Maternal antibody interference, immunisation schedules, and protective antibody levels in infants.

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Vaccination programmes have been a cornerstone of public health policy globally for many decades. Infant immunisation programmes prevent millions of infections worldwide each year. In many cases infection is not just prevented in the immunised infants, but also in the wider population through the induction of herd effects.

Whilst the efficacy and cost-effectiveness of infant immunisation programmes is well-established, the design of such programmes varies greatly from country to country. Within any single country, changes to the programme occur over time, when new vaccines are introduced, when the type or manufacturer of vaccines changes, or when there are changes to the number of doses, or the spacing of doses.

In this thesis I explore the waning of maternal transplacental antibody, its effect on the immunogenicity of infant vaccines, and the timing and spacing of infant doses. I review the statistical methods for determining an antibody correlate of protection for vaccine-induced protection against disease and use new methods to derive serotype-specific correlates of protection for pneumococcal conjugate vaccines against the acquisition of carriage in infants.

Using secondary analysis of serological data from 49 infant immunogenicity trials, I show that maternal antibody inhibits the immunogenicity of most infant vaccines. Higher antibody responses are observed in infants who commenced vaccination at an older age, independent of the interference caused by maternal vaccination. Current methods for determining antibody correlates of protection are not fit for purpose. Using generalised additive models of serological data I show that the antibody level associated with protection against pneumococcal carriage varies across serotypes, and is higher in low- and lower-middle income countries.

These findings on key factors that influence the immune response in infants will help policy-makers in the UK and worldwide to make informed evidence-based decisions to protect the population against disease.

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2. Voysey M, Kelly DF, Fanshawe TR, Sadarangani M, O'Brien KL, Perera R, Pollard AJ. The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses: An Individual Participant Meta-analysis. *JAMA Pediatr*. 2017 Jul 1;171(7):637-46. doi:10.1001/jamapediatrics.2017.0638
3. Voysey M, Sadarangani M, Pollard AJ, Fanshawe TR. Threshold antibody levels of protection: an assessment of methodological bias. PLoS One (submitted)
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2. Voysey M, Pollard AJ, Perera R, Shresthna S, Thorson S, Fanshawe TR. Use of weighted multivariate estimates in trials of multi-serotype vaccines to simplify interpretation of treatment differences. PLOS ONE 2018 Apr. 13(4): e0196200. https://doi.org/10.1371/journal.pone.0196200
3. Voysey M, Pollard AJ. Sero-efficacy of Vi-polysaccharide tetanus-toxoid typhoid conjugate vaccine (Typbar-TCV). Clinical Infectious Diseases. 2018 Jan. https://doi.org/10.1093/cid/cix1145

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- The effect of maternal antibody and age at vaccination on antibody responses in infants.

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2.3 LIST OF ABBREVIATIONS

AOM	acute otitis media
CI	confidence interval
CRM	diphtheria-toxin mutant carrier protein
DTaP-IPV	diphtheria, tetanus, acellular pertussis, and inactivated polio vaccine
DTP	diphtheria-tetanus-pertussis containing vaccine
DTwP	diphtheria, tetanus toxoid and whole cell pertussis vaccine
ELISA	enzyme-linked immunosorbent assay
FHA	filamentous haemagglutinin
GAM	generalised additive model
GBS	group B *Streptococcus*
GMC	geometric mean concentration
GMR	geometric mean ratio
GSK	GlaxoSmithKline
H/UMIC	high or upper middle income countries
Hep B	hepatitis B
Hib	*Haemophilus influenzae* type b
HID	non typeable *Haemophilus influenzae* protein D
HR	hazard ratio
IgA	immunoglobulin A
IgG	immunoglobulin G
IPD	invasive pnuemococcal disease
IPV	inactivated polio vaccine
IU	international units
L/LMIC	low or lower middle income countries
LCL	lower confidence limit
LLD	lower limit of detection
LLQ	lower limit of quantification
NIAID	National Institute of Allergy and Infectious Diseases
PCV	pneumococcal conjugate vaccine
PE	protective efficacy
PRN	pertactin
PRP	polyribosylribitol phosphate
PT	pertussis toxoid
ROC	receiver operator characteristic
RSV	respiratory syncytial virus
SAGE	strategic advisory group of experts
Td	low-dose diphtheria-tetanus toxoid vaccine
Tdap	low-dose diphtheria, tetanus toxoid and acellular pertussis vaccine
TT	tetanus toxoid vaccine
UCL	upper confidence limit
UK	United Kingdom
VE	vaccine efficacy
WHO	World Health Organisation

