Title: **Determinants of Severe Pertussis Infection in Australian Children and Awareness and Uptake of Pertussis Vaccination in Adults.**

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ABSTRACT

Despite long standing immunisation programs, pertussis remains a challenging disease in Australia and globally, with young infants most at risk of death and severe disease. Globally, the emergence of pertactin negative strains of *Bordetella pertussis* has been observed, with implications unclear. Adults are a common reservoir of infection and a source of transmission to young infants, and vaccine coverage in this group is largely unknown. In this research program, we aimed to:

1. Describe the clinical severity of *Bordetella pertussis* infections in Australian children and investigate factors associated with severe disease;
2. Describe the impact of genotypic variants lacking pertactin expression on severity of pertussis infections in children; and
3. Assess community knowledge and awareness of pertussis infections and predictors of uptake of recent pertussis vaccination in the South Australian population.

Methods:

1. Medical, laboratory and vaccination records were reviewed for children admitted to hospital with a diagnosis of pertussis at any of eight participating tertiary paediatric hospitals around Australia. A severity scoring system was designed and used to determine predictors of severe pertussis disease in the enrolled children.

2. To assess the influence of emerging *B. pertussis* variants deficient in pertactin antigen, clinical details were collected from medical records of children presenting to, or admitted to one of three major paediatric hospitals in Australia (Women's and Children's Hospital, Adelaide, Princess Margaret Hospital, Perth, Children's Hospital at Westmead, Sydney) during 2008-2012 with a confirmed pertussis infection and an isolate available for determination of pertactin expression and genotyping.

3. A cross-sectional survey of randomly selected households was conducted in South Australia by Computer Assisted Telephone Interviews to ascertain pertussis (whooping cough) vaccination history and predictors of recent vaccine uptake (within previous five years) amongst South Australian adults. Knowledge, perceptions and attitudes towards pertussis infections and prevention were evaluated. Log binomial models were fit to assess predictors of awareness of adult pertussis vaccine availability

Results:

1. One hundred and twenty children who were hospitalised with pertussis were enrolled nationally. Over 40% of cases were in children less than two months of age. A pertussis severity score (PSS) was determined for all cases with the majority of children classified as not severe (PSS ≤ 5). Young age (< two months, p=0.014), presence of fever at admission (p=0.030), presence of co-infection (p=0.004) and prematurity (p=0.024) were associated with more severe disease.

2. A total of 199 *B. pertussis* isolates collected during 2008-2012 were identified from children presenting to, or admitted to, one of the three participating hospitals. One third of these isolates (35.7%; 71/199) were pertactin deficient. Over one third of cultured cases were from children less than three months of age (n=82/199; 41.2%). Most severe disease occurred in young, unimmunised infants. Adjusting for pertussis toxin promoter allele, vaccination status and age category, Prn status was not associated with disease severity (Risk Ratio=0.97, 95%CI: 0.57-1.62, p=0.90).

3. From 3124 randomly sampled contactable households, 1967 interviews with individuals aged 18-93 years were conducted (participation rate 63%), including 608 parents of children aged < 18 years. Recent adult pertussis vaccine coverage was low, with only 10% of respondents reporting pertussis vaccination in the previous

five years. Predictors of recent pertussis vaccination included higher education, larger household size, perception of greater disease severity and discussion with a family physician about pertussis vaccination. The majority of respondents (97%) had heard of pertussis (whooping cough) and many (73%) considered whooping cough to be highly contagious and severe for infants (89%). Whilst 61% of respondents were aware of the availability of an adult pertussis booster vaccine, only 8% (n=154) reported their general practitioner had discussed it with them. If provided free, 77% agreed that they would be more likely to accept a booster pertussis vaccination.

Conclusion:

B. pertussis infections continue to pose a threat to Australian children, particularly infants too young to have received direct protection through vaccination. Children admitted to hospital with *Bordetella pertussis* and fever or co-infection should be closely monitored, particularly if they have a history of prematurity. The rapid emergence of pertactin negative *Bordetella pertussis* variants do not appear to be associated with any increased severity of disease for children, however the impact of strain evolution on vaccine efficacy and transmissibility requires further investigation. Whilst knowledge regarding transmission and severity of *Bordetella pertussis* was high in the general community, uptake of adult pertussis vaccination is remarkably low amongst South Australians. Improved awareness regarding the availability of a booster pertussis vaccine through general practitioners and/or provision of funded pertussis vaccination for adults has the potential to improve pertussis vaccine coverage and provide greater protection for vulnerable infants.

THESIS DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed: _____ Date: _____

PUBLICATIONS DURING CANDIDATURE

This thesis contains three manuscripts, which have been published by international peer-reviewed journals, 'Paediatric Infectious Diseases Journal'-published; 'Journal of Infection'- and 'Vaccine'.

1) Marshall H, Clarke M, Rasiah K, Richmond P, Buttery J, Reynolds G, et al. Predictors of disease severity in children hospitalized for pertussis during an epidemic. *The Pediatric Infectious Diseases Journal*. (Impact Factor 3.135)

2) Clarke M, McIntyre P, Blyth C, Wood N, Octavia S, Sintchenko V, Giles L, Quinn H, Hanly G, Hill V, H, Lan R, Marshall H. Impact of pertactin deficient *B. pertussis* variants on clinical severity of pertussis disease in Australian children**.** *Journal of Infection* (Impact Factor: 4.441)

3) Clarke M, Thomas N, Giles L, Marshall H. Community awareness and predictors of uptake of pertussis booster vaccination in South Australian adults. *Vaccine*. (Impact Factor: 3.492)

Statement of contribution to manuscripts

For the first manuscript, Michelle Clarke is second author, having contributed to data collection and the statistical analysis plan with Helen Marshall as lead author conceiving and leading the national research study. The manuscript was co-authored by Helen Marshall and Michelle Clarke with statistical input and guidance from Data Management and Analysis Centre (Tom Sullivan and Suzanne Edwards) and the Women's and Children's Hospital Public Health Research Unit (Kate Dowling and Peter Baghurst). Michelle completed the final presented statistical analyses and prepared reports of statistical tables for review and discussion with above named statistical advisors. Other national co-authors contributed to editing and review of the manuscript and the final data analysis plan.

For the second manuscript, Michelle Clarke collected data with regards to clinical outcomes for children enrolled at the Women's and Children's Hospital, prepared national data collection tools and collated and cleaned data from all three participating sites. Michelle Clarke designed the analysis plan and performed all statistical analyses with Lynne Giles guiding and advising for more complex statistical analyses. Michelle Clarke prepared the first draft of the manuscript with guidance from Helen Marshall and Lynne Giles. HM, LG and co-authors from sites in NSW and WA assisted with study design, and/or contributed to review and editing of the manuscript.

For the third manuscript, Michelle Clarke performed all statistical analysis with guidance from supervisors, Helen Marshall and Lynne Giles. MC prepared the first draft of the manuscript under the direct supervision of HM and LG. HM and NT assisted with study design, and contributed to, reviewed and edited the manuscript.

LG provided guidance and advice to MC for statistical analyses, and contributed to, reviewed and edited the manuscript.

I confirm that all three manuscripts have been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all authors. I acknowledge the assistance of Chris Heath, Research Nurse and friend within the Vaccinology and Immunology Research Trials Unit (VIRTU) for her assistance with proof reading manuscripts and this thesis.

PRESENTATIONS DURING CANDIDATURE

During candidature, I presented work related to this Master's thesis at the School of Population Health Seminar series, the Robinson Research Institute Symposium (2014) and the National Public Health Association of Australia Communicable Diseases Conference (2015).

Oral presentations

1) Clinical severity comparisons between pertactin deficient and pertactin positive *Bordetella pertussis* variants. Communicable Diseases Conference, Brisbane, 01 June 2015
2) Community understanding of whooping cough and vaccine uptake in South Australian adults. University of Adelaide School of Population Health Seminar, 30 April 2015
3) Pertussis: a continuing challenge. Robinson Research Institute Symposium – invited oral presentation, 06 November 2014

Poster presentations

1) Community knowledge and uptake of booster pertussis vaccination – poster. Robinson Research Institute Symposium, Adelaide, 06 November 2014
2) Clinical severity comparisons between pertactin deficient and pertactin positive *Bordetella pertussis* variants. Florey postgraduate conference 24 September 2015 and Robinson Research Institute Symposium, Adelaide, 04 November 2014

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"There is no trust more sacred than the one the world holds with children. There is no duty more important than ensuring that their rights are respected, that their welfare is protected, that their lives are free from fear and want and that they can grow up in peace."

-- Kofi Annan

LIST OF ABBREVIATIONS

ABS	Australian Bureau of Statistics
ACIR	Australian Childhood Immunisation Register
ACT	Adenyl Cyclase Toxin
ATAGI	Australian Technical Advisory Group on Immunisation
CATI	Computer Aided Telephone Interview
CI	Confidence Interval
CPAP	Continuous positive airway pressure
DNT	Dermonecrotic Toxin
DTP	Diphtheria-Tetanus-Pertussis
DTPa	Diphtheria- Tetanus-acellular Pertussis
DTwP	Diphtheria-Tetanus-whole cell Pertussis
FP	Family Physician
GCP	Good Clinical Practice
GP	General Practitioner
HDU	High Dependency Unit
HREC	Human Research Ethics Committee
ICD	International Classification of Diseases
ICU	Intensive Care Unit
Ig	Immunoglobulin
IQR	Interquartile range
IV	Intravenous
LOS	Length of stay
NIP	National Immunisation Program

NNDSS	National Notifiable Disease Surveillance System
NSW	New South Wales
OR	Odds Ratio
PBAC	Pharmaceutical Benefits Advisory Committee
PCR	Polymerase Chain Reaction
PRN	pertactin
PROS	Population Research and Outcomes Studies
PSS	Pertussis Severity Score
PT	Pertussis Toxin
PTXP	Pertussis Toxin Promoter
RR	Risk Ratio
RSV	Respiratory Syncytial Virus
SA	South Australia
SD	Standard deviation
TCT	Tracheal Cytotoxin
UK	United Kingdom
US	United States
VIRTU	Vaccinology and Immunology Research Trials Unit
WA	Western Australia
WCH	Women's and Children's Hospital
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Bordetella pertussis infections remain a public health concern despite longstanding vaccination programs. Pertussis, also known as whooping cough, is the most common vaccine preventable disease in Australia, responsible for more than 24,000 cases in 2012, and as many as 38,000 cases at the peak of the most recent epidemic in 2011 [1]. Pertussis infection in children, particularly young infants, can be severe and potentially even fatal [2-4]. Adequate immunity against pertussis is unlikely to develop in an infant until they have received at least two doses of a pertussis containing vaccine (approximately 4 months of age) [2-4]. The most severe consequences of pertussis infection have been seen in the very young; ten of the 11 pertussis deaths occurring in Australia between 2006 and 2011 were in infants less than six months of age [5]. Despite a strong childhood immunisation program, where over 90% of children have received three doses of pertussis containing vaccine by 12 months of age [6], notification rates for pertussis reached 550 cases/100,000 children aged five to nine years during the latest pertussis epidemic in Australia [1]. A review of notified pertussis infections in South Australia during 2008-2009 showed that although the majority of notifications for pertussis occurred in adults, the highest notification rate and hospitalisation rate occurred in infants less than one year of age [7]. Post epidemic national notification rates for 2013 and 2014 indicate that notification rates reduced and have remained stable at approximately 100 cases per 100,000 children for young children (0-4y) and school aged children (5-14y) [1].

During the recent epidemic, various strategies were considered or implemented in attempts to reduce the burden of circulating disease and provide greater protection for vulnerable infants. This included bringing forward first vaccinations to 6 weeks

of age (instead of 8 weeks) and bringing forward the 4 year old booster pertussis vaccine to 3.5 years. Changes to recommendations for vaccination of adults have also occurred. Previously a single pertussis booster in adult life was all that was recommended for adults. Recently, recommendations for Australian adults involved with the care of, or household contacts of, young infants has changed to include booster pertussis vaccinations every 10 years. Furthermore, women planning a pregnancy who have not received a pertussis containing vaccine within five years are also recommended to be vaccinated prior to pregnancy, during the third trimester or following delivery [8]. Maternal immunisation as a strategy for preventing pertussis in infants is a very recent recommendation in the Australian context and its effectiveness for preventing pertussis in Australian infants is, as yet, unclear, although emerging data from the United Kingdom is promising. 'Cocooning', or vaccinating household contacts of newborns, is a strategy that was primarily implemented during the recent epidemic, but with varied programs and delivery models throughout Australia. In South Australia, this program was only funded for families who held a health care card, whereas some other states provided free pertussis booster vaccines for all families. Regardless of funding, it is unclear how valued or important pertussis booster vaccination is to the general community. The research program outlined in this thesis is aimed at improving protection of infants from severe pertussis disease through refining our understanding of predictors of severity and investigating the potential to reduce transmission to vulnerable infants through improved vaccine coverage in adults.

Vaccination programs are most effective when uptake is high and herd immunity interrupts transmission and therefore provides some protection for those who are unable to be directly vaccinated themselves [9]. Whilst the rate of uptake of adult pertussis vaccination is unclear, a 2009 adult vaccination survey suggested that only

11% of Australians aged 18 years or over reported having received a pertussis vaccination as an adolescent or adult [10].

Improving adult vaccination rates may increase protection for newborns as pertussis is commonly transmitted to infants from their parents [11-13], but there are currently limited data on adult pertussis vaccine uptake. The reasons why adults do not elect to receive a pertussis booster vaccine remain largely unknown. It is possible that awareness of such vaccines is low, or that adults do not perceive pertussis as an important disease to protect themselves against. Furthermore, the general community may not be aware of how pertussis is transmitted, or the severity of disease in infants, and may not understand that they could potentially protect their infant by being vaccinated themselves. Cost may also be a barrier to vaccination. Our current research aims to address these uncertainties and provide information on community knowledge and awareness of pertussis disease and prevention strategies.

Understanding what contributes to the severity of pertussis in children is important for reducing the burden of disease and protecting individuals and the community. A study investigating predictors of fatal pertussis infections in Canada found that pneumonia and leucocytosis were independent predictors of death in infants hospitalised with pertussis [14]. A small Australian study describing pertussis in infants requiring intensive care (n=49) also suggested that pneumonia and leucocytosis were predictors of poorer outcome [15].

Recently, several investigators have reported changes to the *Bordetella pertussis* genotype, demonstrating a shift in the compatibility between the vaccine antigens and circulating strains which may be contributing to the poor control of pertussis infections [16-20]. As pertactin is one of the antigens included in all Australian acellular pertussis vaccines, *B. pertussis* variants lacking expression of this antigen

may have an advantage for evading vaccine induced immunity. We will assess the impact of pertussis genotype on severity of clinical disease and also determine whether clinical symptoms at presentation to hospital can predict more severe disease and outcomes. Results of this research can be used to inform health policy and contribute to improved protection against severe pertussis for Australian children.

1.2 RESEARCH QUESTIONS

The overarching aim of our research is to investigate predictors of severe pertussis in children and determine whether changes to circulating *B. pertussis* genotypes, particularly the rapid expansion of *B. pertussis* variants that are pertactin deficient, are associated with altered severity. An additional aim is to evaluate community knowledge and awareness of pertussis infections and vaccination practices in the South Australian community to determine need and opportunity for improved protection for infants through increased vaccine coverage in adults.

We addressed the following research questions:

1. What factors are associated with increased severity of disease for children hospitalised with pertussis infections? (Chapter 4)
2. Will the rapid emergence of pertactin deficient strains of *B. pertussis* have an impact on severity of pertussis for Australian children? (Chapter 5)
3. What does the South Australian community understand about pertussis disease and prevention strategies and what proportion of South Australian adults have received a recent pertussis booster vaccine? (Chapter 6)

It is important to understand clinical implications of evolutionary changes to circulating pathogens, particularly for pertussis which has been responsible for more than 10 infant deaths in Australia over the last decade. Our study results can be used to inform decisions about vaccination policy. Our investigation of community knowledge and awareness of pertussis can provide insight into inadequacies with current pertussis prevention programs and useful information to guide policy makers and immunisation educators in the development of strategies to promote and improve vaccine awareness and uptake in adults.

1.3 THESIS OUTLINE

This thesis is structured as follows. Chapter 1 provides a brief introduction to the background and aims of the research. Chapter 2 outlines the current and key literature around *B. pertussis* infection and control, describes previous studies, and outlines the rationale for the research projects. The third chapter describes the methods used in the conduct of the research projects.

Chapter 4 (paper 1) provides results of the national study investigating clinical characteristics and predictors of severity of pertussis infections in a nationally representative sample of children hospitalised with confirmed pertussis during an epidemic (2008-2009). A manuscript has been published in *The Paediatric Infectious Diseases Journal* (see Appendix 2). In Chapter 5 (paper 2), associations between *Bordetella pertussis* pertactin (Prn) antigen expression and pertussis disease severity for children presenting to, or admitted to hospital between 2008 and 2012 are described. A manuscript has published in *Journal of Infection* (see Appendix 2). Chapter 6 (paper 3) describes community awareness of pertussis and predictors of uptake of adult pertussis vaccination in South Australia. The corresponding manuscript has been published in the international peer-reviewed journal *Vaccine* (see Appendix 2).

Chapter 7 summarises the research findings, discusses some of the strengths and limitations and suggests further research goals.

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CHAPTER 2: LITERATURE REVIEW

Pertussis, commonly referred to as 'whooping cough', is an acute bacterial respiratory infection caused primarily by the bacterium *Bordetella pertussis*. *Bordetella pertussis* infections remain a public health concern despite longstanding vaccination programs. The following literature review will highlight and discuss key scientific knowledge of the microbiology, epidemiology, disease burden, prevention strategies and clinical implications of *B. pertussis* infections.

2.1 Pertussis (whooping cough) – Aetiology and clinical features

Pertussis, or whooping cough, is the illness that arises from infection with the small gram negative encapsulated bacterium, *Bordetella pertussis* and to a lesser extent, *Bordetella parapertussis*. *B. pertussis* and *B. parapertussis* are both within the genus Bordetella in the Alcaligenaceae family, with *B. pertussis* responsible for the majority of pertussis disease, particularly severe disease [1, 2] and the primary focus of this thesis. *B. pertussis* has no known animal or environmental reservoir and is solely a pathogen of humans [3]. The organism in a fastidious aerobe, surviving only a few hours in respiratory secretions and requires specialised medium for culture (Bordet-Gengou medium or charcoal-horse blood agar) [2].

B. pertussis was first isolated in 1906 by Jules Bordet and Octave Gengou [4], however the illness was named 'pertussis', meaning violent cough, by Sydenman in 1679 [5] and epidemics have been recognised and attributed to pertussis as early as 1578 [6].

B. pertussis is highly contagious and transmission occurs readily between humans through respiratory droplets. Therefore atypical or mild cases in older children and adults can be important in spread of the infection [7].

Pertussis has an incubation period of 7 to 20 days [8] and disease progression is often classified into three phases – catarrhal, paroxysmal and convalescent [9]. The early symptoms may appear similar to a common cold which can result in a delayed diagnosis during this highly infectious period. This catarrhal phase is then followed by the paroxysmal phase, in which paroxysms of cough become more frequent and intense, often worse at night, before paroxysmal coughing subsides (see Table 1).

Table 1: Clinical features of classical pertussis:
Adapted from source: http://www.cdc.gov/pertussis/clinical/features.html

	Length	Clinical Features
Stage 1: Catarrhal	Usually 7-10 days; (range 4-21)	Characterised by: Coryza Low-grade fever Mild, occasional cough (gradually becomes more severe)
Stage 2: Paroxysmal	Usually lasts 1-6 weeks, but may persist for up to 10 weeks	Characterised by: Paroxysms of numerous, rapid coughs due to difficulty expelling thick mucus from the tracheobronchial tree Long aspiratory effort accompanied by a high-pitched "whoop" at the end of the paroxysms Cyanosis Vomiting and exhaustion Paroxysmal attacks: Occur frequently at night, with an average of 15 attacks per 24 hours Increase in frequency during the first 1-2 weeks, remain at the same frequency for 2-3 weeks, and then gradually decrease
Stage 3: Convalescent	Usually 7-10 days; range of 4-21	Characterised by: Gradual recovery Less persistent, paroxysmal coughs that disappear in 2-3 weeks Paroxysms often recur with subsequent respiratory infections for many months after the onset of pertussis.

Whilst classical pertussis presents with a characteristic paroxysmal cough with inspiratory whoop, atypical features are common for very young infants and

previously immunised adults [10]. Young infants may present with apnoea and cyanotic episodes and a cough without the characteristic whoop [10]. Pulmonary hypertension and extreme leucocytosis have also been reported complications of pertussis infection in infants [9].

Death due to pertussis infection is rare in older children and adults, with the majority of pertussis deaths occurring in infants less than six months of age [9, 11]. The case fatality rate in infants less than six months of age has been estimated to be 0.8% [8]. The most common cause of death in persons with pertussis infection is pneumonia, and severe cases can be complicated by pneumonia seizures and hypoxic encephalopathy [9], [12].

Clinical presentation of pertussis in vaccinated children and older adults may vary from asymptomatic infection [13, 14] or prolonged coughing illness with or without post-tussive vomiting to intracranial haemorrhage and rib fracture [10].

A German study reporting clinical features of pertussis in adolescents and adults demonstrated that the dominant symptom of pertussis in adults was a persistent cough, however complications including pneumothorax, inguinal hernia, and carotid artery dissection have been reported [15].

2.2 Pertussis transmission and virulence

B. pertussis is considered highly contagious, with up to 90% of unvaccinated persons within a household likely to become infected from an index case [16]. Untreated cases can remain infectious for up to three weeks, although the use of antibiotics can reduce this to as little as five days [10]. Neither vaccination nor natural infection provide long-term protection and repeat infection can occur [16-19].

Adults have been recognised as an important reservoir for pertussis [17, 20, 21]. Adults often have a milder or atypical illness and are less likely to be diagnosed and

treated than children. This in combination with the likelihood of waned vaccine immunity allows for increased opportunity for transmission to vulnerable infants and unvaccinated children [22]. It has been estimated that *B. pertussis* accounts for up to 7% of cough illnesses per year in adults [8]. Asymptomatic pertussis has been widely reported [13, 14, 23].

Studies investigating the source of transmission of *B. pertussis* to young infants consistently identify parents and siblings as the most important sources [24-30]. A UK study of children hospitalised with pertussis identified parents as the most common source of transmission (14/33, 42%) [28]. Two Australian studies have found that siblings and parents were frequently identified sources of transmission to infants, with young siblings the most important transmitters [26, 29]. A recently conducted review of sources of transmission of pertussis to infants in high income countries found that over half of identified sources of infant pertussis infection were a parent, with mothers approximately twice as likely as fathers to be an identified source [25].

Bordetella pertussis readily colonises the mucosal membranes of the human respiratory tract [2]. The organism has various virulence factors that enable it to attach to ciliated epithelia, replicate effectively and evade host defences (Figure 1). *Bordetella pertussis* primarily causes diseases in the human host through release of toxins, including Pertussis Toxin (PT), Tracheal Cytotoxin (TCT), Dermonecrotic Toxin (DNT) and Adenyl Cyclase Toxin (ACT) [17]. Adhesion factors include Pertactin (Prn), Filamentous Haemagluttinin (FHA) and Fimbriae (Fim 2,3). These adhesion factors allow colonisation of the respiratory tract and enhance bacterial replication whilst the secreted toxins (PT, TCT and ACT) damage local tissues and inhibit the immune response by suppressing the activation and chemotaxis of T cells and blocking phagocytosis [17, 31].

Bordetella pertussis is the only species of the Bordetella genus to secrete PT giving it the ability to induce lymphocytosis in mammals. PT was one of the earliest identified virulence factors and most extensively characterised [31].

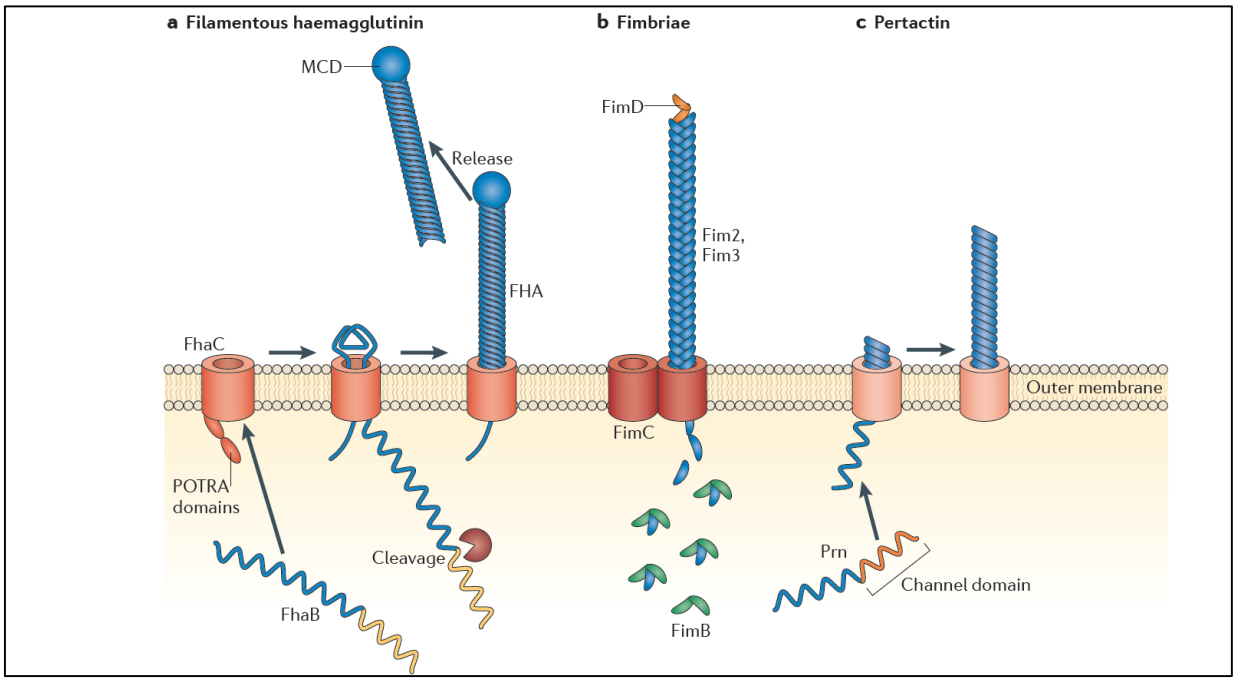

Figure 1: Presentation of filamentous haemagglutinin, fimbriae and pertactin on the *Bordetella* cell surface. Source: Melvin JA, et.al. *Bordetella pertussis* pathogenesis: current and future challenges. Nature Reviews Microbiology [31]

2.3 Pertussis epidemiology

2.3.1 Global epidemiology

B. pertussis continues to cause morbidity and mortality worldwide [32]. In 2008, the World Health Organization (WHO) estimated that approximately 195,000 children died as a result of pertussis infection [33]. A resurgence of pertussis has been recognised over the last decade [34-37]. In the US, this increase in pertussis incidence has been demonstrated in all age groups (Figure 2a and 2b).

