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Newborn vaccination: How early can we protect and how long does protection last?

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'Discovery consists of seeing what everybody else has seen and thinking what nobody else has thought.'

Jonathon Swift, *Gulliver's Travels*

'There was no telling what people might find out once they felt free to ask whatever questions they wanted to.'

Joseph Heller, *Catch 22*

STATUTORY DECLARATION OF ORIGINALITY

This thesis was carried out at the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases at The Children's Hospital at Westmead. I hereby testify that this thesis is my own, original research.

I wrote the literature review for Chapters 1, 2 and 4. I was involved in the original planning of the newborn pertussis vaccine study (Chapter 3) and wrote the ethics applications (The Children's Hospital at Westmead, Westmead Hospital) and successful Financial Markets Foundation for Children grant application. I worked closely with my supervisor, Professor Peter McIntyre on both the grant and ethics applications. I enrolled the infants and their parents into the newborn pertussis vaccine study. I conducted study visits for all infants from birth to 8 months old in Sydney, along with the study research nurses. I entered the immunogenicity and adverse event data and analysed the results for this study. I discussed the analysis and results with my supervisor, Professor Peter McIntyre.

I wrote the ethics applications (The Children's Hospital at Westmead, Sydney South West Area Health Service, Menzies School of Health Research) for study 1 and 2 in Chapter 5. I wrote the successful National Health and Medical Research Council New Investigator project grant application (396700) for Study 2 (Chapter 5). I enrolled and conducted study visits (vaccine administration, blood sample collection) for participants in Study 1 (Chapter 5), with the assistance of Dr Leon Heron and study research nurses at Sydney South West Area Health Service. I entered the data and analysed immunogenicity results for study 1 in Chapter 5. I enrolled and conducted study visits (vaccine administration, blood sample collection) for participants in Study 2b in Darwin and remote Northern Territory communities and surrounds (Tiwi Islands, Wadeye, Kakadu), with the assistance of Kobi Schutz and Belinda Davison, of the Aboriginal Birth Cohort study team at Menzies School of Health Research. I entered the data and analysed immunogenicity results for study 2a and 2b in Chapter 5. I was responsible for and ensured that the 3 vaccine studies were the conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki and ethics committee approval.

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ABSTRACT

Newborn vaccination: how early can we protect and how long does protection last?

Background

The challenge for infant vaccination is to protect as early as possible and to maintain immunity as long as possible. At present only three vaccines are given at birth, oral polio vaccine (OPV), hepatitis B (HBV) and Bacille Camille Guerin (BCG).

Although the youngest infants are at greatest risk of death or hospitalisation from pertussis, the earliest age the first pertussis containing vaccine is currently given is at 6 weeks. Few studies examining newborn responses to acellular pertussis vaccine exist and no studies have examined 2 doses of monovalent acellular pertussis (Pa) vaccine before 8 weeks old.

The first groups to receive HBV vaccine in Australia were Indigenous infants and those born to mothers from countries with high endemicity of HBV. The duration of protection afforded by infant HBV vaccination is not definitively known beyond 15 years and its determination has important worldwide implications on whether booster doses are required following infant vaccination. No studies have examined long-term (> 10 years) HBV immunity following infant HBV vaccination in Australia.

Aim

The aim of this thesis is to examine responses to early life (neonatal) vaccination, in particular acellular pertussis vaccine, and the longevity of immunity, in particular following HBV vaccine in infancy.

Methods

Clinical vaccine trials examining early life responses to acellular pertussis vaccine and longevity of HBV immunity were conducted.

Neonatal responses to acellular pertussis (Pa) vaccine: 76 newborns were randomized to 3 different vaccine groups: (1) Pa at birth and one month or (2) at birth only or (3) controls. All received Hepatitis B vaccine (HBV) at birth and combination vaccines which included diphtheria, tetanus, *Haemophilus influenzae* type b, Hepatitis B and polio antigens with conjugate pneumococcal vaccine at 2, 4 and 6 months. IgG antibody responses to pertussis toxoid (PT), filamentous hemagglutinin (FHA) and pertactin (PRN) were measured in maternal serum and at 2, 4, 6 and 8 months of age. Antibody responses to concomitant antigens, hepatitis B, Hib, diphtheria and tetanus were measured at 8 months. Cell mediated immunity to pertussis was also measured on the 8 month sample. Adverse events were recorded by parental diary and active telephone follow-up for 7 days after each vaccination.

Long term immunity to HBV vaccine: Clinical vaccine studies were conducted in two different cohorts

(i) Study 1: Children and adolescents over 10 years of age born to 'at risk' mothers targeted for HBV vaccination because of their ethnicity (n=120)

(ii) Study 2: Indigenous adolescents, age 16-20 years, in the Northern Territory, Australia (n=437)

Duration of HBV protection was measured by rates of chronic HBV infection in those vaccinated in infancy and booster responses to HBV vaccine. HBV serology was measured at baseline, including hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc) and hepatitis B surface antigen (HBsAg) as a marker of the prevalence of HBsAg carriage. Anamnestic booster response was defined as a rise in anti-HBs level to >10mIU/ml in those with non detectable levels pre-booster or a 4 fold increase in anti-HBs level in those with detectable levels pre booster. HBV vaccine history was collected from individual child health records, healthcare provider/ community healthcare clinic records and electronic immunisation registers. Indigenous adolescents (study 2) were asked additional questions relating to current or past substance use (cigarettes, alcohol marijuana, petrol sniffing). Anamnestic responses were measured 2-4 weeks after a booster dose of a recombinant 10ug HBsAg vaccine.

Results

Neonatal responses to acellular pertussis (Pa) vaccine: Infants receiving Pa at birth and one month had significantly higher IgG antibody to PT, FHA and PRN at 2 months than those who received Pa at birth only and controls. At 8 months of age, following 5, 4 and 3 doses of Pa-containing vaccines respectively, IgG antibodies to PT, FHA and PRN were equivalent. Concomitant antigen responses to Hib and hepatitis B vaccines were non significantly reduced in the birth and one month old Pa vaccine group. Receipt of Pa vaccine at birth resulted in a significant increase in IL5 and IL13 cytokine (TH2) responses to pertussis vaccine antigens at 8 months compared to those who commenced Pa vaccine later. There were no differences in IFNγ and cytokine (TH1) responses to other vaccine antigens between groups. The Pa birth dose was well tolerated with no significant systemic or local reactogenicity.

Long term immunity to HBV vaccine: Two thirds of adolescents were non immune to hepatitis B (anti-HBs <10mIU/ml) 14 to 18 years after infant vaccination, 62% in study 1 and 64% in study 2. Few participants were HBsAg positive, nil in study 1 and 1.6% in study 2. Nearly one fifth (n=99/437) of the total Indigenous cohort had serological evidence of past HBV infection (anti-HBc positive) and 2.7% were anti-HBc positive alone. Nearly all adolescents (98%) born to 'at risk' mothers in study 1 demonstrated an anamnestic response, compared with 78% of Indigenous adolescents in Study 2. Current users of alcohol, cigarettes and marijuana were less likely to demonstrate an anamnestic response compared to those who were non users.

Conclusion

Neonatal responses to acellular pertussis (Pa) vaccine: Data in this thesis suggest that Pa vaccine at birth and one month induces significantly higher IgG antibody against pertussis antigens by 2 months of age without reducing subsequent pertussis antibody responses. Reduced concomitant antigen responses may reflect interference from the newborn Pa vaccine and needs further investigation. Hepatitis B vaccine is routinely given at birth in most of the world and this approach, if efficacy and safety is confirmed by longer term follow-up in larger studies, could have wide application in rich and poor countries

Long term immunity to HBV vaccine: This thesis has documented low rates of HBsAg positivity and majority demonstrating anamnestic responses in adolescents over 10 years old for the first time in Australia. However, interpreting anamnestic responses in long-term follow up studies of adolescents who were vaccinated in infancy is complex. Numerous factors, either alone or in combination, such as age, sex, nutritional status, immune competence, HLA type, substance use and environmental HBV endemicity, may influence HBV vaccine effectiveness and immune memory.

KEY TO ABBREVIATIONS

ACIR	Australian Childhood Immunisation Register
Anti-HBs	Hepatitis B surface antibody
Anti-HBc	Hepatitis B core antibody
BCG	Bacille Camille Guerin
CI	Confidence interval
CMI	Cell mediated immunity
CRM_{197}	Genetically modifed diphtheria toxin
DTPa	Diphtheria, tetanus, acellular pertussis vaccine
DTPw	Diphtheria, tetanus, whole cell pertussis vaccine
ELISA	Enzyme linked immunsorbent assay
FHA	Filamentous haemagglutinin
GMC	Geometric mean concentration
HBIG	Hepatitis B immunoglobulin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
Hib	*Haemophilus influenzae* type b
HLA	Human leucocyte antigen
IFNγ	Interferon gamma
IgG, IgM	Immunoglobulin G, Immunoglobulin M
IL	Interleukin
NCIRS	National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases
NHMRC	National Health and Medical Research Council
NT	Northern Territory
OPV	Oral polio vaccine

Pa	Monovalent acellular pertussis vaccine
PCR	Polymerase chain reaction
PRN	Pertactin
PRP – OMP	Polyribosylribitol phosphate –Neisseria menigitidis outer membrane protein complex conjugate
PRP-T	Polyribosylribitol phosphate –tetanus conjugate
PT	Pertussis toxin
TH1, TH2	T helper 1, T helper 2
SSWAHS	Sydney South West Area Health Service
US	United States
WHO	World Health Organisation

PUBLICATIONS ARISING FROM THIS THESIS

McIntyre P, Wood N. Pertussis in early infancy: disease burden and preventive strategies. *Curr Opin Infect Dis*. 2009; 22: 215-223

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ABSTRACTS AND PRESENTATIONS FROM THIS THESIS

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Immunogenicity of birth and one month old acellular pertussis vaccine. Public Health Association of Australia 10th National Immunisation Conference 2008, Gold Coast, Queensland, Australia

Estimates of chronic hepatitis B infection in the Northern Territory. Coalition for research to Improve Aboriginal Health (CRIAH) conference 2008, Sydney, New South Wales, Australia

Estimates of chronic hepatitis B infection in the Northern Territory. Indigenous Immunisation workshop. Menzies School of Health Research 2007, Darwin, Northern Territory, Australia

Long term persistence of hepatitis B immunity in children who received hepatitis B vaccinations in infancy. Australasian Society of Infectious Disease Annual Scientific meeting 2007, Hobart, Tasmania, Australia

Perinatal hepatitis B infection: an update. Paediatric Infectious Diseases Special Interest group satellite meeting. Australasian Society of Infectious Disease Annual Scientific meeting 2007, Hobart, Tasmania, Australia

Predictors of hepatitis B immunity in an Aboriginal birth cohort. 5th International Congress on Developmental Origins of Health and Diseases 2007, Perth, Western Australia, Australia

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A Guide to the Thesis

The ideal vaccine would be a single dose given at birth, preferably orally, and provide immediate and long lasting protection against multiple diseases. At present only three vaccines are given at birth, oral polio vaccine (OPV), hepatitis B (HBV) and Bacille Camille Guerin (BCG). Current vaccine schedules require several doses in infancy, meaning that protection is often delayed for several months and in addition may need subsequent booster doses in childhood and or adolescence to maintain immunity.

The challenge for infant vaccination is to protect as early as possible and to maintain immunity as long as possible. For some infections early protection following birth is important, for example young infants with pertussis are at an increased risk of death or hospitalisation than infants infected at an older age while infants exposed to HBV from vertical transmission (mother to infant during birth) have a high chance of becoming chronically infected with HBV and subsequent associated risk of cirrhosis and liver cancer. For pertussis the earliest the first vaccine is currently recommended to be given is at 6 weeks old and most infants are not reliably protected until after the second dose at 10-16 weeks of age.

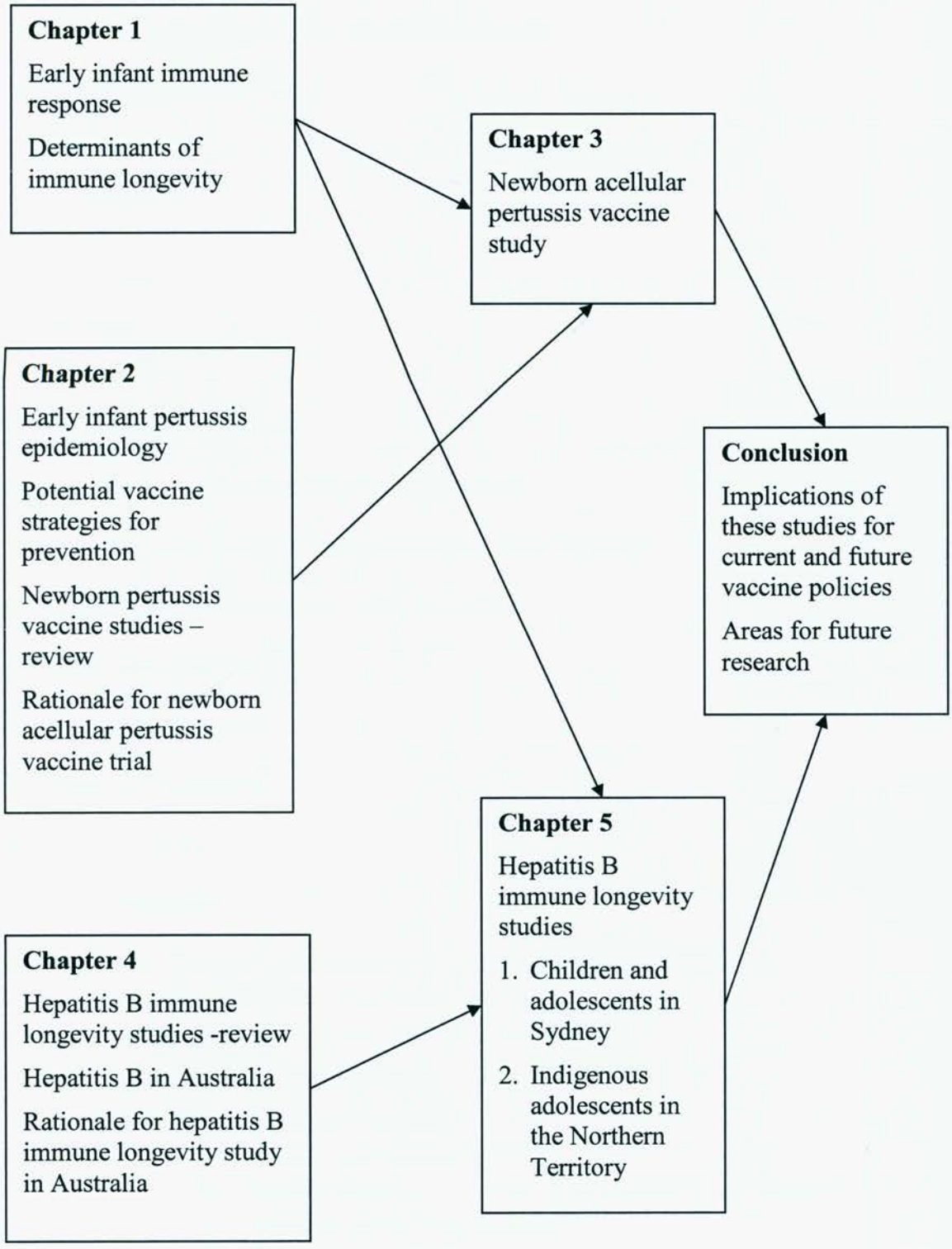
The aim of this thesis is to examine responses to neonatal vaccination, in particular acellular pertussis vaccine, and the longevity of immunity, in particular following hepatitis B vaccine. These vaccines were chosen because protection from birth is important for both pertussis and hepatitis B and each allows exploration of different issues. For pertussis, the issue is how early presumptive protection can be achieved. For hepatitis B, correlates of protection can be examined among adolescents and young adults vaccinated in early life.

There are two main research questions

1. Is acellular pertussis vaccine immunogenic when given at birth?

2. How long does protective immunity against hepatitis B last following birth and infant vaccination?

This thesis is divided into six chapters. (Figure 1.1) Chapter 1 examines immunology of early life vaccination and factors that impact on the ability of a neonate to respond, as well as the determinants of immune longevity. Chapter 2 discusses the epidemiology of early infant pertussis, potential strategies for prevention and results of past newborn pertussis vaccine trials. Both chapters 1 and 2 provide background for the rationale, study design and interpretation of results of the newborn acellular pertussis vaccine trial discussed in chapter 3. Chapter 4 discusses the results of international studies examining hepatitis B immune longevity and why this is important in the Australian context. Both chapters 1 and 4 provide background for the rationale, study design and interpretation of results of two immune longevity studies discussed in chapter 5. Chapter 6 is the conclusion and discussion of areas for future research.



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CHAPTER 1: EARLY LIFE VACCINE RESPONSES AND IMMUNE LONGEVITY

1.1 Introduction

Each year more than 2 million children worldwide, aged between 1-6 months old, die from acute respiratory infections or diarrhoeal diseases, many of which are potentially vaccine preventable. [1] The ideal vaccine would be a single dose given at birth, preferably orally, and provide immediate and long lasting protection against multiple diseases. At present only three vaccines are given at birth, oral polio vaccine (OPV), hepatitis B (HBV) and Bacille Camille Guerin (BCG).

The challenge for infant vaccination is to protect as early as possible and to maintain immunity as long as possible. There are several obstacles and uncertainties to achieving this including the ability of the infant's immune system to respond to vaccines given at birth without negative immunological or clinical effects. Infants under 6 months of age have the highest rates of pertussis infection and the highest morbidity and mortality from pertussis.[2,3] The earliest the first pertussis vaccine is currently recommended to be given is at 6 weeks old and most infants are not reliably protected until after the second dose at 10-16 weeks of age. One question that arises is: can we protect the most vulnerable infants against pertussis earlier than 6 weeks old by vaccinating them earlier?

The World Health Organization (WHO) recommends a 3 dose HBV infant vaccine schedule with the first dose commencing at birth. The aim of this vaccine schedule is to prevent mother to child transmission in the peri-partum period because infection at this age is more likely to result in chronic carriage of HBV and risk of subsequent chronic liver disease and hepatocellular carcinoma than infection at an older age. However, a second period of high risk exposure to HBV virus occurs during adolescence, with high risk of clinical hepatitis, and therefore maintenance of immunity following infant vaccination into this age is very important. Another question that arises is how long does immunity against HBV persist following newborn and infant vaccination and are subsequent booster doses required?

An understanding of the functional maturity of the newborn immune system with respect to response to vaccines is important for the design and interpretation of clinical vaccine trials examining early life responses (pertussis) (Chapter 3) and immune longevity (hepatitis B) (Chapter 4 and 5). In this chapter the literature pertaining to early life vaccine responses and determinants of immune persistence is reviewed in two sections. The first summarises the immunological factors that determine the newborn's antibody and T cell responses to vaccines, with particular emphasis on pertussis and HBV vaccines. The second summarises the determinants of immune persistence following birth and infant vaccination, with particular emphasis on HBV vaccine.

1.2 Early life vaccine responses

1.2.1 B cell immune response

One of the ways vaccines protect is by induction of antigen specific antibodies, produced by B cells, which allow neutralisation of pathogens (or their toxins) at mucosal surfaces or soon after invasion. This is particularly important for extracellular pathogens, such as encapsulated bacteria like *Streptococcus pneumoniae* and *Haemophilus influenza*e type b. Nearly all vaccines need several doses to elicit sufficient antibody concentrations for protection.[4]

There are many factors that determine the quality and quantity of the early infant antibody response, including; the maturity of the infant's immune system, presence of immunodeficiency, genetic factors, type of vaccine, number of subsequent doses, and influence of maternal antibodies.[4,5] In general, neonates have a reduced ability to respond to vaccines compared to older infants and children and this has several implications. The first is that the ability to protect as early as possible has limitations and second the longevity of immunity is reduced. This is because immunity longevity is related to the magnitude of the initial immune response. [4,6] Infants who commenced vaccination at an older age have higher antibody responses following completion of the primary vaccination series than infants commencing at younger ages. Older infants with higher antibody levels post vaccination are therefore more likely to retain antibody for a longer period than younger infants. The follow on implication of reduced immune longevity is that booster doses may be required.

In this section the factors that affect the quality and quantity of early infant antibody responses will be summarised and include the following:

a) B cell immunity

b) Vaccine schedule: number, type and timing of vaccines.

c) Influence of maternal antibodies

d) Existence of neonatal priming

e) Influence of residual vaccine induced antibodies

f) Existence of immune tolerance, hyporesponsiveness and vaccine interference

a) B cell immunity

Following birth the infant's immune system undergoes postnatal maturation that results in better responses to vaccination at an older age compared to vaccination at birth. Infants at birth have the following innate factors that account for the reduced antibody response to vaccines in early life.

Poor response to Thymus (T) independent polysaccharides

Polysaccharide antigens preferentially localise to the marginal zone of the spleen.[7] Infants have an immature architecture of the marginal zone of the spleen which results in reduced 'natural memory' cells, and along with reduced levels of expression of CD21/CR2 complement receptors required for B-cell activation, which leads to poor responses to T-independent polysaccharides.[5,8] This accounts for the well known inability of infants to mount adequate immunity following exposure to polysaccharide capsular antigens, such as those of *Neisseria meningiditis, Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib).[9] Both pertussis and hepatitis B vaccines do not rely on polysaccharide antigens. Pertussis vaccines consist of 3-5 proteins including pertussis toxin (PT), pertactin (PRN), filamentous haemagglutinin (FHA) and fimbrial proteins, while hepatitis B vaccine uses hepatitis B surface antigen.[10]

Reduced antibody response to Thymus (T) dependent antigens

Infant B cells produce a reduced antibody response to most T dependent protein or conjugate vaccine antigens compared to adults. The primary reason for reduced antibody responses in the first 4 weeks of life is most likely the time taken for germinal centres to mature in the postnatal period. By 4 months of age germinal centres usually contain sufficient follicular dendritic cells to enable activation of antigen specific B cells and subsequent differentiation into plasma and memory B cells.[5,10] The implication is that administration of pertussis vaccine at birth may not be immunogenic due to immaturity of germinal centres.

The induction of significant adult-like primary IgG responses occurs after 2-3 months of age, once germinal centres mature. IgG responses are higher with increasing age of

vaccination.[4,10] This has been demonstrated for antibody responses to Hib conjugate vaccines following a single dose at 2-3 months vs 4-6 months vs 8-17 months, where the later age at commencement resulted in higher antibody levels.[9] The proportion of infants who seroconvert, defined as achieving antibody to hepatitis B surface antigen (anti-HBs) > 10mIU/ml, following a single dose of hepatitis B vaccine at birth is estimated at 10-20%.[11] A second dose is required 1-2 months after the birth dose to achieve adequate and long lasting immunity against HBV infection.[11-13] Of note however, despite low or absent measurable antibody production, a single dose of hepatitis B vaccine at birth can protect from vertical hepatitis B transmission, this may be related to the virus-like particle nature of hepatitis B surface antigen used in the vaccine.[11,14]

Reduced quality of antibody responses

In addition to reduced quantity, antibody produced by the newborn also has qualitative differences, including shorter duration compared to older infants, different IgG subclasses produced and reduced avidity. These factors are summarised below.

Short duration – The level of antibody produced following infant vaccination declines rapidly.[8] One example is meningococcal C conjugate vaccine, which when given following a schedule of 2, 3, 4 months, has antibody levels reaching a peak at 5 months and declining rapidly by 1 year of age.[15-17] It is likely that the rapid decline is due to the absence of factors, such as chemo-attractant and adhesion molecules on stromal cells in the bone marrow, that are necessary for prolonged survival of plasma cells.[18] This is consistent with studies in mice where it was found that early life bone marrow stromal cells failed to provide sufficient survival signals to plasma cells reaching bone marrow niches.[10] The rapid decay in antibodies in early life is due to the reduced ability of the infant bone marrow to sustain plasma cells that are capable of producing antibodies.

IgG subclass – In addition to a rapid decline in antibody levels, there are qualitative differences in the type of IgG antibodies produced.[10] Infant IgG subclass responses are characterised by high IgG1 and IgG3 antibodies and low IgG2 antibodies. It is known that IgG antibody responses to diphtheria toxin and tetanus toxoid are predominantly IgG1, while responses to *Haemophilus influenzae* type b (Hib) and other polysaccharide

antigens are mainly IgG2.[4,6,7] The low IgG2/IgG1 ratio may reflect a tendency for neonates and infants to preferentially induce T helper 2 (TH2) versus T helper 1 (TH1) responses. It is not until approximately 24 months old that IgG2 production fully matures.[19]

Avidity – In addition to the quantity of antibody, the functional capacity of antibody is important in mediating clinical protection. Antibody affinity is the term used to describe the strength of binding of an antibody to a specific epitope at the surface of an antigen.[10] Antibody avidity is the 'sum of epitope specific affinities for a given antigen' and is one of several measures shown to correlate with protection against bacteria, especially those with polysaccharide capsules and viruses.[10,20,21] Binding of antibody to bacterial polysaccharide capsule activates the classical pathway of the complement system and leads to opsonophagocytosis. In general, higher-avidity antibodies are more effective than lower-avidity in mediating opsonophagocytosis.[20,22] Infant antibody responses are characterised by lower avidity compared to adult responses and maturation of avidity occurs during the first year of life.[23-25]

Genetic factors

There are known associations between genes and immune responses to vaccines.[26] The human leucocyte antigen (HLA) class II locus has been shown to be an important factor in antibody responses to measles, pneumococcal polysaccharide and hepatitis B vaccines.[27] Immune responses to BCG, DTPw and hepatitis B vaccines have been compared in monozygotic and dizygotic infant twins in the Gambia.[26,27] HLA genes influenced BCG responses, while non HLA genes influenced hepatitis B and OPV responses.[27] Evidence exists for certain HLA subtypes and low response to hepatitis B vaccines, as is discussed in chapter 5.[28-31] Antibody responses are likely to be affected by gene polymorphisms in molecules critical for B and T cell differentiation and activation.[10] In addition, it is likely that as yet unknown genetic factors also influence B cell responses to vaccines.

b) Vaccine schedule – number, type and timing of vaccines

The level of infant antibody response to a vaccine is also influenced by vaccine and schedule factors. These include the type of vaccine, timing of the first dose and dosing interval.

Type of vaccine and antigen dose

There are a number of differences between response to live versus inactivated/subunit vaccines. In general, antibody responses to live viral vaccines, such as measles and varicella, are limited in the first 6 months of life, due in part to interferences by maternal antibodies as discussed below. Beyond this early infant period, when maternal antibody levels wane, attenuated viral vaccine replication results in higher and longer antigen presentation to B cells and very good antibody responses. Inactivated or protein vaccines, such as pertussis and hepatitis B, can successfully elicit strong antibody responses following repeated doses in early infancy. In general the higher the antigen content the more B cell binding and activation with resulting higher antibody levels. Higher amounts of hepatitis B surface antigen in HBV vaccines, given in infancy, have been shown to increase anti-HBs levels achieved.[13]

Timing of first dose and dosing interval

The earlier the age of commencement of vaccination and the closer subsequent doses are following birth, the lower the antibody response generated in comparison to later age of commencement and wider spacing between doses. In general, a short inter-dose schedule, (example 2, 3, 4 months) will result in lower geometric mean antibody concentrations compared to a longer inter-dose schedule (2, 4, 6 months), as shown in Hib and hepatitis B vaccine studies.[8,9,11,32,33] Higher antibody levels to Hib are seen following a 3, 5, 9 months Hib schedule compared to a 2,3,4 months schedule.[33,34] Similarly, higher antibody levels after hepatitis B (HBV) vaccine at 0,1 and 6 months are achieved compared to a 0,1 and 2 or 3 month schedule.[12,35] A minimum interval of 3-4 weeks between doses is needed to avoid competition between several waves of primary immune responses, a principle used to inform the design of the newborn pertussis trial in Chapter 3.[10] In addition, a minimal interval of 4 months between priming and boosting allows time for affinity maturation of B cells to occur with

subsequent higher secondary responses.[10] A good example of such a priming and boosting schedule is the hepatitis B vaccine schedule of 0, 1 and 6 months.[36]

c) *Influence of maternal antibodies*

Infants receive variable amounts of antibody transplacentally depending on the physical characteristics of the antibody and placental characteristics, as summarised below. Maternally derived antibody can protect the infant from early disease but may also interfere with active immunisation in the infant. A good example of protection is maternal immunisation against tetanus which has been very successful in preventing neonatal tetanus.[37] The level of passive transfer of maternal antibodies differs between antibody types and transfer of one type of antibody is not the same in all infants. In the case of pertussis, antibody transfer is active and in general levels are higher in the neonate than in the mother.[38] However, in other studies infants born to mothers with similar pre delivery pertussis antibody levels have varying level of placental transfer and as a result differing levels at birth.[39-41]

Maternally derived antibodies can interfere with response to vaccines in infancy, as discussed below. It is important to understand that maternal antibodies may influence infant vaccine responses as potential strategies to prevent early infant infections, for example pertussis and pneumococcus, include maternal and neonatal immunisation.[42-44] It is possible that maternal immunisation in the last trimester of pregnancy with resultant antibody production in the mother and passive transfer to the neonate will influence neonatal and infant vaccine responses. In addition it is likely that the recent strategies of adolescent and young adult booster vaccination prior to pregnancy, example pertussis and hepatitis B, means that antibody levels are likely to be higher at birth in infants born to these women compared to infants born to women who have not received booster vaccines. This has as yet unknown implications for infant vaccine schedules and immune responses.[4]

There are two main factors to consider when examining the influence of maternal antibodies:

i) Factors affecting maternal transfer of antibodies to infant

ii) Factors affecting infant response to vaccines in the presence of maternal antibodies

i) Factors affecting maternal transfer of antibodies to infant

Maternal IgG levels

The amount of antibody transferred to infants is partly dependent on baseline maternal IgG levels. In immune competent women, maternal IgG concentration to various vaccine antigens is a product of past exposure to the virus or bacteria and past vaccine history. In addition, during pregnancy antibody levels are affected by oestrogen, progesterone, cortisol and interleukin levels and as a result of haemodilution, maternal IgG levels are often lower than levels pre-pregnancy.[41] Interestingly Englund reports that higher total maternal IgG levels may result in lower placental transfer of specific IgG as antibodies compete for a limited number of placental receptors needed for transport, a form of competitive inhibition.[41]

Gestational age

IgG is actively transported across the placenta, mediated by a specific receptor, with IgG1 and IgG3 preferentially transported over other IgG subclasses. Placental transfer commences at 17 weeks gestation and increases through gestation, however most transfer occurs between 30 to 40 weeks.[40] IgG levels between mother and foetus are equivalent at 33 weeks gestation while foetal levels are usually higher at term (40 weeks) due to active placental transport. Thus the gestation of the infant influences the amount of placentally transferred antibody, with premature infants having less maternally derived antibody at birth than term infants.

Placental integrity

Transfer of antibodies necessitates a functioning placenta. The function of the placenta can be affected by infections, such as malaria and HIV, which decrease placental transport. In a study by Mulholland et al placental transfer of Hib antibodies, following Hib vaccination in pregnancy, was reduced by active placental infection with malaria and higher maternal antibody equated to less efficient placental transfer, suggesting competitive inhibition, as discussed above.[45] In other studies, ascending infections from the birth canal have been shown to increase placental transfer of antibodies.[44,46]

ii) Factors affecting infant response to vaccines in the presence of maternal antibodies

As mentioned previously, maternally derived antibodies may provide direct protection to the infant but their presence can impact on the infant's response to the primary series of vaccines. The heterogenous level of maternal antibodies and placental transfer in a given infant population makes it difficult to accurately predict their influence on infant antibody responses. However, it is well known that maternal antibodies can suppress infant vaccine responses, for example measles, hepatitis A and whole cell pertussis.[39] There are several possible hypotheses to explain how maternal antibodies may suppress infant responses to live or subunit vaccines, discussed below.

Live viral vaccines and maternal antibodies

Maternal antibodies in sufficient concentration can inhibit replication of vaccine viruses, thus reducing the amount of antigen available to stimulate the immune system with subsequent reduction in antibody produced. However, T cell responses are not reduced suggesting that in vivo replication has occurred. It seems that the ratio of maternal antibody to viral load or vaccine antigen is more important. When there is maternal antibody excess then there is suppression of viral replication and reduced B cell responses, while the opposite occurs when there is excess viral load.[4,8]

Inactivated / subunit vaccines and maternal antibodies

Maternal antibodies also bind to infant B cells and mask epitope sites available for vaccine antigen, with consequent reduction in the B cell response. The ratio of maternal antibody to vaccine antigen is important. In the presence of excess maternal antibody, B cell antigen masking occurs and B cell responses are inhibited. In contrast, when there is an antigen excess, B cell epitope masking does not occur and B cell responses are preserved. Studies with hepatitis A and tetanus vaccine illustrate that as maternal antibody levels decrease, resulting in an increased ratio of antigen: antibody, infants are able to produce antibody.[47,48] This leads to the suggestion that higher doses of vaccine antigen may overcome high levels of maternal antibody, as has been shown with hepatitis A vaccine.[47,48] Although B cell responses are reduced T cell responses are preserved. Vaccine antigen – maternal antibody complexes are taken up by macrophages and dendritic cells and small peptides are displayed on the cell surface of

the antigen presenting cells thus allowing CD4 and CD8 T cells to bind and become activated.

Maternal antibodies and response to hepatitis B vaccine

HBV vaccine at birth is immunogenic even in the presence of high levels of maternally derived hepatitis B antibodies and with the addition of HBIG at birth.[49-51] However, one recent study in China compared hepatitis B vaccine response in infants born to HBV seropositive and seronegative mothers and suggested that high levels of maternal HBV antibodies (>1000mIU/ml) ameliorated the infant HBV antibody response.[49]

Maternal antibodies and response to pertussis vaccine

High cord blood anti-pertussis toxin levels have been shown to negatively impact on infants responses to whole cell pertussis vaccine, both historically in studies in the 1950s and more recently, however responses to acellular pertussis vaccine seem not to be affected.[39,40,52,53] Of the maternally derived antibodies, anti-pertussis toxin appears to have the greatest suppressive effect on infant whole cell pertussis vaccine responses, and less so for other antibodies (pertactin, and FHA).[39]

In summary the ratio of maternal antibody to viral load or antigen is the most important determinant of how maternal antibodies influence infant vaccine responses. Of note, maternal antibody decays relatively quickly post delivery. The mean half life of IgG1 is 30 days and so each month post vaccination antibody levels approximately halve. In the case of pertussis antibodies levels this means levels are negligible in infants by 2 months old.[38,40,52] This means that infant responses to pertussis vaccine commencing at or after 2 months old are not likely to be affected by maternal antibodies. However, the immunogenicity of acellular pertussis vaccine given prior to 2 months old may be affected by maternal antibody and has not yet been adequately studied. In chapter 2, the influence of maternal antibody on responses to whole cell pertussis vaccines given at birth is discussed. In chapter 3 the influence of maternal antibody on responses in infants who were given acellular pertussis vaccine at birth is discussed.

d) Existence of neonatal priming

The inherent limitations of the newborn's immune system, the influence of maternal antibodies and the vaccine type and schedule used mean that protective immunity, such as measurable antibody above protective levels, following a single dose at birth is not achieved for most of the current vaccines in use.

Despite the factors above that lead to reduced antibody responses following early life vaccination, it is clear that neonatal priming can be achieved. Neonatal priming means that exposure to vaccine antigens at birth can effectively prime B cells to mount increased antibody responses after the second dose. Initial exposure to the antigen induces B cell activation and differentiation into memory cells and results in enhanced antibody responses following a second exposure. The rise and kinetics of the antibody response is higher and faster in infants primed compared to unprimed infants of the same age.

There are several examples of successful neonatal priming following birth vaccination, based on both immunologic and epidemiological evidence. Another important uncertainty is whether priming the immune system at birth results in reduced antibody responses to subsequent doses of the antigen or other concomitantly administered antigens and as a result reduction in clinical protection.

Immunologic evidence of neonatal priming

Clinical vaccine trials have demonstrated successful immune priming following exposure to oral polio vaccine (OPV) Hib and HBV vaccines at birth. Interestingly, not all vaccine antigens/formulations are able to induce immune memory and effectively prime neonatal B cells, for example diphtheria and tetanus.[6,54] Examples of successful neonatal priming are discussed below.

Oral polio virus (OPV) vaccine – In a study by Dong et al in China, neonates were given OPV at birth, 60 and 90 days and antibody responses were compared to control infants who received a first dose of OPV at 60 days old.[55] At 2 months of age, the proportion of infants in the birth vaccine group (after 1 dose) with detectable antibodies to poliovirus types 1, 2, 3 was higher than the control group where antibody levels had

significantly declined. This indicates that the birth dose of OPV was able to stop the decay of maternally derived antibodies.[55] This is supported by other OPV vaccine studies in India and Brazil.[56] In 1984, WHO recommended a birth dose of oral polio vaccine in polio endemic countries.[56,57] A review of OPV vaccine at birth found that 70-100% of newborns develop local intestinal immunity and 30-50% develop serum antibodies to one of the 3 OPV types after the birth dose.[58] In a recent study in Egypt, one month following a birth dose of monovalent OPV (type1) vaccine 55% of infants were shown to have seroconverted and nearly half of these infants produced antibodies in the presence of maternal antibodies. Failure to detect excretion of virus in the stool following challenge with poliovirus indirectly demonstrates the presence of intestinal immunity. When challenged with a further dose of monovalent OPV at one month old, only one quarter of infants in the Egypt study were found to excrete the type 1 OPV, indicating that the birth dose was immunogenic, and likely to be effective if the infant was exposed to wild type OPV.[59]

Haemophilus influenzae type b (Hib) vaccine – Only a few studies have investigated neonatal Hib vaccination. In a study by Kurrika et al, neonates were given Hib polysaccharide conjugated to tetanus toxoid (PRP-T).[60] At 4 months old infants given PRP-T at birth did not demonstrate the expected decline in antibody (reduction to 8% of the neonatal value because of maternal antibody decay) indicating active antibody production in the neonate. In addition, investigators challenged infants with a Hib polysaccharide vaccine, which was not expected to elicit antibody response at this age, to test for immune priming. Approximately one third of infants had a 4 fold rise in antibody level compared to 10% of unprimed infants indicating that priming had occurred.[60,61]In another study by Lieberman et al, neonates were given diphtheria and tetanus toxoid (DT) or Hib polysaccharide conjugated to a genetically modified diphtheria toxin (CRM_{197} protein) (HibOC vaccine). Infants given HibOC vaccine at birth and 2 months old had significantly higher PRP antibody levels at 4 months old (after 2 doses) than infants who had only received HibOC vaccine at 2 months (after 1 dose).[54] This also suggested that immune priming occurred, however higher antibody levels did not persist with similar results seen between groups at 6 months of age. In a third study by Ward et al, Alaskan native infants given Hib polysaccharide conjugated to a *Neisseria meningitidis* outer membrane protein complex (PRP-OMP) vaccine at

birth demonstrated higher PRP antibody levels at 2 months old compared to controls, however levels were significantly lower than controls after completion of the vaccine series.[62] Kurikka et al concluded that neonatal Hib vaccination warranted further study, while Lieberman et al and Ward et al concluded neonatal vaccination was not likely to result in earlier Hib protection and raised concerns about reduced responses to later doses.

Hepatitis B – Seroconversion, as indicated by anti-HBs > 10mIU/ml, after the first dose of hepatitis B vaccine at birth, is estimated at 10-20%.[12] Following a second dose at 1 month old approximately 50-70% seronconvert, although subsequent doses are required for prolonged protection.[12] The seroconversion rate after the second dose, 1 month following the birth vaccine, is higher than infants given their first dose at 1 month of age and thus is indicative of successful priming.

Epidemiological evidence of neonatal priming

Although the above studies demonstrate neonatal priming immunologically, they do not provide evidence that this priming is protective from infection. Challenging an infant with a vaccine and demonstrating antibody rise does not necessarily equate to protection from bacterial or viral exposure, where the intensity of transmission is different, the method of exposure is often mucosal and it takes time from colonisation to invasion to occur. However, there is epidemiological evidence of protection against disease after receiving some vaccines at birth, for example hepatitis B and BCG, to support the concept of successful immune priming.

Hepatitis B – the highest risk of transmission of hepatitis B virus (HBV) from a HBV carrier mother to her infant is during the peri-partum period. The current strategy to prevent this transmission is administration of hepatitis B immunoglobulin (HBIG) and HBV vaccine within 1 week of birth.[63]As noted above, approximately 10% of infants seroconvert following the birth HBV vaccine and demonstration that vaccine alone at birth, in the absence of HBIG, is 70-80% effective in preventing transmission from mother to infant, at a time when the risk is highest, is an indication that neonatal priming has been achieved.[50]One could surmise that the birth HBV vaccine primes the immune system such that on exposure to HBV, during delivery or immediately post

partum, immunity generated by vaccination is able to prevent viral replication and development of the chronic carrier state. In this case the incubation period and replication cycle of natural HBV following exposure is slower than the production of immunity from vaccination and protection can be achieved from birth hepatitis B vaccination.[13,14]

BCG – In general, protective efficacy of a birth dose of BCG vaccine has been measured through follow up of vaccinated cohorts and case control studies of infants rather than by immunogenicity measurements, as no surrogate for protection exists.[10,64] There does not appear to be a correlation with clinical protection afforded by BCG vaccine and the presence of a positive post-vaccination tuberculin skin test.[10] The protection afforded varies according to the form of tuberculosis (TB), for example pulmonary, lymphadenitis, miliary or meningitis, in general increasing with disease severity and degree of dissemination. An early case control study in 1987 suggested that birth BCG protective efficacy ranged from 20% for primary pulmonary TB to 80% for disseminated TB.[64] In addition studies in Sweden and Czechoslovakia have shown increases in TB incidence when BCG vaccination was ceased.[64,65] Two recent meta-analyses have found the protective efficacy of BCG vaccine at birth against TB meningitis is 64 -73% and miliary TB is 78-77%.[65,66] BCG vaccine at birth is recommended by WHO for countries with a high burden of TB.[67]

In summary there is immunologic and epidemiologic evidence that administration of several vaccines at birth including OPV, hepatitis B and BCG, is able to successfully prime the immune system to respond more briskly to the second dose and in some cases provide a degree of clinical protection after the birth dose alone. However, neonatal exposure may result in lower antibody levels following further vaccine doses, as seen in the Hib vaccine examples compared to infants who had no neonatal exposure, and is discussed later. In this thesis, responses to neonatal acellular pertussis vaccine are examined in Chapter 3, both for evidence of immunologic priming and effect on antibody responses after subsequent doses in later infancy.

e) *Influence of residual vaccine induced antibodies*

As shown above, maternal antibodies at the time of neonatal vaccination influence the infant's antibody response to the birth vaccine dose. Maternal antibodies are short-lived, with a half life of 30 days, and thus their influence on antibody response to vaccine induced doses in later infancy wanes with time. However, responses to subsequent doses are also influenced by the antibody level present at the time of vaccine receipt. Residual vaccine antibody can form antigen/antibody complexes which reduce the antigen available for B cell binding, and also provide negative feedback to B cells and plasma cells via Fc gamma receptors, which limits the amount of antibody produced.[10] This type of biological feedback phenomenon is postulated to prevent immune system overload from excessive antibody production.[10] This is important as vaccinees with residual antibody titres may show a relative increase in their level which is lower than the 2 to 4 fold increase seen in those with low pre-existing levels. This emphasises the importance of the proportions of vaccine recipients reaching a threshold for protection, when this is well established, as fold increase is strongly influenced by the residual antibody titre present at the time of vaccination.

f) *Existence of immune tolerance, hyporesponsiveness and vaccine interference*

Immune responses induced by early life vaccination can also be influenced by both hyporesponsivess to later doses and interference from other simultaneously administered vaccines, as summarised below.

Immune tolerance and hyporesponsiveness

Immune tolerance originates from the recognition that cellular responses to allo-antigens are suppressed because of neonatal exposure to that alloantigen (tolerization).[10] In the case of vaccines this means that neonatal vaccination would result in tolerance and the abrogation (absence) of specific immune responses to the vaccine antigen. Siegrist suggests that neonatal immune tolerance/paralysis has been misused to characterise the lower level neonatal responses seen at completion of the primary vaccine series in those given whole cell pertussis containing vaccines at birth compared to controls (no whole cell pertussis vaccine at birth), which are more correctly termed 'hyporesponsiveness'. (Personal communication: CA Siegrist) Hyporesponsiveness

refers to the blunting of responses following re-exposure to the same antigen, however there is no accepted international definition.

Hyporesponsiveness and polysaccharide vaccines

The term hyporesponsiveness traditionally refers to responses to repeated doses of polysaccharide vaccines and describes the fact that booster doses elicit serum antibody concentrations lower than those elicited by primary immunisation.[68]
Hyporesponsiveness has been reported following re-exposure to meningococcal C and pneumococcal polysaccharide vaccines.[68-70] The potential mechanism behind hyporesponsiveness is initial polysaccharide exposure forces B cells to differentiate into antibody secreting cells without generating memory cells, thus depleting the B cell pool, with the consequence that on re-exposure to the polysaccharide responses are limited.[68,71] In addition, it is likely that dendritic cells and suppressor T cells have a role in hyporesponsiveness.[68].

Hyporesponsiveness and whole cell pertussis vaccines

Hyporesponsiveness has also been reported after neonatal administration of lipopolysaccharide containing whole cell pertussis vaccine in mice.[72] Mice were immunised with DTPa or DTPw vaccines at birth and pertussis antibody responses were compared to control mice first vaccinated at 3 weeks old. Following the second dose at 3 weeks old responses increased rapidly in mice given DTPa vaccine at birth but were comparatively blunted in mice who received DTPw vaccine at birth. At 4 weeks old PT specific antibody levels were higher in mice who received their first dose at 3 weeks than mice who had received a birth and 3 week old DTPw vaccine. These results mimic those found in DTPw vaccine studies in humans where neonatal DTPw vaccine did not result in primary antibody responses after the first dose and interfered with responses to subsequent doses of DTPw. Neonatal priming with DTPw vaccine in infants resulted in lower antibody levels post completion of the vaccine series compared to infants who had not been primed with DTPw vaccine.[73-75] The neonatal DTPw vaccine studies are discussed in more detail in Chapter 2.

Although Roduit et al showed blunted antibody responses after birth DTPw vaccine in mice the protective efficacy, as measured by response to respiratory challenge with

B.pertussis, remained intact.[72] Mice primed with DTPw and a second dose at 3 weeks old were then given DTPa vaccine at 12 weeks old to assess whether hyporesponsiveness following the neonatal dose was still present. No evidence of hyporesponsiveness was found, as pertussis specific antibodies significantly increased 21 days after the DTPa vaccine booster.[72] This demonstrates that DTPw priming in the neonatal period resulted in hyporesponsiveness and not permanent tolerance.

Vaccine interference

Antibody production is not only influenced by the timing of vaccination but also by the type and number of vaccine antigens concomitantly administered. There is evidence that antibody responses to one antigen may be influenced by responses to other concomitantly administered antigens. This is known as 'vaccine interference'. Siegrist defines it as the 'modulation of vaccine responses that result from the concurrent or sequential administration of several distinct vaccines'.[71] Vaccine interference is modulated by interactions, some known and some not yet defined, occurring between vaccine induced antigen presenting cells, T and B lymphocytes. The interference can be positive, responses to one antigen enhanced by another or negative, responses to one antigen reduced by the presence of another antigen. The administration of BCG vaccine at birth has been shown to positively influence the response to hepatitis B vaccine administered at the same time.[76] BCG vaccine given at the same time as the priming dose of HBV vaccine at birth resulted in increased cytokine production (IFN gamma, IL5 and IL13) and hepatitis B surface antibody, at 4-5 months old compared to a control group who were not given BCG vaccine.[76] The likely mechanism for this positive interference is enhanced activation of T lymphocytes by antigen presenting cells.[76] In contrast, numerous studies have shown that the addition of Hib to DTPa vaccine results in lower Hib antibody levels following administration of the combination vaccine compared to responses when Hib and DTPa are given separately.[10,77] Lower Hib antibody levels following DTPa-Hib combination vaccines is one of the contributory factors for the increase in invasive Hib disease seen in the UK, as well as an absence of a Hib booster dose in the second year of life.[78]

One of the mechanisms for vaccine interference is carrier induced epitope suppression.[71,79] Conjugate vaccines are based around linking a polysaccharide (the

hapten in this case), for example Hib-PRP, to a carrier, such as tetanus toxoid or diphtheria toxoid. Carrier-specific B cells and naive B cells compete for the same hapten. Carrier-induced epitope suppression occurs when competition results in better responses to the carrier and reduced responses to the hapten (polysaccharide).[80] In addition, carrier-specific T regulatory cells also interfere with anti-hapten responses when a conjugate vaccine is given to those who have pre-existing anti-carrier immunity. Combination vaccines containing up to 6 antigens are now in use and there is an increasing need to understand the complex interactions between concomitantly administered antigens and the influence of carrier priming on antigen responses. Diphtheria toxoid and tetanus toxoid are the most frequently used carriers and vaccines using diphtheria toxoid or genetically modified diphtheria toxin (CRM_{197}) as the carrier protein are most likely to be associated with vaccine interference.[81]

Another mechanism for vaccine interference, which is less well understood, is 'bystander interference.' It is proposed that active immune responses to one antigen in a lymph node interfere with responses to another simultaneously administered antigen at the same site.[81] It is likely that vaccine induced T lymphocytes affect responses to bystander antigens through changes in the balance of TH1, TH2 and T regulatory cells.[71]

The immunological mechanisms behind vaccine interference remain to be fully defined and are complex. Vaccine interference is important to consider with respect to administration of an acellular pertussis vaccine at birth as there is the potential for effects on other antigens, either administered concurrently or sequentially, as seen in Chapter 3.

In addition to B cell and antibody responses, T cell responses in early infancy are quantitatively and qualitatively different, as discussed in the next section.

1.2.2 T cell immune response

In this section, early infant T cell responses in 2 main areas are described – innate/adaptive immunity and T helper cell responses. Infant T cell responses to

vaccines differ from older children and adults in reduced adaptive cellular capacity and prolongation of a T helper type 2 (TH2) bias as is explained below.

a) Limited innate and adaptive immunity

It is well known that neonates are particularly susceptible to infection with intracellular pathogens, including herpes simplex virus, respiratory syncytial virus, mycobacterium tuberculosis, chlamydia and listeria monocytogenes.[10] Protection from these pathogens is primarily dependent on T cell lymphocytes. This susceptibility is due the newborn's limited innate (dendritic cells, natural killer cells, polymorphonuclear cells) and adaptive (CD8 cytotoxic T cells and CD4 Th1 cells) cellular immunity.[82-84] In addition, maternal T lymphocytes do not cross the placental barrier in the same way maternal antibodies do, so that passive protection from T cells is not afforded.[83] Following birth there is a progressive maturation in the ability of the infant to mount T cell responses with increasing age and most infants can mount adult like T cell responses by 12 months old.[4] This has been demonstrated by comparing T cell responses to BCG vaccine at birth and 2- 6 months old, whereby improved T cell proliferative responses occurred with vaccination at an older age[85]and also with measles vaccination at 6, 9 or 12 months of age, whereby higher proliferative T cell responses were seen with later vaccination.[6]

b) T helper cell preference

There are two main types of T helper response (type 1 and 2) activated in response to vaccination. T helper 1 (TH1) responses are characterised by Interferon (INF) gamma and interleukin (IL) 2 cytokine production and promote macrophage activation and cytotoxic T cell responses. T helper 2 (TH2) responses are characterised by IL4, IL5 and IL13 cytokine production and promote antibody responses.[83,84] In theory, well balanced TH1 and TH2 responses would provide the best means of protection. TH1 responses are required for protection against mycobacteria and viruses.[83] In general, neonatal cellular immune responses are characterised by a TH2 bias.[5,83] During pregnancy there is a preferential TH2 drive which probably serves to protect the infant from inflammatory reactions at the placental materno-foetal interface and may explain the neonatal tolerance to alloantigens. Limited TH1 responses are seen in early life, related to some or all of the following, persistence of TH2 bias, limited exposure to

pathogens and microbes and a reduced co-stimulation from antigen presenting cells (APC's) to T helper lymphocytes.[6] Limited TH1 responses and a TH2 bias may mean that cytotoxic T cell responses are impaired, reducing protection against intracellular pathogens, such as respiratory syncytial virus, herpes simplex and chlamydia.

Several studies have investigated T helper responses following newborn vaccination, and found the TH1 vs TH2 response is not the same for all vaccines. Factors that influence whether TH1 or TH2 responses are induced include antigen dose, type of adjuvant and route of administration.[83,84] At present only BCG, OPV and HBV are routinely administered at birth. Ota et al reported that HBV vaccine at birth induced lower IFN gamma (TH1) responses, and higher TH2 memory responses in newborns compared to adults.[86] A similar study by Vekemans et al showed infants given OPV vaccine at birth have reduced IFN gamma (TH1) response compared to adults after OPV vaccine at birth.[87] However, the bias towards TH2 responses is not universal. Studies have shown newborns can mount TH1 responses to BCG vaccine given intradermally and whole cell pertussis vaccine at birth.[83,88] The TH1 responses that Pw and BCG vaccines induce may relate to their dendritic cell activating properties which are not yet completely defined.[83] In general, neonatal dendritic cells have a lower capacity to produce cytokines that drive TH1 responses compared to adult dendritic cells.[5,83,89]

T helper cell responses to pertussis

T cells specific to pertussis antigens, PT, PRN and FHA cells have been reported after natural pertussis infection and are thought to protect by facilitating intracellular clearance of *B. pertussis* through activating macrophages and neutrophils.[90-92] A recent report on histopathology of the lung in children who died from pertussis confirms the presence of *B. pertussis* intracellularly within alveolar macrophages and ciliated epithelium, thus clearance of intracellular organisms is important for recovery.[3] The presence and type of T cell immunity induced by pertussis vaccines appears important for protection. Murine studies suggest whole cell pertussis vaccines preferentially activate TH1 responses.[91,93] However, little data exists on human infant T cell responses following whole cell or acellular pertussis vaccines. TH1 responses have been demonstrated in infants following whole cell pertussis (Pw) immunisation.[83,92]

Responses to acellular pertussis vaccine seem to be different to whole cell pertussis vaccine. Zepp et al measured cell mediated immunity in infants who received DTPa at 3,4, 5 months and found both TH1 and TH2 cells were activated, with a preference to TH1 cytokine production.[91] Ausiello et al showed preferential TH1 responses in infants vaccinated with whole cell pertussis vaccines compared to both TH1 and TH2 responses in acellular pertussis vaccines.[92] Rowe et al measured cell mediated immunity after Pa vaccine, as part of a combination DTPa vaccine, in 2-18 month old infants.[94] Peripheral blood mononuclear cell responses to the tetanus toxoid antigen were skewed towards TH2 cytokine production. This group were slightly younger than Zepp et al and this may have influenced the bias to TH2 responses.[91,94]

In summary, cell mediated immunity (CMI) following vaccination is likely to be important for clinical protection against pertussis No studies have reported CMI following neonatal Pa vaccination and further study is needed.

1.2.3 Conclusion

In summary, there are many factors that can affect the quality and quantity of early infant antibody and T cell responses. This includes the following: maturity of the innate infant immune system, vaccine schedule (number, type and timing of vaccines), influence of maternal antibodies, existence of neonatal priming, vaccine interference, hyporesponsiveness and T helper cell preferential activation.

Summary of immune responses following early infant vaccination:

- Reduced antibody response to vaccines given under 4 weeks old
- Antibody levels following early infant vaccination rapidly decay
- Repeated doses are needed to elicit strong antibody responses
- Shorter intervals between doses result in lower antibody responses
- Maternal antibodies may interfere with primary antibody response in the infant
- Neonatal vaccine can successfully prime the immune system and a single dose at birth has been shown to be clinically protective for hepatitis B and BCG vaccine

- Antibody responses to select antigens have been modified by responses to other concomitantly administered antigens, known as vaccine interference
- Neonatal T cell responses have a TH2 bias

At present, only BCG, OPV and HBV vaccines are routinely administered at birth and shown to have good protective efficacy. For some infections such as pertussis, earlier protection from birth would be desirable, given the high morbidity and mortality of early infant pertussis. The earliest the first pertussis vaccine is given is 6 weeks old and responses to Pa administration earlier than this age may be affected by the above immune system factors, however it is not known, therefore, this thesis examines responses to acellular pertussis vaccine starting at birth. For HBV, persistence of immunity following infant vaccination into adolescence is important and one of the principal determinants of this is the initial primary infant response. An understanding of each of the immunological factors, discussed in this chapter, has assisted with informing the design and analysis of the neonatal acellular pertussis vaccine trial (Chapter 3) and long-term hepatitis B follow up studies (Chapter 5).

1.3 Longevity of immunity

Sustained vaccine efficacy beyond infancy into adolescence and adulthood relies on persistence of immune memory, which determines whether booster vaccinations are required later in life to maintain protection. In particular, the ability to provide long lasting protection following infant doses is important for developing countries where provision of booster doses in later life is logistically challenging and expensive. The principal determinants of immune persistence and memory are discussed in this chapter, with specific reference to hepatitis B immune longevity. In chapter 5, a clinical trial examining the persistence of hepatitis B immunity following birth and infant vaccination is presented and interpretation of the trial results requires a theoretical understanding of the major determinants of immune longevity.

1.3.1 B cell immune longevity

The principal determinants of B cell immune longevity include:

a) Level of post primary immunity

b) Level of plasma cell production during primary vaccination series

c) Memory B cell persistence

d) Persistence of antibody over time

e) Immune memory

f) Kinetics of immune memory reactivation and microbial invasion

a) Level of post primary immunity

In general, the higher the antibody level post primary immunisation the longer the persistence of circulating antibody. Higher antibody levels following primary vaccination series are associated with greater induction of immune memory B cells. Vaccination at birth or in early life may result in reduced post primary antibody levels, with consequent reduction in antibody persistence, and lower memory B cell induction compared with vaccination commenced later. This is important for hepatitis B, where persistence of immunity into adult life after birth and infant vaccination is necessary for

ongoing protection. In addition, if neonatal vaccination results in interference with concomitant antigen responses this may further compromise immune persistence.

b) Level of plasma cell production and survival

Following primary vaccination, long lived plasma cells migrate to survival niches in bone marrow. The duration of antibody response is proportional to the number of long lived plasma cells created during primary vaccination.[10,16] In general bone marrow in the neonate has a lower capacity to sustain plasma cell survival compared to older children.

c) Memory B cell persistence

Memory B cells are produced during primary immunisation and on re-exposure to antigen differentiate into plasma cells with rapid production of antigen specific antibody with high affinity, this is known as immune memory re-activation.[95] Similar to plasma cells, the level of memory B cells produced during primary immunisation is dependent on the degree of germinal centre activation.[16] However, factors that drive memory B cell formation and persistence are not well understood. As noted, neonate and early infant germinal centre activation is limited and so subsequent memory B cell production is reduced compared to immunisation at an older age.

Other factors known to influence the generation of memory B cells include – lower antigen content of vaccine at priming which preferentially induces memory B cells rather than plasma cells, persistence or re-exposure to antigen, (for example by natural boosting) and a longer interval between doses in the primary schedule (4 to 6 months between doses 2 and 3). Blanchard –Rohner et al showed there was a strong correlation between the presence of meningococcal C specific memory B cells, after primary vaccination, and the persistence of antibody at 1 year post infant meningococcal C vaccination.[16] Interestingly, the success of immunisation programs, resulting in reduced disease burden (low endemic countries), with reduced potential for natural boosting, may mean vaccine booster doses are required to replace natural exposure.

d) *Persistence of antibody over time*

As mentioned the half life of most antibodies is approximately 30-40 days, but some vaccine induced antibodies persist in the circulation for much longer periods than this. The persistence of antibody in the circulation is especially important for protection against those pathogens where replication is faster than immune memory activation, such as Hib or meningococcal infection, and is discussed below in the section on kinetics. The reason for antibody persistence is not fully established. The persistence of antibody is most likely related to the number of long lived plasma cells produced following immunisation. In addition, antibody levels are determined by memory B cell reactivation into short lived plasma cells.[10] Three theories are outlined by Pichichero.[95] First, it may relate to persistence of memory B cells, some of whom continue to regularly mature into plasma cells, leave the bone marrow and produce small amounts of antibody in the serum. Second, there is the 'treadmill of antigen hypothesis' where small amounts of antigen are retained in antigen-presenting cells and continue to stimulate antibody production. A third hypothesis is that polyclonal B cell activators maintain the memory B cell pool.[95] It is also likely that natural boosting, through subclinical reinfection, colonisation or exposure to an organism with a cross reacting antigen continues to drive small amount of antibody production. A good example of this is natural rises in hepatitis B surface antibody in those living in hepatitis B endemic areas and is discussed in chapter 4.

e) *Immune memory*

Antibody levels wane with increasing time since vaccination and the rate at which they decline is dependent on many factors, as outlined above. However, absence of antibody does not necessarily equate with absence of protection, because of immune memory (anamnestic) activation. On re-exposure to a foreign antigen, memory B cells differentiate into plasma cells and antibody production ensues. Thus, measurement of antibody levels alone is not sufficient to determine immune longevity and surrogate measures of immune memory are used. Immune memory can be measured in vitro or in vivo.

In vitro

In vitro memory can be demonstrated by measuring the number of memory B lymphocytes retaining the ability to produce antibody following stimulation by an antigen, using an assay known as the spot-ELISA.[96,97] In vitro T cell memory can be measured by isolating peripheral blood mononuclear cells and assessing the proliferative response of T lymphocytes to an antigen.[98] In vitro measurements of hepatitis B memory T and B cells demonstrate persistence of memory for 10 years post HBV vaccination.[96-100] Studies examining in vitro memory T and B cells are hampered by limited sensitivity of the currently available methods to detect the low numbers of memory cells in circulation in peripheral blood.[101] In vitro memory B and T cell responses are not the subject of this thesis.

In vivo

In vivo, measurements of immune responses following 'challenge' with an antigen, usually a vaccine, are conducted. Administration of an antigen (booster vaccine) and assessment for rapid antibody rise, within days, post vaccination, is used as a marker of immune memory and specifically presence of memory B cells.[10,95] (Figure 1.2)

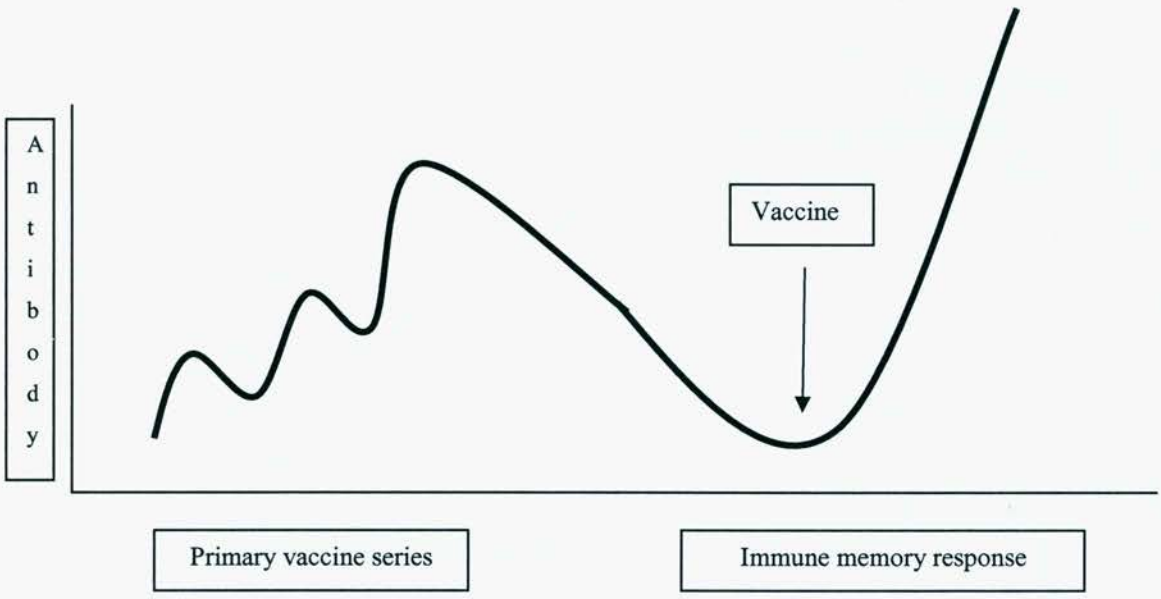
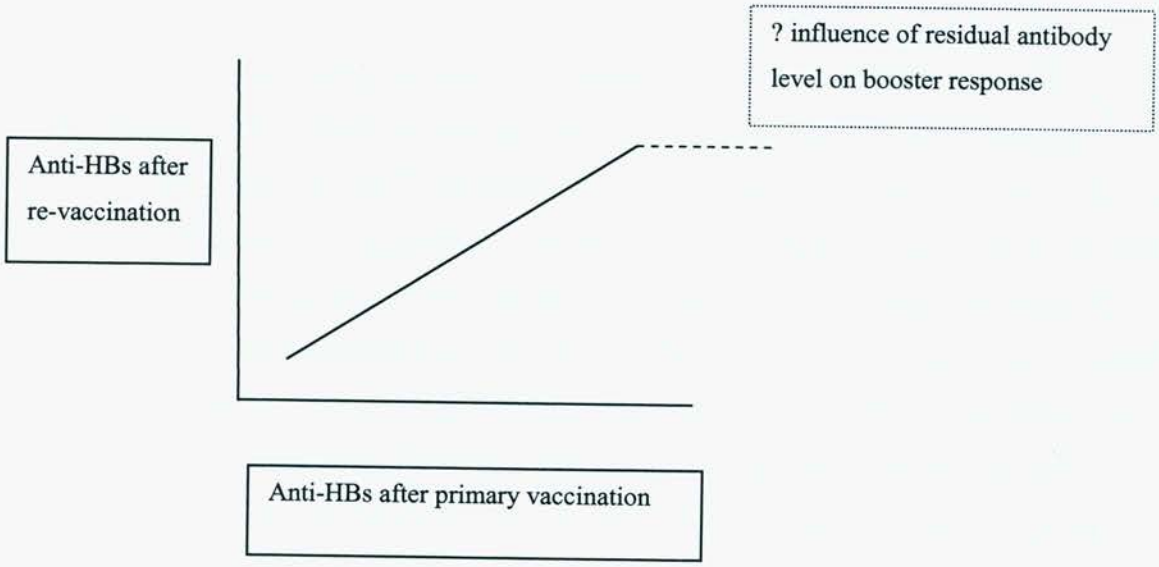


Figure 1.2 Immune memory response following primary vaccination

Slower and lower antibody rises may indicate that memory B cell induction, persistence and/or reactivation was insufficient or has waned.[10] Of note, challenge with a parenterally administered vaccine, containing adjuvant, does not completely mimic natural exposure, which is often by the mucosal route (respiratory, gento-urinary, gastrointestinal).[95] In addition, studies measure serum antibody where for some infections, such as *S. pneumoniae*, mucosal antibody responses may be more important.[102] Vaccine challenge studies to measure immune memory have been conducted for many vaccines, including hepatitis B, as outlined in chapter 4.[15,103,104 105]

In general, individuals with high antibody levels post primary vaccination show high antibody rises following booster vaccination indicating that more memory B cells were generated by the primary vaccine series. This association has been shown for hepatitis B vaccines. (Figure 1.3) However, it may be possible that higher residual antibody levels at the time of booster vaccine 'challenge' result in antigen-antibody complex formation, meaning reduced free antigen is available for B cell binding and possible antibody mediated negative feedback on B cells, as discussed earlier in this chapter. This could lead to reduced antibody production in the memory B cell response.