3 INTRODUCTION

3.1 INFANT AND MATERNAL IMMUNISATION

It is difficult to overstate the global impact that routine infant immunisation has had on reducing morbidity and mortality from infectious diseases in the last century. However, the design of immunogenic schedules for routine immunisation is increasingly challenging due to multiple factors including: the number and variety of licensed vaccines available and the potential for interactions between them; the potential for interactions between infant and maternal prenatal immunisation programmes for some pathogens; and changes in the epidemiology of infections over time and between regions. A detailed understanding of the variables that influence the immunogenicity of vaccines is therefore of great importance in order to maximise the impact vaccination programmes can have on the health of populations.

Infant vaccination programmes

Globally, there is substantial variation in the way vaccine programmes for infants are implemented. The World Health Organisation (WHO) recommends infant vaccination commence at 6 weeks of age as a minimum, and that 3 doses of a diphtheria-tetanus-pertussis (DTP) containing vaccine be administered in infancy, with 4 to 8 weeks between doses.[1] A booster dose in the second year of life is also recommended. These recommendations allow for, and indeed expect, that vaccination implementation will differ between countries.

Widespread vaccination against diphtheria has been in place since the 1940s, and vaccination against tetanus and pertussis since the 1950s,[2] The administration of these three vaccines in combination has become the backbone of infant immunisation schedules worldwide. However, the number of doses, age at first vaccination, and the spacing between doses varies across countries. European nations administer either 2 or 3 doses of DTP vaccine in infancy, and start at either 2 or 3 months of age. Most European countries administer a booster dose in the second year of life however, in the UK no booster dose is

given. Some countries, such as the Netherlands, the Czech Republic, and Germany, administer the booster dose earlier, at 10 or 11 months of age (Table 1). In contrast, many low income countries start earlier and vaccinate according to a 6-10-14 week schedule.[3] The reasons for variations in vaccination schedules are multi-factorial and can be due to epidemiological variation in the age and incidence of disease, resource limitations, social, and logistical constraints. Where available, access to country-specific data on disease epidemiology and vaccine efficacy can also influence decision-making about vaccine implementation. When new vaccines are introduced into an existing programme, the number and spacing of doses given is often largely influenced by the number and spacing of doses currently being administered for other vaccines as the cost of adding additional visits to current schedules for infants is prohibitive. Thus if a country has an infant immunisation programme that starts at 2 months of age and has doses at age 2, 4 and 6 months, a new vaccine would be introduced into the schedule that fits with the timing of the current programme. Although variation in vaccine schedules between countries is expected, it is unclear how such variation affects the protective efficacy of the vaccines given.

Maternal prenatal vaccination programmes

Neonatal Tetanus Infections

In many countries in Asia and Africa where neonatal tetanus is a major cause of death, pregnant women receive a tetanus vaccine in order to protect newborns from infection.[4,5] Vaccination during pregnancy induces antibodies which are transferred to the infant across the placenta and protect infants in the first months of life.[6] These passively-acquired antibodies gradually decay over time. Immunisation with a tetanus toxoid vaccine during pregnancy was first shown to be effective in preventing cases of neonatal tetanus in a 1961 study in Papua New Guinea. Women were vaccinated during pregnancy and followed up for birth outcomes in a non-randomised fashion. Those receiving only 0 or 1 doses of vaccine had higher rates of neonatal tetanus (10%) than those who received 2 doses (3.4%), or 3 doses (0.6%) of tetanus toxoid during pregnancy.[7]