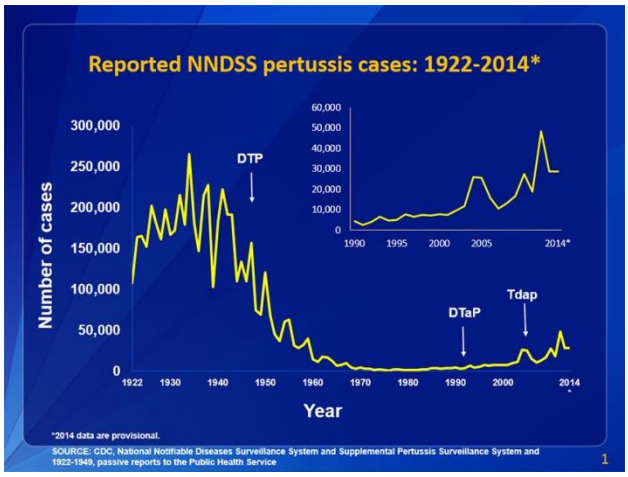

Figure 2a: Reported NNDSS pertussis cases in USA: 1922-2014.
Source: CDC – available at: http://www.cdc.gov/pertussis/surv-reporting.html

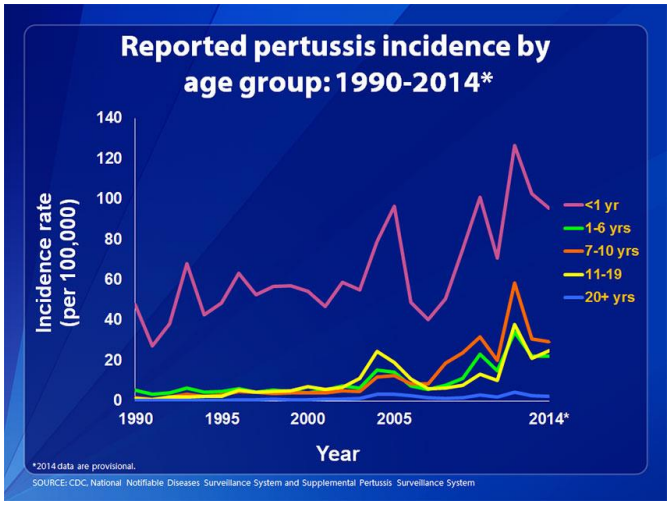

Figure 2b: Reported pertussis incidence in USAby age group-1990-2014.
Source: CDC – available at: http://www.cdc.gov/pertussis/surv-reporting.html

The resurgence of pertussis may be explained by several factors, including improved detection through more sensitive Polymerase Chain Reaction (PCR) diagnostic tests, ease of testing [38] and reduced duration of immunity following the introduction of acellular vaccines in Australia in 1997 [16, 18, 19].

Questions have also arisen regarding the impact of a change from whole cell pertussis vaccines to acellular vaccines on the duration of pertussis immunity and evolution of *Bordetella pertussis*. The most prominent recent changes in circulating *B. pertussis* strains are polymorphisms in the promoter of the pertussis toxin promoter operon (*ptxP*), and the non-production of pertactin [35, 37, 39-43]. Previously, *ptxP3* has been shown to be associated with increased pertussis toxin production as a result of a single base mutation in the *ptxP* and with increased hospitalisations in the Netherlands [37].

In Australia, *ptxP3 B. pertussis* isolates predominated during the 2008-2012 epidemic [42]. Similar findings have been demonstrated in European countries and in the Netherlands [39, 44]. There is also increasing evidence worldwide of emergence of *B. pertussis* variants that are deficient in the pertactin (Prn) protein [10, 18-22].

2.3.2 Pertussis epidemiology in Australia

Pertussis epidemics occur every 3-4 years in Australia [8], with the most recent epidemic between 2008 and 2011, the worst since the introduction of pertussis vaccination in the 1940s (Figure 3).

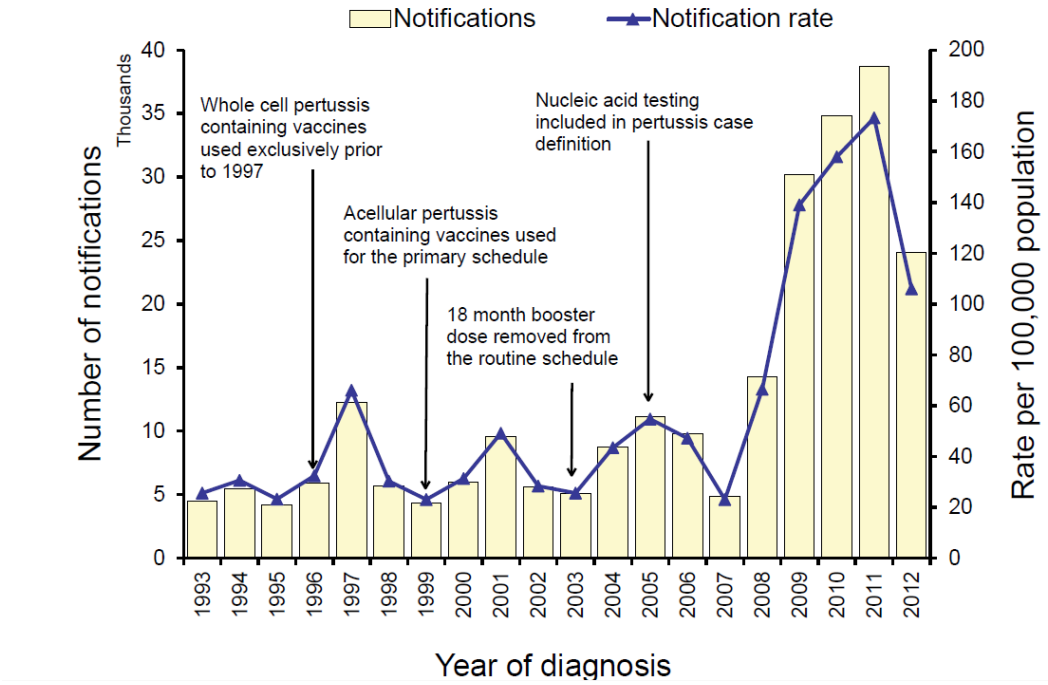

Figure 3: Notifications and notification rates for pertussis, Australia, 1993 to 2012
Source: Australia's notifiable disease status, 2012: Annual report of the National Notifiable Diseases Surveillance System. NNDSS Annual Report Writing Group [45]

At the peak of the Australian epidemic in 2011, almost 38,000 cases of pertussis were notified, with incidence rates increasing to 450/100,000 for infants and young children [46]. Notification rates for pertussis between 2007-2012 by age and sex indicate a slight female predominance across all age groups for notified pertussis. Rates in children are at least twice that of notification rates for all other age groups (Figure 4)[45].

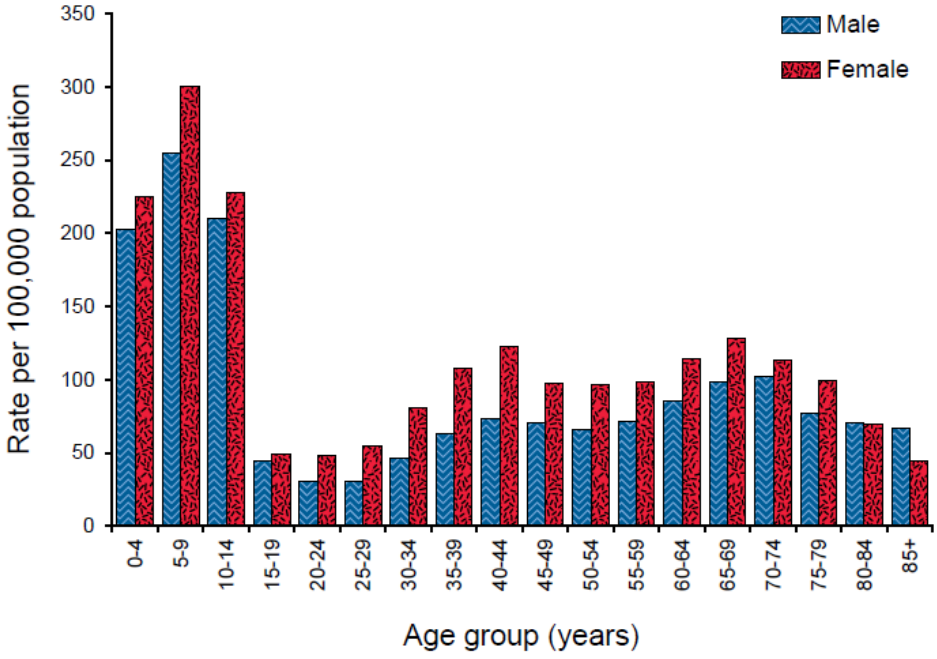

Figure 4:
Notification rate for pertussis, 2007 to 2012, by age group and sex
Source: Australia's notifiable disease status, 2012: Annual report of the National Notifiable Diseases Surveillance System NNDSS Annual Report Writing Group[45]

Approximately 200 infants are hospitalised each year in Australia, the majority being less than five months of age and notification rates often higher in Aboriginal than non-Aboriginal individuals. A report including pertussis epidemiology for 2005-2010 describes the overall indigenous to non-indigenous rate as 2.9:1[47]. Between 2006 and 2011, 11 pertussis deaths were reported, with 10 of these occurring in infants less than six months of age [48].

An Australian Paediatric Surveillance Unit study conducted in 2001 found that 65% of hospitalisations for pertussis were in children too young to be vaccinated [49]. Whilst infants are most at risk of severe pertussis requiring hospitalisation, a review of pertussis epidemiology in South Australia revealed that during the last pertussis epidemic (Jul 2008-Dec 2009), the majority (66%) of notified pertussis infections occurred in adults over 24 years of age [20]. Hence, strategies to reduce the prevalence of pertussis infections in adults may provide greater protection for infants through herd immunity and reduced potential for transmission.

2.4 Pertussis vaccination

A dramatic reduction (>90%) in pertussis incidence and mortality was observed in the industrialized world following the introduction of pertussis vaccination during the 1950s - 1960s. Pertussis vaccination has been part of the WHO Expanded Program on Immunization since its inception in 1974 [33]. Pertussis vaccination programs are well received in many countries with global coverage for three doses of DTP vaccines by 12 months of age estimated to be 86% [50].

Pertussis immunisation was introduced in Australia more than 60 years ago, initially as a whole cell pertussis vaccine and subsequently as an acellular vaccine. Presently, the National Immunisation Program (NIP) recommends that infants receive a dose of acellular pertussis vaccine at 2, 4 and 6 months of age, with a booster dose recommended and funded at 4 years of age and again during adolescence [8]. An additional booster dose at 18 months of age will also be reinstated from October 2015. Childhood pertussis vaccination programs have excellent uptake in Australia, with more than 90% of children having received all three doses of primary pertussis vaccine before 12 months of age [51]. Previous literature suggests that protection against pertussis requires at least two doses of the current acellular vaccine for effective protection against pertussis [21]. An Australian study published in 2009 on adolescent acellular pertussis vaccine reported vaccine effectiveness of at least 78% against pertussis [52]. A recent Australian study has suggested that the vaccine erffectiveness of 3 doses of DTaP against reported pertussis was 83.5% (95% CI, 79.1%–87.8%) between 6 and 11 months, 70.7% (95% CI, 64.5%–75.8%) between 2 and 3 years of age and declines to 59.2% (95% CI, 51.0%–66.0%) for children between 3 and 4 years of age [19]. Various studies have assessed vaccine efficacy for whole cell and acellular pertussis vaccines [53-56]. Duration of immunity and effectiveness of vaccination

against pertussis may be lower with acellular pertussis vaccines compared with the previous whole cell vaccines, with a recent US case control study finding that teenagers who had received four doses of the whole cell DTwP (Diphtheria – Tetanus – whole cell Pertussis) vaccine were five times less likely to have a positive pertussis PCR compared to teenagers who had received four doses of the acellular DTaP (Diphtheria-Tetanus-acellular Pertussis) vaccine during a pertussis outbreak [57].

Pertussis vaccination is recommended for any adult who wishes to reduce the likelihood of illness from pertussis, but it is not currently funded as part of the Australian National Immunisation Program. Vaccination against pertussis is strongly recommended for healthcare workers, childcare workers and household contacts/carers of young infants with boosters suggested every 10 years [8]. Women planning a pregnancy who have not received a pertussis containing vaccine within five years are also recommended to be vaccinated prior to pregnancy, during the third trimester or following delivery. Research has also shown that parents and siblings are an important source of transmission of pertussis infection to vulnerable newborns [25] and therefore adult/adolescent vaccination may provide protection against pertussis for the individual as well as other vulnerable populations.

With infants most at risk of hospitalisation and death from pertussis, potential strategies investigated to reduce severe pertussis disease in infants have included earlier direct vaccination, including potentially vaccinating newborns [58], ‘cocooning’ whereby household members or close contacts of newborns are provided a booster pertussis vaccination to reduce likelihood of transmission to infants [25], and more recently, immunisation during pregnancy [8]. Whilst the most effective strategies are still not clear, pertussis vaccination of mothers during the

third trimester of pregnancy has been recently recommended in several countries including Australia, New Zealand, UK and the US [8, 59-61].

Vaccine uptake for pertussis booster vaccination is largely unknown due to a lack of systematic monitoring but a 2009 national survey of adult vaccination suggested that approximately 11% of Australians aged 18 years or over reported having received a pertussis vaccination as an adolescent or adult [62]. This national survey was prior to the large, recent pertussis epidemic in Australia, and was unable to address reasons for low uptake, or barriers or facilitators for improved uptake.

A study published in 2004 conducted in Canada suggested that the majority of surveyed adults (59%) indicated willingness to receive pertussis immunisation if provided free and 87% reported that they would be likely to receive a pertussis vaccine if it were recommended to them by their nurse or doctor. This study was limited by the potential for substantial selection bias with a very low response rate (15%) [63]. A small, recent study assessing the knowledge and acceptability of adult pertussis immunisation in Korean women of childbearing age demonstrated that most women were not aware of the availability or effectiveness of pertussis vaccination and were not provided with any recommendation to receive the vaccine [64]. In Australia, with the exception of pregnant women, pertussis booster vaccination is not provided free for adults. Therefore, cost may also be a barrier to uptake. Future studies to understand barriers to provider vaccine recommendations around adult pertussis vaccination have been recognised as an important step towards developing interventions to improve adult coverage and reduce pertussis infection burden [65].

With the greatest burden of severe pertussis disease apparent in infants too young to be vaccinated, strategies to interrupt transmission or provide protection through transfer of maternal antibodies are essential for improved control of severe pertussis.

Increasing coverage in the population, particularly those in contact with young infants, has the potential to save infant lives. To achieve this, increasing awareness of pertussis disease and the availability of adult booster vaccines in the community is an important strategy as vaccine programs in Australia rely on public confidence. Improved vaccine uptake has been linked to awareness and education in previous studies [66, 67].

2.5 Severe pertussis

Aside from strategies to optimise our current immunisation program for protection against pertussis, through maximising uptake and delivering vaccines to the most at risk of either severe disease, or transmission to vulnerable populations, it is important to understand other risk factors associated with increased severity of disease. Previous research has suggested that leucocytosis (elevated white cell counts) is indicative of more severe disease and poorer outcomes, along with the presence of pneumonia in a cohort of patients hospitalised with pertussis infection [9, 12]. Indigenous status has been considered a risk factor for more severe disease as indicated by the significantly higher rate ratio for hospitalisation in indigenous Australians compared with non-indigenous Australians [68]. Establishing predictors of poorer outcomes, or more severe disease in infants infected with pertussis will allow improved management and potentially prevention of infant deaths.

Whilst recent literature highlights significant changes to the circulating *B. pertussis* genotypes, including altered pertactin and pertussis toxin antigens, the clinical consequences of these evolutionary changes are unclear [41, 43, 69-72]. Previously, *ptxP3* carrying strains have been shown to be associated with increased pertussis toxin production as a result of a single base mutation in the ptxP allele and with increased hospitalisations in the Netherlands [37]. Murine studies have indicated that Prn deficient strains remain virulent, with similar invasion and cytotoxicity

properties compared with strains expressing Prn [73]. *B. pertussis* mutants which do not express Prn may persist longer in the epithelia than Prn expressing variants [74]. Two recent studies examining clinical findings in children and associations with Prn status of isolates suggest symptoms and clinical course are similar or reduced, with no apparent difference in requirement for hospitalisation or presence of symptoms (with the exception of apneoa which was less likely in Prn deficient infections) when compared to Prn positive cases [41, 69]. Our research adds to this literature by providing additional evidence from Australian children, with larger numbers of young infants, who are most at risk of severe disease to support the findings that infections with pertactin deficient *B. pertussis* strains are not more severe than pertactin positive strains. In addition, our research highlights the potential for *ptxP3* strains to cause more severe disease, which supports epidemiological findings from the Netherlands [37].

This thesis addresses the gaps in current knowledge required to prevent severe pertussis disease in infants through increasing our understanding of the clinical features and predictors of severe pertussis, providing data and evidence regarding the impact of evolutionary changes on severe pertussis disease in children, and estimating pertussis knowledge, prevalence of recent pertussis vaccination, and predictors of uptake in South Australian adults.

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CHAPTER 3: METHODOLOGY.

The main objectives of this research program were to:

- Describe clinical features and predictors of increased disease severity for Australian children hospitalised with confirmed pertussis;
- Determine the relationship between emerging *B. pertussis* variants and pertussis disease severity in Australian children; and
- Estimate the awareness and uptake of pertussis booster vaccination in adults.

In this Chapter, the broad overall approach and more detailed description of methods of data collection and statistical analysis are presented. In subsequent chapters, an overview of these details is presented in each of the manuscripts which together comprise the research that addressed the main objectives of this thesis.

Study 1: To achieve the first objective, a national, multicentre cross-sectional observational study was designed to determine the clinical severity of pertussis infection and epidemiological risk factors in Australian children admitted to hospital in one epidemic season (May 2009-April 2010) using a novel and simplified objective scoring system. To maximise sample size and national representativeness, children who were admitted to hospital with confirmed pertussis at any of the eight tertiary paediatric hospitals around Australia during the study period were eligible for enrolment. To minimise any influence of misclassification bias, children admitted with pertussis were only eligible for inclusion in this study if they met the Australian National Case Definition for a confirmed case of pertussis (Table 2).

Table 2. Australian National Case Definition for pertussis
Source: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-casedefs-cd_pertus.htm

Confirmed case A confirmed case requires either: • Laboratory definitive evidence, OR • Laboratory suggestive evidence AND clinical evidence.
Laboratory definitive evidence 1. Isolation of *Bordetella pertussis* , OR 2. Detection of *B. pertussis* by nucleic acid testing. 3. Seroconversion in paired sera for B.pertussis using whole cell or specific B.pertussis antigen(s) in the absence of recent pertussis vaccination.
Laboratory suggestive evidence In the absence of recent vaccination, 1. Significant change (increase or decrease) in antibody level (IgG, IgA) to ***B. pertussis*** whole cell or ***B. pertussis*** specific antigen(s) 2. Single high IgG and/or IgA titre to Pertussis Toxin (PT) 3. Single high IgA titre to Whole Cell ***B.pertussis*** antigen.
Clinical evidence 1. A coughing illness lasting two or more weeks, OR 2. Paroxysms of coughing OR inspiratory whoop OR post-tussive vomiting.
Probable case A probable case requires clinical evidence AND epidemiological evidence
Epidemiological evidence An epidemiological link is established when there is: 1. Contact between two people involving a plausible mode of transmission at a time when: a. one of them is likely to be infectious (from the catarrhal stage, approximately one week before, to three weeks after onset of cough) AND b. the other has an illness which starts within 6 to 20 days after this contact AND 2. At least one case in the chain of epidemiologically linked cases (which may involve many cases) is a confirmed case with either laboratory definitive or laboratory suggestive evidence.

Note: Both **confirmed cases** and **probable cases** should be notified.

A data collection tool was designed to capture relevant information from admission records or parents (where applicable) in a standardised way to enable scoring of disease severity according to a pertussis severity scoring (PSS) system designed by Helen Marshall and Kavita Rasiah for the purpose of this study (Figure 2). Using this scoring system, the minimum score achievable was 2 and the maximum score achievable was 15 (highest PSS=15). Based on the distribution of the severity scores for the overall study, cases were classified as severe if their assigned score was greater than the median group score (PSS=5).

Table 3. Pertussis Severity Scoring System

Pertussis severity score for children with pertussis infection				
	Grade 0	Grade 1	Grade 2	Grade 3
Duration of hospitalisation	Not hospitalised	< 24 hours	1-7 days	> 1 week
Level of hospitalisation	Not hospitalised	General ward	HDU	ICU
Hydration	Oral – breast/bottle	Nasogastric	IV hydration < 48hrs	IV hydration ≥ 48hrs
Respiratory support	None required	Suction/ oxygen	CPAP	ventilation
Presence of complications	none	Dehydration/ Hypoxia Chest X-ray changes	Pneumonia Clinical or radiological	Encephalopathy/ cardiomyopathy (complications requiring ICU admission)

De-identified questionnaires were all sent to the lead site (Adelaide) where pertussis severity scores were determined by two investigators. To minimise reporting bias, vaccination history for enrolled participants was verified using the Australian Childhood Immunisation Register.

The pertussis severity scoring system was internally validated by assessing the median severity score for groups included in each possible score for individual parameters. To explore associations between the ordinal indicators of severity, Pearson correlation coefficients were calculated and to assess internal consistency, Cronbach's alpha was calculated for the overall sum of the items and for the sum excluding items one at a time.

Cronbach's alpha was calculated to be 0.75, indicating reasonable internal consistency between the items within the Pertussis Severity Score.

Univariate and multivariable logistic regression models were used to assess predictors of a binary outcome (PSS ≤ 5 “not severe” or PSS > 5 “severe”). Results were reported as Odds Ratios with 95% Confidence Intervals. Consideration was given to clinical importance and relevance for variables retained in the final model and models were compared to maximize the fit of the regression model. Statistical analyses were performed using either SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) or STATA version 11 (StataCorp, Texas).

Study 2: We investigated the relationship between *B. pertussis* genotype and pertussis disease severity, using a multicentre observational study design. We identified children who had *B. pertussis* isolates that had been sent to NSW for genotyping during the period 2008-2012. Cases were eligible for inclusion in this study if they were less than 18 years of age, if they had genotyping data available for either expression of Pertactin (Prn) or ptxP promoter, and if they either presented to, or were admitted to one of three participating paediatric tertiary hospitals (WA, SA, NSW). Clinical data such as admission requirement, duration of admission and evidence of complications such as pneumonia were captured from hospital records

and vaccination data were collected from the Australian Childhood Immunisation Register. All data were entered onto a master database by sites. The master database was assessed and cleaned to ensure only independent and eligible cases remained. This involved verifying and removing duplicate samples from the same patient, verifying patients attended an emergency department or were admitted to the hospital for pertussis symptoms and ensuring all cases met the age definition (less than 18 years). Severe disease was defined as a requirement for long admission (greater than seven days), admission to an intensive care unit, or if death occurred.

As this primary outcome variable was binary and was not rare (almost 20% of eligible cases were classified as severe), log binomial regression was used to measure associations between various predictor variables and likelihood of severe disease. Results were expressed as Risk Ratio estimates and 95% Confidence Intervals. *B. pertussis* genotype (Prn and *ptxP3*) were included as predictor variables in a multivariable binomial regression model along with potential confounding variables. All statistical analyses were performed using STATA 11 with statistical significance defined as $p<0.05$. Potential confounding variables included age, vaccination status, ptxP3 promoter type, Aboriginality. These were selected and included based on potential association with more severe disease either through previous literature or clinical opinion. As several predictor variables in the final model were correlated, and sample size did not allow for modelling interactions between these variables (i.e. age and vaccination history), we investigated any variance inflation issues that might arise in such situations. Models with and without each of the correlated variables were fit and compared. As each of the models provided similar conclusions for direction and magnitude of association, and standard errors were not greatly inflated (variance inflation factor <4), all correlated

variables of interest (Prn, *ptxP3*, age, vaccination history) were retained in the final multivariable model.

Chi square tests of association or Fisher's exact test, as appropriate, were used to assess differences in proportions of Prn deficient vs Prn positive isolates with specific characteristics of interest (i.e. co-infection, absence of prior vaccination). T-tests (or Mann-Whitney, as appropriate) were used to assess differences in means/medians between Prn deficient vs Prn positive isolates.

Study 3: To estimate uptake and awareness of adult pertussis booster vaccination, a survey of South Australian households was conducted. This cross-sectional study was carried out as part of the 'Health Monitor' program conducted by the Population Research and Outcomes Studies (PROS) unit, University of Adelaide, South Australia (SA). The component of the study was approved by the Human Research Ethics Committees of the Women's and Children's Hospital and the University of Adelaide. The Health Monitor survey uses a random sampling process based on the South Australian electronic White Pages household telephone listings in both metropolitan and rural areas and selects the household contact (aged ≥ 18 years) who most recently had a birthday as the desired respondent. The interviews were conducted by the Computer-Assisted Telephone Interviewing (CATI) method with up to six call-backs made to interview the identified individual. Phone calls were made to households at different times between 9am and 9pm over seven days per week to maximise response rate. A pilot study of 50 randomly selected households was completed in March 2011 to test the question formats and sequence prior to commencement of the main study.

The structured survey was designed to determine the level of knowledge and community awareness of pertussis and the recommendation and availability of the adult pertussis booster vaccine (Appendix 2). Respondents were asked about prior

pertussis vaccination, knowledge and experience with pertussis infections. For simplicity, the term ‘whooping cough’ was used throughout the survey rather than ‘pertussis’.

For the purpose of the current study, a sample size of >1500 respondents enabled the proportion within the community who had knowledge of pertussis, or who had been vaccinated against pertussis in the last five years, to be estimated with a ± 2.5% precision at a 95% confidence level.

All survey responses were weighted to ensure that survey findings were applicable to the South Australian population. Survey weights were calculated from the inverse probability of selection of a household and re-weighted to sex, age and geographical area profile (metropolitan or rural) according to ABS 2009 Estimated Residential Population data for South Australia.

