials considered at considerable risk of bias (Aloia & Li-Ng 2007; Jorde et al. 2012) according to Cochrane Collaboration’s trial assessment tool, only one of which was included by Mao et al. Overall, the less stringent inclusions by Bergman et al. led to the inclusion of four extra trials; the shortcoming of which was greater heterogeneity among the data in the analysed studies. Attentively, Bergman et al. showed that vitamin D supplementation was efficacious against respiratory tract infections even without the inclusion of the two high-risk studies. As such, the major differences between reviews were the three studies where participants had pre-existing respiratory conditions and the weighting applied to each study included in the meta-analyses.
The three studies included in the Bergman et al. review where pre-existing respiratory conditions existed were: a trial of 124 adults with baseline asthma (meta-analysis weighting 8.1%) (Bergman et al. 2012), a small trial of 48 children with newly diagnosed asthma (meta-analysis weighting 3.2%) (Majak et al. 2011) and a trial of 453 children with baseline pneumonia (meta-analysis weighting 11.5%) (Manaseki-Holland et al. 2010). While together these trials contributed approximately 20% of the pooled outcome, influence analysis showed that neither could influence the outcome alone. Both systematic reviews gave the greatest weighting to the 2012 trial by Manaseki-Holland et al., a large randomised controlled trial of over 3000 children which showed no benefit of 3 monthly bolus doses of vitamin D (100,000 IU) against pneumonia incidence among children (1-11 months of age) in socioeconomically disadvantaged inner-city districts of Kabul where both pneumonia and vitamin D deficiency are common (Manaseki-Holland et al. 2012). Bergman et al. applied 15% weighting to this study based on the inverse of the standard error while Mao et al. applied 74% without describing the weighting methodology. While there are other differences between these reviews, for example the use of odd ratios (Bergman et al. 2013) compared to risk ratios (Mao & Huang 2013), this large difference stands out as the major reason for the opposing outcomes and is an important example of why detailed methodology is required to justify the outcome of such meta-analyses.
Figure 5.4  Systematic review of vitamin D supplementation for the prevention of respiratory infections, Mao 2013.

Reproduced with permission from the authors (Mao & Huang 2013). RR: relative risk.
Figure 5.5  Systematic review of vitamin D supplementation for the prevention of respiratory infections, Bergman 2013.

Reproduced with permission from the authors (Bergman et al. 2013). OR: odds ratio.
5.13 Vitamin D for the prevention of childhood respiratory infection

Six trials have considered vitamin D supplementation for the prevention of respiratory infections among paediatric populations (mean age <3 years), two considered in the aforementioned systematic review and four subsequent trials (Appendix A: Search 9). The blinded randomised placebo controlled trials from the systematic review were both conducted in Kabul, Afghanistan, and delivered 100,000 IU doses of vitamin D to infants; a single dose in the 2010 trial (Manaseki-Holland et al. 2010) and quarterly doses for 18 months in the 2012 trial (Manaseki-Holland et al. 2012). In the 2010 trial, infants had pneumonia at baseline and were followed regularly by paediatricians (<fortnightly) for 3 months. The single bolus of vitamin D resulted in fewer subsequent pneumonia episodes compared to controls (45% versus 58%; p=0.010) and a longer time to the event (Manaseki-Holland et al. 2010). In the 2012 trial, healthy infants received regular bolus doses of vitamin D for 18 months during which time they were also followed regularly (<fortnightly) by paediatricians during the supplementation period. There was no difference in the incidence of pneumonia episodes between supplemented infants and placebo controls (Risk Ratio 1.06; 95% CI 0.89 to 1.27) (Manaseki-Holland et al. 2012). In 2012, Choudhary & Gupta showed that 5 days of vitamin D supplementation (1000 IU/day <1 year of age and 200 IU/day >1 year of age) did not significantly improve the resolution time (vitamin D: 72 hours versus placebo: 64 hours) of children (<5 years) hospitalised with severe pneumonia (Choudhary & Gupta 2012). A trial by Goldring et al. examined maternal vitamin D supplementation for improvements in childhood respiratory health. In this randomised study, 800 IU/day of vitamin D from 27 weeks gestation until birth did not alter the odds of childhood ALRI or URTI to 3 years of
age, while, a single bolus dose of 200,000 IU at 27 weeks resulted in an increased adjusted odds of ALRI (Goldring et al. 2013). Although only 15% and 3% of children in the respective daily and bolus dose groups achieved vitamin D sufficiency, these data support previous observations that large bolus doses of vitamin D may have an immunosuppressive effect (Coussens et al. 2012; Khoo et al. 2011; Kimball et al. 2011). Further, ALRI was not the primary outcome of this trial, nor was it clear how ALRI diagnosis was determined. In a trial of vitamin D for the prevention of otitis media, four weeks of vitamin D supplementation (1000 IU/day) among children aged 1-5 years (n=116) restored 25OHD levels to over 75 nmol/L and was associated with fewer episodes of AOM over a 6 month follow-up period (45% versus 66%; p=0.030), particularly AOM without perforation (Marchisio et al. 2013). Recently in New Zealand, 260 mother/infant pairs were randomised to receive either: placebo/placebo (n=87), 1000 IU/400 IU vitamin D per day (n=87; low dose) or 2000 IU/800 IU per day (n=86; high dose) from 27 weeks gestation until infant age 6 months. High-dose vitamin D infants had fewer acute respiratory infections (primary care records) compared to placebo control infants (87% versus 99%; p=0.004) (Grant et al. 2014a).

These were well conducted trials, in terms of randomisation, blinding and follow-up making it difficult to interpret whether or not vitamin D is beneficial for preventing infant respiratory infections. Four of the studies employed clinical follow-up and investigated respiratory infection as the primary outcome; two were supportive (Manaseki-Holland et al. 2010; Marchisio et al. 2013) and two were not. Three studies enrolled children with pre-existing conditions or a history of pre-existing conditions, pneumonia (Choudhary & Gupta 2012; Manaseki-Holland et al. 2010)
and otitis media (Marchisio et al. 2013). Of these, only the trial by Choudhary & Gupta was not supportive but the study was of short duration, the children had severe pneumonia and the outcome was time to disease resolution. Vitamin D dose and dosing schedule was the factor most likely to determine outcome. While a single bolus dose of vitamin D proved able to reduce subsequent pneumonia episodes in the 2010 trial by Manaseki-Holland et al. (Manaseki-Holland et al. 2010), bolus dosing was unsuccessful for prevention of pneumonia in the 2012 trial by Manaseki-Holland (Manaseki-Holland et al. 2012) and the maternal supplementation trial by Goldring (Goldring et al. 2013). Where daily infant vitamin D doses of 800-1000 IU were used for four to six months (Grant et al. 2014a; Marchisio et al. 2013) the data are supportive of a benefit against respiratory infection. Concordant with this finding, subgroup analysis in the systematic review by Bergman et al. showed daily vitamin D dosage was significantly more effective than bolus dose supplementation against respiratory infection (Bergman et al. 2013). This subgroup analysis also suggested that baseline vitamin D status, pre-existing disease and age had no influence on the outcomes.

In summary, vitamin D insufficiency is associated with the risk of respiratory infection; however, the evidence that vitamin D supplementation prevents respiratory infection is inconsistent. Studies using daily infant vitamin D doses of 800 IU or higher for extended periods are promising. As vitamin D is theoretically necessary for optimal respiratory immunity, insufficiency is a potentially causal risk factor for infection. A better understanding of vitamin D’s regulatory role in both systemic and local respiratory immunity is essential to tease out potential pathogen specific effects and deeper consideration should be given to population-level differences, such as
vitamin D receptor genotypes, with respect to optimising dosage and dosing regimens.

5.14 Gaps in the knowledge

Sufficient vitamin D levels are required to maintain optimal respiratory immunity and provide resilience against a broad range of pathogens. Currently, little is known about the vitamin D status of Indigenous populations. Studies are needed to characterise the vitamin D status of Indigenous mothers and infants in northern Australia and to investigate whether insufficiency is associated with the risk of ALRI as have been shown in similar populations in India, Bangladesh and New Zealand.
CHAPTER 6

Cord blood vitamin D status and the risk of acute lower respiratory infection for Indigenous infants in the Northern Territory of Australia during their first year of life.
Chapter 6: Cord blood vitamin D status and the risk of acute lower respiratory infection for Indigenous infants in the Northern Territory of Australia during their first year of life.

6.1 Context

Globally it is estimated that one billion people have suboptimal vitamin D levels (<75 nmol/L) (Holick 2007). The discovery that vitamin D receptors are widely distributed throughout human tissues and that several cell types, such as those of the immune system, can synthesise the active vitamin D metabolite (1,25OH2D) from the vitamin D circulating metabolite (25OHD3) has prompted recently renewed interest into the role of vitamin D. Of particular interest to this chapter is the link between vitamin D and respiratory infections. Sunshine exposure is the most important factor influencing vitamin D status. As such, there has been little consideration of vitamin D and disease in the tropical north of Australia.

6.2 Abstract

Background

Several international studies have described that low cord blood vitamin D concentrations are associated with an increased risk of infant acute lower respiratory infection (ALRI). Indigenous infants in the Northern Territory of Australia have a high burden of ALRI yet their vitamin D status is unknown.
Methods
In a cohort of 109 mother-infant pairs we measured the vitamin D levels in available bloods from 33 mothers during pregnancy (30-36 weeks), 106 mothers at birth, 84 cord specimens (<72 hours of birth) and 37 infants at age 7 months. Infants underwent 12 months of follow-up for ALRI hospitalisations identified using International Classification of Diseases coding (ICD-10-AM; J09-J22 & A37-A37.9) recorded during admissions. We investigated the relationship between cord blood vitamin D levels and the risk of ALRI hospitalisation.

Results
There was a 48% relative difference in the 25OHD3 concentration between mothers at 30-36 weeks gestation and cord blood (104 nmol/L and 54 nmol/L respectively). At birth, 80% (67/84) of cord bloods had 25OHD3 levels <75 nmol/L. Mean cord blood 25OHD3 was 37 nmol/L among the 7 infants subsequently hospitalised for an ALRI compared to 56 nmol/L among the 77 infants who were not hospitalised with and ALRI (p=0.025).

Conclusions
Among this small cohort of Indigenous mothers and infants in the Northern Territory, sufficient maternal vitamin D during the third trimester of pregnancy did not ensure adequate vitamin D concentrations in cord blood. Lower cord blood vitamin D concentrations were found among infants subsequently hospitalised with an ALRI compared to those who were not. These data should be interpreted with caution due to the small number of ALRI outcomes.
6.3 Background

One fifth of Indigenous infants born in the Northern Territory of Australia are hospitalised with an acute lower respiratory infection (ALRI) in the first year of life (O'Grady, Torzillo & Chang 2010). An inverse relationship between cord blood vitamin D levels and infant respiratory infections has been described in several international studies (Belderbos et al. 2011; Camargo et al. 2011; Mohamed & Al-Shehri 2012; Shin et al. 2013) yet there is a paucity of vitamin D data in this region. We hypothesised that vitamin D levels below 75 nmol/L would be common among Indigenous mothers and infants in the Top End of the Northern Territory despite the tropical climate and be associated with the risk of infant ALRI hospitalisation.