Table 1 Infant vaccination programmes in Europe for diphtheria

Months of age	2	3	4	5	6	10	11	12	13	14	15	16	18
Austria		D		D				D					
Belgium	D	D	D								D		
Bulgaria	D	D	D									D	
Croatia	D		D		D			D					
Cyprus	D		D		D						D		
Czech Republic	D	D	D			D							
Denmark		D		D				D					
Estonia		D	D		D								
Finland		D		D				D					
France	D		D				D						
Germany	D	D	D				D				D		
Greece	D		D		D						D		
Hungary	D	D	D										D
Iceland		D		D				D					
Ireland	D		D		D								
Italy		D		D			D						
Latvia	D		D		D			D					
Liechtenstein	D		D		D						D		
Lithuania	D		D		D								D
Luxembourg	D	D	D						D				
Malta	D	D	D										D

Netherlands	D	D	D				D						
Norway		D		D				D					
Poland	D	D		D								D	
Portugal	D		D		D								D
Romania	D		D				D						
Slovakia	D		D			D							
Slovenia		D	D		D			D					
Spain	D		D				D						
Sweden		D		D				D					
United Kingdom	D	D	D										

European Centre for Disease Prevention and control http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx.[8]

D=diphtheria vaccine administration

Effectiveness was also demonstrated in Bangladesh in a study in the late 1970s in which the rate of neonatal death from tetanus was 7.8% in infants from unvaccinated mothers, and 4.3% in infants from mothers who received two doses of vaccine during pregnancy.[5]

Today, immunisation in pregnancy against neonatal tetanus is done using a single antigen tetanus-toxoid vaccine (TT) or a combination vaccine, usually low-dose diphtheria-tetanus toxoid vaccine (Td).[9] Due to more advanced maternity care in high-income countries, immunisation against neonatal tetanus is not necessary, and is not included in European programmes. However, many women now receive tetanus vaccine during pregnancy anyway, as part of vaccination programmes against pertussis.

Infant Pertussis Infections

In recent decades a worldwide resurgence in the incidence of pertussis has been observed, despite globally high vaccination rates.[10,11]

The WHO Strategic Advisory Group of Experts (SAGE) reviewed the data on pertussis incidence in 2014 and noted clear evidence of an increase in pertussis in 5 of 19 countries with appropriate data (Australia, Chile, Portugal, USA and UK).[12] Evidence of pertussis re-emergence has been subsequently reported elsewhere. [13-15]

The resurgence of pertussis is due in part to the widespread change from whole-cell vaccines to less-immunogenic acellular pertussis vaccines, but also due to changes in detection and reporting practices, and potentially the evolution of *B. pertussis* strains over time.[16-19]

Infants are at particular risk of severe complications from pertussis during the period when they are still too young to have received the full series of infant pertussis vaccinations. The UK was the first country to introduce a pertussis vaccination programme in pregnancy in 2012.[20] Prenatal vaccination programmes have subsequently been widely introduced in many other countries.

Vaccination in pregnancy has been highly effective in preventing pertussis in young infants.[21-25] Effectiveness was first demonstrated by Public Health England

1 year after the introduction of the programme in the UK to vaccinate women in pregnancy.[21] The UK observational cohort study included 26 684 women and 82 cases of laboratory confirmed pertussis in infants aged < 3 months. Vaccine efficacy (VE) was calculated using the screening method as 1 minus the odds ratio (odds of maternal vaccination in cases divided by the odds of vaccination in the population). VE was 91% in infants aged < 3 months and 90% in those aged < 2 months.[21] A second, smaller case-control study in the UK also estimated VE as 91% in those aged < 2 months.[22] In an American retrospective cohort study of 148 981 newborns, Baxter *et al* estimated the effectiveness (1 minus hazard ratio (HR)) of maternal Tdap administration in pregnancy as 91% for infants age < 2 months.[24] A matched case-control study in Valencia, Spain, also estimated VE of 91%, using VE calculated as 1 minus the odds ratio from an adjusted conditional logistic regression model.[25] Whilst variation in study design and computational methods means that these apparently identical estimates are not quite the same, they all consistently indicate high effectiveness of maternal prenatal pertussis vaccination programmes.