Estimates of population percentages for respondent characteristics with 95% CIs were determined. Univariate log binomial regression models were used to assess factors associated with awareness and uptake of pertussis booster vaccination with outcomes reported as risk ratios (RR) with 95% CI. Multivariable models were developed to assess adjusted RRs. All analyses were carried out using STATA version 11 (StataCorp, Texas).

All three studies were conducted in accordance with National Statement for Ethical Conduct in Human Research and The Australian Code for the Responsible Conduct of Research. Studies were performed in accordance with Human Research Ethic Committee’s requirements and applicable policies of the Women’s and Children’s Hospital, The University of Adelaide and the South Australian Department of Health.

CHAPTER 4: Manuscript 1 - Predictors of disease severity in children hospitalised for pertussis during an epidemic.

In order to prevent severe infections in children, we first need to clearly understand key clinical characteristics of these infections in our population, and determine clinical, demographic and environmental factors which may influence the likelihood of severe disease. Recognising factors associated with pertussis disease severity is vital for improving medical management of infected children, developing programs targeted at those most at risk, and for providing the best protection possible for those within our population who are most vulnerable.

The manuscript, “Predictors of disease severity in children hospitalized for pertussis during an epidemic”, reports results of a national study describing the burden of disease, clinical characteristics and predictors of severity of pertussis disease in children admitted to one of eight participating hospitals during a 12 month period (May 2008-April 2009). The manuscript has been published in *The Paediatrics Infectious Disease Journal* (Appendix 2).

This manuscript uses a novel pertussis severity scoring system to objectively classify cases and assess predictors of severe disease. This manuscript provides relevant recent data concerning the clinical characteristics of children hospitalised in Australia with pertussis.

Statement of Authorship

Title of Paper	Predictors of disease severity in children hospitalised for pertussis during an epidemic.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Publication Style
Publication Details	Marshall H, Clarke M, Rasiah K, Richmond P, Buttery J, Reynolds G, et al. Predictors of disease severity in children hospitalized for pertussis during an epidemic. Pediatr Infect Dis J. 2015;34(4):339-45. Epub 2014/09/2

Principal Author

Name of Principal Author (Candidate)	Michelle Clarke
Contribution to the Paper	MC assisted with data collection at the least site in South Australia. MC designed analysis plan in conjunction with Helen Marshall and statistical units within Adelaide University and Women's and Children's Hospital (WCH). MC performed statistical analysis and interpretation with assistance from Data Management and Analysis Centre (University of Adelaide) and Women's and Children's Hospital public health unit. MC co-authored the manuscript with Helen Marshall with additional revision and editing from co-authors. MC was second author for this manuscript.
Overall percentage (%)	40%
Signature	Date 10 Aug 2015

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Helen Marshall
Contribution to the Paper	HM was the chief investigator of this national study and guided and supervised the project from concept to completion. HM was corresponding author providing a first draft and input into the final manuscript.
Signature	Date 10 Aug 2015

Name of Co-Author	Kavita Rasiah
Contribution to the Paper	KR assisted in the study design and concept and development of the severity score. Kavita assisted in data collection at WCH and contributed to review and editing of the final manuscript.
Signature	Date 28/8/15

Name of Co-Author	Peter Richmond
Contribution to the Paper	PR was the study investigator at the Perth site and contributed to revision and editing of manuscript
Signature	Date: 28 Aug 2015

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Contribution to the Paper	JB was the study investigator at Melbourne site and contributed to revision and editing of manuscript
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Contribution to the Paper	PM was the study investigator at the Sydney site. PM contributed to the analysis and interpretation of study results and to the revision and editing of the manuscript.
Signature	Date 26/8/15

Please cut and paste additional co-author panels here as required.

4.1 ABSTRACT

Background: Australia recently experienced its worst pertussis epidemic since introduction of pertussis vaccine into the National Immunisation Program. This study aimed to determine factors associated with severe pertussis in hospitalized children during an epidemic using a novel pertussis severity scoring (PSS) system.

Methods: This prospective, observational, multicenter study enrolled children hospitalized with laboratory confirmed pertussis from 8 tertiary pediatric hospitals during a 12 month period (May 2009 – April 2010). Variables assessed included demographics, clinical symptoms and relevant medical and immunisation history. Cases were scored using objective clinical findings with cases classified as either severe (PSS >5) or not severe (PSS ≤5). Logistic regression models were used to predict variables associated with severe disease.

Results: 120 hospitalized children 0-17 years of age were enrolled with a median PSS of 5 (IQR 3-7). Most (61.7 %) were classified as not severe with 38.3% (46/120) severe. Most severe cases (54.3%) were <2 months of age. Presence of co-infection (OR: 4.82, 95%CI 1.66-14.00), < 2 months old (OR: 4.76, 95%CI 1.48-15.32) fever >37.5C (OR: 5.97, 95%CI 1.19-29.96) and history of prematurity (OR 5.00, 95%CI 1.27-19.71) were independently associated with severe disease. Of the 70 cases in children ≥ 2 months of age, almost a third (n=23) had not received pertussis vaccine.

Conclusions: Most severe pertussis occurred in young, unimmunized infants, although severe disease was also observed in children > 12 months of age and previously vaccinated children. Children admitted with pertussis with evidence of co-infection, history of prematurity or fever on presentation need close monitoring.

4.2 INTRODUCTION

Despite high immunisation coverage, Australia had the largest annual reported pertussis cases in 2010, since introduction of diphtheria-tetanus-pertussis vaccine to the National Immunisation Program (NIP) in 1954, with 34,790 cases notified [1]. The highest notification rate was 424/100,000 for children 5-9 years of age [1]. The Australian NIP includes primary pertussis immunisation at 6-8 weeks, 4 and 6 months using a combination vaccine, DTPa-HepB-IPV/Hib (Diphtheria-Tetanus-acellular Pertussis (DTPa), Hepatitis B, Inactivated Poliovirus (IPV) and Haemophilus influenzae type b), with boosters at 4 years, (DTPa-IPV) and 11-13 years of age (dTpa). An 18 month DTPa booster was used until 2003 and prior to 1999, a whole cell pertussis vaccine manufactured in Australia was used for all primary doses. Vaccine coverage has remained consistently around 92% to 12 months of age over the past decade.

Although notification is significantly increased by the use of polymerase chain reaction (PCR) testing of throat and nasopharyngeal specimens, recorded rates of hospitalisation for pertussis in Australian children also increased during the epidemic period and were the highest in the developed world (28.4/100,000 for non-Indigenous and 93.2/100,000 for Indigenous children) [2-5]. However, with pertussis increasingly being included as part of a respiratory panel in PCR-based laboratory testing, the range of disease severity among children hospitalized with pertussis is likely to be less uniform than when diagnosis was almost exclusively clinical or by positive culture.

This study aimed to evaluate severity and factors associated with severity in children hospitalized with pertussis. A scoring system for pertussis severity based on disease symptoms has been reported from a developing country setting at community level

[6] but no scoring system applicable to hospitalized children in developed settings is otherwise available. We developed a scoring system based on objectively measured variables which could be ascertained from case notes which we believe has applicability to clinical predictive algorithms, epidemiological comparisons, vaccine trials and economic analyses where accurate disease burden measurement is needed.

4.3 METHODS

4.3.1 Setting and participants

Eight metropolitan tertiary paediatric hospitals participated in the study during a 12 month pertussis epidemic period (01 May 2009 – 30 April 2010). Children 0-17 years of age were eligible for enrolment in the study if they were admitted to hospital with a pertussis diagnosis which met the Australian national case definition for a confirmed case (Laboratory definitive evidence or laboratory suggestive evidence and clinical diagnosis of pertussis)[7]. Laboratory definitive evidence includes isolation of *B. pertussis*, detection of *B. pertussis* by nucleic acid testing or seroconversion in paired sera for *B. pertussis* in the absence of recent pertussis vaccination. Laboratory suggestive evidence includes significant change in *B. pertussis* antibody level or single high IgG or IgA to Pertussis Toxin or *B. pertussis* antigen in the absence of recent vaccination.

Research nurses actively identified cases and a structured questionnaire which included demographic details, clinical features, and management was completed by the research nurse/doctor after informed parental consent. Clinical parameters at the time of presentation to the Emergency Department and maximum parameter score during the hospital stay were recorded. Presence of any co-infection as

measured on routine respiratory panel on admission or any additional testing ordered during clinical management was collected. Immunisation status was confirmed using the Australian Childhood Immunisation Register.

Severity of disease was scored by two investigators at the lead centre after data collection had been completed, to ensure consistency. The study was approved by each Human Research Ethics Committee at participating study centres.

4.3.2 Pertussis severity score (PSS)

The PSS was designed by co-investigators Marshall and Rasiah following a review of the literature with identification of objective parameters associated with severity of disease and discussion with academic and treating physicians and then refined following statistical validation of the included parameters [8-10].

Objective parameters considered important for reproducibility and severity assessment, such as duration of stay, level of hospitalisation and use of oxygen and intravenous therapy were included in the PSS as well as clinical features such as presence of complications (Table 1). Grading of these parameters allowed a total score to be calculated for each individual child enrolled. Lymphocyte count was initially included in the PSS based on the finding that a raised lymphocyte is associated with severe disease [10], however only 53% (n=64) of children had a complete blood picture requested and the likelihood of being testing was distributed unevenly different between non severe and severe groups so it was subsequently removed.

Each of the five final parameters for the PSS was given a score of 0-3, with 0 being absence of parameter with increasing severity from 1-3 with a possible total score of 15. The maximal grade of the parameter during the hospital admission was used

to determine the severity score. The final PSS assigned to each individual was the sum of the five individual parameter scores (Table 1). Based on the distribution of severity scores in the final dataset, cases were classified as severe if the final PSS was greater than the median severity score (PSS=5). The cut-off value was chosen using the median severity score (PSS=5) for the overall cohort. This cut-off was further supported by the finding that all of the children requiring HDU and/or ICU management were scored >5 and all cases requiring only short stay admissions (n=10) scored <5.

Table 1: Pertussis severity scoring system for children hospitalized with pertussis infection.

Variable	Grade 1	Grade 2	Grade 3
Duration of hospitalisation score	< 24 hours 1	1-7 days 2	> 1 week 3
Level of hospitalisation score	General ward 1	HDU 2	ICU 3
Hydration Requirement score	Nasogastric 1	IV hydration < 48hrs 2	IV hydration ≥ 48hrs 3
Respiratory support score	Suction/oxygen 1	CPAP 2	Ventilation 3
Presence of complications score	Dehydration Hypoxia Chest x-ray changes# 1	Pneumonia Pneumothorax Aspiration 2	Encephalopathy Cardiomyopathy 3

Chest x-ray changes including atelectasis, interstitial infiltrate/opacity but not diagnosed as pneumonia

*Parameters measured are scored according to the highest recorded during the period of hospitalisation

4.3.3 Data collection and analysis

The completed data collection questionnaires were provided by each site to the lead centre (Adelaide - Marshall). A master database was created to capture each variable of interest. The database was checked for consistency and accuracy and data queries generated and resolved prior to analysis. Data were analysed using descriptive and inferential statistics. Logistic regression models were used to test for associations with severe disease and identify potential predictors of pertussis severity. Univariate analysis of factors such as age, immunisation status, presenting signs and co-infection identified potential variables to be included in a multivariable analysis in which logistic regression was used. Variables were included in the initial model if the univariate analysis for that variable returned a p-value of ≤0.2. Variables were removed one by one from the model starting with the highest p-value, until only variables with p-values of <0.05 remained. Consideration was given to clinical importance and relevance for variables retained in the final model and models compared to maximize the fit of the regression model.

4.3.4 Ethics

The study protocol, information sheet and consent forms and the severity tool (questionnaire) were approved by the Children, Youth and Women's Health Service Human Research Ethics Committee (CYWHS HREC #2157)

The study was conducted in accordance with GCP guidelines, the National Statement for Ethical Conduct in Human Research and applicable Women's and Children's Hospital and Department of Health policies. Written informed consent was obtained from participants' parent/guardian prior to study enrolment.

4.4 RESULTS

4.4.1 Study population

A total of 140 children hospitalized with pertussis were enrolled in the study. Twenty children were excluded from the according to protocol analysis: 5 did not meet eligibility criteria and 1 inpatient with congenital heart disease was asymptomatic despite pertussis DNA detected on PCR testing. A further 14 were excluded as they did not meet the case definition for confirmed pertussis. Questionnaires were completed for all participants, with 120 (100%) sufficiently complete to determine a PSS.

Children enrolled ranged from 10 days to 17 years of age (median age: 2 .5 months; IQR: 1.2– 15.1 months). Of the 120 children enrolled, 50 (41.7%) were < 2 months of age, 29 (24.2%) were 2 - <6 months of age, 9 (7.5%) were 6 - <12 months of age, 14 (11.7%) were 1 - < 4 years old, and 18 (15.0%) were 4 – 17 years old. Slightly more were male (n=65, 54.2%) and 12 (10.0%) were identified as being of Aboriginal or Torres Strait Islander ethnicity. The majority of children enrolled (73/120; 60.8%) had received no prior dose of pertussis containing vaccine (Table 2).

Table 2; Doses of pertussis vaccine received by age category.

Doses pertussis	**Age category**					
vaccine received	<2m	2-6m	6-12m	12m-4y	4+	TOTAL
0	50	12	3	5	3	73
1	0	13	2	0	0	15
2	0	4	2	1	0	7
3	0	0	2	6	3	11
4	0	0	0	1	9	10
5	0	0	0	0	1	1
Unknown	0	0	0	1	2	3
% Confirmed unvaccinated	100.0%	37.9%	22.2%	35.7%	16.7%	58.3%

Clinical Features

Most children had a reported cough (93.3%), 56.7% had post-tussive vomiting, 44.2% had an inspiratory whoop and 32.5% had apnoeic episodes. Significant differences in clinical features were detected between younger and older children (Table 3). Of the 120 hospitalized pertussis cases, 28 children (23.3%) had documented complications. Pulmonary complications were the most common, including lower respiratory tract involvement with pneumonia (n=9; 7.4%, atelectasis and interstitial changes (n=27; 22.5%) confirmed on chest X-ray and pneumothorax (n=1; 0.8%).

Table 3: Clinical characteristics of children hospitalized with pertussis infection by age (01 May 2009 – 30 April 2010)

Clinical features	< 2 months (N=50) n (%)	2-11 months (N=38) n (%)	1-17 years (N=32) n (%)	Total (N=120) n (%)	Difference in proportions P value
Cough	48 (96.0)	32 (84.2)	31 (96.9)	112 (93.3)	0.063
Post-tussive Vomiting	27 (54.0)	23 (60.5)	18 (56.3)	68 (56.7)	0.828
Whoop	24 (48.0)	15 (39.5)	14(43.8)	53 (44.2)	0.726
Coryza	21 (42.0)	18 (47.4)	13 (40.6)	52 (43.3)	0.825
Apnoea	23 (46.0)	13 (34.2)	3 (9.4)	39 (32.5)	0.002
Cyanosis	20 (40.0)	15 (39.5)	6 (18.8)	41 (34.2)	0.100
Fever	9 (18.0)	6 (15.8)	14 (43.8)	29 (24.2)	0.010
Wheeze	5 (10.0)	10 (26.3)	12 (37.5)	27 (22.5)*	0.012
Bradycardia	10 (20.0)	1 (2.6)	0	11 (9.2)	0.002
Seizures	0	1 (2.6)	1 (3.1)	2 (1.7)	0.477
Respiratory Distress	17 (34.0)	15 (34.5)	16 (50.0)	48 (40.0)	0.352
O_2 saturation <96%	8 (16.0)	7 (18.4)	7 (21.9)	22 (18.3)	0.798

Note: *6/27 of children reporting wheeze had a respiratory co-infection (5 positive for RSV, 1 positive for Rhinovirus, one positive for H1N1 Influenza)

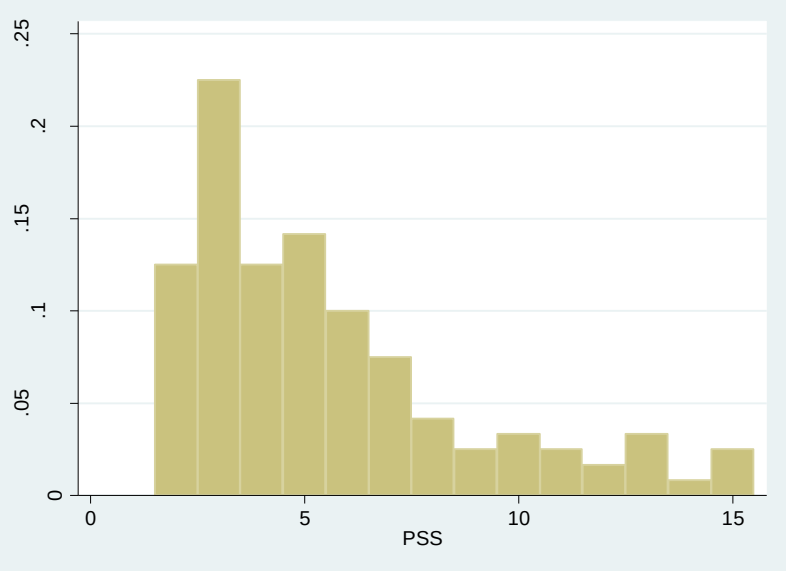
Laboratory testing

Of the 120 enrolled cases, 119 were PCR positive, of which 13 were also culture positive. One additional patient was confirmed by culture only. The 14 culture positive cases were all from children aged <4.5 months who were mostly unimmunized (10/14).

4.4.2 Pertussis severity scoring system

A pertussis severity score was calculated for all of the eligible cases (n=120). The majority of children (n=74, (61.7%)) had a PSS of ≤5 (not severe). Infants < 2 months of age had the highest proportion (50.0%) with a severe score, PSS ≥ 5 (Table 4). A maximum score of 15 was only seen in three infants, all aged <12 months of age. Distribution of severity scores are shown in figure 1.

Figure 1: Relative frequency distribution for pertussis severity scores (n=120)



4.4.3 Clinical characteristics and management

The median duration of hospitalisation was 4 days (IQR: 2–10 days) with a range of 1-48 days. Eighty six (64.2%) children were admitted for less than one week, 29 (21.6%) required admission for 7–14 days and 19 (14.2%) required admission for over 2 weeks. Twenty children (16.7%), with ages ranging from 3 weeks to 9 years (median=1 month) required critical care management in a High Dependency Unit (median PSS:11 (IQR:10-12), range:7-13) or ICU (median PSS:11 (IQR 10-14), range 7-15). The remaining 100 children were managed in a general paediatric ward (n=90) or a short stay ward (n=10) and had a median PSS of 4 (IQR: 3-6, range: 2-13). Almost all children (95%, n=113/120) were treated with antibiotics including erythromycin (38%), azithromycin (31%), clarithromycin (10%), amoxicillin/augmentin (4%), bactrim (2%) and other antibiotics (15%).

4.4.4 Risk factors for severe pertussis

Clinical parameters collected on presentation to the Paediatric Emergency Departments in each of the paediatric hospitals were complete for almost all participants (96-99%). Univariate analyses of predictor variables indicated that age <2 months old, fever >37.5°C at presentation, history of premature birth and identified co-infection were independently associated with severe disease as defined by a PSS >5. Presence of fever was the strongest predictor of severe disease with febrile children almost six times more likely to be scored severe than afebrile children (OR: 5.97 CI 1.19 - 29.96, p=0.03) in a multivariate analysis adjusted for co-infection, prematurity and age.

The presence of apnoea/bradycardia and physician documented respiratory distress were associated with more severe disease in univariate analysis, but did not remain significant in multivariate models. Lymphocytosis was associated with

more severe disease in univariate analysis, with patients with absolute lymphocyte counts >20,000, 10 times more likely to be scored severe than patients with absolutely lymphocyte counts lower than 20,000. (OR: 10.59, CI 1.28-87.96, p=0.029). The majority of patients with lymphocytosis were scored as severe (11/12) however, as lymphocyte count was measured for less than 60% of the enrolled patients, we were unable to include this variable in the multivariate model (Table 5).

The relationship between immunisation and severity was investigated. A small, (but not statistically significant) reduction in risk of severe disease was found when either ≥ 1 vs 0 doses were compared (OR: 0.56, CI 0.25-1.26, p=0.164). When univariate analysis was restricted to cases aged less than 12 months of age at the time of admission, infants who had received at least one dose of pertussis containing vaccine were half as likely to have severe disease compared with infants who had not received any prior pertussis vaccination, however this reduction was also not statistically significant (OR: 0.46, CI 0.16-1.34, p=0.155). Whilst sample size is limited for this group (n=88), only 6/23 vaccinated infants (at least one dose of pertussis vaccine) were considered severe (26.1%) compared with 26/62 (41.9%) of unvaccinated infants.

Co-Infection

Children with a co-infection were more likely to have severe disease (OR:4.82 CI 1.66 – 14.00; p=0.004). A co-infection was identified on respiratory screening by PCR in 29 enrolled children, with an age range of 2 weeks to 9 years (median=2.3 months). The majority (n=25) of co-infections were viral, including rhinovirus (n=12), respiratory syncytial virus (RSV) (n=8), adenovirus (n=3), human metapneumovirus and H1N1 influenza (n=2). Other infective agents included Klebsiella pneumoniae, Streptococcus pneumoniae, Mycoplasma pneumoniae and Staphylococcus aureus.

Three children had two additional co-infecting agents identified; adenovirus and rhinovirus, RSV and adenovirus, RSV and parainfluenza virus. The median PSS for children with laboratory identified co-infections was 6 (IQR 4-9), compared with a median PSS=4 (IQR 3-6) for children without an identified co-infection. Furthermore, 65.5% (19/29) of children who had laboratory evidence of *Bordetella pertussis* and an additional co-infecting pathogen were considered severe compared with 29.7% (21/91) of children who either had no evidence of co-infection or had unknown co-infection status (p=0.001). Co-infections were apparent in all age groups with 13/41 (31.7%) of infants <2 months of age, 9/35 (25.7%) of infants 2-12 months of age and 7/30 (23.3%) of children >1 year old having evidence of a co-infection.

Prematurity

Overall, 15 of 103 (14.6%) children of known gestational age were born prior to 37 weeks, including 7 infants less than 32 weeks gestation. For children older than 1 year requiring hospitalisation for pertussis, the proportion with a history of prematurity was even higher at 33% (9/27). Over 50% (8/15) of admissions for premature children were scored severe compared to 32% (31/88) for children born at >37 weeks, although this difference in proportions was not statistically significant (Pearson chi2 (1) = 1.7857 Pr = 0.181), possibly due to the small sample size. Prematurity was not significantly associated with more severe disease in the univariate logistic regression, but an association became evident in a multivariate model (OR: 5.19 CI 1.24, 26.67; p=0.04) adjusting for age category, fever at presentation and presence of co-infection (Table 5).

Immunisation status

A total of 88 infants (73.3%) admitted with pertussis were < 12 months of age. Of these, 65 (73.9%) had not received any prior pertussis containing vaccine, including 50 (56.8%) who were below the Australian NIP recommended age (8 weeks) for first vaccination at the time of the study (Table 2). More recent recommendations in Australia include immunisation from 6 weeks of age. Under this recommendation, 12 additional children, including 5 who were assessed as severe cases (PSS >5) would have been eligible to receive a first dose of vaccine. There were 12 infants of Aboriginal or Torres Strait Islander ethnicity with documented immunisation history, with the majority (n=9) < 2 months of age. Among older children, 2 were up to date with immunisations and 1 child was eligible to have received a third dose.

In children 1-17 years of age, 20/32 (62.5%) had received 3 or more pertussis immunisations, with a median time between last immunisation and hospital presentation of 3 years and 6 months (IQR: 2 years, 6 months – 6 years, 6 months).

Table 4a: Pertussis severity score (PSS) by age at admission (n=120)

Age	Number of children	Median PSS	IQR	PSS range (2-18)	PSS ≤ 5 (not severe)		PSS >5 (severe)	
					n	%	n	%
<2months	50	5.5	4-9	(2-15)	25	50.0	25	50.0
2-11 months	38	4	3-5	(2-15)	29	76.3	9	23.7
1-17 years	32	4	3-6	(2-15)	20	62.5	12	37.5
Total	120	5	3-7	(2-15)	74	61.7	46	37.3

Table 4b: Pertussis severity score (PSS) by doses of pertussis vaccine received (n=117 with confirmed immunisation status)

Doses of pertussis vaccine received	Number of children	Median PSS	IQR	PSS range (2-18)	PSS ≤ 5 (not severe)		PSS >5 (severe)	
					n	%	n	%
0	73	5	3-7	(2-15)	42	57.5	31	42.5
1-2	22	3.5	3-5	(2-8)	18	81.8	4	18.2
2+	22	3.5	3-9	(2-15)	13	59.1	9	42.9

Table 5: Risk factors associated with severe disease (n=120)

Variable	Level	n	Odds ratio (95% CIs)	P-value	% severe	Adjusted Odds ratio (95% CIs)	P-value
Age Group	< 2 months	50	3.22 (1.27,8.17)	**0.047**	50.0	4.76 (1.48-15.32)	**0.014**
	2-11 months	38	1.9 (0.69,5.44)		23.7	0.87 (0.21,3.67)	
	≥12 months	32	1		37.5	1	
Prematurity	< 37 weeks	15	2.10 (0.70, 6.34)	**0.188**	53.3	5.19 (1.24, 21.67)	**0.024**
	≥ 37 weeks	88	1		35.2	1	
No. children in household	> 2	49	1.99 (0.92, 4.30)	**0.080**	46.9	-	-
	≤ 2	65	1		30.8		
Immunisation status	1+	44	0.56 (0.25, 1.26)	**0.164**	29.5	-	-
	0 doses	73	1		42.5		
Apnoea/ Bradycardia on presentation	Yes	41	2.42 (1.10, 5.30)	**0.027**	51.2	-	-
	No	76	1		30.3		
Fever (>37.5^0 C at presentation	Yes	14	7.44 (1.95-28.38)	**0.003**	78.6	5.97 (1.19, 29.96)	**0.030**
	No	106	1		33.0	1	
Whoop	Yes	53	0.56 (0.26,1.20)	**0.133**	30.2		-
	No	64	1		43.8		
Respiratory distress #	Yes	48	2.54 (1.18, 5.44)	**0.017**	52.1	-	-
	No	70	1		30		
Co-infection	Yes	29	4.75 (1.91, 11.82)	**0.001**	65.5	4.82 (1.66-14.00)	**0.004**
	No	77	1		28.6	1	
Lymphocyte count > 10,000*	Yes	26	2.86 (0.98, 8.33)	**0.055**	73.1	-	-
	No	39	1		48.7		
Lymphocyte count > 20,000*	Yes	12	10.59 (1.28, 87.96)	**0.029**	91.7	-	-
	No	53	1		50.9		

Note: Univariate predictors with p value <0.2 were included into multiple regression model. Least significant variables removed sequentially until only significant predictor variables remained.

on presentation as noted by physician. * n= 65 for lymphocytosis as this was not tested for all patients therefore not included in multiple regression analyses.