Ref: Adapted from Banatvala et al[103]

Figure 1.3 Association of antibody response following booster vaccine with antibody level achieved following completion of the primary vaccine series

Successful demonstration of in vivo immune memory is thought to equate with protection following natural exposure, however the kinetics of memory response is important.[10,95]

f) Kinetics of immune memory reactivation and microbial invasion

Following exposure to a microbe, in an individual who has been primed by prior vaccination, immune memory is reactivated and antibody production ensues. If exposure to the microbe results in production of effector cells before the end of the microbes' incubation period clinical disease may be averted.[10] For example, hepatitis B exposure in a previously vaccinated individual results in antibody production faster (within 2 weeks) than the HBV incubation period (4-12 weeks) and HBV chronic carriage is prevented, although evidence of infection may be seen (hepatitis B core antibody positive).[95] This is particularly important for HBV, where a second high risk period of infection commences during adolescence with onset of sexual activity and therefore the persistence of immune memory for more than 10 years following birth/infant vaccination is necessary. Several studies in high and low HBV endemic countries have examined the persistence of HBV immunity and are described in chapter 4. Of note, it is possible that with increasing time since birth/infant vaccination immune memory may wane.

However, there are examples where the kinetics of memory cell activation and demonstration of antibody production may not be fast enough to prevent clinical disease. This includes Hib[106] and meningococcal C disease[17] where protection following an infant schedule (2, 3, 4 months), without booster vaccination, was higher in the first year after completing the vaccine schedule than during the following three years. Of note, in both these examples from the UK the infant schedule is completed at 4 months of age as opposed to 6 months in Australia and North America. The lower protection rate after the first year may relate to a reduced pool of memory B cells produced following primary vaccination due to a shorter interval (1 month) between vaccine doses in the schedule or earlier age of completion of the primary schedule (4 months).

1.3.2 T cell memory

There are 2 broad types of memory T cells – effector and central.[10,95] Following the primary vaccine course, T cells become primed and memory T cells are formed, such that on re-exposure to an antigen, cytotoxic T cells and natural killer cells are activated.[10] The main determinant of the number of memory T cells is the amount of antigen present during primary vaccination.[10] *Effector memory cells* are located within non lymphoid tissues, monitor for the presence of foreign antigens, and on exposure are cytotoxic.[10] *Central memory T cells* are located in lymphoid tissue and spleen, have a high proliferative capacity and on exposure differentiate quickly, within days, into large numbers of effector cells.[10,95] In the case of hepatitis B, these effector cells are able to attack any hepatocytes infected with hepatitis B virus.[10,107] Marchant et al showed that TH1 responses after BCG vaccine at birth persisted until 1 year old and indicate that memory T cells were activated by the initial dose at birth.[88] This is important as successful demonstration of T cell memory means that birth vaccination may continue to provide protection into later life with no booster dose requirement.

1.4 Summary and relevance to thesis

In summary, neonatal vaccination is characterised by reduced T and B cell responses compared to vaccination later in life. This has subsequent implications on how early vaccine schedules can afford protection and the length of protective immunity, as discussed above. The epidemiology of some diseases, such as pertussis, has driven a desire to protect as early as possible through either maternal or neonatal vaccination. An understanding of the immunology of early life vaccination is important in interpreting results from neonatal vaccine trials. If we consider vaccinating earlier (at birth) with the possibility that immune memory may be reduced, then long term follow up studies examining the persistence of immunity are needed. The remaining sections of this thesis examine early life pertussis vaccine responses and longevity of immunity following neonatal and infant hepatitis B vaccination.

There are many determinants of immune response to vaccines in early infancy, as shown in figure 1.4. These include both infant B and T cell immune system factors and vaccine / schedule factors. Immune responses to early life vaccination can be influenced by vaccine interference, due to concurrent or sequential administration of several distinct vaccines, and result in enhanced or reduced antibody responses. In addition, neonatal priming may result in blunting of responses (hyporesponsiveness) to subsequent doses of the same antigen. Although hyporesponsiveness has been demonstrated as an immune phenomenon following polysaccharide revaccination and early DTPw birth vaccine studies in humans, it is not clear whether this reduced response has clinical implications for disease prevention. Any investigation of neonatal vaccination needs to include assessment for vaccine interference and hyporesponsiveness.

Early infant cellular responses to vaccines are limited by their intrinsic adaptive cellular capacity. T helper responses vary between vaccine type and age at vaccination, with vaccination at a younger age more likely to result in a TH2 preference. Acellular pertussis vaccines in infancy appear to induce both TH1 and TH2 T cell responses, with the latter being preferentially induced. Administration of BCG at birth induces TH1 responses and limited studies on OPV and hepatitis B vaccine at birth suggest TH2

responses predominate. No studies have examined the cellular response to acellular pertussis vaccine when given at birth and this is examined in Chapter 3 of this thesis.

In conclusion, an understanding of immunological factors affecting responses to vaccines in early infancy and longevity of immunity is needed to inform the design and analysis of the neonatal acellular pertussis vaccine trial and long-term hepatitis B follow up studies in chapters 3 and 5 in this thesis. However, the design and results from previous newborn pertussis vaccine trials and hepatitis B immune longevity studies also assist in informing the studies conducted in this thesis, as discussed in chapter 2 and 4.

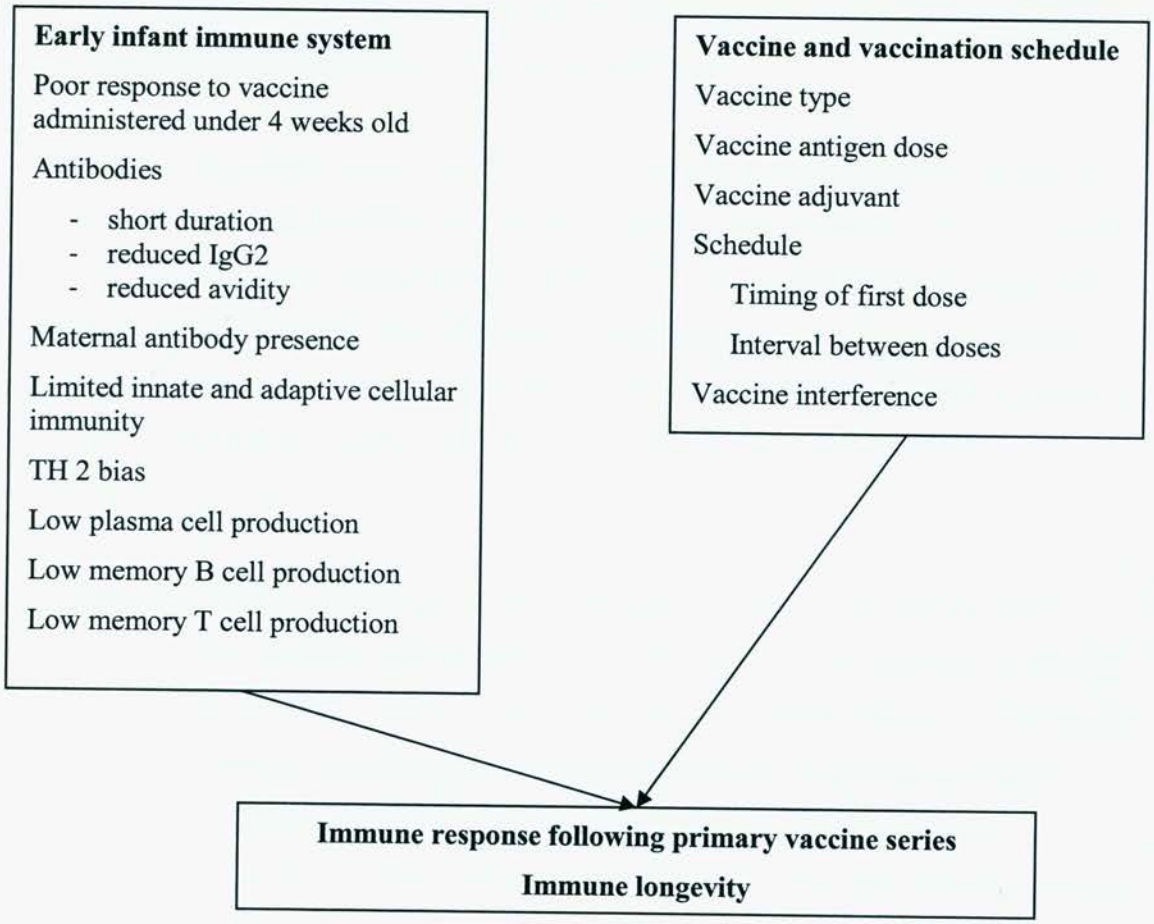


Figure 1.4 Summary of determinants of immune response to vaccines in early infancy and longevity

CHAPTER 2: EPIDEMIOLOGY AND STRATEGIES FOR THE PREVENTION OF EARLY INFANT PERTUSSIS

2.1 Introduction

Infants under 6 months of age, have the highest rates of pertussis infection and the highest morbidity and mortality from pertussis.[108,109] One of the UN Millenium Development Goals is to reduce child mortality by one third by 2015 and achieving this includes the necessity to reduce pertussis deaths.[110] In countries with well-developed childhood pertussis immunisation programs, adults and adolescents are now the primary sources of infection for infants.[111-113] In developing countries, high coverage for 3 doses of pertussis-containing vaccines at 6, 10 and 14 weeks remains the key challenge and transmission from older children is still the primary issue.

The true incidence of pertussis is poorly defined because of varying levels of clinician suspicion and reporting practices and limited diagnostic test sensitivity, even for newer tests, such as polymerase chain reaction (PCR).[114-117] This is particularly true for older populations with less typical and often later clinical presentation. Currently, the true incidence of pertussis is generally considered to be substantially higher than reported by either notifications or hospitalisations in both developed and less developed countries.[118]

This chapter reviews the current global epidemiology of pertussis in infancy, the reasons behind the changing epidemiology and compares potential strategies to prevent pertussis infection in infants. There are two broad ways to protect infants – indirectly by preventing transmission of pertussis to infants and directly by vaccinating mothers during pregnancy (passive immunity) or infants at or shortly after birth (active immunity) to prevent disease when exposed to pertussis.[119] Indirect protection strategies include booster vaccination every 10 years throughout life, which would probably prevent most disease but is challenging because of cost and implementation problems. A more targeted indirect strategy, which targets parents, grandparents and other close contacts of new babies, including healthcare workers, is the 'cocoon' strategy. It is less costly and has potential advantages for implementation. It is recommended in France,[120]

Australia, USA, Germany and Austria but has not been successfully implemented at population level in any country.[121-123] Direct protection of the infant through maternal pertussis vaccination and transplacental passage of pertussis antibodies (passive immunity) is likely to be protective, but faces significant practical and scientific hurdles as reviewed in this chapter. Direct protection by early infant pertussis vaccination was investigated in the 1950s and 1960s with whole cell vaccines because of the high burden and morbidity of early infant pertussis. This chapter reviews previous studies of early whole cell pertussis vaccine use and the concerns regarding immune 'paralysis' or hyporesponsiveness which discouraged further pursuit of this strategy. The arrival of acellular pertussis vaccines has led to questions as to whether they can be used neonatally. This chapter concludes with the issues behind neonatal acellular pertussis vaccination as a strategy to prevent early infant pertussis disease and factors influential in study design of the acellular pertussis vaccine trial in chapter 3.

2.2 Background epidemiology

Bordetella pertussis, the cause of pertussis or whooping cough, is an exclusively human pathogen.[10] Disease elimination by vaccination should be therefore be possible, but has proved elusive. Pertussis is a significant cause of mortality in early infancy worldwide. Nearly 300 000 deaths occur each year, most in developing countries, but deaths are probably under-estimated in both rich and poor countries.[108,109] Soon after whole cell pertussis vaccines were introduced in industrialised countries from the late 1940s there was a marked decline in pertussis disease.[10] However many industrialised countries are currently seeing a resurgence of pertussis, despite universal vaccination programs with high uptake. For example, there has been an increase in pertussis notifications in the past decade in the United States, France, Canada and Australia, particularly among adolescents and adults.[124-127] *Bordetella pertussis* is highly infectious, is spread by respiratory droplets and causes epidemics every 3 to 4 years. Fewer adults and older children develop classic pertussis symptoms, with diagnosis often delayed and capacity to transmit infection potentially lasting several weeks.[10]

Comparison of pertussis incidence between countries is problematic due to differences in case definitions, access to diagnostic tests, clinician awareness and reporting practices and whether pertussis is notifiable under public health legislation. Differences in immunisation strategies and historical levels of immunisation coverage within and between countries also affect the epidemiology of pertussis. Clinical recognition of disease due to *B. pertussis* infection is often poor, especially in adults and older children, who are more likely to present with atypical findings, on a background of waning immunity from infection or prior vaccination.[128]

In countries with well-developed immunisation programs, pertussis is now a problem in two broad age groups – those over the age of 10 years and those under the age of 5 months.[129-131] The former group were often born in an era of low immunisation coverage and in those who were vaccinated susceptibility is exacerbated by waning immunity. Infants under 5 months are too young, under most immunisation schedules, to have reliably received two or more doses of diphtheria, tetanus, acellular pertussis (DTPa) vaccine. Infection in young infants is a particular concern because infants are most likely to have severe disease, resulting in hospitalisation or death.[2,132] The most

common cause of death in infants is pertussis pneumonia, at times complicated by apnoea, seizures and encephalopathy.[2,3,108,133] Most deaths are associated with the presence of pneumonia, and when circulatory support is required, most infants do not survive even with extracorporeal membrane oxygenation, underlining the importance of preventive strategies.[3,132,134]

2.2.1 Changing epidemiology of pertussis

The resurgence of pertussis has been well documented in many industrialised countries.[116,125,130,131,135] A review by the Global Pertussis Initiative noted that the increase in infant, adolescent and adult pertussis has been seen despite presumed under-reporting of varying degrees.[136] The spectrum of current pertussis epidemiology is summarised below by country:

Australia: Available data on pertussis in Australia include notifications to public health, hospitalizations and deaths.[126,137] Pertussis notification rates in infants aged less 6 months remain higher than any other age group, despite maximal pertussis vaccine coverage in older children.[126] Infant hospitalisations average 400 per year, more than 80% occur before 5 months of age and over 50% before 8 weeks of age. (Figure 2.1) There has been a significant reduction in pertussis-related hospitalisations in the most highly vaccinated age group (5 months to 9 years), corresponding to changes in vaccination schedules and vaccine coverage over time.[126,137] However, the incidence of hospitalisations for pertussis under the age of 5 months in New South Wales, the most populous state in Australia, has remained unchanged.[138] This pattern is also seen in national data from the Australian Paediatric Surveillance Unit (APSU) which facilitated active monthly surveillance of infant pertussis hospitalisations in 2001 when an acellular pertussis vaccine was exclusively used.[111] Of 140 cases identified, approximately half (48%) were under 2 months of age and therefore were not eligible to receive any pertussis-containing vaccines according to the current Australian vaccination schedule. In 68% of cases where there was a presumptive source, infection was acquired from an adult.[111] Infants under 2 months of age accounted for approximately 30% of pertussis deaths in the 1960s and now account for over 80% of deaths. (Unpublished data: Australian Institute of Health and Welfare Mortality database) Of the 18 deaths coded as due to pertussis in Australia 1993-2004, all but two

were less than 6 months of age.[126,137,139] In the APSU study 18% of infants required intensive care (56% including ventilation) and 4 deaths were reported, all in infants under the age of 6 weeks.[111]

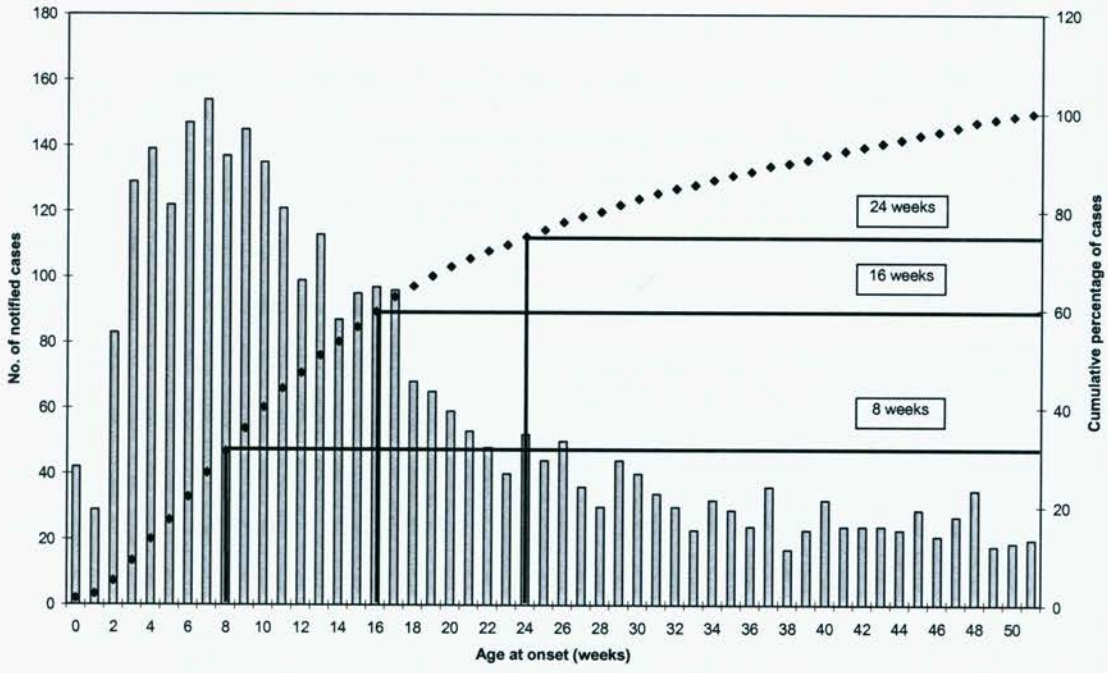


Figure 2.1 Australian pertussis hospitalisations under 12 months of age 1994-2004 (n=4114)[140]

Europe: Similar patterns in age-specific pertussis incidence are also seen in Europe. In many countries the highest incidence rates are in infants, although rates are not increasing.[108,141-143] However, the European Community-funded surveillance project, EUVAC-NET recorded a 115% increase in incidence rates in persons over 14 years old between 1998 and 2002. Of 32 deaths recorded during this same period, 87% occurred in infants under 6 months of age.[141] Denmark saw an increase in pertussis incidence, for both notifications and hospitalisations in infants (0-5 months), following introduction of an acellular pertussis vaccine in 1997.[144] (Table 2.1) Evidence from the UK suggests there may be 3 times more hospitalisations and deaths in infants than is recognised.[2] France saw a resurgence of cases in the early 1990s, mostly in partially or unvaccinated infants under 1 year of age, with adults the main source of infection.[125]

North America: In the USA the highest pertussis incidence, between 1997 to 2000, was in infants (approximately 55 per 100 000).[145-147] Tanaka notes the mean annual incidence of pertussis in infants under 4 months of age increased from 63.4 cases per 100 000 in the 1980s to 88.7 cases per 100 000 in the 1990s.[130] From 2001-2003, the highest annual incidence was in infants under 6 months of age (98.2 per 100 000). This was much higher than the annual incidence in infants aged 6-11 months (12.3 per 100 000).[148] Pertussis rates have also been documented to be on the increase in adolescents (aged 10-19 years), from 5.5 per 100 000 in 2001 to 10.9 per 100 000 in 2003.[148] In 2004, the incidence of pertussis in infants aged <1 year was 84 per 100,000 and in children aged 1-10 years was 18 per 100,000.[149] Another recent US study of pertussis hospitalisations noted that nearly 90% of infants hospitalised in the US in 2000 and 2003 were under 3 months old and the hospitalisation rate was highest in those aged 1-2 months (239 per 100 000 births).[150] Similar but higher hospitalisation rates have been seen in American Indian and Alaskan native infants, with the highest rates in those under 6 months old of 234 per 100 000 from 1980 to 2004.[151] In the US, 103 pertussis related deaths were reported between 1990 and 1999, an increase from the previous decade and 82% were in infants under 4 months old.[152] In Canada, infants have the highest incidence rates and 89% of infants admitted with pertussis between 1991 and 2001 to tertiary hospitals nationally, were under 6 months of age.[153]

Africa, Asia, South America: Recent reports of pertussis epidemiology in Asia, Africa and South America are limited.[154] However, the World Health Organization (WHO) documents demonstrate that these countries have high disease burdens.[109,116] Estimating rates of pertussis are difficult in these countries because of lack of access to diagnostic methods and possible misdiagnoses, variable rates of underreporting, and use of different reporting criteria between countries. In Brazil no increase in pertussis was documented following introduction of pertussis vaccination in the 1980s.[155] In Argentina there is a lack of epidemiological studies on pertussis.[156] Despite these constraints, it is clear that pertussis remains one of the top ten causes of death in children under 1 year old worldwide with an estimated 10 million cases and as many as 400 000 pertussis-related deaths annually, 90% in developing countries and mostly in infants.[108,109,147]

Table 2.1: Pertussis hospitalisation rates for children aged <1 year in industrialised countries

Country	Year	Hospitalisation rate under 12 months (per 100000 population)	Source
USA	1990-1999 1993-2004 1980-2004	30.8 65 234	Tanaka et al [130] Cortese et al[150] Murphy et al[151]
Catalonia, Spain	1997-2001	118	Moraga et al[157]
Denmark	1996-1996 1998-2001	194 (0-5 months) 53.5 (6-23 months) 278.6 (0-5 months) 12.6 (6-23 months)	Hviid et al[144]
Canada	1989-2000 1990-1998	182.7 270	Galanis et al[153] Ntezeyabo et al[124] Betinger et al[158]
Australia	1998-2000 2001-2002	82 154	NCIRS [126,137]
England	1995-1997	164 (0-2 months) 76 (3-5 months) 17 (6-11 months)	Van Buynder et al[159]
France	1993-1994	95	Baron et al[125]
Austria	1996-2003	71.2	Rendi-Wagner et al [160]

In summary, there is a global trend for pertussis to predominantly affect adolescents/adults and partially or unvaccinated infants. Possible reasons for this are examined in the following section.

2.2.2 Explanations for changing epidemiology

An understanding of the possible reasons for the changing epidemiology is important to focus initiatives and strategies that may impact on reducing the rates of infant pertussis. There are a range of factors to consider including; the duration of protection and waning immunity, incomplete protection from vaccination, the infection source for infants, strain polymorphism, reporting trends and the development of newer diagnostic methods which allow detection of cases that were previously not able to be identified.

a) *Duration of protection and waning immunity following vaccination*

Possible contributory factors to the changing epidemiology of pertussis include waning immunity following infant vaccination, resulting in susceptibility to pertussis disease during adolescence and reduced opportunities for boosting immunity due to reduced circulation of pertussis.[146,161] Estimates of the duration of protection following whole cell pertussis vaccination range from 4-14 years and following acellular pertussis vaccination (based on limited studies) approximately 5-6 years.[161-163] However, these estimates are predominantly based on vaccine efficacy trials and there is no definitive serologic marker for protective immunity. Many factors complicate interpretation of serological data, including circulating levels of pertussis in the community, variations in vaccine type and schedules, and differences in surveillance and reporting methods between countries. However despite this, age cohorts experiencing increased incidence of pertussis have been identified in Canada related to waning immunity from a less effective vaccine used with high coverage[124] and in Australia related to low vaccine uptake of a moderately effective whole cell vaccine.[164]

b) *Incomplete protection from vaccination*

Two or more doses of a pertussis-containing vaccine appear to be needed for protection.[142,165] Infants under 5 months are too young, under most immunisation schedules, to have reliably received two or more doses. In Australia, half of all pertussis hospitalisations between 1994 to 2004 occurred in infants under 12 weeks of age, eligible to receive only one dose of pertussis-containing vaccine at 8 weeks of age.[129] In a multinational study, approximately 75% of hospitalised infants had received nil or 1 dose only of pertussis vaccine.[112]

c) *Infection source for infants*

Adults, particularly parents, are the most important source of infection for infants.[119,166] Adults and adolescents usually present late in the course of the infection, often after 4 or more weeks of coughing,[115,167] though diagnostic delay by clinicians is also frequent.[146] Pertussis has been found to be the cause in up to 13-20% of adults with prolonged cough.[108] This result will be highly dependent on the study population, diagnostic method and whether it is an epidemic year or not. In the UK, 37% of consecutive children, aged 5 to 16 years, presenting to primary care with cough more than 14 days

had well-documented serological evidence of pertussis.[168] These data formed part of the evidence base for introduction of a 4th dose of DTP vaccine at 4 years in the UK schedule.[168] A probable scenario is that adults and adolescents, who become infected because of lack of immunity, waning vaccine induced immunity or reduced natural boosting, along with delayed diagnosis, act as reservoirs for infection and transmit infection to unvaccinated or partially vaccinated infants.[116]

Adults, especially parents, were first noted in the US in the 1970s to have replaced older siblings as the major source of pertussis infection in young infants.[166] In Australia this was also demonstrated by a national study in 2001, where a presumptive source of infection could be identified (60% of hospitalised infants), 68% were adults and 60% parents.[111] Parents were also identified as the single most important source in a study in French hospitals.[125] In a household transmission study in Brazil three quarters of infants under 6 months of age were infected by people over 11 years 6 months old.[169] In addition to parents, grandparents and healthcare workers (HCW) are also responsible for transmission.[111,119,170-172]

d) *Strain polymorphism*

It has been suggested that *Bordetella pertussis* has adapted to express pertussis toxin and pertactin distinct from the vaccine strains, with consequent reduction in vaccine effectiveness, and has been investigated in the Netherlands and Finland.[131,173,174] Recent studies in Finland note that *Bordetella pertussis* is evolving and suggested that conformational changes in region 1 of pertactin and reduced vaccine induced immunity to this strain may account for the observed increase in pertussis rates.[175] However despite evidence of polymorphism for pertussis toxin and pertactin in several countries, no direct link to vaccination programs or their efficacy has been proved.[131]

e) *Increased diagnosis and reporting*

In developed countries improved diagnostic techniques and reporting has led to increased notification rates for pertussis.[116] Increased press reports and scientific literature on the 'resurgence of pertussis', associated to reports about clinical vaccine trials and subsequent licensure of low dose acellular pertussis vaccines for use in adults are likely to have led to an increase in clinician awareness and reporting.[115,116] In

addition the increase in reporting is likely to have been driven by increasing use of PCR based assays.[176] In infants, increased rates of pertussis may be related to vaccination strategies and infection sources as well as the availability of PCR for diagnosis, with significantly increased sensitivity over culture. In older children and adults, the use of serology dramatically increases detection of pertussis, as delayed presentation makes other diagnostic methods such as culture and PCR much less likely to be positive.[123,177,178] Globally however, under-recognition, underreporting and misdiagnosis still result in a substantial underestimation of the true burden of pertussis.[118]

2.3 Potential strategies for prevention of pertussis in early infancy

The Global Pertussis Initiative is a multidisciplinary panel of experts created in 2001 to evaluate potential pertussis vaccine strategies to reduce early infant pertussis.[118,136] This initiative included over 30 scientific experts from 17 countries who were divided into three regional sub groups – Europe, North America and International. The focus of the group was to raise global awareness of pertussis as an important preventable disease and to develop and communicate evidence-based recommendations for immunisation based strategies to slow the increasing trend in pertussis incidence.[118,136] These strategies include indirect protection (via herd immunity), vaccination of older age groups and direct protection of the infant. Indirect protection may be from universal pertussis immunisation programs at the population level which aim to achieve timely and high coverage. This could range from primary programs in older infants to boosters in the pre-school, adolescent and adult age group. Indirect protection may also be achieved through targeted vaccination programs aimed at parents and possibly others in close contact with the newborn, such as grandparents, as well as healthcare workers. This indirect targeted vaccine program is known as the "cocoon" strategy. Direct protection against pertussis for the infant may be achieved either though earlier active infant immunisation (prior to one month of age) or immunisation during pregnancy aimed at producing passive protection through maternal antibody. Each of these approaches, which are not mutually exclusive, has important advantages, disadvantages and uncertainties which will be summarised below.

2.3.1 Indirect protection

a) Universal adolescent and adult vaccination

The likelihood of indirect protection from immunisation of older age groups will depend first on the level of population coverage obtained and second on the amount of contact between young infants and these various age groups which may vary according to social practices and population demographics between and even within countries. In Sweden, resumption of mass vaccination according to a 3, 5, 12 month schedule resulted in a reduction in pertussis notifications between 1979 and 1995 in both vaccinated and non vaccinated infants.[179] However, in Canada while the total number of

hospitalisations due to pertussis fell from 1174 during the whole-cell vaccine era to 842 after the introduction of acellular vaccines, the residual proportion of young infant cases increased from 26 to 39%.[158]

Recently, a low dose, adult-formulated diphtheria, tetanus, acellular pertussis (dTpa) vaccine was licensed and recommended in several countries for use in adults and adolescents. (Table 2.2) A reduction in the adult/adolescent reservoir could be achieved by administering a booster dose of a low dose pertussis vaccine (dTpa). The only adult efficacy data come from the Acellular Pertussis Trial (APERT) in the USA, with a point estimate for vaccine protection against symptomatic pertussis disease, proven by culture or PCR, of 92%, (95% CI 32-99) although protection against less severe coughing illness is likely to be only 50-60%.[180] It was also possible in the APERT trial to demonstrate, using serial serological tests over a 12 month period, lower infection rates in vaccine recipients versus controls, which would be expected to translate into reduced rates of pertussis transmission. Interestingly, among controls in the APERT trial over a 12 month period, 0.4 to 2.7% had increases in paired pertussis antibody titres of various types and degrees, and 20%-46% had prolonged cough illnesses.[181] If extrapolated to the population level, this illustrates how common coughing illnesses in adults are, and while that few of them can be demonstrated to be pertussis there are nevertheless likely to be substantial opportunities for transmission to infants.

As immunity wanes following completion of the primary infant vaccine series, in many countries, booster doses of a pertussis containing vaccine are given at pre school or adolescence or both. (Table 2.2)

Impact of pre-school booster vaccination:

A few studies have measured the impact of introducing a pre-school DTPa booster on reducing early infant pertussis. A study by Torvaldsen et al suggested no downward trend in infant rates following the introduction of a 5th DTPa dose at pre-school,[182] and studies in the US [130,152] and New Zealand [183] do not support this strategy. In contrast the introduction of the pre-school DTPa booster in the Netherlands reduced the hospitalisation rate in the under 6 month olds by 40% between 1998-2001 and 2002-

2005.[135] Modelling data from Hviid et al estimates an 18% reduction in pertussis hospitalisations in infancy following a pre-school DTPa booster introduction.[184]

Table 2.2 Pertussis vaccination primary and booster schedules in selected countries

Country/Organisation	Type of pertussis containing vaccine	Primary immunisation schedule	Adolescent dTp booster recommended
WHO	DTPw	6, 10, 14 weeks	No
Australia	DTPa-combination	2, 4, 6 months	Yes
Brazil	DTPw-combination	2, 4, 6 months	No
Canada	DTPa-combination	2, 4, 6 months	Yes
Egypt	DTPw-combination	2, 4, 6 months	No
France	DTPa-combination	2, 3, 4 months	Yes
Germany	DTPa-combination	3, 4, 5 months	Yes
Italy	DTPa-combination	3, 5, 11 months	No
South Africa	DTPw-combination	6, 10, 14 weeks	No
Sweden	DTPa-combination	3, 5, 12 months	Yes
UK	DTPa-combination	2, 3, 4 months	No
USA	DTPa-combination	2, 4, 6 months	Yes

Source: http://www.who.int/immunization_monitoring/en/globalsummary/countryprofileresult.cfm Accessed March 2008

Impact of universal adolescent/adult vaccination:

Data on the potential reduction in infant pertussis following universal adult/adolescent low dose pertussis vaccine (dTpa) are scarce. One study in Canada showed that the introduction of an adolescent booster dose decreased rates in the target group but also in infants under 12 months old, suggesting that this strategy was beneficial.[185] An as yet unanswered question is the timing of booster doses for this cohort of adolescents, many of whom have received dTpa vaccines aged 15-18 years old.

b) Targeted adult and parental vaccination

As there is good evidence that parents are the commonest source of infection for infants, it seems plausible that booster vaccination aimed specifically at new parents might be an effective means to reduce infant exposure to pertussis.[169,186] However, the source of

infant infection is often not identified. In a multinational study of severe infant pertussis, at least one household contact was found as the probable source of infection in only 27% of 88 infants admitted to intensive care, most commonly mothers.[112] Other studies have not identified the source in the majority of infant cases, although in countries with high levels of childhood immunisation, this is usually an adult.[111,113,125]

There are no field data to confirm that the cocoon strategy reduces the frequency of transmission to unprotected infants despite the fact that many countries, including Australia, France, USA, Germany and Austria recommend that all parents of newborns receive a dTpa booster shortly after delivery of their child.[120-122] However, two years after the launch of the pertussis cocoon strategy in France, coverage of eligible young parents was low.[120] Various approaches have been taken to model the impact of cocoon strategies and universal adult boosters against pertussis. One model suggested that routine adult vaccination every 10 years, commencing at age 20 years, combined with selective vaccination of household contacts of newborns would yield the greatest reduction in infant pertussis incidence.[187] Another modelling study examined a more readily implementable strategy where the cocooning was combined with a single dose for all adults rather than ten yearly doses and suggested a substantial impact from this approach.[188] Governmental support for implementation of the cocoon strategy through either funding or distribution is likely to be needed for this strategy to have an impact on early infant pertussis. In 2009, the NSW government funded dTpa booster vaccination for carers of children under 12 months old for a limited period. A recent study has demonstrated high levels of parental support for such an initiative.[189] In this study nearly 90% of parents of newborns admitted to a US neonatal intensive care unit agreed to be given a dTpa booster vaccination following explanation about risks and benefits.[189]

Another source of infection for infants is healthcare workers. Estimates of the annual incidence of pertussis among healthcare workers range from 1 to 3.6%.[190] Nursery outbreaks where infants have been exposed to one or more health care workers with pertussis have led to vaccination of healthcare workers in contact with infants being either strongly recommended or mandatory.[122,172]

A US study suggested that the costs involved with investigating and managing such an outbreak were such that universal health care worker pertussis boosters were likely to be cost-effective.[191] Implementation of such a strategy would require well-prepared educational materials. A recent Australian study showed both substantial knowledge gaps among health care workers with respect to pertussis and vaccination and that 12% would be unwilling to be immunised.[192]

Protection following adolescent/adult booster vaccination wanes and additional boosters may be required. Current studies suggest that antibody and cell mediated immunity persist for 3-5 years post dTpa vaccination.[162,163,193] while a study modelling known patterns of antibody decay and projecting these suggested booster doses may be required at 10 years.[194]

However, high vaccination coverage for both universal adult/adolescent and 'cocoon' vaccination is difficult to achieve unless funding and distribution is supported, which is not currently the case in Australia, except in NSW, or elsewhere. Such implementation issues are likely to limit the impact of targeted 'cocoon' strategies and there are no studies that have conclusively shown a reduction at the population level. Direct protection via either birth or maternal vaccination seems more likely to have a significant impact on early infant disease.

2.3.2 Direct protection

Direct protection of the infant is either by passive transfer of immunity (antibody) through maternal vaccination or by active immunity (antibody and cellular immunity) generated in the infant following vaccination at birth or in infancy. Direct protection from immunising the infant (active immunity) is limited until at least two doses of a pertussis-containing vaccine has been received.[134,142,150,158,165] Some protection against the most severe disease, resulting in death or the need for respiratory support, may be achieved by only one dose.[134] This means that under the WHO EPI schedule (6, 10, 14 weeks), the earliest age for significant protection is approximately 12 weeks. Under some European (2, 3, 4 month) schedules, it would be 14 weeks and under schedules in North America and Australia (2, 4, 6 months) would be 18 weeks, if it is assumed that at least 14 days is required for an adequate immune response. A US modelling study by

Shinall et al suggested that giving the first Pa containing vaccine at 6 weeks instead of 8 weeks (2 months) could reduce deaths and hospitalisations in under 3 month olds by up to 5-10%.[195] In addition, it is important not to delay administration of Pa containing vaccines, especially the first dose, as delay has been associated with increase risk of pertussis.[196,197] However, even with accelerated schedules, the majority of severe disease due to pertussis in infants, and especially deaths, occur under 12 weeks old and these are largely not preventable by direct immunisation under current schedules. The high infant morbidity and mortality from pertussis in infants was recognised more than 60 years ago[10,58] and led to trials of maternal[42] and neonatal vaccination[74,75,198] with whole cell vaccine preparations which are reviewed below.

a) Maternal pertussis vaccination

The recent availability of dTpa vaccine, its demonstrated safety and efficacy in the adult population[180] has prompted researchers to question whether maternal vaccination in the last trimester of pregnancy could provide temporary protection until the primary DTPa vaccine series in infancy is completed. This potential strategy was recently reviewed by Mooi et al and is an attractive proposition for several reasons.[42] First, most cases of pertussis in infants are acquired prior to completion of the primary vaccination series, from household contacts, particularly mothers.[186] Second, dTpa vaccine is highly immunogenic in adults, who achieve antibody levels after a single booster dose, 2-5 times higher than infants following completion of the primary series.[180] Third, pregnant mothers frequently visit healthcare centres where the vaccine program can be successfully delivered. Fourth, the dTpa vaccine is safe and use in pregnancy is not deemed contraindicated by the US Advisory Committee on Immunisation Practices,[121] although it is not recommended.

In summary, the theory is that maternal pertussis vaccination boosts antibody levels in the mother which are then transported to the infant across the placenta, predominantly in the last trimester, providing passive immunity (pertussis IgG antibodies) to the infant at birth. There are many factors that can effect the placental transfer of antibodies, as outlined in chapter 1. A good demonstration of this theory in practice is maternal tetanus vaccination which has been extremely successful in preventing neonatal tetanus in developing communities.[37,199] In the developing world, WHO recommends two doses

of tetanus toxoid in the first pregnancy and one in each subsequent pregnancy up to a total of 5 doses.[37,199]

Studies of maternal vaccination in the 1940s and 1950s showed that pertussis antibody was passively transferred to the infant, sometimes at higher levels than the mother, and that the vaccine was safe for both mother, although fever and injection site reactions were seen, and newborn.[42] No studies of maternal dTpa vaccination have been reported, but two clinical trials are currently registered, supported by the NIH in the US and by industry sponsors in Canada.[200] Despite this, maternal vaccination against pertussis has significant hurdles, particularly in developed countries, and many unanswered questions as outlined below.

First, licensure of any therapeutic substance in pregnancy is difficult and even when recommended often has low uptake, such as seen with maternal influenza vaccination, with rates of 3.5 to 7.5% in one study in the US.[201] Additional barriers include liability issues for manufacturers, a need to educate obstetrical care providers regarding the benefits of maternal pertussis immunisation and optimisation of delivery methods.[46]

Second, although there is suggestion that passively transferred maternal antibody may have been higher and provided more protection in the pre-vaccine era, protection was nevertheless imperfect and levels declined rapidly after birth.[40,41,46] Studies in the 1930s and 1940s revealed that not all infants had higher antibody levels following maternal pertussis vaccination compared to infants whose mothers did not receive pertussis vaccination in pregnancy.[40] In other studies active placental transfer of maternal specific IgG pertussis antibodies has been demonstrated.[52,202,203] There are no data available on the quality and type of antibodies transferred placentally following maternal vaccination with dTpa vaccine and there is the possibility that due to competitive inhibition there could be a maximum antibody concentration able to be transferred, as outlined in Chapter 1.

Third, maternal levels wane rapidly and so are not likely to provide protection to infants beyond 6-8 weeks of age.[38,40] Edwards et al have demonstrated that the mean half life of pertussis antibodies transferred to infants ranged from 36 days (PT), 40 days (FHA) to

55 days for agglutinins.[40,202] It seems that most infants have no detectable pertussis antibodies by the age of 2-6 months following maternal transfer in the absence of active pertussis vaccination in infancy.[40,202] Ideally passive immunity should continue to provide protection until active immunity can be generated. This is not until after the second dose of Pa vaccine, which is at the earliest at 10 weeks old under the EPI schedule and not until 16 weeks in the USA and Australia. Thus, passive transfer of post vaccination maternal antibody can provide protection but it is not absolute or reliably generalisable, and is entirely dependent on both maternal response to dTpa and placental transfer, which has many potential interferences, such as gestational age and placental integrity, as reviewed in Chapter 1.

Fourth, while maternal antibody transferred to the infant provides temporary protection it may interfere with the infant's response (active immunity) to the primary vaccine series. The main reason for this interference is epitope masking by maternal antibodies, preventing antigen binding by infant B cells, as outlined in Chapter 1. It is not known whether maternal dTPa vaccination will suppress the infant's response to the primary DTPa series in infancy, as has been shown with maternal Pw vaccination. Several studies in the 1940s and more recently suggested that administration of whole cell pertussis vaccines to infants with pre-existing high levels, following maternal transfer, was associated with a reduced response to the primary course.[39,73,74,204] In contrast, a recent study by Saffar et al reported similar seroconversion following completion of a DTPw vaccine schedule in infants with pre-existing antibody and those without.[53] Several studies have shown that Pa vaccines remain immunogenic when given to those infants with pre-existing high titres.[39,52] The reason behind this apparent lack of maternal suppression with Pa vaccines is not known.[202] However, as a booster Pa vaccine may induce substantially higher antibody levels than are seen with natural immunity, the possibility of interference remains.

Fifth, antibody levels in mothers following a dTpa booster persist for up to 5 years, but levels rapidly wane in the first 12 months post booster, declining to pre booster levels at 5 years.[193] It is not known what level of maternal antibody will result in infant protection or if this would last until the infant could be protected directly. Repeated doses with subsequent pregnancies may be required.

As noted, there are two studies of maternal Pa vaccination that have been registered on an international clinical trials registry (Clinical trials.gov), both are in North America and currently recruiting and so no results are available. One is in Texas – 'Pertussis vaccine in healthy pregnant women, registration: NCT00707148' and the other in Canada – 'Pertussis maternal immunization study, registration: NCT00553228'.[200]

In summary, some direct protection of infants through placental transfer of pertussis IgG antibodies following maternal dTpa vaccination is likely and does not seem to suppress the infant's response to the primary DTPa series. However, the protection will be temporary (up to 6-8 weeks old) and may not persist until active immunity is generated. In addition, several unanswered questions remain, including the quality and quantity of placental antibody transferred to neonates and the need for repeated doses in subsequent pregnancies, along with regulatory and practical implementation issues.

Given the above uncertainties, I chose to investigate further whether direct protection through newborn Pa vaccination could be achieved. Direct protection, by relying on active immunity generated in the newborn, has several advantages over maternal vaccination. This is because it does not rely on passive placental antibody transfer, and its potential limitations. Infant vaccination in general is feasible, practical and effective, and may be given from birth as with hepatitis B and BCG vaccine.

b) Newborn pertussis immunisation

Newborn whole cell pertussis vaccination

Vaccines using inactivated whole *Bordetella pertussis* organisms (Pw) of 3 serotypes were first developed in the 1930s and subsequently combined with diphtheria and tetanus toxoids for widespread use in immunisation programs.[205]

Early studies of Pw vaccines given at birth (Table 2.3) were first conducted in the 1940s and 1950s and used very different vaccine schedules, measured only pertussis agglutinins and had small numbers of subjects. Often, data on responses prior to dose 3 were not stated or antibody titres were determined at younger ages in those given birth vaccine, meaning that direct comparison of results between groups was not possible.[58]

One small study raised concerns that Pw vaccine given in the first week of life might be followed by immune 'paralysis'.[74] The authors referred to inadequate serologic responses resulting from DTPw vaccination at birth and suggested that protection might be lessened by vaccinating too early. Antibody responses to the birth DTPw vaccine were seen but they were blunted in comparison to the control group (no birth DTPw vaccine) and therefore met the definition of hyporesponsiveness, as discussed in chapter 1. This study was influential in discouraging further pursuit of early vaccine schedules and the emphasis shifted to commencing whole cell vaccines later in the first year, even though the validity of these concerns was later questioned. [58]

In their 1984 review, Galaska and Halsey noted that DTPw vaccine given in the first 2 weeks of life resulted in approximately 15-25% of subjects achieving pertussis agglutinin titres >1:320, which was considered to be the protective level at the time.[58] One study by Butler et al in which infants were given plain or aluminium adjuvant pertussis vaccine at birth compared to controls was able to show a reduction in clinical pertussis cases, 1.5 cases per 1000 month years (birth pertussis vaccine) versus 5.1 cases per 1000 month years (controls).[206] Galaska and Halsey concluded that the administration of 'three or more doses of adsorbed pertussis vaccine has been demonstrated to induce protection against disease when the first dose was given after one week of age'. In addition they concluded immunisation with DTP 'beginning in the first three weeks of life results in lower antibody titres than immunisation beginning at older ages'.[58] In a more recent study comparing a 2, 3, 4 month DTPw schedule with a 3, 5, 9 month schedule in the UK, diphtheria and tetanus antibody titres were lower for the accelerated schedule but the pertussis toxin antibody titre was non-significantly higher.[32] This suggests that hyporesponsiveness to pertussis antigen following an accelerated DTPw schedule (2, 3, 4 months vs 3, 5, 9 months) is not a major problem.[32] It is important to note there is no accepted definition of immune 'paralysis' or hyporesponsiveness, as discussed in Chapter 1, and while some of the early whole cell pertussis birth vaccine studies describe lower pertussis agglutinins following completion of the primary vaccine series they were not able to measure responses to individual pertussis antigens (for example pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN)). The clinical significance of lower agglutinin levels is not known.

Table 2.3 Studies of whole cell pertussis vaccine at birth

	USA[207]	UK[198]	Switzerland[75]	USA[208]
Journal publication year	Pediatrics 1949	BMJ 1955	Journal Pediatrics 1958	Public Health Reports 1958
Sample size	n= 471	n=468	Not available	n=93
Birth vaccine used	Saline suspended H. Pertussis ($5x10^{10}$ to $10x10^{10}$cells)	DTP (60Lf diphtheria toxoid, 12LF tetanus toxoid, $4x10^{10}$ H.pertussis)	DPT – alum precipitated	DTP ($3x10^{10}$ H.pertussis) 1. Aluminium phosphate adjuvant 2. Aluminium hydroxide adjuvant
Manufacturer	Cutter laboratories, USA	Not available	Cutter	Not available
Birth dose (mean age days)	5	7 days	<14 days	<14 days old
Vaccine schedule	Birth, 1, 2 months	Birth, 6 weeks, 14 weeks	<2 weeks, 4-8, 8-12 weeks	Birth, 4 weeks, 8 weeks
Serology measured	Serum pertussis agglutinins	Only diphtheria and tetanus antitoxin titres measured	Serum pertussis agglutinins	Serum pertussis agglutinins
Serology timing	Cord, 1, 2,3,6 months	6, 12 months	Birth, 4-8 weeks, 8-12 weeks, 12-16 weeks	3,8,15 months
Pertussis antibody level post dose 2 or 3 in birth group compared to control	15-25% above 1:320 titre after 3rd dose in birth group compared to 48-62% in birth group	Not available	Lower levels in birth group compared to infants commencing vaccination at 1 month or 6-26 months old (>1:320)	No control group available for comparison 19% above 1:320 after 3rd dose in birth group
Reduced pertussis antibody post completion of primary schedule in birth group	Not available	Not available	yes	Not available

	USA[207]	UK[198]	Switzerland[75]	USA[208]
Transplacental antibody causes immune suppression	No	Not available	Not available	Not available
Author conclusions	Schedule should commence at 4-8 weeks of age	Not relevant to pertussis	"preferable not to vaccinate the infant against pertussis until the minimum age of 1 month"	Aluminium phosphate adjuvant superior to aluminium hydroxide

	USA[209]	UK[206]	USA[74]	USA[73]
Journal publication year	Pediatrics 1962	Lancet 1962	New England Journal Medicine 1965	Pediatrics 1984
Sample size	n= 633 (Birth group n=179)	n=502	n=74	N= 91
Birth vaccine used	DTP (Quadrigen (DTP-Polio with 11-19 mouse antigenic units of pertussis per 1.5mls)) or (Triogen – 20 mouse antigenic units of pertussis per 1.5mls)	Plain pertussis vaccine (20x109 organisms per ml) and aluminium phosphate adjuvant pertussis vaccine (10x109 org. per ml)	H. pertussis and/or DTP	DTPw
Manufacturer	Parke-Davis	Glaxo laboratories		Wyeth
Birth dose (age in days)	2	7 days	<1 day	3.5
Vaccine schedule	Birth, 4, 8, 12 weeks	1, 6, 12 weeks	Birth, 4, 8 weeks for pertussis	Birth, 2,4,6 months

	USA[209]	UK[206]	USA[74]	USA[73]
Serology measured	Serum pertussis agglutinins	Serum pertussis agglutinins	Serum pertussis agglutinins	IgM and IgG antibodies to FHA, Lymphocytosis promoting toxin (LPT) by ELISA Serum pertussis agglutinins
Serology timing	Cord, 1, 2,3,4 months	Birth, 24 weeks	Birth, 4, 8, 12 weeks	Cord, 4,6,9 months
Pertussis antibody level post dose 2 or 3 in birth group compared to control	Median titre in birth group significantly lower compared to infants with first dose at 1-2 mos. old	Plain vaccine GMT – 28 Aluminium vaccine GMT – 131	No control group 0% >1:160 titre after 3 doses of H. pertussis vaccine (aged 12 weeks)	Only IgM FHA sig. higher at 4 mos. IgG FHA, IgM LPT, IgG LPT – nil sig. difference
Reduced pertussis antibody post completion of primary schedule in birth group	Not available	Not seen Pertussis agglutinin levels increased after 12-15 month old booster	Results suggest "immune paralysis"	No, except infants in birth group with low baseline (cord) levels had sig. lower IgG LPT at 9 mos.
Transplacental antibody causes immune suppression	Not seen but very few infants had detectable maternal antibody at birth	Not available	Not seen but <75% infants had detectable maternal antibody at birth	Yes
Author conclusions	Pertussis agglutinin response better when the initial vaccine is given at 3 months or older compared to birth vaccination	"plain or adsorbed pertussis vaccine is effective in infants even 1 week old"	Pertussis antigen alone or combined, be used cautiously in neonates and that immunisation should probably not be attempted under 3 weeks of age	If IgM FHA is protective then birth vaccination may be beneficial

These early whole cell pertussis vaccine study results are not likely to be directly comparable to acellular pertussis vaccines used today.

In summary, early whole cell pertussis vaccines studies demonstrated that newborns can make antibody responses to pertussis which may be protective but levels might be lower post completion of the schedule than infants who commenced vaccination at older ages raising concerns about hyporesponsiveness. As a result of these studies, most vaccine schedules were recommended to commence at 6 weeks of age or later and this set the scene for vaccine schedules around the world for the next four decades. The arrival of acellular pertussis vaccines, coupled with a resurgence of pertussis in infants less than 3 months old has prompted further interest in examining whether neonatal acellular pertussis vaccination is immunogenic and safe.

Newborn acellular pertussis vaccination

In Australia, North America and much of Europe, diphtheria, tetanus, acellular pertussis (DTPa) vaccines have replaced DTPw vaccine in national primary vaccination schedules because of a better safety profile compared to Pw vaccines. In many countries pertussis antigens are now included in multivalent vaccines which may contain diphtheria, tetanus, hepatitis B, *Haemophilus influenzae* type B, and poliovirus.

There have been several murine studies investigating newborn Pa vaccination but prior to commencement of work on this thesis, the only published human study testing the effect of Pa vaccination at birth was one conducted in Italy in 1999.[210]

Newborn acellular pertussis (Pa) vaccine studies: Animals

The first studies examining Pa vaccines in newborns were conducted in mice. In an infant mouse model, where immunologic maturity corresponds closely to human newborns, published work suggests good protection against *B. pertussis* respiratory challenge after a birth dose of either Pa or Pw.[72] The mouse model has been used to compare immunogenicity of 1 week old (immune maturity equivalent to newborn human) and 3 week old (immune maturity equivalent to human infant) vaccination using DTPa and Pa vaccines.[211] In these studies 3 week old mice have been shown to have higher pertussis antibodies after the first dose compared to 1 week old mice but

similar responses to the second dose indicating that successful priming had occurred in neonatal and infant mice. In addition, Schallert et al demonstrated that while 1 week old mice had lower levels of anti-PT antibodies following one dose of DTPa vaccine, the avidity index of the anti-PT antibody was the same as adult mice following a single dose of DTPa vaccine.[24] DTPa and Pa vaccinated and naive mice have been challenged with intranasal *B. pertussis* and lung bacterial counts found to be lower in both 1 and 3 week old mice compared to naïve mice suggesting protective efficacy. DTPa and Pa vaccination in newborn mice well tolerated and there was no increased reactogenicity compared to 3 week old vaccination. This murine data suggests neonatal DTPa and Pa can induce successful priming and is protective from intranasal challenge with *B. pertussis*.[211]

Newborn Pa vaccine studies: Humans

In the Italian study, published in 2001, newborns were randomised to receive a birth dose of Pa vaccine (comprising PT 5mcg, FHA 2.5mcg, and PRN 2.5mcg) or no vaccination.[210] Both groups then received doses containing the same amounts of pertussis antigens at ages 3, 5 and 11 months. A total of 91 infants were enrolled (45 in birth Pa group and 46 in control) and had antibodies to PT, FHA and PRN measured at 3, 5, 6 and 12 months old. Results demonstrated birth Pa vaccine produced statistically significantly higher antibody levels and higher proportions with a 4-fold increase at 5 months (after 2 doses) to PT, PRN and FHA compared to controls (after 1 dose). (Table 2.4)

Infants in both groups reached measurable antibody levels after 2 doses of pertussis containing vaccine. However at age 12 months, the antibody responses in those vaccinated at birth were significantly lower for anti-PT ($p<0.0001$), significantly higher for anti-FHA ($p=0.002$) and the same for anti-PRN compared to the control group. (Table 2.5) In addition, the authors reported that there did not appear to be any influence from maternal antibodies on infant response to Pa vaccines and that 'no side effects were observed in children of either group'.[210]

Table 2.4 Percentage with 4-fold increase in pre-vaccination (birth) antibody levels according to group[210]

	Group 1#				Group 2#			
	n	Anti-PT	Anti-FHA	Anti-PRN	n	Anti-PT	Anti-FHA	Anti-PRN
At birth	45				46			
3 months	23	8.7	4.3	13	21			
5 months	**17**	**41.2***	**29.4***	**70.6***	**25**	**14.3**	**0**	**14.3**
6 months	23	60.9	39.5	82.6	21	81	9.5	76.2
12 months	40	87.5	42.5	85	43	83.3	27	89.6

Group # – Group 1 received Pa vaccine at birth followed by Pa combination vaccine at 3, 5, 11 months. Group 2 received Pa combination vaccine at 3, 5, 11 months

*4-fold increase statistically significantly higher in group 1 vs group 2

Table 2.5 Geometric mean titre for each pertussis antigen according to group[210]

	Group 1#				Group 2#			
	N	Anti-PT GMC^	Anti-FHA GMC	Anti-PRN GMC	N	Anti-PT GMC	Anti-FHA GMC	Anti-PRN GMC
At birth	45	4.5	16.6	4.6	46	5.5	23.2	4.5
3 months	23	2.8	7.7	4.3	21	4.1	5.8	2.2
5 months	**17**	**19.8***	**20.9***	**26.7***	**25**	**6.2**	**3.6**	**7.9**
6 months	23	42.5	45.8	116.1	21	59.1	12.7	49.1
12 months	40	53.5*	61.6	194.8	43	108.8[&]	30.8	172.1

Group # – Group 1 received Pa vaccine at birth followed by Pa combination vaccine at 3, 5, 11 months. Group 2 received Pa combination vaccine at 3, 5, 11 months

^ GMC – refers to geometric mean concentration

*GMC statistically significantly higher in group 1 vs group 2

[&]GNC statistically significantly higher in group 2 vs group 1

The Italian study concluded that pertussis 'immunisation at birth may be important for earlier prevention of the pertussis disease in infants under 6 months old, especially in Italy where the recommended ages for Pa vaccine administration are 3, 5 and 11 months' and concluded that further studies are needed.[210]

2.4 Issues relating to neonatal acellular pertussis vaccination

There are several issues to consider further when pursuing newborn Pa vaccination including; how to measure pertussis immunity following vaccination, both antibody and cellular immunity, the timing of the schedule after the birth dose, particularly how early can the second dose be given and safety. It is these factors that were crucial elements in the design of the study in Chapter 3.

2.4.1 Measures of pertussis immunogenicity and clinical protection

The currently available acellular pertussis vaccines (Pa) include up to 5 antigens. All manufacturers include **pertussis toxin (PT)**, as it is only present in *B. pertussis*, but the quantity and inactivation processes differ according to vaccine manufacturer. In addition, **filamentous haemagglutinin (FHA)** and **pertactin (PRN)** both attachment factors for *B. pertussis* are contained. Several manufacturers also include **fimbrial proteins (FIM1 and FIM2)**.

Since the early neonatal Pw vaccine trials, newer laboratory methods using ELISA techniques to measure antibodies against PT, FHA, PRN and fimbrial proteins have become well established. In murine studies it has been shown that PT protects both to intracerebral inoculation of *B. pertussis* or challenge by the respiratory route. FHA immunisation and passive acquisition of FHA antibodies protect mice against respiratory but not intracerebral challenge with pertussis and PRN is protective in a mouse respiratory challenge model.[72]

However, no definite single serological correlate of protection in humans has been established for pertussis and neonatal effectiveness trials are not feasible due to the large sample sizes required. This necessitates reliance on surrogate serological measures of protection.[212]

Evidence to support the validity of surrogate serologic measures comes from both animal studies, as above and vaccine trials.[72,213,214] As mentioned previously the composition of currently available Pa vaccines was determined by detailed immunogenicity studies under the auspices of the US National Institutes of Health.[215]

Human data on the correlation between antibody and protection come from household contact studies, allowing rates of pertussis infection following known exposure to be correlated with post-vaccination antibody.[213,214,216,217] A Swedish study found a statistically significant efficacy of 75% (95% CI 0-96%) against typical pertussis when measurable antibody levels to both PT and PRN were present post primary vaccination.[216] A German study found protection significantly correlated with PRN.[217] Despite limitations in evidence for specific antibody thresholds for protection against disease, these measures are deemed sufficient for global licensure by all regulatory bodies and the overwhelming body of evidence suggests antibody to both PT and PRN has a role in protection. In conclusion, PT and PRN IgG antibodies are considered to be the current best surrogates for protection.

In addition to antibody responses the cellular immune response needs to be examined. As discussed in chapter 1, theoretical concerns exist regarding qualitative aspects of vaccine cell mediated immunity induced in neonates due to the intrinsically TH2-polarised nature of immune responses (default to production of IL-4, IL-5 and IL-13) in this age group, and this has the potential to antagonise development of TH1-dependent sterilising immunity.[4,91,92,218] For example, studies of RSV infection in both neonatal mice [219] and human neonates [220] concluded that early infection commits the immune system to development of strong primary TH2 immunity, and moreover this may influence symptomatology associated with future infections via the presence of an excessive component of pro-inflammatory TH2 cytokines in the resultant memory response.[220] Cell mediated immunity has not yet been systematically addressed in the context of neonatal pertussis vaccination.

In summary, antibodies to PT, FHA and PRN are the best studied immune correlates of protection against pertussis and their measurement needs to be included in the study design. In addition maternal antibody may interfere with infant responses to birth Pa vaccine and subsequent Pa doses. Little is known about cell mediated immunity (CMI) following newborn Pa vaccination and so a measure of CMI also needs to be included in the study design.

2.4.2 Timing of vaccine schedule

As discussed in Part 1, higher IgG responses to pertussis vaccines are seen with later age of commencement, greater spacing of doses and the use of certain adjuvants. Speed of antibody loss may increase with earlier dosing. There is evidence of good immune responses to a single dose of vaccine at birth only for the BCG vaccine (a live vaccine where later commencement gives better responses) and Hepatitis B vaccine (where a second dose is required for good responses and is given from 1 month old).

Importantly, the Italian study showed that there was no antibody increase after the first dose of Pa vaccine at birth or 3 months old and it was the second dose in both groups that resulted in increased antibody levels.[210] This led to the idea that it may be possible to successfully prime the infant with monovalent Pa vaccine at birth and then produce adequate antibody levels if the second dose was given earlier than 3 months old. However, it is not known whether administering the second dose earlier than 3 months would be as immunogenic as later administration.

The fact that two doses could be protective is supported by observational studies. In Germany, protective efficacy against infant hospitalisation was 68% after the first and >90 % after the second dose of DTPa.[142] In Sweden, the incidence of pertussis fell from 230-235 (cases per 100 000 person years) after zero or one dose of pertussis vaccine to 52 after two doses.[165]

At present pertussis vaccination schedules differ significantly around the world but none currently start earlier than 6 weeks of age. (Table 2.2) WHO recommends a schedule 6, 10, and 14 weeks in the Expanded Program on Immunisation (EPI) against pertussis [36] while a 2, 3, 4 month schedule is used in many European countries. In Australia and the USA, the primary immunisation schedule is DTPa in combination vaccines at 2, 4 and 6 months. The second dose is given the earliest at 10 weeks of age under the EPI, and at 12 weeks in some European countries and 16 weeks elsewhere.[108] As a result, even if optimally delivered, current pertussis immunisation schedules cannot provide direct protection to infants under 8 weeks of age. When delays in immunisation are taken into account, protection is often delayed even more.[196,197]

The ideal schedule may therefore include a second dose given earlier than 6 weeks of age, however, there are no data on immune responses or safety to two doses of Pa vaccine given before 6 weeks old. Therefore, the study in Chapter 3 examines the immune response following two doses before 6 weeks old compared to one or no doses of Pa vaccine.

2.4.3 Safety

In addition to enhancing the immune response, the possibility of enhanced reactogenicity (adverse effects) with earlier vaccination must be considered.[58] In 1941 Sauer wrote 'because whooping cough is fatal only during the first 2 years of life ... active immunisation, if it is to be effective, should be attempted as early as immunity can be conferred. The vaccine should not elicit severe local or systemic reactions, nor should it sensitize the individual'.[221] There were no significant safety concerns identified in the early whole cell pertussis vaccine studies. Baraff et al reported similar rates of local reactions in control and birth vaccine groups (16.7% vs 18.2%) and no increased rate of systemic adverse events after birth DTPw vaccine.[73] Belloni et al reported no adverse events from birth Pa vaccination.[210]

In addition to clinical adverse events an assessment of vaccine interactions, as discussed in chapter 1, needs to be included. Little is known about potential alterations, either enhancement or suppression, of immune responses to other vaccine antigens, (diphtheria, tetanus, hepatitis B) administered following neonatal Pa vaccine. It is possible that earlier antibody loss in children vaccinated at birth may mean additional boosters are required after 6 months of age. This is important with respect to implementation of a birth dose, because additional schedule changes would significantly add to the program cost.

Thus, the study design must measure clinical safety, response to concomitant antigens and potential maternal antibody interference.

2.5 Summary and relevance to thesis

Despite widespread vaccination, with good uptake in infancy in many industrialised countries, there has been a resurgence of pertussis, with highest rates in the youngest infants. Death and hospitalisation from pertussis predominantly occur in infants too young to have received more than one dose under current vaccine schedules. Two doses of a pertussis-containing vaccine provide significant protection against severe disease, and even one dose may provide some protection against death.

Two broad vaccine strategies to prevent early infant pertussis exist – indirect and direct prevention. Indirect protection relies on increasing herd immunity by vaccinating older children, adolescents and adults or a more targeted 'cocoon' strategy of vaccinating those in closest contact with the infant such as parents, grandparents and healthcare workers. Although recommended by several countries, limited data exist on the protection afforded by the indirect strategies, they rely on achieving high vaccination coverage in the target group and there are difficulties in their implementation, including funding and distribution. Direct protection in early infancy relies on passive immunity through maternal vaccination or active immunity by vaccinating the infant earlier than 6 weeks old. Both of these approaches were studied in the 1940s and 1950s. Maternal vaccination has numerous scientific hurdles, including, level of antibody transferred, potential for interference with infant responses, need for booster doses in subsequent pregnancy and licensure issues. Two studies investigating maternal Pa vaccination are currently recruiting and results are not yet known. Early whole cell pertussis vaccine studies demonstrated that newborns can make antibody responses to pertussis but levels were lower than infants who commenced vaccination at older ages and raised concerns about immune hyporesponsiveness.

The potential strategy of accelerated acellular pertussis (Pa) vaccine schedules in early infancy, with the first dose administered at birth, may prevent early infant pertussis infection and needs further exploration. Such a strategy could provide direct protection to infants and does not rely on achieving indirect protection through vaccinating adults and others who come into close contact with infants.

Studies of neonatal Pa vaccines should examine:

- Humoral and cellular immune responses
- Best timing of the second dose
- Safety

In summary, at the commencement of work for this thesis, promising animal data on neonatal Pa vaccine and one small human newborn vaccine trial were published and encouraged pursuit of neonatal Pa vaccination as a strategy. This thesis was able to examine several questions including, optimal timing of subsequent doses after birth dose, especially timing of the second dose, the immunogenicity of two doses of Pa vaccine before 8 weeks old, additional safety data, potential influence of maternal antibodies and interference with concomitant antigen response.

The following table 2.6 combines information from chapter 1 on basic immunology and chapter 2 which was used to inform the study design of the newborn Pa vaccine trial conducted in chapter 3.

Chapter 3 describes a neonatal Pa vaccine trial which was conducted to examine the above critical points, reviews the literature on two other similar trials in other countries at the same time as this thesis and compares results between the trials.