Unlike vaccination in pregnancy against tetanus, there is no licenced vaccine that contains pertussis alone. Therefore pertussis vaccination in pregnancy is done using a combination vaccine. In the UK the vaccine used contains diphtheria, tetanus, acellular pertussis, and inactivated polio (DTaP-IPV) and there are two licensed versions of the vaccine. Repevax, (Sanofi Pasteur) was used from 2012 until Summer 2014, then Boostrix-IPV (GSK) has been in use since 2014.[26] Other countries, such as the USA, also use a combination vaccine but the vaccine used does not contain the inactivated polio component (Tdap).

Other vaccines aimed at protecting infants against disease through maternal prenatal immunisation are currently in development, such as those for respiratory syncytial virus (RSV)[27] and group B streptococcus.[28] In addition, maternal prenatal vaccination against influenza is recommended in many countries. Maternal prenatal vaccination programmes are likely to become increasingly common.

Maternal antibody interference in immunogenicity of infant vaccines

Whilst passively-acquired maternal antibody protects infants against disease in the early months of life, high concentrations of maternal antibody in infants at the time they are vaccinated are thought to inhibit the immune response to vaccination against some antigens.[29-37] However, there is inconsistent evidence on the extent of this inhibition and a lack of consensus regarding which antigens or vaccines are affected. In particular, there are conflicting reports on whether pre-existing passive antibody interferes with responses to acellular pertussis vaccines. This was examined in the early 1990s in the large National Institute of Allergy and Infectious Diseases (NIAID) Multicenter Acellular Pertussis Trial, in which a licensed whole cell pertussis combination vaccine (DTwP) was compared with 12 other acellular pertussis vaccines which varied in their components and immunogenicity.[38] Englund et al examined the relationship between the antibody response to vaccination and the pre-existing levels of maternal antibody in the NIAID study and concluded that "*The [pertussis toxoid] PT antibody response to DTaP, unlike DT[w]P, is not adversely affected by preexisting antibody*."[32] This analysis however, did not adjust for the differences between vaccines included in the study. A more recent, yet small, study of acellular pertussis vaccine reported 'modest' interference in 43 infants (of which 15 infants were born to mothers vaccinated in pregnancy).[39] Additionlly, Ladhani et al report a significant inverse relationship between pre-vaccination and post-vaccination pertussis toxoid antibody titres in an analysis of 129 infants from mothers vaccinated with acellular pertussis vaccine in pregnancy.[36] Post-vaccination antibody levels in that study were lower by 11% for every 2-fold increase (doubling) of maternal antibody (Geometric mean ratio (GMR) 0.89, per 2-fold increase in pre-vaccination concentration; 95% CI, 0.81–0.98; P = 0.023).[36]

However, some studies appear to confirm that maternal vaccination does not cause inhibition of infant antibody responses to acellular pertussis vaccination. In a randomised controlled trial in Vietnam, Hoang et al reported no difference in anti-pertussis toxoid responses in infants from mothers who received Tdap during pregnancy compared with TT.[37] Similarly, there was no significant difference in pertussis antibody responses to infant immunisation according to

antibody levels at birth in a study of 36 infants.[35] Other studies show mixed results likely due to the small numbers of samples tested at different time points.[40]