4.5 DISCUSSION

Consistent with other studies, our findings indicate that the most severe disease occurs in infants < 2 months of age who are ineligible to receive pertussis vaccine [11, 12]. Additional factors associated with increased risk of severe pertussis disease identified in our study included fever, co-infection and a history of prematurity. Routine PCR testing (84% of cases) was done at centres contributing the highest number of cases, however not all children were tested for co-infections at all centres. Our data may be biased in that children who appeared more unwell may have received additional respiratory testing at several of the centres without routine testing. However, in a stratified analysis limited to only the cases enrolled at the Women's and Children's Hospital (n=37) where testing for co-pathogens was conducted for 95% of participants, the association between presence of co-infection and severe disease remained (OR: 4.82, CI 1.66-14.04, p=0.004). Whilst fever and co-infection were both independent predictors of severity in the multivariable regression model, it is possible that cases with fever also had the presence of an undetected co-infection. As testing practices for routine respiratory panel testing varies between states, and respiratory pathogen testing doesn't include all potential co-infecting agents, it is possible that cases with fever in absence of identified co-pathogens may be indicative of presence of an unidentified co-infection.

In contrast to our findings, Nuolivirta et al [13] found no difference in clinical disease severity, including duration of clinical support and ICU admission between children admitted with Respiratory Syncytial Virus (RSV) positive bronchiolitis and those admitted with bronchiolitis who were co-infected with RSV and *B. pertussis*.

Children who present with fever associated with signs of pertussis and especially those < 2 months of age should be admitted to hospital and monitored closely for signs of deterioration or consideration given to admitting them and those with a co-infection, directly to a higher level care unit.

Prematurity may predispose children to increased risk of vaccine preventable diseases such as pertussis [14]. A history of prematurity was found to be an independent predictor of more severe pertussis, although the sample size for premature infants was small. We investigated whether potential bias from higher likelihood of, or earlier threshold for ICU management was evident for premature vs term cases, but found no difference in proportions reporting ICU hospitalisation. Langkamp et al [15] showed that low birth weight infants were at increased risk of both reported pertussis and pertussis hospitalisation in the first 2 years of life. Increased severity may be due to underlying poor health, increased exposure to pertussis, immunological immaturity and/or lower levels of maternal antibody transfer [16, 17]. Vaccine responses have been shown to be similar between premature and term infants following acellular pertussis vaccination [18, 19].

A severity scoring system has potential for use in clinical trials to identify changes in clinical disease that may relate to changes in virulence of circulating pertussis genotypes. Mooi et al [20] have shown a recent emerging genotype associated with more severe disease in The Netherlands. The PSS described here is being used in another Australian study to assess any association between disease severity and circulating pertussis genotypes including pertactin negative genotypes and

genotypes containing the *ptxP3* allele, associated with increased pertussis toxin production [21, 22]. This scoring system is most likely to be useful for assessment of infants < 1 year of age who carry the burden of the most severe pertussis disease. This scoring system could be used to monitor disease severity in future epidemics and inform health care utilization and costs. Early detection of severe cases may inform public health interventions for the next pertussis epidemic.

The dilemma for pertussis control is that those at highest risk of severe disease are too young to be protected by current immunisation schedules which start at 6 weeks of age. As the youngest infants are the most vulnerable for severe disease, vaccine strategies aimed at providing earlier protection through direct or indirect protection are warranted and currently either under investigation (immunisation at birth) or recently recommended (maternal immunisation and cocooning strategies) [23-28]. Many of the children admitted to hospital in this study were unimmunized and although immunisation status did not predict severe hospitalized cases, other studies have shown that unimmunized children are at higher risk of severe disease [13, 29-31]. Immunisation status is highly correlated with age with the majority of infants in our study too young to be immunized which may explain why it was not a significant variable on univariate testing in addition to the limitations of a small sample size. Multiple pertussis vaccinations did not prevent hospitalisation which may, in part, be due to waning immunity as shown by the median interval of 3.5 years between last immunisation and hospitalisation in children who had previously received at least 3 doses of pertussis vaccine and supported by other studies [32].

This is an observational study and therefore subject to selection bias. In addition, as not all paediatric hospitalisations in Australia were captured during this period,

particularly rural hospitals, the data may not be generalizable to all hospitalized cases, although collection of data from all Australian mainland states was achieved.

Pertussis remains a vaccine preventable disease of significant concern due to severe epidemics that continue to occur globally. Identifying predictors of severe disease will improve management protocols and assist in determining better strategies for prevention of deaths and disease from pertussis.

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CHAPTER 5: Manuscript 2- The relationship between Bordetella genotypes and clinical severity of pertussis disease in Australian children.

Over the past decade, literature from Australia and globally has highlighted the genetic evolution of *Bordetella pertussis*, with particular concern around the increasing predominance of strains that do not express the outer membrane protein, pertactin (Prn). As Prn is one of the components of all acellular pertussis vaccines licensed in Australia, it is important to understand any clinical implications of these antigenic changes in the circulating *B. pertussis* strains. It has also been recognised that the majority of *B. pertussis* strains are now carrying a *ptxP3* allele, which has been shown to result in greater production of pertussis toxin and which may also lead to increased virulence. To address these questions and in collaboration with colleagues in other Australian states who had genotyped several hundred *B. pertussis* isolates collected from Australian hospitals, we sought to review clinical features of children presenting to, or admitted to hospital with confirmed pertussis at three major paediatric hospitals.

The manuscript "The relationship between *Bordetella* genotypes and clinical severity of pertussis disease in Australian children" reports results of a national study investigating associations between pertactin deficient *B. pertussis* isolates and severity of disease in a cohort of children presenting to, or admitted to one of three participating hospitals. The manuscript is published in Journal of Infection.

This manuscript reports the first Australian data, and is one of only three studies worldwide that investigates the clinical implications of Prn deficient *B. pertussis* infections in children. Due to the re-emergence of pertussis globally, and the limited control of pertussis infections despite long standing immunisation program, these data are essential for informing public health policy.

Statement of Authorship

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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5.1 ABSTRACT

Background: Pertussis control remains a challenge despite pertussis immunisation programs. Changes in circulating *Bordetella pertussis* genotypes, including a novel pertussis toxin promoter *ptxP3* allele and absence of pertactin (Prn) antigen, have been reported from several countries but limited data on relative severity are available. We compared markers of disease severity in children with *B. pertussis* infection due to strains of differing genotype.

Methods: Culture confirmed cases presenting to tertiary paediatric hospitals in three Australian states between 2008 and 2012 were classified as severe if they required a hospital stay greater than seven days, were admitted to intensive care, or if death occurred. Associations between age, vaccination, genotype and severity were assessed.

Results: Of 199 pertussis cases, 81 (41%) were <3 months, including 32/39 (82%) of severe cases. The proportion of isolates from these cases that were Prn deficient increased markedly between 2008 and 2012. Of *B. pertussis* isolates, the proportion considered severe was similar for Prn positive (27/128, 21%) and Prn deficient (12/71, 17%) cases but only 1/22 (4.5%) of non *ptxP3* cases were severe versus 38/177 (21.4%) *ptxP3* positive. Adjusting for *ptxP* type, vaccination status and age, disease severity was not significantly associated with Prn status (RRA: 0.95, [0.57-1.56]; p=0.83).

Conclusions: In children, we found no relationship between Prn status and markers of severe pertussis. An increased proportion of severe disease in isolates with the *ptxP3* allele was observed. Further research on the influence of the *ptxP3* allele on pertussis disease severity is warranted.

5.2 INTRODUCTION

Pertussis remains a clinical and public health burden in Australia and globally. Despite long standing immunisation programs in most countries, epidemics continue to occur every three to four years in high resource countries. The incidence of pertussis in young infants is particularly concerning, with infants at highest risk of death and severe disease [1-3]. The most recent pertussis epidemic in Australia was the largest reported in the vaccination era, both in terms of number of cases and duration of the epidemic [2]. At the peak of that epidemic, pertussis notification rates in young children reached 333 per 100,000 children less than five years of age. The highest notification rate was observed in children 5-9 years of age (550 pertussis notifications per 100,000 children) [2]. Ten of the 11 pertussis deaths in Australian between 2006 and 2011 were in infants less than six months of age [4].

The resurgence of pertussis in Australia may be explained by several factors, including improved detection through more sensitive PCR diagnostic tests, ease of testing [5] and reduced duration of immunity following the introduction of acellular vaccines in 1997 [6-9]. Questions have also arisen regarding the impact of a change from whole cell pertussis vaccines to acellular vaccines on the duration of pertussis immunity and evolution of *Bordetella pertussis* [6, 10, 11]. The most prominent recent changes in circulating *B. pertussis* strains are polymorphisms in *ptxP*, the promoter of the Ptx operon and the non-production of pertactin [12-18] *ptxP3* was shown to be associated with increased pertussis toxin production as a result of a single base mutation in the *ptxP* and with increased hospitalisations in the Netherlands [16]. In Australia, *ptxP3 B. pertussis* isolates predominated during the 2008-2012 epidemic [17]. There is increasing evidence worldwide *of* emergence of *B. pertussis* variants that are deficient in the pertactin (Prn) protein [10, 18-22], an

outer membrane protein involved in adhesion to epithelial cells [23]. The clinical consequences of these evolutionary changes are unclear [10, 15, 18, 21, 24, 25]. Murine studies have indicated that Prn deficient strains remain virulent, with similar invasion and cytotoxicity properties compared with strains expressing Prn [20]. *B. pertussis* mutants which do not express Prn may persist longer in the epithelia than Prn expressing variants [26]. Two recent studies examining clinical findings in children and associations with Prn status of isolates suggest symptoms and clinical course are similar or reduced, with no apparent difference in requirement for hospitalisation or presence of symptoms (with the exception of apneoa which was less likely in Prn deficient infections) [15, 25].

As the current acellular pertussis vaccines used in Australia contain the pertactin antigen [27], the absence of this outer membrane protein in circulating *B. pertussis* strains may allow the bacteria to “escape” vaccine induced immune protection increasing susceptibility to infection despite immunisation.

In Australia, vaccination against pertussis is funded for infants and children through the National Immunisation Program (NIP) offering free Diphtheria-Tetanus-acellular Pertussis (DTPa) vaccinations at 2, 4 and 6 months of age, with two booster doses at four years and 10-15 years of age [27]. In attempts to improve protection against pertussis, an 18 month booster dose will be re-introduced with federal funding from 01 October 2015. More recently, vaccination of women in their third trimester of pregnancy has been recommended and provided as part of a state funded vaccine program in all Australian states [27]

Whilst it is clear that the prevalence of *B. pertussis* variants that do not express Prn has increased over recent years [10, 18, 21], the clinical implications of this change in epidemiology are less clear. Understanding the clinical relevance of the

increasing prevalence of Prn deficient strains is important for future control through vaccination strategies and management of pertussis infections in children.

This study aimed to assess the impact of emerging *B. pertussis* variants on severity of pertussis disease by comparing clinical disease parameters for Australian children infected with Prn deficient and Prn positive *B. pertussis* isolates between 2008 and 2012. Since the novel pertussis toxin promoter *ptxP3* allele has been associated with increased hospitalisations in the Netherlands [16], we also examined the effect of the *ptxP3* allele on disease severity.

5.3 METHODS

5.3.1 Study design and population

The genotypes and Prn expression of *B. pertussis* isolates from cultures of children presenting to three participating Australian paediatric hospitals (Sydney Children's Hospital at Westmead (New South Wales - NSW), Princess Margaret Hospital (Western Australia - WA) or Women's and Children's Hospital (South Australia - SA) between 2008 and 2012 have been determined in our previous studies [10],[17]. The availability of isolates for genotyping was based on routine pertussis diagnostic practices at each of the participating hospitals. The majority of genotyped isolates were from NSW and WA where bacterial culture remains a diagnostic standard in conjunction with PCR. In SA, PCR testing was the routine diagnostic test for pertussis during the study period with culture only on specific request with very few samples collected for culture from 2010 onwards.

Medical and laboratory data were collected for children under 18 years of age who either presented to, or were admitted to one of the three participating hospitals and had an isolate available for determination of Prn expression. Clinical and

demographic variables were collected to assess the relationship between these variables and Prn expression. Immunisation history was obtained from the Australian Childhood Immunisation Register (ACIR) or hospital records. Previous vaccine was defined as documented receipt of pertussis-containing vaccine at least 14 days prior to diagnosis of pertussis. The presence of a respiratory co-pathogen was defined as laboratory evidence of any respiratory organism within seven days of pertussis diagnosis. Various methods of respiratory pathogen detection including PCR, culture and antigen detection panels are employed by the three participating hospitals and only SA routinely tests for a range of respiratory pathogens (respiratory PCR panel) with each sample requesting pertussis diagnostic testing. The presence of lymphocytosis was determined according to local age specific laboratory reference ranges for cases where absolute lymphocytes were measured as part of the medical management of the child. A case was classified as 'severe' if the patient required a hospital admission for greater than seven days, intensive care unit management, or died.

5.3.2 Data collection and analysis

Chi-square tests and Mann-Whitney U or t-tests were used to assess differences in proportions (categorical) and median/mean (continuous) data between Prn deficient and Prn positive groups. Univariate and multivariable log binomial regression models were used to assess association between Prn status and severity of pertussis. Multivariable models were adjusted for other potential confounders and variables of interest, including *ptx* promoter type (*ptxP3* or not *ptxP3*), age (<2 months; 2 to <4 months or ≥4 months) and vaccination (0 or ≥1 dose). Associations were reported as risk ratios (RR) with 95% confidence intervals (CI). All statistical

analyses were performed using STATA 11 with statistical significance defined as P<0.05.

5.3.3 Ethics

The study was approved by the Human Research Ethics Committee's at the respective sites (WCHN HREC #2391).

5.4 RESULTS

5.4.1 Study population and antigen expression

A total of 199 isolates from children with pertussis infection during 2008-2012 were identified for children presenting to, or admitted to one of the three participating hospitals where results were available for absence or presence of Prn expression. The majority of isolates were collected in NSW and WA (NSW: n=91, 45.7%; WA: n=85; 42.7%; SA: n=23, 11.6%). A third of these isolates (35.7%; 71/199) were Prn deficient with the remaining 128 samples expressing Prn. A higher proportion of isolates from WA were Prn deficient (40/85; 47.1%) compared with NSW (26/91; 28.6%) and SA (5/23; 21.7%).

The proportion of isolates that were Prn deficient increased over the period of the study; only 1 of the 29 isolates (3.4%) collected in 2008 identified as Prn deficient, compared with 28/39 (71.8%) and 29/35 (82.9%) identified as Prn deficient in 2011 and 2012 respectively (Figure 1). Isolates with the *ptxP3* promoter type, were predominant throughout each year of the study, with virtually all isolates (96%) in 2011 and 2012 identified as *ptxP3* type (Figure 1). Almost all Prn deficient isolates (70/71, 98.6%) had the *ptxP3* allele compared with 107/128 (83.6%) of Prn positive isolates (p = 0.001).

Isolates were collected from all months of the year although the majority of pertussis cases in this study occurred during Spring and Summer (n=147/199, 73.9%) compared with Autumn and Winter months (n=52/199, 26.1%) (p<0.001).

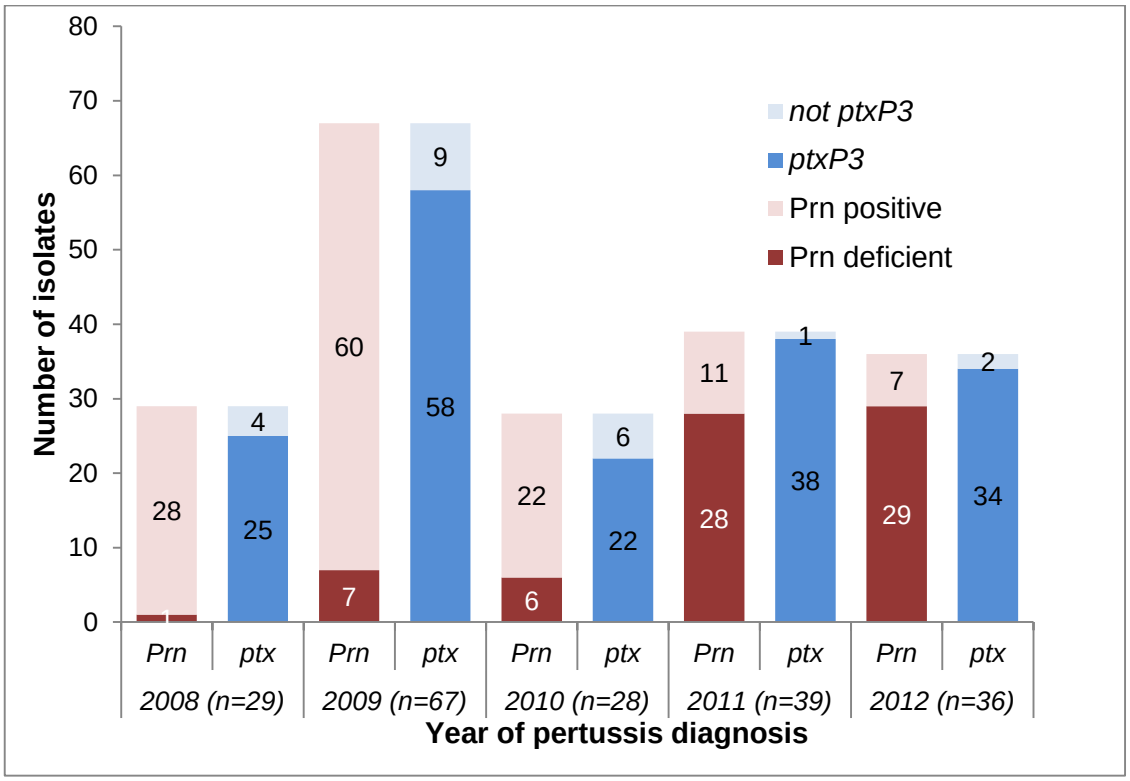

Figure 1: Isolates by year and pertactin (Prn) expression/pertussis toxin promoter (*ptxP*) type

5.4.2 Demographic and Clinical comparisons between Prn-ve and Prn+ve cases

The median age at pertussis diagnosis was three months (interquartile range [IQR] 2-18 months, range 13 days to 12.9 years). Over a third of cases were less than three months of age (n=82/199; 41.2%). Almost half of the cultured cases were female (98/199, 49.2%) and almost 10% identified as Aboriginal or Torres Strait Islander children (19/199; 9.5%). There were no significant differences between the distributions for age, gender or Indigenous status for children infected with Prn positive versus Prn deficient *B. pertussis* variants (Table 1).

Table 1: Cohort demographics and characteristics

		Prn deficient (n=71)	Prn positive (n=128)	P value
Gender	Male	35 (49.3%)	66 (51.6%)	0.76
	Female	36 (50.7%)	62 (48.4%)	
Aboriginal/Torres Strait Islander	No	64 (90.1%)	116 (90.6%)	0.91
	Yes	7 (9.9%)	12 (9.4%)	
Age category	<3m	29 (40.8%)	53 (41.4%)	0.36
	3-<6m	13 (18.3%)	24 (18.8%)	
	6-<12m	9 (12.7%)	5 (3.9%)	
	12m-<2y	9 (12.7%)	21(16.4%)	
	>=2y	11 (15.5%)	25 (19.5%)	
Age (months)	(median /IQR)	4m (1-16)	3m (2-19)	0.79
Hospital admission	Yes	40 (56.3%)	71 (55.5%)	0.91
Admission duration	<2 days	40 (57.1%)	66 (53.2%)	0.82
Intensive care management	Yes	5 (7.0%)	16 (12.5%)	0.23
Death	Yes	0 (0.0%)	3 (2.3%)	0.19
Pneumonia	Yes	1 (1.4%)	8 (6.3%)	0.12
Lymphocytosis^ (n=56)	Yes	10/21 (47.6%)	14/35 (40.0%)	0.58

IQR: Interquartile range
P values are chi-square or Mann-Whitney U tests as appropriate
*<admission duration (n=194): excludes 5; 3 died/2 transferred."<2 days" includes cases that presented without admission (n=88)
^ includes only those with white cell counts performed (n=56; Prn deficient n=21, Prn positive n=35)

Death

Three deaths occurred in the study cohort. All three deaths occurred in infants less than four months of age with no evidence of prior immunisation. *B. pertussis* isolates from these infants were all Prn positive and had the *ptxP3* allele.

Admission to hospital:

Overall, 55.8% (n=111) of the isolates were from children who were admitted to hospital as opposed to presentation to an emergency department only. There was no significant difference between the proportion of children with Prn deficient infections who were admitted to hospital (40/71, 56.3%) compared with Prn positive infections (71/128, 55.5%) (p=0.91).

Data on duration of admission was available for 106 of the 111 pertussis cases that were hospitalized (3 died and 2 were transferred). For these 106 cases, the median duration of hospital stay was similar for Prn deficient cases (n=39; median 4 days, IQR 2-9 days) and Prn positive cases (n=67; median 5 days, IQR 2-10 days). The proportion of children hospitalized for more than seven days was not statistically different between Prn deficient and Prn positive groups (11/70, 15.7% vs 23/124, 18.5%; (p=0.62))

A lower, but not statistically significant proportion of Prn deficient pertussis cases were admitted to Intensive Care Units compared with Prn positive pertussis cases (excluding five cases where requirement for Intensive Care Unit management was unknown) (5/69; 7.2%, vs 16/125; 12.8%; (p=0.23))

Pneumonia

Chest X-rays were performed for 54 (27.1%) of the cases included in this study. Similar proportions of children received a chest X-ray for both Prn deficient and Prn positive isolates (21/71; 29.6% vs 33/128 25.8%). Chest X-ray confirmed pneumonia was observed less frequently in Prn deficient cases compared with Prn positive cases, however this difference was not statistically significant (1/71; 1.4% vs 8/128 6.3%, p= 0.12).

Lymphocytosis

White cell counts were measured and available for 21 Prn deficient and 35 Prn positive cases. A similar proportion of Prn deficient cases (with white cell counts available) had elevated lymphocytes (10/21; 47.6%) compared with Prn positive cases (14/35, 40.0%), p=0.58. The mean absolute lymphocyte count was also similar between Prn deficient and Prn positive cases (12.6 (SD 6.9) vs 12.8 (SD 7.3) $x10^9$/L; p=0.91).

Co-infection

Co-infecting pathogens were reported for 40 of the 125 cases tested for other respiratory pathogens of the total pertussis cases included in this study (32.0%) with the most common co-pathogens being rhinovirus (n=19) followed by parainfluenza type 3 (n=7), respiratory syncytial virus (n=6), adenovirus (n=4) and human influenza virus (n=4). Of children tested for the presence of a co-pathogen, there was no difference in the proportion of cases with a respiratory co-infection identified between Prn deficient infections (14/48; 29.2%) and Prn positive infections (26/77; 33.8%) (p=0.59).

Immunisation history

Previous immunisation history was available for 59/71 Prn deficient cases (83.1%) and 112/128 Prn positive cases (87.5%). Almost a quarter of cases (n=43) were too young to have received any pertussis vaccine at least 14 days prior to diagnosis. The proportion of cases less than 56 days of age at time of diagnosis was similar for Prn deficient and Prn positive pertussis cases (15/71; 21.1% vs 28/128; 21.9%, p=0.90).

Of the 171 cases with known immunisation history, almost half had not received any pertussis vaccination at least 14 days prior to pertussis diagnosis (72/171; 42.1%). There was no difference in the proportion that had received 1 or at least 2 doses of acellular pertussis vaccine more than 14 days prior to pertussis diagnosis between Prn deficient and Prn positive cases (Table 2).

Table 2: Immunisation history by status of pertactin (Prn) in *B. pertussis* isolate

	Prn deficient (n=71)	Prn positive (n=128)	P value
Known immunisation history	59 (83.1%)	112 (87.5%)	0.39
No prior valid pertussis vaccine dose	24/59 (40.7%)	48/112 (42.9%)	0.78
Prior receipt of 1 valid pertussis vaccine dose	16/59 (27.1%)	28/112 (25.0%)	0.76
Prior receipt of at least 2 valid pertussis vaccine doses	19/59 (32.2%)	36/112 (32.1%)	0.994

*A valid dose was defined as documented evidence of pertussis containing vaccine at least 14 days prior to diagnosis of pertussis infection

5.4.3 Predictors of severity

Cases were classified as severe if they met any of the following conditions: hospital admission longer than seven days; intensive care management or death occurred. Overall, 39/199 (19.6%) were classified as severe according to this definition.

There was a lower proportion of Prn deficient *B. pertussis* infections classified as severe compared with Prn positive isolates (12/71; 16.9% vs 27/128 21.1%); however the difference was not statistically significant (p=0.48).

Almost 40% of infants less than three months of age were classified as severe compared with 9.6% of children between 3 and 12 months of age and 3.0% of children over 12 months of age (Table 3). Proportions of isolates deficient in Prn or carrying *ptxP3* allele were similar between the three age categories. Most children over 12 months of age (98.0%) had received at least one dose of acellular pertussis vaccine, whereas most infants under three months of age (80.8%) were unvaccinated (Table 3).

Table 3: Characteristics by age category.

	Overall	Age <3 months (n=81)	Age 3-<12 Months (n=52)	Age ≥ 12 months (n=66)	P value[#]
Characteristic		N (%)	N (%)	N (%)	
Prn deficient	71/199 (35.7%)	29/81 (35.8%)	22/52 (42.3%)	20/66 (30.3%)	0.401
ptxP3	177/199 (88.9%)	74/81 (91.4%)	48/52 (92.3%)	55/66 (83.3%)	0.223
Severe	39/199 (19.6%)	32/81 (39.5%)	5/52 (9.6%)	2/66 (3.0%)	<0.001
Died	3/199 (1.5%)	2/81 (2.5%)	1/52 (1.9%)	0/66 (0.0%)	0.486
ICU	22/197 (11.2%)	17/81 (21.0%)	3/50 (6.0%)	2/66 (3.0%)	0.001
Admitted for more than 7 days[(i)]	34/194 (17.5%)	29/79 (36.7%)	3/49 (6.1%)	2/66 (3.0%)	0.000
Pneumonia[(iii)]	9/199 (4.5%)	4 (4.9%)	1(1.9%)	4(6.1%)	0.625
Unvaccinated[(ii)*]	72/171 (42.1%)	63*/78 (80.8%)	8/44 (18.2%)	1/49 (2.0%)	<0.001

i) Admission duration unknown for n=5
ii) Vaccination history unknown for n=28
iii) pneumonia defined as chest X-ray confirmed pneumonia
P value is chi square or fisher's exact test for difference in proportion by age category
* includes 43 who were too young to have received any valid pertussis vaccine dose prior to diagnosis (<56 days)

A higher proportion of young infants were classified as severe if they were infected with an isolate with the *ptxP3* promoter type, regardless of Prn status compared with infants infected with isolates that were not *ptxP3*. For older children (≥3 months), only those infected with Prn-positive/*ptxP3* variants were classified as severe (Table 4).