As described in Chapter 5.2, vitamin D is produced endogenously in the skin following exposure to sunlight. Subsequent hydroxylation by the liver yields the dominant circulating vitamin D metabolite, 25OHD3. The less common metabolite, 25OHD2, is generally only present following consumption of supplements containing vitamin D synthesized by fungi and yeast. Together, circulating 25OHD3 and 25OHD2 are referred to as 25OHD. Generally, vitamin D deficiency is defined by serum 25OHD concentrations <50 nmol/L and vitamin D insufficiency as 50-75 nmol/L (Holick et al. 2011; Vieth 2011), yet in Australia the insufficiency definition is not recognised and levels ≥50nmol are considered sufficient (Paxton et al. 2013). National population surveys demonstrate a variable prevalence of 25OHD <50 nmol/L during pregnancy, from 10% among women in southeast Queensland (McLeod et al. 2011) to over 80% of dark skinned and/or veiled women in Melbourne, Victoria (Grover & Morley 2001) yet little information exists regarding the vitamin D status of expectant Indigenous mothers. One small study of pregnant
women (non-Indigenous n=93, Indigenous n=23) in far north Queensland screened for vitamin D mid-gestation and found little evidence of insufficiency <75 nmol/L (7%; 8/116) (Bendall et al. 2012). While little is known about the vitamin D status of pregnant Indigenous women, dark skin is a risk factor for deficiency and/or insufficiency (Paxton et al. 2013) and our recent data showed that around 40% of hospitalised Indigenous infants (median age 7 months) in the Northern Territory had 25OHD3 levels <75 nmol/L (Binks et al. 2014).

Vitamin D is required for innate (antimicrobial peptide production) and adaptive immune responses (favours Th2 response) (Mora, Iwata & von Andrian 2008) which may be particularly important in the developing infant and explain the demonstrated relationship between cord blood vitamin D and the risk of respiratory infection (Belderbos et al. 2011; Camargo et al. 2011; Mohamed & Al-Shehri 2012; Shin et al. 2013).

The aims of this study were to describe the relationship between vitamin D levels of Indigenous mothers and infants and to investigate whether low vitamin D levels at birth (represented by the cord blood vitamin D metabolite, 25OHD3) were associated with an increased risk of infant ALRI hospitalisation in the first year of life.

6.4 Methods

6.4.1 Participants and study design

Using available data from participants in the PneuMum randomised controlled trial of maternal pneumococcal vaccination (Chapter 3), we established a cohort of 109 Northern Territory Indigenous mothers-infants pairs who were recruited from 2006
to 2011 and followed over several visits from the third trimester of pregnancy until infant age 7 months. In this cohort, blood was available from 33 mothers during pregnancy (30-36 weeks), 106 mothers at birth, 84 cord specimens (<72 hours of birth) and 37 infants at age 7 months. We measured vitamin D levels in each of these blood samples to establish the vitamin D status of the cohort, to describe the temporal trends in vitamin D over the birth period, and to establish the exposure of interest (cord blood vitamin D status) prior to ascertaining the primary outcome, ALRI hospitalisation before 12 months of age. To examine the association between vitamin D and ALRI, we compared the cord blood 25OHD3 concentration of infants that were subsequently hospitalised with an ALRI (<12 months of age) against the concentrations of those infants that were not hospitalised with an ALRI.

6.4.2 Vitamin D measurements

Serum 25OHD3 was measured using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) as previously described (Binks et al. 2014; Maunsell, Wright & Rainbow 2005). Low, medium and high commercial controls (UTAK, Australia) were used to monitor assay precision and sample identity was concealed during testing. We considered 25OHD3 levels <75 nmol/L as insufficient and <50 nmol/L as deficient.

6.4.3 Identifying ALRI hospitalisations, the primary outcome.

As described in Chapter 4.4.3, infant ALRI hospitalisations (<12 months of age) were identified by International Classification of Diseases coding (ICD-10-AM; J09-J22 & A37-A37.9) recorded during admissions to Royal Darwin or Alice Springs hospital, the major hospitals servicing the study area (National Centre for
Classification in Health 2009). Diagnoses made during the birth admission (Z37.0-Z39.2) or related admissions within 7 days were excluded from analysis.

6.4.4 Analysis

Vitamin D levels were described firstly for the available samples at each time point and subsequently for matched bloods taken during pregnancy and at birth to monitor the intra-participant trends. Participant characteristics were described according to cord blood vitamin D categories (<50 nmol/L, 50-74 nmol/L, >75 nmol/L) to investigate potential confounders the exposure. Fishers exact test (proportional data) and the Kruskal Wallis test (continuous data) were used to assess trends. The primary analysis was a comparison of the cord blood vitamin D levels of those infants who were subsequently hospitalised with an ALRI and those who were not. The Student’s t-test was used to compare the continuous vitamin D data (which were normally distributed) and two-tailed p-values <0.05 were considered statistically significant. With 84 cord blood samples, a broadly assumptive mean cord blood 25OHD3 concentration of between 50-75 nmol/L (standard deviation 30nmol/l) for healthy infants and the expectation that 20% of infants would be hospitalised with an ALRI (O’Grady, Torzillo & Chang 2010), we had the power to detect a difference in 25OHD3 concentration of approximately 20 nmol/L among the expected fraction of ALRI hospitalised infants.

6.4.5 Ethics

The study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC
Written consent was obtained for access to each child’s medical records and use of their blood samples.

6.5 Results

6.5.1 Participant characteristics

In general, participant characteristics were similar across the cord blood 25OHD3 categories (Table 6.1), however, remote dwelling was associated with lower cord blood 25OHD3 levels while maternal smoking prevalence at 1 month was incongruous across the categories and unlikely to be of importance. The median age of mothers at recruitment was 24 years. Over one third reported smoking during pregnancy and the uptake of the influenza vaccine during pregnancy was low (14%). Most infants (91%) had received 3 doses of the pneumococcal conjugate vaccine (7vPCV or PHID-10CV) by 12 months of age.

6.5.2 Validity of vitamin D assay

The ID-LC-MS/MS vitamin D assay fulfilled the quality control acceptance criteria. In triplicate, the respective low, medium and high commercial controls yielded data with a coefficient of variance of 3.7%, 2.4% and 4.5% and an accuracy of 97%, 99% and 104% across 6 runs. This data was within 3 standard deviations of the historical data for the corresponding quality control lot number.
Table 6.1  Participant characteristics by cord blood 25OHD3 status.

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>n=84</th>
<th>&lt;50</th>
<th>50 - 74</th>
<th>≥75</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood 25OHD (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median maternal age (range), years</td>
<td>24 (17-37)</td>
<td>25 (17-37)</td>
<td>23 (17-33)</td>
<td>26 (17-33)</td>
<td>0.197</td>
</tr>
<tr>
<td>Household occupancy (range), people</td>
<td>4 (1-11)</td>
<td>5 (2-11)</td>
<td>4 (1-11)</td>
<td>4 (3-10)</td>
<td>0.117</td>
</tr>
<tr>
<td>Remote community residence</td>
<td>14 (17)</td>
<td>12 (32)</td>
<td>2 (7)</td>
<td>0 (0)</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Smoker</td>
<td>36 (43)</td>
<td>19 (51)</td>
<td>8 (27)</td>
<td>9 (53)</td>
<td>0.086</td>
</tr>
<tr>
<td>Influenza vaccine in pregnancy</td>
<td>12 (14)</td>
<td>6 (16)</td>
<td>5 (17)</td>
<td>1 (6)</td>
<td>0.613</td>
</tr>
<tr>
<td><strong>Infant characteristics at birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (56)</td>
<td>20 (54)</td>
<td>17 (57)</td>
<td>10 (59)</td>
<td>0.960</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500 grams)</td>
<td>2 (2)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1 (6)</td>
<td>0.491</td>
</tr>
<tr>
<td>Premature (&lt;37 weeks)</td>
<td>1 (1)</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Special or intensive care admission</td>
<td>9 (11)</td>
<td>4 (11)</td>
<td>4 (13)</td>
<td>4 (6)</td>
<td>0.901</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exclusively breast fed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>48 (47)</td>
<td>18 (49)</td>
<td>18 (60)</td>
<td>12 (71)</td>
<td>0.290</td>
</tr>
<tr>
<td>2 months</td>
<td>34 (40)</td>
<td>14 (38)</td>
<td>12 (40)</td>
<td>8 (47)</td>
<td>0.807</td>
</tr>
<tr>
<td>7 months</td>
<td>31 (37)</td>
<td>16 (43)</td>
<td>9 (30)</td>
<td>6 (35)</td>
<td>0.536</td>
</tr>
<tr>
<td>Mother smoking*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 month</td>
<td>35 (46)</td>
<td>19 (61)</td>
<td>7 (25)</td>
<td>9 (53)</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>2 months</td>
<td>31 (45)</td>
<td>15 (56)</td>
<td>9 (32)</td>
<td>7 (50)</td>
<td>0.196</td>
</tr>
<tr>
<td>7 months</td>
<td>38 (55)</td>
<td>17 (57)</td>
<td>13 (50)</td>
<td>8 (62)</td>
<td>0.856</td>
</tr>
<tr>
<td>2 doses of PCV*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>by 7 months</td>
<td>57 (68)</td>
<td>25 (68)</td>
<td>22 (73)</td>
<td>10 (59)</td>
<td>0.595</td>
</tr>
<tr>
<td>3 doses of PCV*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>by 12 months</td>
<td>68 (91)</td>
<td>31 (89)</td>
<td>24 (96)</td>
<td>13 (87)</td>
<td>0.591</td>
</tr>
<tr>
<td>23vPPV in pregnancy</td>
<td>24 (29)</td>
<td>8 (22)</td>
<td>9 (30)</td>
<td>7 (41)</td>
<td>0.322</td>
</tr>
<tr>
<td>23vPPV at birth</td>
<td>29 (35)</td>
<td>11 (30)</td>
<td>14 (47)</td>
<td>4 (24)</td>
<td>0.221</td>
</tr>
</tbody>
</table>

*Smoking prevalence data were unavailable for 8 mothers at 1 month and 15 mothers at 2 and 7 months post-partum. #PCV includes the 7-valent pneumococcal conjugate vaccine (7vPCV) or the 10-valent pneumococcal *Haemophilus influenzae* protein D-conjugate vaccine (10vPHID-CV). p (p-value) for trend was calculated using the Fisher’s exact test for proportional data and the Kruskal Wallis test for continuous data.
6.5.3 Vitamin D levels

Among mothers, the prevalence of vitamin D insufficiency or deficiency (25OHD3 <75 nmol/L) (Table 6.2) was 21% (7/33) in maternal venous blood at 30-36 weeks gestation (median gestation 32 weeks, range 28-36 weeks) and 45% (48/106) at birth (median gestation 39 weeks, range 34-41 weeks). In cord blood, the prevalence of 25OHD3 <75 nmol/L was 80% (67/84) (median gestation 39 weeks, range 36-41 weeks) with 10% (8/84) <25 nmol/L. Among infants at the 7 month visit (median age 7.1 months, range 6.6-8.1 months), the prevalence of 25OHD3 <75 nmol/L in venous blood was 22% (8/37).