Table 2.6 Factors informing design of neonatal acellular pertussis vaccine trial

Immune system factor	Significance to newborn acellular pertussis (Pa) vaccine trial design	How factor informed trial design
Immune system factors in newborn		
Reduced antibody response to T dependent antigens	Limited antibody response after birth dose likely	Measure antibody response after birth dose
Reduced quality of antibody response • short duration • IgG subclass • Decreased avidity	Need multiple doses after birth dose	Measure antibody response after birth dose and subsequent doses

Immune system factor	Significance to newborn acellular pertussis (Pa) vaccine trial design	How factor informed trial design
Vaccine schedule		
Type of vaccine and antigen dose	Higher antigen content and adjuvant elicit better responses	Use adjuvanted Pa vaccine
Interval between doses	Minimum of 4 weeks between doses	Earliest second dose can be given is 4 weeks old
Timing of second Pa dose	Not known how early second Pa dose can be given	Include an arm of 2 doses prior to 8 weeks old
Maternal antibodies		
Maternal transfer of antibodies dependent on: • maternal health • gestational age • placental integrity	Healthy mother with intact placenta transfers increased antibody Increasing gestation equates to increasing antibody transfer	Healthy mother Normal pregnancy Term gestation (>36 weeks)
Influence of maternal antibody on infant response	Maternal antibody may suppress response to birth Pa vaccine	Need to measure maternal antibody at birth Need to measure infant antibody levels at regular intervals during primary vaccine series
Neonatal priming		
Vaccine trial and epidemiological data	Evidence exists for successful priming • OPV • Hib • Hepatitis B • Whole cell pertussis vaccine Does acellular pertussis vaccine at birth successfully prime the newborn's immune system?	Need to measure response after birth and second dose of Pa vaccine
Immune hyporesponsiveness	Birth Pa vaccine may result in lower antibody levels post completion of primary vaccine series	Need to measure antibody levels at 6 and 8 months
Interference with concomitant antigen response	Birth Pa vaccine may reduce responses to concomitant antigens	Need to measure responses to Hib, hepatitis B, diphtheria and tetanus
T cell immunity	Limited Th1 responses with Th2 bias in the newborn	Need to measure cell mediated immunity

CHAPTER 3: NEWBORN ACELLULAR PERTUSSIS VACCINE TRIAL

3.1 Introduction

Unimmunised infants less than 3 months old are at highest risk of hospitalisation and death from pertussis but cannot be protected by current vaccination schedules. The earliest the first pertussis containing vaccine is administered is 6 weeks old and antibody levels rise significantly after the second dose, usually given at a minimum of 4 weeks later. At the onset of this thesis only one study in Italy had examined acellular pertussis (Pa) vaccine at birth followed by a second dose at 3 months old.[210] Pertussis antibody levels were higher in the birth vaccine group at 5 months old, after the second dose, compared to controls. This positive immunogenicity data suggested starting the pertussis vaccine schedule at birth may protect infants earlier than the current Italian vaccination schedule. However, the study was small (91 infants), the second dose was not given until 3 months old, antibody responses to other vaccine antigens were not measured and there was a suggestion that post completion of the vaccine series antibody levels to FHA and PRN were lower in the birth vaccine group.[210]

On this basis, a clinical vaccine trial was designed to further examine neonatal pertussis vaccination and the timing of the second dose as a potential strategy to reduce early infant pertussis. This trial measured pertussis immunogenicity after birth acellular pertussis vaccine, compared to controls given the standard immunisation schedule, and also whether two doses of Pa vaccine before 2 months old (birth and one month old) were immunogenic. Antibody responses to vaccine antigens administered at the same time and cellular immunity to pertussis were included in the study design. The aim of this study was to provide further evidence on the immunogenicity of birth Pa vaccine, explore the optimal number and spacing of Pa doses between birth and six months old, investigate concomitant antigen responses and record vaccine safety.

3.2 Aims and hypotheses

3.2.1 Aims

a) Primary

- To establish if the administration of acellular pertussis vaccine commencing at birth produces antibody responses to pertussis consistent with protection from severe disease due to *B. pertussis* infection earlier than current vaccination schedules commencing at 2 months of age

- To determine the optimal number and spacing of doses between birth and six months of age.

b) Secondary

- To examine the incidence and severity of local and systemic adverse reactions following all doses up to 8 months of age, in early commencing vaccine schedules.

3.2.2 Hypotheses

1. **By 2 months of age**, the IgG antibody responses to pertussis following Pa vaccine at birth and 1 month of age (Group 1) will be significantly higher than following Pa vaccination at birth alone (Group 2) and the residual levels of transplacental antibodies (Group 3).

2. **By 4 months of age**, the IgG antibody responses to pertussis following Pa vaccine at birth and 1 month and 2 months of age (Group 1) will be significantly higher than following Pa vaccination at birth and 2 months of age (Group 2) and vaccination at age 2 months only (Group 3).

3. **At 8 months of age**, IgG antibody responses to pertussis in Group 1 and/or Group 2 will equal or exceed those in the Group 3.

4. Local and systemic reactions at each vaccination point do not differ significantly between infants receiving Pa vaccine at birth and others

3.3 Methods

3.3.1 Design and study population

This pilot study was a randomised, non-blinded trial of administration of monovalent acellular pertussis (Pa) vaccine to newborn infants. Although lack of blinding limited the ability of the study to examine the incidence of non-specific adverse reactions, such as irritability and abnormal crying, the use of a placebo was not justified according to current guidelines for trial conduct.[222] This study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki 1999 and had the approval of three ethics committees (The Children's Hospital at Westmead (Appendix 1), Westmead Hospital, and the Children, Youth and Women's Health Service, Adelaide). Written informed consent was obtained from parents/guardians before the enrolment of infants.

a) Study groups

Neonates were randomly assigned to one of three groups.

5. **Group 1** (early vaccination) received vaccinations containing pertussis antigens at the following ages: birth, 1 month, 2 months, 4 months and 6 months.

6. **Group 2** (early vaccination) received vaccinations containing pertussis antigens at the following ages: birth, 2 months, 4 months, and 6 months.

7. **Group 3** (standard vaccination) received vaccinations containing pertussis antigens at the following ages: 2 months, 4 months, and 6 months

All groups received hepatitis B vaccine at birth followed by the standard vaccine antigens at 2, 4 and 6 months old. These were given as a combination DTPa-HepB-Hib-IPV vaccine (*Infanrix Hexavalent* ™, GlaxoSmithKline Pharmaceuticals) as well as 7 valent pneumococcal conjugate vaccine (*Prevenar TM, W*yeth Pharmaceuticals)

Thus overall, subjects in group 1, 2 and 3 received 5, 4 and 3 doses respectively of a pertussis-containing vaccine by 6 months of age

Parents who were eligible for a diphtheria or tetanus booster were invited to receive a licensed and recommended low dose dTpa booster vaccine (Boostrix™ - GlaxoSmithKline Pharmaceuticals) at the birth of their child, as recommended in the Australian Immunisation Handbook 2003 (8th edition).[223]

The date and batch numbers of vaccines administered at birth, 2, 4 and 6 months old were recorded in the Child's Personal Health Record. Parents were asked who was their regular general practitioner normally responsible for administering the routine 2, 4 and 6 month old immunisations. A copy of the immunisation record and the Australian Childhood Immunisation Register encounter form was sent to this general practitioner, who was then able to claim the immunisation incentive.

b) Subjects

Eligible subjects were healthy infants, who had completed at least 36 weeks gestation, were born after an uncomplicated pregnancy to mothers seronegative for hepatitis B surface antigen (HBsAg) and were enrolled within 120 hours of birth.

c) Recruitment of subjects

English-speaking women in the last trimester of pregnancy or who have recently given birth to a healthy infant were approached at Westmead Hospital, Sydney and the Women's and Children's Hospital, Adelaide. They were provided with written information about the study and trained personnel discussed the study with them. Written information (in English only) about the study was also available at antenatal clinics and labour wards and through local private obstetricians. Women indicating interest in the study were provided with detailed information in writing and orally and had the opportunity to discuss the study with the researchers and had questions they may have regarding the study answered to their satisfaction. (Appendix 2) Interested women also had time to discuss issues associated with participation in the study with family members, their medical advisers or any other persons they wished, before consenting to participate. Parents were informed that participation was voluntary and that it was possible to withdraw from the study at any time without penalty or need to give reason.

d) Exclusion criteria

Enrolment in the study was excluded by any of the following known contraindications to vaccination as per the Australian Immunisation Handbook 2003[223]

- administration of immunoglobulins or blood products preceding the first dose of study vaccine or their planned administration during the study period
- any confirmed or suspected immunosuppressive or immunodeficient condition in the parent or child
- major congenital defects or serious chronic illness

Continuing participation was excluded by use of any investigational or non-registered drug or vaccine; administration of a vaccine not foreseen by the study protocol during the period from 30 days before any dose of vaccine(s) to 30 days after; administration of immunoglobulins and/or any blood products; administration of immunosuppressants or other immune-modifying drugs; or diagnosis or suspicion of an immunosuppressive condition.

The study was conducted in Sydney and Adelaide, Australia between February 2005 and March 2007.

The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN012605000013662).

e) Vaccines

A single dose of investigational Pa vaccine (0.5ml) containing pertussis toxin (PT) 25ug, pertactin (PRN) 8 ug, filamenous haemagglutinin (FHA) 25 ug and 0.5 mg aluminium as hydroxide salts was supplied by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. All infants received 10ug Hepatitis B surface antigen (HbsAg) with 0.25mg aluminium hydroxide adjuvant (*Engerix B*) at birth (within 120 hours old). The Pa vaccine was administered intramuscularly into the right anterolateral thigh and the HBV vaccine into the left anterolateral thigh in Groups 1 and 2 prior to 120 hours of

age. The antigen composition of the Pa vaccine used at birth and one month was identical to that in the combined DTPa-HBV-IPV/Hib vaccine (*Infanrix Hexa*) in routine use. As indicated above, routine scheduled vaccines at 2, 4 and 6 months included *Infanrix Hexa* and 7 valent pneumococcal conjugate vaccine (*Prevenar* – Wyeth pharmaceuticals).

The composition is as follows: *Infanrix hexa* (DTPa-HBV-IPV-Hib) containing 30 IU diphtheria toxoid, 40 IU tetanus toxoid, 25 ug pertussis toxoid, 25 ug filamentous haemaglutinin, 8 ug pertactin, 10 ug recombinant hepatitis B surface antigen, 40 D-antigen units inactivate poliovirus type 1, 8 D-antigen units type 2, 32 D-antigen units type 3 absorbed onto aluminium hydroxide/phosphate and a vial containing 10ug Hib polysaccharide conjugated to 20-40 ug tetanus toxoid. Prevenar – 7 valent pneumococcal conjugate vaccine containing 2ug of pneumococcal serotypes 4, 9V, 14, 18C, 19F, 23F and 4ug of serotype 6B conjugated to non-toxic diphtheria toxin (CRM_{197}) carrier protein absorbed onto aluminium phosphate.[223]

Infanrix Hexa was administered intramuscularly in the right thigh and *Prevenar* in the left thigh at 2, 4 and 6 months of age by study nurses.

f) Financial support

This study was supported by a grant from the Financial Markets Foundation for Children, Australia (S112 – 2004). GSK Biologicals provided the investigational Pa vaccine and performed all serologic assays.

3.3.2 Assessment of immunogenicity

In total, 5 blood samples were collected. To reduce the number of blood samples required from the infant, the first sample was obtained from the mother at the same time as the infant received the first vaccination (Pa and HBV or HBV alone). Subsequent samples were collected from infants at 2, 4, 6 and 8 months of age. Samples were centrifuged, serum separated and stored at -80C. Samples were sent in batches to GSK Biologicals laboratory in Belgium. All serologic assays were performed at GSK Biologicals, Belgium (GSK).

a) *Antibodies to pertussis*

Pertussis toxin (Anti-PT), pertactin (anti-PRN), and filamentous haemagglutinin (anti-FHA) IgG antibody concentrations were measured at each sampling point by enzyme linked immunosorbent assay (ELISA: cut-off 5 EL.U/ml) using standard assay methods at the GSK laboratory developed for licensure of DTPa vaccines.

b) *Antibodies to other vaccine antigens*

Anti-diphtheria (cut-off 0.1 IU/ml), anti-tetanus (cut-off 0.1 IU/ml), and anti-PRP (cut-off 0.15 ug.ml) IgG antibodies were measured by ELISA on the sample taken at 8 months of age (2 months after the final vaccine dose). Hepatitis B surface antibodies (anti-HBs) were measured by ELISA (AUSAB, Abbott Laboratories) as per the manufacturer's recommendations (cut-off 10 mIU/ml) on samples collected at 8 months of age. The laboratory was blind to the study assignment of subjects.

c) *Cellular immune responses*

Cellular immune responses to pertussis antigens, hepatitis B, diphtheria, and tetanus were measured on samples collected at 8 months old. Analysis was performed at the Institute for Child Health Research, University of Western Australia.

Blood was collected in Sydney or Adelaide and placed into transport tubes containing RPMI 1640 with 20 Units/ml Heparin and transported by courier to the Institute for Child Health Research, University of Western Australia.

Peripheral blood mononuclear cells (PBMC) were isolated and cryopreserved. At the time of cell culture, PBMC were thawed and resuspended at 1 x 10^6 viable cells/ml in either RPMI supplemented with 5% pooled human AB serum (for vaccine antigens) or AIM-V serum-free medium for PHA.

Cryopreserved PBMC were batch analysed in groups of 8 within a short period and with identical reagents. Aliquots of 0.5 x 10^6 cells were cultured for 96 hours alone or together with; 1 μg/ml PT + 1 μg/ml FHA + 1 μg/ml PRN (Mix, all generously supplied by GSK), or 1 μg/ml PT, or 1 μg/ml FHA, or 1 μg/ml PRN, or 0.5 Lf/ml tetanus toxoid (TT, CSL, Melbourne, Australia), or 1.0 Lf/ml diphtheria toxoid (DT, CSL), or 2.5

μg/ml Hepatitis B surface antigen (ProSpec-Tany TechnoGene Ltd., Rehovot, Israel), or for 48 hours alone or together with PHA (1 μg/ml, Murex Biotech Ltd., Kent, UK).

The levels of IL5, IL6, IL13 and IFNγ in culture supernatants were measured by in-house time-resolved fluorometry assays. The cytokine values for each stimulus are displayed in picograms per millilitre (pg/ml) after subtraction of background control values. Significant differences shown between the groups were determined by Mann-Whitney U test for unpaired responses using SPSS software package (SPSS Inc., Chicago, USA).

3.3.3 Assessment of reactogenicity

After administration of each vaccine, all infants were observed for 30 minutes. Vaccine reactogenicity and safety was assessed using a 7 day diary card after each vaccination. (Appendix 3) Parents were given a thermometer, instructed in its use, and asked to record temperature and any solicited adverse reactions 3 and 6 hours after injection and at bedtime each evening for 7 days. Solicited adverse reactions included: fever (axillary temperature > 38°C by electronic thermometer and use of antipyretics); drowsiness (unusually sleepy or inactive); irritability scored as normal, periodically more irritable than usual but with normal activity [mild], prolonged crying and refusal to play [moderate], prolonged crying and unable to be comforted [severe]; anorexia defined as 'unusually poor appetite'; vomiting judged to be greater than a posset; redness and swelling at the vaccination site each measured in mm; pain scored as none, minor light reaction to touch [mild], crying or protesting to touch [moderate], or crying when the leg is moved [severe]. All unsolicited adverse events occurring within the time interval between vaccinations were recorded by parent/guardian and/or study physician at each study visit. Telephone contact was made with parents/guardians on days 2 and 7 to enquire about adverse events and encourage completion of the diary cards following vaccination. The duration of safety follow-up was 2 months following the final vaccine dose at 6 months. Any serious adverse event, including hospitalisation, was assessed by an independent vaccine safety committee.

3.3.4 Statistical analysis

Only subjects who had completed the vaccine schedule according to protocol and had at least two assay results available, including the maternal baseline sample, were included in the immunogenicity analysis. For pertussis antigens, antibody geometric mean concentrations (GMC) with 95% confidence intervals (CI) were calculated from the anti-log of the mean of the log transformed values. Values below the laboratory assay cut-off were assigned a value half of the cut-off value in order to calculate the GMC. The GMC was calculated due to the non normal distribution of antibody levels as is the normal practice for interpreting antibody data in clinical vaccine trials.[224,225]

The primary objective of the study was to assess if IgG antibody to PT and PRN was significantly higher in group 1 at 2 months of age (after 2 Pa doses) compared to one dose in group 2 and no prior doses of pertussis-containing vaccine in group 3. As no universally agreed serologic correlate of protection exists for pertussis, serological response (seronconversion), defined as a 4-fold increase from the pre-vaccination antibody level, was examined. This is based on data from the National Institutes of Health (NIH) sponsored comparative immunogenicity trials for acellular pertussis vaccines, where seroconversion to PT and PRN measured by ELISA was defined as a 4x increase in pre-immunisation ELISA Unit (EU) value, to at least 4x the minimum detectable level.[215]

a) Reverse cumulative distribution curves

Data were examined using reverse cumulative distribution curves. The horizontal axis of the graph represents antibody levels in a logarithmic scale. The vertical axis represents the percent of subjects having at least that level of antibody, ranging from 0 to 100%. Each reverse cumulative distribution curve is constructed by plotting against the vertical axis the percentage of subjects having an antibody concentration equal to or greater than the level shown at each point on the horizontal axis.[226] If the total curve is shifted to the right, this indicates better antibody responses compared to subjects included in the curve to the left.

b) Comparison of antibody levels post completion of primary vaccination series

As discussed in chapter 2, whole cell pertussis vaccine studies found significantly lower levels of pertussis agglutinins following completion of the primary vaccine series. Belloni et al found significantly lower anti-PT GMC at 12 months in the birth vaccine group compared to controls, while anti-FHA was significantly higher and anti-PRN not significantly different.[210] Previous studies have compared the proportions achieving detectable antibody and/or a four-fold increase in antibody level post completion in those who were initially seronegative or compared proportions with antibody levels greater than one quarter of the initial level in those who were seropositive at baseline.

In this study pertussis antibody levels post completion of the primary vaccine series were assessed in two ways:

1. Comparison of pertussis antibody GMC in birth vaccine groups 1 and 2 with group 3 when measured 2 months post completion of the routine vaccination schedule at 8 months of age.

2. Comparison of proportion of detectable pertussis antibodies at 8 months in those in birth vaccine groups 1 and 2 who were initially seronegative with group 3.

For diphtheria, tetanus, Hib and hepatitis B, serological response was defined as any level above the lower limit for detection in the assay used for each antibody (0.1 IU/ml, 0.1 IU/ml, 0.15 ug/ml and 10 mIU/ml respectively).

Antibody responses between groups were compared using log-transformed data by the independent samples t test with a p value <0.05 indicating a possible group difference. The proportion of study group subjects with a serological response and local and systemic reactions after vaccination in study groups were compared by Fisher exact test.

c) Power calculations

Pertussis IgG antibodies

There are no definitive serologic correlates of immunity to pertussis antigens to assist with sample size calculations. I based the following sample size calculations on the

differences in proportions of birth vaccinees compared to controls achieving a four fold increase in antibodies to PT from baseline to post 2 doses of Pa vaccine. I have chosen antibody to PT because Pa efficacy trials conclude PT and PRN IgG antibodies are the current best surrogate for protection, although it is not clear what difference in proportion is clinically significant.

Four-fold increase in antibodies to PT

The proportion achieving a four fold increase from baseline to post second dose in the study by Belloni et al was 14.3%.[210] The sample sizes required to detect a 30%, 40%, 50% difference from this baseline proportion with 80% power and a significance of 5% is shown in the following Table 3.1.

Table 3.1 Sample size estimates needed in each group to detect a difference in proportions achieving a 4-fold increase in anti-PT from birth to post second dose

Proportion (1)*	% Increase in Proportion (1) to Proportion (2)	Proportion (2)*	Sample size
10%	30%	13%	40
10%	40%	14%	25
10%	50%	15%	20
20%	30%	26%	45
20%	40%	28%	30
20%	50%	30%	20

Proportion (1)* – refers to the proportion of subjects who achieve a 4-fold increase in anti-PT level from baseline to post second Pa vaccine. This proportion is adjusted upwards by 30%, 40% or 50% to result in proportion (2)* and the samples sizes required are shown.

To detect a significant difference for the primary outcome of four fold increase in anti-PT antibody after the second dose, and to allow for drop-outs and failure to obtain some specimens by venipuncture, 25 subjects per group were aimed to be recruited.

3.4 Results

3.4.1 Study population

A total of 76 eligible newborns were enrolled. (Table 3.2) All were at least 36 weeks gestation, 59% were male and there was no significant difference in birth weight between groups. (Table 3.3) Sixty-two infants were recruited in Sydney and 14 in Adelaide. A total of 68 infants remained enrolled to completion of the vaccination schedule at 6 months and 64 infants until the completion of safety follow-up, with 62 providing a blood sample at 8 months.

Table 3.2 Enrolment and withdrawal according to group

	Group 1	Group 2	Group 3
Number	(n)	(n)	(n)
Enrolled	27	23	26
Withdrew before 2 months	2	1	5
Reason for withdrawal before 2 months	Social concern Declined venipuncture	Moved out of area	Inadvertent vaccination Declined venipuncture
Completed 6 months	25	22	21
Completed 8 months	23	20	19
Reason for withdrawal before 8 months	Moved out of area and declined venipuncture		

Table 3.3 Characteristics of study subjects according to group

	Group 1	Group 2	Group 3
Enrolled subjects	n=27	n=23	n=26
Mean birthweight (g) (range)	3454 (2840-4215)	3306 (2575-4205)	3560 (2600-4370)
Mean gestation weeks (range)	39.8 (38-41.3)	39.4 (37.2-41.3)	39.7 (37-41.5)
% male (n)	68% (17)	55% (12)	55% (12)
% vaccinated day 0-2 (n)	36% (9)	50% (11)	n/a
% vaccinated day 3-5 (n)	64% (16)	50% (11)	n/a

Eight infants, two from Group 1, one from Group 2, and five from Group 3 withdrew from the study after enrolment and before the first blood sample at 2 months for varied reasons including relocation (1), declining blood tests (4) and inadvertent vaccination with non study vaccines (3) (Table 3.2).

3.4.2 Immunogenicity

a) Antibody responses to pertussis vaccination

At enrolment, the GMC of maternal IgG to pertussis toxin (PT), pertactin (PRN) and filamentous haemagglutinin (FHA) was not significantly different among groups, except infants randomised to group 2 had significantly higher maternal anti-PT IgG than those randomised to group 3 (GMC 6.2 EL.U/ml vs 3.3 EL.U/ml, p=0.04). In all groups, IgG antibody levels to the 3 antibodies measured (PT, PRN, FHA) increased from baseline to 8 months (Tables 3.4, 3.5, 3.6).

Reverse cumulative distribution curves for anti-PT, anti-FHA and anti-PRN at 2, 4, 6 and 8 months old according to group are shown below. These indicate that responses to PT, PRN and FHA were higher in group 1 infants at 2 and 4 months old compared to group 2 and 3 infants and antibody responses to all three pertussis antigens converge by 6 and 8 months old.

Table 3.4 Antibody responses to pertussis toxin (PT) according to age and group

	Group 1*				Group 2*				Group 3*			
	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)
Maternal	25		32	3.9 (2.9-5.3)	22		50	6.2 (3.9-9.9)	21		14.3	3.3 (2.2-4.9)
2 months	25	2	88	16.1+ (10.5-24.7)	21	1	43	5.0 (3.4-7.6)	20		15	3.2 (2.3-4.6)
4 months	21	3	100	41.3+ (29.8-57.1)	22	2	91	21.1$ (14.4-30.9)	19	1	68.4	8.0 (5.2-12.3)
6 months	22	4	100	55.3+ (40.7-75.3)	20	3	100	30.5 (22.2-42)	20	2	100	35.6 (26.6-47.8)
8 months	23	5	100	55.0 (40.9-73.9)	20	4	100	36.4 (27.6-48)	19	3	100	40.3 (29.1-55.8)

*Group 1 – Pa vaccine at birth and one month, then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

number – number of according to protocol subjects who had blood sample collected for antibody analysis

Pa doses received – the GMC at each time period reflects the number of doses of Pa vaccine the subject had received before the blood draw

% detectable – anti-pertussis toxin antibody > 5 EL.U/ml

^ GMC – geometric mean concentration (EL.U/ml)

+ Group 1 significantly higher than Groups 2 and 3; $p<0.05$

$ Group 2 significantly higher than group 3; $p<0.05$

Table 3.5 Antibody responses to pertactin (PRN) according to age and group

	Group 1*				Group 2*				Group 3*			
	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)
Maternal	25		56	6.9 (4.5-10.7)	22		23	4.1 (2.6-6.3)	21		50	6.1 (3.5-10.5)
2 months	25	2	100	17.3+ (12.2-24.7)	21	1	33	4.0 (2.8-5.7)	20		30	4.4 (2.7-7.1)
4 months	21	3	100	44.5$ (30.8-64.3)	22	2	95	26.4& (17.2-40.6)	19	1	74	12.2 (7.1-20.8)
6 months	22	4	100	79.3 (56.8-110.8)	20	3	100	54.8 (39.8-75.5)	20	2	95	48.2 (26.9-86.3)
8 months	23	5	100	126.9 (95-169.4)	20	4	100	99.6 (77.6-127.8)	19	3	95	78.7 (44.4-139.5)

*Group 1 – Pa vaccine at birth and one month, then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

number – number of according to protocol subjects who had blood sample collected for antibody analysis

Pa doses received – the GMC at each time period reflects the number of doses of Pa vaccine the subject had received before the blood draw

% detectable – anti-pertactin antibody > 5 EL.U/ml

^ GMC – geometric mean concentration (EL.U/ml)

+ Group 1 significantly higher than Groups 2 or 3; $p<0.05$

$ Group 1 significantly higher than Group 3; $p<0.05$

& Group 2 significantly higher than Group 3; $p<0.05$

Table 3.6 Antibody responses to filamentous haemagglutinin (FHA) according to age and group

	Group 1*				Group 2*				Group 3*			
	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)	number	Pa doses received	% detectable #	GMC^ (95% CI)
Maternal	25		76	13.3 (7.8-22.6)	22		81.8	13.9 (8.5-22.63)	20		95	17.6 (11.1-27.8)
2 months	25	2	100	87.4+ (59.0-129.5)	21	1	95.2	14.7 (10.3-20.8)	20		75	9.4 (5.9-15.2)
4 months	21	3	100	177.4$ (126.1-249.5)	22	2	90.9	92.2& (61.7-137.6)	19	1	100	15.0 (11.2-20.1)
6 months	22	4	100	278.7 (206.5-376.2)	20	3	100	168.7 (119.8-237.4)	20	2	100	105.3 (75.8-146.4)
8 months	23	5	100	353.0 (269.6-462.3)	20	4	100	233.0 (172.6-314.5)	19	3	100	152.3 (115.2-201.4)

*Group 1 – Pa vaccine at birth and one month then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

number – number of according to protocol subjects who had blood sample collected for antibody analysis

Pa doses received – the GMC at each time period reflects the number of doses of Pa vaccine the subject had received before the blood draw

% detectable – anti-filamentous haemagglutinin antibody > 5 EL.U/ml

^ GMC – geometric mean concentration (EL.U/ml)

+ Group 1 significantly higher than Groups 2 or 3; $p<0.05$

$ Group 1 significantly higher than Group 3; $p<0.05$

& Group 2 significantly higher than Group 3; $p<0.05$

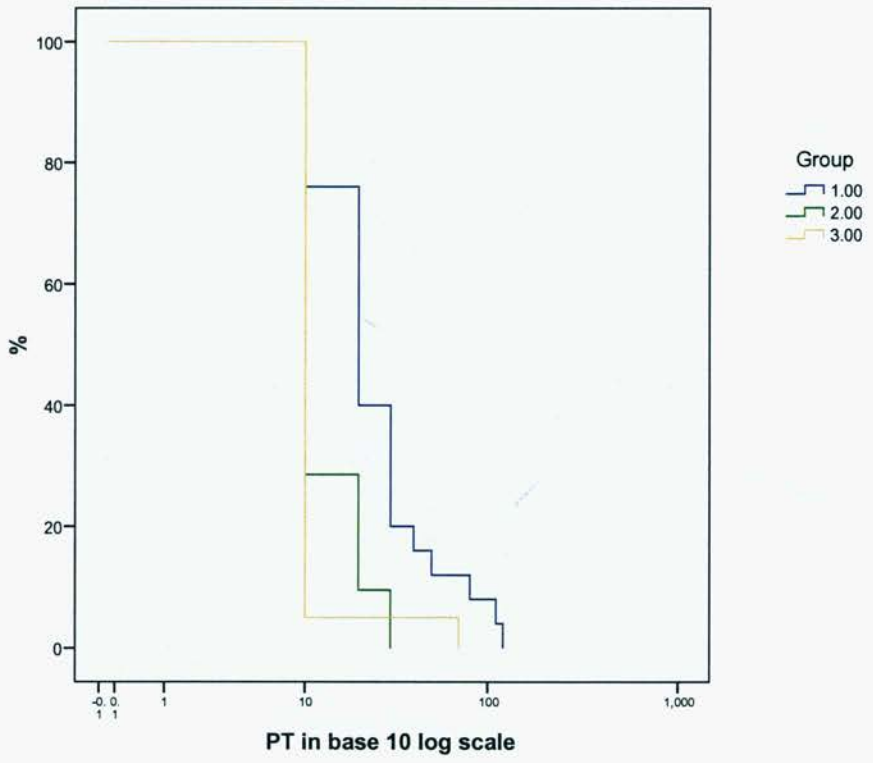


Figure 3.1 **Reverse cumulative distribution curve by group for antibody to PT at 2 months old**

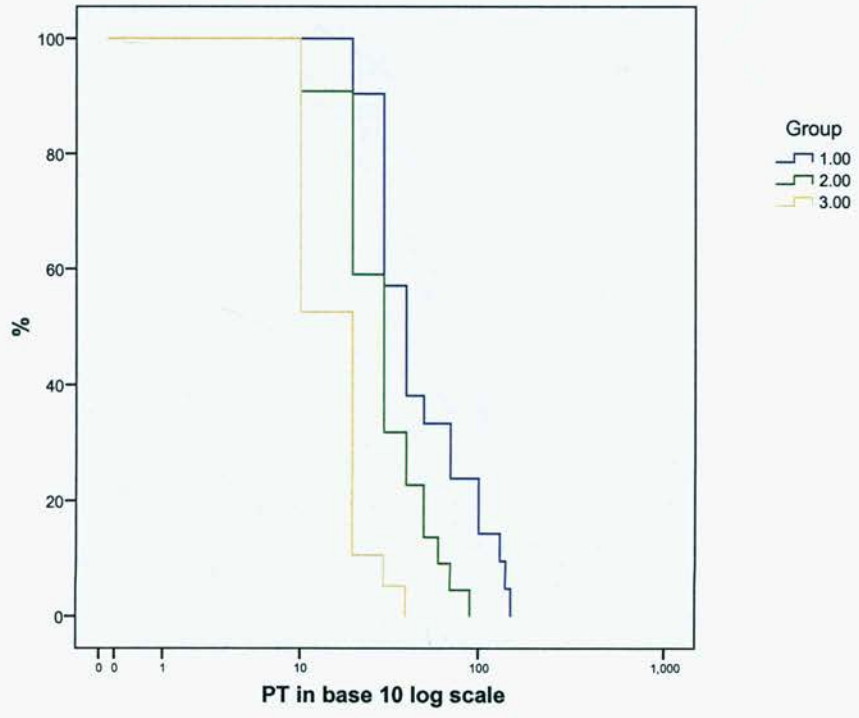


Figure 3.2 **Reverse cumulative distribution curve by group for antibody to PT at 4 months old**

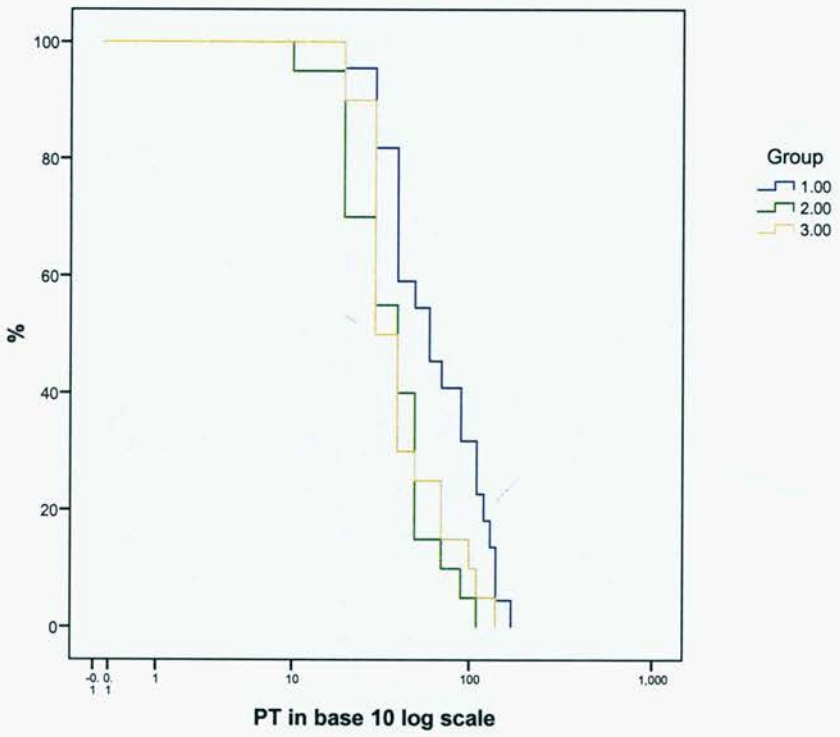


Figure 3.3 **Reverse cumulative distribution curve by group for antibody to PT at 6 months old**

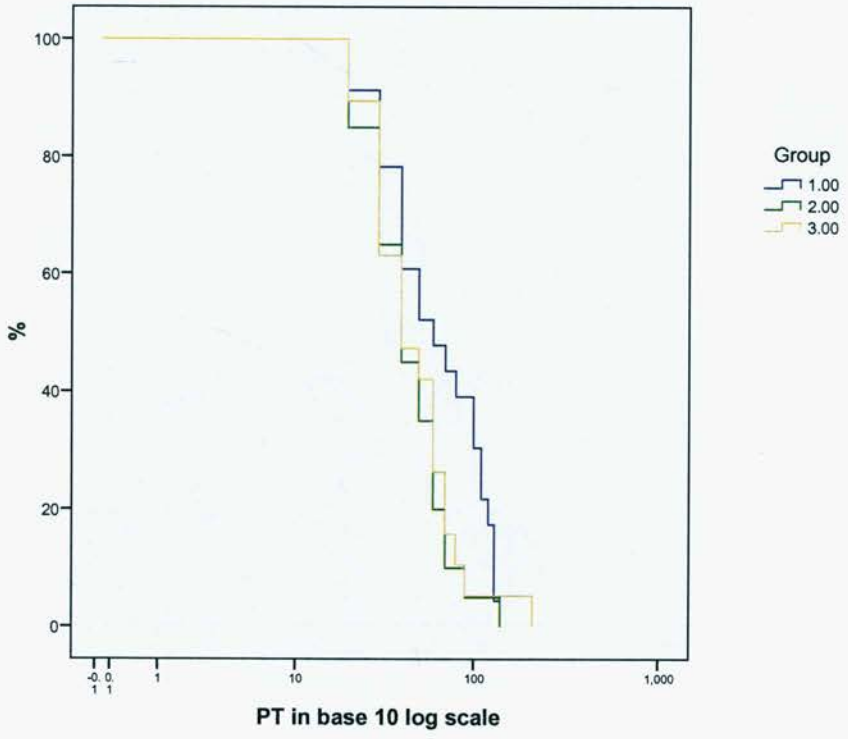


Figure 3.4 **Reverse cumulative distribution curve by group for antibody to PT at 8 months old**

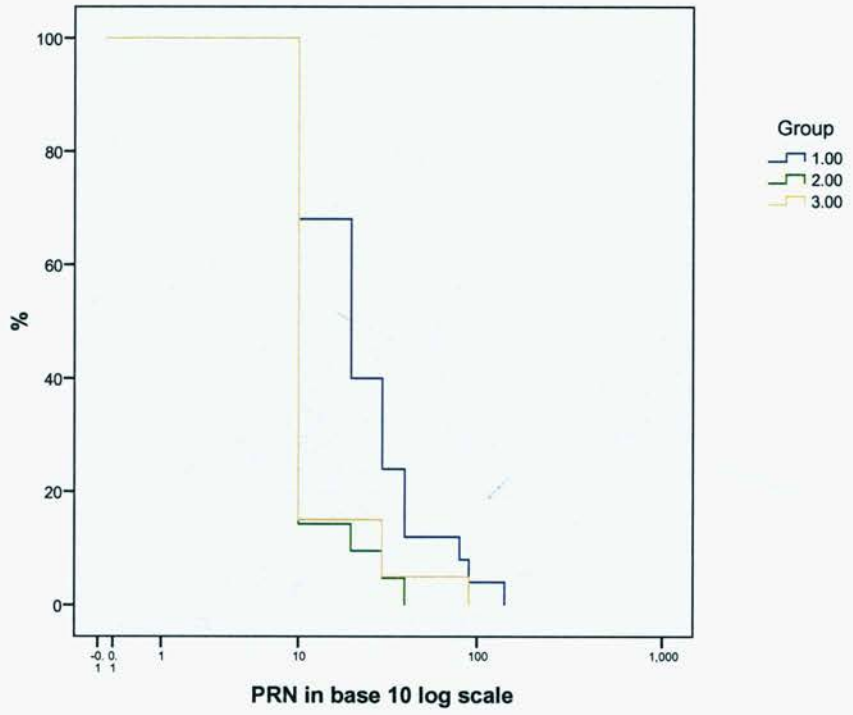


Figure 3.5 **Reverse cumulative distribution curve by group for antibody to PRN at 2 months old**

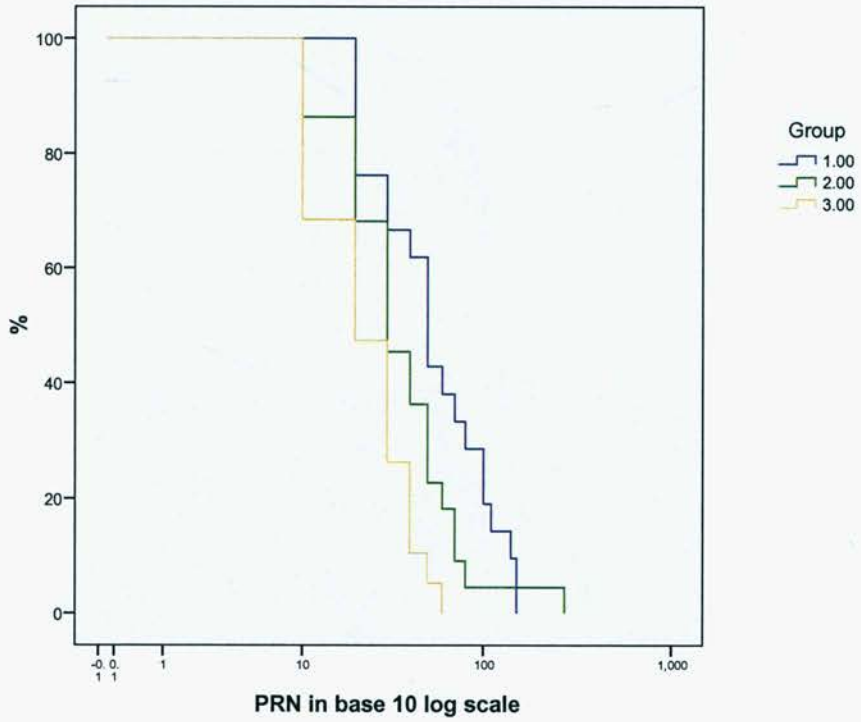


Figure 3.6 **Reverse cumulative distribution curve by group for antibody to PRN at 4 months old**

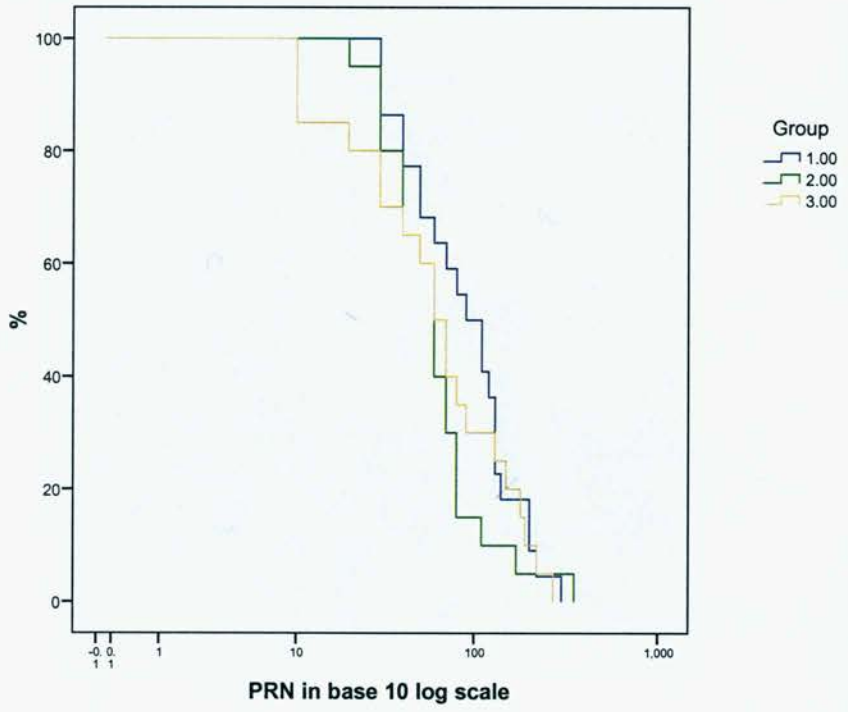


Figure 3.7 **Reverse cumulative distribution curve by group for antibody to PRN at 6 months old**

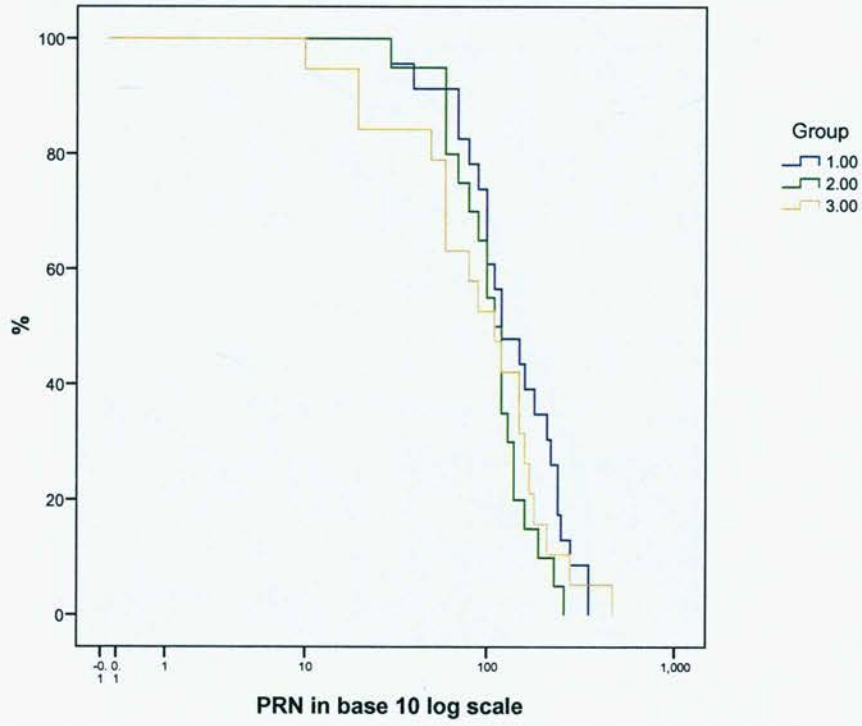


Figure 3.8 **Reverse cumulative distribution curve by group for antibody to PRN at 8 months old**

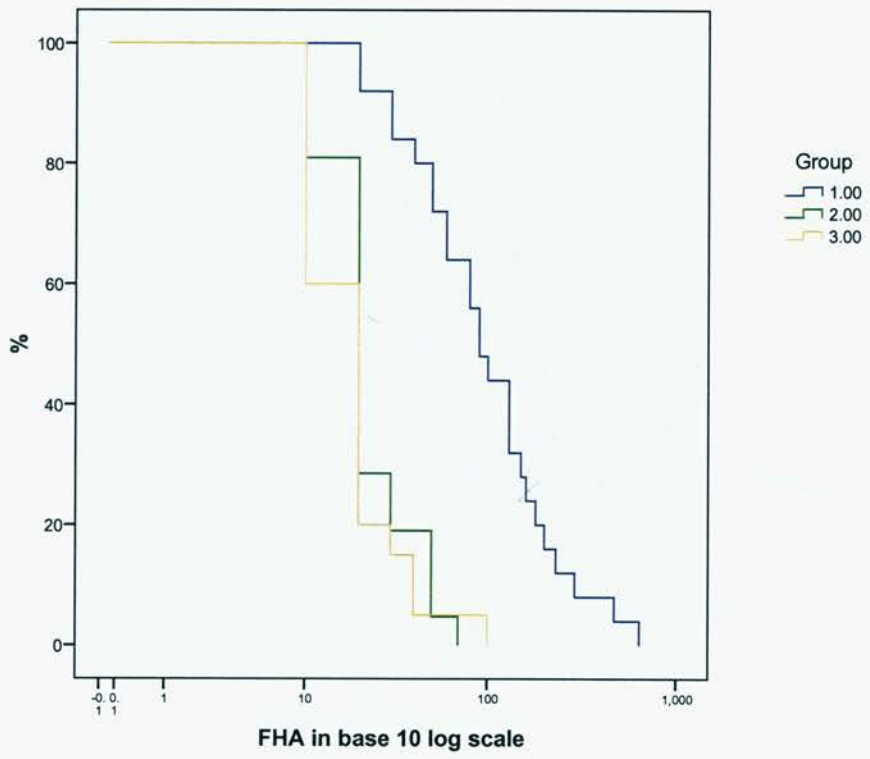


Figure 3.9 **Reverse cumulative distribution curve by group for antibody to FHA at 2 months old**

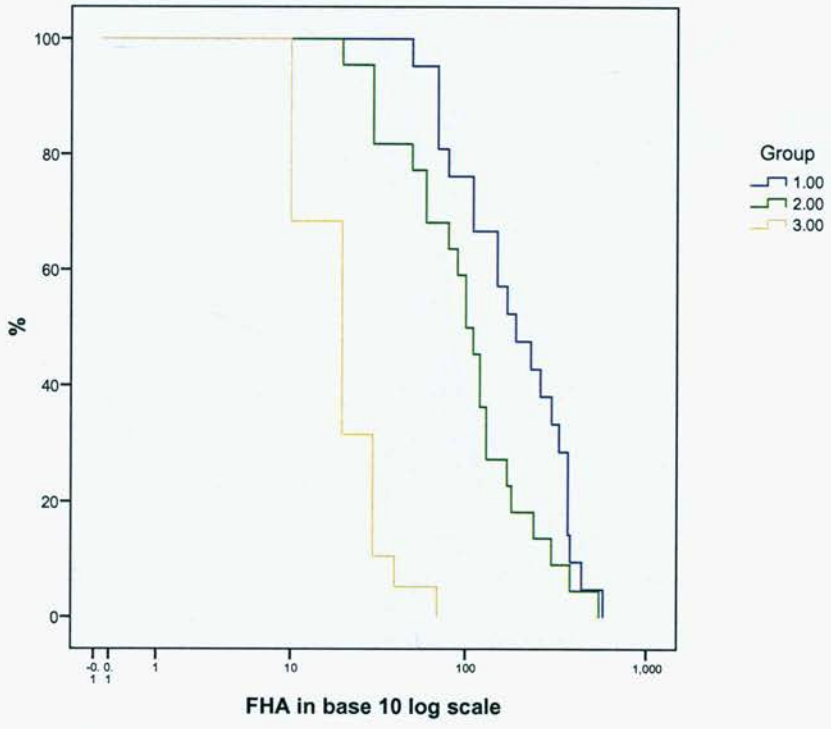


Figure 3.10 **Reverse cumulative distribution curve by group for antibody to FHA at 4 months old**

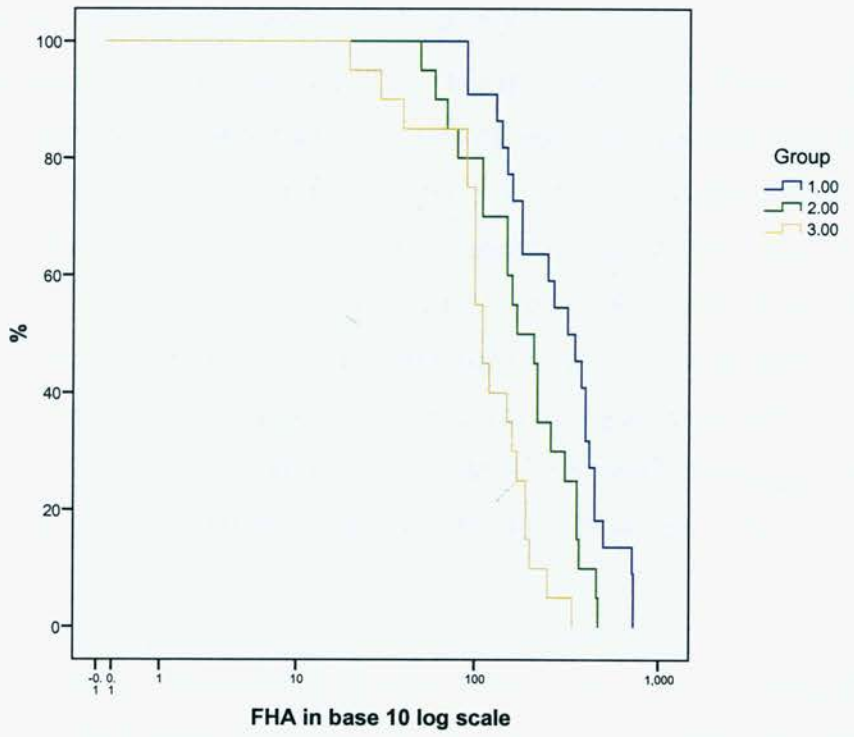


Figure 3.11 Reverse cumulative distribution curve by group for antibody to FHA at 6 months old

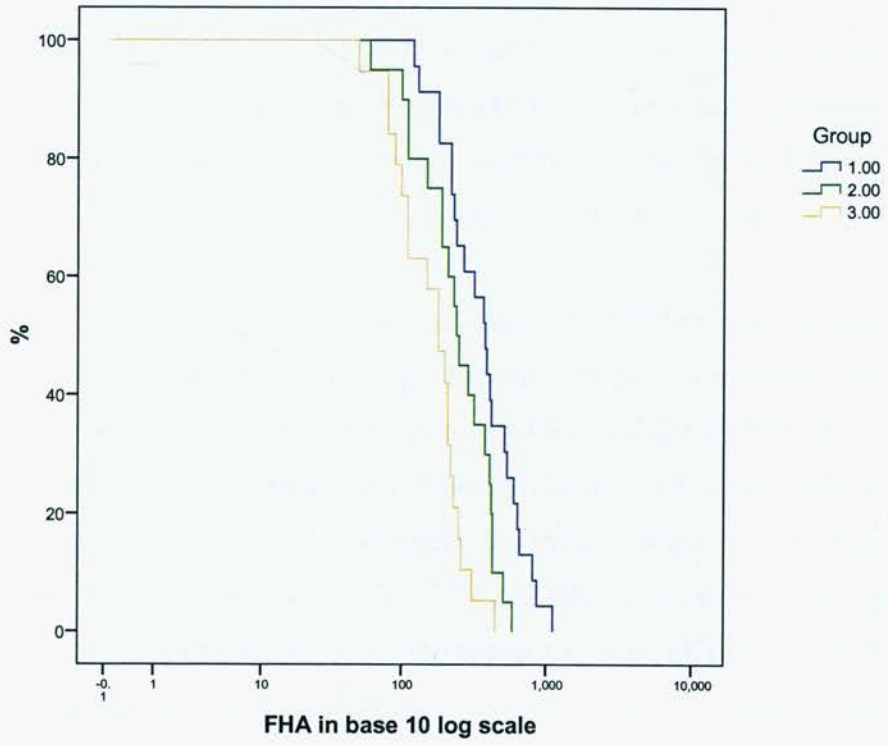


Figure 3.12 Reverse cumulative distribution curve by group for antibody to FHA at 8 months old

Comparison of antibody responses between groups

With respect to GMCs, at two months, following two doses of Pa, Group 1 infants had statistically significantly higher GMCs for anti-PT, anti-FHA and anti-PRN IgG compared to both Group 2 and 3 infants (Table 3.4, 3.5, and 3.6). For anti-PT IgG, levels remained significantly higher in group 1 compared to groups 2 and 3 at 4 and 6 months. (Table 3.4 and figure 3.13). For anti-PRN IgG, at 4 months of age, after 3 doses of a pertussis–containing vaccine, levels were significantly higher in group 1 compared to groups 2 (2 doses) and 3 (one dose) but there was no significant difference at 6 or 8 months of age. (Table 3.5 and figure 3.13) For anti-FHA, levels were significantly higher in groups 1 and 2 compared to group 3 at 4 months of age. (Table 3.6 and figure 3.13)

With respect to the proportion above the limit of detection, at 2 months old, after 2 doses of a pertussis–containing vaccine, 22 (88%) of group 1 infants had a level of IgG to PT above 5 EL.U/ml compared to 9 (43%) of those in group 2 (one dose) and 3 (15%) of group 3 (no doses). Similarly, all group 1 infants had detectable antibody (>5 EL.U/ml) to PRN one month after the second dose of Pa at 2 months, compared to 33% for those in group 2 who had received a dose at birth only and 30% for controls. Significantly more infants in group 1 had a 4 fold rise in anti-PT IgG from maternal levels at 2 months (56% vs 5 and 0% respectively for groups 2 and 3, $p<0.02$). This proportion remained significantly higher at 4 months (86% vs 55% vs 47%, $p<0.05$) compared with infants in groups 2 and 3. (Table 3.7 and figure 3.14)

Tables 3.4, 3.5, 3.6 and figure 3.13 also demonstrate the heterogeneity of antibody responses to PT, PRN and FHA according to group. The highest antibody levels achieved in all 3 groups were anti-FHA, then anti-PRN and anti-PT had the lowest GMC. At 8 months Group 1 had non significantly higher anti-PT, anti-PRN GMCs and significantly higher anti-FHA GMC compared to Group 3 infants. At 8 months, Group 2 infants had a non significantly higher GMC for anti-PRN and anti-FHA antibodies and non significantly lower anti-PT compared to group 3 infants. Of note, responses to the third dose of Pa vaccine also varied between pertussis antibody categories. Group 2 and 3 infants had minimal anti-PT responses, as measured by change in GMC, to the third

dose but higher anti-FHA and anti-PRN responses. In contrast, Group 1 infants had higher pertussis antibody responses to all three antigens after the 3rd dose.

Of note, anti-pertussis antibody levels seem to converge by 6 months when all groups had received at least 2 doses, despite groups 1 and 2 having higher levels earlier at 2-4 months (Figure 3.13).

Table 3.7 Proportion achieving four-fold increase in anti-pertussis antibody level according to age and group

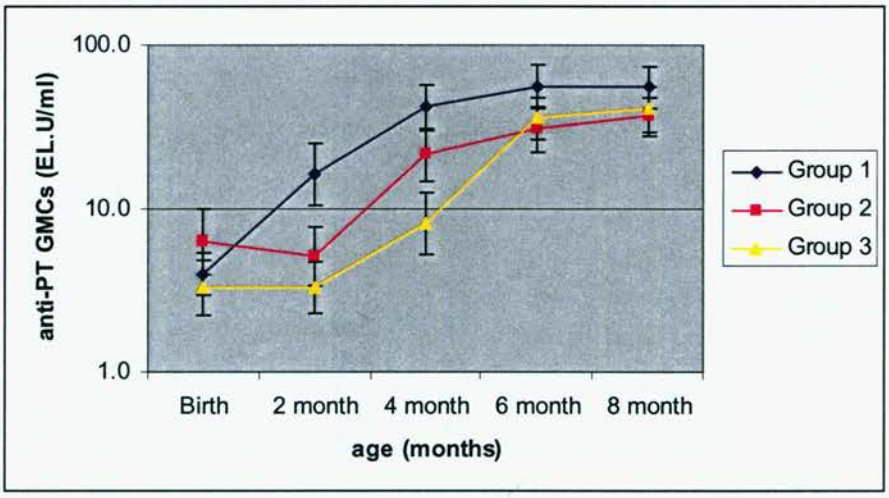
	Group 1*					Group 2*					Group 3*				
	n[&]	Pa doses received	Anti-PT %	Anti-FHA %	Anti-PRN %	n[&]	Pa doses received	Anti-PT %	Anti-FHA %	Anti-PRN %	n[&]	Pa doses received	Anti-PT %	Anti-FHA %	Anti-PRN %
Maternal to 2 months	25	2	56	56	44	21	1	4.8	14	5	20		0	0	5
Maternal to 4 months	21	3	86	81	67	22	2	55	59	73	19	1	47	16	42
Maternal to 6 months	22	4	86	86	73	20	3	65	70	80	20	2	90	60	75
Maternal to 8 months	23	5	87	87	87	20	4	70	85	85	19	3	95	68	74

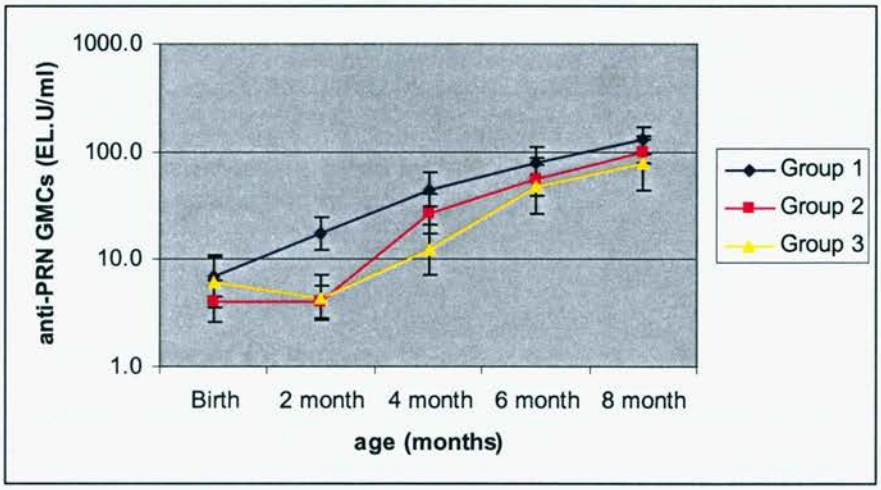
*Group 1 – Pa vaccine at birth and one month then Infanrix Hexa at 2, 4 and 6 months of age

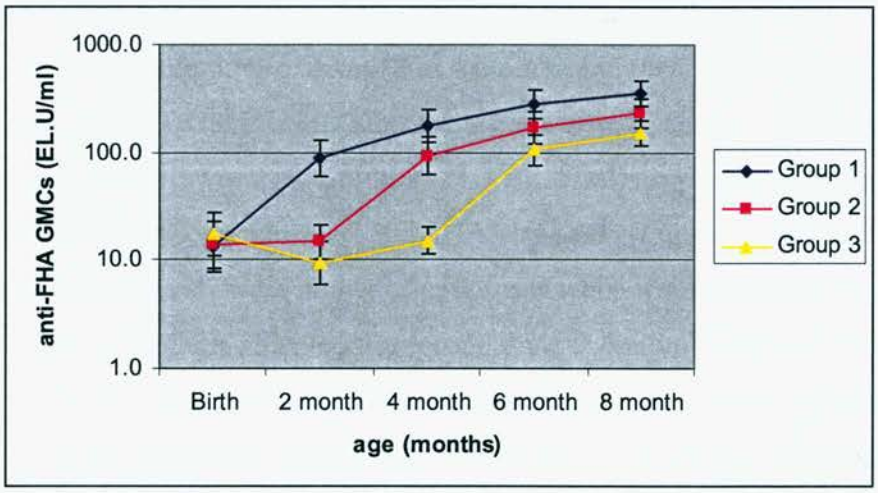
*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

n[&] – number of according to protocol subjects who had blood sample collected for antibody analysis







Birth – the birth GMC refers to the maternal antibody level taken at birth of the infant.

Each time point GMC has 95% confidence interval bars shown.

Group 1 – acellular pertussis (Pa) vaccine at birth and one month old, Group 2 – Pa vaccine at birth only, Group 3 – nil Pa vaccine at birth. All groups received hepatitis B vaccine at birth and Infanrix Hexa at 2, 4 and 6 months old.

Figure 3.13 Anti-pertussis antibody geometric mean concentrations (GMCs) from birth until 2 months after completion of primary vaccination

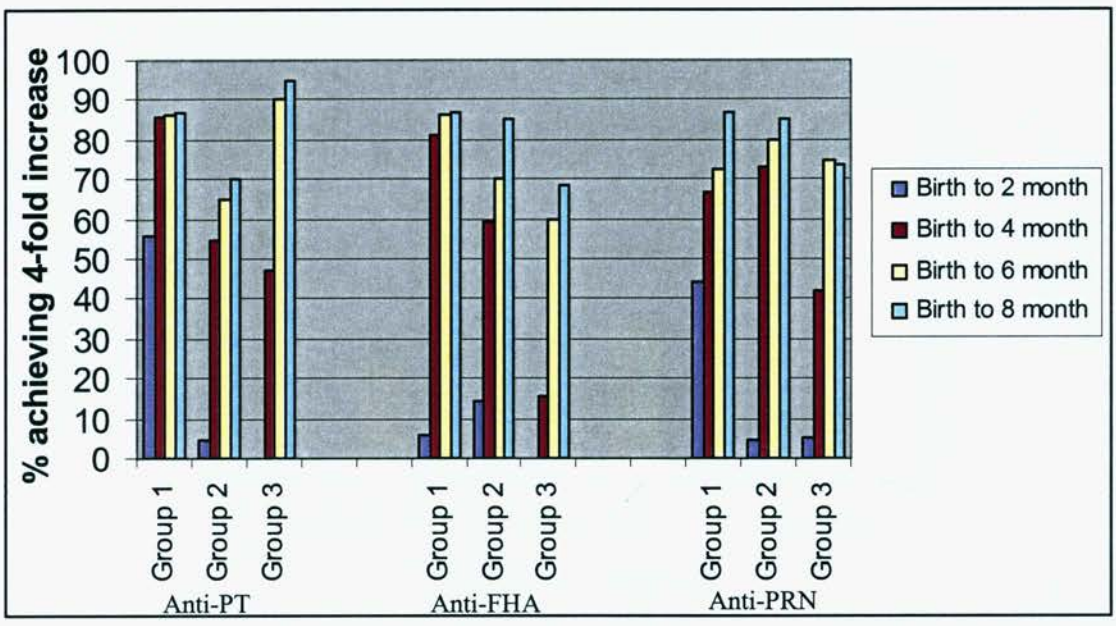


Figure 3.14 Proportion achieving 4-fold increase in anti-pertussis antibody level according to age and group

Response to second dose of Pa vaccine

Irrespective of age, antibody levels increased substantially following the second dose of a pertussis containing vaccine in all three groups. In the case of anti-PT IgG, after the second dose, a four-fold increase in anti-PT levels was seen in 56% of group 1 infants at 2 months, compared with 55% of group 2 at 4 months and 90% of group 3 infants at 6 months of age. Anti-PT GMC levels after the second dose in Group 1 (16.1 EL.U/ml) were non significantly lower than Group 2 (21.1 EL.U/ml) and Group 3 (35.6 EL.U/ml). A similar pattern in responses to PRN and FHA was seen. (Figure 3.15) For all three pertussis antibodies, levels were higher after 2 doses when it was given at an older age, for example group 3 infants received the second dose at 4 months old and the pertussis antibody GMCs after the second dose were higher than group 1 infants who received the second dose at 1 month old and group 2 infants who received the second dose at 2 months old. This is a reflection of the maturity of the immune system with age. The older the infant when given the second Pa vaccine the higher the antibody response.

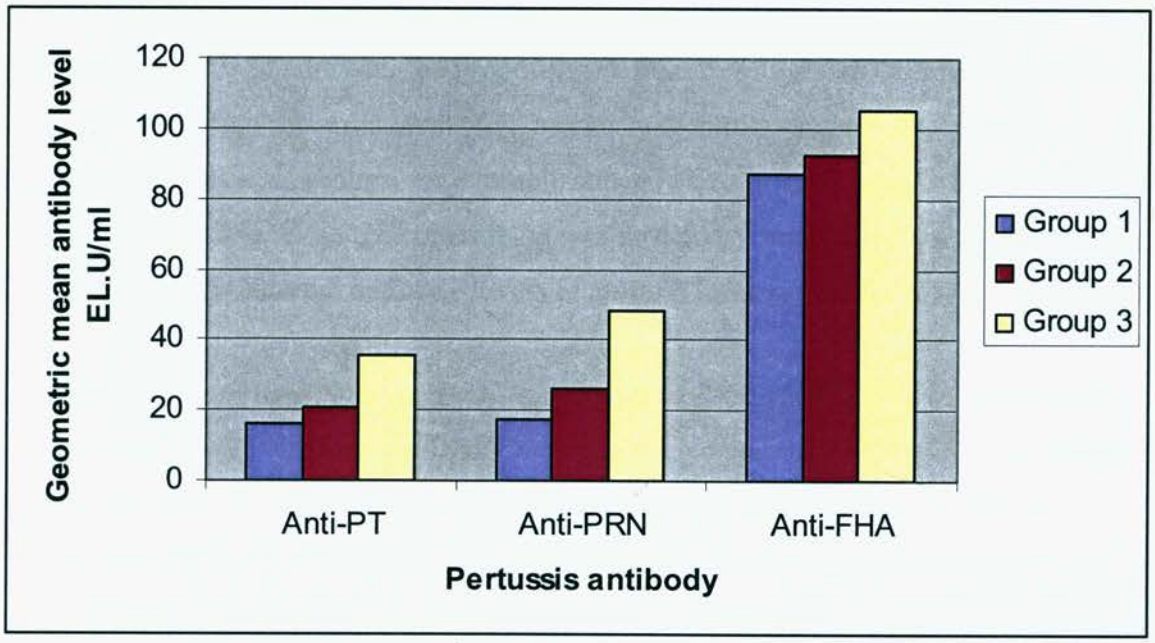


Figure 3.15 Pertussis antibody responses after second dose of acellular pertussis vaccine according to group

Response to the third dose of Pa vaccine

Anti-PT GMC levels were similar in all three groups after the third dose (Group 1 at 4 months: 41.3 EL.U/ml, Group 2 at 6 months: 30.5 EL.U/ml, Group 3 at 8 months: 40.3 EL.U/ml). (Table 3.4) Similar to earlier doses a heterogeneity of responses to pertussis antigens was seen. Anti-FHA levels after the third doses progressively decreased from group 1 (177.4 EL.U/ml) to group 2 (168.7 EL.U/ml) to group 3 (152.3 EL.U/ml). (Table 3.6) In contrast, anti-PRN levels after the third dose progressively increased from group 1 (44.5 EL.U/ml) to group 2 (54.8 EL.U/ml) to Group 3 (78.7 EL.U/ml). (Table 3.5)

Comparison of post primary vaccination antibody levels at 8 months old

Post primary pertussis antibody levels in each group were compared by GMC and proportion with detectable levels.