Table 4: Genotypic variants and severity by age category and vaccination status

Variant	Prn deficient/ *ptxP3* (n=70)	Prn positive/ *ptxP3* (n=107)	Prn deficient/ Not -*ptxP3* (n=1)	Prn positive/ Not –*ptxP3* (n=21)
Age category	n (%) severe	n (%) severe	n (%) severe	n (%) severe
<3 months	12/28 (42.9%)	19/46 (41.3%)	0/1 (0.0%)	1/6 (16.7%)
>=3 months	0/42 (0.0%)	7/61 (11.5%)	0/0 (0.0%)	0/15 (0.0%)
Vaccination status	n (%) severe	n (%) severe	n (%) severe	n (%) severe
0 doses	11/23 (47.8%)	18/42 (42.9%)	0/1 (0.0%)	1/6 (16.7%)
1 or more	1/35 (2.9%)	6/53 (11.3%)	0/0 (0.0%)	0/11 (0.0%)
2 or more	0/19 (0.0%)	2/28 (7.1%)	0/0 (0.0%)	0/8 (0.0%)

Univariate binomial regression models for predictors of severe pertussis demonstrated that younger age, no prior pertussis vaccination, and identification as Aboriginal/Torres Strait Islander were statistically significantly associated with more severe pertussis (Table 5). In multivariable analysis including the above variables as well as Prn expression and *ptxP* promoter type, Prn deficiency was not associated with any altered risk of severity (RR 0.95 [0.57-1.56], p=0.83). However, the *ptxP3* variants were more than three times more likely to be classified as severe (RR 3.344 [0.59-20.00], although this association was not statistically significant

(p=0.17). Compared with infants over 4 months of age, infants less than 2 months of age were significantly more likely to be classified as severe (RR 4.62 [1.28-16.73], p=0.02 There was no evidence of a statistically significant association between identification as Aboriginal/Torres Strait islander and severe pertussis in the multivariable model (RR 1.31, [0.72-2.37], p=0.38). Children who had not received any prior pertussis vaccination were twice as likely to be classified as severe, (RR 2.10 [0.73-5.99] however this was not statistically significant (p=0.16).

Table 5: Univariate and multivariate associations with severe outcome#

Variable	Level	n	Univariate Risk Ratio (95% CI)	p value	Adjusted Risk Ratio (95% CI)	p value
Prn status	Positive	128	1.0		1.0	
	Deficient	71	0.80 (0.43-1.48)	0.48	0.97 (0.57-1.62)	0.90
ptxP status	Not *ptxP3*	22	1.0		1.0	
	ptxP3	177	4. 72 (0.68-32.72)	0.12	3.35 (0.55-20.34)	0.19
Aboriginal/Torres Strait Islander	No	180	1.0		1.0	
	Yes	19	2.44 (1.32-4.53)	0.01	1.31 (0.72-2.37)	0.38
Age cat	3m+	118	1.0		1.0	
	<3m	81	6.65 (3.09-14.35)	<0.001	2.85 (1.00-8.19)	0.05
Vaccine doses	1+	99	1.0		1.0	
	0	72	5.89 (2.74-12.66)	<0.001	2.62 (0.97-7.09)	0.06

Outcome was considered severe if Intensive care unit management or hospital admission duration greater than seven days was required or if death occurred.

5.5 DISCUSSION

Results from this study suggest that Prn deficient *B. pertussis* variants caused disease which was similar in severity with infection caused by Prn positive strains, with point estimates suggesting that infection from Prn deficient variants may be less severe, resulting in fewer cases of pneumonia and admission to Intensive Care Units. This is in agreement with other reports in the literature on Prn deficient *B. pertussis* infections which have found no difference in proportions reporting symptoms such as apneoa, duration of coughing illness or requirement for admission between infections with Prn deficient vs Prn positive infections [15, 25]. Our study adds to previously reported data [15, 25] with the inclusion of greater numbers of younger infants and thus provides reassurance that the rapid emergence of Prn deficient *B. pertussis* variants is unlikely to contribute to any greater risk of death or severe outcomes from infections in young, vulnerable infants.

In contrast with Martin *et al*. [15], but similar to Bodilis *et al.* [25], we did not find any association between Prn status and history of vaccination. This may be due to our relatively small sample size with only 35 of the 71 Prn deficient cases having evidence of any prior vaccination. In agreement with Bodilis *et al.* [25], our data also demonstrated that vaccination was associated with reduced risk of severe disease.

There is no evidence from this study that Prn deficient *B. pertussis* infections increase susceptibility of hosts to co-infections, although it is important to recognize that not all cases were routinely tested for the presence of respiratory co-pathogens and thus it is possible that co-infections were undetected in children in this cohort.

Bacterial load may be an important factor associated with severity of disease as shown in previous research [28]. We do not have PCR results to get a measure of

bacterial loads for our cases, however, all eligible cases required successful culture. As culture success has been linked to higher bacterial load [29], it is likely that bacterial loads were similar between Prn deficient and Prn positive cases included in this study.

Examining factors associated with more severe *B. pertussis* infection using univariate models reconfirmed that young age and absence of prior vaccination were important predictors of more severe disease. Interestingly, our data suggests that although there was no evidence that Prn deficient isolates were related to more severe disease, strains carrying the *ptxP3* promoter allele may have an impact on disease severity. However, the small number of non-*ptxP3* cases (n=22) and wide confidence intervals for estimated risk ratios limits the ability of this study to provide conclusive evidence on the impact of *ptxP3* and disease severity. In our study, only two cases infected with a non-*ptxP* strain had lymphocytes measured so we were unable to examine the relationship between these *ptxP* variants and lymphocytosis. Further investigation on the importance and prevalence of *ptxP3* on severity of pertussis disease is warranted. These results agree with recent literature suggesting that *ptxP3* strains are more virulent in humans than *ptxP1* strains based on death and hospitalisation data in the Netherlands during two time periods with low and high *ptxP3* frequencies [16]. The proportion of cases that were *ptxP3* in our sample (which included isolates from 2008-2012) was high (89%), with similar predominance of *ptxP3* strains evident around Australia [17] and globally [13, 28].

Our results provide reassurance that the recent evolutionary changes in *B. pertussis* (increasing proportions of Prn deficient isolates) are not significantly impacting on disease severity. Some evidence, including our study findings, suggest that Prn

deficient genotypes may be associated with less severe disease, which may lead to increased transmission due to delayed or under diagnosis of the infected cases. Surveillance of pertussis genotypes and the impact of the rapid emergence of *B. pertussis* variants on effectiveness of vaccination programs should be closely monitored.

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CHAPTER 6: Manuscript 3 - Community awareness and predictors of uptake of pertussis booster vaccination in South Australian adults.

The majority of severe pertussis occurs in young children, particularly those too young to be protected by direct vaccination. There is increasing evidence that *B. pertussis* is commonly transmitted from parents to young infants, and recent evidence that the majority of notified pertussis occurs in adults. As neither natural infection nor vaccination affords lifelong protection against pertussis, unvaccinated adults play a significant role in transmission of pertussis to vulnerable infants. Furthermore, whilst adult pertussis booster vaccinations are recommended for any adult wishing to reduce the likelihood of pertussis, this prevention strategy is not funded (with the exception of recent introduction of free pertussis vaccination for pregnant women in their third trimester). Adults also often present atypically, often with milder symptoms and may therefore have a longer time between onset and diagnosis or treatment. Unrecognised, untreated pertussis facilitates transmission and therefore increasing the vaccine coverage in adults may improve interruption of transmission to infants. As there are currently no systematic methods of recording adult vaccination, data on pertussis booster vaccination coverage in Australian adults is very limited. We aimed to assess the level of knowledge in the general community about pertussis (whooping cough) and their attitude towards, awareness of, and uptake of an adult booster pertussis vaccination. The manuscript reports finding from a large cross sectional survey assessing knowledge, attitudes, awareness and predictors of adult pertussis booster vaccination. The manuscript has been published in the journal "*Vaccine".*

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Name of Principal Author (Candidate)	Michelle Clarke
Contribution to the Paper	MC designed and performed all data analysis. MC interpreted finding and authored the manuscript.
Overall percentage (%)	75%
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	HM assisted in study design and provided supervision and guidance through the development of the manuscript. HM contributed to review and editing of the final manuscript.		
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Please cut and paste additional co-author panels here as required.

6.1 ABSTRACT

Objective: Pertussis is a highly virulent vaccine preventable disease that remains a global challenge. This study aimed to assess community knowledge of pertussis infection and transmission as well as awareness and uptake of preventative strategies, such as a pertussis booster vaccination for adults.

Methods: A cross-sectional survey was conducted of randomly selected households in South Australia by Computer Assisted Telephone Interviews in 2011. Survey data were weighted to the age, gender and geographical area profile of the population.

Results: From 3124 randomly sampled contactable households, 1967 interviews were conducted (participation rate 63%) with individuals aged 18-93 years, including 608 parents of children aged <18 years. The majority of respondents (97%) had heard of pertussis (whooping cough) and 18% reported that a household member had previously experienced whooping cough infection. Most respondents (73%) considered whooping cough to be highly contagious and severe for infants (89%). Over half (51%) of those surveyed were aware that family members commonly transmit pertussis to infants. Despite high knowledge, pertussis vaccine uptake was low, with only 10% of respondents reporting pertussis vaccination in the previous five years. Whilst 61% of respondents were aware of the availability of an adult pertussis booster vaccine, only 8% (n=154) reported their family physician had discussed it with them. If provided free, 77% agreed that they would be more likely to accept a booster pertussis vaccination. Independent predictors of recent pertussis vaccination included higher education, larger household size, perception of greater disease severity and discussion with a family physician about pertussis vaccination.

Conclusions: Whilst knowledge regarding transmission and severity of *Bordetella pertussis* was high, uptake of pertussis vaccination for adults is remarkably low

amongst the South Australian community. Improved awareness regarding the availability of a booster pertussis vaccine through family physicians and/or provision of funded pertussis vaccination for adults has the potential to improve pertussis vaccine coverage.

6.2 INTRODUCTION

Despite long standing immunisation programs, control of pertussis continues to be a challenge globally with a substantial disease burden of morbidity and mortality. In 2008, the World Health Organization (WHO) estimated that approximately 195,000 children died as a result of pertussis infection [1]. Pertussis epidemics occur every 3-4 years in Australia [2], with its recent epidemic between 2009 and 2011 the worst since the introduction of pertussis vaccination in the 1940s. At the peak of the Australian epidemic in 2011, almost 38,000 cases of pertussis were notified, with incidence rates increasing to 450/100,000 for infants and young children [3]. Between 2006 and 2011, 11 pertussis deaths were reported, with 10 of these occurring in infants less than six months of age [4].

In Australia, vaccination against pertussis is funded for infants and children through the National Immunisation Program (NIP) offering free pertussis-containing vaccinations at 2, 4 and 6 months of age, with a booster vaccination at four years and again for adolescents when 10-15 years of age. Pertussis vaccination is also recommended for toddlers at 18 months of age and any adult who wishes to reduce the likelihood of illness from pertussis, but these recommendations are not currently funded as part of the NIP [2]. To reduce the risk of pertussis occurring in infants or others at increased risk of severe pertussis, healthcare workers, childcare workers and adult household contacts/carers of young children are strongly recommended to receive vaccination against pertussis with boosters every 10 years. At the time of

the study, women planning a pregnancy, pregnant (third trimester) or post-partum and those in close contact with infants and children were also recommended to receive a single dose of pertussis containing vaccine if five or more years have elapsed between prior pertussis vaccination and expected delivery date. Recently, these guidelines have been updated to include a recommendation for pregnant women to receive pertussis vaccination during their third trimester of pregnancy, with this vaccine provided as part of a funded program in most states of Australia [2].

Epidemiological evidence indicates that adults are a significant reservoir for pertussis infection and transmission. A review of South Australian data has shown the majority of cases of notified pertussis that occurred during the first 18 months of the 2009-11 epidemic, occurred in adults, with 66% of the notifications for individuals over 24 years of age [5]. It has also been established that the most common source of transmission of pertussis infection to vulnerable young infants are parents and siblings [6, 7]. Strategies proposed to reduce the burden of pertussis disease, particularly in infants who are at most risk of severe disease and/or death, have included the cocooning strategy, which involves vaccinating members of the household to reduce the risk of transmission to newborn babies [8], or more recently, maternal immunisation to facilitate maternal antibody transfer to vulnerable infants during their first few months of life [9].

High uptake of effective vaccines is necessary for successful infectious disease prevention programs. Neither infection nor vaccination provide long term immune protection against *Bordetella pertussis*, with duration of immunity following acellular pertussis vaccines estimated at less than five years [8, 10, 11]. Vaccine coverage in Australian infants is high, with national data indicating that more than 92% of

infants have received the recommended three doses of pertussis containing vaccine by 12 months of age [12]. There are no current systematic processes for capturing adult population immunisation rates and therefore pertussis vaccine uptake in Australian adults remains largely unquantified. As adults are a common reservoir for transmission of infection to vulnerable infants, it is important to understand their knowledge of pertussis disease and immunisation strategies and estimate pertussis vaccine coverage and associated factors.

6.3 METHODS

6.3.1 Study design and population

This cross-sectional study was conducted as part of the 'Health Monitor' program conducted by the Population Research and Outcomes Studies (PROS) unit, University of Adelaide, South Australia (SA)[13]. The random sampling process was based on the South Australian electronic White Pages household telephone listings in both metropolitan and rural areas. The household contact identified the adult in the household (aged ≥ 18 years) who most recently had a birthday. The interviews were conducted by the Computer-Assisted Telephone Interviewing (CATI) method. Up to six call-backs were made to interview the identified individual if they were not available at the time of the telephone call. Phone calls were made to households at different times between 9am and 9pm over seven days per week. A pilot study of 50 randomly selected households was completed in March 2011 to test the question formats and sequence prior to commencement of the main study.

The structured survey was designed to determine the level of knowledge and community awareness of pertussis and the recommendation and availability of the adult pertussis booster vaccine. Respondents were asked about prior pertussis

vaccination, knowledge and experience with pertussis infections. For simplicity, the term ‘whooping cough’ was used throughout the survey rather than ‘pertussis’. Participants were asked to rate how severe whooping cough infection was in infants aged less than six months on a scale from 1 (very mild) to 10 (extremely severe). Respondents were also asked to rate the ease of spread of pertussis from person to person on a scale of 1-10, with 1 being not at all contagious and 10 being extremely contagious. Participants were also nominated who they thought young infants were most likely to catch whooping cough from (with multiple responses allowed).

6.3.2 Data collection and analysis

For the purpose of the current study, a sample size of >1500 respondents enabled the proportion within the community who had knowledge of pertussis, or who had been vaccinated against pertussis in the last five years, to be estimated with a ± 2.5% precision at a 95% confidence level.

All survey responses were weighted to ensure that survey findings were applicable to the South Australian population. Survey weights were calculated from the inverse probability of selection of a household and re-weighted to sex, age and geographical area profile (metropolitan or rural) according to ABS 2009 Estimated Residential Population data for South Australia [14].

Estimates of population percentages for respondent characteristics with 95% confidence intervals (95% CIs) are presented. Univariate log binomial regression models were used to assess factors associated with awareness and uptake of pertussis booster vaccination with outcomes reported as risk ratios (RR) with 95% CI.

Multivariable models were developed to assess adjusted associations and including univariate predictor variables with a p value ≤0.1. All analyses were carried out using STATA version 11 (StataCorp, Texas). A two-tailed p-value of less than 0.05 was considered to be statistically significant.

6.3.3 Ethics

The study was conducted in accordance with Good Clinical Practice (GCP) guidelines, the National Statement for Ethical Conduct in Human Research and the applicable Women's and Children's Hospital, The University of Adelaide and the Department of Health policies.

Written information was supplied to the participant prior to telephone contact. This written information explained the study and the nature of their involvement. It also included a telephone number participants could telephone if they preferred to opt out of the survey. Verbal informed consent was obtained over the telephone from each participant prior to the survey beginning.

This study was reviewed and approved by the Women's and Children's Hospital Human Research Ethics Committee (HREC # 2352)

6.4 RESULTS

6.4.1 Study population (weighted data)

From 4,400 households selected to participate (from a total of 660,461 households in South Australia) [15], 3,124 were able to be contacted. From these households, 1,967 adults completed a computer aided telephone interview during March and April 2011 with a participation rate of 62.9% (Figure 1).

Figure 1: Sample flowchart

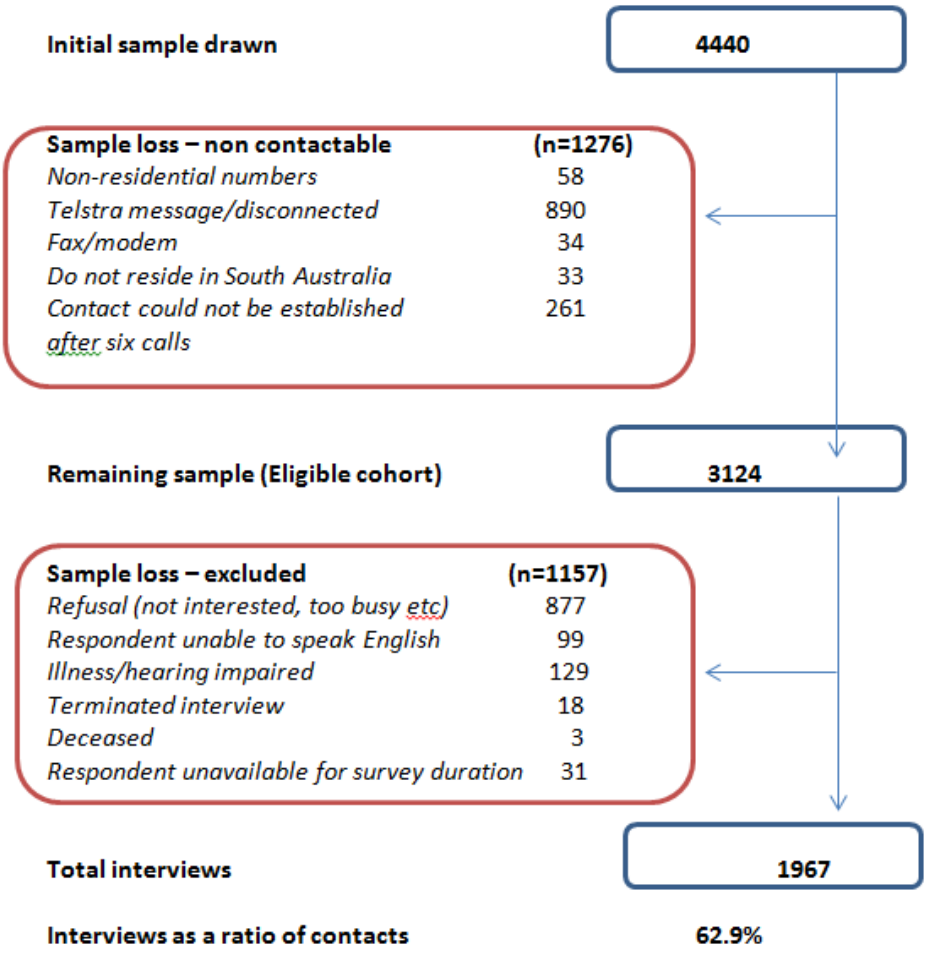

Table 1: Respondent Characteristics (Raw and Weighted)

		Raw Data		Weighted Data		
Variable	Level	Total	%	Weighted number	Weighted %	95% Confidence Limits
Sex	Male	776	39.45	962.04	48.89	46.20-51.58
	Female	1191	60.55	1006.0	51.11	48.42-53.38
Age category	18-34	308	15.66	566.19	28.77	25.93-31.61
	35-54	617	31.37	705.63	35.86	33.31-38.40
	55-74	762	38.74	504.03	25.61	23.64-27.58
	75+	280	14.23	192.11	9.76	8.55-10.98
Area	Metropolitan	1440	73.21	1451	73.73	71.41-76.05
	Rural	527	26.79	516.99	26.27	23.95-28.59
Aboriginal/TSI	Yes	11	0.56	12.48	0.64	0.22-1.05
	No	1951	99.44	1950	99.36	98.95-99.78
Number of children < 18 years in household	0	1424	72.39	1234.0	62.70	60.02-65.38
	1	210	10.68	287.72	14.62	12.54-16.70
	2	240	12.20	328.58	16.70	14.56-18.83
	3+	93	4.73	117.74	5.98	4.71-7.26
Parent with child <18y in household?	Yes	543	27.61	607.52	30.95	28.40-33.50
	No	1424	72.39	1355.0	69.04	66.50-71.60
Employment group	Employed	1040	53.03	1245.0	63.27	60.82-65.72
	Unemployed	27	1.38	35.88	1.82	1.06-2.59
	Home duties	99	5.05	101.71	5.17	3.99-6.34
	Retired	674	34.37	423.68	21.53	19.77-23.29
	Other	121	6.17	161.56	8.21	6.59-9.83
Country of birth	Australia	1526	77.58	1559.0	79.23	77.08-81.37
	UK and Ireland	238	12.10	196.61	9.99	8.50-11.48
	Other	203	10.32	212.19	10.78	9.06-12.50
Income group	Up to $20,000	288	14.64	173.78	8.83	7.67-9.99
	$20,000 - $40,000	544	27.66	486.80	24.74	22.53-26.94
	$40,000 - $80,000	717	36.45	891.90	45.32	42.63-48.02
	$80,000 -$100,000	159	8.08	132.30	6.72	5.53-7.92
	$$100,000 +	259	13.17	283.18	14.39	12.32-16.46
Highest education	School	918	46.81	851.54	43.42	40.64-45.90
	Trade/Apprenticeship	616	31.41	632.2	32.24	29.61-34.64
	Bachelor degree	427	21.77	477.36	24.34	21.87-26.64

The age of respondents ranged from 18 to 93 years with a mean age of 47.5 years. Almost half of adults were male (47.4%, n=962) and 608 respondents (30.9%) interviewed were parents with at least one child (aged less than 18 years) residing in the household. The majority of respondents were born in Australia (79.2%) and lived in metropolitan Adelaide (73.7%) with the remainder (26.3%) living in rural South Australia. Educational attainment varied amongst the respondents with 9.8% having left school at the age of 15 years or less, 32.1% with a trade, certificate or diploma and 24.3% attaining a bachelor degree or higher (Table 1).

6.4.2 Knowledge: severity and transmissibility

The majority of respondents (97.1%) had previously heard of whooping cough and almost 1 in 5 (361/1967, 18.4%) reported that at least one person in their household had previously experienced whooping cough, with 39 (2.0%) respondents reporting a household member had experienced whooping cough within the previous 12 months.

Almost 90% (n=1741) of respondents rated the severity of pertussis infection in infants as high (≥7), 5.4% (n=105) reported that they did not know, and only 1.0% (n=20) of respondents rated this question with a score of ≤3. Participants nominated who they thought young infants were most likely to catch whooping cough from with 50.7% (n=998) of respondents correctly suggested that mothers and fathers were a likely source of infection. Siblings and other children were also considered a likely source of infection by 29.5% (n=581) of respondents. Grandparents were only considered a likely source of transmission for 10.9% (n=215) of respondents. Most respondents (73.1%; n=1437) rated pertussis as highly contagious (score ≥7) with less than 1% (n=13) suggesting that whooping cough was not contagious (score=1).

6.4.3 Awareness of adult pertussis vaccine availability

Overall, 38.1% (750/1967) of the respondents were NOT aware that a vaccine was available to protect against whooping cough in adults. Of respondents who were aware that a vaccine was available, only 12.9% (n=154) reported that their GP had discussed whooping cough booster vaccination for adults. In multivariate log binomial regression analysis, male gender, younger age, lower educational attainment, metropolitan area of residence and being an Aboriginal or Torres Strait Islander were all associated with a reduced likelihood of awareness of vaccine availability. There was no significant difference in awareness of pertussis vaccine availability for adults by either parental status, household income or household size (Table 2).

Table 2: Predictors of awareness of pertussis vaccine availability

Variable	Levels	n^	Univariate regression			Multivariable regression		
			RR	CI	p value	RR	CI	p value#
Gender	Female	1005	1.0		<0.001	1.0		<0.001
	Male	962	0.83	0.76-0.91		0.83	0.75-0.91	
Age category	18-34	566	1.0		<0.001	1.0		<0.001
	35-54	705	1.07	0.93-1.23		1.03	0.90-1.18	
	55+	696	1.22	1.08-1.39		1.20	1.06-1.36	
Area	Metropolitan	1450	1.0		0.068	1.0		0.007
	Rural	517	1.09	0.99-1.20		1.13	1.03-1.23	
Aboriginal/ TSI	Non ATSI	1547	1.0		0.004	1.0		0.005
	ATSI	12	0.12	0.03-0.51		0.13	0.03-0.54	
Parental status	Non parent	1355	1.0		0.987			
	Parent	608	1.0	0.91-1.10				
Country of birth	Australia	1558	1.0		0.605			
	UK/Ireland	196	1.06	0.93-1.20				
	Other	208	1.03	0.90-1.19				
Income group	<$80,000	885	1.0		0.600			
	>=$80,000	667	0.99	0.89-1.09				
	Refused	415	0.94	0.83-1.06				
Highest education	School or less	851	1.0		0.088	1.0		0.042
	Trade/apprentice/ certificate/Diploma	632	1.02	0.92-1.13		1.06	0.96-1.18	
	Bachelor or higher	477	1.12	1.01-1.25		1.15	1.03-1.29	
Household size	1	232	1.0		0.385			
	2-3	1036	1.05	0.95-1.15				
	4-5	621	0.97	0.86-1.09				
	6+	79	0.89	0.61-1.29				
Household experience with whooping cough	Yes	361	1.0		0.004	1.0		0.321
	No	1476	0.95	0.86-1.05		1.00	0.90-1.24	
	Unk/missing	130	0.59	0.43-0.81		0.78	0.55-1.10	

^ weighted n in cohort

multivariable model included all variables with univariate associations with awareness of pertussis vaccine availability returning a p value ≤0.1.