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Visit</th>
<th>n</th>
<th>mean (95%CI)</th>
<th>RD (%)</th>
<th>25OHD3 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;50 n (%)</td>
</tr>
<tr>
<td>Maternal</td>
<td>Pregnancy</td>
<td>33</td>
<td>104 (93-115)</td>
<td>base</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Maternal</td>
<td>Birth</td>
<td>106</td>
<td>80 (74-86)</td>
<td>-23</td>
<td>18 (17)</td>
</tr>
<tr>
<td>Cord</td>
<td>Birth</td>
<td>84</td>
<td>54 (50-59)</td>
<td>-48</td>
<td>37 (44)</td>
</tr>
<tr>
<td>Infant</td>
<td>7 months</td>
<td>37</td>
<td>93 (86-101)</td>
<td>-10</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

**RD:** relative difference in vitamin D concentrations compared with maternal vitamin D during pregnancy (1 minus the vitamin D ratio). Pregnancy: 30-36 weeks gestation.
Considering all samples (unmatched), the relative difference in mean 25OHD3 concentrations (Table 6.2; Figure 6.1A) was 23% between maternal venous bloods at 30-36 weeks and birth (104 nmol/L and 80 nmol/L respectively) and 33% between maternal venous and cord bloods at birth (80 nmol/L and 54 nmol/L respectively). Overall, there was a 48% relative difference in the 25OHD3 concentration between mothers at 30-36 weeks gestation and cord blood (104 nmol/L and 54 nmol/L respectively).

This trend in relative difference is similarly followed among matched samples. There were 32 matched maternal venous bloods at 30-36 weeks gestation and at birth and the relative difference in 25OHD3 concentrations was 18% (104 nmol/L and 84 nmol/L respectively), while there were 81 matched maternal venous and cord bloods at birth and the relative difference in 25OHD3 levels was 33% (81 nmol/L and 54 nmol/L respectively). Overall, there was a 44% relative difference in 25OHD3 levels between the 22 matched maternal venous bloods at 30-36 weeks gestation and cord blood (106 nmol/L to 59 nmol/L respectively). The median time difference between collection of the matched maternal bloods in the third trimester and at birth was 6.4 weeks (range 1.3-11).

At birth, the 25OHD3 concentrations of the 81 matched maternal venous and cord blood samples exhibited a linear correlation \( r=0.84; \ p<0.001 \) (Figure 6.1B).
Figure 6.1 Maternal and infant vitamin D levels.

A: Vitamin D levels were measured in pregnancy (30-36 weeks gestation; n=33), at birth (maternal venous, n=106; cord, n=84) and at infant age 7 months (n=37). Dashed lines indicate 50 nmol/L (deficiency) and 75 nmol/L (insufficiency) 25OHD3 reference values.

B: Correlation between maternal venous and cord blood vitamin D levels at birth. r: Pearson’s correlation coefficient. n: number of matched maternal and infant bloods.
6.5.4 Vitamin D level among urban and remote participants

Mean vitamin D levels were lower among remote compared to urban dwelling participants during pregnancy, at birth and at infant age 7 months (Table 6.3). The relative difference between maternal vitamin D levels at 30-36 weeks gestation and cord blood vitamin D levels was 57% (87 nmol/L and 37 nmol/L respectively) among remote participants compared to 46% (108 nmol/L and 58 nmol/L respectively) among urban participants. All (100%; 14/14) cord blood 25OHD3 concentrations of remote infants were <75 nmol/L and most (86%; 12/14) were <50 nmol/L.

Table 6.3 Vitamin D among urban and remote dwelling participants.

<table>
<thead>
<tr>
<th>Blood sample</th>
<th>Visit</th>
<th>Urban</th>
<th>Remote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean (95%CI)</td>
<td>n</td>
</tr>
<tr>
<td>Maternal</td>
<td>Pregnancy</td>
<td>26 108 (95-122) base</td>
<td>7 87 (68-107) base</td>
</tr>
<tr>
<td>Maternal</td>
<td>Birth</td>
<td>85 86 (79-92) -23</td>
<td>21 57 (49-66) -34</td>
</tr>
<tr>
<td>Cord</td>
<td>Birth</td>
<td>70 58 (53-63) -46</td>
<td>14 37 (30-43) -57</td>
</tr>
<tr>
<td>Infant</td>
<td>7 months</td>
<td>33 94 (86-101) -13</td>
<td>4 90 (56-124) +3</td>
</tr>
</tbody>
</table>

RD: relative difference in vitamin D concentrations compared with maternal vitamin D during pregnancy (1 minus the vitamin D ratio). Pregnancy: 30-36 weeks gestation.

6.5.5 Vitamin D levels according to ALRI hospitalisation outcome.

Maternal venous and cord blood vitamin D levels at birth were lower among infants hospitalised with an ALRI compared to those who were not hospitalised with an ALRI in the first 12 months of life (Figure 6.2).
**Figure 6.2** Vitamin D levels in pregnancy, birth and at infant age 7 months according to ALRI hospitalisation outcome.

Note: Only 1 maternal vitamin D measurement during pregnancy (30-36 weeks); was related to an infant ALRI hospitalisation; hence no confidence interval for the open triangle. Only 2 infants with vitamin D measurements at 7 months were hospitalised with an ALRI; hence, the upper and lower confidence boundaries around the open triangle were wide (exceeding the graph scale) as indicated by the arrows.
Among the 84 infants with a cord blood sample, the period prevalence of the primary outcome, ALRI hospitalisation during the first 12 months, was 8% (7/84) and all of these infants had cord blood 25OHD3 <75 nmol/L. In our primary analysis (Figure 6.2), mean cord blood 25OHD3 was 37 nmol/L among the 7 infants subsequently hospitalised for an ALRI compared to 56 nmol/L among the 77 infants not hospitalised for an ALRI (p=0.025). In a sensitivity analysis, the mean cord blood 25OHD3 concentration of the 7 infants hospitalised with an ALRI was 37 nmol/L compared to 56 nmol/L among the 65 infants who were not hospitalised for any reason (p=0.022).

Among infants of the 106 mothers with a venous blood sample at birth, the period prevalence of ALRI hospitalisation during the first 12 months was 8% (8/106) and 7 of the 8 had mothers with venous blood 25OHD3 <75 nmol/L at birth. In our secondary analysis (Figure 6.2), mean maternal blood 25OHD3 was 61 nmol/L among mothers of 8 infants subsequently hospitalised for an ALRI compared to 82 nmol/L among the 98 infants not hospitalised for an ALRI (p=0.066). In a sensitivity analysis, the mean 25OHD3 concentration of the 8 mothers of infants hospitalised with an ALRI was 61 nmol/L compared to 83 nmol/L among the 82 infants who were not hospitalised for any reason (p=0.052).

6.5.6 ALRI among remote dwelling infants.

Compared to urban infants, remote infants had proportionally more ALRI hospitalisations (3/70; 4% versus 4/14; 29%; p=0.013). The number of infant ALRI hospitalisations (i.e. n=7) were insufficient to allow for a model including remote dwelling as a confounder (Peduzzi et al. 1996).
6.6 Discussion

This is the first study to longitudinally describe vitamin D among pregnant Indigenous mothers and their infant offspring. We found that vitamin D concentrations in cord blood were approximately half (48%) that of maternal blood at 30-36 weeks gestation, and that cord blood vitamin D levels were lower among infants who were subsequently hospitalised with an ALRI compared to those infants who were not (37 nmol/L versus 56 nmol/L; \( p=0.025 \)). The comparison of cord blood vitamin D levels by ALRI hospitalisation outcome should be interpreted with caution due the small number of events (n=7) and inability to investigate remote dwelling as a confounder. Replete vitamin D concentrations among Indigenous mothers during the third trimester of pregnancy may not insulate against neonatal vitamin D insufficiency and an increased risk of ALRI.

6.6.1 Maternal vitamin D during pregnancy

Physiologic changes in vitamin D metabolism occur during pregnancy to support the increased calcium demands of the foetus, though the specific mechanisms are not fully understood. Vitamin D binding protein and the active vitamin D metabolite, 1,25OH2D, are shown to increase steadily during pregnancy, while concentrations of 25OHD3 are generally reported to remain stable (Brannon & Picciano 2011; Papapetrou 2010). Among the participants in our study, 25OHD3 concentrations fell both late in pregnancy (104 nmol/L to 80 nmol/L; -23%) and across the placenta (80 nmol/L to 54 nmol/L; -33%). The observed differential in 25OHD3 concentrations between venous and cord blood at birth is consistent with other studies (Waiters, Godel & Basu 1999); however, few studies have specifically characterised 25OHD3 late in pregnancy. In 2003, a small study of 20 Hungarian women showed no
difference in maternal 25OHD3 concentrations between 22-24 weeks gestation and birth (More et al. 2003), while in France a study of 14 healthy women that showed a 21% decline in 25OHD3 levels between 36 weeks gestation and birth (Salle et al. 2000). The latter data are consistent with our findings and suggest that a supplementation strategy beginning in the third trimester of pregnancy may be required to prevent neonatal vitamin D insufficiency. With little seasonal 25OHD3 variation in the tropical Northern Territory (Binks et al. 2014)(Chapter 7), the drop in late pregnancy may be a consequence of increased demand by the growing foetus or the emergence of risk factors (Paxton et al. 2013) and increased body mass index or more time spent indoors. We did not measure post-partum maternal 25OHD3 levels but they are also important. Breast fed infants rely almost exclusively on maternal vitamin D and are almost 6 times more likely to have vitamin D levels <75 nmol/L (risk ratio, 5.7; 95% CI, 2.7–10.2) than formula fed infants (Grant et al. 2009). Approximately half of the Indigenous infants in our study were exclusively breast fed at 1 month of age.

6.6.2 Infant vitamin D from birth

Our cord blood vitamin D data suggest that four out of the five Indigenous study infants were born with vitamin D insufficiency (<75 nmol/L) and one in ten were severely deficient (<25 nmol/L). Vitamin D status was largely restored by infant age 7 months, the next sampling point, where 25OHD3 levels climbed to 93 nmol/L and only 21% had vitamin D insufficiency. Concordant with our data, Grant et al. showed that non-supplemented New Zealand infants born with a mean cord blood 25OHD3 of 33 nmol/L, had a steady increase in 25OHD3 from birth (sampled at 2, 4 and 6 months) and reached sufficiency (≥75 nmol/L) by 4 months of age (Grant et al. 2009).
2014b). This is one of the few studies to document the natural history of 25OHD3 from pregnancy through to infancy in an otherwise healthy population, albeit as the placebo group of a randomised controlled trial. In this trial (Grant et al. 2014b), compliant maternal (1000 IU/day from 27 weeks until birth) and infant (400 IU/day from birth until 6 months) supplementation strategies improved cord blood 25OHD3 to 48 nmol/L compared to 33 nmol/L among placebo infants and maintained infant vitamin D sufficiency at each of the 2, 4 and 6 months sampling points.

6.6.3 Other considerations

Analysis of the matched samples in our study showed vitamin D levels of a similar magnitude and trend across the sampling points suggesting that our unmatched dataset was representative of a complete set of mother-infant pairs. Further we were cognisant of the potential influence of remote dwelling on vitamin D. The general trends in vitamin D concentration were comparable in both urban and remote participants. However, remote dwelling mothers had lower 25OHD3 during pregnancy and at birth (21-29 nmol/L) and the relative difference concentrations between 30-36 weeks gestation and cord blood was greater than among their urban counterparts (-57% remote compared to -48% urban). As the climate and time spent outdoors are likely to be similar between the urban and remote participants, factors, other than exposure to sunlight, are most likely responsible for this difference. This requires further investigation.