1. *GMC in birth vaccine groups 1 and 2 compared to group 3 at 8 months old* – Antibody levels (GMC) at 8 months of age, 2 months after completion of the vaccine schedule, did not significantly differ from control infants. Indeed, group 1

infants had non-significantly higher GMCs compared to the other two groups. (Figure 3.13) In addition, the proportion of infants with a four fold rise in PT or PRN IgG from maternal levels to 8 months was similar and not significantly different between groups, for example anti-PT (Group 1: 87% vs Group 2: 70% vs Group 3: 95%), although comparison was limited by small sample size and the lower mean maternal antibody levels in group 3 infants. (Table 3.7)

2. *Proportion of detectable pertussis antibodies at 8 months in those in birth vaccine groups 1 and 2 who were initially seronegative compared to group 3* – All infants in groups 1, 2, 3, who were initially seronegative at baseline, had detectable antibodies to PT, PRN, FHA at 8 months old and there was no significant difference between groups. At 8 months the GMC to all three pertussis antigens in those who were seronegative at baseline was higher in group 1 infants compared to group 2 and 3 infants.

Proportions of infants in groups 1, 2, 3 whose anti-PT level increased following each Pa dose were examined. (Table 3.8) In the majority of group 1 subjects, anti-PRN and anti-FHA levels increased after the 5th dose (6 to months 8 old), (anti-PRN (86% increased, n= 9/22), anti-FHA (82% increased, n=18/22)), however only one third (36%, n=8/22) had increase in their anti-PT. (Table 3.8). In contrast higher proportions of group 2 (65%) and group 3 (79%) infants increased their anti-PT level after the 6 month dose. (Table 3.8)

The definition of hyporesponsiveness used in this thesis refers to significantly lower pertussis antibody levels (GMC or detectable) in birth vaccine groups at 8 months old compared to group 3 infants. Using this definition, and within the limits of sample size, no significant hyporesponsiveness was found. This definition does not refer to changes in pertussis antibody levels from dose to dose. In only one third of group 1 infants did the addition of a 5th dose at 6 months old result in higher anti-PT levels at 8 months, however over two thirds had higher anti-FHA and anti-PRN levels. This indicates lessening immune responses with later doses in the birth vaccine group and seems to affect anti-PT responses more than antibody responses to the other pertussis antigens.

Table 3.8 Comparison of proportion of infants whose anti-PT levels were higher according to doses received and group

	Dose 1 to 2	Dose 2 to 3	Dose 3 to 4	Dose 4 to 5	6 to 8 months old
Group 1					
Age	Birth to 2 months*	Birth to 2 months old	2 to 4 months old	4 to 6 months old	6 to 8 months old (after 5th Pa dose)
% (n) anti-PT increased	84% (21/25)	75% (20/25)	100%	86% (18/21)	**36%** (8/22)
Group 2					
Age	Birth to 2 months	2 to 4 months old	4 to 6 months old	not applicable	6 to 8 months old (after 4th Pa dose)
% (n) anti-PT increased	14% (3/21)	71% (15/21)	77% (17/22)		**65%** (13/20)
Group 3					
Age	2 to 4 months	4 to 6 months old	not applicable	not applicable	6 to 8 months old (after 3rd Pa dose)
% (n) anti-PT increased	10% (2/20)	100% (19/19)			**79%** (15/19)

*In group 1 infants the first blood sample was collected at 2 months old after receipt of 2 doses (birth and one month old). No sample was collected at one month old. The percentage increase in the table refers to the change in anti-PT level from birth (maternal) to 2 months old (after 2 doses).

Influence of maternal pertussis antibody levels at birth

At baseline, anti PT was detected in 32%, 50% and 14.3% of Group 1, 2, 3 infants respectively. (Table 3.4) At baseline anti-PRN was detected in 56%, 23% and 50% of Group 1, 2, 3 infants respectively. (Table 3.5) At baseline anti-FHA was detected in 76%, 81% and 95% of group 3 infants respectively. (Table 3.6) The most susceptible infants to pertussis are likely to be those with non detectable antibody levels to all pertussis antigens. Nearly one in five (19%) infants in group 1 had absent antibodies to all three pertussis antigens (anti-PT, anti-PRN and anti-FHA). Results for the other antigens are shown in table 3.9.

Table 3.9 Combination of absent pertussis antibodies according to group at baseline (maternal level)

Baseline	Anti-PT and anti-PRN Not detectable (<5 EL.U/ml)		Anti-PT, anti-PRN, anti-FHa Not detectable (<5 EL.U/ml)	
Group	n	%	n	%
Group 1 (n=27)	5	19	5	19
Group 2 (n=23)	6	26	3	13
Group 3 (n=26)	12	46	1	4

At 2 months of age, antibody levels to all three pertussis antigens in groups 2 and 3 were similar or slightly lower than maternal levels, consistent with waning of maternal antibodies. (Table 3.4, 3.5, 3.6). Pertussis antibody responses according to group and presence or absence of detectable maternal antibodies are shown in Table 3.10, 3.11, 3.12. In all three groups infants who had detectable maternal anti-PT (>5 EL.U/ml) at birth had higher GMCs at 2 months of age compared to those with no detectable maternal anti-PT at birth. (Table 3.10) Of the 8 infants in Group 1 who had detectable anti-PT IgG in maternal sera, 6 (75%) showed an increase in anti-PT between birth and 2 months of age compared to 1 (7%) of the infants in groups 2 and 3 combined who had detectable maternal antibody. Thus, the second dose of Pa at 1 month of age in group 1 infants appeared to overcome the decline in anti-PT levels from birth to 2 months old. However, antibody levels in group 2 infants declined from birth to 2 months old despite the birth dose of Pa vaccine.

Table 3.10 Antibody responses to pertussis toxin (PT) according to baseline level (maternal antibody) and group

	Group 1*			Group 2*			Group 3*		
Age	Pa doses	Anti-PT detectable at baseline# GMC^ (95% CI) n=8	NO Anti-PT detectable at baseline GMC^ (95% CI) n=17	Pa doses	Anti-PT detectable at baseline# GMC^ (95% CI) n=11	NO Anti-PT detectable at baseline GMC^ (95% CI) n=11	Pa doses	Anti-PT detectable at baseline# GMC^ (95% CI) n=3 (Individual results shown for these 3 subjects)	NO Anti-PT detectable at baseline GMC^ (95% CI) n=18
Maternal	0	10.4 (6.8-15.8)	2.5	0	15.4 (9.8-24.4)	2.5	0	5 11 96	2.5
2 months	2	25.4 (11.2-57.6)	13.0 (7.6-22.0)	1	7.5 (3.9-14.4)	3.3 (2.2-4.9)	0	2.5 2.5 60	2.8 (2.4-3.4)
8 months	5	49.1 (24.2-99.3)	57.8 (40.5-82.5)	4	36.1 (24.4-53.5)	36.6 (23.2-57.7)	3	30 58 28	41.0 (27.9-60.4)

*Group 1 – Pa vaccine at birth and one month, then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

detectable – anti-pertussis toxin antibody > 5 EL.U/ml at baseline

^ GMC – geometric mean concentration (EL.U/ml)

Table 3.11 Antibody responses to pertactin (PRN) according to baseline level (maternal antibody) and group

	Group 1*			Group 2*			Group 3*		
Age	Pa doses	Anti-PRN detectable at baseline# GMC^ (95% CI) n=14	NO Anti-PRN detectable at baseline GMC^ (95% CI) n=11	Pa doses	Anti-PRN detectable at baseline# GMC^ (95% CI) n=5	NO Anti-PRN detectable at baseline GMC^ (95% CI) n=17	Pa doses	Anti-PRN detectable at baseline# GMC^ (95% CI) n=3	NO Anti-PRN detectable at baseline GMC^ (95% CI) n=18
Maternal	0	15.5 (10.4-23.1)	2.5	0	21.5 (7.6-60.6)	2.5	0	14.9 (7.1-31.3)	2.5
2 months	2	12.9 (7.9-21.0)	25.0 (14.8-42.2)	1	6.5 (1.3-33.7)	3.5 (2.6-4.5)	0	6.3 (2.6-14.9)	3.1 (1.9-5.0)
8 months	5	119.0 (80.0-176.9)	137.9 (83.0-229.3)	4	85.4 (23.4-312.5)	103.5 (80.6-132.8)	3	49.6 (18.6-132.2)	131.3 (76.1-226.4)

*Group 1 – Pa vaccine at birth and one month, then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

detectable – anti-PRN antibody > 5 EL.U/ml

^ GMC – geometric mean concentration (EL.U/ml)

Table 3.12 Antibody responses to filamentous haemagglutinin (FHA) according to baseline level (maternal antibody) and group

	Group 1*			Group 2*			Group 3*		
Age	Pa doses	Anti-FHA detectable at baseline# GMC^ (95% CI) n=19	NO Anti-FHA detectable at baseline GMC^ (95% CI) n=6	Pa doses	Anti-FHA detectable at baseline# GMC^ (95% CI) n=18	NO Anti-FHA detectable at baseline GMC^ (95% CI) n=4	Pa doses	Anti-FHA detectable at baseline# GMC^ (95% CI) n=19	NO Anti-FHA detectable at baseline GMC^ (95% CI) n=1[$]
Maternal	0	22.5 (13.9-36.2)	2.5	0	20.3 (13.6-60.3)	2.5	0	19.5 (12.7-29.8)	2.5
2 months	2	84.9 (51.7-139.4)	96.1 (45.2-204.2)	1	15.5 (10.9-22.1)	11.5 (1.8-74.8)	0	10.1 (6.3-16.3)	2.5
8 months	5	330.6 (237.4-460.3)	447.2 (270.0-740.7)	4	218.0 (1525-311.8)	303.7 (140.7-655.6)	3	148.4 (111.0-198.4)	244

*Group 1 – Pa vaccine at birth and one month, then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

detectable – anti-FHA antibody > 5 EL.U/ml

^ GMC – geometric mean concentration (EL.U/ml)

$ n=1, actual antibody levels are shown for this patient.

At 2 months of age, the majority of infants in group 2 (n=8/10, 80%) and group 3 (n= 15/17, 88%), who had no detectable maternal antibody still had anti-PT levels <5 EL.U/ml. In contrast, only 18% (n=3/17) of infants in group 1 remained below the detectable threshold. This suggests that the majority of infants in group 2 and 3 remained susceptible at an age when infection is likely to be severe.

Antibody responses to PT, FHA and PRN according to maternal antibody levels are shown in Tables 3.10, 3.11. 3.12.

At 8 months of age, the GMCs for anti-PT, anti-PRN and anti-FHA in infants (who had detectable maternal IgG at baseline) in all three groups were lower compared to infants in each of the 3 groups whose mothers had no detectable IgG antibodies to these antigens.(Table 3.10, 3.11, 3,12). However, the small sample size in each sub-group means the interpretation of this result is problematic and so antibody responses at 8 months were compared when group 1, 2 and 3 data were combined. (Table 3.13) Pertussis antibodies (PT, PRN, FHA) were non significantly higher ($p>0.05$), in combined group data at 8 months, for those with absent maternal antibody at baseline compared to those with detectable maternal antibody. (Table 3.13)

Table 3.13 Pertussis antibody response at 8 months old for combined group (1, 2, 3) data according to detectable or non detectable maternal antibody at baseline

Baseline	Maternal antibody detectable (>5 EL.U/ml)		Maternal antibody not detectable (<5 EL.U/ml)	
Pertussis antibody	n	GMC^ (95% CI)	n	GMC^ (95% CI)
Anti-PT	19	40.5 (30.5-53.9)	43	45.3 (36.5-56.2)
Anti-PRN	27	81.9 (54.2-123.9)	35	119.1 (96.9-147.0)
Anti-FHA	52	220 (180.8-268.8)	10	360.6 (260.4-499.3)

^ GMC – geometric mean concentration (EL.U/ml)

Anti-PT and anti-PRN responses, in those with detectable pertussis antibodies at birth, were also assessed after 3 doses (Group 1 aged 4 months, Group 2 aged 6 months and

group 3 aged 8 months) and compared to those in groups 1, 2, 3 with no detectable maternal antibody at birth after 3 doses (Table 3.14). Anti-PRN and anti-FHA responses were significantly higher in infants with no detectable maternal antibody compared to those with detectable maternal antibody. (Table3.14)

Table 3.14 Pertussis antibody responses after 3 doses for combined group (1 2, 3) # according to detectable or non detectable maternal antibody at baseline

Baseline	Maternal antibody detectable (>5 EL.U/ml)		Maternal antibody not detectable (<5 EL.U/ml)	
Pertussis antibody	n	GMC^ (95% CI)	n	GMC^ (95% CI)
Anti-PT	18	31.8 (21.9-55.2)	42	39.5 (32.1-48.7)
Anti-PRN	25	36.4* (24.0-55.2)	35	78.9* (61.7-100.8)
Anti-FHA	49	151.2^ (123.8 -184.6)	11	253.5^ (187.2-343.4)

^ GMC – geometric mean concentration (EL.U/ml)

Combined groups after 3 doses

Antibody responses after 3 doses (group 1 – aged 4 months, group 2 – aged 6 months, group 3 – aged 8 months) in those with detectable maternal antibody were combined

Antibody responses after 3 doses (group 1 – aged 4 months, group 2 – aged 6 months, group 3 – aged 8 months) in those with no detectable maternal antibody were combined

*- anti-PRN GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs non detectable antibody. (p=0.002)

^- anti-FHA GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs non detectable antibody. (p=0.02)

b) Antibody responses to other vaccine antigens

Two months after completion of the primary immunisation schedule, 100% of subjects in all groups had IgG levels to diphtheria and tetanus above those usually associated with protection (0.1 U/ml), with no significant difference between the groups (Table 3.15). Reverse cumulative distribution curves for responses to concomitant antigens (diphtheria, tetanus, hepatitis B and Hib) are shown (Figure 3.16. 3.17. 3.18. 3.19). These demonstrate similar responses to diphtheria and tetanus for all groups and show lower antibody responses in group 1 compared to groups 2 and 3 for Hib and hepatitis B.

Table 3.15 Immune responses two months after completion of primary vaccination for concomitant antigens according to group

Antibody	Threshold	Group 1*			Group 2*			Group 3*		
		n#	% > threshold	GMC^ (95% CI)	n#	% > threshold	GMC^ (95% CI)	n#	% > threshold	GMC^ (95% CI)
Hepatitis B	>10 mIU/ml >100 mIU/ml	20	100 80.0	292.9 (14.2-604.1)	19	100 95.0	540.5 (301.8-967.8)	15	100 100	821.8 (488.2-1383.3)
Haemophilus Influenzae type b	>0.15 ug/ml >1 ug/ml	23	65.2 26.0	0.39 (0.2-0.75)	20	95.0 45.0	1.03 (0.47-2.22)	19	89.5 47.4	0.8 (0.41-1.58)
Diphtheria	>0.1 IU/ml >1 IU/ml	23	100 78.2	1.64 (1.2-2.24)	20	100 75.0	1.7 (1.18-2.46)	19	100 84.0	1.97 (1.3-2.98)
Tetanus	>0.1 IU/ml >1 IU/ml	23	100 47.8	0.84 (0.55-1.28)	20	100 55.0	1.46 (0.98-2.17)	19	100 78.9	1.34 (0.89-2.04)

*Group 1 – Pa vaccine at birth and one month then Infanrix Hexa at 2, 4 and 6 months of age

*Group 2 – Pa vaccine at birth then Infanrix Hexa at 2, 4 and 6 months of age

*Group 3 – Infanrix Hexa at 2, 4 and 6 months of age

#n – number of according to protocol subjects who had blood sample collected at 8 months old for antibody measurement

^GMC – geometric mean concentration

There was a non-significant trend to reduced anti-Hepatitis B surface antibody GMC responses in infants who received the Pa vaccine at birth (Group 1 and 2 vs Group 3), however all were above the anti-HBs level associated with protection (10 mIU/ml). Similarly, Group 1 infants had non-significantly lower GMCs against Hib and a lower proportion with anti PRP IgG above 1ug/ml, compared to Group 2 and 3 infants (26% vs 45% vs 47%; Table 3.15). The Hib GMC in both birth vaccine groups 1 and 2 combined was non significantly lower than group 3, (group 1 and 2: anti-Hib GMC = 0.61 (95% CI 0.37-1.01) vs group 3: anti-Hib GMC = 0.8 (95%CI 0.41-1.58, p=0.5).

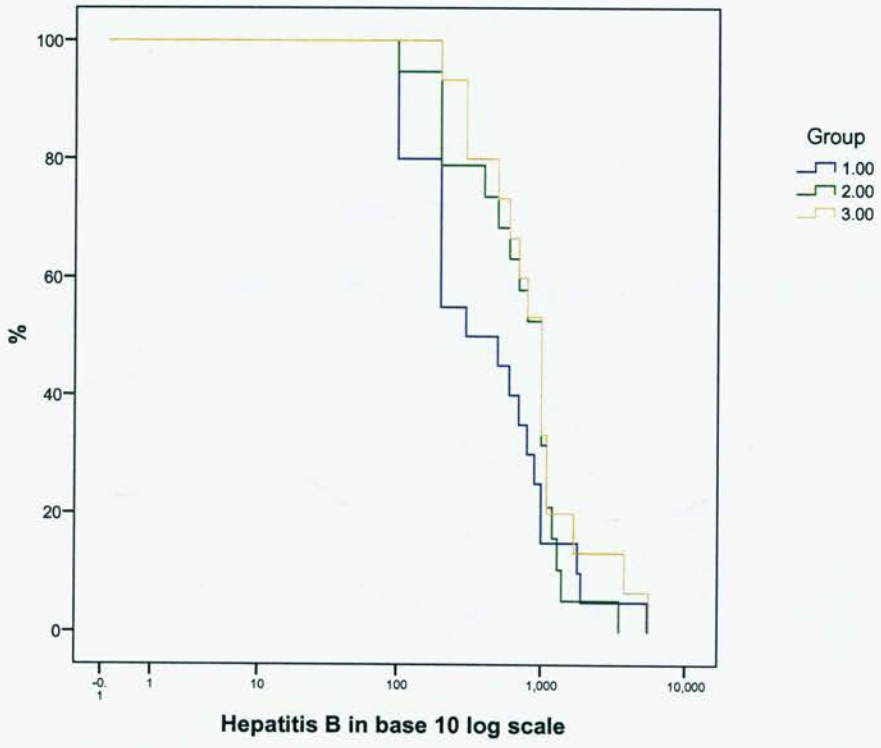


Figure 3.16 Reverse cumulative distribution curve by group for antibody to hepatitis B at 8 months old

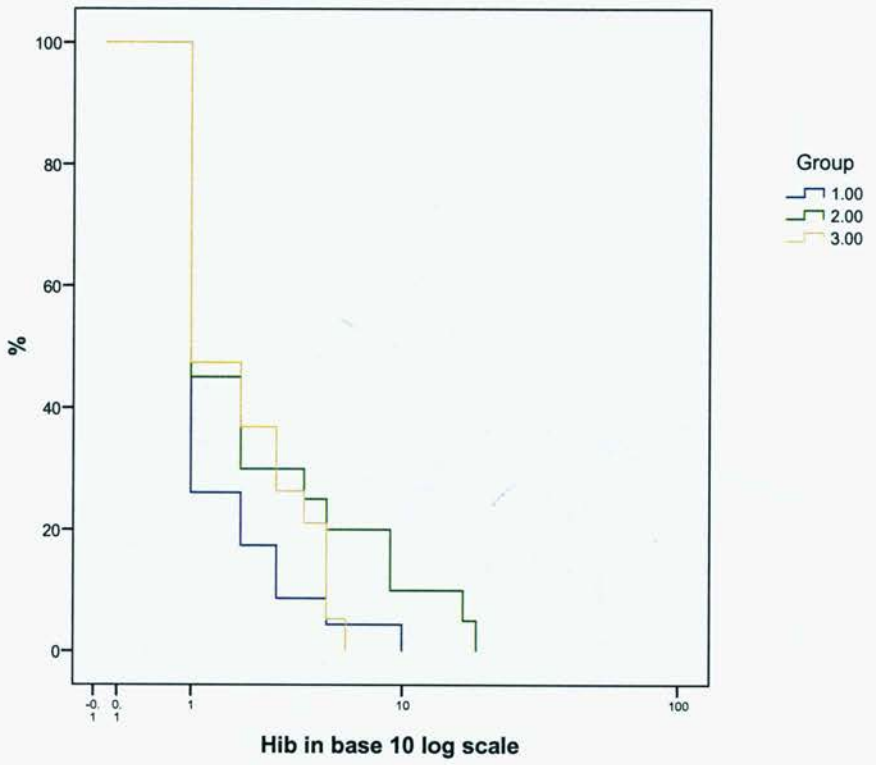


Figure 3.17 Reverse cumulative distribution curve by group for antibody to Hib at 8 months old

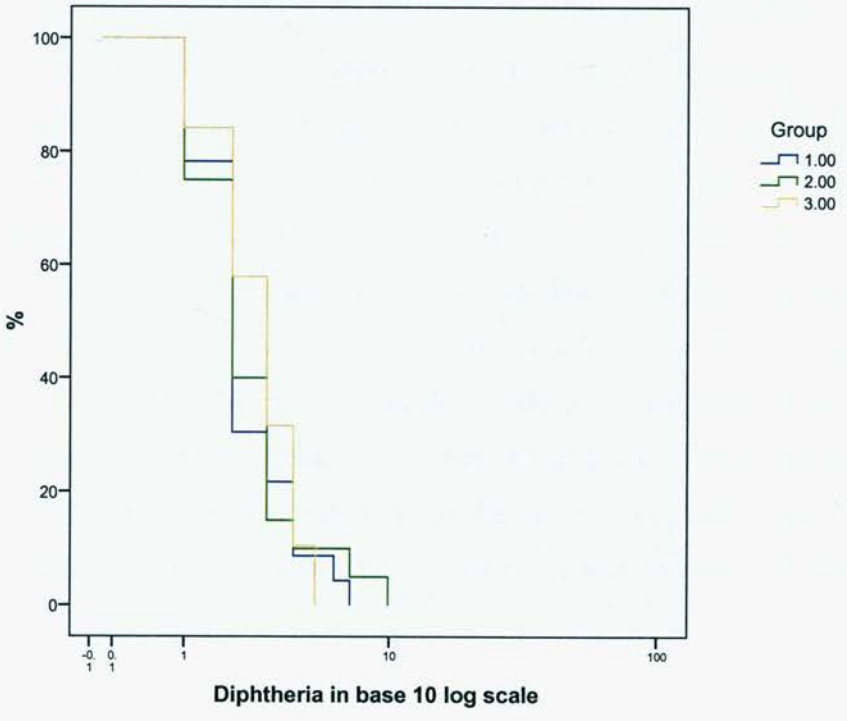


Figure 3.18 Reverse cumulative distribution curve by group for antibody to diphtheria at 8 months old

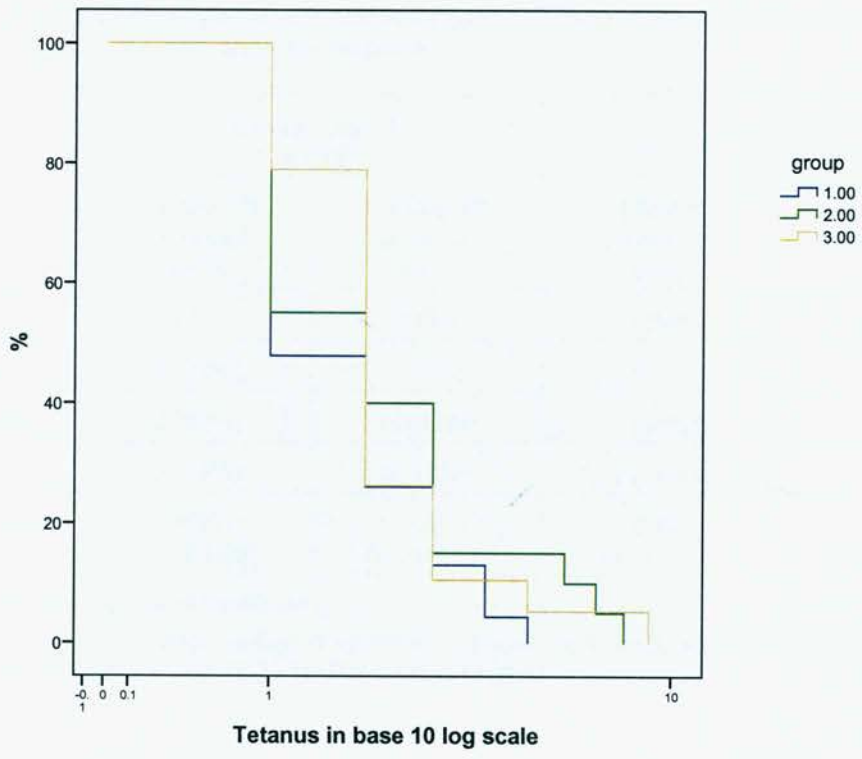


Figure 3.19 Reverse cumulative distribution curve by group for antibody to tetanus at 8 months old

Association of pertussis antibody response and concomitant antigen response

Lower Hib and hepatitis B responses were seen in the birth vaccine group 1 infants who had received more Pa doses and had higher pertussis antibody levels at 2 and 4 months old compared to controls. To explore if there was an association between the degree of pertussis antibody response and Hib response, infants in group 1 and 2 were divided into those who had a >4 fold increase in anti-PT level from 2 months to 4 months old (Group 1: after 3 Pa doses, Group 2: after 2 Pa doses) and those with a < 4 fold increase in anti-PT level from 2 months to 4 months old. (Table 3.16) Infants in group 1 and 2 combined who had a > 4 fold increase in PT response after the 2 month old dose had a non significantly higher anti-Hib GMC at 8 months old ($p>0.05$) compared to Group 1 and 2 infants with <4 fold increase in PT. The same was seen in group 3 infants. (Table 3.16)

Table 3.16 Association of pertussis antibody (anti-PT) response after Pa vaccination at 2 months old and anti-Hib response

	Group 1 and 2 n= 42		Group 3 N=21	
	< 4 fold PT increase* n=24	> 4 fold PT increase* n=18	< 4 fold PT increase* n=11	> 4 fold PT increase* n=10
Hib	n (%)	n (%)	n (%)	n (%)
<0.15 ug/ml	8 (33%)	1 (6%)	2 (18%)	0
>0.15-1 ug/ml	8 (33%)	11 (61%)	5 (45%)	3 (30%)
>1 ug/ml	8 (33%)	6 (33%)	4 (36%)	7 (70%)
GMC^ (95% CI)	0.50 (0.23-1.06)	0.68 (0.34-1.33)	0.52 (0.21-1.3)	1.02 (0.24-4.30)

^ GMC – geometric mean concentration (EL.U/ml)

*fold increase – refers to >4 or <4 fold increase in anti-PT level between 2 to 4 months old (after 3rd Pa dose in group 1 infants, 2nd Pa dose in group 2 infants and 1st Pa dose in group 3 infants)

Exploration for a similar association with four fold increase in anti-PT responses after the 2 month dose (measured at 4 months old) and hepatitis B responses was also conducted (Table 3.17). Similar to anti-Hib responses infants who had less than a 4 fold increase in anti-PT after the 2 month dose in all groups were non significantly more likely to have lower hepatitis B responses (Table 3.17).

Table 3.17 Association of pertussis antibody (anti-PT) response after Pa vaccination at 2 months old and anti-hepatitis B response

	Group 1 and 2 n= 38		Group 3 N=15	
	< 4 fold PT increase* n=21	> 4 fold PT increase* n=17	< 4 fold PT increase* n=7	> 4 fold PT increase* n=8
Hepatitis B	n (%)	n (%)	n (%)	n (%)
>10-100 mUI/ml	3 (14%)	2 (12%)	0	0
>100-1000 mIU/ml	15 (72%)	10 (59%)	7 (100%)	3 (38%)
>1000 mIU/ml	3 (14%)	5 (29%)	0	5 (62%)
GMC (95% CI)	313.0 (165.2 -593.4)	498.3 (237.9-1043.4)	508.7 (331.6 – 825.1)	1250.2 (519.5 – 3008.5)

^ GMC – geometric mean concentration (EL.U/ml)

*fold increase – refers to >4 or <4 fold increase in anti-PT level between 2 to 4 months old (after 3rd Pa dose in group 1 infants, 2nd Pa dose in group 2 infants and 1st Pa dose in group 3 infants

c) *Cellular immune responses*

Cellular immune responses in infants who received Pa vaccine at birth (Groups 1 and 2) differed markedly from those receiving the standard DTaP schedule (Group 3). Cytokine production profiles in response to *in vitro* stimulation of their PBMC with a mixture of the three major pertussis vaccine antigens or with PT or FHA alone revealed significantly increased levels of TH2 cytokines (IL5 and IL13) in group 1 and 2 infants compared to group 3 infants (Table 3.18). This was not the case for IL6 or the TH1 cytokine IFNγ, where no significant differences were seen between groups.

Table 3.18 Cytokine responses to *in vitro* stimulation of PBMC with vaccine and control antigens at 8 months of age

Stimulus	Group	IL5	IL13	IFNγ	IL6
Mix (PT,FHA, PRN)	1	493 (0, 1008) *[0.008]*	460 (0, 1220) *[0.010]*	8 (0, 92) *[n.s.]*	278 (0, 1562) *[n.s.]*
	2	137 (0, 492) *[0.003]*	193 (0, 644) *[0.004]*	15 (0, 347) *[n.s.]*	147 (0, 1412) *[n.s.]*
	1,2	163 (0, 1008) *[0.001]*	220 (0, 1220) *[0.001]*	11 (0, 347) *[n.s.]*	209 (0, 1562) *[n.s.]*
	3	0 (0, 55)	23 (0, 108)	8 (0, 32)	100 (0, 1947)
PT	1	30 (0, 421) *[n.s.]*	63 (0, 244) *[n.s.]*	0 (0, 211) *[n.s.]*	11 (0, 295) *[n.s.]*
	2	30 (0, 145) *[0.012]*	49 (0, 232) *[0.043]*	0 (0, 21) *[n.s.]*	17 (0, 1044) *[n.s.]*
	1,2	30 (0, 421) *[0.016]*	59 (0, 244) *[0.043]*	0 (0, 211) *[n.s.]*	14 (0, 1044) *[n.s.]*
	3	0 (0, 18)	6 (0, 57)	3 (0, 25)	2 (0, 147)
FHA	1	61 (0, 406) *[0.04]*	137 (0, 452) *[n.s.]*	59 (0, 475) *[0.014]*	44 (0, 4989) *[n.s.]*
	2	60 (0, 553) *[0.016]*	88 (0, 691) *[0.016]*	16 (0, 79) *[n.s.]*	8 (0, 279) *[n.s.]*
	1,2	61 (0, 553) *[0.007]*	102 (0, 691) *[0.011]*	37 (0, 475) *[0.027]*	28 (0, 4989) *[n.s.]*
	3	8 (0, 21)	5 (0, 48)	0 (0, 34)	11 (0, 423)

Stimulus	Group	IL5	IL13	IFNγ	IL6
PRN	1	0 (0, 9) *[n.s.]*	3 (0, 29) *[n.s.]*	0 (0, 0) *[n.s.]*	66 (0, 487) *[n.s.]*
	2	0 (0, 0) *[n.s.]*	0 (0, 21) *[n.s.]*	0 (0, 0) *[n.s.]*	180 (17, 1867) *[n.s.]*
	1,2	0 (0, 9) *[n.s.]*	0 (0, 29) *[n.s.]*	0 (0, 0) *[n.s.]*	123 (0, 1867) *[n.s.]*
	3	0 (0, 40)	0 (0, 0)	0 (0, 0)	10 (0, 425)

Data are median cytokine concentrations in pg/ml (min, max) after subtraction of value for unstimulated cells. *[p value]* = Mann-Whitney U test to compare difference between either Group 1, or Group 2 or Groups 1 and 2 combined, with Group 3. *[n.s.]* = not significant at the 95% level. Mix = mixture of three *Bordetella pertussis* vaccine antigens, pertussis toxoid (PT), filamentous haemagglutinin (FHA) and pertactin (PRN). TT = Tetanus toxoid. PHA = phytohaemagglutinin.

3.4.3 Adverse events

Within the limitations of the small sample size, birth Pa vaccination was well tolerated, with no vaccine-related severe adverse events detected. After the birth dose, only two infants had redness or swelling > 10mm and none had fever >38C. Following the 6 month vaccination, there was no difference in the proportion of infants with swelling or redness >10mm between group 1 (after 5 doses, 17% (n=4)), group 2 (after 4 doses, 14% (n=3)) or group 3 (after 3 doses, 22% (n=4)) (p>0.5). Similarly, the proportion with reported systemic reactions or fever >38C was similar between the groups at 2, and 6 months. (Figure 3.20 and 3.21) There were two serious adverse events. Two infants required hospitalisation for pyloric stenosis, one aged 4 weeks in Group 2 and the other aged 6 weeks in group 3.

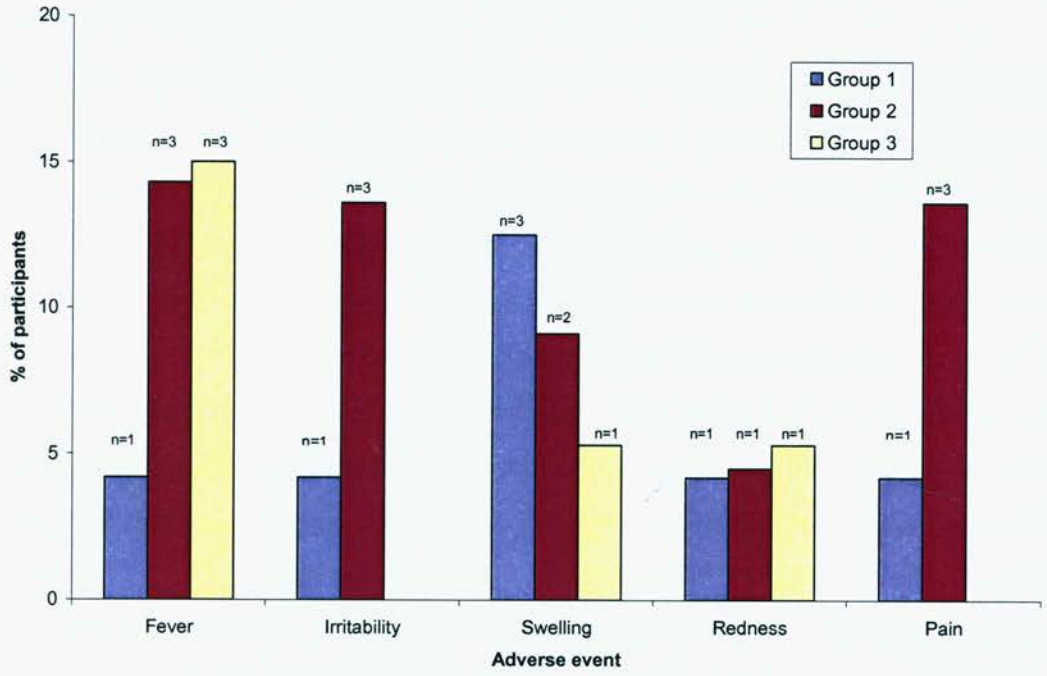


Figure 3.20 Solicited symptoms during 7 day follow-up after 2 month old dose

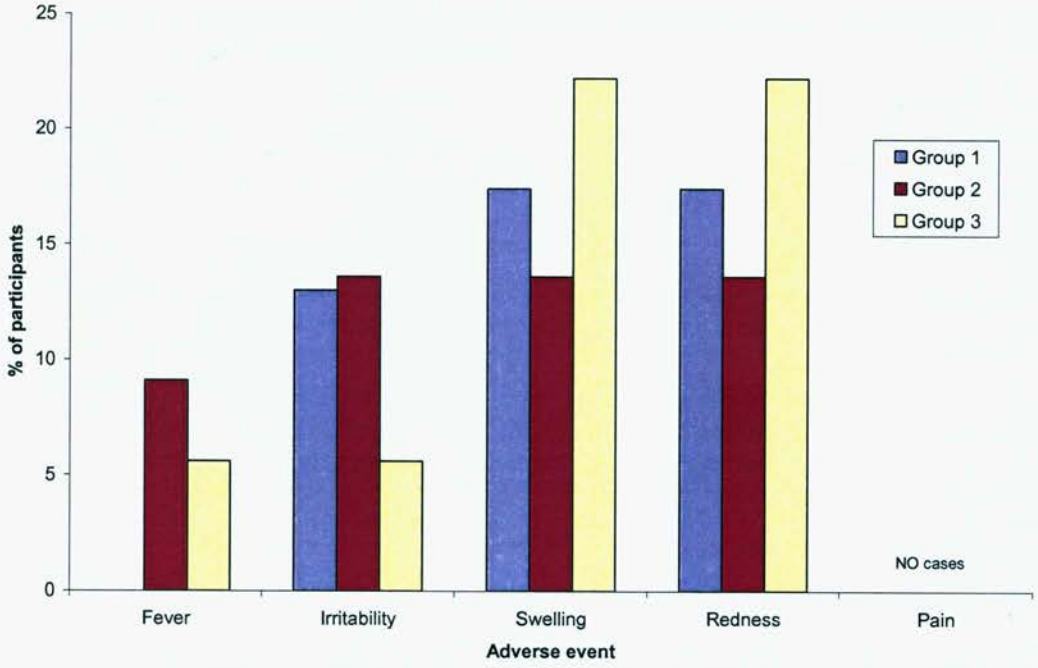


Figure 3.21 Solicited symptoms during 7 day follow-up after 6 month old dose

Pertussis infection

One male infant in group 1 who had received 3 doses of a pertussis-containing vaccine (birth, 1 and 2 months of age) developed symptoms of mild fever, cough and rhinorrhea at 115 days (30 days after the third dose). Pertussis was identified by PCR from a nasopharyngeal aspirate on day 117 but pertussis culture was negative. A maternal aunt with contact with the infant was reported to have a cough consistent with pertussis commencing approximately 14 days before onset of symptoms in the infant, and had positive single titre whole cell IgA on serology. This infant had a mild clinical course and did not require hospital admission. All anti-pertussis antibodies at two months of age, measured after 2 doses and 49 days prior to onset of symptoms were detectable (anti-PT 15 EL.U/ml, anti-FHA 198 EL.U/ml and anti-PRN 39 EL.U/ml). Convalescent anti-pertussis antibodies at 4 months (after 3 doses of Pa and 11 days post diagnosis of infection) increased two-fold for anti-PT and anti-PRN and nearly two fold for anti-FHA. (Table 3.19) Interestingly anti-pertussis antibody levels decreased from 6 months to 8 months after the 5 dose of Pa vaccine.

Table 3.19 Pertussis antibody responses in the infant in group 1 according to age and timing of pertussis infection

Acellular pertussis vaccine dose	Age (days)	Anti-PT EL.U/ml	Anti-PRN EL.U/ml	Anti-FHA EL.U/ml
1	2	7	2.5	7
2	33			
3	68	15	39	198
Diagnosed with pertussis Age – 117 days				
4	128	38	133	361
5	187	51	124	316
2 months post dose 5	250	33	116	174

3.5 Discussion

This is the first study to assess the immunogenicity and reactogenicity of two doses of Pa vaccine (birth and 1 month) given before 2 months of age. The study is also unique in that all infants received HBV vaccine at birth, thus allowing direct comparison of the potential influence of birth Pa vaccine on concomitant hepatitis B responses.

There have now been a total of 4 trials worldwide to date examining Pa vaccines at birth in a total of 327 infants. (Table 3.20) [210,227,228] The trials differ in the vaccines used (Pa – 3 studies, DTPa – 1 study), vaccine manufacturer, timing of blood collection, laboratory used for analysis and only slightly in the vaccine schedules used. (Table 3.20)

Table 3.20 Studies of Pa vaccine at birth

	Italy[210]	USA[227]	Germany[228]	Australia: Thesis
Sample size	n= 91	n= 50	n=110	n=76
Birth vaccine used	Pa	DTPa	Pa	Pa
Manufacturer	Chiron Acelluvax	Sanofi Aventis Daptacel	GSK Monovalent Pa	GSK Monovalent Pa
Pertussis antigen content	PT 5 mcg PRN 2.5 mcg FHA 2.5 mcg	PT 10 mcg PRN 3 mcg FHA 3 mcg Fimbrial 5 mcg	PT 25 mcg PRN 8 mcg FHA 25 mcg	PT 25 mcg PRN 8 mcg FHA 25 mcg
Birth vaccine mean age days (range)	4	3.2 (2-14)	2.9 (2-5)	2.9 (0- 5)
Schedule	3, 5, 11 months	2, 4, 6 months	2, 4, 6 months	1, 2, 4, 6 months
Immunogenicity measurement	0, 3, 5, 6 12 months	0, 6, 7, 17, 18 months	0, 3, 5, 7 months	0, 2, 4, 6, 8 months
Site of ELISA testing	Not stated	Vanderbilt University, US	GSK Biologicals, Belgium	GSK Biologicals, Belgium
Antibody response post birth dose only	Measured	Not measured	Not measured	Measured
Antibody post dose 2 (2 to 3months old)	Measured	Not measured	Measured	Measured
Antibody response at 7-8 months	Measured	Measured	Measured	Measured
Concomitant antigen response measured	Not measured	Measured	Measured	Measured
Cellular immunity reported	No	No	No	Yes

All measured safety and antibodies to PT, FHA and PRN. The German study is most similar in design to this study, as it used the same Pa vaccine and the same laboratory for pertussis serology.[228]

In the discussion I will compare the results of this study with the 3 other newborn Pa trials in the following areas, as well as the potential implications of these results in each area:

3.5.1 Immunogenicity to pertussis

a) Antibody response to the birth dose of Pa vaccine
b) Antibody response to the second Pa dose
c) Antibody response to subsequent doses
d) Results post completion of primary vaccine series and hyporesponsiveness
e) Influence of maternal antibodies
f) Cellular immune response

3.5.2 Immunogenicity to concomitant antigens

3.5.3 Birth Pa vaccine and safety

3.5.1 Immunogenicity to pertussis

a) Antibody response to the birth dose of Pa vaccine

Data on antibody response following a single Pa dose at birth are only available in 2 studies, the Italian and this study.[210] The German study first measured pertussis antibodies at 3 months old after 2 doses (birth and 2 months), while the US study first measured immunity at 6 months old after 3 doses (birth, 2 and 4 months old). In both the Italian study and this one no significant increases were seen in antibody levels after the first dose at birth. In fact in this study there was no significant difference at 2 months old between groups 2 (birth dose only) and group 3 (controls) to all 3 pertussis antigens. The levels in groups 2 and 3 declined from maternal levels at birth to 2 months old, as was shown in the Italian study.[210,227,228]

Implications:

This is the second study to report antibody data on responses to the birth Pa vaccine. The absence of a statistically significant increase in antibody level following the birth dose does not preclude successful priming. The evidence for this is shown in the response to the second dose.

b) Antibody response to the second Pa dose

Despite its small sample size, this study showed statistically significant higher anti-PT, anti-PRN and anti-FHA IgG antibody GMC at 2 months of age in Group 1 infants who received Pa vaccine at birth and one month of age, compared to those receiving Pa vaccine at birth only (Group 2) and those who had not been vaccinated (Group 3). A similar statistically significant higher GMC was seen after the second dose in group 2 infants compared to group 3 infants. The following table shows the response after the second dose in the 3 studies that measured pertussis antibodies (Italian, German, Australian) at this time point. (Table 3.21)

Table 3.21 Geometric mean concentrations of pertussis antibodies after second acellular pertussis vaccine (Pa) dose in birth groups compared to control group at same age

	Italy[210]		Germany[228]		Australia: Thesis		
Age	5 months		3 months		2 months*	4 months	4 months
Pa Vaccine schedule	Birth (n=21)	Control (n=23)	Birth (n=50)	Control (n=48)	Birth and 1 month (n=25)	Birth (n=22)	Control (n=20)
Pa doses received	2	1	2	1	2	2	1
GMC^							
Pertussis Toxin	19.8	6.2	31.0	4.2	16.1	21.1	8.0
Pertactin	26.7	7.9	28.1	15.8	17.3	26.4	12.2
Filamentous haemagglutinin	20.9	3.6	186.4	18.8	87.4	92.2	15

^GMC – geometric mean concentration EL.U/ml

*Sample was collected at 2 months old in these infants after 2 doses of Pa vaccine (birth and 1 month)

The German study also demonstrated a significantly higher GMC of anti-pertussis IgG to PT, PRN and FHA in infants after 2 doses of Pa at birth and 2 months compared to controls.[228] In the earlier Italian study, where a Pa vaccine manufactured by Chiron was given at birth and 3 months of age, higher PT IgG was seen in these infants at 5 months compared to controls.[210]

All these studies suggest a first dose at birth primes the immune system, with a significant increase in antibody after the second dose, whether given at one, two or three months of age. In general, higher antibody levels to PT, PRN and FHA were seen in the German study compared to the other two studies and the reason for this difference is not clear. Observational studies[143,165] suggest some protection against severe pertussis from even one dose, possibly related to rapid antibody production following natural exposure in a primed infant, as is suggested by these immunogenicity results.

In summary, these results suggest successful immune priming occurred following the birth Pa vaccine. Two doses of Pa vaccine, the first as soon as possible after birth and the second four to 12 weeks later, resulted in higher antibody levels compared to infants who had received only one dose at 2-3 months old.

Implications:

This study has made important contributions to the international literature as it is the first study to examine birth and one month old Pa vaccine and significantly this data assists with determining optimal timing of the second Pa dose. The results indicate that administration of a second dose earlier than 2 months is immunogenic. Thus, a dose of monovalent Pa vaccine at birth followed by a Pa combination vaccine at 6 weeks of age should result in similar antibody results. This schedule is more feasible and practical than a second dose at 4 weeks old and current combination vaccines including Pa are licensed from 6 weeks old. In Australia, administration of the second dose at 6 weeks is considered a valid dose by the Australian Childhood Immunisation Register (ACIR). A second dose at 6 weeks of age would be feasible and practical because it aligns with the currently recommended age (6 weeks) for routine maternal postnatal visit and baby checks in Australia and is consistent with the current WHO Expanded Program on Immunisation (EPI) schedule. If pertussis vaccine given at birth was to be included in

the WHO EPI schedule, infants would then receive 3 doses of a pertussis-containing vaccine by 10 weeks of age (0, 6, 10 weeks). At present, most developing countries use whole cell pertussis (Pw) vaccine in combination with diphtheria and tetanus in the primary immunisation schedule and as yet no data exist about the immunogenicity and reactogenicity of Pw alone at birth.

c) *Antibody response to subsequent doses*

Antibody responses to subsequent doses are presented as proportions above a detectable threshold and proportions achieving a four fold increase in antibody level. The latter is influenced by the residual antibody titre present at the time of vaccination, as discussed below.

Proportions with detectable pertussis antibodies

In this study levels of anti PT and anti PRN IgG achieved after 3 doses of a pertussis-containing vaccine at birth, one and two months of age were all above the cut off (5 EL.U/ml) and were similar to those seen with 3 doses administered at 0, 2, 4 or the conventional 2, 4 and 6 months of age (Table 3.22). Pertussis antibody results after the third dose in this study and the other three published studies are shown in Table 3.22. In addition, pertussis antibody levels from the GSK DTPa vaccine that was evaluated in the Multicenter Acellular Pertussis Trial (MAPT) following doses at 2, 4, 6 months[215] are shown in Table 3.22. In the MAPT trial, one month following DTPa immunisation at 7 months old, anti-PT was 54 (46-64), anti-PRN 185 (148-231) and anti-FHA 103 (88-120), similar to the other studies. (Table 3.22)

In this study nearly all Group 1 infants at 2 months old had anti-PT above detectable (88%) and all were above detectable for anti-PRN and anti-FHA. (Table 3.4, 3.5, 3.6) It was not until after the second dose in group 3 infants, aged 6 months, that similar proportions above detectable levels for all three pertussis antigens was seen. All infants in the German study who received the birth Pa vaccine were also above the cut off values (>5 EL.U/ml) for anti-PT, anti-FHA and anti-PRN after three doses.[228]

Table 3.22 Geometric mean concentration of antibodies to pertussis antigens after third Pa dose in birth vaccine group compared to controls after three doses

	Multicentre Acellular pertussis trial [215]	Italy[210]		US[227]		Germany[228]		Australia: Thesis		
Age	7 mo	6 mo	12 mo	6 mo	7 mo	5 mo	7 mo	4 mo	6 mo	8 mo
Pa vaccine schedule	Control n=107	Birth n=23	Control n=21	Birth n=25	Control n=25	Birth n=50	Control n=48	Birth and 1 month n=25	Birth n=22	Control n=20
Pa doses received	3	3	3	3	3	3	3	3	3	3
GMC^										
Pertussis toxin	54	42.5	108.8	12	27	n/a*	61.5	41.3	30.5	40.3
Pertactin	185	116.1	172.1	51	442	n/a*	149.4	44.5	54.8	78.7
FHA	103	45.8	30.8	18	26	n/a*	324.2	177.4	168.7	152.3

^GMC – geometric mean concentration EL.U/ml

*n/a – GMC results at 5 months not published in the German study.

As mentioned in chapter 2 there is some evidence from a household contact study in Sweden where a statistically significant clinical efficacy of 75% (95% CI 0-96%) against typical pertussis when detectable antibody levels to both PT and PRN were present post primary vaccination.[216] In this Swedish study vaccine efficacy increased to 85% when detectable antibody levels to al three pertussis antigens (PT, PRN, FHA) were present. Thus, as infants given the birth Pa vaccine achieved detectable pertussis antibodies earlier than control infants this potentially means earlier clinical protection. However, it is possible that persistence of detectable maternal antibodies could influence the proportion of infants with detectable antibodies at 2 months old.

Proportions with four-fold increase in pertussis antibody level

As discussed earlier the composition of currently available Pa vaccines was determined by detailed immunogenicity studies under the auspices of the US National Institutes of Health (NIH).[215] Despite limited evidence for association between specific levels of individual antibodies and protection against disease, there has been a pragmatic need to measure antibody markers of immune response to Pa vaccines. In NIH sponsored comparative immunogenicity trials for acellular pertussis vaccines, seroconversion to PT and PRN measured by ELISA was defined as a four-fold increase in pre-immunisation ELISA Unit (EU) value, to at least four times the minimum detectable level.[215] The minimum detectable levels in NIH studies were 2 EL.Uml for PT and PRN. In this study the minimum detectable level was 5 EL.U/ml for all three pertussis antigens. In the Italian study the minimum detectable level for anti-PT, anti-FHA was 1.5 EL.U/ml and for anti-PRN 3 EL.U/ml.[210] In the US study the minimum detectable antibody level were anti-PT (2 EL.U/ml), anti-FHA (3 EL.U/ml) and anti-PRN (not specified).[227]

One of the difficulties with using fold increase is that if the baseline is high, significant increase above this level following doses can be difficult to achieve. As discussed in chapter 1, responses to subsequent doses are influenced by the residual antibody titre present at the time of vaccination. Higher residual titres may reduce the fold increase after vaccination and this need to be taken into account when interpreting results.

Using NIH thresholds for comparison, proportions achieving a four-fold increase in antibody from baseline to post 3 doses in this study, MAPT, US, Italian and NIH studies are shown in Table 3.23. Four-fold increase results from the German study were not reported. In this study proportions achieving a four-fold increase in PT and FHA after the third dose were comparable between group 1 infants, aged 4 months and control infants and similar to infants in the MAPT, aged 7-8 months. However, in the US study, proportions achieving a four-fold increase after 3 doses are lower than the other 3 studies in both the birth vaccine (DTPa) and control groups.[210,215,227]

Implications:

There is limited data on immunological correlates of protection against clinical pertussis and no accepted international definition. Possible correlates include proportions with detectable pertussis antibodies and proportion with a four-fold increase in pertussis antibodies.

Proportions with detectable pertussis antibodies

As discussed above one household contact study in Sweden suggested presence of detectable antibodies to all three pertussis antigens conferred clinical protection.[216] All infants in group 1 had detectable antibody to all three pertussis antigens at 4 months old, which was earlier than control infants and suggests earlier clinical protection.

Proportions with four-fold increase in pertussis antibody level

Infants in birth vaccine groups also had similar proportions achieving a four-fold increase in pertussis antibodies after three Pa doses (aged 4 months in Group 1, aged 6 months in Group 2) to older control group 3 infants at 8 months old. Similar findings were seen in the Italian study, while results in the US study were lower.[210,227] This raises the prospect of achieving serological protection, particularly against severe pertussis, at least 4 months earlier than under current vaccination schedules, subject to the caveat that antibody correlates of protection against pertussis disease of different severities in infants have not been clearly established. Using NIH thresholds, birth Pa vaccination resulted in comparable proportions with a four-fold increase in pertussis antibody levels after 3 doses, aged 4-6 months, to control groups aged 8 months.

Table 3.23 Proportion of infants achieving a fourfold increase (seroconversion) in pertussis antibodies from baseline (at birth) after three Pa doses

	Multicellular Acellular pertussis trial[215]	Italy[210]		US[227]		Australia: Thesis		
Age	7 mo	6 mo	12 mo	6 mo	7 mo	4 mo	6 mo	8 mo
Pa vaccine schedule	Control n=107	Birth n=23	Control n=21	Birth n=25	Control n=25	Birth and 1 month n=25	Birth n=22	Control n=20
Pa doses received	3	3	3	3	3	3	3	3
% four fold increase	%	%	%	%	%	%	%	%
Pertussis toxin	90.7	60.9	83.3	13	23	85.7	65	94.7
Pertactin	85	82.6	89.6	43	59	66.7	80	73.7
Filamentous haemagglutinin	83.2	39.5	27.1	26	27	81	70	68.4

This study is important as the additional dose at 1 month in group 1 infants indicates that comparable protection could be achieved at the earliest time point (4 months) studied in the literature.

d) *Results post completion of primary vaccine series and hyporesponsiveness*

In this study antibody results at 8 months old, following completion of 5, 4 or 3 doses of pertussis containing vaccine, are equivalent between study groups 1, 2, 3, however lower than results in the German study where the same vaccine and laboratory were used to measure immunogenicity.[228] (Table 3.24) In this study receipt of 5 doses of Pa vaccine was associated with higher pertussis antibody levels at 8 months old compared to 3 or 4 Pa doses.

There was heterogeneity in pertussis antibody responses. In this and the Italian study, anti-PT responses were lower at 8 months old while anti-PRN and anti-FHA were higher in the birth only vaccine group. (Table 3.24) In particular, the US study found significantly lower pertussis antibody levels in infants who received DTPa at birth persisted to 18 months of age.[227] While levels were lower in the birth vaccine group compared to controls in the US and Italian studies after completion of the primary vaccine series, antibody levels increased from dose to dose in the birth vaccine group. This indicates that infant responses were not 'tolerised' (absent) by the birth Pa vaccine exposure.

Hyporesponsiveness

Hyporesponsiveness, or 'immune paralysis', a concern of early studies,[74] was not seen in this study or in the German study.[228] Equivalent anti-pertussis antibody levels at 8 months with or without a birth dose were reported by both studies. (Table 3.24) Interestingly, despite higher anti-pertussis antibody levels at 2-4 months in these two studies, levels seem to converge between birth Pa vaccine groups and control groups at 8 months old, suggesting that the higher antibody level was short lived. The addition of an extra 1-2 doses, at birth and 1 month old, did not result in a significantly higher anti-pertussis levels at 7-8 months old.

Table 3.24 Pertussis antibody geometric mean concentration at 7-12 months of age after completion of the primary vaccine series

Country	Italy[210]		USA[227]		Germany[228]		Australia: Thesis		
	Birth	Control	Birth	Control	Birth	Control	Birth and 1 month	Birth	Control
Age	12 months		7 months		7 months		8 months		
Number of Pa doses received	4	3	4	3	4	3	5	4	3
Pertussis toxin GMC^	*53.5**	*108.8*	*17**	*27*	60.3	61.5	55	36.4	40.3
Pertactin GMC^	194.8	172.1	*161**	*442*	145	149.4	126.9	99.6	78.7
FHA GMC^	61.6	30.8	25	26	437	324.2	353.0	233.0	152.3
Interpretation	*PT significantly lower in birth group		*PT and pertactin significantly lower in birth group		No significant difference seen		No significant difference seen		

^GMC – geometric mean concentration EL.U/ml

Although it has not been defined as hyporesponsiveness there was a lessening immune response with later doses in the birth group. Following the fifth dose of DTPa in group 1 infants, anti-PT levels only increased in one third of infants, whilst levels increased in 65-79% of infants in group 2 (after 4th dose) and 3 (after 3rd dose) respectively. This most likely relates to the biological feedback phenomenon, discussed in chapter 1, and relates to achieving a 'ceiling' of antibody level designed to protect the body from immune overload due to excessive antibody production.

In summary, two of the recent neonatal Pa vaccine trials (Germany, Australia) demonstrated equivalent levels between groups post completion of the primary vaccination series, while two other studies, Italy and US suggested lower antibody levels in birth vaccinees.[210,227,228]

The reason for the difference between these studies is not clear and may relate to any or a combination of different composition of the pertussis antigens in the GSK (3 component), and Sanofi Aventis (5 component) vaccine, aluminium adjuvant used, or a vaccine interference effect of concomitant diphtheria and tetanus toxoid contained in the vaccine used in the US study.

Vaccine antigen content and aluminium adjuvant

The Sanofi Aventis DTPa vaccine (Daptacel), used in the US study, has a lower antigen content of three pertussis antigens (PT, FHA and PRN) compared to the GSK Pa vaccine used in this and the German study, however only the former vaccine contains fimbrial proteins. (Table 3.20) In addition, reduced immune responses have been seen after use of aluminium phosphate adjuvant (Daptacel in the US study) compared to aluminium hydroxide adjuvant used in the Pa vaccine in this and German studies.[230] However, in contrast an older study by Goerke et al suggested superior responses with DTPw vaccine adjuvanted with aluminium phosphate compared to aluminium hydroxide adjuvant.[208] It seems more likely that vaccine interference, rather than antigen content or adjuvant, is the mechanism for the reduced post primary response in the US study.

Vaccine interference

In the US study, antibody levels were not measured before 6 months of age, so it is not possible to assess whether the birth dose was immunogenic earlier than 6 months old. Siegrist notes lower levels in the US study post completion are unlikely to be due to the inhibitory influence of maternal antibodies, as discussed below, because both groups had similar levels at baseline.[71] One potential reason for the reduced levels post completion of the primary series in the birth DTPa group in the US study is 'vaccine interference', as discussed in Chapter 1. Siegrist defines it as 'the modulation of vaccine responses that result from the concurrent or sequential administration of several distinct vaccines'.[71] It is possible that the addition of diphtheria and or tetanus to Pa in the combination DTPa vaccine in the US trial was responsible for the reduced antibody responses (to diphtheria, PT, PRN and fimbrial proteins) through vaccine interference. Interestingly, the interference was specific for the previously noted antigens as it did not affect tetanus toxoid or FHA responses. The addition of diphtheria and tetanus may have caused interference with antigen presentation or B lymphocyte priming resulting in lower pertussis antibody levels. This is supported by Baraff et al who found lower anti-PT and anti-FHA levels at 9 months in those infants given DTPw at birth compared to controls.[73] Finally, vaccine interference seen in the US study seems to have influenced induction of both naive and memory B cells as not only were levels lower at 7 months they persisted to 17 months and remained significantly lower after a DTPa booster at 18 months.

Implications:

This is the second study to show equivalent levels of pertussis antibodies after birth vaccination and the first to investigate the effect of five Pa doses by 6 months old. The possibility of lower post primary vaccination antibody levels needs to be considered in any vaccine strategy commencing with the first dose at birth. Although the US study showed lower levels resulted from birth DTPa vaccine, none of the three published studies, nor this one, was adequately powered for this end point. In developed countries the epidemiology of diphtheria and tetanus suggests that protection is not required earlier than 2-3 months of age.[126,231] Thus, a birth dose of monovalent pertussis vaccine, without diphtheria and tetanus, is a more likely candidate vaccine for the schedule.

Future Pa vaccine studies need to be adequately powered to detect significant antibody differences and their clinical relevance post completion of the primary vaccine series, as well as research the mechanisms for vaccine interference.

As trials to measure disease endpoints in neonates are infeasibly large, all studies of Pa-containing vaccines given at birth must rely on antibody responses as surrogate markers of protection. Using the difference in proportion of infants with detectable antibodies after 3 doses the following sample size can be estimated. To detect a 5% difference in proportion of infants, who were initially seronegative, and subsequently developed detectable antibodies, at 80% power, a sample size of 280 per group (birth and control) is required.

e) *Influence of maternal antibodies*

Previous studies have shown that high levels of maternal antibodies to pertussis can interfere with subsequent infant responses.[39,53] In this study, post primary vaccination antibody levels in all groups were lower in those infants with detectable maternal antibodies at baseline, however the impact of maternal antibody has not been adequately evaluated, particularly with respect to higher levels of maternal antibody, as the sample size was small and few mothers had detectable antibody.

The US study reports no interference from maternal antibody levels on infant responses to DTPa at 2, 4, and 6 months old, but comments that pre-vaccination pertussis antibody levels were low in birth and control groups.[227] Similarly, the Italian study reported no correlation between maternal antibody levels at delivery and infant antibody levels at 3, 5, and 6 months, although data were not shown.[210] The German study did not report on the influence of maternal antibodies on infant response to Pa vaccine.[228]

The effect of pre-existing maternal pertussis antibodies, particularly higher levels resulting from recent dTpa immunisation, on responses to Pa vaccines at birth or subsequently is uncertain. Data from this study suggest that 2 doses of Pa vaccine (at birth and 1-2 months old) are immunogenic in the presence of detectable maternal antibody, but study subjects did not include mothers immunised with dTpa vaccine.

Implications:

This is the first study to report the possible influence of pre-existing maternal antibody interfering with birth and 1 month old Pa vaccine responses. Larger studies, especially among women with higher pertussis antibody levels following receipt of pertussis-containing vaccine as adolescents or adults prior to pregnancy, are needed. With increasing use of dTpa vaccine as an adult booster in many countries, the potential influence of higher maternal antibodies on both infant pertussis disease and responses to pertussis-containing vaccines will become more important.[3] Future mothers are likely to have higher antibody levels either through receipt of adult or adolescent booster doses as recommendations for their use currently exist in many countries. In NSW, Australia, the state health department has required, since 2007, all healthcare workers with patient contact be given dTpa booster vaccination and non-mandatory guidelines are in place in other jurisdictions. In addition, the Australian Immunisation handbook 2008 recommends that parents planning to become pregnant receive dTpa vaccine prior to pregnancy.[122] Few studies are underway examining the strategy of maternal immunisation during pregnancy. Irrespective of the results of maternal immunisation studies, the question of persistence of adequate antibody in subsequent pregnancies remains.

f) Cellular immune responses

This is the only study to have included detailed study of cellular immune responses following birth Pa vaccination. This pilot study showed, in a sub-sample of infants (n=30), receipt of Pa vaccine at birth resulted in a significant increase in IL5 and IL13 cytokine (TH2) responses to pertussis vaccine antigens at 8 months compared to those who commenced Pa vaccine later. There were no differences in IFNγ and cytokine (TH1) responses to other vaccine antigens between groups. This suggests that birth Pa vaccine results in a TH2 bias, restricted to pertussis responses, compared to controls and raises the question of altered responses following subsequent natural pertussis exposure. In addition, TH2 skewed memory to DTPa antigens following infant vaccination has been associated with an increase risk for injection site reactions subsequent to booster pertussis vaccination when given at 4-6 years old.[232]

Implications:

This is the first study to report on T cell responses after birth Pa vaccination and to document the TH2 bias that resulted. Detailed profiling of infant T-cell immunity in response to neonatal pertussis vaccination needs further investigation. Altered responses following subsequent natural pertussis exposure or increased potential for development of atopic responses are potential negative effects of vaccination in early life which requires evaluation.[94,232]

3.5.2 Immunogenicity to concomitant antigens

Other antigens given together with pertussis antigens in combination vaccines in the 4 studies included diphtheria, tetanus, polio, Hepatitis B and *Haemophilus influenzae* type b (Hib). Only this study and two of the others report concomitant antigen responses. (Table 3.25)

Table 3.25 Comparison of concomitant antigen responses at 7-8 months old between three studies

	Germany[228]		USA[227]		Australia: Thesis	
GMC	**Birth n=53**	**Control n=55**	**Birth n=23**	**Control n=22**	**Birth# n=43**	**Control n=19**
Diphtheria IU/ml	0.923	1.175	1.64	3.0 (p=0.002)	1.7 (n/s)	1.34
Tetanus IU/ml	2.05	2.85	4.25	3.76 (p=0.58)	1.1 (n/s)	1.97
Hepatitis B mIU/ml	310	1684 (p<0.05)*	427	439 (p=0.96)	394.8 (n/s)	821.8
Hib ug/ml	0.942	2.353 (p<0.05)	3.49	1.77 (p=0.14)	0.61 (n/s)	0.8

*In the German study – infants in the control group received 4 doses of hepatitis B vaccine in contrast to the birth group who only received 3 doses of hepatitis B. This is the reason for the significantly lower anti-HBs responses in the birth vaccine group

^GMC – geometric mean concentration

#Birth – results for group 1 (Pa vaccine at birth and one month old) and group 2 (Pa vaccine at birth only) are combined

n/s – not significant

In the US study, infants who had received DTPa at birth had significantly lower diphtheria and pneumococcal serotype 14 antibody levels compared to controls at 7 months old.[227] However, the US study could not comment on other pneumococcal responses as it only measured pneumococcal serotypes 6B, 14 and 23F. In the German study, attainment of anti-PRP IgG antibody responses consistent with short-term protection (>0.15ug/ml) was significantly less after the first 3 doses in the birth compared to control groups (88% vs 98%).[228] In our study, anti-PRP IgG levels appeared to be lower only in infants who received two doses of Pa vaccine prior to 2 months of age but power to detect even a substantial difference was low. Reduced anti-PRP IgG responses have been associated with DTPa-Hib combination vaccines, but this has only been documented as a likely contributor to increased susceptibility to invasive Hib disease in the second year of life in one country, the United Kingdom, leading to introduction of a Hib booster in late infancy.[78] However, it is likely that such a phenomenon following the primary series of vaccination will not be clinically relevant if a booster is routinely given, as is the case currently in Australia.