Variable level p values were based on likelihood ratio tests

6.4.4 Pertussis vaccination and predictors of uptake

Overall, only 202 (10.3%) of the respondents reported that they had received a whooping cough vaccine within the preceding five years, although 1518 (77.2%) considered that they would be likely to accept the whooping cough booster vaccine if it was free. A slightly higher proportion of parent respondents (with a child under 18 years of age residing in the household) reported recent pertussis booster vaccination compared with non-parents (69/608, 11.3% vs 133/1355; 9.8%), although this difference was not statistically significant (χ^2 p=0.296).

In univariate analysis, gender, education level, household size, perception of severity of whooping cough in infants, perception of contagiousness of whooping cough and having had a Family Physician (FP) discussion about pertussis were all associated with higher likelihood of having received a booster pertussis vaccination within the prior five year period (Table 3). Of these, education level, perception of severity in infants and FP discussion remained independent, significant predictors of uptake in a multivariable model.

Any association between Indigenous status and vaccine uptake could not be established in this dataset as only 11 responders identified as Aboriginal or Torres Strait Islander and none had received the pertussis vaccine within the previous five years. It is important to note that whilst the largest risk ratio of uptake was associated with FP discussion, with respondents who had discussed whooping cough vaccination with a FP 3 times more likely to have been vaccinated (RR 2.95, CI 2.21-3.96; p<0.001), it cannot be determined whether this discussion preceded the decision whether or not to receive the vaccine.

Table 3: Predictors of pertussis vaccination within the preceding five years

		n^	Univariate binomial regression			Multivariable binomial regression		
Variable	**Levels**		RR	CI	p-value	RR	CI	p-value
Gender	Female	1005	1.0		0.011	1.0		0.574
	Male	962	0.65	0.47-0.91		0.92	0.68-1.23	
Age cat	18-34	566	1.0		0.939			
	35-54	705	1.06	0.68-1.64				
	55+	696	1.01	0.66-1.53				
Area	Metropolitan	1450	1.0		0.641			
	Rural	517	0.93	0.67-1.28				
Aboriginal/TSI	Non ATSI	1547	1.0					
	ATSI	12	NA*					
Parental status	Non parent	1355	1.0		0.383			
	Parent	608	1.16	0.83-1.63				
Country of birth	Australia	1558	1.0		0.354			
	UK/Ireland	196	0.73	0.42-1.29				
	Other	208	0.72	0.39-1.33				
Income group	<$80,000	885	1.0		0.106			
	>=$80,000	667	1.44	1.02-2.01				
	Refused/not stated	415	1.09	0.73-1.64				
Highest education	School	851	1.0		<0.001	1.0		0.014
	Trade/apprentice/ Cert/Diploma	632	1.36	0.94-1.97		1.24	0.89-1.72	
	Bachelor or higher	477	2.30	1.58-3.33		1.66	1.18-2.34	
Employment status	Employed	1245	1.0		0.191			
	Unemployed/ student	288	0.64	0.37-1.11				
	Retired	423	0.83	0.60-1.13				
Household size	1	231	1.00		0.005	1.0		0.061
	2-3	1036	1.79	1.24-2.56		1.48	1.05-2.08	
	4+	699	1.31	0.83-2.05		1.20	0.78-1.83	
Perception of severity for infants (scale 1-10)	High (7+)	1741	1.0		0.003	1.0		0.041
	Low/Mod (<6)	122	0.67	0.32-1.37		0.74	0.36-1.53	
	Don't know	104	0.19	0.07-0.52		0.23	0.07-0.76	
Perception of contagiousness (scale 1-10)	High (7+)	1437	1.0		<0.001	1.0		0.128
	Low/Mod (<6)	399	0.37	0.20-0.66		0.52	0.31-0.99	
	Not contagious /Don't know	130	0.31	0.11-0.83		0.82	0.31-2.16	
Household experience with whooping cough	Yes	361	1.0		0.477			
	No	1476	1.13	0.74-1.72				
	Unk/missing	130	0.71	0.29-1.7				
FP discussed pertussis	No	1766	1.0		<0.001	1.0		<0.001
	Yes	154	7.85	5.7-10.7		3.06	2.29-4.06	

^ weighted n in cohort

*ATSI –This variable could not be included in regression models because no ATSI respondent had received a pertussis vaccination in the previous five years.

6.5 DISCUSSION

The results of this research indicate that whilst the population are well informed about whooping cough disease and infection, including the vulnerability of young infants to severe consequences and adults/parents being a highly likely source of transmission, their awareness of the availability of pertussis booster vaccines for adults is relatively low. Almost 40% of adults were not aware that a pertussis booster vaccine was available for purchase and only 10% of South Australian adults had been vaccinated with a booster vaccine within the last five years. This uptake percentage is slightly higher than reported in the 2009 Australian Adult Vaccination Survey [16] which estimated only 7.8% of South Australian adults had received a pertussis vaccine during adulthood. This slight improvement in vaccination rate could be attributed to increased awareness of pertussis and the potential for vaccination in the community as the epidemic progressed.

The results of this survey demonstrated a significant association between FP discussion about whooping cough booster vaccine recommendations and vaccine uptake. Several studies have demonstrated that a recommendation from a physician can improve vaccine uptake in both the adolescent and adult community [17-19]. Overall, however, the vast majority of respondents reported that their FP had not discussed pertussis booster vaccines, despite the study being conducted immediately following a global pertussis epidemic. Increasing Family Physician education around the value of adult vaccination or encouraging FPs to discuss immunisation as part of their routine assessment may be valuable for improving adult pertussis vaccination coverage.

Adult booster pertussis vaccination was also significantly more likely for people with a higher level of education and a greater perception of disease severity, indicating that appropriate awareness campaigns and information around the potential consequences of pertussis infection could be beneficial for improving coverage rates in our adult population. Although there were few Aboriginal/Torres Strait islander respondents in this survey, results suggest that awareness uptake of pertussis vaccination in these respondents was low compared with non-Aboriginal/Torres Strait Islander respondents. Reducing disparities in disease burden between Aboriginal and non-Aboriginal people requires equal access and information about preventative health measures such as vaccination and this survey indicates that disparities continue to exist.

The majority of respondents in this study also reported that they would be more likely to accept the booster pertussis vaccine if it was provided at no cost. This suggests that cost of pertussis booster vaccinations may be a potential barrier and that providing vaccination either for no or low cost may improve uptake of booster vaccination in adults.

As prior pertussis vaccination history was ascertained from adult self-report, some recall or misclassification bias may exist. There are very few adult immunisations recommended in Australia, however, and pertussis vaccination would have required an act to purchase, therefore any recall bias is likely to be minimal. In SA, healthcare workers were provided with a pertussis containing vaccine at no cost during 2009 and from October – December 2010 parents and grandparents of infants aged <6 months, who held a health-care or pension concession card which entitles low income individuals/families to government subsidised health care, were

eligible for free pertussis vaccination. Therefore there is the potential that the coverage rate determined in this study is higher than during non-epidemic periods and where pertussis boosters were not funded for targeted parts of the population.

Whilst the data gathered for this research are limited to the South Australian population, the findings clearly suggest that more can be done to improve control of pertussis; many of the findings are likely to be transferrable to other populations with similar education and health systems. It is important to note that this survey was conducted in 2011, following a global pertussis epidemic, when pertussis infections were being frequently discussed in the media and health departments. In March 2015, the South Australian Health Department introduced free pertussis vaccine for pregnant women in each pregnancy to improve protection for vulnerable infants. This policy change, and the increased awareness and discussion around adult pertussis vaccination may lead to higher pertussis vaccination coverage rates in the SA community than was determined in this survey.

In conclusion, our findings suggest that campaigns to improve awareness of pertussis booster vaccinations that target adults, particularly through FP practices, may improve uptake of preventative vaccination strategies aimed at reducing the burden of pertussis disease and greater protection for vulnerable infants.

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CHAPTER 7: CONCLUSION

7.1 Key findings and significance

Our research findings provide comprehensive and detailed information on the burden of severe pertussis infections in Australian children, provide reassurance regarding the impact of antigenic evolution of *Bordetella pertussis* and offer potential strategies for improved protection for infants from this potentially life threatening infection.

1) Severe pertussis occurs in infants too young to be vaccinated.

   With a detailed investigation into the clinical features and predictors of severity for Australian children hospitalised with *B. pertussis*, we were able to demonstrate that the greatest challenge for prevention of severe pertussis is that the majority of severe disease occurs in infants too young to have received protection through vaccination. We were also able to demonstrate that presence of a co-infection, presence of fever (which may be a surrogate marker of co-infection in children where presence of respiratory co-pathogens is not tested) or a history of prematurity are associated with increased risk of severe disease.

   These findings may assist with improving medical management, including admission or closer monitoring for infants with these indicators presenting to emergency departments with pertussis symptoms. Immunisation during pregnancy has subsequently been introduced as the most severe disease occurs in young infants.

2) Earlier, timely infant vaccination may improve, but does not guarantee, protection against severe pertussis.

Through both the national pertussis severity study and the pertussis genotypes study, we were able to demonstrate that the majority of severe pertussis occurs in young, unvaccinated infants. In the first study, of the 70 cases in children over two months of age, almost a third (n=23) had not received pertussis vaccine. Similarly in the genotype study which included 199 culture confirmed pertussis cases across a five year period, more than 20% of children over two months of age had not received any prior pertussis vaccination. Over 40% of unvaccinated children were classified as severe compared with 6% of children who had received at least one dose of pertussis vaccine prior to diagnosis.

Our research findings clearly highlighted that pertussis requiring hospitalisation also occurs in children who have received prior pertussis vaccination with 29/120 (24%) children in our pertussis severity study having received at least two prior doses of an acellular pertussis vaccine prior to hospitalisation. Several studies have shown that the duration of immunity following a primary course of acellular pertussis vaccination may be lower than first anticipated. This could explain the presence of fully vaccinated children requiring hospitalisation for pertussis as seen in our first study as the median interval between the third dose of pertussis vaccine and hospitalisation was 3.5 years. Therefore strategies aimed at earlier vaccination, reduced transmission or improved vaccines with greater efficacy against infection are warranted.

Genotypic changes in the circulating bacteria were described during the last epidemic. The major change observed was the loss of Prn antigen expression, a component of all pertussis vaccines licensed in Australia, with the potential to

be related to altered disease severity or reduced vaccine protection against pertussis.

3) The rapid emergence of Pertactin deficient *B. pertussis* strains are unlikely to be affecting disease severity in Australian children – although the impact of strains carrying *ptxP3* alleles warrants further research.

We compared markers of severity between pertactin deficient and pertacin positive strains and found that there was no increased risk of severe pertussis for children infected with Prn deficient strains compared with Prn positive strains. (RR 0.97, 95%CI 0.57-1.62, p=0.90). Prn deficient isolates have rapidly emerged in Australia. Only one of the 29 isolates (3.4%) collected in 2008 was identified as Prn deficient, compared with 28/39 (71.8%) and 29/35 (82.9%) of isolates identified as Prn deficient in 2011 and 2012 respectively. Strains containing the *ptxP3* allele are also predominant with more than 80% of isolates carrying the *ptxP3* allele between 2008 and 2012.

Although sample size limited the statistical power to investigate interactions between pairs of variables, there was some suggestion that strains carrying *ptxP3* alleles were associated with more severe infection; 38/177 (21%) of children with *ptxP3* carrying isolates were classified as severe compared with 1/21 (5%) of non-*ptxP3* isolates.

Whilst our research demonstrated that the shift to Prn deficient isolates is unlikely to affect disease severity, the association between Prn and *ptxP3* warrants further investigation, as does the impact of *ptxP3* strains on disease severity. Although our study did not show any statistically significant association between Prn and vaccination status of the child, our sample size was relatively small. An investigation of whether Prn deficient strains are able to evade

vaccine protection or are more easily transmitted amongst vaccinated hosts than Prn positive strains is needed. Interrupting transmission is an important objective of vaccination programs and a key to protecting infants who are the most vulnerable to life threatening consequences from infection with *B. pertussis.*

4) Community understanding of pertussis is high, but awareness of, and uptake of adult pertussis booster vaccination is low.

Our community survey, which included almost 2000 adult respondents from South Australia during the peak of the most recent pertussis epidemic (2011) demonstrated a high awareness of pertussis in the community. Most respondents (97%) were aware of pertussis, and considered it a highly contagious and severe infection for infants. Over half of the respondents (51%) also correctly identified that young infants are likely to contract pertussis from their parents. However, awareness and uptake of adult booster vaccination for pertussis was sub-optimal.

Whilst adult pertussis booster vaccination was an available and recommended option at the time of the survey, and despite the survey being done during the peak of a pertussis epidemic, only 10% of respondents reported receiving a pertussis vaccination in the previous five years. Whilst this estimate may be influenced by recall bias, it is likely that vaccination coverage in adults is very low. However, if provided free, 77% agreed that they would be more likely to accept a booster pertussis vaccination. Whilst adult pertussis booster vaccination was recommended in the Australian Immunisation Handbook, it was not funded at the time of the survey for adults in South Australia.

Over 40% of adult respondents were unaware of the availability of an adult pertussis booster vaccine highlighting a challenge within our recommended but not funded vaccine programs. It is important that individuals are not disadvantaged through lack of opportunity to take up potentially life-saving vaccines, and strategies to enhance communication and information about such vaccine programs need attention. Delivery of information about preventative health opportunities might be expected to occur through general practitioners, however in our cross sectional study, less than 8% of respondents reported that their General Practitioner (GP) had discussed pertussis vaccination.

Independent predictors of recent pertussis vaccination included higher education, larger household size, perception of greater disease severity and discussion with a GP about pertussis vaccination. Our research suggests there are various strategies that could be implemented to increase adult pertussis vaccination coverage. These include increased opportunities for awareness, and potentially a funded program at recognition of epidemic onset to attempt to reduce transmission and limit the epidemic spread.

7.2 Strengths and Limitations

7.2.1 Strengths

This program aimed to determine factors associated with severe pertussis disease in Australian children and estimate uptake and awareness of pertussis booster vaccination for adults. Strengths of this research program included the national contribution to risk factors for severity, and the completeness of clinical data capture for both the severity study in hospitalised children and the study of associations between genotype and disease severity.

The community study of pertussis vaccine uptake and awareness used rigorous proven methods in survey collection, allowing for random selection of households, with data weighted to ensure representativeness to the South Australian population. In addition, the large sample size for this survey (n= 1967 respondents) allowed for precise population estimates (+/- 2.5% at a 95% confidence level) for variables of interest, including the proportion of adults who had received a pertussis vaccination in the previous five years.

All three studies provided new evidence for the current body of scientific literature around risk factors for severe disease and pertussis vaccine coverage amongst adult populations. The study of risk factors for more severe disease in hospitalised children with confirmed pertussis was consistent with previous research demonstrating that the youngest infants are most at risk of severe disease, but also indicated that fever, co-infection and a history of prematurity were additional important factors to consider. Furthermore, this study reports a novel pertussis severity score which can be used as an objective, sensitive tool to measure changes in severe disease. The study of the relationship between emerging genotypes and disease severity is one of only three papers world-wide to report on clinical outcomes of Prn deficient infections and provides data for a large number of young

infants compared with other published studies, adding robust evidence of where the majority of severe disease burden lies.

7.2.2 Limitations

7.2.2.1 – Determining risk factors for disease severity

The findings of a positive association between fever and co-infection at presentation and more severe pertussis disease in children hospitalised with pertussis was limited by the sample size of the enrolled cohort and variations in ascertainment of presence of co-infecting pathogens between sites. As testing practices for routine respiratory panel testing varies between Australian states, and respiratory pathogen testing does not include all potential co-infecting agents, it is possible that cases with fever in absence of identified co-pathogens may be indicative of presence of an unidentified co-infection. Not all children were tested for co-infections at all centres. Therefore our results may be biased in that children who appeared more unwell may have received additional respiratory testing at several of the centres without routine testing. However, in a stratified analysis limited to only the cases enrolled at the Women's and Children's Hospital (n=37) where testing for co-pathogens was conducted for 95% of participants, the association between presence of co-infection and severe disease remained (OR: 4.82, CI 1.66-14.04, p=0.004). Associations for fever/co-infections and severe disease whilst statistically significant, had imprecise estimates of magnitude of association.

Further research to explore the relationship between fever/co-infection and disease severity is warranted because of the small numbers of cases with these predictors (n=14 with fever at presentation, n=29 with identified co-infection) and the potential for misclassification of these variables in this study.

A further limitation was the inability to include lymphocytosis in the severity scoring system, as this is an established and specific indicator of pertussis disease severity. Lymphocyte counts were only measured for 54% (65/120) of the enrolled cases and therefore this parameter was removed from the scoring system.

7.2.2.2 – Associations between B. pertussis genotype and disease severity.

In the study of the relationship between pertussis genotype and *B. pertussis* disease in children presenting to hospital, we were limited by the sample size. As Prn status was the primary predictor variable of interest, we could only include cases where Prn status had been determined and this was dependent on isolate availability. This meant that the study was only powered (80%) to significantly detect approximately 20% or greater difference in proportions classified as severe between Prn deficient and Prn positive isolates (i.e. 15% vs 35%). This meant that interaction effects between variants (i.e. Prn deficient or positive and presence or lack of *ptxP3* allele) could not be investigated. Similarly, whilst data suggested *ptxP3* may play a role in increased disease severity, the small number of non-*ptxP3* isolates (n=22) limited our ability to provide any conclusive evidence to support the relationship between *ptxP3* and severity.

Young age and vaccination status were strongly correlated predictor variables, but our sample size limited any ability to explore any interaction effects in the multivariable models. Correlation between predictor variables may also result in inflated variance and imprecise point estimates, however upon thorough examination of variance inflation factors and various models including and excluding correlated variables, it was apparent that any effects of correlation did not greatly change the point estimates or conclusions from models.

As this study included three sites (SA, WA, NSW), it is possible that the isolates included in this study are not representative of all children with pertussis disease presenting to hospital because only cases with an isolate available and successfully genotyped were able to be included. Over the last decade, there has been a move away from culture and increased use of PCR for diagnosis of pertussis, therefore limiting the number of genotyped isolates available for this study.

7.2.2.3 – Estimating uptake of recent adult pertussis booster vaccination

Whilst this research program allowed us to estimate uptake of recent adult booster pertussis vaccination in the South Australian community, there was no ability to verify the accuracy of self-report pertussis vaccine receipt and therefore the findings may be affected by recall or misclassification bias. It is expected that such bias would be minimal in this study however as there are very few adult immunisations recommended in Australia, and pertussis vaccination would have required an action to purchase. The use of an automated telephone survey methodology would also minimise any social desirability bias which may occur when respondents feel compelled to answer in an affirmative way rather that truthfully. There were also some vaccine program changes in response to the epidemic which may influence reported pertussis vaccine coverage. In South Australia, healthcare workers were provided with a pertussis containing vaccine at no cost during 2009 and from October – December 2010 parents and grandparents of infants aged less than 6 months, who held a means-tested health-care or pension concession card, were eligible for free pertussis vaccination. Therefore there is the potential that the coverage rate determined in this study is higher than during non-epidemic periods and where pertussis boosters were not funded for targeted parts of the population. At the time of the survey, there was no funded pertussis vaccination program for

adults (apart from the three month cocoon strategy for new parents/grandparents who were health care card holders).

Whilst the number of respondents for this study was high (n=1967), some selection bias may also exist as the participation rate (interviews as a percentage of eligible contactable households) was 62.9%. Therefore it is possible that potential responses from those who refused to participate or were unable to speak English to a level sufficient for the survey completion) may have differed from those who agreed to participate.

Despite these limitations and potential for bias, study findings strongly suggest that recent pertussis vaccination in the South Australian adult population has substantial room for improvement.

It is important to note that this survey was conducted in 2011, following a global pertussis epidemic, when pertussis infections were being frequently discussed in the media and health departments. In March 2015, the South Australian Health Department introduced free pertussis vaccine for pregnant women in each pregnancy to improve protection for vulnerable infants. This policy change, and the increased awareness and discussion around adult pertussis vaccination may lead to higher pertussis vaccination coverage rates in the South Australian community than was determined in this survey.

7.3 Future directions

Following this research program, and with the recent introduction of vaccination of pregnant women in the third trimester of pregnancy, it will be important to monitor the impact of this policy on disease burden in young infants as well as vaccine

responses following infant's primary vaccination programs. We plan to perform a systematic review on the safety of pertussis vaccines during pregnancy and will continue to evaluate evidence related to current and future strategies for protecting infants from pertussis. We intend to examine vaccine effectiveness for the maternal pertussis vaccination program and to evaluate vaccine effectiveness against Prn deficient strains to determine whether the current vaccines in use are providing adequate protection for our infants and more broadly in the community. We are currently investigating barriers to pertussis vaccination in pregnant women and their providers to supplement our data for the general adult community, and will investigate the impact of pertussis vaccination during pregnancy on vaccine coverage, attitudes and impact on infant vaccination schedules. As we have also highlighted issues around suboptimal awareness of recommended but not funded vaccine programs, we also aim to explore opportunities to improve communication about preventative health strategies so as to reduce health disparities between groups through information asymmetry.

We will continue to endeavour to improve protection for infants from infectious diseases such as pertussis and to ensure that those at greatest risk of severe disease are identified early and provided with the best care and protection available.

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APPENDIX 1: Health Monitor Survey questions

Pertussis Questions - WCH

The next questions relate to the disease whooping cough. We are interested to find out how much you know about whooping cough disease and about vaccines available for whooping cough.

A.1 Have you heard of whooping cough before today?
(Single response, don't read options)

1. Yes [ ]
2. No [ ]
3. Don't know [ ]
4. Refused [ ]

A.2 How many people in your household, including yourself, have had whooping cough before?
(Single response)

1. …… [ ]
   (Participant to state number)
2. Don't know [ ]
3. Refused [ ]

A.3 How many have had whooping cough in the last 12 months?

1. …….. [ ]
   (participant to state)
2. Don't know [ ]
3. Refused [ ]

A.4 Has anybody in your household, including yourself, had a coughing like illness last two or more weeks within the last 12 months?
(Single response. Interviewer note: if yes ask how many)

1. Yes, specify how many_____
2. No [ ]
3. Can't remember [ ]
4. Don't know [ ]
5. Refused [ ]

A.5 On a scale of 1 to 10 how easy do you think it is for whooping cough to spread from person to person, with 10 being extremely contagious and 1 being not contagious?

(Single response, state number)

1. …… [ ]
2. Don't know [ ]
3. Refused [ ]

A.6 On a scale of 1 to 10 how easy do you think it is for babies less than 6 months of age to catch whooping cough, with 10 being extremely easy and 1 being very unlikely to catch it?

(Single response, state number)

1. …… [ ]
2. Don't know [ ]
3. Refused [ ]

A.7 On a scale of 1 to 10 how severe do you think the disease is in infants less than 6 months of age with 10 being extremely severe and 1 being very mild?

(single response, state number)

1. ……[ ]
2. Don't know [ ]
3. Refused [ ]

A.8 Who do you think infants under the age of six months are most likely to catch whooping cough from?

(Can have multiple responses)

1. Grandparents [ ]
2. Mother [ ]
3. Father [ ]
4. Healthcare worker [ ]
5. Childcare worker [ ]
6. Siblings [ ]
7. Don't know [ ]
8. Refused [ ]

A.9 Are you aware there is a vaccine available to prevent whooping cough in adults?

(Single response)

1. Yes [ ]
2. No [ ]
3. Don't know [ ]
4. Refused [ ]

A.10 Have you ever been vaccinated against whooping cough?

(Single response)

1. Yes [ ]
2. No [ ]
3. Don't know [ ]
4. Refused [ ]

A.11 When were you last vaccinated against whooping cough?

1. Within last 12 months [ ]
2. 12 months to 5 years ago [ ]
3. More than 5 years ago [ ]
4. Part of Childhood Immunisations [ ]
5. Do not remember/don't know [ ]
6. Refused [ ]

A.12 Why did you receive the whooping cough booster vaccine?

1. ..... [ ]
   (allow participant to state)
2. Do not remember/don't know [ ]
3. Refused [ ]

A.13 Why did you NOT receive the whooping cough booster vaccine?

1. ..... [ ]
   (allow participant to state)
2. Do not remember/don't know [ ]
3. Refused [ ]

A.14 Has your GP discussed the recommendation for adults to have a whooping cough booster vaccination?

1. Yes [ ]
2. No [ ]
3. Didn't need to, already vaccinated [ ]
4. Don't know [ ]

A.15 Would you be more likely to accept the whooping cough booster vaccine if it was for free?

1. Yes [ ]
2. No [ ]
3. Don't know [ ]
4. Refused [ ]

APPENDIX 2: Manuscripts

1) Marshall H, Clarke M, Rasiah K, Richmond P, Buttery J, Reynolds G, et al. Predictors of disease severity in children hospitalized for pertussis during an epidemic. *The Pediatric Infectious Diseases Journal*. 2015;34(4):339-45. (Impact Factor 3.135)

2) Clarke M, McIntyre P, Blyth C, Wood N, Octavia S, Sintchenko V, Giles L, Quinn H, Hanly G, Hill V, H, Lan R, Marshall H. Impact of pertactin deficient *B. pertussis* variants on clinical severity of pertussis disease in Australian children**.** *Journal of Infection. 2015 Dec 8. doi: 10.1016/j.jinf.2015.11.004* (Impact Factor: 4.441)

3) Clarke M, Thomas N, Giles L, Marshall H. Community awareness and predictors of uptake of pertussis booster vaccination in South Australian adults *Vaccine*. 2015 Dec 16;33(51):7337-43. (Impact Factor: 3.492)

Marshall, H., Clarke, M., Rasiah, K., Richmond, P., Buttery, J., Reynolds, G., et al. (2015). Predictors of disease severity in children hospitalized for pertussis during an epidemic.
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The relationship between *Bordetella pertussis* genotype and clinical severity in Australian children with pertussis☆

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KEYWORDS
Pertussis;
Hospitalization;
Children;
Pertactin;
Severity

Summary *Objectives:* Changes in circulating *Bordetella pertussis* genotypes, including a novel pertussis toxin promoter *ptxP3* allele and absence of pertactin (Prn) antigen, have been reported from several countries but limited data on relative severity are available. We compared markers of disease severity in children with *B. pertussis* infection due to strains of differing genotype.
Methods: Culture confirmed cases presenting to tertiary paediatric hospitals in three Australian states between 2008 and 2012 were classified as severe if they required a hospital stay greater than seven days, were admitted to intensive care, or if death occurred. Associations between age, vaccination, genotype and severity were assessed.
Results: Of 199 pertussis cases, 81 (41%) were <3 months, including 32/39 (82%) of severe cases. The proportion of isolates from these cases that were Prn deficient increased markedly between 2008 and 2012. Of *B. pertussis* isolates, the proportion considered severe was similar for Prn positive (27/128, 21%) and Prn deficient (12/71, 17%) cases but only 1/22 (4.5%) of non *ptxP3* cases were severe versus 38/177 (21.4%) *ptxP3* positive. Adjusting for *ptxP* type, vaccination status and age, disease severity was not significantly associated with Prn status (RRA: 0.95, [0.57–1.56]; p = 0.83).
Conclusions: In children, we found no relationship between Prn status and markers of severe pertussis. An increased proportion of severe disease in isolates with the *ptxP3* allele was observed.