6.6.4 Vitamin D and ALRI hospitalisations

Overall, there were fewer infant ALRI hospitalisations in the first 12 months of life (8%; 7/84) than historically predicted in this region (22% (O'Grady, Torzillo &
Regardless, cord blood 25OHD3 levels were lower among infants that were hospitalised with an ALRI compared to those that were not (Figure 6.2). While we could not reliably perform logistic regression or subgroup analysis to account for remote dwelling as a confounder due to the low number of ALRI hospitalisations, these unadjusted findings are consistent with several other studies. In the Netherlands, cord blood 25OHD3 levels were lower in infants who developed RSV diagnosed ALRI within 12 months compared to controls (65 nmol/L versus 84 nmol/L; p=0.009) (Belderbos et al. 2011) while in New Zealand, lower cord blood 25OHD3 levels were associated with an increased risk of any respiratory infection by 3 months of age (odds ratio: 1.00 [reference] for ≥75 nmol/L, 1.39 for 25-74 nmol/L, and 2.16 for <25 nmol/L) (Camargo et al. 2011). In a recent Korean study, 90% (472/525) of cord bloods tested had vitamin D levels below 75 nmol/L, with descending cord blood vitamin D levels strongly associated with the risk of acute nasopharyngitis within the first 6 months of life (Shin et al. 2013). Despite the growing evidence, not all studies support an association between vitamin D insufficiency and ALRI (Roth et al. 2009).

6.7 Conclusions

Cord blood vitamin D insufficiency was common (80%) among Indigenous mother-infant pairs in the Northern Territory with concentrations half that of maternal venous vitamin D during the third trimester of pregnancy. Infants that were hospitalised with an ALRI during the first 12 months of life had lower mean cord blood vitamin D levels than those not hospitalised with an ALRI but larger studies are needed to confirm these findings. Supplementation strategies may be necessary to maintain optimal vitamin D levels among Indigenous infants in the Northern
Territory and reduce the risk of ALRI. These data are essential to leverage funding for future longitudinal studies of vitamin in this region or to support randomised controlled trials of vitamin D supplementation for the prevention of infant ALRI. The recently published New Zealand trial by Grant et al. showing that daily vitamin D supplementation during the third trimester of pregnancy and early infancy reduced primary care respiratory infection visits is an ideal model for future prospective trials though compliance with a daily regime is a potential limitation (Grant et al. 2015).
CHAPTER 7

Vitamin D insufficiency among hospitalised children in the Northern Territory
Chapter 7: Vitamin D insufficiency among hospitalised children in the Northern Territory

7.1 Context

This chapter reports vitamin D data from a cross-sectional sample of hospitalised children in the Northern Territory. At the time this study was conducted, no local vitamin D data were available and the outcomes were used to obtain funding for the longitudinal investigation of vitamin D levels among pregnancy Indigenous mothers and their infant offspring presented in Chapter 6. This work was recently published in the Journal of Paediatrics and Child Health (Binks et al. 2014).

7.2 Abstract

Introduction

ALRI’s are the most common reason for hospitalisation of young children in the Northern Territory of Australia. International studies have linked vitamin D deficiency with increased risk of ALRI in paediatric populations but this has not been explored in tropical regions such as the Top End of the Northern Territory.

Aim

To determine the prevalence of vitamin D insufficiency amongst children hospitalised with ALRI in the Northern Territory.

Methods
Vitamin D serum metabolite (25OHD3) levels were retrospectively measured using liquid chromatography–mass spectrometry in 74 children (64% Male; 57% Indigenous) aged less than 3 years admitted to Royal Darwin Hospital in the Northern Territory of Australia between May 2008 and May 2010.

**Results**

There were 44 (59%) ALRI classified hospitalisations and 30 (41%) non-ALRI classified hospitalisations. The most common ALRI diagnoses were bronchiolitis (n=22, 30%) and pneumonia (n=21, 28%) whilst the most common non-ALRI diagnosis was gastroenteritis (n=20, 27%). Overall, 24/74 (32%) children had 25OHD3 levels <75 nmol/L (insufficiency). For children hospitalised with ALRI, 23% (10/44) had vitamin D insufficiency compared with 47% (14/30) among children hospitalised for other reasons (OR (odds ratio) 0.34, 95%CI 0.11 to 1.03; p=0.043). Twelve of the 20 (60%) children hospitalised for gastroenteritis had vitamin D insufficiency.

**Conclusions**

Vitamin D insufficiency was observed in almost one third of these hospitalised children. Children hospitalised with an ALRI were less likely to have vitamin D insufficiency compared to children hospitalised for other conditions (predominantly gastroenteritis).
7.3 Introduction

Respiratory diseases are a significant health problem in the Northern Territory representing the largest cause of preventable mortality in infants (Li et al. 2007). Between 1999 and 2004, over 22% of Indigenous infants were hospitalised at least once with an ALRI before 12 months of age (O’Grady, Torzillo & Chang 2010). High rates of bronchiectasis among this population are mostly related to recurrent ALRI (Chang et al. 2002).

Several studies have demonstrated an inverse association between vitamin D levels and ALRI in children even in regions with abundant sunshine (Roth et al. 2010; Wayse et al. 2004). In Bangladesh where infants (1-18 months) have serum vitamin D metabolite (25OHD3) levels <50 nmol/L despite abundant year round sunshine (Roth et al. 2010), conditional logistic regression showed the odds of ALRI hospitalisation among children halved for each 10 nmol/L increase in 25OHD3. In the cooler, seasonal location of New Zealand, cord blood 25OHD3 levels below 75 nmol/L were associated with a higher risk of respiratory infection at 3 months of age (Camargo et al. 2011). More recently, a randomised controlled trial showed that daily ingestion of vitamin D fortified milk (300 IU) reduced the risk of acute respiratory infections in Mongolian children with deficient baseline 25OHD3 levels (Camargo et al. 2012). Not all studies support a role for vitamin D in preventing respiratory infection. A large randomised controlled trial (n=3046) showed no benefit of 3 monthly bolus doses of vitamin D (100, 000 IU) against pneumonia incidence among children (1-11 months of age) in inner-city districts of Kabul where both pneumonia and vitamin D deficiency are common (Manaseki-Holland et al. 2012).
There is ongoing controversy about which vitamin D cut-offs should be used. In the United States serum 25OHD3 levels below 50 nmol/L are considered deficient, 50-74 nmol/L as insufficient and ≥75 nmol/L as optimal (Vieth 2011), whereas in Australia and the United Kingdom levels above 50 nmol/L are generally considered adequate (Paxton et al. 2013; Pearce & Cheetham 2010). The evidence does suggest however, that an increased risk of respiratory infection exists for 25OHD3 levels up to 75 nmol/L (Belderbos et al. 2011; Bergman et al. 2013; Camargo et al. 2011). Known risk factors for vitamin D deficiency include obesity, premature birth, pigmented skin, low sun exposure or southerly latitude, and malabsorption (Paxton et al. 2013). Breast fed infants of vitamin D deficient mothers may also be at increased risk of deficiency or insufficiency (Liang et al. 2010).

Despite the respiratory disease burden among Australian children in the Northern Territory, there are no published data relating vitamin D status to ALRI in this setting. We describe 25OHD3 levels among 74 children (<3 years old) who were hospitalised in the Northern Territory with an episode of ALRI or for other conditions.

### 7.4 Methods

#### 7.4.1 Design and Setting

Cross-sectional study of a convenience sample of children aged <3 years who were admitted to Royal Darwin Hospital, Northern Territory of Australia, from May 2008 - May 2010.
Seventy four children hospitalised with respiratory or other illnesses had sufficient stored blood collected (≥100µl at -80°C) for vitamin D testing. Testing was performed following approval by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC 07/33).

7.4.2 Hospitalisation diagnosis

We investigated all diagnostic ICD-10-AM codes (National Centre for Classification in Health 2009) recorded in the Northern Territory hospital discharge dataset at Royal Darwin Hospital for the episode of hospitalisation corresponding with each serum sample. All 74 samples were from first hospitalisation episodes. ICD-10-AM codes J09-J18.9, J20-J22 or A37.0 were used to define ALRI.

7.4.3 Serum vitamin D measurements

Serum 25OHD3 levels below 75 nmol/L were used to define vitamin D insufficiency (Vieth 2011). Vitamin D assays were performed by Royal Melbourne Institute of Technology Drug Discovery Technologies Pty Ltd (Melbourne, Australia) in January 2011. Levels of 25OHD3 were measured in 100 µl of serum using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) as previously published (Maunsell, Wright & Rainbow 2005). Assay precision was determined using low, medium and high commercial controls (UTAK, Australia) and sample identification was concealed during testing.
7.4.4 Statistical analysis

For the primary analysis we compared the proportions of vitamin D insufficiency in children hospitalised for an ALRI (versus no ALRI) and also for children hospitalised with gastroenteritis (versus no gastroenteritis). Odds ratios are reported, Fisher’s exact test p-values <0.05 were considered statistically significant. Comparisons of serum 25OHD3 levels were assessed using the Student’s t-test. Weight (at hospital discharge) for age z-scores (standard deviations) were calculated with reference to World Health Organization child growth standards using the STATA module “zscore06” (Leroy 2011). A weight for age z-score >2 standard deviations below the World Health Organization reference median (World Health Organisation 2006) was considered as severely underweight and indicative of undernutrition. Haemoglobin values were considered low if <110 g/L for all ages (World Health Organization 2011). All statistical analyses were performed using STATA 12 (Stata Corp, USA).

7.5 Results

7.5.1 Hospitalisation diagnosis

Among the 74 hospitalised study children there were 124 ICD-10-AM discharge diagnoses (average 1.7 diagnoses per child). Two participants coded solely with ICD-10-AM codes R05 (cough) and J98.4 (other lung disorders) were classified as ALRI following a clinical note review. The most common reasons for hospitalisation were: ALRI (59%), gastroenteritis (27%), upper respiratory tract infection (19%) and anaemia (18%). The most common ALRI diagnoses were bronchiolitis (30%) and pneumonia (28%) (Table 7.1).
Table 7.1 Reason for hospitalisation of study participants (n=74 children)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ICD-10-AM codes</th>
<th>Children n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALRI</td>
<td>J09-J18.9, J20-J22, R05, J98.4, A37.0</td>
<td>44</td>
<td>59</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>J21-J21.9</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>J12-J18.9</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Non-specific ALRI</td>
<td>J20-J20.9, J22, J98.4, R05</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Confirmed influenza</td>
<td>J09</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pertussis</td>
<td>A37.0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>A00-A09, B79.0, R11.0</td>
<td>20</td>
<td>27</td>
</tr>
<tr>
<td>URTI</td>
<td>J00-J06.9, H66.3, H66.9, J72.9</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Anaemia</td>
<td>D50-D53.9</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>N39.0, P39.3</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Pyoderma (skin sores)</td>
<td>L00-L08.9, L20.0-L30.0, L23.1</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Febrile illness</td>
<td>R50-R50.9</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Unspecified viral infection</td>
<td>B34-B34.9</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sepsis</td>
<td>A40-A41.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory obstruction</td>
<td>T17-T17.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>I42.1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

There were 124 ICD-10-AM (National Centre for Classification in Health 2009) discharge diagnoses among the 74 children at an average of 1.7 diagnoses per child. There were 51 ICD-10-AM diagnoses among the 44 ALRI hospitalised children (italics) dominated by bronchiolitis (n=22) and pneumonia (n=21). ICD-10-AM codes were obtained from Royal Darwin Hospital by the medical records department.