There was no significant difference in response to diphtheria and tetanus antibody responses. This study did not measure responses to polio or pneumococcal antigens, but no significant difference in polio responses were found in two studies measuring them 1 month post completion of primary vaccination.[227,228] Hepatitis B vaccine (HBV) was only given to the control group in the German study[228] whereas in this study, similar to routine practice in the US and as recommended by WHO, all participants received HBV vaccine at birth. Although a reduced anti HBs antibody GMC was seen in infants receiving Pa at birth, all participants achieved anti-HBs levels above the protective level (anti-Hbs >10 mIU/ml) at 8 months of age.

Reasons for reduced responses to concomitantly administered antigens are not definitively known. One possibility is 'bystander interference.'[81] The first dose of DTPa-HepB-Hib-IPV at 2 months old, in this and the German studies, may have reactivated strong pertussis responses that interfered with the induction of primary Hib responses.[228] Knuf et al speculate that strong secondary T lymphocyte–specific pertussis responses after the first dose of combination DTPa vaccine potentially interfered with induction of CD4+ T-cell help that was specifically required for Hib responses but not

other antigens.[228] There is no definition of what constitutes a strong pertussis response. However, those infants who had > 4 fold increase in anti-PT response after the 2 month old dose (time of first combination DTPa-Hib vaccine), potentially indicating a strong pertussis response, had non significantly higher anti-Hib levels at 8 months, in contrast to those with < 4 fold increase in pertussis antibodies. The higher the fold increase (> 4 fold vs < 4 fold) in anti-PT response the higher the Hib level, in contrast to the above suggested mechanism for bystander interference where strong pertussis responses interfere with Hib responses. The major limitation in this study is the small sample sizes and it is not possible to be absolute about vaccine interference. The exact mechanisms for vaccine interference remain to be elucidated.

The reduced diphtheria responses in the US study possibly relate to vaccine interference generated by the use of DTPa at birth.[227] Interestingly, in the US study responses to Hib were not affected by the use of DTPa at birth, however slightly lower levels of pneumococcal serotype 14, which was conjugated to CRM_{197} were seen and may relate to a form of vaccine interference known as carrier induced epitope suppression, as discussed in chapter 1.

Implications:

In summary, other vaccine antigens co-administered with Pa-containing vaccines include diphtheria, tetanus, polio, hepatitis B and *Haemophilus influenzae* type b (Hib). In three of the recent neonatal Pa vaccine trials the possibility of reduced antibody response to concomitant antigens following birth Pa vaccination was suggested. None of these trials was adequately powered to satisfactorily examine this issue. This is the first study to suggest hepatitis B vaccine responses were lowered by birth Pa vaccination. The immunological mechanism behind such interference is not clear and needs further investigation. A reduced post-primary vaccine response has implications for longevity of antibody, as discussed in Chapter 1. Importantly, recognition of the possibility of vaccine interference means that these cohorts of children should be followed long-term to assess if reduced antibody responses result in reduced protection and to assess their response to routine booster vaccines.

3.5.3 Birth Pa vaccine and safety

a) Local and systemic reactions

Similar to other studies, monovalent acellular pertussis vaccine given at birth was well tolerated with no increase in reactogenicity identified at birth or following later vaccine doses compared to infants receiving the routine vaccine schedule.[210,227,228] Importantly, in this study four doses of a Pa-containing vaccine within 4 months of birth was not associated with any severe systemic adverse events and only two subjects had redness/swelling >10mm.

One participant, who had received 3 doses of a pertussis containing vaccine (0, 1, 2 months), developed laboratory-proven pertussis infection at 3 months of age but this was clinically mild and may have been substantially attenuated by earlier Pa vaccination.

In the Italian trial 'no side effects were observed' in 45 subjects given Pa vaccine at birth. Specifically, no large local reactions were observed after the fourth dose.[210]

In US trial, the birth dose of DTPa was well tolerated, no infant developed a fever >38C and there were no serious adverse events in 25 infants. No statistically significant differences in local or systemic adverse events or serious adverse events were reported in either birth DTPa or control groups.[227]

In the German trial, most similar in design to this study, the birth dose of Pa was also well tolerated, in particular no increased rate of fever with the birth dose was noted.[228]

Large injection site reactions have been reported after multiple doses of DTPa vaccines.[233,234] However, after 4 doses in 4 months in this study only 8% (n=2/24) patients had redness/swelling >10mm. This result is supported by the German study where no increased rate of injection site reaction (redness or swelling >50mm) was seen in recipients of 4 Pa doses by 6 months.[228]

b) Serious adverse events

There were 14 serious adverse events seen in the German study and none were considered to be vaccine related. Seven in the birth Pa vaccine group – acute respiratory infection (2), pneumonia, pharyngitis, gastroenteritis, anal abscess and failure to thrive. Seven in the control group – neonatal ophthalmia, perianal abscess, gastroenteritis (2), neonatal jaundice, pyloric stenosis and failure to thrive.[228] In this study there were two serious adverse events and similarly none were felt to be vaccine related.

Implications:

This is the first study to measure the safety profile of birth and one month old Pa vaccine. To date the 4 Pa trials, including 202 infants given Pa at birth, have not demonstrated an increase risk of local or general adverse events. However, the small sample size necessitates that larger studies be performed to more accurately define the risk.

3.5.4 Study limitations

One of the major limitations of this study is the small sample size. It was designed to gather immunogenicity data on responses after Pa vaccine at birth, in particular two doses before 2 months old, in order to inform the design of a larger trial. The sample size of infants who received Pa vaccine at birth (n=47) is similar to the other 3 trials, however is not powered adequately to confirm the positive immunogenicity data, or detect clinically important levels of vaccine interference or vaccine related adverse events. The reduced antibody response to Hib and hepatitis B in Groups 1 and 2 compared to group 3 was not significant, however this could be due to a Type 2 error. In addition, the loss to follow up, (n=8, prior to 2 month old immunisation), even though the majority were in the control group, also further reduces the sample size and immunogenicity data interpretation.

This study did not include a placebo group, as its use was not justified according to current guidelines for trial conduct (Declaration of Helsinki 2008). The Declaration states 'the benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention".[222] Including a group that received

only Pa vaccine and no hepatitis B vaccine (HBV) at birth would have allowed for assessment of the effect of HBV vaccine on responses to pertussis. However, it was unethical to omit HBV vaccine at birth because it is currently recommended in Australia. It was more important to assess the effect of concomitant Pa and HBV vaccines on HBV response.

Another major limitation relates to immunological correlates of protection for pertussis. In this study birth and one month Pa vaccine resulted in higher anti-pertussis antibody levels at 2 and 4 months than infants receiving the standard schedule. However, whether these higher antibody levels mean improved protection from pertussis is not able to be determined at present. In fact, one infant, aged 3 months, was not protected from pertussis infection despite having received 3 doses (birth, 1, 2 months) of Pa vaccine. The clinical course of pertussis infection in this infant was mild, however not all infants aged 3 months with pertussis experience severe disease.[150] In addition, it is possible that cell mediated immunity (CMI) is more important for protection than antibody levels. The sample size of infants who had CMI studied at 8 months old is not sufficient to be definitive about the TH2 bias seen in the birth Pa vaccine group. There is some evidence that CMI is important in protection from pertussis. Human Immunodeficiency virus infected patients with persistent pertussis infection have been reported and murine studies suggest that IFN gamma has an important role in pertussis clearance. CMI has been found to persist in the absence of pertussis antibodies and one outbreak study in a school in Finland suggested that students with persisting CMI were protected from infection, whereas no correlation between antibody levels and protection was seen.[10]

The subjects in this study were followed to 8 months old and therefore I cannot comment on the persistence of antibody levels into later childhood and the response to the DTPa booster at 4-6 years old. However, ethical approval to measure pertussis immunogenicity at 2 years old and both pertussis and concomitant antigen responses at the 4 year old DTPa booster has been obtained and recruitment has commenced.

At birth, blood was collected from the mother and not from the infant or from a cord sample. It is possible that levels in the infant at birth were significantly higher than maternal levels due to active placental transport. If this was the case in only group 1 or 2

infants at birth, then the higher levels seen at 2 months old compared to group 3 infants may be due to higher maternal immunity and not due to birth Pa vaccine. Group 2 infants had significantly higher anti-PT at birth compared to group 3, however there was no difference at 2 months old, indicating that despite any role of active placental transport immunity waned at least as fast as in group 2 infants. In addition, levels in group 1 infants' increased significantly between birth and 2 months old, consistent with priming from the birth dose and effect of a second dose at 1 month old.

3.6 Conclusion

Nearly three quarters of a century ago, studies attempted pertussis vaccination at birth and in pregnant women to prevent pertussis in early infancy. Current global epidemiological data indicate that pertussis remains a significant problem in early infancy and new strategies are needed.[136] The availability of acellular pertussis vaccines, with reduced reactogenicity, has led to renewed interest in both neonatal and maternal pertussis vaccination. The latter has significant scientific hurdles and few studies are underway examining this strategy. Therefore, direct protection for newborn vaccination was examined in this thesis as it seems a more feasible and promising strategy, and has not been widely examined to date.

3.6.1 Overall summary of neonatal acellular pertussis vaccine trials internationally

Since commencement of this thesis, a total of 202 infants worldwide including 47 in this study, have received monovalent Pa vaccine or DTPa vaccine at birth in 4 studies.[210,227,228]

Despite the varying immunogenicity data referred to above, no severe adverse events related to vaccination have been reported. Interpretation of immunogenicity data from these studies suggest the following conclusions, as outlined in Table 3.26. All the Pa or DTPa birth vaccine studies to date have not been powered to sufficiently determine the immunogenicity of birth Pa vaccination and areas of uncertainty requiring further study remain, as outlined in Table 3.27.

Table 3.26 Summary of immunogenicity results from international trials of acellular pertussis vaccines at birth

Positive immunogenicity data	Negative immunogenicity data
• Birth Pa vaccine successfully primes the immune system • Second Pa dose earlier than 2 months old is immunogenic • No influence of birth Pa vaccine on post primary vaccination antibody levels (2 studies)	• Lower post primary vaccination levels seen following completion of primary immunisation schedule (2 studies) • Additional dose at birth did not result in higher antibody levels post primary vaccine series • Antibody levels post primary vaccine series non significantly lower in infants with detectable maternal antibody at birth (1 study)

Positive immunogenicity data	Negative immunogenicity data
• Maternal antibodies do not influence response to Pa at birth and DTPa vaccines in infancy (2 studies)	• Reduced response to selected concomitant antigens • Cellular immunity skewed towards TH2 bias
Limitations of studies	
• Small samples sizes (n=202) precludes generalisation of the immunogenicity and safety data • Studies differ in vaccines used at birth – DTPa (1 study), Pa (3 component PT, PRN, FHA) Chiron vaccine (1 study) and GSK vaccine (2 studies) • Studies differ in primary immunisation schedule – 2, 4, 6 months (3 studies) and 3, 5, 11 months (1 study) • Studies differ in vaccine used in primary schedule – Acellulovax DTPa (1 study), Infanrix hexa (2 studies) and separate vaccines (Hib (ActHIb), Hepatitis B (Recombivax HB), poliovirus (IPOL) (1 study)) • Studies differ in timing of immunogenicity measurement (Table 3.20) and no definite serologic correlate of protections, rely on surrogate measures • Studies differ in laboratory used to measure immunogenicity (Table 3.20) • This study is the only one to report cellular immune responses • Data on maternal antibody levels at baseline and subsequent influence on infant responses not reported in all studies	

Table 3.27 Areas of uncertainty arising from newborn acellular pertussis trials and proposed trial designs for further research

Summary: Areas of uncertainty	Planned trial design and other considerations
Humoral immune responses (a) Presence or absence of successful immune priming. (b) Presence or absence of hyporesponsiveness.	(a) (b) (c) Larger sample size to allow comparison between responses with greater accuracy. If superiority of birth pertussis vaccine is defined as a reduction of 12% or more in the proportion of subjects lacking antibody (no measurable PRN or PT) compared with controls a sample size of 220 in each maternal arm will have 80% power to detect a reduction of this magnitude at a two-sided alpha level of 0.05. (Sample Power V2 www.spss.com)
(c) Concomitant antigen responses.	(c) Need to include measurement of diphtheria, tetanus, hepatitis B and Hib in study protocol post primary vaccination.
(d) Influence of maternal antibodies on infant response especially in resource poor settings where natural maternal immunity may influence responses.	(d) Include mothers with receipt of dTpa booster within 5 years prior to birth and those with no history of dTpa vaccine in study design. Infants born to mothers, given dTpa in the previous 5 years, are likely to have higher baseline antibody levels and subsequent passive transfer to the neonate. Responses to birth and infant DTPa vaccine in both these groups will allow assessment of the influence of maternal antibody on pertussis responses.

Summary: Areas of uncertainty	Planned trial design and other considerations
(e) Follow up for immune longevity.	(e) Studies should include follow-up to 4 years. allowing assessment of immune longevity and response to booster dose
Cell mediated immunity	Planned studies should aim to include CMI measurement early after 1-2 doses of Pa vaccine and post primary vaccination
Vaccine administration (a) Optimal timing of second Pa dose. (b) Whole cell pertussis vaccine.	(a) Timing of second doses should be between 4-8 weeks old. (b) DTPw is still used in many countries globally. Pw vaccine at birth would be feasible and potentially implementable in resource poor settings. Consideration to including Pw in the study design.
Vaccine safety (a) Adverse reactions following birth Pa vaccine. (b) Adverse reactions following DTPa booster vaccines in later childhood.	(a) (b) Large sample sizes need to be included to give a more accurate picture of adverse events post birth Pa vaccine (b) Studies need to include follow for reactogenicity to 4 years old in Australia because of pre-school DTPa booster
Immunological correlates Determination of immunological correlate of protection for pertussis would assist in interpreting clinical vaccine trial results	Surveillance for coughing illnesses or pertussis infection in study participants and correlation with antibody levels • Measure pertussis hospitalisations in study cohort. • Potentially nasal carriage studies for pertussis PCR and culture could be included. • Large sample size required –similar surveillance study was performed as part of the APERT study in the US and enrolled 2781 subjects for surveillance.[180]

CHAPTER 4: HEPATITIS B IMMUNE LONGEVITY

4.1 Introduction

Hepatitis B virus (HBV) infection is a major global health concern with over 350 million people chronically infected.[235] HBV is transmitted from inoculation or mucosal contact with blood or sexual secretions from a HBV carrier, and newborns can be infected at birth from carrier mothers. Most of the morbidity and mortality associated with chronic HBV infection is due to liver cirrhosis and cancer that occurs decades after initial infection. The risk of becoming a chronic carrier is highest if exposure occurs early in life, and 25% of children who become chronic carriers later die from either hepatocellular carcinoma or cirrhosis.[10,63]

In 1990, the World Health organization (WHO) recommended the introduction of HBV vaccine in all national immunisation programs and that infants born in HBV endemic areas receive HBV vaccine at birth, 1 and 6 months old.[236] The principal aim of this strategy is to prevent mother to child transmission in the peri-partum period, as infection at this age results in the highest risk of becoming chronically infected.[10]

The first countries to introduce mass HBV immunisation programs were Taiwan[237] and in USA (Alaska).[238] Studies in these two areas have shown reductions in chronic HBV infection rates and importantly in Taiwan reduced rates of hepatocellular carcinoma following the introduction of mass HBV immunisation.[239-241]

In Australia, universal HBV immunisation was recommended by the National Health and Medical Research Council (NHMRC) in May 2000. Prior to this recommendation a targeted high-risk HBV immunisation program existed and was implemented at different times in each jurisdiction in Australia. In the 1980s, infants of mothers known to be carriers of HBV were recommended to receive hepatitis B immunoglobulin (HBIG) within 12 hours of birth, as well as HBV vaccine, as they have highest risk of vertical transmission. In 1986, the NHMRC recommended HBV immunisation of 'at risk' infants in addition to infants of known carrier mothers. This included Aboriginal and Torres Strait Islander children and those born to mothers from high-risk groups where at least 2% of the population was hepatitis B surface antigen (HBsAg) carriers.[310]

Monitoring of the impact of this vaccination program continues in Australia and recent HBV notification rates have decreased in children under 15 years old.[126]

In the US and Australia most acute HBV infections currently occur during young adulthood related to sexual exposure or injecting drug use.[63,126,242-244] Therefore, a strategy that focuses on infant vaccination, without later booster doses, needs to ensure that protection lasts into this risk period. The duration of protection afforded by infant HBV vaccination is not definitively known but has important implications for whether booster doses following infant vaccination are needed.[245] At present booster doses of HBV vaccine in later childhood or adolescence following infant vaccination are not recommended in the US, Europe, Australia or by WHO.

Multiple studies have examined the longevity of immunity up to 10 years following birth and infant HBV vaccination, but limited data are available beyond 10 years and no studies have been conducted in Australia.

In this chapter, I present the

- Evidence relating to the importance of maintaining immunity against HBV into later childhood and adult life
- Methods used to measure HBV immune persistence/longevity
- Results of international studies examining HBV immune longevity for more than 10 years after infant HBV vaccination
- Rationale for the long term HBV immune persistence studies in this thesis

4.2 Long term immunity to hepatitis B infection following birth and infant vaccination

4.2.1 Importance of maintaining HBV immunity

Persistence of protection against HBV into childhood, adolescence and adulthood following birth and infant vaccination is important for several reasons. First, following infancy a second period of high risk exposure occurs in adolescence with the onset of sexual activity and in some drug injecting individuals.[244] Indeed, in some high endemic countries the risk period commences in early childhood through horizontal transmission. Occupational exposure for some groups, such as healthcare workers, remains a potential source of infection. Continued migration of people from high endemic countries that have not yet implemented HBV vaccine programs will sustain the HBV carriage rate and are a potential source of infection in the community.[243] Second, long-lasting protection may reduce transmission, with consequent reduction in HBV carriage rates in the community. Third, durable protection means that booster doses are not required. Administration of booster doses is costly and logistically difficult for many countries. Fourth, some countries have introduced both universal infant and catch up adolescent vaccine programs with intention to cease the adolescent program once the infant cohort reached the age for adolescent vaccination, for example Australia and Italy.[246] The adolescent HBV vaccine program in Australia will continue until all children born since 2000 reach adolescence, which for some states and territories will be in 2011. In Italy vaccination of adolescents stopped in 2003 after the first cohort of infants vaccinated in 1991 reached this age.[246] Once adolescent programs are discontinued ongoing protection will rely on immunity persisting after infant vaccination.

4.2.2 Methods used to measure HBV immune persistence

Following 3 doses of HBV recombinant vaccine in infancy over 95% of healthy vaccinees achieve antibody to surface antigen (anti-HBs) levels $\geq$ 10mIU/ml, considered to be the protective level.[14,99,246] Subsequently, anti-HBs levels decline rapidly in the first year and thereafter more slowly such that 10 years post vaccination 10-50% of vaccinees have anti-HBs levels below 10mIU/ml.[100,247-249] The rate of decline of anti-HBs is independent of peak antibody concentration achieved post primary vaccination and seems to be equivalent in most individuals.[99] This means

individuals who start at higher post primary levels will retain antibody for longer periods, as discussed in Chapter 1.

However, decrease in anti-HBs to levels <10 mIU/ml does not necessarily equate with loss of protection following exposure to HBV. This is because immune memory may exist in the absence of detectable or low level anti-HBs.[107,245,250] As discussed in Chapter 1, immune memory and its reactivation involves T and B memory cells, memory cytotoxic lymphocytes and antigen/antibody complexes. It is assumed that the presence of immune memory equates to protection from chronic infection on exposure to HBV.[245]

a) Definition of HBV infection

It is important to define what is meant by HBV infection. The persistence of hepatitis B surface antigen (HBsAg positivity) for more than 6 months following exposure indicates the individual has become a chronic carrier.[10] HBsAg detection in a HBV vaccinated individual is referred to as a clinically significant breakthrough infection. The detection of antibody to hepatitis B core antigen (anti-HBc) alone indicates evidence of past infection. In an individual who has received the HBV vaccine, detection of anti-HBc indicates a clinically benign breakthrough infection. The aims of the HBV vaccine program are to prevent the development of acute symptomatic HBV infection and chronic HBV carriage (persistent HBsAg positivity), thereby preventing chronic liver disease and reduce the pool of chronic carriers and subsequently transmission.[250]

As it is not possible to use anti-HBs levels to measure HBV immune persistence and longevity of protection, other methods are used, including in vitro and in vivo assessments of immune memory as outlined below. Immune memory can be demonstrated in vitro using peripheral blood lymphocytes to measure both T and B cell memory responses, in vivo by measuring anamnestic antibody responses following exposure to HBV antigen and at the population level by cohort studies measuring rates of HBV infection in vaccinated cohorts.

b) *In vitro immune memory to HBV*

In vitro memory to HBV can be demonstrated by measuring the number of memory B lymphocytes retaining the ability to produce anti-HBs following stimulation by HBsAg via the spot-ELISA assay.[96,97,99] T cell memory can also be measured in vitro by isolating peripheral blood mononuclear cells and assessing the proliferative response of T lymphocytes to HBsAg.[98,100] Studies in adults have shown T cell proliferative responses are maintained 10 years after vaccination despite the absence of detectable antibody and seem to correlate with anamnestic anti-HBs responses.[98,100] Studies examining in vitro memory T and B cells are hampered by limited sensitivity of the currently available methods to detect a low frequency of memory cells in circulation in peripheral blood.[101] In vitro memory B and T cell responses are not the subject of this thesis.

c) *In vivo immune memory to HBV*

In vivo memory to HBV can be demonstrated through long term cohort studies monitoring rates of HBV infection (HBsAg and anti-HBc) in those who were vaccinated as infants or by booster HBV vaccine trials.

i) Cohort studies in vaccinated populations

Many long term cohort studies up to 15 years have shown that chronic HBsAg positivity is uncommon in successfully vaccinated individuals, despite evidence in some studies of clinically benign breakthrough infections (anti-HBc positivity).[99,107,248] These cohort studies differ in the length of follow up, type, schedule or vaccine used in the primary vaccine course, HBV prevalence in the country studied, and the frequency of testing for HBV infection. Fitzsimons et al reviewed long term protection studies in vaccinated persons in 2005 and presented data on cohort studies with 15 years of follow up.[248] Since that time several studies with follow up periods to 24 years following vaccination have been published and are summarised in Table 4.1 below. Only two long-term follow up studies in Australia, up to 5 years, have been conducted and included in the table for comparison. In addition, a long-term follow up (10 years) study in Italy has also been included for comparison as it is the longest from a low endemic country.[246] Most of the studies come from higher endemicity countries, such as China, Taiwan, USA (Alaska) and Gambia. (Table 4.1)

Search strategy

A medline and EMBASE search for English language publications between 2002 – 2008 using the following key words was used – hepatitis B, hepatitis B vaccines, immunization, immunization programs, preventive health services, time factors, immunity, immunologic memory, hepatitis B antibodies. Only cohort studies with follow up 14 or more years after infant vaccination were included. The following table summarises results from 20 long-term follow-up cohort studies identified. (Table 4.1)

Table 4.1 Long-term follow up cohort studies 14 or more years after primary infant hepatitis B vaccination and published since 2002*

Author	Country	Study year	Primary vaccine and schedule	Age for primary vaccination	Years of follow up	Number of subjects	HBsAg positivity	Anti-HBc positivity	Comments
Yiu-Kuen But et al[253]	Hong Kong, China	2006	3 groups 1. Recombinant 5ug 2 doses 1 month apart 2. Recombinant 10ug 0,1,6 months 3. Plasma 10ug 0,1, 6 months	3 months to 11 years	22	55	0%	5.5%	
Yuen et al[254]	Hong Kong, China	2002	3 groups 1. Recombinant 5ug 2 doses 1 month apart 2. Recombinant 10ug 0,1,6 months 3. Plasma 10ug 0,1, 6 months	3 months to 11 years	18	88	0%	3.4%	
Young et al[255]	Hong Kong, China	1999	6 groups of 3 doses of plasma derived +/- HBIG	< 1 year	16 years	1112	3.5%	8.9%	
Bialek et al[256]	Federated States of Micronesia	2006	Recombinant vaccine 5 ug at birth, 2.5 ug at 2 and 6 months old	<1 year old	15	105	0%	7.6%	
Lin et al[257]	Taiwan	2001	Plasma derived 5ug at 0,1,2,12 months	<1 year old	14	458	0%	2.4%	Booster doses aged 7 given to 246 participants
Lu et al[258]	Taiwan	1999	2 groups 1. Plasma derived 5ug – 4 doses at 0,1,2,12 months and HBIG 2. plasma derived 5ug 4 doses in infancy	<1 year old	15	191	2.6%	16.2%	
Wang et al[259]	Taiwan	2003	Plasma derived 5ug – 4 doses at 0,1,2,12 months	<1 year old	15-16	540	0%	2.2%	
Su et al[260]	Taiwan	2006	Plasma derived 10ug 4 doses at 0,1,2,12 months	<1 year old	18-19	843	1.4%	4.1%	

Author	Country	Study year	Primary vaccine and schedule	Age for primary vaccination	Years of follow up	Number of subjects	HBsAg positivity	Anti-HBc positivity	Comments
Lu et al[261]	Taiwan	2004	Plasma derived 10ug 4 doses at 0,1,2,12 months	<1 year old	15-17	5981	1.6%	4.1%	Vaccine history of total cohort (n=5981) not available
Ni et al[262]	Taiwan	2004	Plasma derived 10ug 4 doses at 0,1,2,12 months	< 1 year old	15-17	6531	1.5%	2.6%	Vaccination coverage estimated at 83% in this age group
Whittle et al[263]	Gambia	1998-1999	3 groups in total – 3 doses 2 groups given intradermally plasma derived vaccine	1-5 years old	~14	171	2.9%	37.4%	
Van der Sande et al[264]	Gambia		3 groups in total – 3 doses 2 groups given intradermally plasma derived vaccine	1-5 years old	20-24	96	2.1%	26%	
Dentinger et al[265]	Alaskan natives	2001	3 doses of plasma or recombinant derived vaccine 0,1,6 month	< 1 year	12-16 years	334	0%	1.8%	
Samandari et al[266]	Alaskan natives	2001-2004	3 doses recombinant or 3 doses of plasma derived vaccine	< 1 year	10-15	212	0%	0.5%]	
McMahon et al[267]	Alaskan natives	1996	3 doses Plasma derived 10ug	0-4 years	15	246	0.4%	1.6%	
Hammitt et al[242]	Alaskan natives	Not recorded	3 doses recombinant vaccine at 0, 1-3, 6-9 months	<1 year	15	37	Not available	0%	
Zanetti et al[246]	Italy	2003	3 doses of recombinant at 3,5,11 months	< 1 year	10	1212	0%	0.08%	Note: only follow up at 10 years but included as low endemic country

Author	Country	Study year	Primary vaccine and schedule	Age for primary vaccination	Years of follow up	Number of subjects	HBsAg positivity	Anti-HBc positivity	Comments
Da Villa et al[268]	Italy	2006	3 doses of recombinant at 3,5,11 months	<1 year	18	112	0%	2.7%	
Hanna et al[252]	Indigenous Australians Far North Queensland	Not recorded	10ug recombinant at 0,1,6 months	<1 year	2 Range 14 -40 months	96	0%	1%	
Hanna et al[251]	Indigenous Australians Far North Queensland	Not recorded	10ug recombinant at 0,1,6 months	<1 year	5 Range 3.7-7.7 yrs	239	1.7%	6%	

*Includes 3 studies with follow up less than 14 years either because they are Australian studies or from low endemic countries

(i) Two Australian studies by Hanna et al[251,252]

(ii) Italian study by Zanetti et al as it is from a low endemic country[246]

Data from these additional 16 cohort studies, with follow up ≥14 years, published since 2002 and not reviewed by Fitzsimons,[248] continue to support the findings of earlier studies. However, with increasing time since vaccination, the size of the cohort in most studies reduces, making interpretation and generalisation of the results more problematic. In addition, most studies have been conducted in intermediate to high endemicity countries. Interpretation of results from these cohort studies is influenced by several other factors, including maternal HBsAg status, differing ethnicities and associated HLA types, lack of documentation of post-immunisation response, other co-existing infections, especially HIV and nutritional status of the vaccinees.[269] In low endemic countries clinically significant breakthrough infections are rare and therefore it is difficult to use this measurement as a marker of waning immunity.

In Australia, there are no long term (>10 years) cohort studies of children vaccinated as infants. In 1992/1993, Wan et al measured anti-HBs levels in Indigenous children in the Northern Territory (NT), aged 1- 4 years, following 3 doses of recombinant vaccine in infancy.[270] Of 612 children, 61.3% (95% CI 57.3-65.1) had anti-HBs levels >100 mIU/ml. The proportion with anti-HBs sero-positivity, defined as >100 mIU/ml, decreased by 7.8% per year after the first HBV vaccine dose, from 75% sero-positive at 1 year to 52% at 4 years. Large birth weight and increasing time interval between birth and first vaccine dose were good predictors of higher anti-HBs levels. There were regional differences in anti-HBs sero-positivity, with higher proportions in Katherine region (76.8%) compared to Darwin region (52%). There was no explanation of the reasons behind this difference, but as the ages of children in each region were similar it may relate to vaccine storage and in particular cold chain maintenance.[270]

Another study in Far North Queensland followed children up to 5 years after infant immunisation.[251,252] When surveyed at a mean age of 2 years, 54% had retained anti-Hbs levels >10mIU/ml, none were HBsAg positive and only 1 child was anti-HBc positive.[252] The proportions retaining anti-HBs >10mUI/ml at 2 years is lower than other studies, where higher proportions ranging from 74-95% were found 4 to 12 years post infant vaccination.[107] This suggested the possibility of a sub-optimal primary response to HBV vaccine in this cohort. Possible reasons for sub-optimal primary responses include inadequate vaccine transport and storage practices, nutritional status

and HLA genetic determinants.[269,271] On review at 5 years, nearly 60% of 239 Indigenous children had no detectable immunity (anti-HBs<10mIU/ml), 6% were anti-HBc positive and there were 4 cases (1.7%) of chronic HBsAg carriage.[251]

In summary, the longest cohort studies internationally suggest development of clinically significant breakthrough infection (HBsAg positivity) is <5%, no cases of symptomatic acute HBV have been reported[272] and proportions with anti-HBc positivity ranging from 0 to 37.4%. This data suggests long term protection from HBsAg carriage, despite anti-HBs levels declining below 10 mIU/ml. In Chapter 5 two cohorts of adolescents, vaccinated as infants more than 10 years previously, have HBV serology performed to detect clinically significant and benign breakthrough infections, as well as responses to booster HBV vaccine doses, for the first time in Australia.

HBV booster vaccine trials

In the clinical trial setting, measuring antibody responses following a booster dose of HBV vaccine to look for anamnestic response has been studied and is discussed below. As it is not ethical to expose individuals to natural HBV, measuring response to HBsAg, administered as a vaccine, is used to mimic viral exposure and look for immune memory. A rise in anti-HBs level following booster vaccination is considered indicative of immune memory persistence and likely protection following any natural exposure, as discussed in detail below. This is based in part on the known protective effectiveness of administration of passive antibody (HBIG) within 2 weeks of exposure.[95] In addition, long-term follow up of vaccinated children in the Gambia reports those with anti-HBs> 10mIU/ml, some of whom had rises in anti-HBs levels from natural boosting, are less likely to become chronic HBsAg carriers than those with levels <10 mIU/ml.[263] It is important to note that vaccine challenge may not be directly comparable to natural exposure. First, it does not directly mimic the route of exposure (intramuscular injection of vaccine vs sexual or percutaneous live HBV exposure) and second HBsAg, in a vaccine, is administered with an adjuvant used to stimulate an immune response.[95]

Definition of anamnestic response in clinical HBV booster vaccine trials

For most studies an anamnestic (memory) response is indicated by a rise in anti-HBs levels from <10 to >10 mIU/ml following vaccination.[96,99,242,246,266] The use of

proportions achieving a rise in anti-HBs level to >10mIU/ml as evidence of immune memory necessitates that such a rise be sufficiently different to levels achieved after primary vaccination in adolescence. One to 2 months after a single dose of recombinant HBV vaccine, (5ug or 10ug) only 10-30% of vaccine-naive adolescents aged 11-19 years old achieve anti-HBs levels >10mIU/ml.[273] This primary response rate is significantly lower than the proportions (>90%) achieved following a booster dose in those adolescents who have been primed in infancy supporting the above definition.[245]

Other definitions include:

1. Rise in anti-HBs titre to >100 mIU/ml if the previous anti-HBs titre was <10mIU/ml[253]

2. 4 fold increase in anti-HBs titre following vaccination in those with a pre-booster titre of >10mIU/ml[242,256,266,274]

In general, booster vaccine studies measure anti-HBs levels prior to and 1-4 weeks after HBV vaccination. A rapid rise in anti-HBs level following a booster vaccine indicates that an HBsAg-specific pool of memory lymphocytes has been established[99,103,245] and that antibody can be produced fast enough to prevent infection or render it transient if the individual was exposed to HBV. In general, studies that demonstrate antibody rise at the shortest interval (days vs weeks) following booster vaccination provide the best measure of immune memory.[95,99,103]

In summary, in vivo anamnestic responses rely on two criteria. First, level of anti-HBs achieved and second, rapidity of the rise, usually within 4 weeks.

In the two booster vaccine trials in this thesis, anamnestic response was therefore defined as:

1. Rise in anti-HBs levels >10 mIU/ml following booster vaccination in those with a level <10mIU/ml pre vaccination measured within 4 weeks post booster

2. 4 fold increase in anti-HBs level following booster vaccination in those with a pre-booster level of >10mIU/ml measured within 4 weeks post booster (Note: Chapter 5: Study 1 only)

4.2.3. Results of booster vaccine trials in Australia and internationally

In most studies, over 90% of non Indigenous children vaccinated as infants, more than 5-10 years previously, demonstrate anamnestic responses on blood samples collected 1-4 weeks following a booster dose.[245] There have been only two booster vaccine studies in Indigenous children in Australia. Wan et al measured the response following a recombinant booster vaccine in 17 Indigenous children, with pre-booster anti-HBs<100 mIU/ml, in the NT in 1992/1993. Fourteen (82.4%) of the 17 had anti-HBs >100 mIU/ml post booster vaccine.[270] In the only other booster study in Australia, 16% of 113 Indigenous children five years after primary HBV vaccination, with no detectable immunity prior to a booster, failed to respond to a booster dose of the HBV vaccine.[251] The authors concluded that, as the majority (84%) retained immunological memory, booster doses were not required at school entry, however 'whether they require booster doses later in life remains to be determined'.[251] In addition, the authors performed molecular typing for HLA-A, B and DR in a subset of children in this booster study. Non responders were significantly more likely to have the HLA DR 14 allele than responders (56% (95%CI 21-86%) vs 12% 95%CI 1.5-36%) p<0.05).[271] A similar association between HLA DR14 and low response to HBV vaccine has been found in other studies, although the biological mechanism has not been defined.[269,275]

In a similar study in Alaskan Indigenous children, 77% had no detectable immunity 5 years following vaccination with the hepatitis B vaccine from birth and approximately 10% failed to respond to a booster dose.[276] The authors concluded that additional long term studies are needed to determine the duration of protection and the need for and optimum timing of booster doses. In the United Kingdom, a low endemicity country similar to Australia, 50% of adolescents had no detectable immunity 15 years after infant vaccination. Most (89%) responded to a booster dose of the vaccine, however those who did not were more likely to have received HBIG at birth.[277] Approximately one quarter (27%, n=8/29) of children who received vaccine plus hepatitis B immunoglobulin at birth did not respond to the booster compared to 6% (n=2/39) of the

children who only received HBV vaccine at birth. The authors postulated that receipt of HBIG might interfere with the formation of memory B cells and the development of immune memory and should be investigated further.[277]

Fitzsimons et al has summarised the findings of booster vaccine trials internationally.[248] Over 90% of participants responded to a booster vaccine administered 10-13 years after infant vaccination, indicating the majority had retained immune memory which was likely to be protective following natural HBV exposure. The response rate seems to relate to level of antibody achieved post primary vaccination and the antigen dose in the booster. Higher anti-HBs titres post primary vaccination result in higher titres post booster. Since this review, additional booster vaccine trials examining responses 10 or more years after infant vaccination have been published and are summarised in the following table.

Search strategy

A medline and EMBASE search for English language publications between 2002 – 2008 using the following key words was used – hepatitis B, hepatitis B vaccines, immunization, immunization programs, preventive health services, time factors, immunity, immunologic memory, hepatitis B antibodies. Only studies of booster vaccination administered 10 or more years after infant vaccination were included.

The following table summarise results from 15 long term booster vaccine trials (14 of which were not reviewed by Fitzsimons et al).[248]

Table 4.2 Long term follow up studies of booster vaccine administered 10 or more years after infant hepatitis B vaccination and published since 2002*

Author	Country	Primary vaccine and schedule	Age at primary vaccination	Years of follow up	Booster vaccine used	Post booster sample (days)	Number of subjects	Anti-HBs <10 mIU/ml pre booster	Anti-HBs <10 mIU/ml post booster *(NON memory response)*	Comments
Bialek et al[256]	Federated States of Micronesia	Recombinant vaccine 5 ug at birth, 2.5 ug at 2 and 6 months old	<1 year old	15	5ug recombinant	14	96	92.3%	52%	Half subjects no response – may indicate waning immunity
Lu et al[258]	Taiwan	2 groups (a). Plasma derived 5ug – 4 doses at 0,1,2,12 months and HBIG (b). plasma derived 5ug 4 doses in infancy	<1 year old	15	20ug recombinant	28	(a) 77 (known primary vaccine responders born to HBV carrier mothers) (b) 109 (unknown primary responders)	(a) 29.9% (b) 62.4%	(a) 2.6% (2/77) (b) 2% (2/109)	
Su et al[260]	Taiwan	Plasma derived 10ug 4 doses at 0,1,2,12 months	<1 year old	18-19	20ug recombinant	28	316	100%	25% (78/316)	Vaccine records verified
Lu et al[261]	Taiwan	Plasma derived 10ug 4 doses at 0,1,2,12 months	<1 year old	15-17	20ug recombinant	28	872	100%	29.2%	Vaccine history of 4 infant doses confirmed

Author	Country	Primary vaccine and schedule	Age at primary vaccination	Years of follow up	Booster vaccine used	Post booster sample (days)	Number of subjects	Anti-HBs <10 mIU/ml pre booster	Anti-HBs <10 mIU/ml post booster *(NON memory response)*	Comments
Samandari et al[266]	Alaskan natives	3 doses recombinant or 3 doses of plasma derived vaccine	< 1 year	10-15	5ug recombinant	14 28	212	65%	24% (14 days post) 20.5% (28days post) Less memory response in those given plasma vaccine in infancy	Increased response in children aged 5-7 years may mean waning immune memory in adolescents
Hammitt et al[242]	Alaskan natives	3 doses recombinant vaccine at 0, 1-3, 6-9 months	<1 year	15	5ug recombinant	10-14 28	37	95%	49% 38%	Half subjects no response – ?indicate waning immunity
Zanetti et al[246]	Italy	3 doses of recombinant at 3,5,11 months	< 1 year	10	10ug recombinant	14	342	100%	3%	low endemic country
Patel et al[278]	Palau	3 doses recombinant at 0,1,6 months	<1 year	10-12	recombinant	14	75	100%	11%	Highly endemic area
Lin et al[269]	Taiwan	Plasma or recombinant at 0,1,2,12 months	<1 year	15-18	20ug recombinant	56	304	100%	19% (59/304)	

Author	Country	Primary vaccine and schedule	Age at primary vaccination	Years of follow up	Booster vaccine used	Post booster sample (days)	Number of subjects	Anti-HBs <10 mIU/ml pre booster	Anti-HBs <10 mIU/ml post booster *(NON memory response)*	Comments
Petersen et al[276]	Alaska natives	3 doses plasma derived vaccine	<1 year	12	2.5ug or 5ug recombinant	56	17 (maternal HbsAg neg) 16 (maternal HBsAg pos)	76% 69%	33% 10%	Small sample size
Boxall et al[277]	United Kingdom		<1 year	6-18	recombinant	28	64 (primary response known) 52 (primary response not known)	52% 46%	11% 23%	Suggestion that HBIG at birth reduced memory response
Jafarzadeh et al[279]	Iran	Recombinant vaccine (10ug) at 0,1,9 months	<1 year	10	Recombinant (Heberbiovac)	28	94	74.5%	4.3%	
Wang et al[280]	Taiwan	plasma or recombinant 4 doses at 0,1,2,12 months	<1 year	16	20ug recombinant		395	100%	22.8%	Cigarette smoking and Betel nut chewing associated with reduced booster responses

Author	Country	Primary vaccine and schedule	Age at primary vaccination	Years of follow up	Booster vaccine used	Post booster sample (days)	Number of subjects	Anti-HBs <10 mIU/ml pre booster	Anti-HBs <10 mIU/ml post booster *(NON memory response)*	Comments
Saffar et al[281]	Iran	Recombinant 3doses at o,1.5 and 9 months	<1 year	10	10ug recombinant IM (n=57) 5ug recombinant IM (n=52) 2.5ug recombinant intradermally (n=56)	14	57 52 56	100%	5.3% 10.7% 21.4%	Intradermal booster response less than intramuscular
Hanna et al[251]	Indigenous Australian	10ug recombinant at 0,1,6 months	<1 year	5 Range (3.7-7.7 yrs)	10ug recombinant	21-28	113	100%	16%	

*Includes single Australian vaccine booster study published[251]

Summary of long term follow up booster studies

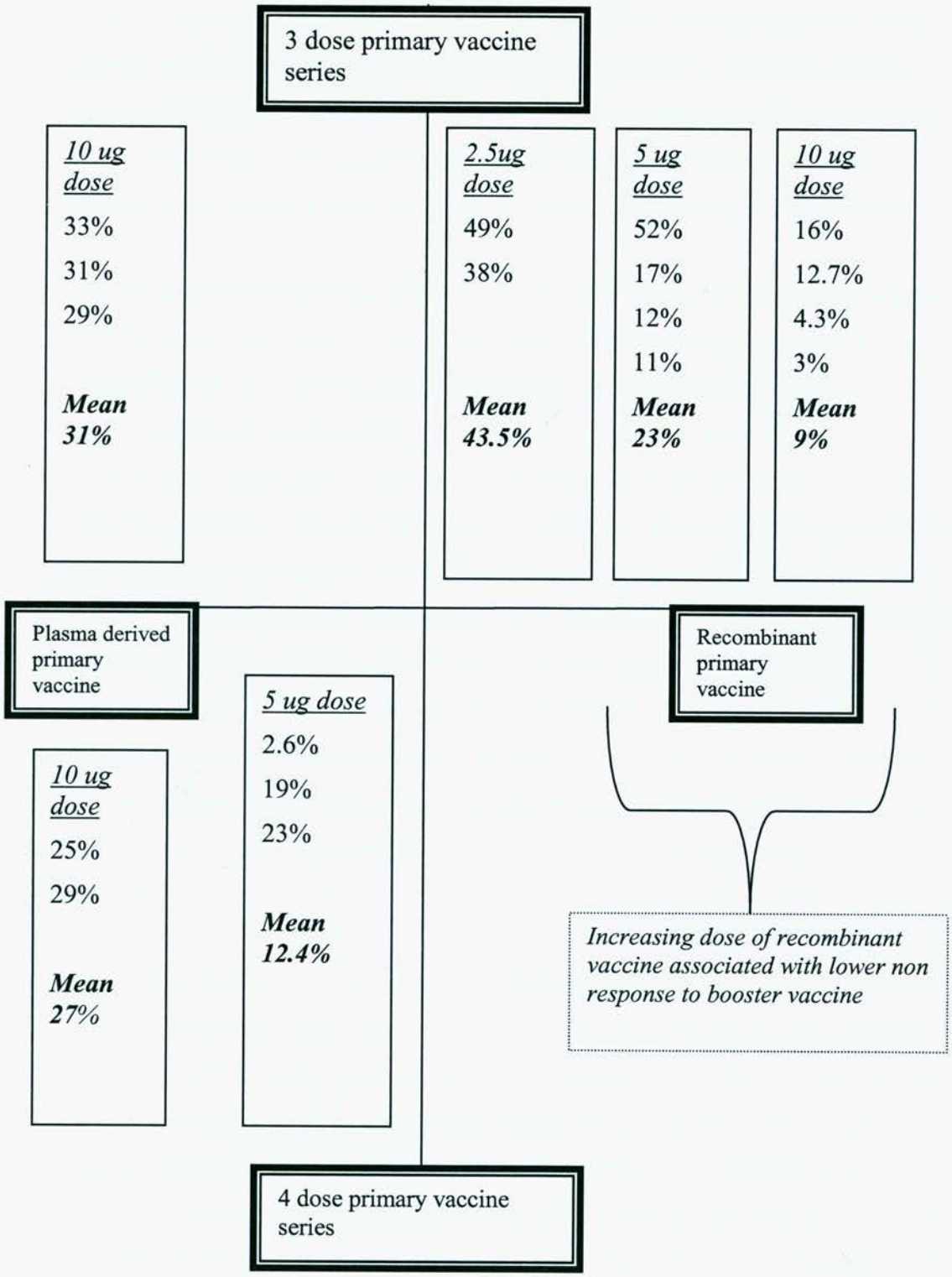
The studies in Table 4.2 differ in setting (country and HBV endemicity), primary vaccine type used (recombinant or plasma derived HBV vaccine), number of doses in primary vaccine schedule (3 or 4 doses) and years of follow up, as is discussed further below. There was considerable variation in the range of absent memory (anamnestic) responses (2 to 52%).

4.2.4 Comparisons between booster vaccine trials

The design of the two booster studies in this thesis was informed by understanding the differences, strengths and weaknesses of previous studies, as discussed below.

a) Vaccine and schedule factors

Number, type (plasma or recombinant), antigen dose and schedule of vaccine used in primary vaccine schedule. All these factors influence the primary vaccine response and subsequently immune memory generation. Higher antigen doses during the primary HBV series equate with higher post-primary anti-HBs levels[103,247,265,282] and this is associated with better memory responses. Lower antigen doses in the recombinant vaccine used in the 3 dose primary vaccine series seem to be associated with higher non memory response. In contrast, there is no correlation with antigen dose in plasma derived vaccines. (Figure 4.1)



Notes: Each % value refers to the proportion of non responders following a HBV booster vaccine, according to type and antigen content of the vaccine used in primary vaccination. Individual results are from studies in Table 4.2. Mean values are calculated from individual values in each box.

Figure 4.1 Absent anamnestic response to booster vaccine according to type and number of doses of hepatitis B vaccine used in primary vaccine series

- *Maternal HBsAg status*. Infants born to HBsAg carrier mothers may have their immunity boosted naturally through household exposure in contrast to infants born to HBsAg negative mothers. Natural boosting will influence the persistence of anti-HBs levels,[253,263] most likely through memory cell persistence, and therefore may result in better anamnestic responses following booster vaccine.

- *Concomitant administration of birth HBV dose with hepatitis B immunoglobulin (HBIG).* Passive antibody (in this case HBIG) administered at the same time as antigen (in this case HBsAg) may suppress the primary immune response by masking epitopes, forming antigen/antibody complexes or inhibiting B cell responses, as discussed in Chapter 1.[277] Mouse models predict better immune responses if antigen is in excess of passive antibody, however in the case of HBIG administration, antibody is in excess of antigen and immune responses may be reduced.[248,277] Despite this a meta-analysis reported HBV vaccine and HBIG to be 85-95% effective, higher than HBV vaccine alone (approximately 70%), in preventing chronic HBsAg carriage in infants born to HBV carrier mothers.[14] HBIG at birth may interfere with the generation of memory B cells,[277] and more studies are needed to confirm the long-term efficacy of HBIG and HBV vaccine in humans, but data suggest efficacy against chronic HBsAg carriage is not reduced.

- *Type, use of adjuvant or not, dose and method of administration of HBV booster vaccine.* In general, recombinant vaccines, of differing dosages (2.5ug to 20ug), were used in the booster vaccine studies and administered either intramuscularly or intradermally. Use of a recombinant booster vaccine in those given plasma vaccine in infancy may result in reduced memory response. Samandari et al suggested those given plasma derived vaccine in infancy responded less well to a recombinant booster vaccine.[266] (Table 4.2) In another small study reduced responses were seen in those participants given an intradermal booster compared to an intramuscular booster.[281] Anamnestic responses have also been shown following administration of a non-adjuvanted HBV vaccine to adults, a closer approximation to natural exposure.[283] In this study of adults, low dose (0.6ug HBsAg) elicited lower anamnestic response rates compared to 10ug and 20ug

HBsAg doses. It is not clear which method of administration of booster dose most closely mimics natural HBV exposure. However, it is likely that the mode of administration and presence of adjuvant are not the important determinants of whether an anamnestic response is seen but rather presence of HBsAg.

b) Measurement of anti-HBs

- *Length of follow up*. Increasing time since vaccination equates with increasing possibility of loss of immune memory. Samandari et al showed that children aged 5-7 years had better memory responses than adolescents at 14 years of age.[266]

- *Method and quantitation of anti-HBs measurement*. Most anti-HBs measurements are performed using radioimmunoassays in commercially available ELISA kits, for example, AxSYN AUSAB or ETI-AB-Corek.[284] The level of pre booster anti-HBs is not fully quantified in all studies. In some, anti-HBs concentrations are reported dichotomously as <10 mIU/ml or > 10mIU/ml. In others, anti-HBs levels are defined as <0.1, 0.1-0.9 and 1-9.9 mIU/ml. Pre-booster antibody at the lowest detectable level of 0.1 mIU/ml seems to better equate to lower booster responses than a broad category of < 10mIUml, as shown in several studies.[246,260,266,285]

- *Timing of post booster anti-HBs measurement*. Studies suggest that the earliest antibody begins to rise is 3-5 days post vaccine.[95,103] In general, most studies measured anti-HBs levels 14 to 28 days after booster vaccination.

c) Study design and conduct

- *Post primary seronconversion rates in the cohort*. Some studies report post primary seronconversion (proportion anti-HBs>10mIU/ml) rates in the cohort, while in others the proportion with protective levels following primary vaccination is not known. There are difficulties in comparing booster responses in those who were known to have responded to the primary series versus those in whom primary response is not known. Differences in the booster response rates seem to relate to the degree of primary response. Several studies have shown that a higher primary response equates to a higher booster response.[246,278,280] In studies where the primary response was not measured, interpretation of a low booster

response in subjects with a pre-booster level of < 10mIU/ml is problematic and may not be due to waning immune memory but because no primary response was achieved.

- *Subject administration of HBV vaccine.* The studies also differ in whether HBV booster vaccine was given only to those with pre-booster anti-HBs <10mIU/ml or all available vaccinees, including those with anti-HBs levels >10mIU/ml at the time of booster. This has implications in defining the anamnestic response and interpreting the anamnestic response rate. One of the definitions of a satisfactory anamnestic response is a 4-fold increase in antibody level post booster. Achieving a 4-fold increase post booster is more difficult in those with pre-booster levels >10mIU/ml because the residual vaccine antibody can form antigen/antibody complexes which reduce the antigen available for B cell binding and limit the amount of antibody produced in response to a booster vaccine to below a 4 fold increase, as discussed in chapter 1. However, despite this many studies report 4-fold rises in antibody level following a booster response as a valid measure of anamnestic response.

- *Rates of HBV endemicity.* Different rates of HBV endemicity (low <2%, intermediate 2-7%, high >8%) exist in countries where study participants are enrolled. Living in a highly endemic environment will result in a greater likelihood of 'natural boosting' and mean that subjects may retain anti-HBs for a longer period and have better memory responses.[263]

- *Vaccination history.* The studies differ in the degree of completeness of vaccination records. Some have strict inclusion criteria. For example, subjects must have received 3 doses in infancy according to the vaccine regimen in use in the country at the time. For others the definition of infant vaccine history is not specified and the degree of completeness not discussed.

4.2.5 Summary: Areas of uncertainty

Long- term protection in those infants who responded to the primary HBV vaccine series appears to last up to 15 years, even if anti-HBs levels become undetectable. This

long term protection relies on immune memory. More recent studies suggest that with periods of time since vaccination of > 15 years immune memory may wane, although rates of clinically significant breakthrough infections (HBsAg positivity) remain < 5%. WHO, US Centers for Disease Control and international collaborations, such as the European consensus group recommend that further long-term (>15 years) protection and booster studies in high and low HBV endemic countries be conducted.[63,245,248] Overall, the low rate of HBsAg positivity found in cohort studies of adolescents and adults vaccinated as infants supports a policy of no additional boosters in both high and low endemicity countries, particularly in those who are immune competent and seroconverted (anti-HBs>10mIU/ml) after the primary vaccine series. In Chapter 5 of this thesis in vivo memory responses to HBV vaccine in 2 cohorts of adolescents (>10 years old) are examined for the first time in Australia.

4.3 Long term HBV immunity in Australia

In order to interpret results from the long term HBV immune persistence studies in Chapter 5, an understanding of HBV epidemiology and vaccination programs in Australia is required.

4.3.1 Epidemiology of hepatitis B in Australia

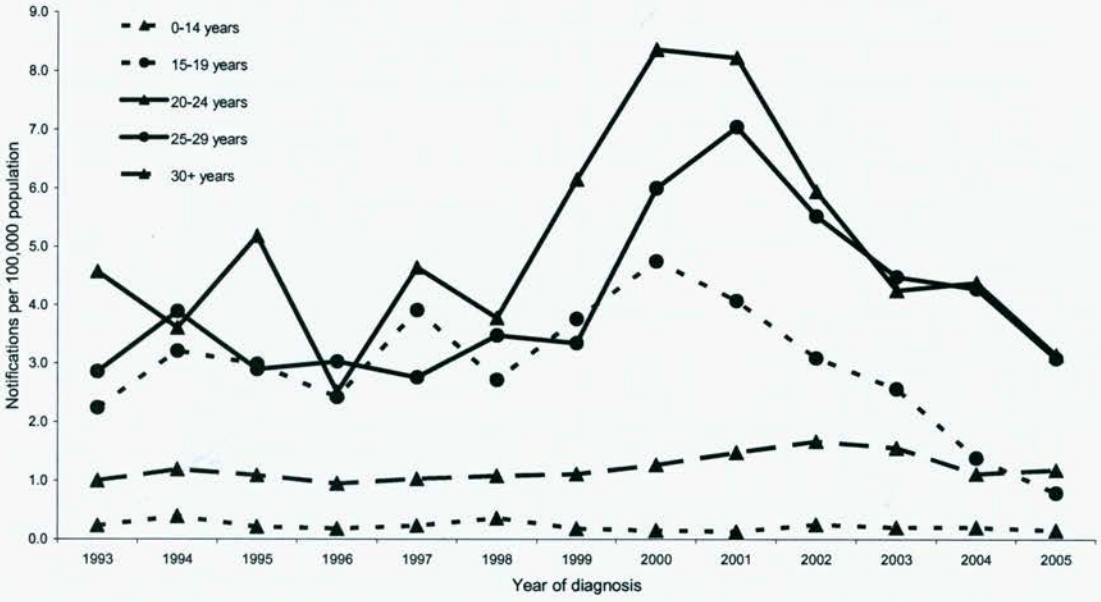
The epidemiology of HBV in Australia is measured through notifications, mandated under public health legislation, hospitalisations and serological surveys. Diagnoses of newly acquired HBV infections are notifiable in all Australian jurisdictions. Cases notified by laboratories, clinicians or hospitals in jurisdictions are complied in the National Notifiable Diseases Surveillance System and population rates of HBV infection by age group are calculated using population estimates from the Australian Bureau of Statistics. In some cases, Aboriginal and Torres Strait islander status is reported along with the HBV notification and this allows estimated rates of Indigenous notification rates to be calculated. Notifications refer to acute HBV infections only and are dependent on levels of HBV testing and reporting.[126,243] Vaccination status is not reliably reported in HBV notifications for persons born before 1996, for which data are not available through the Australian Childhood Immunisation Register. Few large-scale population level serological studies (serosurveys) of HBV prevalence (HBsAg positivity) have been undertaken, particularly in children.

WHO defines communities with a prevalence of chronic HBV infection (HBsAg positivity) of >8% as 'high risk'. The prevalence in Australia is generally low, however specific groups within the community account for the majority of cases.[286,287] This includes immigrants and second/third generation descendants from high risk regions, such as Asia-Pacific countries and Indigenous Australians.[287,288] As a result, Indigenous infants and those born to mothers from high endemicity countries were the first two groups targeted for HBV vaccination as infants in Australia. The epidemiology of HBV at a national level and in these 2 groups is summarised below.

a) National data

Notification rates: The national annual acute HBV notification rate peaked at 2.2 per 100 000 in 2001 and since then has declined to 1.45 per 100 000 persons (2003-2005).[126] (Figure 4.2)

The highest rate was in 20-29 year olds, while rates in 15-24 year olds have significantly declined since 2001.



- Notifications where the month of onset was between January 1993 and December 2005.

Figure 4.2 Acute hepatitis B notification rates, Australia, 1993 to 2005,* by age group[126]

A possible reason for the decline in this latter age group may relate to reducing intravenous drug use since 2000, consistent with trends in hepatitis C notifications, and also the presence of HBV immunised individuals in this age cohort.[126]

Hospitalisation rates: Hospitalisations coded as acute hepatitis B have declined since 1993 and since 1999 seem to have stabilised at a national rate of 0.8 per 100 000.[126]

Serosurveys: National serosurveys estimate a HBV chronic carriage proportion ranging from 0.7% to 5.5%. [287,289] A large serosurvey of adult endoscopy patients (n=2112) in

Sydney between 1999 and 2001, with a median age of 52.5 years, found chronic carriage in 2.1%.[290]

b) Indigenous population

The current epidemiology of HBV infection in Indigenous communities in Australia is inadequately understood. Most studies conducted are population based serosurveys and few have correlated known infant vaccine history with HBV infection rates.

Pre HBV vaccination: Several studies have shown a high prevalence of HBV infection in Aboriginal and Torres Strait Islander Peoples in areas of Australia where a high proportion of the population was Indigenous prior to the commencement of HBV immunisation. (Table 4.3)

Table 4.3 Hepatitis B infection markers in Australian Indigenous populations prior to universal hepatitis B vaccination

Author	Survey year	Location	Age years	Sample size	HBsAg positvity %	Seropositve* for HBV infection
Barrett[294]	1961-1971	Northern Territory Queensland	Children	731	3.4%	n/a
Barrett [291]	1968-1974	Northern Territory	0-10 11-20	376 283	9.6% 13.1%	n/a
Burrell et al[295]	1979-1982	South Australia	10-14 15-19	29 63	23% 23%	72%
Britton et al[296]	1983	Sydney, NSW	Adult	67	9%	54%
Campbell et al[292]	1985	Brewarrina, NSW	0-4 5-9 10-14 15-19	49 73 73 45	23% 19% 29% 22%	59% 55% 80% 87%
Mathews et al[297]	1987	Northern Territory	>16	73	28%	64%
Gill et al[298]	1989	Western Australia	4-9 10-14	91 162	4.5% 6.1%	22% 31%
Gardner et al[299]	1990	Northern Territory	10-15 (rural) 10-15 (urban)		12% 1%	55 19
Gardner et al[293]	1989	Northern Territory	9-17	439	8.2%	41.7%

*refers to detection of anti-HBs or anti-HBc or HBs Ag and reflects natural infection.

One of the earliest reports was by Barrett in 1976, where hepatitis B antigen prevalence was 8.5% in the Northern Territory.[291] This report included hepatitis B antigen prevalence in individual Indigenous communities, such as Maningrida (12.8%), Bathurst Island (3.2%), Yuendemu (12.1%), Papunya (13.3%) and Alice Springs Hospital (10%).[291] In Brewarrina NSW, in 1985, 69% of 0-19 year olds had evidence of prior infection (anti-HBc, anti-HBs positive) and there was a overall chronic HBV carriage rate (HBsAg positive) of 19% with higher rates in certain age groups, such as 10-14 year olds (29%).[292] In this study, 23% of 0-4 year olds were HBsAg positive, most likely reflecting vertical transmission.[292] In another study in the Northern Territory, in 1989, the carriage rate in Indigenous children aged 9-17 years was 8.2% overall, with the highest rates in more remote rural areas.[293] In summary, HBsAg prevalence prior to HBV vaccination, varied between Indigenous communities in Australia, but was likely to be >8% and therefore meet WHO criteria of high HBV endemicity.

Post HBV vaccination; Since the introduction of national HBV vaccination program, the notification rate of newly acquired hepatitis B infection in the Aboriginal and Torres Strait Islander population has doubled from 3 per 100 000 in 2002 to 7 in 2006.[300] In particular, most of the increase was in the age group 20 – 29 years, where the rate doubled from 4.8 in 2002 to 11.2 in 2006. However, the rate in the 13-19 year age group remained stable at 7 per 100 000. The rates were higher in Indigenous males than females.[300]

Several serosurvey studies have been conducted since the introduction of HBV vaccination. The prevalence of chronic HBV carriage among Indigenous Australians varies according to place of residence, with estimates varying from 2% for urban Indigenous populations to 8% for rural Indigenous populations.[287]

Recent prevalence of HBV carriage in 15-19 year olds in the Northern Territory was estimated at 0.8% in sera collected as part of a national serosurvey (1996-1999) while 28% were anti-HBc positive. However in this serosurvey Indigenous and vaccination status were not known but the proportion of Indigenous people was presumed to be high, based on area of residence.[301] In a study by Schultz et al, 3.7% (36/973) of

Indigenous women in the NT, who had HBsAg measured as part of antenatal screening in 2003-2005, were identified as chronic carriers, compared to 1% in non indigenous women.[302] In another study I conducted, a carriage rate of 5.5% (n=522) was found in NT Indigenous women, aged 15 to 39 years, who had HBV serology performed as part of routine antenatal screening.[303] As the vaccine history in these cohorts is not known it was not possible to estimate long term protection.

There are no current data on the prevalence of hepatitis B infection in Indigenous children or children from other high risk ethnic communities in Australia which have been correlated with vaccine history. One of the cohort studies in Chapter 5 provides data on long term protection in Indigenous adolescents by correlating HBV infection, defined serologically, with vaccine history.

c) Adolescents from high endemic region origin

Infants born to mothers from high endemicity regions, such as Asia, Pacific Islands, Mediterranean, Africa and Middle East, were also targeted for birth and infant HBV vaccination, as the risk of vertical (perinatal) and horizontal (household) exposure in these groups was high.[286] The prevalence of HBV infection in immigrants from these regions is similar to the prevalence of HBV in their country of origin.[287] In 2007, the age-adjusted rate of hepatitis B notifications (acute and chronic) in NSW was 38.9 per 100,000 population. The highest rates were in people aged between 15 and 44 years (63.9 per 100,000 population). For the period 2005 to 2007, the highest rates were in the Sydney South West region (77.0 per 100,000 population) reflecting the high number of people born overseas who live in this area and may have contracted HBV in their country of origin.[304]

Few seroprevalence studies have been conducted among selected other ethnic populations in Australia but have been recommended.[243] Burgess et al identified chronic carriage in 1.9% of 2883 primary school children, including a range of low to high risk groups in 1993.[305] Carriage rates in pregnant women from 'at risk' countries in Central Sydney Area Health Service in 1996-1999, varied from 0.5% to a maximum of 5.4% in South East Asian women.[287] A study by Caruana et al found HBsAg positivity in 8-9% of south east Asian immigrants, many of whom did not know their HBsAg status.[306] In

south western Sydney between 1999 and 2002, Maher et al found an HBV carriage rate of 2.7% in injecting drug users, aged 15-47 years, of whom half were from non-English speaking backgrounds.[307]

d) Risk factors for HBV exposure in adolescence

Adolescents who are Indigenous or from high endemicity regions are at significantly increased risk of HBV exposure in later life for several reasons, including engaging in high risk taking behaviours and other social/contextual factors. A report by the Aboriginal Health and Medical Research Council, *Increasing Access to Services in New South Wales for Aboriginal People at Risk of Contracting or Who Have Blood Borne Infection* (2004), identified the following additional risk factors for Indigenous adolescents.[308] These include:

- High proportion of youth in the Indigenous population
- High levels of incarceration
- Mobility of Indigenous people
- Low level of knowledge of blood-borne viruses
- Increase in injecting drug use
- High level of sexually transmissible infections
- High level of violence in some Indigenous communities
- Practices such as non-sterile tattooing and body piercing

Some of the above additional risks also apply to adolescents from high endemicity regions, who have been shown to have poor knowledge of and lower testing rates for blood borne viruses, high rates of injecting drug use, lower socioeconomic status and difficulties accessing health services.[243,307,309]

In summary, although national HBV notification rates have declined since 2001, the highest rates remain in young adults and specific groups in the community, such as Indigenous people and persons from high endemicity regions. These two groups have additional socio-demographic risk factors for HBV infection. Therefore, an assessment

of the long-term protection from HBV vaccination in infancy in Indigenous and 'at risk' adolescents is important.

4.3.2 Hepatitis B vaccination program in Australia

HBV vaccination in Australia commenced in the 1980s, targeting infants born to carrier mothers. (Table 4.4) In 1986, the NHMRC recommended HBV immunisation of 'at risk' infants, in addition to infants of carrier mothers. This included Aboriginal and Torres Strait Islander children, and those born into groups where at least 2% of the source population were hepatitis B surface antigen (HBsAg) carriers. This policy was implemented at different times in each jurisdiction in Australia. In 1985, the Northern Territory (NT) introduced HBV screening for all pregnant women, with vaccination (and HBIG administration) of newborns born to carrier mothers. In 1990, HBV vaccination was introduced in the NT for all Aboriginal and Torres Strait Islander infants and others considered at high risk, such as those born to families from South East Asia. In 1990, universal infant HBV immunisation commenced in Northern Territory. In 1987, the first NSW Health Department Policy regarding the infant hepatitis B vaccination program targeting 'at risk' infants whose parents were born in selected high prevalence countries was issued.[307,310,311] 'At risk' infants referred to children born to parents whose country of origin included, China, Indonesia, Oceania, Thailand, Vietnam and others, while ethnic groups included Australian Aboriginals and Torres Strait Islanders and Maori New Zealanders. A funded universal infant hepatitis B vaccination program commenced in Australia in 2000, as part of global efforts to eradicate hepatitis B.