Introduction

Pertussis remains a clinical and public health burden in Australia and globally. Despite long standing immunization programs in most countries, epidemics continue to occur every three to four years in high resource countries. The incidence of pertussis in young infants is particularly concerning, with infants at highest risk of death and severe disease.[1–3] The most recent pertussis epidemic in Australia was the largest reported in the vaccination era, both in terms of number of cases and duration of the epidemic.[2] At the peak of that epidemic, pertussis notification rates in young children reached 333 per 100,000 children less than five years of age. The highest notification rate was observed in children 5–9 years of age (550 pertussis notifications per 100,000 children).[2] Ten of the 11 pertussis deaths in Australian between 2006 and 2011 were in infants less than six months of age.[4]

The resurgence of pertussis in Australia may be explained by several factors, including improved detection through more sensitive PCR diagnostic tests, ease of testing[5] and reduced duration of immunity following the introduction of acellular vaccines in 1997.[6–9] Questions have also arisen regarding the impact of a change from whole cell pertussis vaccines to acellular vaccines on the duration of pertussis immunity and evolution of *Bordetella pertussis*.[6,10,11] The most prominent recent changes in circulating *B. pertussis* strains are polymorphisms in *ptxP*, the promoter of the Ptx operon, and the non-production of pertactin.[12–18] Previously, *ptxP3* has been shown to be associated with increased pertussis toxin production as a result of a single base mutation in the *ptxP* and with increased hospitalizations in the Netherlands.[16] In Australia, *ptxP3 B. pertussis* isolates predominated during the 2008–2012 epidemic.[17] There is increasing evidence worldwide of emergence of *B. pertussis* variants that are deficient in the pertactin (Prn) protein[10,18–22], an outer membrane protein involved in adhesion to epithelial cells.[23] The clinical consequences of these evolutionary changes are unclear.[10,15,18,21,24,25] Murine studies have indicated that Prn deficient strains remain virulent, with similar invasion and cytotoxicity properties compared with strains expressing Prn.[20] *B. pertussis* mutants which do not express Prn may persist longer in the epithelia than Prn expressing variants.[26] Two recent studies examining clinical findings in children and associations with Prn status of isolates suggest symptoms and clinical course are similar or reduced, with no apparent difference in requirement for hospitalization or presence of symptoms (with the exception of apneoa which was less likely in Prn deficient infections).[15,25]

As the current acellular pertussis vaccines used in Australia contain the pertactin antigen,[27] the absence of this outer membrane protein in circulating *B. pertussis* strains may allow the bacteria to "escape" vaccine induced immune protection increasing susceptibility to infection despite immunization.

In Australia, vaccination against pertussis is funded for infants and children through the National Immunization Program (NIP) offering free Diphtheria-Tetanus-acellular Pertussis (DTPa) vaccinations at 2, 4 and 6 months of age, with two booster doses at four years and 10–15 years of age.[27] In attempts to improve protection against pertussis, an 18 month booster dose will be re-introduced with federal funding from 01 October 2015. More recently, vaccination of women in their third trimester of pregnancy has been recommended and provided as part of a state funded vaccine program in all Australian states.[27]

Whilst it is clear that the prevalence of *B. pertussis* variants that do not express Prn has increased over recent years as demonstrated in several countries including USA, Australia and Europe,[10,18,21] the clinical implications of this change in epidemiology are less clear. Understanding the clinical relevance of the increasing prevalence of Prn deficient strains is important for future control through vaccination strategies and management of pertussis infections in children.

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This study aimed to assess the impact of emerging *B. pertussis* variants on severity of pertussis disease by comparing clinical disease parameters for Australian children infected with Prn deficient and Prn positive *B. pertussis* isolates between 2008 and 2012. Since the novel pertussis toxin promoter *ptxP3* allele has been associated with increased hospitalizations in the Netherlands,[16] we also examined the effect of the *ptxP3* allele on disease severity.

Methods

The genotypes and Prn expression of *B. pertussis* isolates from cultures of children presenting to three participating Australian tertiary paediatric hospitals (Sydney Children's Hospital at Westmead (New South Wales – NSW), Princess Margaret Hospital (Western Australia – WA) and Women's and Children's Hospital (South Australia - SA) between 2008 and 2012 have been determined in our previous studies.[10,17] The availability of isolates for genotyping was based on routine pertussis diagnostic practices at each of the participating hospitals. The majority of genotyped isolates were from NSW and WA where bacterial culture remains a diagnostic standard in conjunction with PCR. In SA, PCR testing was the routine diagnostic test for pertussis during the study period with culture only on specific request with very few samples collected for culture from 2010 onwards.

Medical and laboratory data were collected for children under 18 years of age who either presented to, or were admitted to one of the three participating hospitals and had an isolate available for determination of Prn expression. Clinical and demographic variables were collected to assess relationship between these variables and Prn expression. Immunization history was obtained from the Australian Childhood Immunization Register (ACIR) or hospital records. Previous vaccination was defined as documented receipt of pertussis-containing vaccine at least 14 days prior to diagnosis of pertussis. Infants less than 56 days old at time of diagnosis were recorded as unvaccinated. The presence of a respiratory co-pathogen was defined as laboratory evidence of any respiratory organism within seven days of pertussis diagnosis. Various methods of respiratory pathogen detection including PCR, culture and antigen detection panels are employed by the three participating hospitals and only SA routinely tests for a range of respiratory pathogens (respiratory PCR panel) with each sample requesting pertussis diagnostic testing. The presence of lymphocytosis was determined according to local age specific laboratory reference ranges for cases where absolute lymphocytes were measured as part of the medical management of the child. A case was classified as 'severe' if the patient required a hospital admission for greater than seven days, intensive care unit management, or died.

Chi-square tests and Mann–Whitney U or t-tests were used to assess differences in proportions (categorical) and median/mean (continuous) data between Prn deficient and Prn positive groups. Univariate and multivariable log binomial regression models were used to assess association between Prn status and severity of pertussis. Multivariable models adjusted for other potential confounders and variables of interest, including *ptx* promoter type (*ptxP3* or not *ptxP3*), age (<2 months; 2 to <4 months or ≥4 months) and vaccination (0 or ≥1 dose). Associations were reported as risk ratios (RR) with 95% confidence intervals (CI). All statistical analyses were performed using STATA11 with statistical significance defined as $p < 0.05$.

The study was approved by the Human Research Ethics Committees at the respective sites.

Results

Identified cohort and antigen expression

A total of 199 isolates from children with pertussis infection during 2008–2012 were identified for children presenting to, or admitted to one of the three participating hospitals where results were available for absence or presence of Prn expression. The majority of isolates were collected in NSW and WA (NSW: n = 91, 45.7%; WA: n = 85; 42.7%; SA: n = 23, 11.6%). A third of these isolates (35.7%; 71/199) were Prn deficient with the remaining 128 samples expressing Prn. A higher proportion of isolates from WA were Prn deficient (40/85; 47.1%) compared with NSW (26/91; 28.6%) and SA (5/23; 21.7%).

The proportion of isolates that were Prn deficient increased over the period of the study; only 1 of the 29 isolates (3.4%) collected in 2008 identified as Prn deficient, compared with 28/39 (71.8%) and 29/35 (82.9%) identified as Prn deficient in 2011 and 2012 respectively (Fig. 1). Isolates with the *ptxP3* promoter type, were predominant throughout each year of the study, with virtually all isolates (96%) in 2011 and 2012 identified as *ptxP3* type (Fig. 1). Almost all Prn deficient isolates (70/71, 98.6%) had the *ptxP3* allele compared with 107/128 (83.6%) of Prn positive isolates ($p = 0.001$).

Isolates were collected from all months of the year although the majority of pertussis cases in this study occurred during Spring and Summer (n = 147/199, 73.9%) compared with Autumn and Winter months (n = 52/199, 26.1%) ($p < 0.001$).

Demographic comparisons between Prn deficient and Prn positive cases

The median age at pertussis diagnosis was three months (interquartile range [IQR] 2–18 months, range 13 days to 12.9 years). Over a third of cases were less than three months of age (n = 82/199; 41.2%). Almost half of the cultured cases were female (98/199, 49.2%) and almost 10% identified as Aboriginal or Torres Strait Islander children (19/199; 9.5%). There were no significant differences between the distributions for age, gender or Indigenous status for children infected with Prn positive versus Prn deficient *B. pertussis* variants (Table 1).

Clinical comparisons between Prn deficient and Prn positive cases

Death

Three deaths occurred in the study cohort. All three deaths occurred in infants less than four months of age with no

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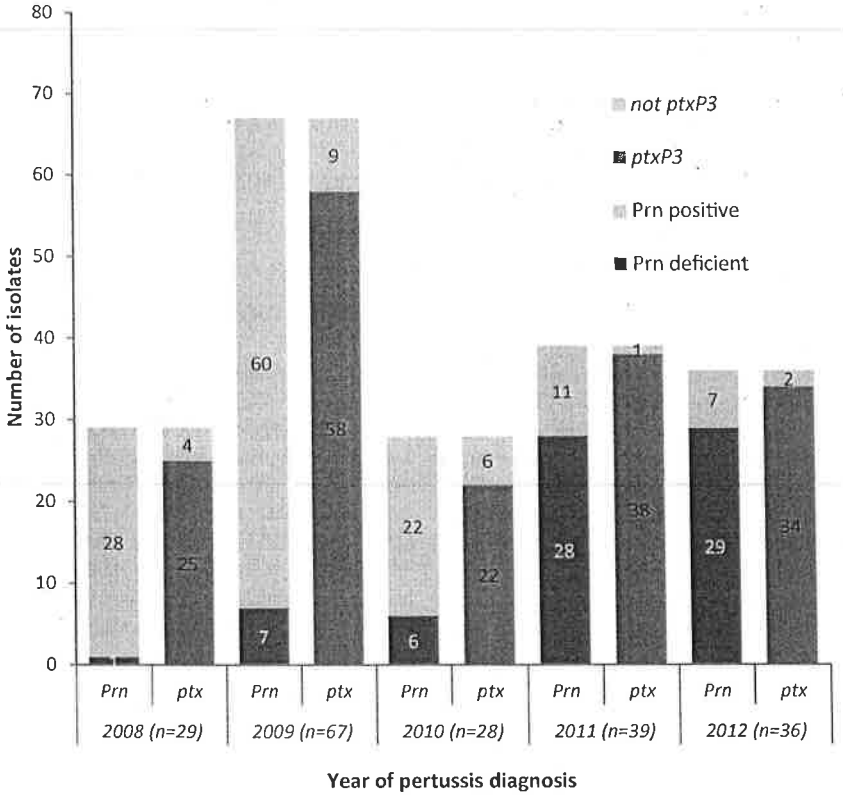

Figure 1 Isolates by year and pertactin (Prn) expression/pertussis toxin promoter (*ptxP*) type.

Table 1 Cohort demographics and characteristics.

		Prn deficient (n = 71)	Prn positive (n = 128)	p Value
Gender	Male	35 (49.3%)	66 (51.6%)	0.76
	Female	36 (50.7%)	62 (48.4%)	
Aboriginal/Torres Strait Islander	No	64 (90.1%)	116 (90.6%)	0.91
	Yes	7 (9.9%)	12 (9.4%)	
Age category	<2m	19 (26.8%)	29 (22.7%)	
	2-<4m	16 (22.5%)	36 (28.1%)	
	4-<6m	7 (9.9%)	12 (9.4%)	0.24
	6-<12m	9 (12.7%)	5 (3.9%)	
	12m-<2y	9 (12.7%)	21 (16.4%)	
	>=2y	11 (15.5%)	25 (19.5%)	
Age (months)	(median/IQR)	4m (1–16)	3m (2–19)	0.79
Hospital admission	Yes	40 (56.3%)	71 (55.5%)	0.91
Admission duration [a](n = 194)	<2 days	40 (57.1%)	66 (53.2%)	0.82
	2–7 days	19 (27.1%)	35 (28.2%)	
	>7 days	11 (15.7%)	23 (18.5%)	
Intensive care management	Yes	5 (7.0%)	16 (12.5%)	0.23
Death	Yes	0 (0.0%)	3 (2.3%)	0.19
Pneumonia	Yes	1 (1.4%)	8 (6.3%)	0.12
Lymphocytosis[b] (n = 56)	Yes	10/21 (47.6%)	14/35 (40.0%)	0.58

IQR: Interquartile range.
P values are chi-square or Mann–Whitney U tests as appropriate.
[a] Admission duration (n = 194): excludes 5; 3 died/2 transferred. "<2 days" includes cases that presented without admission (n = 88).
[b] Includes only those with white cell counts performed (n = 56; Prn deficient n = 21, Prn positive n = 35).

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evidence of prior immunization. *B. pertussis* isolates from these infants were all Prn positive and had the *ptxP3* allele.

Admission to hospital

Overall, 55.8% (n = 111) of the isolates were from children who were admitted to hospital as opposed to presentation to an emergency department only. There was no significant difference between the proportion of children with Prn deficient infections who were admitted to hospital (40/71, 56.3%) compared with Prn positive infections (71/128, 55.5%) (p = 0.91).

Data on duration of admission was available for 106 of the 111 pertussis cases that were hospitalized (3 died and 2 were transferred). For these 106 cases, the median duration of hospital stay was similar for Prn deficient cases (n = 39; median 4 days, IQR 2–9 days) and Prn positive cases (n = 67; median 5 days, IQR 2–10 days). The proportion of children hospitalized for more than seven days was not statistically different between Prn deficient and Prn positive groups (11/70, 15.7% vs 23/124, 18.5%; (p = 0.62))

A lower, but not statistically significant proportion of Prn deficient pertussis cases were admitted to intensive care compared with Prn positive pertussis cases (excluding five cases where requirement for intensive care management was unknown) (5/69; 7.2%, vs 16/125; 12.8%; (p = 0.23))

Pneumonia

Chest X-rays were performed for 54 (27.1%) of the cases included in this study. Similar proportions of children received a chest X-ray for both Prn deficient and Prn positive isolates (21/71; 29.6% vs 33/128 25.8%). Chest X-ray confirmed pneumonia was observed less frequently in Prn deficient cases compared with Prn positive cases, however this difference was not statistically significant (1/71; 1.4% vs 8/128 6.3%, p = 0.12).

Lymphocytosis

White cell counts were measured and available for 21 Prn deficient and 35 Prn positive cases. A similar proportion of Prn deficient cases (with white cell counts available) had elevated lymphocytes (10/21; 47.6%) compared with Prn positive cases (14/35, 40.0%), p = 0.58. The mean absolute lymphocyte count was also similar between Prn deficient and Prn positive cases (12.6 (SD 6.9) vs 12.8 (SD 7.3) $\times 10^9$/L; p = 0.91).

Co-infection

Co-infecting pathogens were reported for 40 of the 125 cases tested for other respiratory pathogens of the total pertussis cases included in this study (32.0%) with the most common co-pathogens being rhinovirus (n = 19) followed by parainfluenza type 3 (n = 7), respiratory syncytial virus (n = 6), adenovirus (n = 4) and human influenza virus (n = 4). Of children tested for the presence of a co-pathogen, there was no difference in the proportion of cases with a respiratory co-infection identified between Prn deficient infections (14/48; 29.2%) and Prn positive infections (26/77; 33.8%) (p = 0.59).

Immunization history

Previous immunization history was available for 59/71 Prn deficient cases (83.1%) and 112/128 Prn positive cases (87.5%). Almost a quarter of cases (n = 43) were too young to have received any pertussis vaccine at least 14 days prior to diagnosis. The proportion of cases less than 56 days of age at time of diagnosis was similar for Prn deficient and Prn positive pertussis cases (15/71; 21.1% vs 28/128; 21.9%, p = 0.90).

Of the 171 cases with known immunization history, almost half had not received any pertussis vaccination at least 14 days prior to pertussis diagnosis (72/171; 42.1%). There was no difference in the proportion that had received 1 or at least 2 doses of acellular pertussis vaccine more than 14 days prior to pertussis diagnosis between Prn deficient and Prn positive cases (Table 2).

Associations with severe pertussis

Cases were classified as severe if they met any of the following conditions: hospital admission longer than seven days; intensive care management or death. Overall, 39/199 (19.6%) were classified as severe according to this definition.

There was a lower proportion of Prn deficient *B. pertussis* infections classified as severe compared with Prn positive isolates (12/71; 16.9% vs 27/128 21.1%); however the difference was not statistically significant (p = 0.48).

Almost 40% of infants less than three months of age were classified as severe compared with 9.6% of children between 3 and 12 months of age and 3.0% of children over 12 months of age (Table 3). Proportions of isolates deficient in Prn or carrying *ptxP3* allele were similar between the three age categories. Most children over 12 months of age (98.0%) had received at least one dose of acellular pertussis vaccine, whereas most infants under three months of age (80.8%) were unvaccinated (Table 3).

A higher proportion of young infants were classified as severe if they were infected with an isolate with the *ptxP3* promoter type, regardless of Prn status compared with infants infected with isolates that were not *ptxP3*. For older

Table 2 Immunization history by status of pertactin (Prn) in *B. pertussis* isolate.

	Prn deficient (n = 71)	Prn positive (n = 128)	p Value
Known immunization history	59 (83.1%)	112 (87.5%)	0.39
No prior valid pertussis vaccine dose	24/59 (40.7%)	48/112 (42.9%)	0.78
Prior receipt of 1 valid pertussis vaccine dose	16/59 (27.1%)	28/112 (25.0%)	0.76
Prior receipt of at least 2 valid pertussis vaccine doses	19/59 (32.2%)	36/112 (32.1%)	0.994

*A valid dose was defined as documented evidence of receipt of pertussis containing vaccine at least 14 days prior to diagnosis of pertussis infection.

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Table 3 Characteristics by age category.

Characteristic	Overall N (%)	Age <3 months (n = 81) N (%)	Age 3-<12 months (n = 52) N (%)	Age ≥ 12 months (n = 66) N (%)	p Value[c]
Prn deficient	71/199 (35.7%)	29/81 (35.8%)	22/52 (42.3%)	20/66 (30.3%)	0.401
ptxP3	177/199 (88.9%)	74/81 (91.4%)	48/52 (92.3%)	55/66 (83.3%)	0.223
Severe	39/199 (19.6%)	32/81 (39.5%)	5/52 (9.6%)	2/66 (3.0%)	<0.001
Died	3/199 (1.5%)	2/81 (2.5%)	1/52 (1.9%)	0/66 (0.0%)	0.486
ICU	22/197 (11.2%)	17/81 (21.0%)	3/50 (6.0%)	2/66 (3.0%)	0.001
Admitted for more than 7 days[a]	34/194 (17.5%)	29/79 (36.7%)	3/49 (6.1%)	2/66 (3.0%)	0.000
Unvaccinated[b,d]	72/171 (42.1%)	63[d]/78 (80.8%)	8/44 (18.2%)	1/49 (2.0%)	<0.001

[a] Admission duration unknown for n = 5.
[b] Vaccination history unknown for n = 28.
[c] P value is chi square or fisher's exact test for difference in proportion by age category.
[d] Includes 43 who were too young to have received any valid pertussis vaccine dose prior to diagnosis (<56 days).

children (≥3 months), only those infected with Prn-positive/*ptxP3* variants were classified as severe (Table 4).

Univariate binomial regression models for predictors of severe pertussis demonstrated that younger age, no prior pertussis vaccination, and identification as Aboriginal/Torres Strait Islander were statistically significantly associated with more severe pertussis (Table 5). In multivariable analysis including the above variables as well as Prn expression and *ptxP* promoter type, Prn deficiency was not associated with any altered risk of severity (RR 0.95 [0.57–1.56], p = 0.83). However, the *ptxP3* variants were more than three times more likely to be classified as severe (RR 3.44 [0.59–20.00], although this association was not statistically significant (p = 0.17). Compared with infants over 4 months of age, infants less than 2 months of age were significantly more likely to be classified as severe (RR 4.62 [1.28–16.73], p = 0.02). There was no evidence of a statistically significant association between identification as Aboriginal/Torres Strait islander and severe pertussis in the multivariable model (RR 1.56, [0.90–2.70], p = 0.12). Children who had not received any prior pertussis vaccination were twice as likely to be classified as severe, (RR 2.10 [0.73–5.99] however this was not statistically significant (p = 0.16).

Discussion

Results from this study suggest that Prn deficient *B. pertussis* variants caused disease which was similar in severity with infection caused by Prn positive strains, with point estimates suggesting that infection from Prn deficient variants may be less severe, resulting in fewer cases of pneumonia and admission to intensive care units. This is in agreement with other reports in the literature on Prn deficient *B. pertussis* infections which have found no difference in proportions reporting symptoms such as apneoa, duration of coughing illness or requirement for admission between infections with Prn deficient vs Prn positive infections.[15,25] Our study adds to previously reported literature[15,25] with the inclusion of greater numbers of younger infants and thus provides reassurance that the rapid emergence of Prn deficient *B. pertussis* variants is unlikely to contribute to any greater risk of death or severe outcomes from infections in young, vulnerable infants.

In contrast with Martin et al.,[15] but similar to Bodilis et al.,[25] we did not find any association between Prn status and history of vaccination. This may be due to our relatively small sample size with only 35 of the 71 Prn deficient cases having evidence of any prior vaccination. In agreement with Bodilis et al.,[25] our data also demonstrated that vaccination was associated with reduced risk of severe disease.

There is no evidence from this study that Prn deficient *B. pertussis* infections increase susceptibility of hosts to co-infections, although it is important to recognize that not all cases were routinely tested for the presence of respiratory co-pathogens and thus it is possible that co-infections were undetected in children in this cohort.

Table 4 Genotypic variants and severity by age category and vaccination status.

Variant	Total n	Prn deficient/*ptxP3* (n = 70)	Prn positive/*ptxP3* (n = 107)	Prn deficient/Not-*ptxP3* (n = 1)	Prn positive/Not-*ptxP3* (n = 21)
Age category		n (%) severe	n (%) severe	n (%) severe	n (%) severe
<3 months	81	12/28 (42.9)	19/46 (41.3)	0/1 (0.0)	1/6 (16.7)
≥3 months	118	0/42 (0.0)	7/61 (11.5)	0/0 (0.0)	0/15 (0.0)
Vaccination status		n (%) severe	n (%) severe	n (%) severe	n (%) severe
0 doses	72	11/23 (47.8)	18/42 (42.9)	0/1 (0.0)	1/6 (16.7)
1 or more	99	1/35 (2.9)	6/53 (11.3)	0/0 (0.0)	0/11 (0.0)
2 or more	55	0/19 (0.0)	2/28 (7.1)	0/0 (0.0)	0/8 (0.0)

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Table 5 Univariate and multivariate associations with severe outcome.[a]

Variable	Level	n	Univariate risk ratio (95% CI)	p Value	Adjusted risk ratio (95% CI)	p Value
Prn status	Positive	128	1.0		1.0	
	Deficient	71	0.80 (0.43–1.48)	0.48	0.95 (0.57–1.56)	0.83
ptxP status	Not *ptxP3*	22	1.0		1.0	
	ptxP3	177	4. 72 (0.68–32.72)	0.12	3.44 (0.59–20.00)	0.17
Aboriginal/Torres Strait Islander	No	180	1.0		1.0	
	Yes	19	2.44 (1.32–4.53)	0.01	1.56 (0.90–2.7)	0.12
Age cat	4m+	99	1.0		1.0	
	2-<4m	52	5.71 (1.94–16.83)	0.002	3.13 (0.96–10.25)	0.06
	<2m	48	11.86 (4.34–32.38)	<0.001	4.62 (1.28–16.73)	0.02
Vaccine doses	1+	99	1.0		1.0	
	0	72	5.89 (2.74–12.66)	<0.001	2.10 (0.73–5.99)	0.16

[a] Cases were classified as severe if they required a hospital stay greater than seven days, were admitted to intensive care or if death occurred.

Bacterial load may be an important factor associated with severity of disease as shown in previous research.[28] We do not have PCR results to get a measure of bacterial loads for our cases, however, all eligible cases required successful culture. As culture success has been linked to higher bacterial load,[29] it is likely that bacterial loads were similar between Prn deficient and Prn positive cases included in this study.

Examining factors associated with more severe *B. pertussis* infection using univariate models reconfirmed that young age and absence of prior vaccination were important predictors of more severe disease. Interestingly, our data suggests that although there was no evidence that Prn deficient isolates were related to more severe disease, strains carrying the *ptxP3* promoter allele may have an impact on disease severity. However, the small number of non-*ptxP3* cases (n = 22) and wide confidence intervals for estimated risk ratios limits the ability of this study to provide conclusive evidence on the impact of *ptxP3* and disease severity. In our study, only two cases infected with a non-*ptxP* strain had lymphocytes measured so we were unable to examine the relationship between these *ptxP* variants and lymphocytosis. Further investigation on the importance and prevalence of *ptxP3* on severity of pertussis disease is warranted. These results agree with recent literature suggesting that *ptxP3* strains are more virulent in humans than *ptxP1* strains based on death and hospitalization data in the Netherlands during two time periods with low and high *ptxP3* frequencies.[16] The proportion of cases that were *ptxP3* in our sample (which included isolates from 2008 to 2012) was high (89%), with similar predominance of *ptxP3* strains evident around Australia[17] and globally.[13,30]

Our results provide reassurance that the recent evolutionary changes in *B. pertussis* (increasing proportions of Prn deficient isolates) are not significantly impacting on disease severity. Some evidence, including our study findings, suggest that Prn deficient genotypes may be associated with less severe disease, which may lead to increased transmission due to delayed or under diagnosis of the infected cases. Surveillance of pertussis genotypes and the impact of the rapid emergence of *B. pertussis* variants on effectiveness of vaccination programs should be closely monitored.