**ALRI**: Acute lower respiratory infection. **URTI**: Upper respiratory tract infection. **ICD-10-AM**: International Statistical Classification of Diseases and Health Related Problems.
7.5.2 Characteristics of study participants

The median age at hospitalisation was 6 months (range 1, 31), 47 (64%) were male, 42 (57%) were Indigenous and the median hospital stay was 5 days (range 1, 32). Those children hospitalised with ALRI were more likely to attend childcare (25% versus 3%, p=0.020) than those hospitalised for other reasons. Whereas those children hospitalised with gastroenteritis were more likely to be Indigenous (85% versus 46%, p=0.003) and more likely to live in a remote location (80% versus 43%, p=0.008) when compared with those children hospitalised for reasons excluding gastroenteritis. No other factors were identified as being associated with ALRI compared to non-ALRI admissions or with gastroenteritis compared to non-gastroenteritis admissions.

7.5.3 Accuracy and validity of the vitamin D assay

The ID-LC-MS/MS vitamin D assay fulfilled the quality control acceptance criteria. The coefficient of variance for the triplicate determinations of low, medium and high commercial controls was 6.4%, 6.9% and 3.9% respectively and the mean for each quality control level was within 3 standard deviations of the historical data for the corresponding quality control lot number. The calibration curve had a regression coefficient ($R^2$) value of 0.996.

7.5.4 Vitamin D levels and participant characteristics

Mean 25OHD3 for all 74 children was 83.9 nmol/L; 24/74 (32%) children were vitamin D insufficient (<75 nmol/L) and 11/74 (15%) were deficient (<50 nmol/L). Mean serum 25OHD3 levels (Table 7.2) were significantly lower for infants who were breast feeding when hospitalised (78 nmol/L versus 93 nmol/L; p=0.029).
There was no difference in mean serum 25OHD3 levels for Indigenous infants who were breast feeding compared to those Indigenous infants who were not, but among the 32 non-Indigenous infants the mean 25OHD3 levels were 102 nmol/L for non-breast feeding children compared with 78 nmol/L for breast feeding children (both mean levels being above the insufficiency cut-off). Mean 25OHD3 levels during hospitalisation were significantly lower for infants with a history of pre-term birth (70 nmol/L versus 91 nmol/L; p=0.013) compared to those who were full-term (not pre-term). The mean 25OHD3 levels for Indigenous compared to non-Indigenous children (78 nmol/L versus 91 nmol/L; p=0.063) and for children living in remote (81% Indigenous) compared to urban settings (78 nmol/L versus 90 nmol/L; p=0.074) were lower but not statistically different. We found no other correlation between the demographics and 25OHD3 levels.
Table 7.2  Mean serum 25OHD3 levels according to participant characteristics

<table>
<thead>
<tr>
<th>General characteristics at hospitalisation</th>
<th>Yes</th>
<th>%</th>
<th>nmol/L</th>
<th>No</th>
<th>%</th>
<th>nmol/L</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>47</td>
<td>64</td>
<td>86</td>
<td>27</td>
<td>36</td>
<td>83</td>
<td>0.618</td>
</tr>
<tr>
<td>Indigenous</td>
<td>42</td>
<td>57</td>
<td>78</td>
<td>32</td>
<td>43</td>
<td>91</td>
<td>0.063</td>
</tr>
<tr>
<td>&lt;6 months of age</td>
<td>34</td>
<td>46</td>
<td>81</td>
<td>40</td>
<td>54</td>
<td>87</td>
<td>0.366</td>
</tr>
<tr>
<td>Breast fed</td>
<td>47</td>
<td>64</td>
<td>78</td>
<td>27</td>
<td>36</td>
<td>93</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>Indigenous</td>
<td>32</td>
<td>43</td>
<td>79</td>
<td>10</td>
<td>14</td>
<td>78</td>
<td>0.985</td>
</tr>
<tr>
<td>Non-Indigenous</td>
<td>15</td>
<td>20</td>
<td>78</td>
<td>17</td>
<td>23</td>
<td>102</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td>Living remote</td>
<td>39</td>
<td>53</td>
<td>78</td>
<td>35</td>
<td>47</td>
<td>90</td>
<td>0.074</td>
</tr>
<tr>
<td>Attending childcare</td>
<td>12</td>
<td>16</td>
<td>94</td>
<td>62</td>
<td>84</td>
<td>82</td>
<td>0.167</td>
</tr>
<tr>
<td>Wet season (Oct-Mar)</td>
<td>38</td>
<td>51</td>
<td>81</td>
<td>36</td>
<td>49</td>
<td>87</td>
<td>0.302</td>
</tr>
<tr>
<td>Severely underweight</td>
<td>5</td>
<td>7</td>
<td>66</td>
<td>64</td>
<td>93</td>
<td>84</td>
<td>0.179</td>
</tr>
<tr>
<td>Haemoglobin &lt;110g/L</td>
<td>29</td>
<td>40</td>
<td>77</td>
<td>44</td>
<td>60</td>
<td>88</td>
<td>0.118</td>
</tr>
<tr>
<td>Hospital stay &gt;5days</td>
<td>31</td>
<td>42</td>
<td>82</td>
<td>43</td>
<td>58</td>
<td>85</td>
<td>0.721</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gestational characteristics</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-term (&lt;37weeks)</td>
<td>14</td>
<td>23</td>
<td>70</td>
<td>46</td>
<td>77</td>
<td>91</td>
<td><strong>0.013</strong></td>
</tr>
<tr>
<td>Low birth weight (&lt;2500g)</td>
<td>12</td>
<td>22</td>
<td>75</td>
<td>42</td>
<td>78</td>
<td>87</td>
<td>0.188</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td>25</td>
<td>34</td>
<td>83</td>
<td>48</td>
<td>66</td>
<td>84</td>
<td>0.850</td>
</tr>
</tbody>
</table>

Mean serum 25OHD3 levels are shown by the occurrence of each participant characteristic. n is the number of children with (Yes) and without (No) each characteristic and % is the proportion of the total (n=74). Weight, haemoglobin and gestational characteristic data were not available for all children: discharge weight (n=69), haemoglobin (n=73), pre-term (n=60), birth-weight (n=54), maternal smoking (n=73). Weight for age and haemoglobin levels were evaluated in relation to World Health Organization defined reference values. Comparisons were performed using the Student's t-test and a p-value of <0.05 was considered statistically significant (bold).
Table 7.3 Proportion of vitamin D insufficiency in children hospitalised with ALRI or gastroenteritis.

<table>
<thead>
<tr>
<th>Hospital diagnosis</th>
<th>25OHD3 (&lt;75nmol/L)</th>
<th>Odds Ratio</th>
<th>95%CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44</td>
<td>10</td>
<td>23</td>
<td>0.34</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>14</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Exclusive Bronchiolitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>7</td>
<td>44</td>
<td>0.89</td>
</tr>
<tr>
<td>non-ALRI</td>
<td>30</td>
<td>14</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Exclusive Pneumonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>2</td>
<td>14</td>
<td>0.12</td>
</tr>
<tr>
<td>non-ALRI</td>
<td>30</td>
<td>14</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>12</td>
<td>60</td>
<td>5.25</td>
</tr>
<tr>
<td>No</td>
<td>54</td>
<td>12</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Proportions and odds ratios of vitamin D insufficiency (<75 nmol/L) by ALRI (versus non-ALRI), exclusive bronchiolitis (versus non-ALRI), exclusive pneumonia (versus non-ALRI) and gastroenteritis (versus no gastroenteritis). Four of the 6 children concurrently diagnosed with ALRI and gastroenteritis had 25OHD3 levels below 75 nmol/L. P-values were calculated using Fisher’s exact test.

7.5.5 Comparison of vitamin D insufficiency by hospitalisation diagnosis

Among 44 ALRI hospitalised children, 10 (23%) had serum 25OHD3 levels below 75 nmol/L compared to 14/30 (47%) non-ALRI children (p=0.043) (Table 7.3).

Among 20 gastroenteritis hospitalised children, 12 (60%) had 25OHD3 levels below 75 nmol/L compared to 12/54 (22%) non-gastroenteritis children (p=0.004). Six children had a co-diagnosis of ALRI and gastroenteritis, of whom four had 25OHD3 levels below 75 nmol/L. Neither the omission of these co-diagnosed children nor the use of a 50 nmol/L cut-off had a meaningful influence on the outcomes.
Within the ALRI group there were 22 (16 exclusive) bronchiolitis diagnoses and 21 (14 exclusive) pneumonia diagnoses. In comparison to the 14/30 (47%) non-ALRI children with insufficient serum 25OHD3 levels (<75 nmol/L), there was a similar proportion of vitamin D insufficiency (<75 nmol/L) among children with an exclusive diagnosis of bronchiolitis (7/16 (44%); p=1.000) but a lower proportion of vitamin D insufficiency (<75 nmol/L) among children with an exclusive diagnosis of pneumonia (2/14 (14%); p=0.049). Care needs to be taken in interpretation of the subgroup analyses due to small numbers and borderline significance.

7.5.6 Vitamin D levels and hospitalisation diagnosis

Mean serum 25OHD3 levels were significantly higher in the ALRI compared to the non-ALRI diagnosed children (90 versus 75 nmol/L respectively; p=0.020) with variation evident among the sub-diagnoses, bronchiolitis (76 nmol/L) and pneumonia (94 nmol/L) (Figure 7.1). In contrast, the mean serum 25OHD3 were significantly lower in the gastroenteritis compared to non-gastroenteritis children (68 versus 90 nmol/L respectively; p=0.003). For the six children where ALRI and gastroenteritis occurred together the median 25OHD3 level was 59 nmol/L.
Figure 7.1 Vitamin D levels in children hospitalised with an ALRI or gastroenteritis.

Mean serum 25OHD3 was significantly higher in children hospitalised with an ALRI diagnosis compared to all other diagnoses. Mean serum 25OHD3 levels associated with an exclusive bronchiolitis diagnosis were comparable to the non-ALRI diagnoses whereas levels associated with an exclusive pneumonia diagnosis were similar to levels in the ALRI group overall. Mean serum 25OHD3 was significantly lower for the major non-ALRI diagnosis, gastroenteritis, compared to all other diagnoses. Six children had concurrent gastroenteritis and ALRI diagnoses. Grey dots represent the individual 25OHD3 levels and black diamonds identify the mean. Box and whisker plots identify the median (white line) interquartile range (black box) and 95% CI’s (lines). P-values were calculated using the Student’s t-test.
7.6 Discussion

Our study is the first to examine the association between ALRI and vitamin D insufficiency in children of the Northern Territory. In this small hospital-based study, we found that almost one-third (32%) of children hospitalised for predominantly infectious causes had suboptimal 25OHD3 levels (<75 nmol/L).