Since 1998 most jurisdictions (New South Wales 2004, NT 1998 (catch up program only), Tasmania 1998, Victoria 1998, South Australia 1999, Western Australia 2002, Australian Capital Territory 1999, Queensland 2004) have also implemented HBV immunisation school based programs for adolescents in years 6, 7 or 8 (aged 12 to14 years). For many states and territories the adolescent program will cease once the cohort of children vaccinated in infancy (since 2000) reaches 12-14 years old. Therefore, in the absence of a booster dose, ongoing protection beyond these ages will rely on immune memory generated by infant vaccination.

Table 4.4 Introduction of hepatitis B vaccine recommendation according to year

Year	Group recommended vaccination
1985	Infants born to HBV carrier mothers
1985	NT commenced universal antenatal HBV screening
1986	NHMRC recommendation: Infants born to Indigenous and 'at risk' (because of maternal ethnicity) nationally. Implemented at different times in each jurisdiction
1987	NSW Health circular recommending infant HBV vaccination according to ethnic risk
1990	Universal infant immunisation in NT
1998	Commencement of adolescent (10-16 year olds) program in states/territories
1998	'Catch up' program for 6-16 year olds in NT
2000	NHMRC recommended universal infant HBV vaccination nationally Booster doses no longer recommended by NHMRC

a) Vaccination coverage in Australia

In 1997 the Australian Childhood Immunisation Register (ACIR) began to record individual vaccine histories for eligible children (registered on Medicare) from birth to 7 years and has been used to provide vaccine coverage estimates. Since the introduction of the universal HBV vaccine program, high coverage for HBV has been achieved. ACIR reports 94.5% coverage for 3 HBV vaccine doses by 12 months and 96% by 2 years old.[126] However, vaccination coverage of the birth HBV dose is not well recorded by ACIR and estimates of coverage must be derived from other sources.

b) Vaccination coverage for Indigenous children

The ACIR reports high HBV coverage (94%) in Indigenous infants up to 12 months as of 2003 comparable to non Indigenous children.[312] There are potential inaccuracies in ACIR data leading to both underestimation (potential under-reporting of vaccinations) and overestimation (incomplete identification of Indigenous children) of true hepatitis B coverage. However, a recent report suggest that over 90% of the estimated number of Aboriginal and Torres Strait Islander children born between June 2001 and December 2002 are recorded as Indigenous on the ACIR.[313] The National Health Survey in 2001 collected data on Indigenous vaccination coverage in children aged 2-6 years but did not include hepatitis B.[312,314] Schultz et al reported 90% of infants born to HBV carrier

mothers in NT received the birth dose in 2003-2005 and 90% completed the primary vaccine course by 15 months old.[302]

Estimates of HBV vaccine coverage in Indigenous children nationally, prior to 2000, are limited and often based on particular regions within states. (Table 4.5) Reported coverage rates in 1993-1994, at two hospitals in the NT, for the birth HBV vaccine dose ranged from 71 to 96%.[315] Several other surveys of immunisation coverage in the 1980s and 1990s did not specifically include hepatitis B.

Table 4.5 Hepatitis B vaccine coverage in Indigenous populations prior to 2000

Author	Survey year	Location	number	Age	HBV coverage %
Guthridge et al[316]	1993	Darwin rural, Katherine, East Arnhem land Northern territory	461	2 years	96% (3 doses)
Merianos et al[317]	1989 to 1996	Top End Northern Territory	Birth cohort for years 1989 to 1996	0 to 7 years	70% (3 doses) 80% in East Arnhem and Katherine
Merianos et al[318]	1989 to 1996	Central Australia Northern Territory	Birth cohort for years 1989 to 1996	0 to 7 years	63% (3 doses)
Thorman et al[319]	1996	Northern Territory	902	12-14 months	83% (3 doses) Range 65-95%) according to district
Hanna et al[320]	1996	Far North Queensland	772	0 to 2 years	~80% (3 doses)

c) *Vaccination coverage for children born to mothers from high endemic regions*

The success of the targeted program in preventing maternal transmission from high endemic region mothers to infants relied on accurate and timely identification of the 'at risk' infant. Several studies suggest that targeted programs result in lower coverage than universal programs mainly because of these difficulties in risk identification and program implementation.[321-324] As a result, universal HBV vaccination in infancy in all countries, regardless of HBsAg endemicity, is recommended by the WHO and was implemented in Australia in 2000.

Estimates of vaccine coverage in targeted 'at risk' infants in the 1990s in Australia are limited. In Victoria, Oman et al reported HBV immunisation rates in infants from targeted ethnic groups in 1992 to be low, with approximately 10% of eligible infants not receiving any HBV vaccine and only 64% completing the 3 dose primary schedule.[311] Another study in Victoria showed that only one third (38%) of targeted infants were fully vaccinated by 12 months.[325] In NSW, documented HBV coverage in targeted infants between 1987-1990 was 60%.[326] Non or incomplete immunisation may be associated with lack of maternal ability to read English.[327] Several studies of HBV vaccination coverage among the Vietnamese community in Sydney have also been published. Reznik reported 78% coverage for 3 doses in infants born to Vietnamese mothers in South Western Sydney one year after introduction of the targeted program.[328] However, a follow up study in 1995 found that only 68% of eligible children, aged 1-7 years, had received 3 doses of HBV.[329]

In summary, there is now an Australian cohort of adolescents aged 15-20 years, who were among the first groups to receive HBV vaccination, albeit with variable coverage, who would be potential participants in studies assessing long term immunity to HBV. To date, there have been no long term (> 5 years) follow up studies of booster vaccine response in Australia. Therefore, chapter 5 examines long term protection and booster vaccine responses in 2 cohorts of children who were the first to receive the HBV vaccine – Indigenous adolescents from the NT and 'at risk' adolescents in Sydney. In this cohort of adolescents born prior to 1997, vaccine history may have been recorded in State/territory databases, individual child personal health records, healthcare providers, local government council clinics or community health clinics or a combination of these locations. As the adolescents in the cohort studies in Chapter 5 were born prior to 1997, before the commencement of ACIR, a search of multiple sites was required to ascertain each participant's HBV vaccine history. In the next section, factors informing the design of the cohort studies in chapter 5 are examined.

4. 4 Factors informing the design of the booster studies in this thesis

The design of cohort studies in Chapter 5 is informed by the literature review, methods and findings of the in vivo memory studies described in this chapter and illustrated in the following table 4.6.

Table 4.6 Factors informing design of cohort studies in Chapter 5

Questions and factors informing cohort study	Thesis cohort study design
Rationale	
No long term (>5 years) cohort studies of Australian children vaccinated in infancy examining: • Rates of chronic infection in low and intermediate endemic regions • Booster vaccine responses	Two cohort studies: 1. Low endemicity region: Adolescents in Sydney (10-14 years old) 2. Intermediate endemicity region: Indigenous adolescents in Northern Territory (18-20 years old)
Vaccine and schedule factors	
Which HBV vaccine was used in the primary schedule?	Plasma and recombinant vaccine used in 1980s in Australia. Recombinant only since 1990.
Is maternal HBsAg status known?	Need to identify in studies
Which booster vaccine type, dose and method of administration to use?	Recombinant vaccine 10ug (HB Vax II) vaccine) given intramuscularly
HBV serology measurement	
Which laboratory and method of measurement?	National serosurvey reference laboratory used for HBV serology measurement Radio-immunoassay measurement (Commercial kit – Abott Architect)
What is the minimum interval (years) between primary vaccine and follow up?	Only enrol participants >10 years post infant vaccination
When should post booster anti-HBs level be measured?	14 and/or 28 days
Study design	
Was primary seroconversion documented?	Ideal to have this data but it is not available for the planned cohorts
Is there a difference between memory and primary responses?	Compare anti-HBs responses in a group vaccinated in infancy vs a vaccine naïve group of adolescents

Questions and factors informing cohort study	Thesis cohort study design
Whom to administer booster HBV vaccine according to baseline anti-HBs measurement?	Adolescents in Sydney – participants with baseline anti-HBs >10 and <10 mIU/ml boosted. This enables assessment of immune memory in those with detectable anti-HBs (4 fold increase) as well as those with non detectable levels (rise in anti-HBs<10 to >10mIU/ml). Indigenous cohort booster vaccine ONLY given to those with baseline anti-HBs <10mIU/ml. Not boosting all participants in this cohort allows future studies to measure natural boosting, rates of anti-HBs decay and appearance of chronic infections.
Is infant vaccination history recorded and available?	Need to have accurate infant vaccine records available in order to correlate serology with vaccine history Multiple locations searched for vaccine history as adolescents in the cohorts born prior to commencement of ACIR

4.5 Summary and relevance to thesis

Children from Indigenous communities and those born to mothers from high endemic region countries are at significant risk of infection from HBV, and were the first groups targeted to receive HBV vaccination in Australia. They currently live in a low endemicity country where there are no recent studies to assess the impact of this selective vaccination strategy on chronic carriage or the long term persistence of anti-HBs. More long term follow up (>10 years) studies of HBV immunity following infant vaccination have been recommended by the European Consensus Group on Hepatitis B Immunity and Viral Hepatitis Prevention Board.[245,248] In addition, there are no recent estimates of carriage rates in Australian children born to carrier mothers, and uncertainty exists as to whether immune memory has been compromised by the administration of HBIG and HBV vaccine at birth.

The cohort studies in Chapter 5 will measure the current prevalence of HBV infection, long term (>10 years) persistence of anti-HBs in adolescents and response to HBV vaccine booster dose as a marker of immune memory for the first time in Australia. These long term follow up data on the first groups to be targeted by the National Immunisation Program are important as adolescence is a time of increased risk of hepatitis B transmission. If clinically significant HBV infections are found to be rare and immunologic memory can be demonstrated in these cohorts, this would provide good evidence to support the argument that booster doses are not required in the Australian context. Internationally, this study will add to the limited information about anamnestic responses to hepatitis B vaccine in adolescents initially vaccinated at birth, especially in Indigenous communities.

Chapter 5 presents the aims, methods, results and conclusions of two cohort studies, Indigenous adolescents in Northern territory and 'at risk' adolescents in Sydney.

CHAPTER 5: HEPATITIS B COHORT STUDIES

5.1 Introduction

Children from Indigenous communities and those born to mothers from regions of high HBV endemicity are at significant risk of infection from HBV, and were the first groups targeted to receive HBV vaccination in Australia. Follow up studies of at least 10 years duration of HBV immunity following infant vaccination have been recommended by the European Consensus Group on Hepatitis B Immunity and Viral Hepatitis Prevention Board. There are no recent estimates of carriage rates in Australian children who received HBV vaccine more than 10 years ago.

The aim of this chapter is to measure the prevalence of serological markers of HBV infection, long term (>10 years) persistence of hepatitis B surface antibody (anti-HBs) in two cohorts of adolescents and response to HBV vaccine booster dose as a marker of immune memory for the first time in Australia. These long term follow up data on the first groups to be targeted by the National Immunisation Program are important, as adolescence is a time of increased risk of hepatitis B transmission. These studies add to the limited information internationally about anamnestic responses to hepatitis B vaccine in adolescents initially vaccinated at birth, especially in Indigenous communities.

In this Chapter, I outline the methods and results of each of the cohort studies separately. The first cohort study describes serologic markers and immune memory responses in adolescents aged over 10 years who were targeted for birth and infant HBV vaccination in the 1990s because they were born to 'at risk' mothers from high HBV endemic countries. The second cohort study describes serologic markers, immune persistence and memory response in Indigenous adolescents in the Northern Territory. There are several areas within the methods section that are common to both studies including; serologic methods, HBV vaccine used in the booster study and assessment of immune memory. These will be discussed in the first cohort study and referred to in the second study. The final section of this chapter is discussion of the data from both cohort studies.

5.2 Hepatitis B cohort studies

Two prospective cohort studies were performed:

Study 1: Adolescents born to 'at risk' mothers in Sydney

Study 2: Indigenous adolescents in the Northern Territory.

Each study has the following aims and hypotheses.

5.2.1 Aims

1. Estimate the prevalence of HBV infection markers hepatitis B core antibody (anti-HBc) and hepatitis B surface antigen (HBsAg) carriage

2. Measure antibody to hepatitis B surface antigen (anti-HBs) as a marker of long-term immunity following HBV vaccine in infancy.

3. Measure the response to a booster dose of the HBV vaccine as a marker of immune memory.

5.2.2 Hypotheses

1. A selective vaccination strategy targeting Aboriginal children and children born to mothers from 'at risk' countries has resulted in lower hepatitis B carrier and infection rates compared to pre vaccination data.

2. Most children who were vaccinated more than 10 years previously will have anti-HBs less than 10mIU/ml but will have an anamnestic (memory) response to a booster dose of HBV vaccine.

5.2.3 Definitions

In chapter 4 discussion about anamnestic (memory) responses led to the following definitions:

Anamnestic (memory) response

- Anti-HBs levels >10 mIU/ml following a single dose of HBV vaccine in those with anti-HBs <10mIU/ml pre-booster vaccination
- 4 fold increase in anti-HBs titre in those with a pre-booster titre of >10mIU/ml

HBV infection

Definition of HBV infection according to HBV serology as below:

- HBsAg positivity = *HBV carrier*
- Anti-HBc positivity = *Past infection*
  - Anti-HBc positive and anti-HBs positive
  - Isolated anti-HBc positivity (anti-HBc positive and anti-HBs negative)

Definition of HBV infection according to vaccine history and HBsAg status:

- Detection of HBsAg with documented receipt of HBV vaccines is termed '*clinically significant breakthrough infection*'
- Detection of anti-HBc with documented receipt of HBV vaccines in the absence of HBsAg is termed '*clinically benign breakthrough infection*'

Anti-HBs measurement

Anti-HBs titres are reported in milli-International Units (mIU) per ml using the WHO international reference standard. Anti-HBs ≥10mIU/ml is considered to be protective against hepatitis B infection.[330]

- Anti-HBs≥10mIU/ml and no detectable anti-HBc is deemed to indicate immunity through vaccination
- Anti-HBs <10mIU/mL and no detectable anti-HBc defines an individual as non-immune

Assessment of vaccination status

Study 1

1. Group 1 subjects – HBV vaccine history met 'on time' modified US Centers for Disease Control (CDC) criteria with completion of HBV vaccine schedule by 9 months old.[331]

   Defined as:

   - 1st dose within 7 days of birth
   - 2nd dose 24 to 120 days (1 to 4 months) following birth, with a minimum interval of 4 weeks after 1st dose
   - 3rd dose 164 to 286 days (6 to 9 months) following birth dose, with a minimum interval of 8 weeks from dose 2

2. Group 2 – never received HBV vaccine

Study 2

Each individual's vaccine history was classified into the following categories:

1. Received 3 doses as per 'on time' CDC criteria –as outlined above OR
2. Received 3 doses under 2 years old OR
3. Received 3 doses at any time but not (a) or (b) OR
4. Received less than 3 documented doses OR
5. No HBV vaccine history recorded

5.3 Study 1: Adolescents born to 'at risk' mothers in South West Sydney

5.3.1 Study design

This study was a cross sectional prevalence study of HBV serologic markers and response to administration of HBV vaccine to adolescents in two groups (see below). This study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki 1999[222] and had the approval of three ethics committees (The Children's Hospital at Westmead, Westmead Hospital, and Sydney South West Area Health Service (SSWAHS) (Appendix 4)).

5.3.2 Study cohort

South Western Sydney has a large ethnically diverse population, with over 1/3 of residents born in non English speaking countries with higher prevalence of HBsAg carriage and among those targeted for infant HBV vaccination in the 1990s.[304,310] A cohort of children in the SSWAHS region aged 10 to 17 years, who were among the first to have received infant HBV vaccination in Australia were targeted for enrolment in Study 1.

Two groups of children and adolescents were enrolled:

Group 1: Adolescents over 10 years old who received HBV vaccine in infancy and no subsequent booster doses

Group 2: Adolescents over 10 years old who had never received HBV vaccine

Identifying the groups

A HBV vaccine uptake study was conducted by Dr Leon Heron (SSWAHS Public Health Unit) in primary schools (SWSAHS HREC Project No. 00/062) in South Western Sydney in 2001. This study recruited children, aged 10 to 12 years, from primary schools in South Western Sydney and asked them about their hepatitis B vaccine history, as well as socio-demographic details, which were recorded in a database. This study was nested within a school based HBV vaccination program being

conducted in NSW. Data collected in this study included; child's date of birth, ethnicity, HBV vaccine status, mother and father's country of birth and contact details and were registered on a database in 2001. The database from this vaccine uptake study was then used to identify adolescents over 10 years old and who were born to mothers or fathers targeted by the HBV vaccine program because of their country of birth. On review of the data this group was then divided into those who received HBV vaccine in infancy and those who did not. These adolescents and their parents were then approached to participate in this study.

From the database on HBV vaccine uptake two groups with the following characteristics were identified (Table 5.1)

Table 5.1 Eligibility and aims of anamnestic (memory) study according to group

Group 1 – HBV vaccination in infancy	Group 2 – no HBV vaccination
Adolescents over 10 years old Known mother and father ethnicity HBV vaccine given in infancy	Adolescents over 10 years old Known mother and father ethnicity HBV vaccine NOT given in infancy
Approximately 750 eligible adolescents on database	Approximately 660 eligible adolescents on database
Aim • Measure HBV serology following infant vaccination • Measure anti-HBs persistence • Measure anamnestic response to a HBV booster vaccine	*Aim* Compare anti-HBs response following single HBV vaccine with group 1

5.3.3 Study recruitment and enrolment

Initial telephone contact was made with parents of subjects on this database to explain the study and recruit interested participants. Approved interpreters were used, if needed. Unsolicited letters requesting study participation were not used because previous experience showed that initial telephone contact more frequently led to study participation than did initial contact via letter. Telephone interviews made it possible for parents to have their questions answered immediately.

Interested parents and adolescents were then enrolled into Group 1 or 2 according to the following criteria.

a) Enrolment criteria

Group 1

Adolescents whose parents' country of birth or ethnicity defined them as eligible for the targeted HBV vaccination program that operated in NSW before May 2000 and who received a course of three HBV vaccine injections as per CDC criteria (as above) and who had no known additional doses of HBV vaccine

Siblings of the subjects in Group 1 were eligible to participate in the study if the following criteria were met:

- Sibling aged 10 or more years
- Received a course of three hepatitis B vaccine injections as per CDC criteria
- No additional doses of hepatitis B vaccine

Group 2

Adolescents whose parents' country of birth or ethnicity defined them as targets for the hepatitis B vaccination program that operated in NSW before May 2000 and who never received any doses of hepatitis B vaccine.

Siblings of the subjects in Group 2 were eligible to participate in the study if the following criteria were met sibling was aged 10 or more years and never received any doses of hepatitis B vaccine

b) Date of birth for oldest children that were included in this study

The first NSW Health Department Policy regarding the infant hepatitis B vaccination program targeting infants whose parents were born in selected high prevalence countries was issued on 20 May 1987.[310] Therefore, no child born before 1 July 1987 was included in this study.

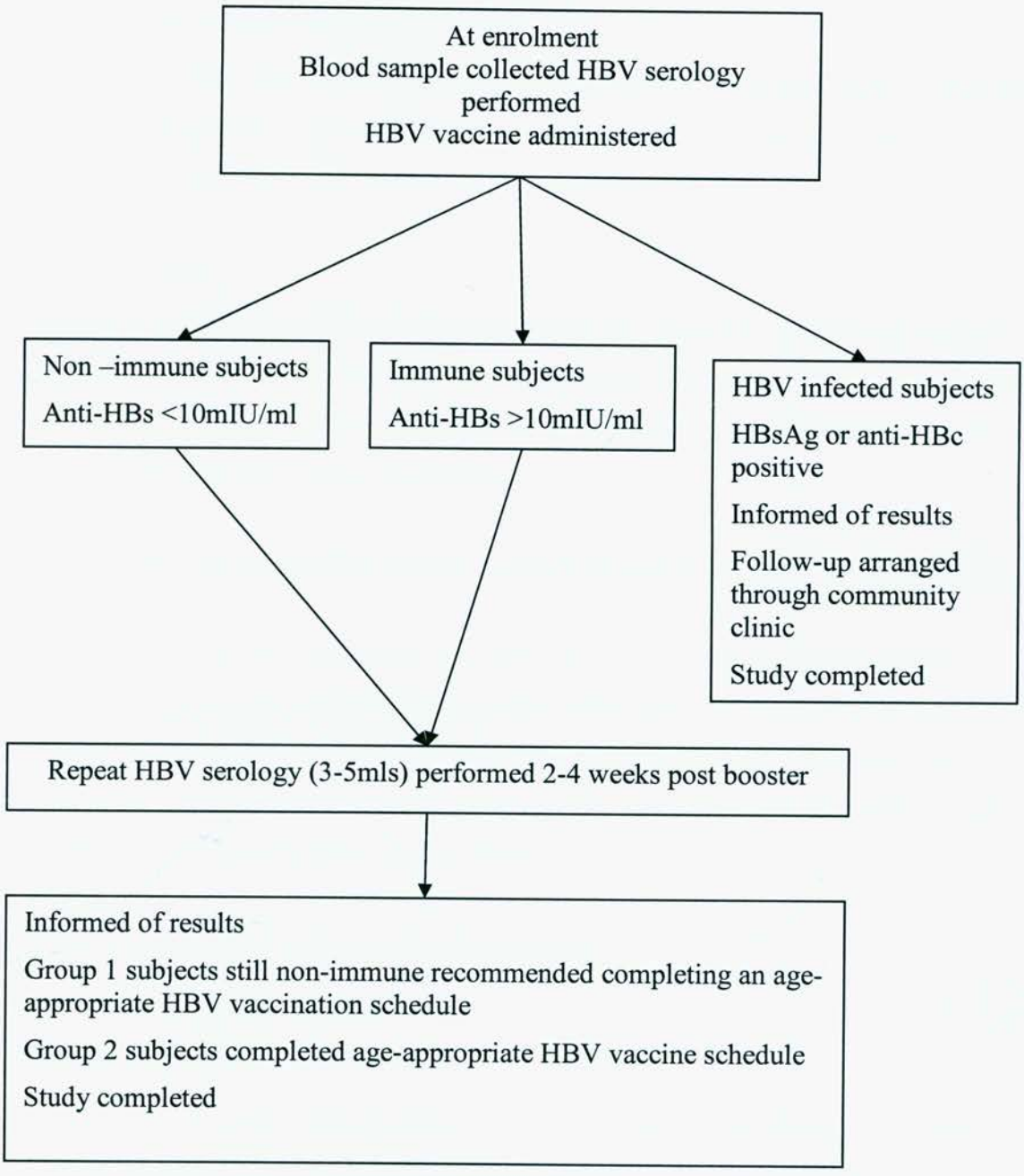


Figure 5.1 Study flow chart for Groups 1 and 2

c) Determination of hepatitis B vaccination status

Hepatitis B vaccine history was confirmed, as much as possible, by written records from child personal health records and GPs associated with the subject.

d) Duration of participation

The duration of the study participation for each participant was approximately 1 month. Those requiring a full course of vaccination (subset of Group 1 and all Group 2 subjects) had additional visits as required according to their age.

e) Consent

Parents and children were given a full explanation, provided with an information sheet and given the opportunity to ask questions. (Appendix 5) Separate written consent was obtained for study participation and for vaccination. Children aged 16 or more years also provided informed consent in writing.

f) Follow-up of families with a subject found to be HBsAg or anti-HBc positive

Research staff asked for permission to contact the mother's GP and hospital birth medical records for the mother's HBsAg status. If no data were available, testing of the mother was arranged through the mother's GP or by research staff if she consented. Appropriate follow-up for a person found to be HBsAg positive was arranged through the person's GP and community health clinic.

5.3.4 Vaccine and vaccine administration

a) Details of the vaccine

Hepatitis B vaccine – H-B-VAX II

H-B-VAX II (CSL/Merck Sharp Dohme) Adult formulation preservative free – each 1ml dose contained hepatitis B surface antigen protein 10ug adsorbed onto 0.5mg aluminium hydroxide.[122] This vaccine is currently in widespread use in Australia and is recommended for use in adolescents.

Exclusion criteria for HBV vaccine

1. Known hypersensitivity to any component of the vaccine
2. Previous anaphylactic reaction to the HBV vaccine

3. Patients with thrombocytopenia or coagulation disorders that prevented intramuscular vaccination

4. Participation in another investigational study

5. Vaccination was delayed in those with severe intercurrent illnesses

b) Vaccine administration

Experienced study medical staff performed vaccination and blood sample collection. The vaccination was deferred if the subject had an acute febrile illness with temperature above 38C. The H-B-VAX II was administered by intramuscular injection in the arm. No other vaccines were administered concomitantly. Subjects were observed for adverse events for 30 minutes after vaccination. Standard immunisation practices were followed and appropriate precautions for anaphylactic reactions were taken.

Parents and subjects were given telephone numbers so that they could report adverse events that were of concern. Subjects for whom adverse events were reported by parents/subjects were followed-up. Adverse events were reported through the standard reporting mechanisms to the NSW Health Department and Australian Drug Reactions Advisory Committee.

The Australian Immunisation Handbook 9th edition recommends that individuals at risk of hepatitis B infection through occupational exposure and who are non immune following primary vaccinations complete a repeat course of hepatitis B vaccination.[122] Group 1 subjects in this study who did not respond to a booster hepatitis B vaccine were recommended to complete a repeat course of the hepatitis B vaccine (H-B-VAX II) consisting of either two doses of H-B-VAX II 10ug separated by 6 months for those aged 11-15 years old or three doses H-B-VAX II at 0,1 and 6 months for those aged 16 years and older.

Individuals in group 2 (never vaccinated) were offered a complete course of hepatitis B vaccine as per the Australian Immunisation Handbook 9th edition [122] consisting of either two doses of H-B-VAX II 10ug separated by 6 months for those aged 11-15 years old or three doses H-B-VAX II at 0,1,and 6 months for those aged 16 years and older. All

Group 2 subjects who consented to vaccination completed an age-appropriate course of vaccination irrespective of the anti-HBs results following vaccine dose 1.

5.3.5 Assessment of immunogenicity

At enrolment a 5-10ml blood sample was collected from each participant. Blood was kept refrigerated until processed by the laboratory. The baseline sample was analysed for anti-HBs and anti-HBc in all subjects. Samples positive for anti-HBc were then assayed for HBsAg. The assays were performed at the South Western Sydney Pathology Service, Liverpool Hospital. HBV serologic markers were measured on Abbott AxSYM automated analyser using test kits from Abbot laboratories. Repeat blood samples were collected at 2 and 4 weeks post booster vaccination for measurement of anti-HBs. (Figure 5.1)

Results are presented according to age, sex, ethnicity (maternal country of origin), and time since infant vaccination. Maternal country of origin was grouped according to regions: Australia, Asia, Middle East, New Zealand/Pacific Islanders, South/eastern Europe and South America.

Group 1 adolescents were further divided into Group 1a (anti-Hbs levels <10mIU/ml) at baseline and Group 1b (anti-HBs levels >10mIU/ml) at baseline. Response to the HBV vaccine booster is presented for group1 as a whole and group 1a and 1b.

5.3.6 Statistical analysis and power analysis

Only subjects who had completed the vaccine schedule according to protocol and had two assay results available, including the baseline sample, were included in the immunogenicity analysis. Serum anti-HBs concentrations were log transformed for statistical analysis and a geometric mean concentrations, with 95% confidence intervals, was calculated for anti-HBs. Proportions positive for anti-HBs, anti-HBc and HBsAg were calculated. The anti-HBs GMC at 2 and 4 weeks following a dose of vaccine is presented.

Values below the laboratory assay cut-off were assigned a value half of the cut-off value in order to calculate the GMC.[332]

Comparisons of antibody concentrations between groups were using log-transformed data by the independent samples t test with a p value <0.05 indicating a possible group difference. The proportions of study group subjects with a serological anamnestic response (as defined above) after vaccination in study groups were compared by Fisher exact test.

Power analysis

The power of the study was limited by availability of subjects on the database from the previous HBV vaccine uptake study.

If the study enrolled 437 subjects in each group, and assuming a HBsAg carrier rate of 2.5% in the unvaccinated group, it would be possible to detect a 90% efficacy of the vaccine for preventing HBsAg carriage with a 95% confidence interval and 80% power.

5.3.7 Results

a) Enrolment

One hundred and twenty eligible subjects were enrolled between March 2005 and December 2005, 70 in group 1 and 50 in group 2. All were over 10 years old and approximately half were male in each group (Table 5.2).

Table 5.2 Demographics of groups enrolled in 'at risk' children and adolescent hepatitis B booster vaccine study

Factor	Group 1	Group 2
Subject number	70	50
Mean age years (range)	14.5 (11.5-16.6)	15.7 (14.2-18.0)
Male (%)	38 (54%)	29 (58%)
Maternal country of birth^		
Australia	8	22
Asia	26	4
Middle east	20	5
S/E Europe	10	16
New Zealand/Pacific Island	4	3
South America	2	0

Factor	Group 1	Group 2
Mean interval between birth and HBV vaccine doses (days)		
HBV dose 1 (range)	2 (0-6)	n/a
HBV dose 2 (range)	45 (24-94)	n/a
HBV dose 3 (range)	201 (161-275)	n/a
Hepatitis B serology		
Anti-HBc (% pos)	2 (2.9%)	1 (2%)
HBsAg (% pos)	0	0
Anti-HBs positive (>10mIU/ml)	26 (38%)	0

Group 1 – subjects with a previous history of hepatitis B vaccine in infancy

Group 2- subjects with no previous history of hepatitis B vaccine in infancy

^Maternal country of birth

Group 1 includes Aboriginal (1), Cambodia (6) Vietnam (14) China (3) India (1)

Indonesia (1) Philippines (1) Lebanon (19) Syria (1) Italy (6) Yugoslavia (1)

Greece (2), Cyprus (1) Fiji (4) Chile (2), Australia (7).

Note: in those 7 cases where mothers were born in Australia, fathers of the child were born in Greece/Cyprus (4), Lebanon (2), Turkey (1)

Group 2 includes: Australia (18), Aboriginal (4), China (2) Philippines (1) Laos (1) Iran (3)

Lebanon (2) Italy (8) Turkey (3) Malta (1) Romania (3) Macedonia (1) Tonga (1) New Zealand (2)

Note: In the 18 cases where mothers were born in Australia, fathers of the child were born in Bosnia (1), Chile (1), China (1), Croatia (1), Greece (1), Indonesia (1), Italy (3), Lebanon 91), Macedonia (1), Malta (2), Slovenia (1), South Africa (1), Yugoslavia (1), Australia (1)

Group 2 subjects were non significantly older than group 1 ($p>0.05$). All subjects in group 1 were born to mothers eligible for inclusion in the targeted HBV vaccine program because of their country of birth and had received the HBV vaccine on time as per CDC criteria (see methods). In group 1, the majority of mothers were from Asian and the Middle Eastern countries of origin. In group 2, nearly half (n=22/50, 44%) had mothers born in Australia.

Three subjects (Group 1 (n=2) (2.9% (95% CI 0.8% – 9.8%)) and Group 2 (n=1) (2% 95% CI 0.4-10.5%)) were anti-HBc positive and were excluded from the immunogenicity assessment for memory response. Individual serologic markers for these participants are shown in table 5.3. All three subjects were born to mothers who were HBsAg negative, but were targeted for the HBV vaccine program based on country of birth.

Table 5.3 Hepatitis B serologic markers for three participants who were positive for hepatitis B core antibody

Age (yrs)	Maternal birth country	Sex	Maternal HBsAg	Anti-HBs (mIU/ml)		
				Baseline	2 weeks after booster	4 weeks after booster
12.4	Vietnam	Male	Negative	75	1917	2040
15	Cyprus	Female	Negative	17	1958	1568
15.4	China	Female	Negative	<10	<10	Not available

b) Hepatitis B immunogenicity

Two thirds (42/68, 68%) of subjects tested in Group 1 had anti-HBs levels <10mIU/ml, and were designated Group 1a. Twenty six subjects tested in Group 1 had anti-HBs levels >10mIU/ml and were designated Group 1b. There was no significant difference between Groups 1a and 1b in mean (14.6 years vs 14.3 years) or median ages or male sex (57% vs 50%). Immunogenicity results are presented separately for groups 1a and 1b.

As expected from the case definition, group 1 geometric mean anti-HBs levels were significantly higher than Group 2 at baseline and group 1b anti-HBs levels were significantly higher than Group 1a at baseline (32.3 vs <10 mIU/ml, $p<0.02$) (Table 5.4)

In groups 1a and 1b anti-Hbs levels were significantly higher than baseline at 2 and 4 weeks after HBV vaccine booster ($p<0.02$). (Table 5.4) Group1b anti-HBs levels were significantly higher at 2 and 4 weeks post booster than group 1a subjects ($p<0.02$). (Table 4 and figure 5.2)

Table 5.4 Anti-HBs levels (geometric mean concentration) according to group and time since hepatitis B vaccine

Group#	n	Anti-HBs		
		Baseline GMC* (95% CI)	2 weeks post GMC* (95% CI)	4 weeks post GMC* (95% CI)
Group 1	68	10.2 (7.9-13.1)	1095 (681-1761.2)	1132.3 (720.3-1780.1)
1a	42	<10	469 (267.5-822.4)$	533.2 (315.1-902.2)$
1b&	26	32.3 (23.3-44.7)	4313 (2500.4-7439.5)	3822.3 (2100.6-6955.2)
Group 2	49	<10	<10 (11, 31)^	<10 (17)^

* GMC – geometric mean concentration (mIU/ml)

^ refers to individual subjects in group 2 with levels > 10 mIU/ml. Anti-HBs (2 weeks post) had two subjects with levels of 11 and 31mIU/ml and one subject with anti-HBs (4 weeks post) level 17mIU/ml

Group

Group 1a – subjects with baseline anti-HBs <10mIU/ml

Group 1b – subjects with baseline anti-HBs >10mIU/ml

Group 2 – subjects with baseline anti-HBs <10mIU/ml and nil previous HBV vaccination

$ Group 1a significantly higher than Group 2 at 2 and 4 weeks post hepatitis B (H-B-VAX II) vaccine ($p<0.02$)

& Group 1b anti-HBs levels significantly higher than groups 1a and 2 at each time point ($p<0.02$)

c) Hepatitis B anamnestic response

Anamnestic memory responses were demonstrated in all subjects in groups 1a and 1b, except for one subject in Group 1a whose anti-HBs remained <10mIU/ml at weeks 2 and 4 (n=1/42; 2.4% (95% CI 0.4-12.3%)). (Table 5.5 and figure 5.2) Over 80% of subjects in group 1a and all subjects in 1b had anti-HBs levels >100mIU/ml two weeks post booster. All subjects in group 1b demonstrated more than 4-fold increase in anti-HBs levels post booster.

Table 5.5 Increase in anti-HBs levels according to proportion >10mIU/ml and fold increase by group

		Anti-HBs										
	n	Baseline		2 weeks post HBV vaccine				4 weeks post HBV vaccine				
Group#		%>10	%>100	%>10	%>100	% 4 fold increase	Mean fold increase (range)	%>10	%>100	%>1000	GMC (95% CI)	Mean fold increase anti-HBs3 from anti-HBs2 (range)
Group 1a	42	0	0	98%[$]	83%	93%	303 (1-2017)	98%	86%	38%	533.2 (315.1-902.2)	1.7 (0.3-9.5)
Group 1b	26	100	15	100%	100%	100%	235* (15-1570)	100%	100%	80%	3822.3 (2100.6-6955.2)	1.5 (0.3-16.7)
Group 2	49	0	0	4%	0	0	n/a	2%	0%	0	<10	n/a

Group =Group 1a – subjects with baseline <10mIU/ml subjects, Group 1b – subjects with baseline >10mIU/ml

Group 2 – subjects with no history of previous HBV vaccination

$ In one subject anti-HBs levels remained < 10mIU/ml at 2 and 4 weeks post booster vaccine

*One subject in group1b had large increase in anti-HBs levels. (Anti-HBs baseline (14 mIU/ml), Anti-HBs 2 weeks post (5394 mIU/ml), anti-HBs 4 weeks post (90004)). If this subject is removed from the group then mean fold increase in anti-HBs in group1b is 230 and 177 at 2 weeks and 4 weeks post vaccine respectively.

The mean fold increase in groups 1a and 1b was over 200 and 80% of Group1b participants had anti-Hbs levels >1000mIU/ml four weeks post booster vaccine. (Table 5.5) Reverse cumulative distribution curves for group 1a and 1b subjects and a plot of pre vs 2 week post HBV immunisation anti-HBs levels demonstrate superior responses in those with pre-existing anti-HBs at baseline. (Figures 5.3, 5.4. 5.5) Only two Group 2 subjects had anti-HBs levels >10mIU/ml at week 2 and by week 4 one of these subjects level had fallen from 31 to <10mIU/ml, while the other had risen from 11 to 17mIU/ml. The remainder showed no anti-HBs response to the first HBV vaccine dose at week 2 and 4.

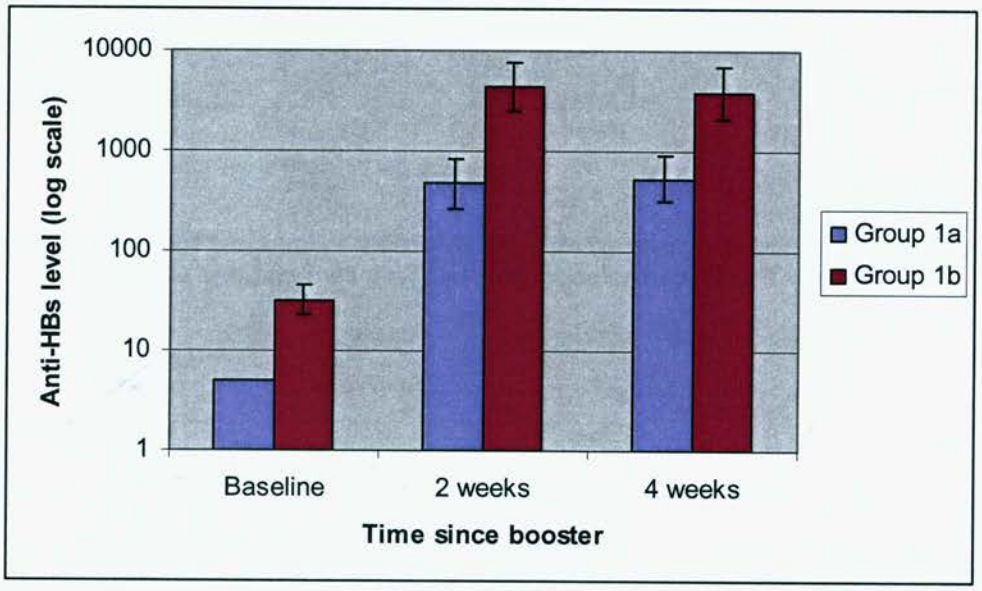


Figure 5.2 **Anti-HBs level in Group 1a and 1b subjects according to time following hepatitis B booster vaccine**

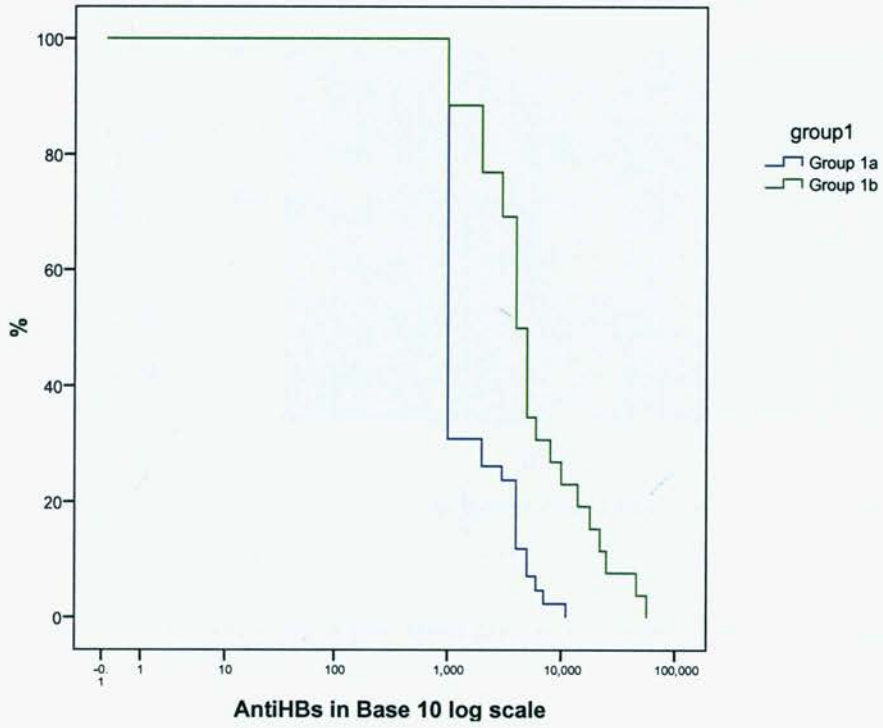


Figure 5.3 **Reverse cumulative distribution curve by group for antibody to hepatitis B surface antigen 2 weeks following booster hepatitis B vaccine**

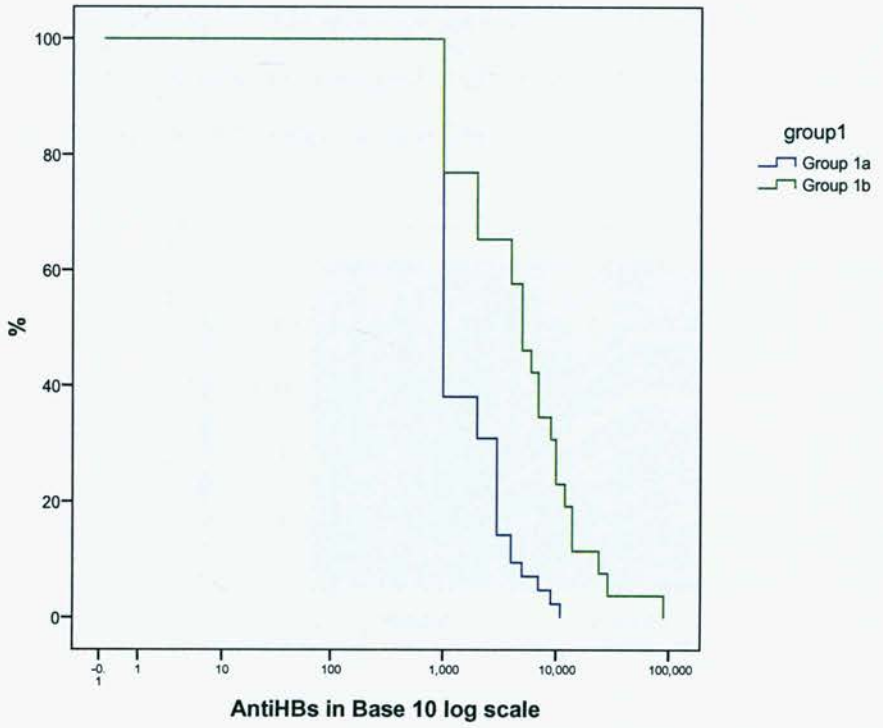


Figure 5.4 **Reverse cumulative distribution curve by group for antibody to hepatitis B surface antigen 4 weeks following booster hepatitis B vaccine**

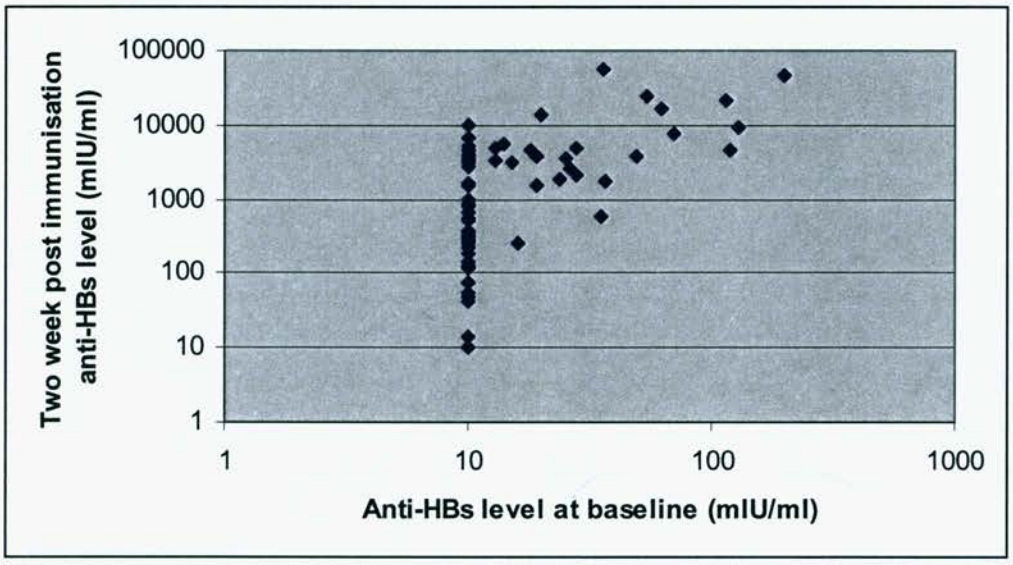
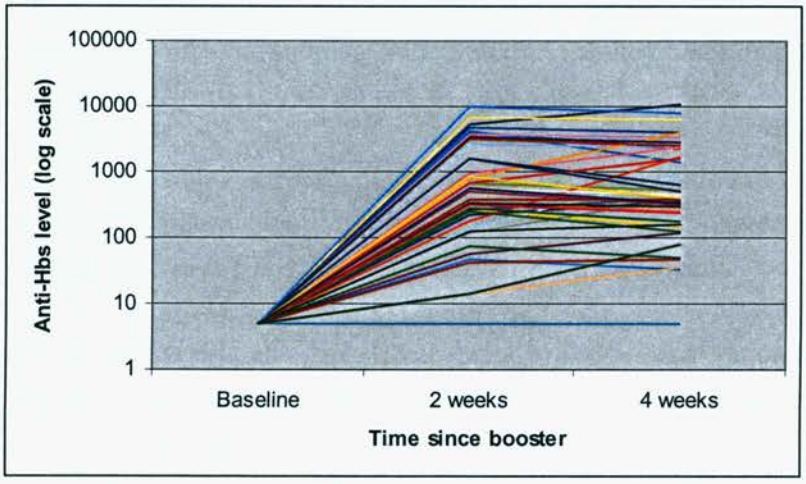


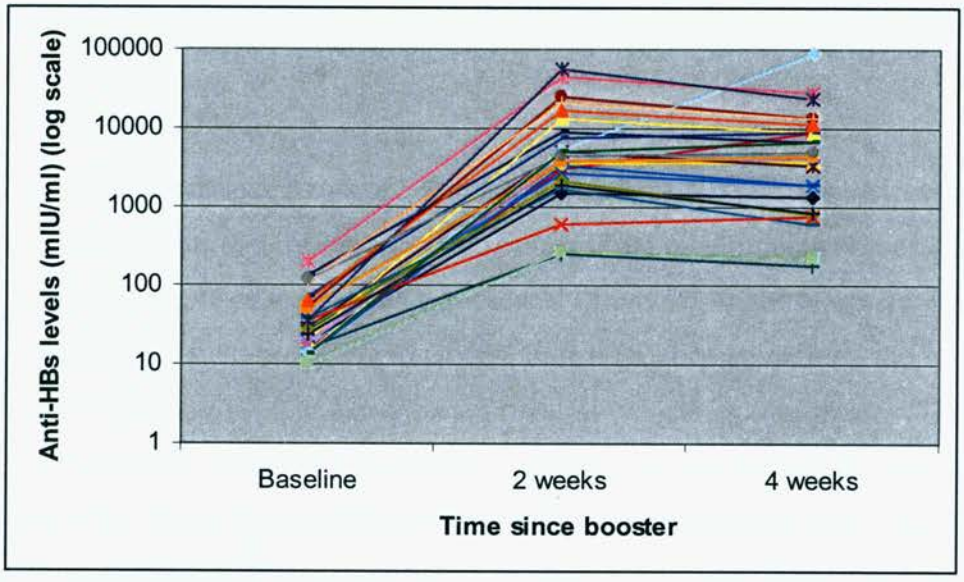
Figure 5.5 Correlation of pre-immunisation anti-HBs level and response 2 weeks following HBV booster vaccine

Figure 5.6 and 5.7 show the individual rises in anti-HBs levels in subjects in groups 1a and 1b. As noted in table 5.5 and shown in the following figures (Figures 5.6, 5.7), anti-HBs levels did not rise significantly between week 2 and 4 after booster vaccine (p=0.94). The mean fold increase between weeks 2 and 4 was 1.7 and 1.5 in groups 1a and 1b respectively. This suggests that most of the memory response occurred in the first 2 weeks after booster vaccination.



Note: each line represents individual results for a subject

Figure 5.6 Anti-HBs levels in Group 1a subjects according to time following hepatitis B booster vaccine



Note: each line represents individual results for a subject

Figure 5.7 Anti-HBs levels in Group 1b subjects according to time following hepatitis B booster vaccine

5.3.8 Summary of results of Study 1

- In the vaccinated group 1 there were no cases of HBsAg positivity (clinically significant breakthrough infection) and only 2.9% (95% CI 0.8-9.8%) had evidence of past infection (clinically benign breakthrough infection). This compares with 2% (95% CI 0.4-10.5%) anti-HBc positive in the vaccine naïve group 2.

- Nearly two thirds (62%) of subjects vaccinated in infancy had non detectable anti-HBs levels (<10mIU/ml) at a mean age of 14 5 years.

- Nearly all (98%) group 1 subjects demonstrated an anamnestic memory response 14.5 years after infant vaccination. This contrast with almost absent responses to a single vaccine dose in the HBV vaccine naïve group 2 subjects.

- Subjects with measurable anti-HBs levels prior to the booster vaccine demonstrated higher levels post booster than those who had non detectable anti-HBs.

- Anamnestic responses were seen in the blood sample collected 2 weeks post booster and did not appear to rise further by 4 weeks.

5.4 Study 2: Australian Aboriginal Birth Cohort

5.4.1 Study design

This study includes measurements of serologic markers for HBV infection in a cohort of Indigenous adolescents (Study 2a) and a non blinded trial of administration of HBV vaccine to a sub-sample of Indigenous adolescents (Study 2b) in the Northern Territory. This study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki 1999 and had the approval of two ethics committees (The Children's Hospital at Westmead, Westmead Hospital, and the Joint Institutional Ethics Committee of the Royal Darwin Hospital and the Menzies School of Health Research, Darwin, Northern Territory including the Aboriginal Ethical Sub-committee Menzies School of Health Research (approval number 05/26). (Appendix 6)

This study was funded by a NHMRC New Investigator grant (396700). (Appendix 7)

5.4.2 Study cohort

A cohort of Indigenous adolescents in the Northern Territory, aged 16 to 20 years, who were among the first to have received infant HBV vaccination in Australia, were targeted for enrolment.

Identifying the cohort

In 1987 an Australian Aboriginal Birth Cohort (ABC) was established to provide a resource for descriptive and analytical studies with particular attention on the causes and determinants of chronic non-communicable disease. The catchment area (Darwin health region) was served by the Royal Darwin Hospital. This includes Darwin, the capital of the Northern Territory (NT), and rural and remote towns and communities across the northern part of the NT and Western Australia. This sparsely populated area (0.2 person/km^2) is approximately 2 million square kilometres. (Figure 5.8)

Six hundred and eighty six babies delivered at the Royal Darwin Hospital between January 1987 and March 1990 to an Aboriginal mother (Wave 1) were enrolled in the ABC study. HBV vaccine was recommended for all Indigenous infants, commencing at birth then 1 and 6 months old, during the period that the birth cohort participants were

born. The ABC study was funded by an NHMRC Project Grant No: 137203 from 2001-3 and since then by an NHMRC program grant and is recognised internationally as the oldest and largest Indigenous cohort study in the world.[333-337] The cohort is also well recognised as being a representative sample of Aboriginal children in Darwin Health region. It includes participants from both urban (Darwin/Palmerston surburbia) and rural/remote areas, has carefully collected demographic and clinical data available, and has a proven high follow-up rate. Importantly, gestational age was measured by a validated postnatal method, because it was not known for many Indigenous women and, in addition to birth weight, is available to be used as a predictor of HBV immunity.[338,339] These children have been prospectively followed in 1998-2001 (Wave 2, n=572), and were reviewed again between December 2006 and January 2008 (Wave 3). Wave 3 data included the following: anthropometric data, nutritional status, respiratory, renal, metabolic and cardiovascular function, haematological indices and psychosocial measures. This cohort study provided a unique opportunity to collect additional information on HBV serology and the principal investigator of the ABC study (Dr Susan Sayers: Menzies School of Health Research) supported the addition of hepatitis B serology to this study in wave 3. Waves 1 and 2 did not measure HBV serology, so comparison of wave 3 results with earlier samples is not possible.

5.4.3 Study recruitment and enrolment

The recruitment of participants for study 2a and 2b required considerable resources, dedicated research staff and was logistically difficult. This is due to the isolated nature of the rural/remote communities, distances between communities, lack of access to telecommunication services, language barriers and the mobility of participants.

a) Study 2a: HBV serology measurement

Subjects who were enrolled in Wave 3 of the ABC study had HBV serology performed as part of their baseline Wave 3 assessment. The preparation and recruitment for Wave 3 follow up (2006-2008) is outlined below.

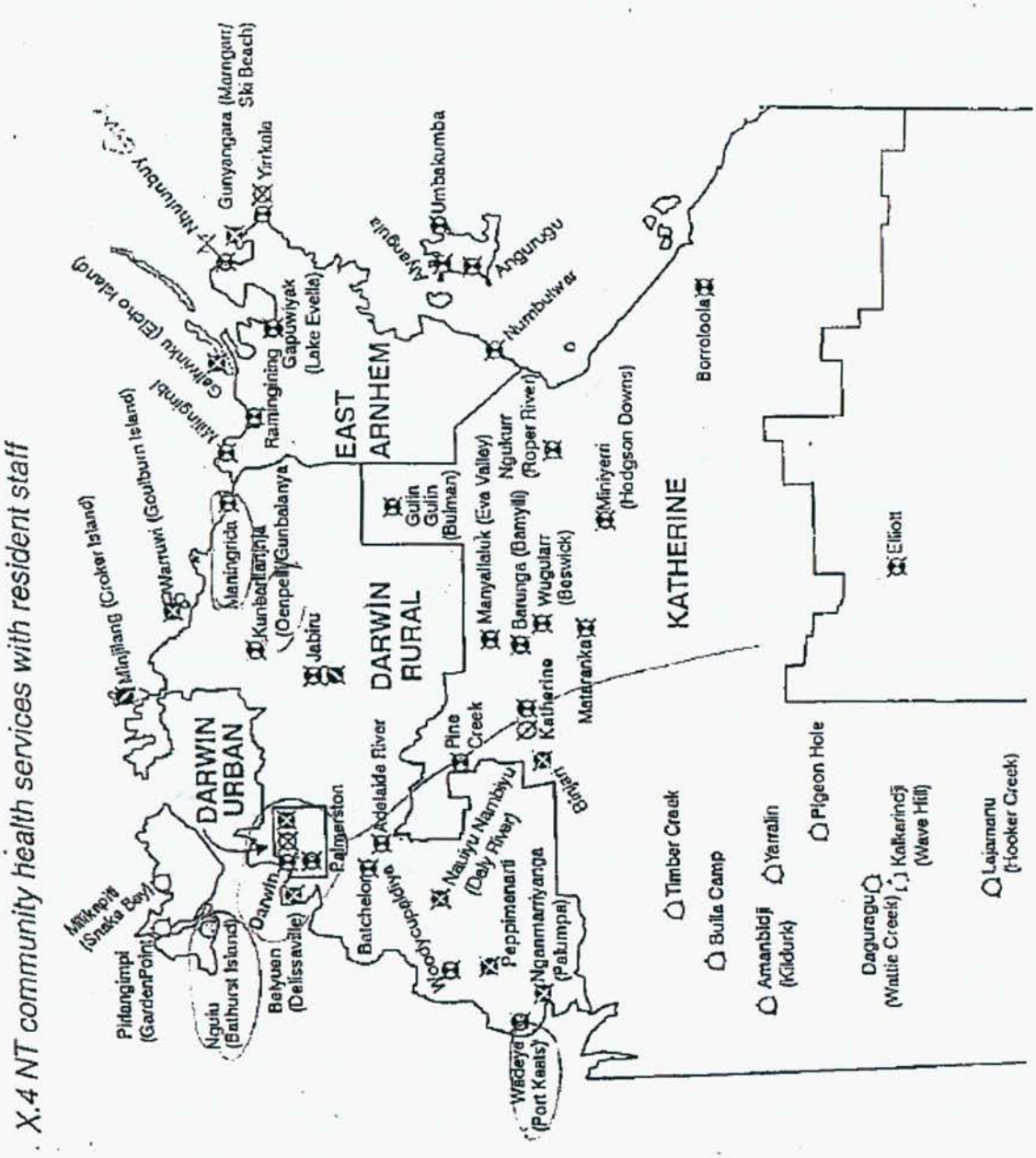


Figure 5.8 Map of Darwin Health region

Preparation

In preparation for follow-up a manual audit of hospital medical records was undertaken to update addresses and communities recorded from Wave 2. Publicity for the study was generated through the local newspaper, radio stations, Northern Territory Health Department publications, Northern Territory Education Department, Aboriginal liaison officers and via the distribution of posters to community health clinics and Aboriginal Health Worker conferences. Electronic matches of names were undertaken with NT and Western Australian Death Registries.

Written approval at the community level was sought from local health boards and community councils prior to visiting each community. To obtain community approval, a letter detailing the aims, methods, benefits and risks of the study was required. (Appendix 8) Local health care clinics or other suitable locations (such as council rooms, community halls or schools)` in each community were used as a site to collect blood samples, perform other investigations in Wave 3 and administer the booster HBV vaccine in the sub study 2b. Flexibility about location and timing, weekends or weekdays, was required.

Individual permits for each researcher were required by community land councils prior to granting access to lands.

Tracing

Tracing was undertaken by one full time and one part time research assistant. Tracing strategies differed between rural and urban participants, as outlined below.

- *Rural participants*: For the rural participants initially a phone call was made to a senior worker of a community health clinic explaining the study and seeking their cooperation. A list of participants last known to be in the target community was faxed to the community health clinic with a request to return the fax with information about participant's current whereabouts and any pertinent information relative to locating the subject. In bigger communities the clinic was also asked to provide the name and contact of local Aboriginal people willing to undertake casual employment, tracing and locating participants in their community. On

visiting community names of participants were shown to key community members in order to locate participants in surrounding communities and outstations.

- *Urban participants*: For urban participants, the last known address was contacted by phone or letter, followed by a personal visit if these were unsuccessful. The urban lists were reviewed on a one on one basis with local Aboriginal people known to the research team and Aboriginal liaison officers and interpreters from the local hospital. The lists were compared with an online telephone directory, the computerized local electoral roll and the local high schools rolls. The remaining names were shown to local Aboriginal urban residential communities and dedicated Aboriginal health services within Darwin with a subsequent snowballing effect from all the urban networks used. These included local Aboriginal hostels, sporting clubs, corrective services, churches, The Larrakai Nation and The Northern Land Council.

b) Study 2b: HBV vaccine booster response

The ABC study included participants from over 60 rural communities and outstations as well as urban areas in Darwin. The ABC study had developed good relationships with many communities and in order to limit costs, individuals from a selection of larger and less isolated communities, who were non immune to HBV, were invited to participate in the vaccine booster sub-study 2b.

Following measurement of HBV serology in the Wave 3 baseline assessment, a cohort of eligible adolescents in communities, who were non-immune to anti-HBs (<10mIU/ml) and negative for anti-HBc and HBsAg, were identified. These adolescents were eligible to participate in the booster study 2b. Due to the time delay between baseline results and enrolment into the booster study 2b, repeat HBV serology was collected at the time of administration of the booster HBV vaccine.

Similar methods to tracing eligible participants in these rural and urban communities in Wave 3 (Study 2a) were also used in Study 2b, as detailed above. Indigenous health

workers and other locals were employed by me to assist in finding eligible participants in the following communities:

- Nguiu – Bathurst Island
- Milikapiti and Pirlangimpi – Melville Island
- Wadeye (Port Keats)
- Maningrida
- Oenpelli
- Kakadu
- Darwin

Location

Communities were grouped according to the following locations/regions, based on their proximity to each other (Figure 5.8):

- *Darwin and surrounds:* Includes the following communities – Darwin, Palmerston, Humpty Doo, Belyuen, Acacia Hills, Mudginberri, Woolaning college
- *Wadeye (Port Keats) and surrounds:* Includes the following communities – Wadeye, Peppimenarti, Palumpa, Daly River (Nauyu Nambiyu), Kununurra, Timber creek, Kalkarindji, Lajamanu
- *Kakadu and surrounds:* Includes the following communities – Kakadu, Jabiru, Katherine, Adelaide River, Oenpelli, Barunga, Beswick, Hodgson Downs
- *Tiwi islands:* Includes the following communities – Nguiu, Milikapiti, Pirlangimpi
- *Maningrida*
- *East Arnhem region and surrounds:* Includes the following communities – Maparru, Galiwinku, Gapuwiyak, Groote Eylandt, Yirrkala, Raminginning, Ski Beach, Minjilang, Warruwi, Milingimbi

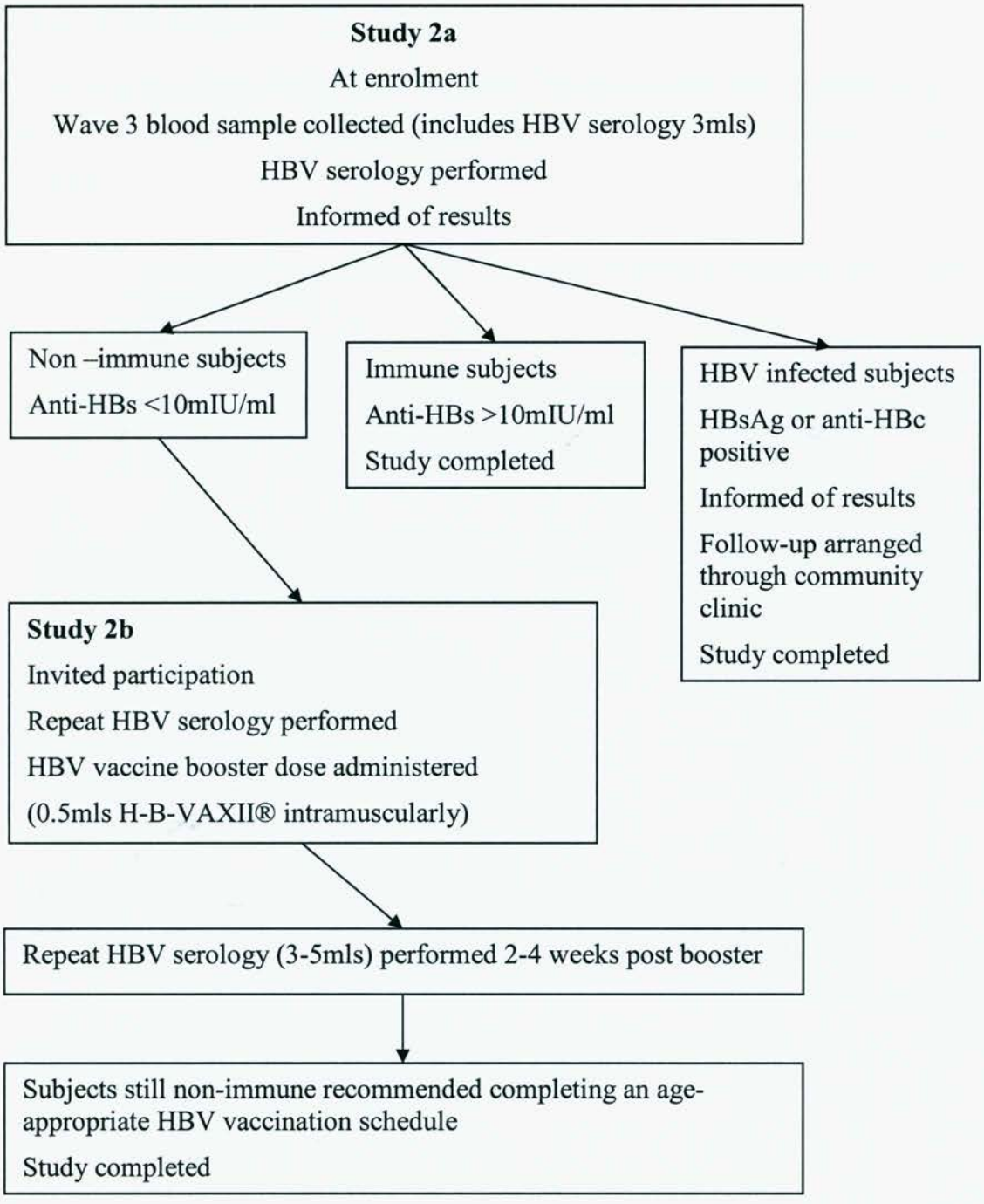


Figure 5.9 Study flow chart for Indigenous adolescent study

c) Enrolment criteria

Subjects who were enrolled in the ABC study were eligible to participate in Study 2a and a sub-sample of anti-HBs negative (<10mIU/ml) participants in the above communities were eligible to participate in Study 2b.

5.4.4 Data collection

Table 5.6 is the list of data collected at wave 3. The procedures took approximately 2 hours for each participant. Figure 5.9 shows the study flow chart with respect to Study 2a and 2b.

Table 5.6 Data collection and source of information in Wave 3 Aboriginal Birth Cohort Study 2006-2008

Data collected	Source of information
Anthropometric and nutritional	
Date of assessment	
Height	Portable stadiometer
Weight	Tannita model TBF 521
Head, mid upper arm, waist hip circumferences	Physical examination
Renal	
Renal ultrasound	Ultrasonography
Random urinary albumen creatinine ratio	Beckman image
Random urinalysis	Urinary dipstick
Metabolic and cardiovascular	
Fasting plasma glucose, total cholesterol, HDL-C, LDL-C, triglycerides	Hitachi 917 Autoanalyser, Roche reagents
Apolipoprotein A-1, Apolipoprotein B, lipoprotein(a)	BN2-Behrring
Fasting plasma insulin	Immunoassay, AIA-PACK
Red blood cell folate	BayerAC 180, Bayer reagents
Blood pressure	Welch Allyn Lifesigns monitor
Haematological and infection	
Full blood count	Coulter Max M
Impetigo, scabies, ear discharge, nasal discharge	Physical examination
Dental	
Questionnaire	
Oral examination	
Non-invasive cardiovascular assessment	
Carotid intimal thickness measure	
Heart rate variability	
Pulse pressure	

Data collected	Source of information
Thyroid Function	
Thyroid ultrasound	
Urinary iodine	
Hepatitis B immunization status	
Hepatitis B surface antigen antibody (anti-Hbs) Hepatitis B core antibody (anti-Hbc) Hepatitis B surface antigen	
Social-emotional well being	
Computer based questionnaire	
Cognitive function	
Computer based Cogstat test battery	
Socio-economic status	
Questionnaire	
Muscle strength	
Grip strength	

Procedures undertaken in wave 2 in 1998-2001

5.4.5 Hepatitis B vaccination history

In the Northern Territory, human serum HBV vaccine was used from 1983 (restricted) until end of 1989, while recombinant HBV vaccine was used from 1st August 1990. Hepatitis B vaccine history was established by adolescent or parental report, confirmed as much as possible, by consulting child personal health records, community health clinic records and Centres for Disease Control (CDC) Immunisation database in Darwin.