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Conflict of interest statement

Peter McIntyre, Nick Wood and Helen Marshall are investigators on studies funded by GSK. All other co-authors have no conflicts of interest to declare.

Contribution statement

All authors have contributed to the design/analysis and/or writing/editing of this manuscript. All authors have reviewed and approved the content for the final version.

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Community awareness and predictors of uptake of pertussis booster vaccine in South Australian adults

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ABSTRACT

Objective: Pertussis is a highly virulent vaccine preventable disease that remains a global challenge. This study aimed to assess community knowledge of pertussis infection as well as awareness and uptake of adult pertussis booster vaccine.
Methods: A cross-sectional survey was conducted of randomly selected households in South Australia by Computer Assisted Telephone Interviews in 2011. Survey data were weighted to the age, gender and geographical area profile of the population.
Results: From 3124 randomly sampled contactable households, 1967 interviews were conducted (participation rate 63%) with individuals aged 18–93 years, including 608 parents of children aged <18 years. The majority of respondents (97%) had heard of pertussis (whooping cough) and 18% reported that a household member had previously contracted whooping cough infection. Most respondents considered whooping cough to be highly contagious (73%) and severe for infants (89%). Over half (51%) of those surveyed were aware that family members commonly transmit pertussis to infants. Despite high knowledge, pertussis vaccine uptake was low, with only 10% of respondents reporting pertussis vaccination in the previous five years. Whilst 61% of respondents were aware of the availability of an adult pertussis booster vaccine, only 8% ($n = 154$) reported their Family Physician had discussed it with them. If provided free, 77% agreed that they would be more likely to accept a booster pertussis vaccination. Independent predictors of recent pertussis vaccination included higher education, larger household size, perception of greater disease severity for infants and discussion with a Family Physician about pertussis vaccination.
Conclusions: Whilst knowledge regarding transmission and severity of *Bordetella pertussis* was high, uptake of pertussis vaccination for adults is remarkably low amongst the South Australian community. Improved awareness regarding the availability of a booster pertussis vaccine through Family Physicians and/or provision of funded pertussis vaccination for adults has the potential to improve pertussis vaccine coverage.

1. Background

Despite long standing immunization programs, control of pertussis continues to be a challenge globally with a substantial disease burden of morbidity and mortality. In 2008, the World Health Organisation (WHO) estimated that approximately 195,000 children died as a result of pertussis infection [1]. Pertussis epidemics occur every 3–4 years in Australia [2], with its recent epidemic between 2009 and 2011 the worst since the introduction of pertussis vaccination in the 1940s. At the peak of the Australian epidemic in 2011, almost 38,000 cases of pertussis were notified, with incidence rates increasing to 450/100,000 for infants and

Abbreviations: ABS, Australian Bureau of Statistics; CATI, Computer Aided Telephone Interview; CI, confidence interval; FP, Family Physician; NIP, National Immunisation Program; PROS, Population Research and Outcomes Studies; SA, South Australia; WHO, World Health Organisation.

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young children [3]. Between 2006 and 2011, 11 pertussis deaths were reported, with 10 of these occurring in infants less than 6 months of age [4].

In Australia, vaccination against pertussis is funded for infants and children through the National Immunisation Program (NIP) offering free pertussis-containing vaccinations at 2, 4 and 6 months of age, with a booster vaccination at four years and again for adolescents when 10–15 years of age. Pertussis vaccination is also recommended for toddlers at 18 months of age and any adult who wishes to reduce the likelihood of illness from pertussis, but these recommendations are not currently funded as part of the NIP [2]. To reduce the risk of pertussis occurring in infants or others at increased risk of severe pertussis, healthcare workers, childcare workers and adult household contacts/carers of young children are strongly recommended to receive vaccination against pertussis with boosters every 10 years. At the time of the study, women planning a pregnancy, pregnant (third trimester) or post-partum and those in close contact with infants and children were also recommended to receive a single dose of pertussis containing vaccine if five or more years have elapsed between prior pertussis vaccination and expected delivery date. Recently, these guidelines have been updated to include a recommendation for pregnant women to receive pertussis vaccination during their third trimester of pregnancy, with this vaccine provided as part of a funded program in most states of Australia [2].

Epidemiological evidence indicates that adults are a significant reservoir for pertussis infection and transmission. A review of South Australian data has shown the majority of cases of notified pertussis that occurred during the first 18 months of the 2009–2011 epidemic, occurred in adults, with 66% of the notifications for individuals over 24 years of age [5]. It has also been established that the most common source of transmission of pertussis infection to vulnerable young infants are parents and siblings [6,7]. Strategies proposed to improve pertussis prevention are targeted at young infants who are at most risk of severe disease and/or death. These have included the cocooning strategy, which involves vaccinating members of the household to reduce the risk of transmission to newborn babies [8]. More recently, maternal immunization to facilitate maternal antibody transfer to vulnerable infants during their first few months of life [9].

High uptake of effective vaccines is necessary for successful infectious disease prevention programs. Neither infection nor vaccination provide long term immune protection against *Bordetella pertussis*, with duration of immunity following acellular pertussis vaccines estimated at less than 5 years [8,10,11]. Vaccine coverage in Australian infants is high, with national data indicating that more than 92% of infants have received the recommended three doses of pertussis containing vaccine by 12 months of age [12]. There are no current systematic processes for capturing adult population immunization rates and therefore pertussis vaccine uptake in Australian adults remains largely unquantified [13]. As adults are a common reservoir for transmission of infection to vulnerable infants, it is important to understand their knowledge of pertussis disease and immunization strategies and estimate pertussis vaccine coverage and associated factors.

2. Methods

This cross-sectional study was conducted as part of the 'Health Monitor' program conducted by the Population Research and Outcomes Studies (PROS) unit, University of Adelaide, South Australia (SA) [14]. The random sampling process was based on the South Australian electronic White Pages household telephone listings in both metropolitan and rural areas. This residential telephone listing comprises primarily of landline number listings which are included by default, with mobile numbers only included upon request of the owner.

The household contact identified the adult in the household (aged ≥18 years) who most recently had a birthday. The interviews were conducted by the Computer Assisted Telephone Interviewing (CATI) method. Up to six call-backs were made to interview the identified individual if they were not available at the time of the telephone call. Phone calls were made to households at different times between 9am and 9pm over 7 days per week. A pilot study of 50 randomly selected households was completed in March 2011 to test the question formats and sequence prior to commencement of the main study.

The structured survey was designed to determine the level of knowledge and community awareness of pertussis disease and the availability and uptake of the adult pertussis booster vaccines. Respondents were asked about prior pertussis vaccination, knowledge and experience with pertussis infections. For simplicity, the term 'whooping cough' was used throughout the survey rather than 'pertussis'. Participants were asked to rate how severe whooping cough infection was in infants aged less than six months on a scale from 1 (very mild) to 10 (extremely severe). Respondents were also asked to rate the ease of spread of pertussis from person to person on a scale of 1–10, with 1 being not at all contagious and 10 being extremely contagious. Participants were also nominated who they thought young infants were most likely to catch whooping cough from (with multiple responses allowed).

For the purpose of the current study, a sample size of >1500 respondents enabled the proportion within the community who had knowledge of pertussis, or who had been vaccinated against pertussis in the last 5 years, to be estimated with a ±2.5% precision at a 95% confidence level.

All survey responses were weighted to ensure that survey findings were applicable to the South Australian population. Survey weights were calculated from the inverse probability of selection of a household and re-weighted to sex, age and geographical area profile (metropolitan or rural) according to ABS 2009 Estimated Residential Population data for South Australia [15].

Estimates of population percentages for respondent characteristics with 95% confidence intervals (95% CIs) are presented. Univariate log binomial regression models were used to assess factors associated with awareness and uptake of pertussis booster vaccination with outcomes reported as risk ratios (RR) with 95% CI. Multivariable models were developed to assess adjusted associations and including univariate predictor variables with a p-value ≤0.1. All analyses were carried out using Stata version 11 (StataCorp, Texas). A two-tailed p-value of less than 0.05 was considered to be statistically significant.

Research ethics approval was obtained from the Women's and Children's Health Network Human Research Ethics Committee and the University of Adelaide Human Research Ethics Committee.

3. Results

3.1. Study population (weighted data)

From 4400 households selected to participate (from a total of 660,461 households in South Australia) [16], 3124 were able to be contacted. From these households, 1967 adults completed a computer aided telephone interview during March and April 2011 with a participation rate of 62.9% (Fig. 1).

The age of respondents ranged from 18 to 93 years with a mean age of 47.5 years (median age 46 years; interquartile range 33–62 years). Almost half of adults were male (47.4%, n=962) and 608 respondents (30.9%) interviewed were parents with at least one child (aged <18 years) residing in the household. The

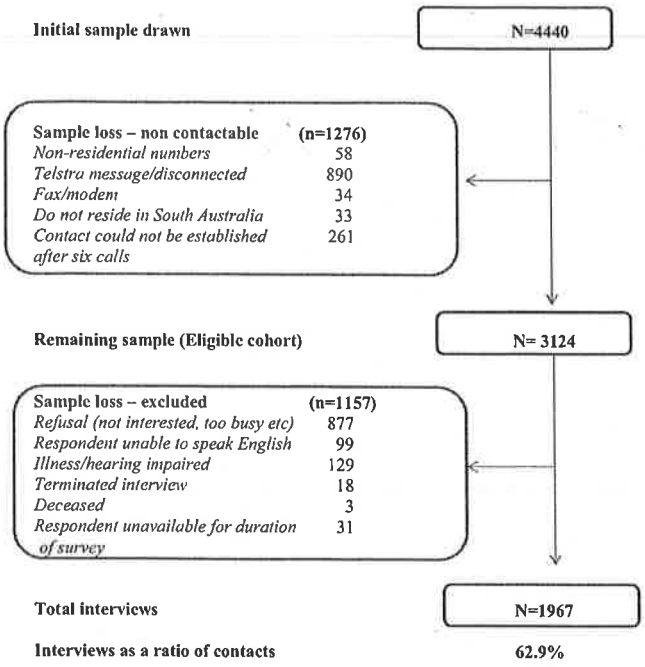

Fig. 1. Sample flowchart.

majority of respondents were born in Australia (79.2%) and lived in metropolitan Adelaide (73.7%) with the remainder (26.3%) living in rural South Australia. Educational attainment varied amongst the respondents with 9.8% having left school at the age of 15 years or less, 32.1% with a trade, certificate or diploma and 24.3% attaining a bachelor degree or higher (Table 1).

3.2. Knowledge of whooping cough, severity in infants and transmissibility

The majority of respondents (97.1%) had previously heard of whooping cough and almost 1 in 5 (361/1967, 18.4%) reported that at least one person in their household had previously experienced whooping cough, with 39 (2.0%) respondents reporting a household member had experienced whooping cough within the previous 12 months. Notifiable disease data for South Australia indicates the prevalence of notified pertussis infections in South Australia for 2010 and 2011 to be 4.5% and 1.4% respectively [3].

Almost 90% ($n = 1741$) of respondents rated the severity of pertussis infection in infants as high (≥7), 5.4% ($n = 105$) reported that they did not know, and only 1.0% ($n = 20$) of respondents rated this question with a score of ≤3. Participants nominated who they thought young infants were most likely to catch whooping cough from (multiple responses allowed). Approximately half of the respondents correctly suggested that mothers

Table 1
Respondent characteristics (raw and weighted).

Variable	Level	Raw data		Weighted data		
		Total number	Percentage	Weighted number	Weighted percentage	95% confidence limits
Sex	Male	776	39.45	962.04	48.89	46.20–51.58
	Female	1191	60.55	1006.00	51.11	48.42–53.38
Age category	18–34	308	15.66	566.19	28.77	25.93–31.61
	35–54	617	31.37	705.63	35.86	33.31–38.40
	55–74	762	38.74	504.03	25.61	23.64–27.58
	75+	280	14.23	192.11	9.76	8.55–10.98
Area	Metropolitan	1440	73.21	1451	73.73	71.41–76.05
	Rural	527	26.79	516.99	26.27	23.95–28.59
Aboriginal/TSI	Yes	11	0.56	12.48	0.64	0.22–1.05
	No	1951	99.44	1950	99.36	98.95–99.78
Number of children <18 years in household	0	1424	72.39	1234.00	62.70	60.02–65.38
	1	210	10.68	287.72	14.62	12.54–16.70
	2	240	12.20	328.58	16.70	14.56–18.83
	3+	93	4.73	117.74	5.98	4.71–7.26
Parent with child <18 years in household?	Yes	543	27.61	607.52	30.95	28.40–33.50
	No	1424	72.39	1355.00	69.04	66.50–71.60
Employment group	Employed	1040	53.03	1245.00	63.27	60.82–65.72
	Unemployed	27	1.38	35.88	1.82	1.06–2.59
	Home duties	99	5.05	101.71	5.17	3.99–6.34
	Retired	674	34.37	423.68	21.53	19.77–23.29
	Other[a]	121	6.17	161.56	8.21	6.59–9.83
Country of birth	Australia	1526	77.58	1559.00	79.23	77.08–81.37
	UK and Ireland	238	12.10	196.61	9.99	8.50–11.48
	Other	203	10.32	212.19	10.78	9.06–12.50
Income group	Up to $20,000	288	14.64	173.78	8.83	7.67–9.99
	$20,000–$40,000	544	27.66	486.80	24.74	22.53–26.94
	$40,000–$80,000	717	36.45	891.90	45.32	42.63–48.02
	$80,000–$100,000	159	8.08	132.30	6.72	5.53–7.92
	$100+ $100,000	259	13.17	283.18	14.39	12.32–16.46
Highest education	School	918	46.81	851.54	43.42	40.64–45.90
	Trade/Apprenticeship Certificate; Diploma	616	31.41	632.2	32.24	29.61–34.64
	Bachelor degree or higher	427	21.77	477.36	24.34	21.87–26.64

[a] Other employment group includes: student/unable to work due to disability/refused.

Table 2
Predictors of awareness of pertussis vaccine availability.

Variable	Levels	n[a]	Univariate binomial regression			Multivariable binomial regression		
			RR	CI	p-Value	RR	CI	p-Value[b]
Gender	Female	1005	1.0		<0.001	1.0		<0.001
	Male	962	0.83	0.76–0.91		0.83	0.75–0.91	
Age category	18–34	566	1.0		<0.001	1.0		<0.001
	35–54	705	1.07	0.93–1.23		1.03	0.90–1.18	
	55+	696	1.22	1.08–1.39		1.20	1.06–1.36	
Area	Metropolitan	1450	1.0		0.068	1.0		0.007
	Rural	517	1.09	0.99–1.20		1.13	1.03–1.23	
Aboriginal/TSI	Non ATSI	1547	1.0		0.004	1.0		0.005
	ATSI	12	0.12	0.03–0.51		0.13	0.03–0.54	
Parental status	Non parent	1355	1.0		0.987			
	Parent	608	1.0	0.91–1.10				
Country of birth	Australia	1558	1.0		0.605			
	UK/Ireland	196	1.06	0.93–1.20				
	Other	208	1.03	0.90–1.19				
Income group	<$80,000	885	1.0		0.600			
	≥$80,000	667	0.99	0.89–1.09				
	Refused/not stated	415	0.94	0.83–1.06				
Highest education	School or less	851	1.0		0.088	1.0		0.042
	Trade/apprentice/certificate/Diploma	632	1.02	0.92–1.13		1.06	0.96–1.18	
	Bachelor or higher	477	1.12	1.01–1.25		1.15	1.03–1.29	
Household size	1	232	1.0		0.385			
	2–3	1036	1.05	0.95–1.15				
	4–5	621	0.97	0.86–1.09				
	6+	79	0.89	0.61–1.29				
Household experience with whooping cough	Yes	361	1.0		0.004	1.0		0.321
	No	1476	0.95	0.86–1.05		1.00	0.90–1.24	
	Unk/missing	130	0.59	0.43–0.81		0.78	0.55–1.10	

[a] Weighted n in cohort.
[b] Multivariable model included all variables with univariate associations with awareness of pertussis vaccine availability returning a p-value ≤0.1.
Variable level p values were based on likelihood ratio tests.

(n = 998, 50.7%) and fathers (921, 46.8%) were a likely source of infection. Siblings and other children were also considered a likely source of infection by 29.5% (n = 581) of respondents. Grandparents were only considered a likely source of transmission for 10.9% (n = 215) of respondents. Most respondents (73.1%; n = 1437) rated pertussis as highly contagious (score ≥7) with less than 1% (n = 13) suggesting that whooping cough was not contagious (score = 1).

3.3. Awareness of pertussis vaccine

Overall, 60.5% (1190/1967) of the respondents were aware that a vaccine was available to protect against whooping cough in adults. Of these, only 12.9% (n = 154) reported that their Family Physician (FP) had discussed whooping cough booster vaccination for adults. Assuming that respondents who were unaware of the vaccine had not had any discussion with their FP about it, the overall sample proportion whose FP had discussed whooping cough vaccination reduced to 7.8% (154/1967).

In multivariate log binomial regression analysis, male gender, younger age, lower educational attainment, metropolitan area of residence and being an Aboriginal or Torres Strait Islander were all associated with a reduced likelihood of awareness of vaccine availability (Table 2). There was no significant difference in awareness of pertussis vaccine availability for adults by either parental status, household income or household size (Table 2).

3.4. Pertussis vaccination and predictors of uptake

Overall, only 202 (10.3%) of the respondents reported that they had received a whooping cough vaccine within the preceding 5 years, although 1518 (77.2%) considered that they would be likely to accept the whooping cough booster vaccine if it was free. A slightly higher proportion of parent respondents (with a child under 18 years of age residing in the household) reported recent pertussis booster vaccination compared with non-parents (69/608, 11.3% vs. 133/1355; 9.8%), although this difference was not statistically significant (χ^2 test, p = 0.296).

In univariate analysis, gender, education level, household size, perception of severity of whooping cough in infants, perception of contagiousness of whooping cough and having had a Family Physician (FP) discussion about pertussis were all associated with higher likelihood of having received a booster pertussis vaccination within the prior 5-year period (Table 3). Of these, education level, perception of severity in infants and FP discussion remained independent, significant predictors of uptake in a multivariable model.

Any association between indigenous status and vaccine uptake could not be established in this dataset as only 12 responders identified as or Torres Strait Islander and none had received the pertussis vaccine within the previous 5 years. It is important to note that whilst the largest risk ratio of uptake was associated with FP discussion, with respondents who had discussed whooping cough vaccination with a FP 3 times more likely to have been vaccinated (RR 2.95, CI 2.21–3.96; p < 0.001), it cannot be determined whether

Table 3
Predictors of pertussis vaccination within the preceding five years.

Variable	Levels	n[a]	Univariate binomial regression			Multivariable binomial regression[b]		
			RR	CI	p-Value	RR	CI	p-Value
Gender	Female	1005	1.0		0.011	1.0		0.574
	Male	962	0.65	0.47–0.91		0.92	0.68–1.23	
Age cat	18–34	566	1.0		0.939			
	35–54	705	1.06	0.68–1.64				
	55+	696	1.01	0.66–1.53				
Area	Metropolitan	1450	1.0		0.641			
	Rural	517	0.93	0.67–1.28				
Aboriginal/TSI	Non ATSI	1547	1.0					
	ATSI	12	NA[c]					
Parental status	Non parent	1355	1.0		0.383			
	Parent	608	1.16	0.83–1.63				
Country of birth	Australia	1558	1.0		0.354			
	UK/Ireland	196	0.73	0.42–1.29				
	Other	208	0.72	0.39–1.33				
Income group	<$80,000	885	1.0		0.106			
	≥$80,000	667	1.44	1.02–2.01				
	Refused/not stated	415	1.09	0.73–1.64				
Highest education	School	851	1.0		<0.001	1.0		0.014
	Trade/apprentice Certificate/Diploma	632	1.36	0.94–1.97		1.24	0.89–1.72	
	Bachelor or higher	477	2.30	1.58–3.33		1.66	1.18–2.34	
Employment status	Employed	1245	1.0		0.191			
	Unemployed/student	288	0.64	0.37–1.11				
	Retired	423	0.83	0.60–1.13				
Household size	1	231	1.00		0.005	1.0		0.061
	2–3	1036	1.79	1.24–2.56		1.48	1.05–2.08	
	4+	699	1.31	0.83–2.05		1.20	0.78–1.83	
Perception of severity for infants (scales 1–10)	High (7+)	1741	1.0		0.003	1.0		0.041
	Low/Mod (<6)	122	0.67	0.32–1.37		0.74	0.36–1.53	
	Don't know	104	0.19	0.07–0.52		0.23	0.07–0.76	
Perception of contagiousness (scales 1–10)	High (7+)	1437	1.0		<0.001	1.0		0.128
	Low/Mod (<6)	399	0.37	0.20–0.66		0.52	0.31–0.99	
	Not contagious/Don't know	130	0.31	0.11–0.83		0.82	0.31–2.16	
Household experience with whooping cough	Yes	361	1.0		0.477			
	No	1476	1.13	0.74–1.72				
	Unk/missing	130	0.71	0.29–1.7				
FP discussed pertussis	No	1766	1.0		<0.001	1.0		<0.001
	Yes	154	7.85	5.7–10.7		3.06	2.29–4.06	

[a] Weighted n in cohort.
[b] Multivariable model included all variables with univariate associations with awareness of pertussis vaccine availability returning a p-value ≤0.1.
[c] ATSI – this variable could not be included in regression models because no ATSI respondent had received a pertussis vaccination in the previous 5 years.

this discussion preceded the decision whether or not to receive the vaccine.

4. Discussion

The results of this research indicate that whilst the population are well informed about whooping cough disease and infection, including the vulnerability of young infants to severe consequences and adults/parents being a highly likely source of transmission, their awareness of the availability of pertussis booster vaccines for adults is relatively low. Almost 40% of adults were not aware that a pertussis booster vaccine was available for purchase and only 10% of South Australian adults had been vaccinated with a booster vaccine within the last 5 years. This uptake percentage is slightly higher than reported in the 2009 Australian Adult Vaccination Survey [13] which estimated only 7.8% of South Australian adults had received a pertussis vaccine during adulthood. This slight improvement in vaccination rate could be attributed to increased awareness of pertussis and the potential for vaccination in the community as the epidemic progressed.

The results of this survey demonstrated a significant association between FP discussion about whooping cough booster vaccine recommendations and vaccine uptake. Several studies have demonstrated that a recommendation from a physician can improve vaccine uptake in both the adolescent and adult community [17–19]. Overall, however, the vast majority of respondents reported that their FP had not discussed pertussis booster vaccines, despite the study being conducted immediately following a global pertussis epidemic. Increasing Family Physician education around the value of adult vaccination or encouraging FPs to discuss immunization as part of their routine assessment may be valuable for improving adult pertussis vaccination coverage.

Adult booster pertussis vaccination was also significantly more likely for people with a higher level of education and a greater perception of disease severity, indicating that appropriate awareness campaigns and information around the potential consequences of

pertussis infection could be beneficial for improving coverage rates in our adult population. Although there were few Aboriginal/Torres Strait islander respondents in this survey, results suggest that awareness uptake of pertussis vaccination in these respondents was low compared with non-Aboriginal/Torres Strait Islander respondents. Reducing disparities in disease burden between Aboriginal and non-Aboriginal people requires equal access and information about preventative health measures such as vaccination and this survey indicates that disparities continue to exist.

The majority of respondents in this study also reported that they would be more likely to accept the booster pertussis vaccine if it was provided at no cost. This suggests that cost of pertussis booster vaccinations may be a potential barrier and that providing vaccination either for no or low cost may improve uptake of booster vaccination in adults.

Whilst there is substantial scope for improving awareness, our research suggests that increasing awareness of the vaccine availability alone is not enough to maximize pertussis vaccine coverage and control, with only 20% of those who were aware of the vaccine availability reporting receipt of pertussis vaccine within the previous 5 years.

As prior pertussis vaccination history was ascertained from adult self-report, some recall or misclassification bias may exist. There are very few adult immunisations recommended in Australia, however, and pertussis vaccination would have required an act to purchase, therefore any recall bias is likely to be minimal. In SA, healthcare workers were provided with a pertussis containing vaccine at no cost during 2009 and from October to December 2010 parents and grandparents of infants aged <6 months, who held a health-care or pension concession card, which entitles low income individuals/families to government subsidized health care, were eligible for free pertussis vaccination. Therefore there is the potential that the coverage rate determined in this study is higher than during non-epidemic periods and where pertussis boosters were not funded for targeted parts of the population.

Whilst the data gathered for this research are limited to the South Australian population, the findings clearly suggest that more can be done to improve control of pertussis; many of the findings are likely to be transferrable to other populations with similar education and health systems. It is important to note that this survey was conducted in 2011, following a pertussis pandemic, when pertussis infections were being frequently discussed in the media and health departments. In March 2015, the South Australian Health Department introduced free pertussis vaccine for pregnant women in each pregnancy to improve protection for vulnerable infants. This policy change, and the increased awareness and discussion around adult pertussis vaccination may lead to higher pertussis vaccination coverage rates in the SA community than was determined in this survey.

In conclusion, whilst awareness of pertussis in the general adult population is very high, knowledge of adult vaccination against pertussis is low. This is reflected by only 10% coverage, which is much lower than that achieved with targeted adult vaccination. Family Physicians play a key role in educating selective adult groups on pertussis booster vaccination and recommending it in order to protect vulnerable infants. Furthermore, eliminating cost as a barrier to uptake of the vaccine has significant potential to improve uptake. Providing funded programs may be an efficient way to improve the uptake in target groups such as pregnant women and families with young children.

Conflict of interest and author declaration

HM would like to declare she has support from a National Health and Medical Research Council Career Development Fellowship No. 1016272. HM has been an investigator on clinical trials of pertussis vaccines and her institution received funding from Sanofi Pasteur to cover the costs of the Health Monitor survey. HM has been a member of vaccine advisory boards for GlaxoSmithKline Biologicals and Wyeth, and has received travel support from CSL, Pfizer, Novartis, and GlaxoSmithKline Biologicals to present scientific data at international meetings. Her institution has received funding for investigator-led research from GlaxoSmithKline, Sanofi-Pasteur, and Novartis Vaccines. MC has received travel support from GSK to present scientific data at a national conference. NT has received travel support from Sanofi-Pasteur to present scientific data at a national conference.

Submission declaration and verification

All authors acknowledge that the paper has not been submitted for consideration elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Contribution and authorship

MC drafted the manuscript. All authors contributed to study design and contributed to the manuscript. MC conducted statistical analysis and interpretation under direction of LG and HM. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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Clinical trial registration

This study has not been registered in a clinical trial registry because it was a population based survey and therefore registration was not required.

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