The Northern Territory has some of the highest rates of ALRI hospitalisation in the world, especially for young Indigenous children (22%) (O'Grady, Torzillo & Chang 2010). Together with studies reporting an association between vitamin D insufficiency, mucosal immunity and the risk of ALRI (Liu et al. 2006; Roth et al. 2010), and the absence of vitamin D data in the Northern Territory, we investigated vitamin D insufficiency among a convenience sample of hospitalised children for whom the most common diagnoses were ALRI and gastroenteritis. We found a high proportion of vitamin D insufficiency among children hospitalised with ALRI (23%) and an even higher proportion among those hospitalised for gastroenteritis (60%).

7.6.1 Vitamin D insufficiency and gastroenteritis

The higher point prevalence of vitamin D insufficiency among the non-ALRI diagnosed children was likely influenced by lower 25OHD3 levels in the non-ALRI children with gastroenteritis compared to without gastroenteritis (65 nmol/L versus 83 nmol/L respectively; p=0.106). Among all 74 children the 25OHD3 levels were significantly lower in gastroenteritis compared to non-gastroenteritis hospitalisations (68 nmol/L versus 90 nmol/L respectively; p=0.003), noting that remote living and Indigeneity were more common among gastroenteritis diagnosed children and may be confounders of this association. Whilst vitamin D insufficiency could be
considered a risk factor for gastroenteritis because most mucosal surfaces rely, at least partly, on vitamin D-mediated immune defence (Lagishetty, Liu & Hewison 2011), poor dietary absorption and a greater burden of illness among these children may also explain the lower vitamin D levels.

7.6.2 Vitamin D insufficiency in northern regions of Australia

In the Top End of the Northern Territory, sunshine hours and UV index are well above the national average; however, population data on vitamin D in this region are lacking. In a study from far North Queensland, only 7% of 116 women presenting for antenatal care were vitamin D insufficient (<75 nmol/L), though Indigenous women had significantly lower 25OHD3 levels, with the median just above 75 nmol/L (Bendall et al. 2012). In 2011, a national survey of Australian adults over 25 years of age (n=11,247) found 73% insufficiency (<75 nmol/L) overall (Daly et al. 2012). A subset of these data representing climatic conditions most closely related to that of the Northern Territory (latitude of <30°S during summer-autumn) revealed that over 35% of males and almost 60% of females were insufficient (<75 nmol/L) (Daly et al. 2012). Another study performed in southeast Queensland (27°S) found 41% and 15% of women were deficient (<50 nmol/L) in winter-spring and summer respectively (van der Mei et al. 2007). Thus, vitamin D insufficiency appears less common in a tropical climate or during the summer, however, the lack of congruency in the data described above suggests that population-level factors, testing bias or study design might also influence reported 25OHD3 levels.
7.6.3 Potential methodological bias

It has been recently demonstrated that routine LC-MS/MS methods can overestimate serum vitamin D levels by as much as 11% compared to reference LC-MS/MS methods, most likely because of failure to resolve the 25OHD3 epimer, 3-epi-25OHD3 (Carter 2011). The Royal Melbourne Institute of Technology Drug Discovery Technologies LC-MS/MS method does not delineate the 25OHD3 epimers yet did correlate well with the commonly used RIA radioimmunoassay (DiaSorin LIAISON®) which does not bind 3-epi-25OHD3. It is therefore possible that our estimates of serum vitamin D insufficiency are conservative.

In our study, the point estimate for mean serum 25OHD3 levels was lower in Indigenous children though not statistically significant (78 nmol/L versus 91 nmol/L; p=0.063). Melanin content of the skin influences the number of photons that reach the lower cellular layers, where 25OHD3 synthesis takes place, and the amount of 25OHD3 produced following equivalent sunlight exposure is lower for darker skinned individuals (Matsuoka et al. 1991). Few studies have investigated 25OHD3 levels in Indigenous Australians. In temperate South Australia, the mean 25OHD3 level among 58 healthy Indigenous adults was 57nmol/L (Vanlint et al. 2011); well below the cut-off for insufficiency (75 nmol/L).

7.6.4 Breast feeding and vitamin D status

Although most children (64%) were breast fed at the time of hospitalisation, we found that those children who were not breast fed had significantly higher levels of 25OHD3. Stratification by Indigeneity highlighted that the effect was limited to the non-Indigenous non-breast fed sub-group where high 25OHD3 levels (102 nmol/L)
were found (Table 7.3). It is difficult to draw a conclusion regarding breast feeding and 25OHD3 levels because of the exclusive nature of the definition (breast feeding at hospitalisation) and the age difference between breast feeding and non-breast feeding children (5 versus 12 months). Age had little influence on vitamin D levels in this study but others have shown breast fed infants are at greater risk of vitamin D insufficiency even in sunny climates (Liang et al. 2010). Greater food intake and/or the use of vitamin D fortified infant formulas among non-Indigenous non-breast feeding mothers and their children are plausible explanations.

7.6.5 Vitamin D supplementation to prevent respiratory infections
Vitamin D has been postulated to be a potential panacea for a range of diseases including diabetes, cancer, heart, infectious and autoimmune diseases such as multiple sclerosis. However, most of these data come from association studies. A recent meta-analysis found that vitamin D supplementation had a net protective effect against respiratory tract infections (Bergman et al. 2013). Importantly, that analysis showed that age and baseline vitamin D status had no effect on the outcome but that daily dosage was significantly more effective than bolus dose supplementation.

7.7 Limitations
This was a small opportunistic cross-sectional study and results should be interpreted with caution. Only children hospitalised for predominantly infectious diseases and with blood drawn for pathology could be investigated and small numbers limited the ability to interpret the relationship between vitamin D and the less common diagnoses. Furthermore, as with any cross sectional study, there was uncertainty
whether vitamin D insufficiency was a cause or an effect of the investigated diseases and recent evidence suggests vitamin D is a negative acute phase reactant that is depleted following an inflammatory insult (Gama et al. 2012).

7.8 Conclusions

This is one of the few reports on vitamin D among Indigenous Australians. Vitamin D insufficiency was evident in one third of these hospitalised children of the Northern Territory with the lowest vitamin D levels seen among children hospitalised with gastroenteritis. If vitamin D insufficiency does increase the risk of ALRI among Indigenous children in this setting, it will be important to conduct a larger population-based study where children with vitamin D insufficiency can be compared against non-hospitalised/healthy controls. The high level of exposure (insufficiency) suggests that either prospective or retrospective cohort studies are feasible options for the future.
CHAPTER 8

Final Discussion
8.1 Overview

While there has been a “closing of the gap” in child and infant mortality rates between Indigenous and non-Indigenous children since 1998, respiratory diseases remain a prominent cause of death, killing twice as many Indigenous as non-Indigenous children (Australian Health Ministers' Advisory Council 2012). In 2009, local cross-sectional surveillance data showed that one in five Indigenous children (<6 years) in remote Northern Territory communities had otitis media with perforation of the tympanic membrane, the same as in 2001 (Leach 2014; Morris et al. 2005). Similarly, one in five Indigenous children (<12 months) were hospitalised with an ALRI in their first year of life between 1999 and 2004 (O'Grady, Torzillo & Chang 2010) and there has been little documented change in the burden of childhood ALRI hospitalisation in this region up until 2010, despite reductions in other Australian states (Jardine, Menzies & McIntyre 2012).

8.2 Study rationale

Driving the high burden of respiratory disease among Indigenous children in the Northern Territory is the early onset of infection, prior to completion of the 3 dose infant vaccination schedule (2, 4 and 6 months) against the common respiratory pathogens (pneumococcus, pertussis, *Haemophilus influenzae* type b; influenza vaccination is only recommended from 6 months of age). Neonates have poorly protective immunity against many pathogens, and conditions in this region are highly
conducive to pathogen transmission (Wiertsema & Leach 2009). As such, Indigenous infants are colonised with the pneumococcus and NTHi (for which there is no licensed vaccine in Australia) within weeks of birth and their carriage prevalence reaches as high as 80% by 3 months of age. This early colonisation is associated with the onset of otitis media (Leach et al. 1994) and the peak frequency of ALRI hospitalisations which occurs at 4 months of age (O'Grady, Torzillo & Chang 2010). Commonly, respiratory disease is associated with a high diversity of pathogens in the nasopharynx and the impact of antibiotic interventions has been modest (McCallum et al. 2013; Morris et al. 2010a). Non-conjugate vaccine pneumococcal serotypes are common (Leach et al. 2009) as are other bacteria such as *Moraxella catarrhalis* and *Staphylococcus* species (Hare et al. 2013) and viral pathogens including human rhinovirus, respiratory syncytial virus and human adenovirus (Binks et al. 2011). In summary, respiratory infections begin in the immunologically naïve infant prior to vaccine protection against common respiratory pathogens and the ensuing inflammation results in complicated polymicrobial infections (Binks et al. 2011) largely outside the therapeutic ability of currently available antibiotic interventions.

Evidence points to early, dense and diverse respiratory infections as relatively unchecked in this region. This thesis focussed on maternal pneumococcal vaccination as a targeted method of inducing early immune protection against, and vitamin D insufficiency at birth as a modifiable risk factor for, ear and respiratory disease among Indigenous infants.
Two broad questions were addressed in this thesis:

1. Can maternal 23vPPV vaccination at birth or at delivery protect infants against otitis media (Chapter 3) and ALRI (Chapter 4) by preventing or delaying nasopharyngeal colonisation by vaccine serotype pneumococci?

2. Does vitamin D insufficiency exist among Indigenous mothers and infants in the Northern Territory and if so (Chapter 6), is infant vitamin D insufficiency associated with a higher risk of ALRI (Chapter 6 and Chapter 7)?

8.3 Principal study findings

8.3.1 Maternal pneumococcal vaccination against infant ear disease

Chapter 3 reported findings from the randomised controlled trial (2006-2011) of maternal 23vPPV vaccination for the prevention of infant middle ear disease among high risk Australian Indigenous infants, the PneuMum study. Mothers received the 23vPPV either in pregnancy (30-36 weeks), immediately post-partum (within 3 days on birth) or at the 7 months study conclusion (controls). Infants were followed-up at ages 1, 2, and 7 months. Post-vaccination maternal systemic immune responses, in terms of vaccine-specific antibody concentrations, were robust for both pregnancy and birth vaccinees at birth as shown previously (Lehmann et al. 2002; O'Dempsey et al. 1996; Quiambao et al. 2003), while breast milk antibody concentrations were higher among pregnancy vaccinees compared to birth vaccinees which has not been described before. Despite high cord blood concentrations to all vaccine serotypes, we had no direct evidence of passively acquired antibodies by the infants. Antibody concentrations among infants of vaccinees were equivalent to background controls by age 7 months, the next sampling point. Modelling studies have estimated that cord blood vaccine serotype specific antibody concentrations of 4.4µg/ml are required for
persistence in the infant to 4 months of age (Quiambao et al. 2007). In our study, cord blood GMC’s were highly variable by serotype (from 0.48 µg/ml for serotype 12F to 17.12 µg/ml for serotype 14) and only serotypes 14, 15B, 19A, 19F and 33F exceeded the 4.4 µg/ml threshold suggesting that most other serotype-specific antibodies waned much earlier than 4 months. Regardless of the robust maternal immunogenicity, questions remain regarding duration of antibodies among Indigenous infants.