The birth dose of HBV vaccine was recorded in the maternal medical records held at Royal Darwin Hospital. Subsequent HBV vaccinations for individuals in communities in the NT were recorded on personal medical records kept at community health clinics. Prior to 2000, hepatitis B vaccination registries were kept on 7 regional databases in the NT. Vaccination data for these regional databases was periodically supplied from individual community health clinics. In 2000/2001, data from these regional registries began to be uploaded onto a Community Care Information System (CCIS) held by the

CDC in Darwin. Since September 2007, all vaccines administered at community health clinics and by other healthcare providers are directly recorded on the CCIS database and no longer transferred from regional databases.

As few participants had child personal health records, vaccine histories were identified from a combination of maternal medical records for birth HBV dose, individual records held at community health clinics, where available, and the CCIS database at the Darwin CDC.

5.4.6 Substance use

Participants were interviewed by the ABC study social researcher about their use of cigarettes, alcohol, marijuana and petrol sniffing. The following questions were asked:

- *Alcohol:* Do you drink alcohol?, How often per week?, How many per day?, What age did you first start drinking?
- *Cigarettes*: Do you smoke tobacco? Number of cigarettes smoked most days? At what age did you start?
- *Marijuana (gunja)*: Do you smoke gunja? How often per week? What age did you start?
- *Petrol sniffing*: Do you sniff petrol? How often per week? What age did you start?

Responses to the above questions were grouped into one of 3-4 categories

- *Substance use*: No or never, Used to, Yes, Missing response
- *Frequency*: < 1 time per week, 2-3 times per week, daily

Responses to the above questions on substance use/abuse were then correlated with baseline hepatitis B serology and response to the booster dose.

Consent

The study participants were given an information sheet which was explained by one researcher with additional visual aids. (Appendix 9) An attempt was made to gender match research assistants with participants. Occasionally a female research assistant dealt with male participants but very rarely vice versa. Written consent was obtained from participants for all procedures, with a staged consent form accommodating refusal for individual procedures.

As the participants were expected to be aged 16-19 years, the 'mature minor' rule was adopted in line with Guidelines for the General Consent of the Royal Darwin Hospital. The mature minor rule allows a child (under 18 years of age) with sufficient intellectual development and capacity to understand the nature and effect of the relevant treatment to be capable of giving consent. For this study, if the primary carer of a participant under 18 years could be located, a separate minor consent form was signed by the carer.

5.4.7 Vaccine and vaccine administration

Details of the Vaccine

Hepatitis B vaccine – H-B-VAX II

CSL/Merck Sharp Dohme Adult formulation preservative free – each 1ml dose contained hepatitis B surface antigen protein 10ug adsorbed onto 0.5mg aluminium hydroxide was used in Study 2b.

The same exclusion criteria and method of vaccine administration as outlined in Study 1 were used.

5.4.8 Assessment of immunogenicity

The same method of assessing immunogenicity as in Study 1 was used. At enrolment a 5-10ml blood sample was collected from each participant. Serum collected in Aboriginal communities in Study 2a and 2b was centrifuged and processed on site by the ABC study team. Serum was stored frozen in the laboratories at Menzies School of Health Research and transported frozen by couriers in batches to Sydney for HBV serology testing.

Each sample was analysed for anti-HBs, HBsAg and anti-HBc in all subjects. In contrast to study 1, serology for HBV was performed by the Institute for Clinical Pathology and Microbiological Research (ICPMR) at Westmead Hospital, Sydney. ICPMR has extensive experience with these serological tests, it performs serological testing for Australian serosurveys and is engaged in quality assurance with the European Seroepidemiology Network. Sera were analysed no the Abbott Architect Automated Analyser i2000SR (chemiluminescent microparticle immmunoassay). This automated system has been found to have equivalent performance to manual ELISA plate testing. (Personal communication: David Dickeson, Hepatitis B serology laboratory ICPMR).

All three HBV markers were tested for each antibody or antigen in sera on the Abbott Architect automated analyser according to the manufacturer's instructions by staff at ICPMR. The upper limit of quantification for the Abbott Architect is 1000 mIU/ml. Values above this level were reported as >1000 mIU/ml. To calculate GMC for those values reported as >1000 mIU/ml, an absolute level of 1000 was used.

5.4.9 Statistical analysis and power calculation

The same method of statistical analysis as in Study 1 was used. Demographic details for each individual enrolled in the study were used to examine hepatitis B serological status by age, gestational age, sex, region, maternal HBV carrier status, vaccination history and time since vaccination.

Only those participants who remained anti-HBs negative (<10mIU/ml) prior to booster HBV vaccination were included in the analysis of anamnestic responses following booster HBV vaccine.

Vaccine efficacy against HBsAg carriage was calculated as 1-relative risk (proportion of HBsAg positive in vaccinated/proportion of HBsAg positive in unvaccinated)

Vaccine efficacy against anti-HBc (Past infection) was calculated as 1- relative risk (proportion of anti-HBc positive in vaccinated/proportion of anti-HBc positive in unvaccinated)

Power calculations

Study 2A: HBV prevalence estimate and long term immunity following vaccination

Sample size: All children in the ABC study (n= 572). Prior to widespread HBV vaccination in the Northern Territory, a school survey detected an HBsAg carriage prevalence of 8.2% in Indigenous children.[293] It was anticipated that the carriage prevalence will have reduced due to HBV vaccination. Sample sizes required to detect differing HbsAg carriage prevalence at an absolute precision of 0.02 with 95% confidence are detailed in Table 5.7.

Table 5.7 Sample size estimates according to HBsAg prevalence

HBsAg carriage prevalence (%)	Sample size (n)
5%	457
3%	280
2%	180

Study 2B: HBV vaccine booster study

Sample size: From the study Hanna et al, in a comparable setting in North Queensland, 60% of Indigenous children vaccinated in infancy were non immune 5 years post vaccination.[251] A minimum of 60% (n=377/572) would be expected in this study, as the children were over 10 years old and antibody levels continue to decline with increasing age. In Hanna et al, 16% of children who were non immune 5 years post infant vaccination failed to demonstrate an anamnestic response to a booster dose of HBV vaccine.[251] In most other studies, the non response rate to a booster dose of the vaccine is less than 10%.[245]

A sample size of 140 subjects would enable estimation of 10% non response rate with a precision of 0.05 and 95% confidence. One of the difficulties is that participants in this study required two visits, one to administer the vaccine and the second to collect sera for anti-HBs measurement 2-4 weeks post vaccine. Tracing participants is a logistically difficult exercise and predicting a loss to follow up rate challenging.

5.4.10 Results

a) Enrolment

Four hundred and thirty seven ABC participants were seen in Wave 3 follow up, with a median age of 18.4 years (range 16 to 20.5 years) and 49% (n=214) were male. Demographics of the wave 3 follow up participants are shown in table 5.8.

There were no significant differences between males and females in their gestational age, birth weight and birth length. Nearly one quarter of participants were located in Wadeye and surrounding communities, with approximately one fifth from Darwin area and Maningrida.

b) Hepatitis B serology

All 437 participants had hepatitis B serology measured during wave 3 follow up. (Figure 5.10) One fifth (n=91, 20.8% (95% CI 17.3-24.9%)) were anti-HBc positive indicating past HBV infection and 7 (1.6%, (95% CI 0.8-3.3%)) were HBsAg positive (3 of whom were HBeAg positive) indicating chronic HBV carriage. (Table 5.8) Twelve subjects (2.8%, 95% CI 1.6-4.7%)) were isolated anti-HBc positive (anti-HBc positive and anti-HBs negative). Males were more likely than females to be HBsAg positive (2.8% vs 0.4%, p=0.05) Of the remaining subjects (n=339), who were neither anti-HBc nor HBsAg positive, two thirds (n=224, 95% CI 60.9-70.9)) were non immune to HBV (anti-HBs <10mIU/ml). (Table 5.8 and Figure 5.10)

Wadeye and surrounds had the highest proportion of participants with past infection (38%, n=35/91), followed by Maningrida (21%, n=19/91), while the lowest location was Darwin and surrounds (6%, n=5/91). The proportion with serological evidence of past infection (anti-HBc positive) was significantly lower in those participants living in Darwin and surrounds compared to those living outside Darwin (5.8% vs 25%, RR 0.23, (95%CI 0.1-0.56), p<0.02). HBsAg carriers were found in 4 different location areas. (Table 5.9)

Table 5.8 Demographics and hepatitis B serology of wave 3 participants (n=437)

	Total n=437		Males n=214		Females n=223	
Age (years)						
Mean	18.3		18.4		18.2	
Median	18.4		18.5		18.2	
range	16-20.5		16-20.5		16.1-20.5	
Gestation (weeks)						
Mean	38.4		38.6		38.3	
Median	39		39		39	
Range	26-41.2		26-41		30-41.2	
< 32 weeks gestation (%)	8 (2%)		3		5	
Birth weight (grams)						
Mean	3043		3165		2926	
Median	3080		3225		2980	
Range	800-5340		800-5340		1010-4900	
< 2000 g (%)	30 (6.9%)		9 (4.2%)		21 (9.4%)	
Birth length (cms)						
Mean	48.7		49.3		48.2	
Median	49		49.5		48.5	
Range	32-56.5		32-56.5		37-55	
Location						
Darwin and surrounds	88		49		39	
Wadeye and surrounds	108		45		63	
Kakadu and surrounds	60		34		26	
Tiwi Islands	53		25		28	
Maningrida	84		42		42	
East Arhnem region	44		19		25	
Hepatitis B serology	n	%	n	%	n	%
Past infection^$	91	20.8	46	21.5	45	20.2
HBV carrier#	7	1.6	6	2.8	1	0.4
Anti-HBs * (n=339)						
<10 mIU/ml	224	66	111	68.5	113	63.8
>10-100 mIU/ml	75	22	32	19.8	43	24.3
>100-1000 mIU/ml	36	10.6	17	10.5	19	10.7
>1000 mIU/ml	4	0.3	2	1.2	2	1.1
Vaccination history	n	%	n	%	n	%
CDC on time	127	29.1	53	24.8	74	33.2
3 doses under 2 years old	82	18.8	42	19.6	40	17.9
3 doses anytime	60	13.7	28	13.1	32	14.3
1 or 2 doses	69	15.8	33	15.4	36	16.1
Nil recorded doses	99	22.7	58	27.1	41	18.4

^ Past infection – refers to all participants who were anti-HBc positive

$Past infection – of the 91 participants who were anti-HBc positive, n=12 were isolated anti-HBc positive meaning they were anti-HBc positive and anti-HBs negative

HBV carrier – refers to all participants who were HBsAg positive

*Anti-HBs – includes only those participants who were negative for anti-HBc and HbsAg and % relates to anti-HBs level as a proportion of this group

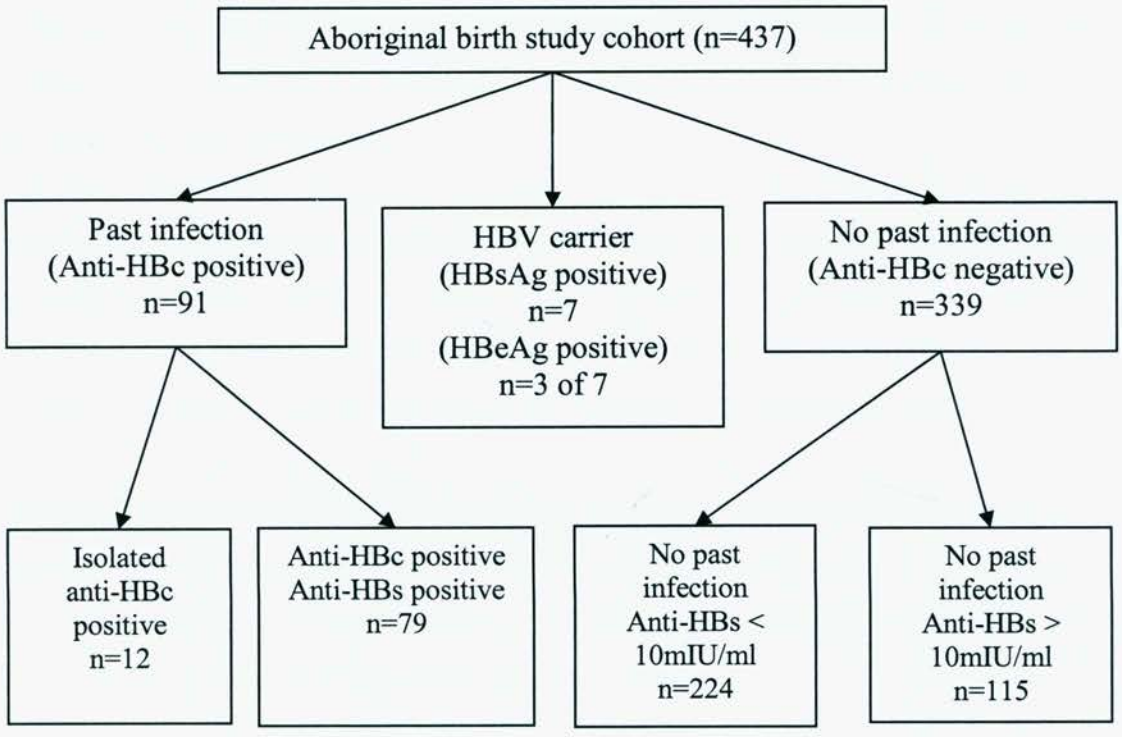


Figure 5.10 HBV serologic markers in Aboriginal birth cohort study participants (n=437)

Table 5.9 Hepatitis B serology according to location of participant at Wave 3 follow up

Location	Past infection n=91 (%)*	Carrier n=7 (%)*	No past infection n=339 (%)*
Darwin and surrounds (n=88)	5 (6%)	2 (2%)	81 (92%)
Wadeye and surrounds (n=108)	35 (32%)	2 (2%)	71 (66%)
Kakadu and surrounds (n=60)	11 (18%)	2 (3%)	47 (78%)
Tiwi Islands (n=53)	12 (23%)	1 (2%)	40 (75%)
Maningrida (n=84)	19 (23%)	0	65 (77%)
East Arnhem region (n=44)	9 (20%)	0	35 (80%)

(%)* – row percentage (proportion from each community/region with past infection, carrier or no past infection)

c) Hepatitis B vaccination history

Only one third of ABC wave 3 participants had received HBV vaccinations on time in infancy, according to CDC criteria, and for approximately one fifth no HBV vaccine record was found in either clinic records or the immunisation database. (Table 5.10)

Males were non significantly less likely to have received HBV vaccines on time than females (24.8% vs 33.2% p = 0.1) and more likely to have no HBV vaccine record found (27.1% vs 18.4%, p=0.03)

Participants who had received HBV vaccines on time tended to have a younger median age compared to those with 1 or 2 doses or no recorded HBV vaccines (17.6 years vs 18.9 years (1 or 2 doses) and 19.2 years (no doses)). There were no significant differences in birth weight or gestational age between vaccine history groups.

Participants from Maningrida had the highest proportion receiving HBV vaccines on time. Participants from Darwin were more likely to have 1 or 2 doses or nil recorded doses than participants from other sites, and less likely to have received vaccines on time as per CDC criteria. (Table 5.10 and Figure 5.11)

Table 5.10 Hepatitis B vaccination history according to age, birth weight, gestational age and birthplace of the participant

		CDC on time n=127		3 doses under 2 years n=82		3 doses anytime n=60		1 or 2 doses n= 69		Nil recorded doses n=99	
Age (years)	Mean Median Range	17.5 17.6 16.1-19.7		17.8 17.9 16.2-19.1		18.9 19.1 16.4-20.5		18.7 18.9 16-20.4		19 19.2 16.4-20.5	
Birth weight (grams)	Mean Median Range	3143 3120 1300-4930		2778 2950 800-3450		3049 3120 1600-4900		3183 3140 1850-5340		3033 3100 1150-5010	
Gestational age (weeks)	Mean Median Range	38.5 39 34-41		37.7 39 26-41		38.3 39 32-41		38.8 39 34-41		38.8 39 31-41.2	
Location^	Total	n	%*	n	%*	n	%*	n	%*	n	%*
Darwin	n=88	10	11	15	17	17	19	21	24	25	28
Wadeye	n=108	40	37	21	19	13	12	21	19	13	12
Kakadu	n=60	16	27	8	13	10	17	11	18	15	25
Tiwi Islands	n=53	16	30	14	26	7	13	5	9	11	21
Maningrida	n=84	36	43	14	17	1	1	4	5	29	35
East Arnhem	n=44	9	20	10	23	12	27	7	16	6	14

^Location – includes regions surrounding the town/community as defined in the methods section

%* – refers to the proportion with vaccine history (CDC on time, 3 doses under 2 years, 3 does anytime, 1 or 2 doses, nil recorded doses) from each location, (eg; number in Darwin with CDC on time/total number of participants from Darwin location)

Interestingly, despite low vaccination completion these participants had the lowest proportion with evidence of past infection, a finding likely to relate to lower HBV carriage rates in Darwin compared to other communities. In contrast, Wadeye participants had the highest proportion of past infection, and the second highest proportion (37%) having received HBV vaccines in infancy on time.

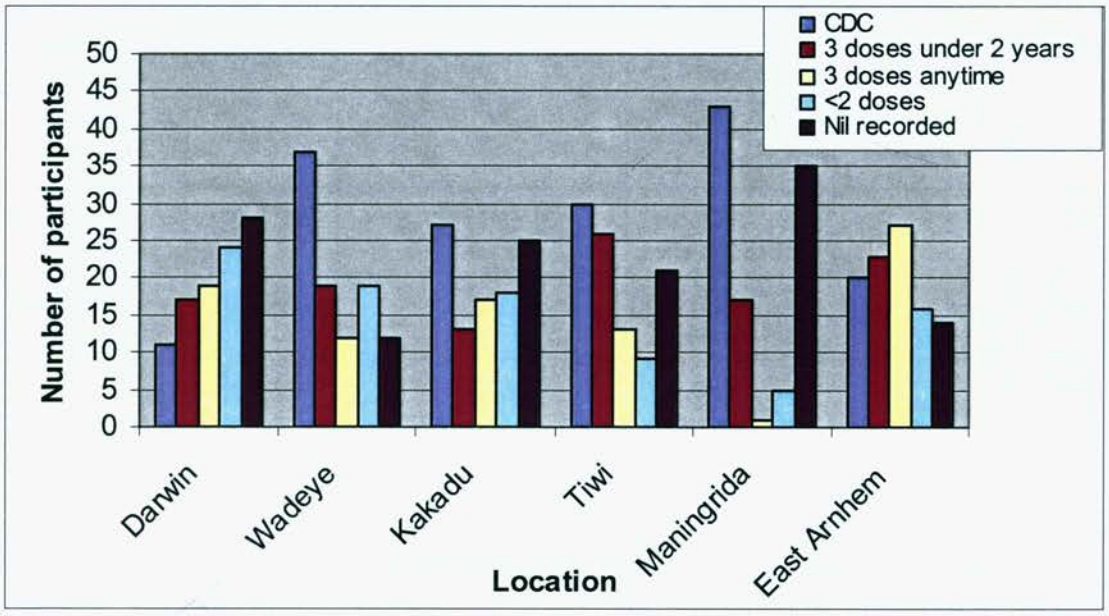


Figure 5.11 Vaccination history according to location

d) Substance use

Participants were asked about alcohol, petrol, marijuana and cigarette use, as outlined in methods. Nearly two thirds reported current cigarette smoking, 40% used alcohol at least 1 time per week, one third used marijuana currently and less than 2% were current petrol sniffers. (Table 5.11) Of those who reported smoking cigarettes, there was equal distribution between the sexes, however males were more likely than females to report current alcohol use (60% vs 40%, RR =1.62 (95% CI 1.3-2.06), p <0.02) and marijuana use (66% vs 34%, RR = 2.11, (95% CI 1.57-2.85), p<0.02). There were no significant differences in median age between those who reported current use of alcohol, cigarettes, or marijuana and those who did not, although they tended to be slightly older (median average 18.6 years vs 18.1 years). Of those who reported current use of marijuana, 41%

(n=55) used it <1 time per week, 31% (n-41) 2-3 times per week and 28% (n=38) used it daily.

Table 5.11 Reported use of alcohol, cigarettes, marijuana and petrol sniffing among wave 3 Aboriginal birth cohort study participants (n=437)

Substance n=437	No/never n (%)	Previous /not current n (%)	Current n (%)	Missing data n (%)
Alcohol	231 (53%)	12 (3%)	173 (40%) <1 / week n=122 2-3 times per week n=47 daily n=4 male n= 104 (60%)	21 (5%)
Cigarettes	119 (27%)	4 (1%)	264 (60%) male n=131 (50%)	50 (11%)
Marijuana	256 (59%)	22 (5%)	136 (31%) male n=90 (66%)	23 (5%)
Petrol sniffing	400 (91%)	10 (2.3%)	7 (1.6%) male n=3 (43%)	20 (4.6%)

Nearly one fifth (17.2%, n=75) of participants reported no or never used alcohol, tobacco, marijuana and petrol sniffing, while 15.8% (n=69) reported current use of multiple substances (alcohol, tobacco and marijuana). One quarter of participants reported current use of combined alcohol and tobacco (27%) and combined tobacco and marijuana (26.5%).

Participants who reported no or never used alcohol, tobacco, marijuana or petrol sniffing were significantly more likely to have received HBV vaccines on time according to CDC criteria than participants who were current users of multiple substances (alcohol, tobacco, marijuana) (46.7% vs 21.7%, RR=2.15, (95% CI 1.29-3.56), p=0.002).

e) Correlation of hepatitis B vaccination history and serologic markers

Most HBsAg positive subjects (n=5/7) had no record of receiving HBV vaccine, and only one was reported to have received HBV vaccine on time as an infant. The latter case is indicative of clinically significant breakthrough infection or vaccine failure, although it is not known whether this patient responded to the primary vaccine series.

Almost one quarter of participants with evidence of past infection had received HBV vaccines on time in infancy compared to nearly one third (29.7%) with no recorded doses. (Table 5.12)

Table 5.12 Correlation of hepatitis B vaccination history with hepatitis B serology collected in wave 3 participants

Vaccine history	Past infection n=91	Carrier n=7	No past infection n=339
CDC on time n=127 (*%)	22 (24.2%)	1 (14.3%)	104 (30.7%)
3 doses under 2 years old n= 82 (*%)	11 (12.1%)	0	71 (20.9%)
3 doses anytime n=60 (*%)	13 (14.3%)	1 (14.3%)	46 (13.6%)
1 or 2 doses n= 69 (*%)	18 (19.8%)	0	51 (15.0%)
Nil recorded doses n=99 (*%)	27 (29.7%)	5 (71.4%)	67 (19.8%)

*% – refers to column percentage

Participants with no vaccines or only 1 or 2 doses recorded were more likely to have evidence of past infection than those with 3 doses recorded (38% vs 21% RR =1.6 (95%CI 1.1-2.3), p=0.01) Of those participants with nil recorded doses (n=99), nearly one third (27/99) had evidence of past infection compared with 17% of those who received vaccines on time (n=22/127, p=0.07) and 13% of those who had 3 doses under 2 years old (11/82, p=0.02). (Table 5.12)

In participants who were negative for anti-HBc and HBsAg (n=339), nearly one third (29.9%. n=20/67) of those with no recorded doses had anti-HBs levels >10mIU/ml at baseline suggesting that they had been exposed to HBV through vaccination despite lack of records. (Table 5.13) This proportion was similar to those who had been vaccinated on time in infancy (35.6%) but lower than those who had received 3 doses at anytime (54.3%). Nearly two thirds (64%) of those who had received vaccines according to 'CDC on time' had anti-HBs levels <10mIU/ml indicating waning anti-HBs levels with time. (Table 5.13)

Table 5.13 Anti-HBs levels at baseline in participants (n=339) with no evidence of past or chronic hepatitis B infection according to vaccine history

Baseline	Hepatitis B vaccination history				
Anti-HBs level (mIU/ml)	**CDC on time n=104 (%*)**	**3 doses under 2 years n=71 (%*)**	**3 doses anytime n=46 (%*)**	**1 or 2 doses n= 51 (%*)**	**Nil recorded doses n=67 (%*)**
<10	67 (64.4%)	51 (71.8%)	21 (45.7%)	38 (74.5%)	47 (70.1%)
>10-100	34 (32.7%)	16 (22.5%)	12 (26.1%)	4 (7.8%)	9 (13.4%)
>100-1000	3 (2.9%)	4 (5.6%)	11 (23.9%)	8 (15.7%)	10 (14.9%)
>1000	0	0	2 (4.3%)	1 (2.0%)	1 (1.5%)

%* – refers to column percentages

Participants with nil recorded HBV doses were significantly more likely to be anti-HBc positive compared to those with any recorded HBV doses (28.7% vs 19%, RR= 0.66, 95% CI 0.45-0.98, p=0.04) (Table 5.14). Vaccine efficacy (1-RR (prevalence in vaccinated/prevalence in unvaccinated)) can be estimated as 34% against anti-HBc positivity, assuming all participants with no recorded doses were actually unvaccinated.

Table 5.14 Clinically benign breakthrough infections (anti-HBc positivity) according to vaccination history

	Anti-HBc positive	**HBsAg negative and anti HBc negative – NO Past infection**	**Total**
Vaccinated (any recorded HBV doses)	64	272	336
No recorded HBV doses	27	67	94
Total	91	339	430

Of those with no recorded doses and HBsAg and anti-HBc negative (n=67), twenty had anti-HBs levels >10 mIU/ml at baseline, suggesting HBV vaccination occurred but had not been recorded. Including these 20 subjects in the group that had any recorded HBV doses slightly increases the vaccine efficacy estimate against anti-HBc. (Table 5.15) Participants with nil recorded doses (excluding those with anti-HBs>10mIU/ml) were significantly more likely to be anti-HBc positive compared to those with any HBV

doses (36.5% vs 17.9%, RR= 0.49, 95% CI 0.34-0.72, p<0.02) and vaccine efficacy of 51% for HBV vaccine protection against HBV infection. (Table 5.15)

Table 5.15 Clinically benign breakthrough infections (anti-HBc positivity) according to vaccination history including those with nil recorded doses who had detectable anti-HBs at baseline

	Anti-HBc positive	HBsAg negative and anti-HBc negative – NO past infection	Total
Vaccinated (any recorded HBV doses)	64	292	356
Nil recorded HBV doses	27	47	74
Total	91	339	430

Similar vaccine efficacy estimates of 45% against HBV infections as measured by anti-HBc are found when comparing only those with no recorded HBV doses and those who have received 3 doses under 2 years old (includes CDC on time and 3 doses under 2 years) (28.7% vs 15.9%, RR = 0.55, 95% CI 0.35-0.86, p<0.02) (Table 5.16)

Table 5.16 Clinically benign breakthrough infections (anti-HBc positivity) according to vaccination history in those with 3 doses under 2 years compared to no recorded doses

	Anti-HBc positive	HBsAg negative and anti-HBc negative – NO past infection	Total
Vaccinated (CDC on time and 3 doses under 2 years)	33	175	208
No recorded HBV doses	27	67	94
Total	60	242	302

Of 437 participants, 339 (78%) were negative for HBsAg and anti-HBc, and of these 274 (81%) had received one or more HBV vaccines. Clinically significant breakthrough infections (HBsAg positive) occurred in only 0.7% (2/274) of participants who had any HBV vaccine doses recorded.

Participants with no recorded HBV doses were significantly more likely to be HBsAg positive compared to those with any recorded HBV doses (6.9% vs 0.7%, RR= 0.11,

95% CI 0.02-0.53, p<0.02) (Table 5.17). Vaccine efficacy (1-RR (prevalence in vaccinated/prevalence in unvaccinated)) can be estimated as 89% against HBsAg positivity, assuming all participants with no recorded doses were actually unvaccinated.

Table 5.17 Clinically significant breakthrough infections (HBsAg positivity) according to vaccination history

	HBsAg positive	HBsAg negative and anti HBc negative – NO Past infection	Total
Vaccinated – (any recorded HBV doses)	2	272	274
Nil recorded HBV doses	5	67	72
Total	7	339	346

Of those with nil recorded doses and no evidence of HBsAg or anti-HBc positivity (n=67), twenty had anti-HBs levels >10 mIU/ml at baseline, suggesting HBV vaccination occurred but had not been recorded. Including these 20 participants in the group that had any recorded HBV doses slightly increases the vaccine efficacy estimate. (Table 5.18) Participants (n=72) with nil recorded doses (excluding those with anti-HBs>10mIU/ml) were significantly more likely to be HBsAg positive compared to those with any HBV doses (9.6% vs 0.7%, RR= 0.07, 95% CI 0.01-0.36, p<0.02) and vaccine efficacy of 93% (95%CI 64%-99%) for HBV vaccine protection against chronic carriage.

Table 5.18 Clinically significant breakthrough infections (HBsAg positivity) according to vaccination history including those with nil recorded doses who had detectable anti-HBs at baseline

	HBsAg positive	HBsAg negative and anti-HBc negative NO past infection	Total
Vaccinated – (any recorded HBV doses)	2	292	294
Nil recorded HBV doses	5	47	52
Total	7	339	346

Similar vaccine efficacy estimates of 92% against HBsAg positivity are found when comparing only those with no recorded HBV doses and those who have received 3 doses under 2 years old (includes CDC on time and 3 doses under 2 years) (6.9% vs 0.6%, RR = 0.08, 95% CI 0.01-0.69, p<0.02). (Table 5.19)

Table 5.19 Clinically significant breakthrough infections (HBsAg positivity) according to vaccination history in those with 3 doses under 2 years compared to no recorded doses

	HBsAg positive	HBsAg negative and anti-HBc negative NO past infection	Total
Vaccinated – (CDC on time and 3 doses under 2 years)	1	175	176
No recorded HBV doses	5	67	72
Total	6	242	302

f) Correlation of hepatitis B serology with birth weight and gestation

Previous reports have suggested reduced primary seroconversion following infant HBV vaccine and birth weight ≤ 2000g and/or gestation ≤ 32 weeks.[63] Participants who had received three HBV vaccine doses on time or under 3 years old (n=209) were assessed for any association with reduced birth weight or gestation with past HBV infection or HBsAg positivity. Only five participants, with receipt of 3 HBV doses prior to 2 years old, had a gestational age less than 32 weeks and none had anti-HBc or HbsAg positivity. Fifteen participants (7.2%, n=15/209), with 3 HBV doses prior to 2 years old, had birth weight less than 2000g. (Table 5.20) None was HBsAg positive and 53% (n=8/15) had anti-HBs < 10 mIU/ml, compared to 57% (n=110/194) of those with birth weight > 2000g. (Table 5.20)

Table 5.20 Hepatitis B serology and relationship to birth weight in participants who had received 3 doses of hepatitis B vaccine under 2 years old (n=209)

	Birth weight	
	≤2000g n =15 (%)	>2000g n = 194 (%)
Past infection	0	34 (17%)
HBsAg positivity	0	1 (0.5%)
No past infection		
Anti-Hbs <10 mIU/ml	8 (53%)	110 (57%)
Anti-HBs 10-100 mIU/ml	6 (40%)	43 (22%)
Anti-HBs >100 mIU/ml	1 (7%)	6 (3.5%)

% refers to column percentage

g) Correlation of hepatitis B serology and substance abuse

Substance use data were available for 6 of the 7 HBV carriers. All reported current use of cigarettes, nearly all used alcohol (83% n=5/6), half used marijuana and none reported petrol sniffing. Approximately one fifth of those who reported current use of alcohol, cigarettes and marijuana had serological evidence of past infection. There was no significant difference in past infection between those using currently using cigarettes or not (24% vs 22%, RR = 0.93, 95% CI 0.62-1.38, p=0.7), and those using marijuana or not (22% vs 21%, RR = 0.95, 95% CI 0.65-1.4, p = 0.8) (Table 5.21). However, non users of alcohol were significantly more likely to have past infection than current users (25.5% vs 16%, RR =1.54, 95% CI 1.03-2.29, p=0.03). Approximately one third of those with past infection reported current use of alcohol and marijuana, while two thirds were current cigarette smokers.

Table 5.21 Hepatitis B serology at baseline according to reported* use of alcohol, cigarettes, marijuana and petrol sniffing among wave 3 Aboriginal birth cohort study participants

	Past infection	Carrier	No past infection
Alcohol	n (%^)	n (%^)	n (%^)
Never/no /used to n=243	62 (25.5%)	1 (0.4%)	180 (74%)
Current use n=173	28 (16%)	5 (2.8%)	140 (81%)
Cigarettes			
Never/no /used to n=123	27 (22%)	0 (0%)	96 (78%)
Current use n= 264	61 (24%)	6 (2%)	197 (75%)
Marijuana			
Never/no /used to N= 278	59 (21%)	3 (1%)	216 (68%)
Current use n=136	30 (22%)	3 (2%)	103 (76%)

*Only includes data where substance use was reported and hepatitis B serology result was available.

^(%) – refers to row percentage

Participants who reported current use of multiple substances were more likely to be HBsAg positive than those who had never used alcohol tobacco, marijuana, however there was no significant difference in past infection (17% vs 28%, p=0.17). (Table 5.22)

Table 5.22 Hepatitis B serology at baseline according to reported* use of combinations of alcohol, cigarettes, marijuana and petrol sniffing compared to no/never used among wave 3 Aboriginal birth cohort study participants

	Past infection	Carrier	No past infection
Alcohol Cigarettes Marijuana Petrol sniffing	n (%^)	n (%^)	n (%^)
Current use n=69	12 (17%)	3 (4%)	54 (78%)
No /never used n=75	21 (28%)#	0	54 (72%)

*Only includes data where substance use was reported and hepatitis B serology result was available.

^(%) – refers to row percentage

Nil significant difference in past infection proportion between no/never used alcohol, tobacco, marijuana, petrol sniffing and multiple substance use (p=0.17).

h) Study 2b: Anamnestic responses following hepatitis B vaccine

Demographics

To be eligible for enrolment in the HBV vaccine booster study, the participant had to be negative for HBsAg, anti-HBc and have anti-HBs levels <10mIU/ml. Of the 339 participants who had no evidence of past HBV infection or chronic carriage, 224 had anti-HBs levels <10mIU/ml and were eligible for enrolment. As outlined in methods section, only participants from the larger communities of Maningrida, Oenpelli, Wadeye, Nguiu, Melville Islands, Darwin and surrounds were approached for enrolment in Study 2b. Of 104 enrolled participants in the booster study from these communities, 69 (66%) completed the study with a second sample collected post booster vaccination for hepatitis B serology measurement. (Table 5.23) Overall study completion was higher in the more remote communities, such as Maningrida and Kakadu compared to less remote, such as Darwin and Tiwi Islands. (Table 5.24)

Table 5.23 Participation in hepatitis B booster vaccine study 2b according to hepatitis B vaccine history

Vaccination history	Total ABC cohort (n)	No HBV infection markers (n)	Uninfected and anti-HBs <10 mIU/ml (n)	Given HBV booster (n)	Post booster vaccine blood sample (n)
CDC on time	127	104	67	41	31
3 doses under 2 years old	82	71	51	24	13
3 doses anytime	60	46	21	8	7
1 or 2 doses	69	51	38	15	8
Nil recorded doses	99	67	47	16	10
Total	437	339	224	104	69

Table 5.24 Participants in the hepatitis B booster vaccine study 2b according to location and completion rate

Location	Eligible*	Enrolled* (received HBV booster vaccine)	Completed* (post booster blood sample)	Completion proportion#
Darwin	61	22	15	68%
Wadeye	35	19	11	58%
Kakadu	33	13	10	77%
Tiwi Islands	26	17	5	29%
Maningrida	50	32	27	84%
East Arnhem	19	1	1	100%
Total	224	104	69	66%

*Eligible – anti-HBc and HBsAg negative and anti-HBs <10 mIU/ml prior to booster on baseline sample

*Enrolled – received HBV booster vaccine

*Completed – blood sample collected 2-4 weeks post HBV booster vaccine

#Completion proportion – proportion of enrolled participants who had a second sample collected 2 to 4 weeks after the booster HBV vaccine

The demographic details of the 104 enrolled participants in study 2b are shown below (Table 5.25). Compared to total ABC study, participants in the booster study were slightly younger (median age 17.9 vs 18.3 years) and more were female (61% vs 51%).

Table 5.25 Participants in the hepatitis B booster vaccine study 2b according to sex, age, birth weight, gestation and vaccination history

	Total N=104	Males N=41	Females N=63
Age (years)			
Mean	17.9	18.1	17.8
Median	17.9	18.1	17.8
range	16-0.20.4	16.0-20.0	16.2-20.4
Gestation (weeks)			
Mean	38.4	38.3	38.4
Median	39	38.6	39
Range	33-41	33-40.7	33-41
Birth weight (grams)			
Mean	3017	3171	2917
Median	3035	3130	2900
Range	1230-4930	1670-4930	1230-3950

	Total N=104	Males N=41	Females N=63
Vaccination history			
CDC on time (%)	41 (39%)	13 (32%)	28 (44%)
3 doses under 2 years old (%)	24 (23%)	11 (27%)	13 (21%)
3 doses anytime (%)	8 (8%)	4(10%)	4 (6%)
1 or 2 doses (%)	15 (14%)	6 (15%)	9 (14%)
Nil recorded doses (%)	16 (15%)	7 (17%)	9 (14%)

Of the 104 participants who received a HBV booster vaccine, 69 (66%) had a repeat anti-HBs level measured 2-4 weeks post vaccine. All participants who had repeat HBV serology performed immediately prior to the booster vaccine remained anti-HBs negative except for three participants who were found to be anti-HBc positive and therefore not included in the assessment of anamnestic responses. As samples were stored and sent in batches the pre-booster results for these three participants were not known at the time of booster vaccination. If their anti-HBc status was known, they would not have been eligible for enrolment in the booster HBV vaccine study. (Figure 5.12)

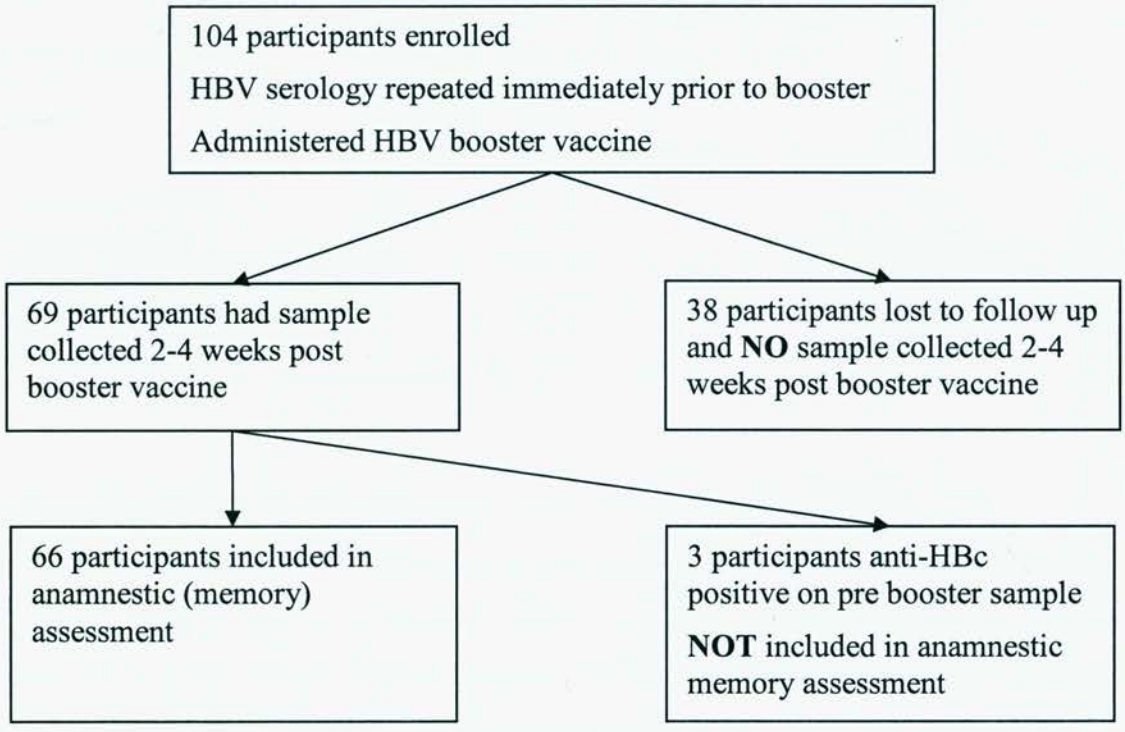


Figure 5.12 Flow chart of participation and loss to follow up for the hepatitis B booster vaccine study 2b

As a result 66 subjects were eligible for inclusion in the assessment of anamnestic (memory) responses. The main reason for loss of follow up (n=38) was an inability to find the participant 2-4 weeks after booster vaccine because he or she was not in the community or had moved out of the community at the time of the second visit. Those who completed the booster study had similar ages, gestation, birth weight and vaccination histories, although were slightly more likely to have received 3 doses of HBV vaccine in total (74% vs 63%, p=0.24), compared to those who did not complete the study. (Table 5.26)

Table 5.26 Comparison of participants who completed the hepatitis B vaccine booster study 2b and those lost to follow up

	Vaccine booster result n= 66	**NO vaccine booster result n= 38**
Age (years)		
Mean	17.9	17.9
Median	17.9	18.0
range	16.2-20.4	16.0-19.7
Gestation (weeks)		
Mean	38.4	38.3
Median	39	38
Range	33-41	34-41
Birth weight (grams)		
Mean	3021	3010
Median	3015	3045
Range	1230-4930	2000-4200
Vaccination history	n=66	n=38
CDC on time (%)	31 (47%)	10 (26%)
3 doses under 2 years old (%)	12 (18%)	12 (32%)
3 doses anytime (%)	6 (9%)	2 (5%)
1 or 2 doses (%)	8 (12%)	7 (18%)
Nil recorded doses (%)	9 (14%)	7 (18%)

Correlation of hepatitis B vaccination status and anamnestic response

The median time interval for collection of post booster sample was 29 days (range 15-92 days). Participants who had received HBV vaccines in infancy according to CDC on time criteria (n=31) had the second sample collected at a median interval of 28 days and the anti-HBs GMC post booster for this group was 24.1 mIU/ml (95% CI 8.9-65.5). (Table 5.27) One quarter of participants (26%, n=8/31, 95% CI 10.4-41.2) in this group failed to demonstrate an anamnestic response. Approximately one fifth (22%, n=10/43,

95% CI 10.4-35.1) participants who received 3 doses, either CDC on time or under 2 years old, failed to demonstrate an anamnestic response. All but one participant with no recorded doses had anti-HBs levels <10 mIU/ml (n=8/9 88%) following the booster consistent with this vaccine history.

Table 5.27 Anti-HBs response to booster hepatitis B vaccine according to vaccination history

Vaccination history	Anti-HBs level (mIU/ml) post HBV booster vaccine							
	<10		10-100		>100-1000		>1000	
	n	*%	n	*%	n	*%	n	*%
CDC on time (n =31)	8	26	16	52	6	19	1	3
3 doses under 2 years old (n= 12)	2	17	6	50	3	25	1	8
3 doses anytime (n = 6)	5	85	0	0	1	15	0	0
1 or 2 doses (n=8)	2	25	1	12.5	4	50	1	12.5
Nil recorded doses (n=9)	8	80	0		1	20	0	
Total (n=66)	25	38%	23	35%	15	23%	3	4.5%

*% – refers to row percentage (proportion of anti-HBs level according to vaccine group, for example anti-HBs <10 mIU/ml divided by total CDC on time participants (=8/31, 26%))

Age and booster anti-HBs response

Older participants (17 and 18 year olds), who had received 3 HBV vaccine doses on time or under 2 years old had non significantly lower anti-HBs GMCs 4 weeks post booster compared to younger participants (16 years old) who had also received 3 HBV vaccine doses on time or under 2 years old (17.8mIU/ml, 95% CI 23.5-171.4) vs 63.5mIU/ml, 95% CI 5.3-59.7). (Figure 5.13)

Correlation of substance use and anamnestic response

Use of alcohol, cigarettes and marijuana in booster vaccine study participants

Information on substance use was available for 64 of the participants who completed the booster study. Nearly one third reported current use of alcohol (n=20/64, 31%) and marijuana (n=18/64, 28%), while more than half smoked cigarettes (n=39/64, 61%) and none reported use of petrol. In those participants who had received 3 doses of HBV

vaccine under 2 years old (n=41), 19% (n=8/41) were current users of alcohol, marijuana (20% (n=9/41)) and cigarettes (56% (n=23/41)).

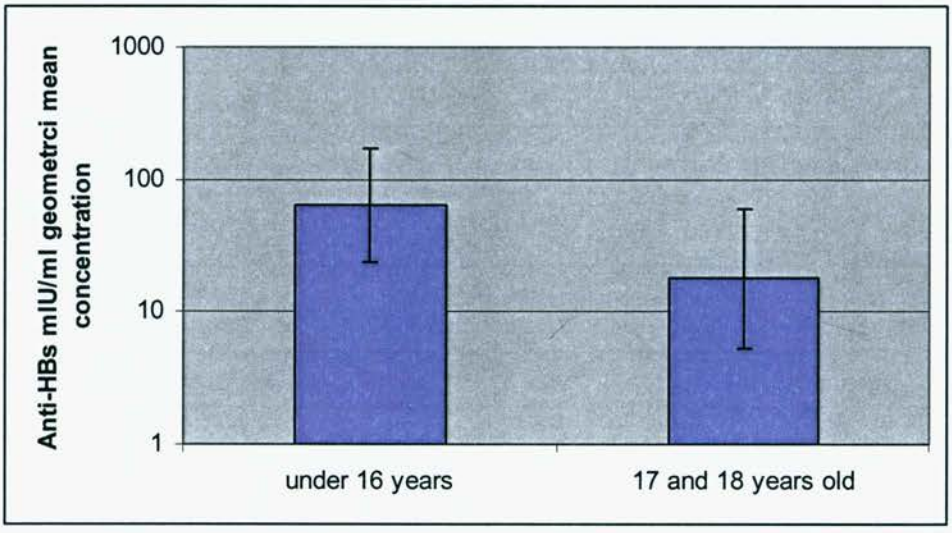


Figure 5.13 Anti-HBs geometric mean concentration post booster vaccine according to age in participants who had received 3 hepatitis B vaccine doses on time or under 2 years old

Anamnestic responses according to substance use

Anamnestic responses in those participants who had received 3 HBV vaccine doses under 2 years old (includes CDC on time and 3 doses under 2 years (n=41)) were compared between those currently using alcohol or cigarettes and non users. (Table 5.28) Current users of alcohol, or cigarettes, were non significantly less likely to demonstrate an anamnestic response compared to non users, while the difference was statistically significant for marijuana users.

The anti-HBs GMCs in those who had received 3 HBV doses either on time or under 2 years old were non significantly lower in substance users compared to non users; alcohol (9.0 vs 31.1mIU/ml, p=0.44), cigarettes (18.5 vs 34.7mIU/ml, p=0.46) and marijuana (3.7 vs 41.4mIU/ml, p=0.12). Comparisons of anamnestic responses between combinations of substance abuse, example tobacco and alcohol vs non substance use, in those who have received 3 HBV vaccine doses under 2 years old is limited by the small samples sizes.

Table 5.28 Anti-HBs response according to use of alcohol, cigarettes and marijuana in participants who had received 3 doses of hepatitis B vaccine on time or under 2 years old

	Anamnestic response			
	Anti-HBs >10 mIU/ml		Anti-HBs <10mIU/ml	
	n	*%	n	*%
Alcohol				
Current use n=8	5	62%	3	38%
Never/no/used to n= 33	26	79%	7	21%
Relative risk = 0.79, (95% CI 0.5-1.4), p =0.34				
Cigarettes				
Current use n= 23	16	70%	7	30%
Never/no/used to n=18	15	83%	3	17%
Relative risk = 0.83, (95% CI 0.6-1.2), p =0.31				
Marijuana				
Current use n=9	3	33%	6	66%
Never/no/used to n=32	28	88%	4	12%
Relative risk =0.38, (95% CI 0.15-0.97), p <0.02				

*% – refers to row percentage

5.4.11 Summary of results of study 2

- Only 1.6% (95% CI 0.8-3.3%) of the total cohort had evidence of HBsAg positivity
- Nearly one fifth of the total cohort had serological evidence of past HBV infection (anti-HBc positive, with or without antiHBs)
- Approximately 2.5% had isolated anti-HBc positivity

- Nearly one third were recorded as receiving HBV vaccines on time in infancy and 62% as receiving 3 doses at any time. These participants were younger than those recorded as having fewer or no doses

- Most HBsAg positive subjects (n=5/7) had no record of receiving HBV vaccine, and only one was reported to have received HBV vaccine on time as an infant. On this basis, clinically significant breakthrough HBV infection (HBsAg positivity) occurred in only 0.7% (95% CI 0.3-1.7) of those who had received any HBV vaccine.

- Of those participants who had received 3 doses of HBV vaccine under 2 years old and were both HBsAg and anti-HBc (n=175), two thirds (67%) had anti-HBs levels < 10mIU/ml indicating waning anti-HBs levels with time.

- Two communities (Maningrida and Wadeye) were more likely to have received HBV vaccine, either on time or 3 doses before the age of 2 years, but were also more likely to have evidence of past infection

- The relative risk of HBsAg positivity in HBV vaccine recipients is 0.09 to 0.11 (95% CI 0.02-0.59). Vaccine effectiveness can be estimated as 89-91% against HBsAg positivity

- Vaccine effectiveness against anti-HBc positivity is estimated at 34 to 51%

- Two thirds of participants who had received HBV vaccines on time in infancy had anti-HBs <10mIU/ml at a median age of 17 years indicating loss of anti-HBs over time

- One quarter of participants, median age 17.9 years, who had received HBV vaccines in infancy on time and who had anti-HBs <10mIU/ml pre HBV booster, failed to demonstrate an anamnestic response.

- Current users of marijuana were significantly less likely to demonstrate an anamnestic response compared to those who were non users, but this was based on small numbers and vulnerable to misclassification.

5.5 Discussion

These cohort studies add important information to the international body of literature regarding long-term follow-up (>10 years) of high-risk groups within a low endemicity country vaccinated against hepatitis B in infancy. WHO, the European consensus group and Viral Hepatitis Prevention Board have called for more long-term follow up studies, particularly in low endemicity countries.[245,248] In this study, anamnestic responses to a dose of HBV vaccine in persons who were initially vaccinated in infancy, 11 to 20 years previously, are presented for the first time in Australia.

In the following section results from both study 1 and 2 are compared with each other and findings in relation to the study hypotheses are presented. These studies add to the international literature in the following areas. First, estimating rates of clinically significant breakthrough HBV infections more than 10 years post infant vaccination in a low endemicity country. Second, making an important contribution to the limited international data on factors that influence anamnestic responses to HBV in previously vaccinated persons and third, confirming anti-HBs responses post booster vaccine are a valid measure of anamnestic response. Potential limitations of each study and future directions are discussed below.

5.5.1 Comparison of Study 1 and Study 2

The two cohorts differ in the age group studied, ethnicity/Aboriginality and anamnestic responses. (Table 5.29) Participants in study 1 had lower HBsAg and anti-HBc positivity rates and higher anamnestic responses compared to study 2. The most likely explanation for the higher proportion with anti-HBc positivity in the Indigenous cohort (study 2) is residence in communities with historically higher HBV endemicity. The reduced anamnestic response in study 2 (78%) compared to study 1 (98%) may relate to the older age of these participants (18.4 vs 14.5 years), potentially indicating waning immune memory, and possibly to reduced rates of primary seroconversion in Indigenous infants compared to non Indigenous infants.[252] HBV serologic markers from waves 1 and 2 in the ABC study are not available, as they were not tested, but would have been useful in measuring waning anti-HBs levels and timing of anti-HBc and HBsAg seropositivity.

Table 5.29 Comparison of hepatitis B serological results in study 1 and 2

	Age median years (range)	HBsAg positivity n (%)	Anti-HBc positivity n (%)	Anti-HBs negative^ (<10mIU/ml) n (%)	Anamnestic response* n (%)	Anamnestic response (GMC)
Study 1 (n=70)	14.5 (11.5 – 16.6)	0	2 (2.9%)	42 (62%) (95% CI 50-73%)	67 (98%)	1095 mIU/ml (95% CI 681 761.2)
Study 2 (n=437)	18.4 (16-20.5)	7 (1.6%)	91 (20.8%)	118 (67%) (95%CI 61-74%)	33 (78%)	24.1 mIU/ml (95% CI 8.9-65.5).

^Anti-HBs negative includes only those participants in study 1 who had received HBV vaccine in infancy according to CDC criteria (n=68) and in study 2 only includes participants who were neither anti-HBc or HBsAg positive and who received 3 doses of HBV vaccine under 2 years old (n=175)

*Anamnestic response (as defined in methods) only includes those participants in study 1 who received HBV vaccine on time according to CDC criteria (n=68) and participants in study 2 who received 3 doses of HBV vaccine under 2 years old and enrolled in booster study (n=43)

5.5.2 Study hypotheses

There were two original hypotheses for both studies.

First, a selective vaccination strategy targeting Aboriginal children and children born to mothers from 'at risk' countries has resulted in lower hepatitis B carriage rates (HBsAg positivity) compared to pre-vaccination data.

Second, most children who were vaccinated more than 10 years previously will have anti-HBs <10 mIU/ml and an anamnestic (memory) response to a booster dose.

a) Hypothesis 1: HBV carrier and infection seroprevalence reduced post HBV vaccination

The results from both these studies are consistent with results from other international studies. Reassuringly rates of clinically significant breakthrough infections (HBsAg positivity) in both studies are below 2% (0% in Study 1 and 1.6% in Study 2), which WHO determines to indicate a country with low HBV endemicity.

Study 1: No chronic carriers were identified in the 'at risk' adolescent cohort in study 1. The small sample size (n=70) and the fact that participants came from several different ethnic groups means that it is not possible to confirm or refute that HBV vaccination has

resulted in a reduction in HBV carrier and infection rates. In the pre –vaccination era, HBsAg prevalence ranged from 0.5% to 9% in different studies, as outlined in chapter 4.[305-307] A larger seroprevalence study with sufficient power and including different ethnic groups is needed to answer this hypothesis.

Study 2: Between 1st January 1987 and March 1990, 1238 Indigenous babies were born at Royal Darwin Hospital, at this time 98% of all births in the region were hospital deliveries, and 4334 Indigenous live births were reported in the Northern Territory.[340] Wave 1 ABC participants were enrolled from 1st January 1987 to March 1990. Thus, Wave 3 ABC participants, (n=437) represent 35.3% (n=437/1238) of total Indigenous births at Royal Darwin Hospital and 10.1% (n=437/4334) of the total Northern Territory cohort for 1987-1990.[337] In addition, the mean birth weight and gestation of ABC participants is similar to Indigenous births in the NT reported from 1987-1990.[340] Average birth weight ranged from 3033 to 3067g and gestation ranged from 38.3 to 38.9 weeks in Indigenous births between 1987 and 1990.[340] Thus, participants in this study could be considered representative of all Indigenous births in the top end of the Northern territory during this period. This is important in generalising these results to the wider Indigenous community in the Northern Territory.

HBV carriage was estimated at 1.6% (95% CI 0.8-3.3%) in 437 Indigenous adolescent participants aged 16 to 20 years in the NT. Vaccine efficacy against HBsAg positivity was estimated at 89-93%, similar to the 96.5% found in Gambian adolescents approximately 19 years after infant vaccination.[264] Lower vaccine efficacy against anti-HBc positivity was found in this study (34-51%) compared to 83.4% (95% CI 79.8-86.6%) in the Gambia, although the 95% confidence intervals overlap.[264]

In the pre-vaccination era, HBsAg prevalence in similar age groups in the Northern Territory was substantially higher, ranging from 8.2% to 23%.[292,293,295,299] The sample size was powered to detect HBsAg carriage of 5% with precision of 0.02. This sample (n=437) equates to 6.5% of the estimated NT Indigenous population aged 15-19 years (n=6677, NT projected population in 2007 based on 2001 census) and 10% of the total NT Indigenous cohort from 1987-1990 and is therefore likely to be an accurate reflection of current HBV carrier prevalence in this age group. The national serosurvey

conducted in 2002 tested 100 serum samples from persons 10-19 years old and found 0% (95% CI 0-3.6%) positive for anti-HBc or HBsAg, but did not include any sera from the NT.[289] The HBsAg positive estimate in study 2 includes men and women and is lower than the 3.7% (95% CI 2.6-5.1%)[302] and 4.8% (95% CI 2.0-9.7%)[303] found in recent seroprevalence studies in a wider age group of Indigenous women in the NT aged 15-39 years. Although participants in study 2 are representative of both urban and remote Indigenous adolescents in the Northern Territory, these results are not likely to be representative of prevalence in other Indigenous communities in Australia, given the diversity of Indigenous communities and the timing of HBV vaccine introduction outside the NT. In Study 2 participants a comparison of the prevalence of HBsAg and anti-HBc positivity by HBV vaccine history indicates the effect of HBV vaccine on reducing HBsAg positivity. (Table 5.30)

Table 5.30 Hepatitis B serologic markers according to hepatitis B vaccine history and comparison with previous studies in the pre hepatitis B vaccine era

Study 2 Vaccination history	**Past infection n (%)**	**HBsAg positivity n (%)**	**Study sample size**
3 doses	46 (17.1%)	2 (0.7%) 95% CI 0.3-1.8	269
< 3 doses	18 (26.1%)	0	69
Nil recorded doses	27 (27.3%)	5 (5.1%) 95% CI 0.7-9.4	99
			Total = 437
Pre vaccination era Study			
Gardner et al[293]	41.7%	8.2%	73
Mathews et al[297]	64%	28%	439
Gill et al[298]	31%	6.1% 95% CI 3.9-9.6	162
Gardner et al[299]	50%	12%	Not reported
Barrett et al[291]		13.1%	283

Summary: Assessing the impact of selective vaccination programs is difficult. The heterogeneity of both 'at risk' ethnic adolescent groups and Indigenous adolescents across the country makes it difficult to confirm a universal reduction in HBsAg positivity post HBV vaccine introduction in all Indigenous and 'at risk' ethnic communities. Study 2 suggests that HBsAg carriage has reduced in the post HBV vaccine era compared to prevalence in the pre vaccine era in Indigenous communities in the NT and is supported by other studies.[302,303] In the next decade, seroprevalence studies and monitoring of HBV notifications/hospitalisations, both nationally and in specific ethnic/Indigenous groups, will be needed to more accurately determine the impact of the HBV vaccination program in Australia.

b) Hypothesis 2: HBV immunity and anamnestic responses

Hepatitis B immunity: persistence of hepatitis B surface antibody

Most participants (two thirds) in study 1 and 2 had anti-HBs <10mIU/ml at 14-18 years post infant HBV vaccination but the majority demonstrated an anamnestic response to a single vaccine dose, thus confirming hypothesis 2.

Summary data from the literature review in Chapter 4 indicates that 10 years or more after infant vaccination, two thirds to three quarters of vaccinees have anti-HBs levels <10mIU/ml. Results in the two studies are similar to this international data and to the 59% (n=132/224) with loss of anti-HBs documented by Hanna et al in the only other long term (5 years) follow-up study in Indigenous Australians.[251]

One fifth of Indigenous adolescents had evidence of anti-HBc positivity (including 16% with three documented doses of HBV vaccine under 2 years of age) (study 2) compared to 3% in adolescents born to mothers from 'at risk' countries (study 1). Of note, three participants had evidence of anti-HBc positivity on the second sample collected at the time of administration of the HBV booster vaccine. One was found to have isolated anti-HBc positivity with anti-HBs negative (<10mIU/ml) and as this may have been a false positive result was excluded from further analysis. Of the remaining two participants, one had no evidence of receipt of HBV vaccines and the other had received 3 doses under 2 years old. In both these cases anti-HBc was positive, HBsAg negative and anti-HBs >100mIU/ml. Both were excluded from further analysis of anamnestic

responses and one, with a past history of HBV vaccine receipt, could be considered as a clinically benign breakthrough infection, who most likely was exposed in the interim period between the baseline visit and HBV booster vaccine administration. Inclusion of HBV serology in subsequent waves of follow up in this cohort is planned and will assist in determining the rate of natural anti-HBs rises and development of anti-HBc or HBsAg positivity. This will be a useful marker of HBV immune persistence following HBV vaccination. The goal of HBV vaccination is to protect the individual from chronic HBsAg carriage and reduce community transmission and absence of HBsAg or clinical hepatitis in vaccinated children is consistent with success. No data on whether participants in the ABC study have experienced clinical acute hepatitis was available.

Isolated anti-HBc positivity

Isolated anti-HBc positivity (anti-HBc alone) was seen in 2.7% (n=12/437) of Indigenous participants (study 2) and no participants in study 1. Serological surveys indicate that isolated anti-HBc positivity is seen in 10-20% of persons with detectable anti-HBc in low endemicity countries.[341] This is comparable to 13% (n=12/91) of Indigenous participants with detectable anti-HBc in study 2.

Anti-HBc alone is due to one of the following: false positive test result, window phase of acute HBV infection, resolved HBV infection or occult chronic HBV infection.[341,342] Occult chronic HBV infection is usually defined as presence of HBV DNA in serum or liver in the absence of HBsAg and its complications include chronic liver disease, such as cirrhosis and hepatocellular carcinoma. HBV DNA has been found in sera of patients with isolated anti-HBc positivity and estimates vary from 0.2% in blood donors, 3% in African immigrants to 47% in IV drug users.[342,343] Therefore, isolated anti-HBc positivity may indicate occult chronic HBV infection and its detection should prompt further investigation. Progression to severe liver disease in those with occult HBV infection is associated with immunosuppression, such as HIV infection, increasing age, hepatitis C co-infection and alcohol use. This has implications for alcohol use in Indigenous adolescents who are anti-HBc positive alone. HBV DNA in sera was not tested in study 2, as consent for this was not sought, and so the number with occult chronic HBV infection is not known. Although it is unlikely, low levels of HBV DNA in sera of these patients are potentially infectious. Management guidelines exist for

isolated anti-HBc positivity and include; confirmatory anti-HBc testing, liver function tests, HBV DNA in sera and possibly liver biopsy.[343]

Anamnestic responses

The majority of participants in both studies demonstrated anamnestic responses (hypothesis 2). However booster responses were higher in younger adolescents from study 1 (98% response), born to mothers targeted by the at risk programs, than slightly older Indigenous adolescents (78%) in Study 2. Not only was the proportion achieving anamnestic response lower in Indigenous adolescents, in those who did demonstrate anamnestic responses their anti-HBs response was quantitatively lower. Two thirds of Indigenous adolescents who received 3 HBV vaccine doses under 2 year old had levels 10-100mIU/ml post booster, with an anti-HBs GMC 24.1 mIU/ml compared to study 1 participants with an anti-HBs GMC 1095 mIU/ml. In contrast, Hanna et al reported 81% of Indigenous children, five years after infant HBV vaccination, achieved anti-HBs levels >100 mIU/ml following a booster HBV vaccine.[251]

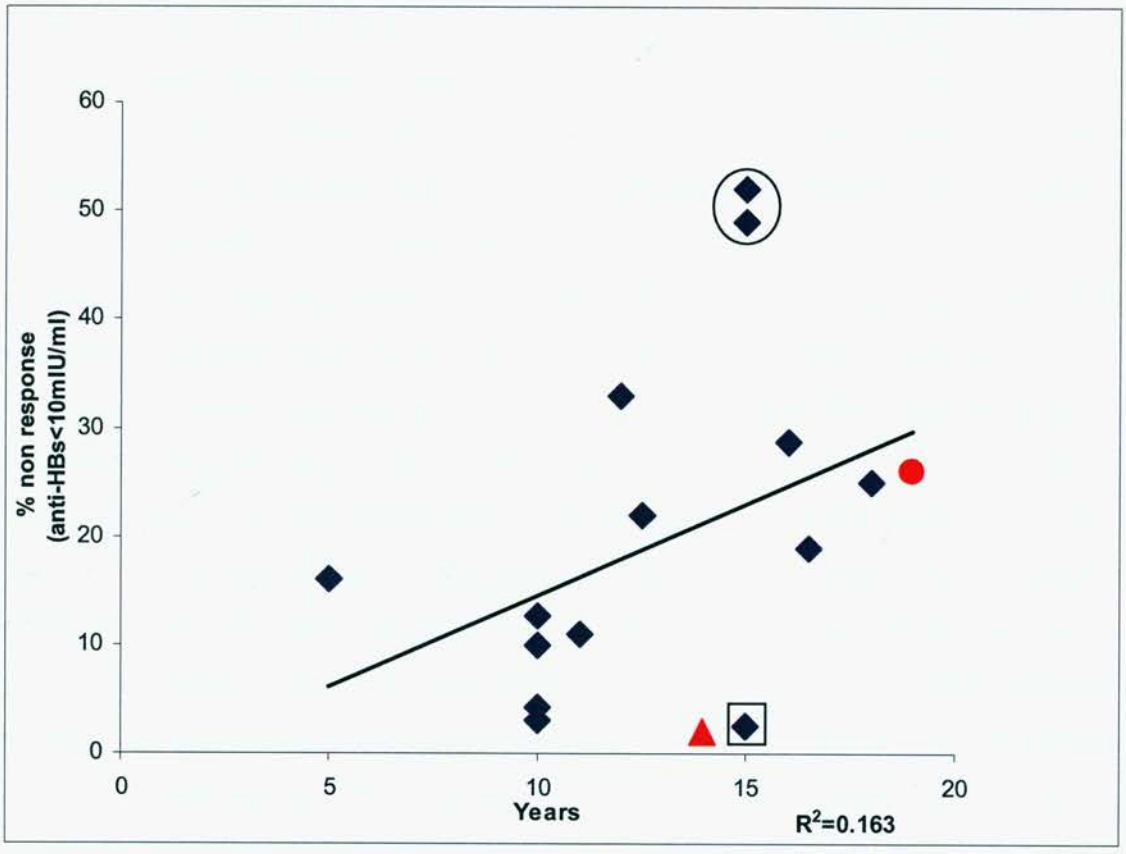
Factors influencing duration of immunity and anamnestic response

There are several factors that contribute to duration of immunity and anamnestic responses, including age, HBV endemicity, primary seroconversion responses, genetic HLA type, and substance use. These factors and their implications for long term immunity are examined below.