Vaccination with the 23vPPV in pregnancy had a non-significant impact against the co-primary outcomes of infant ear disease (VE 12%, 95% CI -12% to 31%) and vaccine serotype nasopharyngeal carriage (VE 30%, 95% CI -34% to 64%) at 7 months of age. The 23vPPV at birth had no effect against infant carriage or ear disease and there were no differences in either outcome between infants of vaccinees and controls at 1 and 2 months of age. These findings are consistent with previous findings in Brazil and the Gambia (O'Dempsey et al. 1996) that saw no significant clinical difference in acute respiratory infections or otitis media respectively and with two studies that showed no significant effect against pneumococcal carriage between 6 (Lopes et al. 2009) and 7 (Munoz et al. 2001) months of age.

Interestingly, there was evidence of a vaccine impact against ear disease concurrent with nasopharyngeal carriage of 23v6A serotypes (23vPPV serotypes and 6A), referred to as 23v6A ear disease. The prevalence of 23v6A ear disease at 7 months was 27% among control infants and 13% among infants of pregnancy vaccinees, equivalent to a 51% relative reduction (95% CI -2% to 76%) and suggestive of a reduction in “vaccine preventable ear disease” even without an overall impact on
carriage or otitis media. Deeper investigation showed that this impact was largely confined to the five most commonly carried 23v6A serotypes associated with infant ear disease (19A, 10A, 6A, 15B or 33F). Strong vaccine antibody responses to serotypes 15B, 19A and 33F, perhaps related to priming by previous exposure, may have contributed to this effect. In summary, whilst the 23vPPV was ineffective against overall ear disease, vaccination in pregnancy was well accepted by the Indigenous community, safe, immunogenic and potentially effective against vaccine serotype-specific ear disease.

8.3.2 Maternal pneumococcal vaccination against infant ALRI

Though ALRI can have a diverse aetiology, the pneumococcus is a leading pneumonia pathogen, and pneumonia hospitalisation rates among Northern Territory Indigenous children are among the highest reported in the world (26.6 per 1000 child-years) (O'Grady et al. 2010c). Chapter 4 investigated infant ALRI hospitalisation and clinic presentations as secondary outcomes of our maternal 23vPPV trial (PneuMum, presented in Chapter 3). In this historically high risk population of urban and remote Indigenous infants, there were fewer than expected ALRI hospitalisations before 12 months of age (11%; 25/225) although the prevalence was 3 times higher for infants living in remote communities (21%; 15/70) where approximately 75% of children presented to their local medical clinic with an ALRI in their first year of life.

For control infants, the incidence of first ALRI hospitalisation approached twice that among infants of pregnancy vaccinates (8 versus 14 per 100 child-years; VE 39%, 95% CI -58% to 80%) and this effect was more pronounced for remote dwelling
infants (9 versus 33 per 100 child-years; VE 72%, 95% CI -22% to 97%) though the study was underpowered and did not exclude no effect. For ALRI clinic presentations (remote dwelling infants only), we saw no differences in the incidence rates between infants of controls and vaccinees. The lack of vaccine effect against ALRI clinic presentations may reflect the non-specificity of the diagnostic algorithm that aimed to identify ALRI of any severity and perhaps encompassed more viral infections. Conversely, the small (non-significant) effect against ALRI hospitalisation, representing the more severe end of the disease spectrum, may reflect a subtle effect against pneumococcal ALRI. Consistent with the preceding maternal pneumococcal vaccine trials that investigated ALRI outcomes (Lopes et al. 2009; O’Dempsey et al. 1996; Riley et al. 1977) our data were largely inconclusive. Carefully planned and larger studies are needed.

8.3.3 Vitamin D and the risk of ALRI

A growing number of reports link cord blood and infant vitamin D insufficiency with the subsequent risk of ALRI (Belderbos et al. 2011; Camargo et al. 2011; Luczynska et al. 2014; Mohamed & Al-Shehri 2012; Roth et al. 2010; Shin et al. 2013) and meta-analysis of randomised controlled trials suggest that vitamin D supplementation may be useful to prevent respiratory infection (Bergman et al. 2013; Mao & Huang 2013). Chapters 6 and 7 explored the relationship between vitamin D and the high burden of respiratory disease among Indigenous children in the tropical north of Australia where vitamin D data are sparse. Chapter 6 demonstrated that vitamin D insufficiency was common at birth (45% of venous and 80% of cord bloods) despite only 21% insufficiency at 30-36 weeks gestation and 22% insufficiency at infant age 7 months. These data suggest that 4 in 5 Indigenous infants in the Northern Territory
are born with vitamin D insufficiency (<75 nmol/L) and almost half with vitamin D deficiency (<50 nmol/L) even though maternal vitamin D status was generally sufficient among mothers in the third trimester. While the prevalence of vitamin D insufficiency at birth was high, fewer than expected infants were hospitalised with an ALRI (8%; 7/84) as we highlighted for this same cohort in Chapter 4. The ALRI hospitalisations all occurred among infants that had cord blood 25OHD3 levels <75 nmol/L (10%; 7/67). As a continuous measure, mean 25OHD3 levels were lower at birth, in both maternal (61 nmol/L versus 82 nmol/L) and cord blood (37 nmol/L versus 56 nmol/L), for the infants who were subsequently hospitalised with an ALRI compared to those who were not. This is consistent with earlier studies demonstrating an association between vitamin D at birth and ALRI (Belderbos et al. 2011; Camargo et al. 2011; Luczynska et al. 2014; Mohamed & Al-Shehri 2012; Roth et al. 2010; Shin et al. 2013); however, these data should be interpreted with some caution because of the low numbers and because remote dwelling was a potential confounder that requires further investigation.

8.3.4 Vitamin D among hospitalised infants

Chapter 7 identified that one in three hospitalised children under 3 years of age (Indigenous and non-Indigenous) in the Northern Territory had vitamin D insufficiency. Factors associated with lower vitamin D levels were breast feeding, pre-term birth, living in a remote community and being of Indigenous ethnicity, though the latter two frequently occurred together and could not be separated. Lower vitamin D levels among Indigenous children are potentially related to darker skin colour. Melanin content of the skin influences the number of photons that reach the lower cellular layers, where 25OHD3 synthesis takes place, and the amount of
25OHD3 produced following equivalent sunlight exposure is lower for darker skinned individuals (Matsuoka et al. 1991). As such, the tropical climate of the Northern Territory may not insulate Indigenous populations against vitamin D insufficiency.

Contrary to our hypothesis, there was less vitamin D insufficiency among children hospitalised with an ALRI (23%) compared to children hospitalised for other reasons, mostly gastroenteritis (60%). While there is little specific literature regarding vitamin D and gastroenteritis, vitamin D is known to influence the production of innate anti-microbial peptides, such as cathelicidin (Liu et al. 2006), on mucosal surfaces (including the gastrointestinal, urinary and respiratory tract) (Lagishetty et al. 2010) and might play a role in enhancing general mucosal immunity.

This study should also be interpreted with caution due to the cross-sectional nature and lack of a healthy control group. Further, hospitalisation itself has been associated with a fall in vitamin D levels among children (Dayal et al. 2014) and some studies purport that vitamin D is depleted during a systemic inflammatory response (Gama et al. 2012).

8.4 Future work to prevent respiratory infections among Indigenous infants

8.4.1 Maternal vaccination

The 51% relative reductions (95% CI -2% to 76%) in ear disease associated with concurrent nasopharyngeal carriage of a 23v6A serotype and the 39% relative
reduction in ALRI hospitalisation incidence (95% CI -58% to 80%) among infants of vaccinees compared to controls are encouraging despite the lack of significance. Post-hoc analysis performed in Chapter 3 suggests that the impact of the 23vPPV in pregnancy against respiratory disease may be confined to pneumococcal serotypes 19A, 10A, 6A, 15B or 33F. The future of maternal 23vPPV in pregnancy may be to target serotypes not contained in the currently used 13vPCV, such as 10A, 15B and 33F. These serotypes are already common causes of mucosal (Chapter 3) and invasive disease (Krause & Cook 2012) among children in the Northern Territory and are likely to increase in prominence (replacement) as 13vPCV serotypes decline. Future trials of 23vPPV in pregnancy need to perform careful risk benefit analysis to determine feasibility. With several underpowered international studies already complete, there is potential for pooled data meta-analysis.

Whether or not future maternal vaccination studies should employ conjugate vaccines is also something to consider for the future. While the 10vPHiD-CV or 13vPCV are more immunogenic than the 23vPPV, inducing memory and mucosal immunity, high 13vPCV coverage rates seen among Indigenous children in this region should induce herd immunity that will essentially negate the benefit of this strategy.

8.4.2 Vitamin D supplementation

While the vitamin D studies conducted in this thesis have several limitations such as small sample size (Chapter 6) and the lack of healthy control group (Chapter 7), they make a valuable contribution to the knowledge base. Chapter 6 established that a high prevalence of vitamin D insufficiency exists among Indigenous mothers and
their infants at birth and found modest evidence supporting an association between cord blood vitamin D and the risk of infant respiratory disease consistent with emerging international studies (Shin et al. 2013). Together with the existing evidence supporting the use of vitamin D supplementation for prevention of respiratory disease (Grant et al. 2014a), moving directly to a randomised controlled trial of maternal or neonatal vitamin D supplementation for the prevention of respiratory infection among an Indigenous population of the Northern Territory is a possibility and will be the subject of a future funding application.

8.4.3 Vitamin D supplementation for enhancing vaccine immunogenicity

Data from *in vitro* and animal models implies that vitamin D augments vaccine immune responses (Enioutina, Bareyan & Daynes 2009) though human studies are less conclusive (Heine et al. 2011; Principi et al. 2013). Based on these findings we hypothesize that vitamin D insufficiency is associated with weaker infant immune responses to influenza, pneumococcal and pertussis vaccinations. Preliminary data (not included in this thesis) from Chapter 6 support this hypothesis with respect to pneumococcal conjugate vaccination and this idea is the subject of an existing research proposal to the National Health and Medical Research Council of Australia. If this association were confirmed, vitamin D supplementation in the first 12 months of life would be a simple and inexpensive public health intervention to augment vaccine immune responses. To date, there have been no studies on the influence of vitamin D on vaccine-induced immunity in young infants (<12 months of age).
8.4.4 Characterising the microbial aetiology of ALRI

Currently, the microbial aetiology of ALRI among Indigenous children is poorly characterised. In the absence of available lung specimens we are currently performing a retrospective matched case-control study (using swabs from local carriage studies, 1996-2004) to investigate the nasopharyngeal microbiology of Indigenous children (aged <2 years) immediately prior to an ALRI episode as determined using hospital and clinic medical records. In addition to routine microbiology, PCR-based assays are being used to screen for respiratory viruses (Arden et al. 2006; Syrmis et al. 2004) and to determine the bacterial loads of the pneumococcus, *Haemophilus influenzae* and three other respiratory bacteria (Binks et al. 2011; Smith-Vaughan et al. 2013). This study will compare the nasopharyngeal pathogen diversity and density in swabs from ALRI cases compared to controls in an attempt to characterise the specific infection dynamics of lower respiratory disease. Data describing the association of vaccine and emerging pneumococcal serotypes with ALRI, will contribute to informed decisions on future pneumococcal vaccination strategies.