Age

With increasing age and therefore time since vaccination, persistence of anti-HBs reduces and anamnestic responses are quantitatively lower, which may indicate waning immune memory. Samandari et al showed that children aged 5-7 years had better responses than adults 14 years old, with higher anti-HBs GMCs 2 weeks post booster in 5-7 years olds (1070mIU/ml) versus 11-14 year olds (148mIU/ml).[266] In these cohort studies the younger 'at risk' adolescents in Study 1, (median age 14.5 years) had anti-HBs GMCs nearly 50 fold higher than Indigenous adolescents (median age 18.4 years). In addition in Study 2, older Indigenous participants (17 and 18 years old) had non-significantly lower anti-HBs GMC compared to younger participants (16 years old) following HBV booster vaccine. Hanna et al found an anamnestic non-response of 16%

(95% CI 10-24%) at 5 years post booster and suggested that, as the majority demonstrated immunological memory, additional booster doses for all Indigenous children at school entry were not required.[251] Study 2 extends the period of follow up by nearly 15 years and has found a higher non response rate of 20-25%. As shown in the following figure 5.14, data from studies in settings of high and low HBV endemicity suggests that increasing age is associated with an increasing prevalence of lack of anamnestic response, likely to be a proxy for waning immune memory.



Each diamond represents % non response detected in individual studies (n=14) from table 4.1 in Chapter 4, however excludes the study by Boxall et al, as follow up ranged from 6- 18 years. The percentage non response from study 1 (triangle) and study 2 (circlel) are also included.

High endemic country studies by Hammit et al (Alaskan natives) and Bialek et al (Micronesia) are circled

Low endemic country study by Zanetti et al (Italy) has a square around it

- Linear regression (trend line) for studies included, $R^2 = 0.1637$

Figure 5.14 Non response to booster hepatitis B vaccine according to time since infant vaccination in studies performed since 2002*

Implication: The presence of anamnestic antibody responses is thought to equate with protection from natural HBV infection. As age increases anamnestic responses decrease implying potential loss of protection requiring additional HBV booster vaccines, however studies to date indicate that HBsAg positivity remains <5% in vaccinated cohorts.

Influence of HBV endemicity

Anamnestic responses seem to relate to age, however other factors may be responsible. There is a suggestion in the graph that HBV endemicity has a role in determining anamnestic responses. Environments with lower HBV endemicity are associated with higher anamnestic responses. Both cohort studies in this thesis were conducted in high risk groups in a low endemic country. Study 1 participants are more likely to come from a lower HBV endemic environment than Indigenous adolescents in study 2, as pre-vaccination data suggests, however the current difference in endemicity is not able to be absolutely quantified due to the limited studies available. However, vaccinated adolescents in these two groups are more likely to have natural HBV exposure than adolescents in lower risk settings in other parts of Australia and as a result experience transient rises in anti-HBs, ideally without developing chronic HBV carriage. This has been termed natural boosting.[263] It has been hypothesised that the duration of protection is longer in those who have intermittent natural HBV exposure, as both cellular and humoral immunity are boosted, compared to those who do not. In a low HBV endemic environment, chances for natural exposure are lower and immunity may not be boosted naturally, suggesting a potentially greater need for booster HBV doses in adolescence.

Exposure to HBV vaccine in the booster vaccine trials is designed to mimic natural exposure, albeit with limitations, such as different route of exposure and administration of adjuvant in vaccine, and should provoke an anamnestic response. Interestingly, anamnestic responses in study 1 participants, who were more likely to be in a lower HBV endemic environment, were higher (98%) than Indigenous adolescents (78%). Supporting data comes from a study in another low endemicity country (Italy) where 97% of children demonstrated anamnestic responses 10 years after infant HBV vaccination[246] In contrast, in high endemicity settings, for example Alaska and

Micronesia, lower proportions of anamnestic responses (48-67%) have been reported.[242,256,276]

Implication: Age and HBV endemicity influence anamnestic responses. Higher HBV endemic environments seem to be associated with lower anamnestic responses and this may have implications for whether additional booster responses are needed. However, HBV endemicity changes over time, in part due to HBV vaccination program success, and therefore its potential influence on natural boosting will also change. Further studies will be needed to clarify the influence of HBV endemicity on anamnestic responses.

Primary seroconversion

One of the main determinants of immune memory persistence is successful primary seroconversion. Factors that reduce primary seroconversion are likely to reduce anamnestic responses. As discussed in Chapter 1, higher antibody levels post primary immunisation result in longer persistence of circulating antibody and greater induction of immune memory B cells which translates into greater subsequent anamnestic responses. In both these cohort studies the post primary seroconversion rates were not known. Reduced anamnestic responses in Indigenous adolescents, compared to study 1 participants, may be due to reduced primary seroconversion. However, HBV seroconversion is known to approximate 95% following the 3 dose infant HBV vaccine series.[63] Although it is not known it is likely that similar seroconversion was achieved in the 'at risk' study 1 adolescents, however was potentially lower in study 2 Indigenous adolescents. There are few studies on rates of primary seroconversion in Indigenous infants after 3 doses of HBV vaccine. As reported by Hanna, a study in Queensland by Sheridan et al found a seroconversion rate of 88% after 3 HBV vaccine doses in 81 Indigenous infants.[344] Hanna reported 54% seroconversion at 10-18 months old in Indigenous infants (n=26) in central Australia, who had received hepatitis B immunoglobulin and HBV vaccine at 0, 1, 6 months old.[344] As discussed in chapter 4, this lower seroconversion rate may reflect potential interference from the co-administered hepatitis B immunoglobulin. Hanna et al also reported 54% of children retained anti-HBs > 10mIU/ml at a median age of 24.5 months after infant (3 dose) vaccination, which was lower than other studies internationally, as discussed in Chapter

4.[107,252] In a comparable study in Thailand, over 90% of children, aged 2 years, vaccinated with the same HBV vaccine and schedule, had anti-HBs >10mIU/ml.[345]

One factor leading to reduced primary seroconversion is reduced vaccine quality because of poor cold chain maintenance. Significant cold chain failures involving the HBV vaccines that these adolescents received during the primary vaccine series may have occurred. A 1990 study by Miller et al investigated the maintenance of the cold chain in the Northern Territory.[346] Nearly half (47.5%) of the hepatitis B vaccine vials were exposed to temperatures of less than 3°C and therefore at risk of losing potency through freezing.[346] In this study there was no clustering by community as might be expected with a single cold chain failure, but it is difficult to evaluate this retrospectively. There were no cold chain failures identified in the storage and handling of the HBV vaccine used in the booster studies.

Another factor known to reduce primary seroconversion is birth weight (<2000g) and gestational age (<32 weeks) and infants with these characteristics are recommended additional doses of HBV vaccine or measurement of their anti-HBs level to determine if seroconversion has occurred.[63,122] In study 2, 209 participants received three doses of HBV vaccine under 2 years old, including only 5 with gestation $\leq$ 32 weeks and 15 with birth weight $\leq$ 2000g, there was no increased risk of past HBV infection or HBsAg positivity compared to participants with higher birth weights and gestation, although the small sample size limits generalisation of this finding.

Another factor influencing primary seroconversion is antigen content of the HBV vaccine used in the infant. The proportion of non response (20-25%) in Indigenous adolescents (Study 2) is lower than that seen in studies in Alaskan natives by Hammit (49%) and Petersen (33%) and in Micronesians by Bialek (52%).[242,256,276] The high rate of non responsiveness in the study by Hammitt et al and Bialek et al may relate to the low dose (HBsAg 2.5ug) of recombinant vaccine used in the primary vaccine series. In contrast, adolescents in study 1 would have received a recombinant 5-10ug HBsAg (HBV) vaccine as this was the vaccine in use at the time. However, the Indigenous adolescents in study 2 received a plasma derived vaccine in infancy and subsequently a recombinant booster vaccine in study 2. One study by Samandari et al suggested

anamnestic responses were lower in those given plasma derived vaccine in infancy followed by a recombinant booster vaccine in adolescence.[266] The mechanism for this has not been defined. At present, all infants in Australia receive a 10ug HBsAg HBV vaccine at birth and 10ug HBsAg in combination vaccines at 2, 4 and 6 months old.

Other factors reducing primary seroconversion include immune deficiency, example HIV infection, premature infants, and haemodialysis patients.[63] Few long term follow up studies have been conducted in immune deficient populations and are thus needed.[63]

Implication: Reduced primary seroconversion equates to reduced anamnestic responses. Vaccine antigen choice and cold chain maintenance can influence primary seroconversion. Long term follow up studies in immune deficient populations vaccinated in infancy are needed to inform booster recommendations

Maternal HBsAg status

Infants born to HBV carrier mothers are at higher risk of chronic HBsAg carriage.[14] Only 7 participants in study 2 and none in study 1 were HBsAg positive. The HBsAg status of mothers of these participants in study 2 is not known and 5 of the 7 had no HBV vaccine doses recorded, and so cannot be considered vaccine failures.

Genetic HLA type

Genetic factors, such as HLA type, can also influence primary HBV responses.[28,30,31] Reduced HBV vaccine responses have been found in those with the following HLA class II alleles –DR3, DR14 and B8.[30,347] Indigenous population in northern Australia has been found to have more commonly an HLA-DR14 allele than non Indigenous people[348] and its presence has been associated with reduced booster HBV responses.[271] Hanna et al report HLA-DR14 allele was present in 56% of non responders compared to 12% of responders ($p<0.05$) to a booster dose of HBV vaccine at 5 years old.[271] A similar association with HLA-DR14 and low responsiveness to primary HBV vaccination and anamnestic responses has also been found in Chinese children [275] and with the absence of HLA A*02, HLA DRB1*08 and presence of HLA B*15 in Indigenous Taiwanese populations.[29,269] However, as rates of HBV infection in Taiwan have markedly decreased since the introduction of HBV vaccination any significant

contribution from HLA alleles leading to reduced vaccine protection is unlikely at a community level.[260]

Implication: Genetic factors have been shown to influence anamnestic booster responses in limited studies. Further studies to investigate this association and underlying mechanism are warranted

Substance use: cigarettes, marijuana and alcohol

Substance use in Indigenous communities is well known in Australia and rates of smoking and alcohol use are considered to be high[349] In 2004/2005 half (52%) of Aboriginal and Torres Strait Islander peoples, aged over 15 years, reported they were current smokers and 23% had used marijuana in the previous 12 months. Rates of cigarette smoking (60%) and marijuana use (31%) in participants in study 2 is similar to data in the Australian Drug Use Statistics report.[349]

Cigarette smoking is known to adversely effect both cell mediated and humoral immune systems in humans[350] and has been shown to reduce both primary and booster response to HBV vaccines in both adults and adolescents.[63,285,351-353] Roome et al investigated the impact of smoking on primary HBV vaccination responses in adult public safety workers in the US.[352] Smokers had a three-fold increase in inadequate anti-HBs (<10mIU/ml) response to 3 doses of HBV vaccine compared to non smokers and there was a dose effect with participants who smoked > 1 pack per day significantly more likely to respond inadequately than smokers of < 1 pack per day.[352] In a study by Wang et al, the impact of substance use on HBV booster vaccine responses was measured in Taiwanese adolescents (n=386) who had received HBV vaccine 15-18 years ago.[285] Those who had smoked cigarettes and chewed betel-quid, in the month prior to the booster, were significantly more likely to not respond to a booster dose of 20ug recombinant HBsAg vaccine than non smokers or non betel-quid users. The association for cigarette smoking and betel quid chewing and non response was higher when analysis was restricted to those with pre-booster anti-HBs levels 0.1-9.9 mIU/ml. The mechanism for reduced responses with betel-quid chewing is not known.[285]

Alcohol has also been shown to reduce primary HBV vaccine responses in adults, with seroconversion rates ranging from 43-70%.[354-356] Anti-HBs levels were non significantly lower in current alcohol users compared to non users in this study. It was not possible to assess if there was a dose effect of alcohol on immune responses because of the small sample size of participants who were current users of alcohol and had received HBV vaccine on time. Study 2 in this thesis is the first to suggest marijuana smoking may reduce anamnestic responses. Mathews et al examined HBV serologic markers and association with kava (intoxicating drink made from root of the pepper plant) use in Arnhem land NT in 1987, and found no increase prevalence of HBsAg positivity in users compared to non users.[297] In this study non significant reductions in anamnestic responses were seen with cigarette and alcohol use, while there were insufficient numbers to measure any contribution from petrol sniffing. The small sample size, particularly participants who had received three HBV vaccine doses prior to 2 years old, precludes definitive conclusions regarding the influence of substance abuse on anamnestic memory responses and subsequent need for booster vaccination.

Implication: Substance abuse, particularly smoking cigarettes, may reduce anamnestic responses as has been demonstrated in limited studies. Future booster studies should include assessment of substance use to enable an accurate assessment of its influence.

Anamnestic responses: validity of measurement

Study 1 provides information on the validity of methods for identifying anamnestic responses by comparing anti-HBs responses following a booster HBV vaccine in a previously vaccinated cohort and a vaccine naive cohort. In the vaccine naive group, only 2 participants (n=2/50) had anti-HBs >10mIU/ml at 14 days post first dose and only one had a low level anti-HBs response persisting at 4 weeks post HBV vaccine. This data was collected from HBV vaccine naive adolescents of similar ages and ethnic background to the vaccinated study group. It adds support to the definition of anamnestic response used in international studies because it confirms that only those who had received prior HBV vaccines are likely to achieve anti-HBs levels >10mIU/ml after a single HBV vaccine dose. In addition, anti-HBs levels post booster in the previously vaccinated cohort were shown to increase significantly by 14 days and only marginally increase further by 4 weeks. Thus, these data indicate measuring an

anamnestic response 14-28 days post booster is optimal as no anamnestic responses would be missed by only measuring at one time point, either 14 days or 4 weeks. These cohort studies do not examine whether anamnestic responses were present before 14 days post booster. In a study by Samandari et al; anamnestic responses were lower 1 week post booster vaccine (56% response) compared to 2 weeks (83%) and 4 weeks (88%).[266]

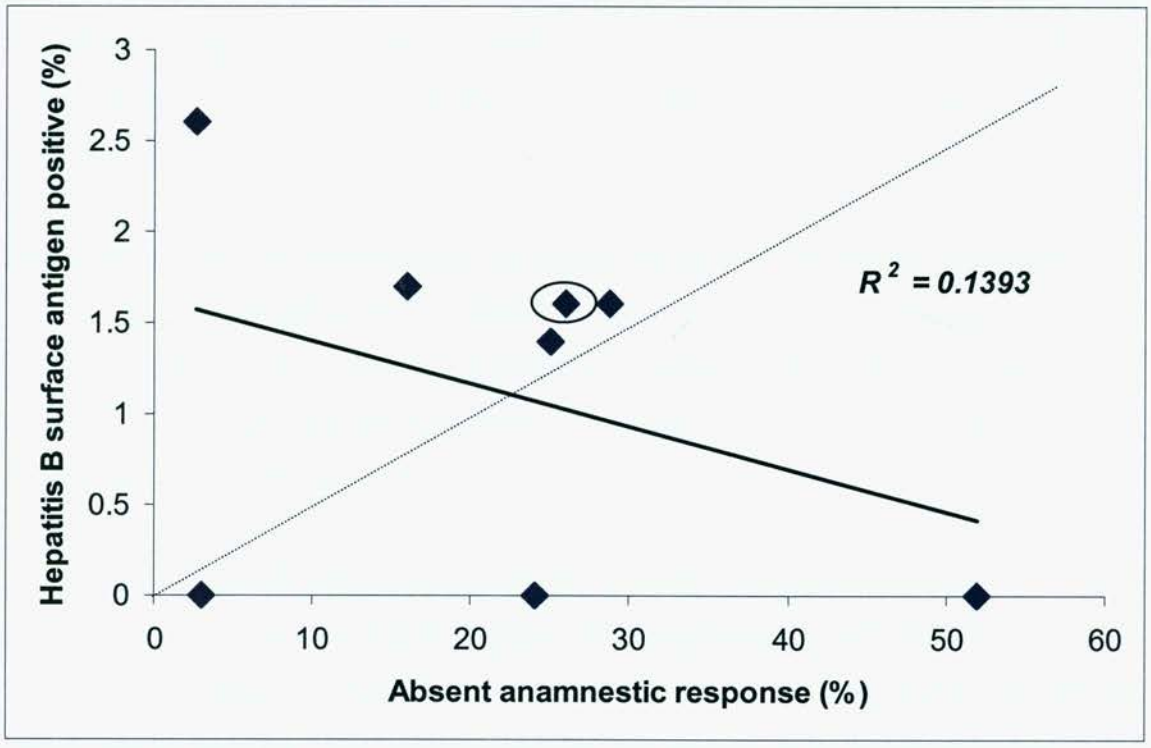
Absent anamnestic responses: Interpretation

Studies reporting absent anamnestic responses are likely to become more common as the length of follow up increases. How do we interpret absent anamnestic responses? The low rates of HBsAg carriage in these 2 cohort studies and from the international literature reviewed in Chapter 4 suggest that protection against clinically significant breakthrough infection may still occur despite the absence of anamnestic response. The prevalence of HBsAg positivity in study 2 was 1.6% and is similar to levels below 5% reported in international literature, despite nearly two thirds of study cohorts having anti-HBs levels < 10 mIU/ml. A long term effectiveness study in the Gambia suggests that protection from chronic HBV carriage continues (vaccine efficacy was estimated at 96.5%) 19 years after commencing vaccination, despite undetectable anti-HBs levels in approximately 50% of known responders to primary vaccination.[264] The rise in antibody to the protective level may not be important if sufficient neutralising antibody, even at levels <10mIU/ml, can be generated to prevent chronic or symptomatic infection during the 4-12 week incubation period of natural HBV.[266]

The relationship between absent anamnestic memory response and prevalence of HBsAg positivity in international studies where both data are presented is shown graphically. (Figure 5.15) The heterogenous nature of each individual study makes it difficult to interpret the relationship between grouped data, but there is no strong association with increasing absence of anamnestic responses and increasing presence of HBsAg positivity.

Ongoing follow up of both these cohorts in Australia and in other vaccinated cohorts internationally is likely to assist in interpreting absent anamnestic responses. In

addition, the contribution of T cell memory in providing protection in the absence of memory antibody responses is not definitively known.[98,100]



Each diamond represents an individual study result. The circled diamond is this thesis study 2 result. Linear trend for this data is shown, with R^2=0.1393. The dashed line would indicate a linear trend to wards increasing HBsAg positivity with increasing absent anamnestic responses.

Figure 5.15 Relationship between absent anamnestic response and hepatitis B surface antigen (HBsAg) positivity in international studies where both HBsAg and response to hepatitis B booster vaccine were measured

5.6 Limitations of study 1 and 2

There are several limitations to data interpretation in both cohort studies. One of the important determinants of immune longevity is successful primary seroconversion. In both studies rates of primary seroconversion were not known and so it is not possible to assess if a lack of anamnestic response is due to failure of primary seroconversion or waning immune memory.

Both studies were conducted in high risk groups living in a low endemicity country. There are significant differences in ethnicity, age, socio-demographic factors, for example substance use, social mixing patterns and rates of HBV infection in each community that means generalising results from both cohort studies to the wider Australian community is problematic.

Samples sizes were not sufficient, particularly in study 1, to enable definitive conclusions about the impact of HBV vaccination on prevalence of HBV infection in the 'at risk' community, especially given ethnic heterogeneity. Study 2 sampled 10% of Indigenous birth cohort (1987-1990) in the NT and is likely to be a more accurate estimate of the true HBV infection prevalence in Indigenous adolescents, particularly as it includes urban and rural participants and the cohort is known to be representative of the Darwin Health region. The sample size of Indigenous adolescents (Study 2 n=66) and 'at risk' adolescents (Study 1 n=70) in the anamnestic response studies is similar to other studies in Indigenous Alaskans[242,276] but smaller than studies in Taiwan.[261,262] In addition, when I only include Indigenous adolescents who had received HBV vaccines according to the correct schedule the sample size becomes smaller (n=43). However, despite the small sample sizes in the booster studies, the results are distinctly different and important, with anamnestic responses in study 1 participants of 98% (95% CI 93-100%) and study 2 participants 78% (95% CI 65-90%).

There was a significant loss to follow up in the booster vaccine study in Indigenous adolescents. This was not unexpected given the known difficulties in research in remote Indigenous communities despite visits to communities on several occasions and use of local contacts for tracing. Of note, however, baseline factors in those who completed the study were not significantly different from those who did not complete the study.

Another major determinant in assessing vaccine effectiveness and anamnestic responses is an accurate vaccine history. In study 1, participant's vaccine histories were obtained from several sources for confirmation, including individual personal child health and healthcare provider records and are likely to be an accurate record. Most study 1 participants had individual child health records which often included HBV vaccine batch numbers available for review. In contrast, no Indigenous adolescents in study 2 had individual personal child health records available and vaccine history was collected from community health clinic records where available and an electronic immunisation register. There are no data available on the quality and completeness or audits of the electronic immunisation register. One fifth of Indigenous participants (n=99/437) had nil immunisation records found, however a proportion of these may have received one or more doses of HBV vaccines. HBV serology data in the 'nil record' group indicate that 30% had anti-HBs positivity alone which reflects past vaccination despite absent immunisation records. The HBV vaccination record data for Indigenous adolescents in study 2 was obtained using multiple sources to improve its accuracy, however it may not be complete.

Another potential limitation in comparing HBV serologic results relates to anti-HBs measurement. HBV serology was measured in different laboratories and by different analysers. Study 1 sera were analysed immediately after collection by South Western Sydney Area Pathology Service using a Abbott AxSymx automated analyser. Anti-HBs results below 10mIU/ml were not quantified and values below this cut off were given an arbitrary value of 5 mIU/ml for measurement of GMC. Study 2 sera were stored frozen and transported in batches for testing at ICPMR, Westmead Hospital at irregular intervals on the Abbott Architect i2000SR automated analyser. The sera were appropriately transported and no freeze/thaw/freeze cycles, which can affect anti-HBs values, occurred. Anti-HBs results above 1000mIU/ml (upper cut off) were not quantified and an arbitrary value of 1000 mIU/ml was used to calculate GMC. In a study in Netherlands, anti-HBs assays on Abbott AxSym and Architect analysers were compared and found to give related but not identical results.[284] AxSym slightly overestimated anti-HBs levels when compared to WHO known reference sera, while Architect results more closely approximated WHO sera.[284]

Substance abuse data in Indigenous adolescents was obtained by participants answering a computer based questionnaire, results were not anonymous and it is likely reporting of illicit drug use to healthcare professionals may be an underestimate. Reported prevalence of cigarette and marijuana use in study 2 participants is similar to prevalence in national drug use surveys. A statistically significant association with reduced anamnestic responses and marijuana use was found despite reporting of its use likely to be underestimated. Higher reported use of marijuana would strengthen the association.

CHAPTER 6: CONCLUSIONS

6.1 Newborn pertussis vaccination

What this study adds and future directions

This is the first study in Australia to investigate Pa vaccination at birth and is the first internationally to measure the immune response and safety profile to 2 doses of Pa vaccine before 2 months old (birth and one month old doses). This is also the first study internationally to measure the impact of birth Pa vaccine on responses to concomitantly administered hepatitis B vaccine and suggested reduced hepatitis B responses at 8 months old.

This is also the first study internationally to report bias towards TH2 cellular immune responses following newborn Pa vaccination. As cell mediated immunity is potentially an important component of protection, and birth Pa vaccine may result in TH2 bias, studies to profile cell mediated immune responses in more detail are needed, including looking for correlations of T cell immunity and antibody responses. In addition, measurement of B cell subsets is warranted. Altered responses following subsequent natural pertussis exposure or increased potential for development of atopic responses are potential negative effects of vaccination in early life which requires evaluation.

This study showed that recruitment for neonatal vaccine trials was successful and demonstrated the feasibility of using specialised transport media for measuring cellular immunity. Despite the small number of participants, this pilot study showed that newborn Pa vaccine is safe and immunogenic. It adds important immunogenicity data to three other small studies performed in the last 10 years. In particular, data presented in this thesis supports the presence of successful neonatal immune priming and absence of immune tolerance. It also raises concerns of reduced concomitant antigen responses and altered cellular immunity following birth Pa vaccine. One of the major limitations of this study is the small sample size, consistent with its status as a pilot study. However, the positive immunogenicity data, safety profile and demonstrated feasibility of recruitment and specimen transport, in this trial has encouraged further study of newborn Pa vaccination in Australia and internationally to evaluate more definitively

the potential for preventing deaths and morbidity from pertussis disease in the youngest most vulnerable infants. Areas of uncertainty requiring further study are outlined in chapter 3. (Table 3.27)

This study provided essential immunogenicity data to inform the design of a planned larger study and contributed to a successful National Health and Medical Research Council (NHMRC) project grant application (Application ID: 570756) in 2008 to fund the larger study. In addition, subjects in this study are currently being followed long-term and will have their pertussis immunogenicity and concomitant antigen responses assessed at 2 and 4 years old.

A combination hepatitis B-Pa vaccine would be of interest in both developed and developing countries, as it involves simpler administration (1 vs 2 injections) at birth, and it is possible the combination formulation will not result in vaccine interference and reduced HBV response. It is also important to ensure that the addition of Pa vaccine does not reduce the early protection afforded by HBV vaccine and increase the risk of vertical transmission in settings where HBV is endemic.

Morbidity and mortality from pertussis in early infancy remains high despite optimum childhood coverage for pertussis vaccines in the acellular era in many in industrialised countries. Strategies to protect the youngest infants include indirect vaccination to increase herd immunity and direct protection, via maternal and neonatal vaccination. Direct protection of the neonate, if proven immunogenic and safe, is the strategy most likely to be implemented in national vaccination programs in rich and poor countries.

6.2 Hepatitis B immune longevity

What this study adds and future directions

The duration of protection afforded by infant HBV vaccination is not definitively known beyond 15 years and its determination has important worldwide implications on whether booster doses are required following infant vaccination.[245,248]

This thesis examined long term (> 10 years) protection and anamnestic responses in adolescents born to 'at risk' mothers from high endemic regions and Indigenous adolescents in the NT for the first time in Australia. Hepatitis B carriage in both cohorts was low indicating effectiveness of HBV vaccines received in infancy. Two thirds of adolescents were non immune to hepatitis B (anti-HBs <10mIU/ml) 14 to 18 years after infant vaccination and the majority demonstrated an anamnestic response to a HBV booster vaccine. Nearly all adolescents (98%) born to 'at risk' mothers demonstrated an anamnestic response, however a lower (78%) proportion of Indigenous adolescents responded. In two other studies even lower anamnestic responses, approximately 50%, have been reported. As time since vaccination increases beyond 20 years it is likely that more studies will demonstrate even lower anamnestic response rates. At some point an anamnestic threshold may be reached, below which clinically significant breakthrough infections may become more common and booster doses of HBV vaccine will be required. At present an anamnestic threshold for determining whether booster doses are required in later life has not been established. Ongoing surveillance, booster vaccine studies and potentially novel methods to measure in vitro immune memory will be needed to inform this decision. Indigenous infants in Australia were among the first groups in the world targeted for HBV vaccination and ongoing monitoring of HBV infection rates and booster responses in this study cohort will provide important data to inform HBV vaccination policy on a global scale. However, interpreting HBV surveillance data and anamnestic responses is complex. Numerous factors, either alone or in combination, such as age, sex, nutritional status, immune competence, HLA type, substance use and environmental HBV endemicity, influence HBV vaccine effectiveness and immune memory. Further studies will be needed to determine the influence of substance use and genetic HLA association on HBV immune memory.

This thesis has made an important contribution to the international literature on longevity of immunity following infant HBV vaccination, raised additional questions and provided data on long-term HBV immunity for the first time in Australia.

Importantly, immunisation at birth and longevity of protection should not be seen in isolation and the two are linked. The desire to protect as early as possible, through birth vaccination, has implications for longevity of immunity and as this thesis has demonstrated exploration and assessment of both are needed. In the future, as new vaccines and schedules are implemented, for example at birth, assessment of their protective efficacy both in the short term and over following decades should be ensured.

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APPENDICES

Appendix 1: The Children's Hospital at Westmead ethics approval letter for the birth acellular pertussis vaccine study

the children's hospital at Westmead

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25 July 2005

Dr Nicholas Wood
National Centre for Immunisation
Research and Surveillance

Dear Dr Wood,

Project Title: **Does early whooping cough (pertussis) vaccination provide earlier antibody protection for infants?**
Project No: **2003/074**

Information Sheet(1):	Version 6, 5 July 2005
Consent Form (1):	Version 6, 5 July 2005
Information Sheet(2):	Version 6, 5 July 2005
Consent Form (2):	Version 6, 5 July 2005
Pamphlet:	Version 3, November 2004
Letter to GP's:	Version 1, 21 July 2004

We refer to your correspondence 11 July 2005.

This is to confirm that final approval has been granted for this study.

We wish you well with your project. Please contact us should you have any queries.

Yours sincerely,

[Redaction]

A O'Neill
Secretary, Ethics Committee

Appendix 2: The Children's Hospital at Westmead parent information sheet for the birth acellular pertussis vaccine study

the children's hospital at Westmead

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Parent Information Sheet

DOES EARLY WHOOPING COUGH (PERTUSSIS) VACCINATION PROVIDE EARLIER ANTIBODY PROTECTION FOR INFANTS?

Investigators

Assoc. Prof. Peter McIntyre, Immunisation Research, The Children's Hospital at Westmead
Ph: 9845 1257
Dr Nicholas Wood, Immunisation Research, The Children's Hospital at Westmead
Ph: 9845 3069
Dr Leon Heron, South West Sydney Public Health Unit Ph: 9828 5944

We would like you to consider your child participating in a research study that will be conducted in the Department of Immunisation Research at The Children's Hospital at Westmead and Westmead hospitals, in conjunction with the National Centre for Immunisation Research and Surveillance.

What is the study about?
In Australia some hospitalisations and deaths from whooping cough (pertussis) occur in babies less than 2 months old. At present pertussis containing vaccines are first given to babies when they are 2 months old. The aim of this study is to see if giving babies the pertussis vaccine (Pa vaccine) earlier than 2 months old means that they are better protected and have less risk of hospitalisation or death from pertussis.

This study is looking at just how early antibodies develop and whether one or two doses before 2 months are needed to develop enough antibody to protect the baby. These results will be used to design a larger study to show definitely whether an early dose like this will protect against whooping cough early.

Who can participate in the study?
Well babies who are less than 4 days old.

What will the study involve?

Study groups
If you agree to your baby participating in this study, he or she will be randomly placed into one of three groups. Two groups will be given two vaccines (Pa vaccine and Hepatitis B vaccine) shortly after birth. Babies in one group will have another dose of Pa vaccine at one month of age. The third group will only be given the Hepatitis B vaccine at birth. All three groups will then be given the recommended vaccines at 2, 4 and 6 months old according to the Australian Standard Vaccination Schedule.

What will I need to do if my child is to participate in this study?

Study timetable

The study timetable is explained in the tables on the following pages. The first visit will take place on the postnatal ward in hospital and last approximately 60-90 minutes. At this time we will discuss whether you would prefer the subsequent visits to occur at home or at The Children's Hospital at Westmead. If you do come to the hospital free parking will be provided. The visits at 2, 4, 6 and 8 months will take approximately 45 minutes each.

Vaccine administration (0, 1, 2, 4, 6 months old)

After birth some babies will be given the study vaccine in one leg and all babies will be given the hepatitis B vaccine in the other leg. These vaccines will be given by an experienced immunisation nurse specialist on the post natal ward. Your baby will be observed for 15 minutes after the immunisation. You will be asked to look for any possible reactions following the immunisation. All infants will then be given the recommended routine vaccinations at 2, 4 and 6 months old by an experienced immunisation nurse specialist.

Diary card (0, 1, 2, 4, 6 months old)

We will give you a diary card asking you to record any redness or swelling at the injection site as well as any irritability or reduced feeding your baby may have after each vaccination. You will be given a thermometer to record your baby's temperature and we will contact you by telephone after each immunisation.

Blood tests (2, 4, 6, 8 months old)

The mother will have a blood test, about 5 mls (2 teaspoons), shortly after the birth of your child. Your child will need to have 4 blood tests taken over 8 months. The blood tests are taken by an experienced nurse immunisation specialist or paediatrician. We will need to take about 2-3 mls of blood (1 teaspoon) from a vein in your child's arm. The first test is at 2 months old and the next ones are at 4, 6, and 8 months of age. This can be painful and bruising can occur. We take the blood to measure the level of antibody in your child's body after the immunisations. We will measure antibodies to pertussis and hepatitis B in this study, and the remainder of the blood will be stored. In the future we would like to use this blood sample to measure other antibodies that your body makes after vaccination, for example antibodies to poliovirus.

Are there any benefits for my child participating in this study?

There are no known benefits to you or your child participating in this study. Pertussis vaccine is currently given only from 2 months of age, when it is too late prevent the most serious cases of pertussis (whooping cough). We hope the results of this study will help to decide whether it is possible to provide earlier protection. You as a parent will be given the opportunity to have a pertussis vaccine also, free of charge.

Are there any side effects and risks associated with this study?

1. Vaccine not working as well when given early

Because we don't know how well giving the pertussis vaccine early will work, there is a chance that the responses may not be as good as current standard practice of starting vaccination at 2 months or that additional doses may be required. You will have access to information about your child's response to the vaccine and the need for any further doses.

2. Side-effects of immunisation
We don't know for sure that there won't be extra side-effects of giving earlier or additional doses, so your baby will be closely monitored for these. Your child may get some of the following side effects from the Pa vaccine. These are usually mild, similar to those seen following vaccination with several routine childhood vaccines. These include being a bit miserable or fussy within 48 hours of the injection, developing a fever or a small lump or some redness where your child had the injection. Lumps can last for a few weeks. There is a very small chance of an allergic reaction occurring after vaccination or of having a convulsion with fever.

There may be other side effects that are not known at this time. You will be made aware of any significant new findings that may affect your decision for your baby to remain in this study.

Other information

What will happen to the data that has been stored?
Following analysis of results, you will be informed of your baby's results. All data collected will be held in strict confidence to protect your privacy. No material which could personally identify you or your child will be used in any reports on this study. Results from the analysis will be stored on computer disc and in written form for a period of 15 years in a locked cabinet in the National Centre for Immunisation Research at The Children's Hospital at Westmead after which time the discs will be erased and any written material will be shredded.

What if you decide not to participate in the study?
Participation in this study is voluntary and if you decide not to take part or decide to withdraw from the study at any time this will not otherwise affect your child's care at the hospital. Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in your child's best interest to continue.

What if you have any questions or concerns about the study?
You have the right to ask questions at any time regarding this study or the potential risks associated with it. You will be informed of any significant new information pertaining to your safety. If you have any concerns about the conduct of the study, please do not hesitate to discuss them with Associate Professor Peter McIntyre (phone: 02-9845 1257) Principal Investigator or Dr Nicholas Wood (phone: 02-9845 3069) or Anne O Neill (phone: 02-9845 1316), secretary of the Ethics Committee which has approved this study.

This information sheet is for you to keep. If you decide to participate in this study a copy of the signed consent form will be given to you.

		Study timetable		
		Group 1	**Group 2**	**Group 3**
		Pertussis vaccine at birth and one month old	**Pertussis vaccine at birth only**	**No pertussis vaccine at birth**
Visit no. and time	**Age**			
1 **60-90 minutes**	**Birth**	Explanation of study Medical history and examination of newborn Consent to participate in study Blood sample taken from mother (5mls or 2 teaspoons) Pertussis and hepatitis B vaccine given to baby Observed after vaccination for reactions Instructions for taking temperature and completing diary card Parents can be given Boostrix® vaccine if desire	Explanation of study Medical history and examination of newborn Consent to participate in study Blood sample from mother (5mls or 2 teaspoons) Pertussis and hepatitis B vaccine given to baby Observed after vaccination for reactions Instructions for taking temperature and completing diary card Parents can be given Boostrix® vaccine if desire	Explanation of study Medical history and examination of newborn Consent to participate in study Blood sample from mother (5mls or 2 teaspoons) Hepatitis B vaccine given to baby Observed after vaccination for reactions Instructions for taking temperature and completing diary card Parents can be given Boostrix® vaccine if desire
Group 1 extra visit **45 minutes**	**1 month**	Infant given pertussis vaccine Observed after vaccination for reactions Instructions for taking temperature and completing diary card	No visit	No visit
2 **45 minutes**	**2 months**	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 2 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 2 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 2 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card

App.6

		Study timetable		
		Group 1	**Group 2**	**Group 3**
		Pertussis vaccine at birth and one month old	**Pertussis vaccine at birth only**	**No pertussis vaccine at birth**
Visit no.	**Age**			
3 **45 minutes**	**4 months**	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 4 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 4 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 4 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card
4 **45 minutes**	**6 months**	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 6 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 6 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card	Medical history Blood sample taken from infant (2-3mls, 1 teaspoon) Infant given standard 6 month old vaccinations Observed after vaccination for reactions Instructions for taking temperature and completing diary card
5 **45 minutes**	**8 months**	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon)	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon)	Medical history and examination of infant Blood sample taken from infant (2-3mls, 1 teaspoon)

Appendix 3: Participant diary card – Birth acellular pertussis vaccine study

Diary Card Birth dose Subject number ____

Pertussis in the Newborn. A study from The New Children's Hospital at Westmead

General Symptoms

Please record, assess and score the occurrence of any of the following signs or symptoms.
Please record the temperature every day.
If the temperature was taken more then once a day please record the highest reading for the day.

Intensity

0 None	No adverse experience
1 Mild	Adverse experience which is easily tolerated
2 Moderate	Adverse experience causing sufficient discomfort to interfere with daily activities
3 Severe	Adverse experience which prevents normal activities and requires a Medical visit

Fussiness Intensity (*)

0 Baby behaves as normal
1 Occasionally more irritable then usual
2 Prolonged crying
3 Persistent crying

General Symptoms	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 14	Date of last day of symptoms (date stopped if > 7 days)
Fever °C (taken under arm)	C	C	C	C	C	C	C	C	Day Month Year
Fussiness/Irritability (*) ☺ ☛									Day Month Year
Unusual crying ⊗ ☛									Day Month Year
Vomiting ⊗ ☛									Day Month Year
Diarrhoea ⊗ ☛									Day Month Year
Loss of appetite ⊗ ☛									Day Month Year
Restlessness/sleeping Less than usual ⊗ ☛									Day Month Year
Sleepiness/sleeping more than usual ⊗ ☛									Day Month Year

Other Symptoms Please give details of any other symptoms or illnesses that your baby experiences between immunisation visits (description-start date-end date-intensity)

_____

_____

Thank you for agreeing to take part in this study which aims to protect your newborn baby from whooping cough. If you need to contact study personnel during business hours please ☎ 9845 3069 or Annemarie's mobile 0418 209 323.

For URGENT contact outside business hours, ring 9845 0000 (Hospital switchboard) to CONTACT Dr Nicholas Wood the Principal Investigator of this Study.

Please contact the study personnel as soon as possible if your baby is admitted to hospital

Diary Card Birth dose Subject number ⌊_⌋⌊_⌋ Sydney

Pertussis in the Newborn. A study from The New Children's Hospital at Westmead

Local symptoms

Please record, assess and measure the occurrence of any of the following signs or symptoms.
Please record these measurements very evening
A diameter of >20 mm is defined as severe.

Pain Intensity ☹

0 Absent
1 Minor light reaction to touch
2 Cries or protests on digital pressure
3 Cries when the limb is moved

Size Please measure the longest diameter

Local symptoms (at the injection site)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Date of last day of symptoms date if > 7 days
Redness ☹ ☛ 	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ ____ ____ Day Month Year
Swelling/Hardness 	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ mm	____ ____ ____ Day Month Year
Pain Intensity ☹ ☛ 0, 1, 2, or 3	---	---	---	---	---	---	---	---	____ ____ ____ Day Month Year

Medications

Please fill in below if your child has taken any medication (even Panadol) since the vaccination

Trade name	Reason	Start date Day Month year	End date or tick if continuing Day Month year
			⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋
			⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋
			⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋

Other Symptoms Please give details of any other symptoms or illnesses that your baby experiences between immunisation visits (description-start date-end date-intensity)

Please do not forget to bring the diary card back at your next visit. Remember ☎ 9845 3069 or Annemarie's mobile 0418 209 323. For URGENT contact outside business hours, ring 9845 0000 (Hospital switchboard) to CONTACT Dr Nicholas Wood the Principal Investigator of this Study

Appendix 4: Sydney South West Area Health Service ethics approval letter for the adolescents 'at risk' hepatitis B study

SYDNEY SOUTH WEST AREA HEALTH SERVICE

INITIAL APPROVAL

Human Research Ethics Committee (Western Zone)
Locked Bag 7017, LIVERPOOL BC, NSW, 1871
Phone: 02 9828 5727
Facsimile: 02 9828 5962

February 23, 2005

SOUTH WESTERN SYDNEY AHS
RECEIVED
25 FEB 2005
PUBLIC HEALTH UNIT

Dr Leon Heron
Public Health Unit
Hugh Jardine Building
Locked Bag 7017
LIVERPOOL BC 1871

Dear Dr Heron,

Project No **05/001 - Long Term Persistence of Hepatitis B Immunity in Children who Received Hepatitis B Vaccinations in Infancy: A Pilot Study'**

The SSWAHS Human Research Ethics Committee wishes to acknowledge receipt of your application with regards to the above project which was reviewed at the meeting held on 31st January, 2005.

Formal approval is hereby granted for this study to proceed as a Category A Project.

Ethics clearance is granted for periods of up to twelve months. This project will be due for renewal on 31st January, 2005 and you must provide a Progress Report (attached) or final report by this date. If no report is supplied, ethics clearance for this project may be cancelled.

Your attention is drawn to the attached document *Guidelines for Investigators* which sets out not only the principles under which research should be conducted, but also **the conditions under which Ethics approval is granted by the Committee**. Also enclosed for your information, is a copy of the document *Guidelines for Responsible Practice in Research and Dealing with Problems of Research Misconduct*.

Please note that the Committee must be notified **IMMEDIATELY** of any untoward or unexpected complications or side affects arising during the project or of any ethical or medico-legal problems that may arise. Also, any changes to the original protocol must be submitted to the Committee for approval.

Would you please quote the above project number in all future correspondence relating to this project.

Yours sincerely,

[Redaction]

PROFESSOR HUGH DICKSON
Chairperson
SWSAHS Research Ethics Committee

For: Dr Diana Horvath, AO
Chief Executive Officer, SSWAHS

Category A: Projects with limited risk potential, including quality assurance surveys.
Category B: Projects with significant patient risks.
Category C: Drug trials (international/national) sponsored by drug companies and already covered for risk evaluation and monitoring of adverse reactions.

Appendix 5: Participant information sheet for the adolescent 'at risk' hepatitis B study

South Western Sydney Area Health Service Public Health Unit

Parent Information Sheet _____
Write child's name here

LONG TERM PERSISTENCE OF HEPATITIS B IMMUNITY IN CHILDREN WHO RECEIVED HEPATITIS B VACCINATIONS IN INFANCY: A PILOT STUDY

Investigators
Professor Peter McIntyre, Immunisation Research, The Children's Hospital at Westmead
Ph: 9845 1257
Dr Nicholas Wood, Immunisation Research, The Children's Hospital at Westmead
Ph: 9845 3069
Dr Leon Heron, Public Health Unit, South Western Sydney Area Health Service
Ph: 9828 5944
For advice after hours contact, The Children's Hospital switch board 9845 0000 and ask them to contact Dr Nicholas Wood.

We would like you to consider if your child will participate in an important research study that will be conducted by the Public Health Unit, South Western Sydney Area Health Service and the Department of Immunisation Research at The Children's Hospital at Westmead.

What the study is about
All children born in Australia since May 2000 have been offered hepatitis B vaccines as babies. Before then, Australian-born babies whose parents came from countries where hepatitis B is common were offered vaccination using three doses of the hepatitis B vaccine, with the first dose given shortly after birth. Studies in other countries have shown that infants given hepatitis B vaccine are protected against hepatitis B infection, but there is still uncertainty about how long the protection lasts and whether extra injections of the hepatitis B vaccine might be needed later in life. This will be the first study in Australia to find out if children vaccinated against hepatitis B when they were babies are still protected. To do this we will need two groups of children who are now aged 10 years or older and whose parents are from countries where hepatitis B is common, to volunteer to help us by giving a blood sample.

Group 1: Children who received 3 injections of the hepatitis B vaccine in infancy and who have not received any more doses of hepatitis B vaccine since.

Group 2: Children who have not had any hepatitis B vaccine.

The blood test will tell if your child is protected against hepatitis B and whether hepatitis B vaccinations might be advisable.

What will happen in the study
From what you have told us, so far, your child qualifies for admission to Group _____ of this study.
Research officer to enter number here

All children in this study.
As part of the study process we will ask to check your child's vaccination and medical records to check your child's hepatitis B vaccinations and we will ask to take a blood sample to determine if your child is protected against hepatitis B or has been infected.

- If the initial blood test shows that your child is protected against hepatitis B, we will tell you the result. There is no need to do anything else for children who are protected.
- If the initial blood test shows that your child has been infected with hepatitis B (this is unlikely) we will tell you and make arrangements, with your family doctor, for your child to have extra advice or treatment.
- If the initial blood test shows that your child does not have the protective antibody (anti-HBs) that prevents hepatitis B infection, we will ask you and your child about progressing to the next step of the study. The next step involves giving a dose of hepatitis B vaccine and taking a blood sample 2 and 4 weeks later to see how fast your child's immune system is responding to the vaccine. Children in **Group 1** (previously vaccinated against hepatitis B) who make a fast response do not need any more hepatitis B vaccinations. Children in **Group 1** who make a slow response and every child in **Group 2** (never vaccinated against hepatitis B) should go on to complete a course of hepatitis B vaccination. We will inform about the results of all tests.

What are the benefits if your child chooses to participate in this study?

- You will know whether your child is protected against hepatitis B infection.
- If your child is not immune, he or she will, if you wish, be given another injection of hepatitis B vaccine.
- Because this is done as part of a study, there is no cost to you.
- Most importantly, your child's participation in this study will help us decide what to do for the other Australian children vaccinated against hepatitis B.

Rights and responsibilities

- You and your child will be fully informed about all aspects of the study. This information sheet is part of the informing process.
- Participation is not compulsory. You and your child are free to choose if he/she will participate in the study. Your child may withdraw from the study at any time. If, for any reason, you wish to withdraw your child from the study, please discuss it with us so that we can help you make the best arrangements for your child.
- If you and your child choose not to participate or if your child withdraws from the study, that choice will not affect any current or future treatment or relationship with any health service, any institution co-operating in this study, or any person involved in the study or treating you or your child.
- When deciding if your child will participate, or at any other time, you can seek advice from anyone you like and you can ask us any questions you like about the study. We will answer your questions truthfully and accurately to your satisfaction.
- You will be informed of any significant new information pertaining to your child's safety.
- The doctors and nurses involved in the study are willing to discuss any concerns that you might have about anything to do with the study, or difficulties you may have attending study appointments. If, at any time you have questions or concerns about anything to do with the conduct of the study, blood test results or vaccination please feel free to telephone us (our telephone numbers are on page 1).
- If we fail to resolve your concerns about the study to your satisfaction you may contact The Executive Officer of the South Western Sydney Area Health Service Ethics Committee (telephone 9828 5727)

Communications
From time to time we will call you by telephone and may send you letters.

Confidentiality
All information provided by you and all test results will remain strictly confidential between you, your child, your family doctor and us. The doctors and nurses involved in the study are bound by law to maintain client confidentiality. If the results of this study are published, participating individuals will **not** be identified in any way. By law we are required to keep all information relating to the study until the youngest child involved in the study is 28 years old. The information for this study will be kept at the South Western Sydney Area Health Service. Once the required storage time has expired, paper records will be shredded and computer records will be deleted. If you need anything from your child's records, please feel free to call one of us.

Taking a blood sample
An experienced specialist immunisation nurse or doctor will take the blood sample(s) from your child. The sample(s) will be small (about 5-10 millilitres – about 1-2 teaspoons).

Side effects of blood sample collection
Sometimes collection of a blood sample can cause discomfort and sometimes a small bruise can develop in the skin at the site where the sample was taken. If your child is concerned about the discomfort of having a blood sample taken, we can, if you and your child wish, apply EMLA gel (a local anaesthetic) to temporarily numb your child's skin at the blood collection site.

About hepatitis B vaccination
If we find that your child may benefit from hepatitis B vaccination, we will inform you about the known risks and benefits of vaccination and ask for your consent. If you agree to your child having hepatitis B vaccine, it will be given by an experienced specialist immunisation nurse or doctor. The person giving the vaccination will assess your child's wellness on the day that vaccination is due. If your child is not well, vaccination may be delayed for a few days. The vaccine we use is used by all family doctors to vaccinate children against hepatitis B. It is approved by the Therapeutic Goods Administration as safe and effective for vaccinating Australian children. It contains purified surface protein from the hepatitis B virus grown in yeast. The vaccine is not a blood product and it does not contain live viruses. It is one of the safest vaccines ever developed. More than 85% of people vaccinated with this vaccine have no side effects at all.

Further information
You may keep this information sheet. Other reliable sources of information about hepatitis B can be found at www.cdc.gov/nip, www.cdc.gov/ncidod/diseases/hepatitis, and www.immunize.org.

If you decide to participate in this study a copy of the signed consent form will be given to you.

About hepatitis B vaccine
The hepatitis B vaccine is one of the safest vaccines ever developed. More than 85% of people vaccinated with this vaccine have no side effects at all. The symptoms of all known and suspected side effects are listed here so that you will know what to expect and what symptoms to see a doctor about. The side effects of the vaccine are far less frequent and far less serious than the effects of hepatitis B disease.

Contraindications to hepatitis B vaccination
People who have had a major allergic reaction (anaphylaxis) after a hepatitis B vaccine injection should not receive the vaccine. Persons who have anaphylactic sensitivity to yeast of to any of the vaccine components (aluminium hydroxide, HBsAg) should not receive the vaccine.

Mild side effects
These reactions may occur after any vaccination. They will disappear within a few days.

- Minor soreness, redness and swelling at the injection site. Sometimes a small hard lump may stay a few weeks.
- Slightly raised body temperature (only 2 or 3 in 100 people). If it is troublesome, it may be treated with Panadol given every 4 hours for 24 hours (maximum 6 doses). Check your child's dose with your chemist or doctor.

Allergic reactions
As with all vaccines given by injection, there is a very small risk of allergic reaction (approximately 1 in 600,000 doses). Most allergic reactions occur less than half an hour after vaccination, during which time your child will be under the observation of the person giving the vaccination.

Immediately contact your doctor or go to the casualty department of your nearest hospital if your child notices any of the following soon after vaccination:

- Swelling of limbs, face, eyes, inside the nose, mouth or throat
- Shortness of breath, breathing or swallowing difficulties
- Hives, itching (especially of the hands or feet), reddening of the skin (especially around the ears), severe skin reactions
- Unusual tiredness or weakness that is sudden or severe

Rare side effects
Always tell your doctor and one of the doctors running this study if your child develops any unusual symptoms soon after any vaccination.

- More than usual pain, redness, swelling or bruising at the injection site
- More than usual headache, unusual tiredness, dizziness or feeling generally unwell.

The symptoms listed below have been reported by some people after hepatitis B vaccination. It is not known if the vaccine caused the symptoms or if the people just happened to get the vaccine at the same time that the disease that caused the symptoms was developing.

- Vomiting or feeling sick, stomach pains, loss of appetite or diarrhoea
- Muscle aches and pains, back pain or neck stiffness
- Swollen glands in the neck, fainting, sweating, flushing or chills

Appendix 6: Menzies School of Health Research ethics approval letter for the Aboriginal birth cohort study

menzies school of health research

PO Box 41096
CASUARINA NT 0811
Building 58
Royal Darwin Hospital C
Rocklands Drive
CASUARINA NT 0810
Ph: 08 8922 8196
Fax: 08 8927 5187
www.menzies.edu.au
ABN 70 413 542 847

2 June 2006

Dr Nicholas Wood
Clinical Research Fellow
National Centre for Immunisation Research and Surveillance
The Children's Hospital at Westmead;
Locked Bag 4001
WESTMEAD NSW 2145

Dear Dr Wood

Re: 06/05 - *Hepatits B immunity in Indigenous children who received hepatitis B vaccination in infancy*

Thank-you for your letter of 30 March 2006.

The HREC Chair has reviewed your submission addressing concerns raised by the Human Research Ethics Committee during the February 2006 meeting. Ethics approval for the project to proceed is now granted from the date of this letter.

The safe and ethical conduct of this project is entirely the responsibility of the investigators and their institution(s). As a condition of ethical approval you should report immediately anything which might affect continuing ethical acceptance of the project, including adverse effects of the project on subjects and the steps taken to deal with these, other unforeseen events, or new information that may invalidate the ethical integrity of the study.

This approval is for twelve months; a progress report is required by 30 March 2007. Approval for a further twelve months will be granted if the HREC is satisfied that the conduct of the project has been consistent with the original protocol.

The Committee must be notified and approve in advance any significant changes to the protocol. The Committee must also be notified at the completion of the project.

Yours sincerely

[Redaction]

Dr Michael Nixon
Chair
Human Research Ethics Committee
of NT Dept of Health & Community Services
and Menzies School of Health Research

Appendix 7: NHMRC New Investigator project grant approval

396700

SCHEDULE

(Note that references are to clauses in the Deed of Agreement, NHMRC Research Funding Schemes Version 3)

A. TYPE OF FUNDING
(subclause 1.1)

NHMRC Project Grant
Project Grant

B. PERIOD OF FUNDING
(subclause 1.1)

This Schedule must be signed and returned to the NHMRC by 31 March 2006 or the Offer is withdrawn.

2 year(s) of funding has been approved for this project

Start date: 01 January 2006
End date: 31 December 2007

Final date by which the Project must commence: 30 June 2006

C. PROJECT & PROJECT PURPOSES
(subclause 1.1 and clause 2)

Application ID: 396700
Administering Institution: The Children's Hospital at Westmead
Project Title: Hepatitis B virus immunity in Indigenous and "at risk" children who received hepatitis B vaccination in infancy

Additional Funding Conditions
Nil

D. FUNDS, FUNDING
(subclause 1.1 and clause 3)

Total Funding amount is: $212,475
Note: GST and an annual indexation may be applied to some components of this budget.

Funding Payments are scheduled quarterly in advance.

E. APPROVED BUDGET

(subclause 1.1 and clause 4)

Year	Year 1	Year 2	Year 3	Year 4	Year 5
TOTAL	$164,100	$48,375	$0	$0	$0

Note: GST and an annual indexation may be applied to some components of this budget.

F. CO-FUNDING

(subclause 1.1 and clause 3)

G. REPORTING

(clause 6)

Annual Financial Reports

Date(s) due: For calendar year ending 31 December, by 31 March following.

Financial Acquittal

Within 6 months after the Period of Funding ends, or the Termination of Funding.

Progress Reports

Date(s) due: For calendar year ending 31 December, by 31 March following.

Final Report

Within 6 months after the Period of Funding ends, or the Termination of Funding.

H. COMMONWEALTH OWNERSHIP

(subclause 10.1)

I. SPECIFIED PERSONNEL

(clause 13)

Chief Investigator(s)

CIA Dr Nicholas Wood

J. NOTICES

(clause 25)

Director, Research Projects Management Section
GPO Box 9848
MDP 33
CANBERRA ACT 2601

Initials of Institutional signatory to Deed:

Initials of Chief Investigator A:
[Redaction]

Appendix 8: Example of letter to an Indigenous community seeking approval to conduct the hepatitis B study on Aboriginal birth cohort participants

NCIRS National Centre for Immunisation Research and Surveillance *of Vaccine Preventable Diseases*

The University of Sydney
ABN 53 188 579 090

The Tiwi Union Chair
The Tiwi Union
Armidale St
Stuart Park 0820
Northern Territory

Dear Chair

I am the principal investigator on a NHMRC funded study (Grant number: 396700 funded 2006-2007) following up immunity to hepatitis B virus in adolescents who had received hepatitis B vaccination in infancy.

Hepatitis B virus (HBV) infection is an important public health issue for Indigenous Australians. Aboriginal and Torres Strait Islander children were among the first groups targeted for hepatitis B vaccination because of high rates of HBV infection. Studies in other countries have shown that babies given hepatitis B vaccine are protected against HBV infection for at least 10 years, but we are not sure how long the protection lasts and whether extra injections of the hepatitis B vaccine might be needed later in life. This will be the first study in Australia to find out if people vaccinated against hepatitis B when they were babies are still protected 10 years or more after vaccination and whether booster doses of vaccine are required.

The study involves 2 parts. The first part is to measure the immunity to HBV by taking a blood sample. The second part is to offer those who have low immunity another hepatitis B vaccine and then check their immunity following this booster dose.The first part is being collected along with other bloods for the Aboriginal Birth Cohort (ABC) study The ABC study was initiated by Dr Sue Sayers and is now in its 20th year following 686 babies throughout their lives. The Tiwi Health Advisory Committee has been very supportive of the ABC study and the ABC team has recently been to the Tiwi Islands.

I am writing to request permission to visit the Tiwi Islands to trace approximately 30 young adults belonging to this cohort and ask if they wish to take part in the second part of the study examining responses to a booster dose of hepatitis B vaccine.

Study method
Thirty four Tiwi island participants in the Aboriginal Birth Cohort study have already had their blood taken by the Menzies School of Health Research team during the recent Wave 3 follow up visit in March and April 2006. In this second part of the study each individual

will be explained his or her hepatitis B immune status. Results of the testing of the Tiwi Island participants indicate no HBV carriers, six participants with evidence of past HBV infection and eight have HBV immunity. Twenty adolescents are now known to have no or low immunity and are being invited to participate in the booster study. A dose of HBV vaccine will be given and then another blood test will be performed 2-4 weeks later to measure the antibody response following vaccination. A rapid rise in antibody level following vaccination indicates immune memory.

This vaccine study will help answer specifically whether children in Australia require booster doses following infant vaccination, because if nearly all are shown to have immune memory, then booster doses are not required.

This study has been approved by Menzies School of Health Research ethics committee and informed written consent will be obtained from each participant. The vaccine used in this study is licensed for use in all Australian adolescents and is currently being used in high school vaccination programs around the country.

We therefore seek your permission to see these Aboriginal Birth Cohort study participants again. I have attached a copy of the study's ethics approval, the information and consent form for your information and review.

I would like to let you and your committee know of the possible benefits that the study will bring to the Tiwi Island communities and others, as well as the resources it requires.

Benefits

- Participants will know whether they are protected against hepatitis B infection.
- Those who have low level immunity will be given another injection of hepatitis B vaccine which may boost their immunity and increase their protection from hepatitis B infection.
- Most importantly, this study will help us decide what to do in the future for other Australian babies vaccinated against hepatitis B.

At the conclusion of the study the study team will return to the Tiwi Islands to report on the study findings from the local communities and feed-back the information gained to the local people.

Resources required

We will employ at least one local Community Research Assistant in each community. They will be employed on a casual basis and paid an hourly rate of $17.80. These assistants will be given the opportunity to learn about the study.

The study will require some working space. We are largely self-sufficient. With your permission, we would prefer that the vaccinations are given by our project Officer (a Nurse) at the Community Health Centre. However, if this is not feasible we will be able to use another location such as a spare school room or recreation hall. We will also need a vehicle when we are on the islands and would be keen to source one locally and

reimburse the owner for the fuel and usage. In addition as I am a paediatrician I would be happy to assist in community health centre consultations as necessary during these visits.

With your approval, we would be on the Tiwi Islands in October. I estimate we would be in Nguiu for 3 days and one day in each of the communities in Melville Island and then return 2-4 weeks later for the same time period in each community.

Please contact me or the ABC study if you require more details or clarification. If you would like me to attend a meeting to discuss the study with you I would be happy to do so.

Yours sincerely,

Dr Nicholas Wood
Principal Investigator and Clinical Fellow
National Centre for Immunisation Research and Surveillance
Phone: 02 9845 1434
Email: nicholw3@chw.edu.au

Enclosed:
Human Research Ethics Committee Approval
Study information and consent Form
Prior approval to conduct the Aboriginal Birth Cohort study on the Tiwi Islands – Wave 3 study

Appendix 9: Participant information sheet for the Aboriginal birth cohort study

NCIRS National Centre for Immunisation Research and Surveillance *of Vaccine Preventable Diseases*

mshr menzies school of health research

Hepatitis B vaccine study

STUDY INFORMATION SHEET

Introduction

You are invited to take part in a research study into **"Hepatitis B immunity in people who were given hepatitis B vaccination as babies"**. Studies in other countries have shown that babies given hepatitis B vaccine are protected against hepatitis B infection for at least 10 years, but we are not sure how long the protection lasts and whether extra injections of the hepatitis B vaccine might be needed later in life. This will be the first study in Australia to find out if people vaccinated against hepatitis B when they were babies are still protected 10 years or more after vaccination and whether booster doses of vaccine are required. To do this we need people who are now aged 10 years or older and who had Hepatitis B vaccine as a baby to volunteer to help us.

The study is being conducted within the Aboriginal Birth Cohort Study, Menzies School of Health Research, Darwin by the National Centre for Immunisation Research and Surveillance (NCIRS) at The Children's Hospital at Westmead, Sydney.

Investigators

Dr Nicholas Wood, National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead
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Dr Susan Sayers, Aboriginal Birth Cohort Study, Menzies School of Health Research
Ph: 08 8922 8006
Professor Peter McIntyre, National Centre for Immunisation Research and Surveillance, The Children's Hospital at Westmead
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What will you need to do?

- If you agree to be part of this study, you will be asked to sign the Consent Form. As part of the study process we will ask to check your hepatitis B vaccination history.
- You agreed for a blood sample to measure immunity to hepatitis B virus to be taken by the Aboriginal Birth Cohort study team when they saw you recently. This blood test shows that you had low levels of an antibody that prevents hepatitis B virus infection.
- We are inviting you to participate in the next step of this study. This involves giving a booster dose of hepatitis B vaccine and taking another blood sample 2-4 weeks later to see how well your immune system responds to the booster vaccine. See table on the next page.

Visit 1	Visit 2 (2-4 weeks after visit 1)
Study explained and any questions answered Consent signed Temperature taken Blood test (1-2 teaspoons) taken from arm Hepatitis B vaccine given in arm Observed for 30 minutes after vaccination	Review of any reactions to vaccine Blood test (1-2 teaspoons) taken from arm Study finished

About hepatitis B vaccination
Hepatitis B vaccine will be given by an experienced specialist nurse or doctor. The person giving the vaccination will assess your wellness on the day that vaccination is due. If you are not well, the vaccination may be delayed for a few days. The vaccine we use is used by all family doctors in Australia to vaccinate children and adults against hepatitis B. It is approved by the Therapeutic Goods Administration as safe and effective for vaccinating Australian people. The vaccine is not a blood product and it does not contain live viruses. It is one of the safest vaccines ever developed. More than 85% of people vaccinated with this vaccine have no side effects at all.

Taking a blood sample
An experienced nurse or doctor will take the blood sample from you before and 2-4 weeks after the booster hepatitis B vaccine. The sample will be small (about 1-2 teaspoon) and taken from a vein in your child's arm. The blood will be taken in sterile conditions and care will be taken not to hurt you. We put on local anaesthetic cream that numbs the skin and the needle is not felt. Blood collection may cause some minor bruising at the site that can last one to two days. We will provide food such as a muffin, fruit and fruit juice during the visits.

Risks
All medical procedures - whether for diagnosis or treatment, routine or experimental – involve some risk of injury. In addition, there may be risks associated with this study that are presently unknown and unforeseeable.

The risks of participating in this study are:
Minor bruising and discomfort at the blood collection site – which will be reduced by the use of local anaesthetic cream
Mild side effects from hepatitis B vaccination including fever and minor soreness, redness or swelling at the injection site which disappears in a few days.

Severe allergic reactions are very rare. Some people have reported the following symptoms after hepatitis B vaccination. It is not known if the vaccine caused the symptoms or if the people just happened to get the vaccine at the same time that the disease that caused the symptoms was developing.

- Vomiting or feeling sick, stomach pains, loss of appetite or diarrhoea
- Muscle aches and pains, back pain or neck stiffness
- Swollen glands in the neck, fainting, sweating, flushing or chills

Benefits

- You will know whether you are protected against hepatitis B infection.
- If you have a low level immunity, you will be given another injection of hepatitis B vaccine which may boost your immunity and protection from hepatitis B infection.

- Most importantly, your participation in this study will help us decide what to do for other Australian babies vaccinated against hepatitis B.

Costs
Participation in this study will not cost you anything, and you will not be paid. However, you will be reimbursed for your travel expenses for study visits.

What are your options?
Participation in this study is entirely voluntary. You do not have to take part in it. If you do take part, you can withdraw at any time without having to give a reason. Whatever your decision, please be assured that it will not affect your medical treatment or your relationship with the staff who are caring for you.

Confidentiality
The data collected is recorded on sheets kept in locked files at the Menzies School of Health Research and entered into computer programs. Information is only available to the investigators and is password protected. When analysing the study data only study numbers are used. The study results may be presented at a conference or in a scientific publication, but individual participants or communities will not be able to be identified.

The doctors and nurses involved in the study are bound by law to maintain client confidentiality. By law we are required to keep all information relating to the study until the youngest child involved in the study is 28 years old. The information for this study will be kept at the Menzies School of Health Research. Once the required storage time has expired, paper records will be shredded and computer records will be deleted. If you need anything from your records, please feel free to call one of us.

Further Information
The ownership of Aboriginal knowledge and cultural heritage is retained by the informant and this will be acknowledged in research findings and in the dissemination of the research.

When you have read this information, Dr Nicholas Wood will answer any questions you may have or discuss any difficulties you may have attending study appointments. If, at any time you have questions or concerns about anything to do with the conduct of the study, blood test results or vaccination please feel free to telephone him (02 9845 1429) or the other investigators (08 8922 8006).

This information sheet is for you to keep.

Ethics Approval
This project has been approved by the Human Research Ethics Committee of the NT Department of Health and Community Services and Menzies School of Health Research, Approval No. 06/05.

Any concerns or complaints should be directed to The Secretary, Human Research Ethics Committee of the NT Department of Health and Community Services and Menzies School of Health Research, telephone 08 8922 7922 or email ethics@menzies.edu.au.