8.4.5 Elucidating the impact of three generations of pneumococcal conjugate vaccine on the incidence of childhood ALRI in the Northern Territory

Despite shifts in serotype hierarchy (Leach et al. 2009), there were no significant reductions in overall nasopharyngeal pneumococcal carriage (Leach et al. 2009) (Hare 2014; Leach 2014) or ALRI hospitalisations (O'Grady et al. 2010b) following 7vPCV introduction in the Northern Territory. There have been no population-based studies of ALRI data from the Northern Territory since 2006. As such, it is not clear
whether the current pneumococcal conjugate vaccines are impacting the burden of ALRI among Indigenous children in the Northern Territory. Using the Northern Territory’s comprehensive and contemporary electronic health and immunisation records we plan to conduct a historical cohort study of Northern Territory Indigenous children aged <12 months living in remote communities between 2006 and 2014. This will elucidate the temporal population level effectiveness of expanded valency pneumococcal vaccines (10vPHiD-CV and 13vPCV) against ALRI among Indigenous children in remote communities. More specifically, a comparison of the ALRI incidence during three periods: 2006-2009 (7vPCV era); 2009-2011 (10vPHiD-CV era); 2011-2013 (13vPCV era) will be performed. This will be done in two ways: by era alone excluding a 3 month uptake period; and separately for those with 3 documented doses of PCV. The relationship of delayed or incomplete schedules will also be investigated to ascertain the effect of vaccine timeliness.

8.4.6 Earlier or combination PCV schedules

Two broader coverage vaccines were introduced in the Northern Territory from 2009, the 10vPHiD-CV targeting 10 pneumococcal serotypes and *Haemophilus influenzae* (protein D) and the 13vPCV targeting 13 pneumococcal serotypes. Each of these vaccines has a unique benefit, based largely on the *Haemophilus influenzae* antigen of 10vPHiD-CV and the pneumococcal serotype 3, 6A and 19A antigens of the 13vPCV, and it remains unclear if one of these vaccines is more efficacious for preventing ALRI among Indigenous children. Currently, two linked randomised controlled trials are underway to compare the immunogenicity and pneumococcal carriage outcomes of a novel early combination schedule of 10vPHiD-CV plus 13vPCV against the standard 3 dose schedules among remote dwelling Indigenous
children of the Northern Territory. As a secondary outcome of this trial we plan to investigate infant ALRI hospitalisations and clinic presentations identified via electronic medical records for all children until 3 years of age.

In these trials, babies aged <6 weeks of age are randomised to receive primary schedules of either: 3 doses of 10vPHiD-CV (1, 2 and 4 months) and 1 dose of PCV13 (6 months); 3 doses of 10vPHiD-CV (2, 4 and 6 months); or 13vPCV (2, 4 and 6 months). At 12 months of age, infants are re-randomised (the second trial) to receive a booster dose of either 10vPHiD-CV or the 13vPCV. This study is well positioned to assess the impact of novel PCV schedules against ALRI in the community, both post-primary and post-booster doses. We are aiming to compare the incidence rate and time to first ALRI hospitalisations and clinic presentations between the combination and single vaccine groups, post primary series (<12 months of age) and post booster doses (<3 years of age) and to investigate more specifically whether the combination schedule can reduce nasopharyngeal carriage of NTHi (based on antibodies to protein D) and pneumococcal serotypes 3, 6A, 19A (13vPCV) as effectively as the single schedules alone.

8.4.7 Next generation pneumococcal conjugate vaccine trials.

Pneumococcal conjugate vaccine trials specifically targeting reductions in ALRI among remote Indigenous children are required in the future. Implementing a trial of this nature would benefit from a deeper knowledge of the ALRI microbiology (proposed in section 8.3.7.1) and efficacy of current and combination PCV strategies (proposed in sections 8.3.7.2-3), together with advances in respiratory vaccines and the established rapport with Indigenous remote communities. At the moment there
are several foreseeable future vaccine options for preventing ALRIs in the Northern Territory. In 2013, neonatal 7vPCV was shown to be safe and immunogenic at 2-3 months of age (Pomat et al. 2013). A birth dose of 13vPCV is an attractive option to prevent early pneumococcal colonisation; however, as mentioned earlier with respect to the use of conjugate vaccines in pregnancy, serotype specific herd immunity from the existing 13vPCV schedule may suffice. A 15-valent PCV has also recently undergone pre-clinical evaluation, is immunogenic against emerging serotypes 22F and 33F, important serotypes in the Northern Territory, and is now being evaluated in clinical trials. Whole cell pneumococcal vaccines (WCV) are a cost-effective option based on non-capsular antigens common to all strains. WCV’s induce serotype-independent immunity and are efficacious against pneumococcal infections in animal models. To address viral ALRI pathogens, routine scheduling of the trivalent influenza vaccine (TIV) or the live attenuated influenza vaccine (LAIV) from 6 months of age are potential options. TIV’s are currently recommended from 6 months of age in Australia but uptake is poor (15%) (Blyth et al. 2014). LAIV’s are not yet licensed in Australia but are more efficacious than TIV’s.

8.4.8 Smoking cessation

Indigenous Australians smoke at a much higher rate than non-Indigenous Australians (Thomas 2012). During the PneuMum randomised controlled trial (2006-2011; Chapters 3), half of all Indigenous mothers reported smoking during pregnancy and this increased post-natally to over 60% by infant age 7 months. Smoking is known to cause numerous chronic health problems and evidence from a meta-analysis shows that maternal smoking is associated with increased risk of infant lower respiratory infection both pre-natally (odds ratio 1.24, 95% CI 1.11 to 1.38) and post-natally
Maternal smoking has also been documented as a risk factor for infant respiratory infection in Australia (Moore et al. 2010). A recent study showed that culturally appropriate, multi-faceted quit programs can be successful among Indigenous populations (Marley et al. 2014). A smoking cessation program for the prevention of infant respiratory disease is also being planned for the future.

8.5 Final conclusions

This thesis showed that vaccinating with the 23vPPV in pregnancy had a non-significant impact against the clinical outcomes of infant middle ear disease and ALRI among high risk Indigenous infants. Regardless, the 23vPPV in pregnancy was safe and immunogenic and the disease rates were encouragingly lower among infants of pregnancy vaccinees, particularly for ear disease associated with concurrent nasopharyngeal carriage of vaccine serotypes. Future studies of 23vPPV in pregnancy are warranted, particularly where there are high rates of early pneumococcal disease. However, with the continually expanding number of serotypes included in pneumococcal conjugate vaccines the extended serotype coverage afforded by the 23vPPV may become less important. For any future trial, care should be taken to ensure adequate study power.

The second part of this thesis highlighted that up to four in five Indigenous infants of the Top End of the Northern Territory had vitamin D insufficiency at birth, despite the tropical climate. Cord blood vitamin D insufficiency was associated with a higher risk of subsequent ALRI and risk factors for lower vitamin D levels included breast feeding, pre-term birth and remote dwelling. Further work is required to strengthen
these findings. In the future, simple vitamin D supplementation strategies should be considered as part of routine ante-natal and pre-natal care for Indigenous mothers and infants.
Appendix A

PubMed literature search strategies
Appendix A: PubMed literature searches

Search 1

(Otitis media OR otitis OR ear disease) AND (Northern Territory) restricted to publications since 1992.

Output 1

Thirty six peer reviewed publications, five of which were relevant to the burden of otitis media among Indigenous children <6 years of age in the Northern Territory.

Search 2

(Otitis media) AND (Pneumococcal vaccines) AND (Australia) restricted to publications since 1990 and children <5 years of age

Output 2

Fourteen peer reviewed publications, two that were relevant to Indigenous children in the Northern Territory.

Search 3

(Aboriginal OR Indigenous) AND (Clinic OR Community) AND (Respiratory) AND (Northern Territory)

Output 3

Six peer reviewed publications, two that were relevant to the burden of clinic presentations for respiratory infections among Indigenous children in the Northern Territory.
Search 4
(Bacteria OR Viruses) AND (Acute respiratory infection OR Respiratory infection) AND (Indigenous OR Aboriginal) AND (Australia)

Output 4
Seventy eight peer review publications, only 3 of which investigated the microbial aetiology of respiratory infections among Indigenous children in the Northern Territory.

Search 5
(Pneumococcal vaccine) OR (Pneumococcal carriage) AND (Northern Territory)

Output 5
Thirty four peer reviewed publications, one of which compared pneumococcal carriage with respect to vaccination status.

Search 6
(Pneumococcal vaccine OR Pneumococcal vaccination OR Pneumococcal immunisation) AND (Pregnancy OR Maternal) OR (Papua New Guinea AND Pneumococcal vaccine) limited to human intervention studies.

Output 6
Thirty four peer reviewed publications comprising 12 relevant studies of 23vPPV in pregnancy with follow-up of maternal and infant outcomes.

Search 7
(Vitamin D) AND (Australia) AND (Indigenous OR Aboriginal)

Output 7
Eleven peer reviewed studies, four describing vitamin D levels in Indigenous Australian adults and one among Indigenous Australian children

**Search 8**

(Vitamin D) AND (Respiratory infection) AND (Cohort) AND (Cord blood)

**Output 8**

Five relevant studies relating cord blood vitamin D to the risk of subsequent respiratory infection.

**Search 9**

(Vitamin D) AND (Respiratory infection OR Pneumonia OR Bronchiolitis OR Otitis media) restricted to clinical trials among children <5 years of age.

**Output 9**

Eleven peer reviewed publications containing six relevant randomised control trials of children.
Appendix B

Geometric mean concentrations of blood and breast milk antibodies against 23vPPV serotypes by visit and randomisation group:
Supplementary data from Chapter 3.
### Appendix B: Geometric mean concentrations (GMC) of blood (IgG) and breast milk antibodies (IgA) against 23vPPV serotypes by visit and randomisation group.

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- **Maternal blood (n)**: Number of samples
- **Breast milk (n)**: Number of samples
- **Cord/Infant blood (n)**: Number of samples
- **Maternal IgG**: Geometric mean concentration
- **Breast milk IgA**: Geometric mean concentration
- **Cord/Infant IgG**: Geometric mean concentration

1. **(10v)**
2. **(7v10v)**
3. **(10v)**
4. **(10v)**
5. **(10v)**

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- **Maternal IgG**: Geometric mean concentration
- **Breast milk IgA**: Geometric mean concentration
- **Cord/Infant IgG**: Geometric mean concentration

1. **(10v)**: Concentrations for 10-valent meningococcal conjugate vaccine.
2. **(7v10v)**: Concentrations for 7-valent pneumococcal conjugate vaccine.
3. **(10v)**: Concentrations for 10-valent meningococcal conjugate vaccine.
4. **(10v)**: Concentrations for 10-valent meningococcal conjugate vaccine.
5. **(10v)**: Concentrations for 10-valent meningococcal conjugate vaccine.

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Serum antibodies (IgG) were measured for all 23vPPV serotypes. Breast milk antibodies (IgA) were measured for 15 of the 23vPPV serotypes.

The presented GMC’s are exponents of the log normal titres. GMC’s among pregnancy and birth vaccinees were compared to controls using a two-tailed t-test. **Bold:** significantly greater than control titres at p<0.05. *(7v10v)*Serotype contained in both the 7vPCV and 10vPHiD-CV. *(10v)*Serotype in 10vPHiD-CV. *#*6A was included as a vaccine-related serotype.
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