These studies which investigate the potential for maternal antibody interference do not provide evidence on the clinical impact of such interference. The most robust data on the clinical impact of maternal pertussis vaccination comes from the study by Armirthalingam et al in which the effectiveness of the maternal pertussis vaccination programme in the UK was investigated 3 years after its introduction.[23] Cases of laboratory-confirmed infant pertussis were collected through the UK national surveillance programme and maternal and infant vaccination status determined through contact with the patient's general practitioner. There were 73 infants with pertussis who had received between 1 and 3 doses of infant vaccines (DTaP-IPV/Hib). The effect of maternal vaccination in 43 cases who had received one dose of vaccine was VE 82% (95% CI, 65%–91%), similar to the maternal VE in children less than 3 months of age who had not received any infant vaccine (VE 91%, 95% CI 88%–94%). For the 12 infants who have received 2 doses, the protection conferred through maternal immunisation was lower (69%, 95% CI 8%–90%), and after three doses was no longer significant.

Maternal antibody interference for co-administered conjugate vaccines

It is not only antibody responses in infants to the same antigens as are contained in the maternal vaccine that may be affected by maternal antibody interference, but also antibody responses to different but related vaccine antigens.

Many vaccines included in infant immunisation programmes are conjugated to either a diphtheria or a tetanus protein. For example, *Haemophilus influenzae* type b polysaccharide (Hib) is conjugated to a tetanus toxoid protein in some vaccines, and the 13-serotype pneumococcal conjugate vaccine (PCV13, Prevenar, Pfizer) has a diphtheria-toxin mutant carrier protein (CRM). 10-valent pneumococcal conjugate vaccine (PCV10, Synflorix, GSK) contains a mixture of different carrier proteins, with the majority of serotypes conjugated to a non-typeable *Haemophilus influenzae* protein and serotypes 18C and 19F conjugated to tetanus and diphtheria toxoids respectively. Maternal prenatal vaccination

programmes using multi-component vaccines containing diphtheria and tetanus, have the potential to affect not just the infant antibody response to diphtheria and tetanus vaccines, but also to affect the immunogenicity of co-administered conjugate vaccines that contain these proteins as carriers. Maertens et al reported the results of a randomised trial of 77 mother-infant pairs and observed lower antibody responses to PCV13 in infants whose mothers had received Tdap (Boostrix, GSK) during pregnancy for 9 of 13 serotypes.[41] Ladhani et al also observed reduced antibody response to PCV13 in infants from mothers who received Tdap/IPV (Repevax; Sanofi Pasteur) during pregnancy, as well as reduced responses to diphtheria, tetanus, and Hib vaccine. However, this study used an historical control for comparisons which may have been a source of bias.[36] In a slightly different study, Madhi et al reported antibody responses in a randomised trial of an investigational Group B *Streptococcus* (GBS) conjugate vaccine with a CRM carrier protein administered during pregnancy. Three concentrations of the GBS vaccine were tested with CRM content of 0.6–3 μg, 3–15 μg, and 6–30 μg per serotype. Antibody responses to diphtheria toxoid vaccination in infants were similar regardless of whether their mother had received the investigational GBS vaccines or placebo.[42] Administration of a conjugate vaccine during pregnancy may, therefore, have less impact on routine infant vaccines than administration of a diphtheria or tetanus toxoid vaccine.

As new vaccines become licensed for administration in pregnancy, maternal antibody interference will continue to be an issue that requires careful consideration in the design of infant programmes. However, consideration of such factors cannot be undertaken until there is clarity on which antigens or vaccines are most affected by maternal antibody and the magnitude of these effects.

Understanding the decay rates of maternal antibodies

Since passively acquired maternal antibody decays over time, the concentration of maternal antibody present in the infant at the time of vaccination will depend to some degree on the age of the child at the time of vaccination, the initial concentration of antibody present at birth, and the rate of decay of the maternal antibody in the infant after birth. Decay rates for passively acquired antibodies

to many antigens are poorly characterised in the medical literature. Estimates of the half-lives of transplacental antibodies are in many cases approximations only, derived from small studies conducted some decades ago. A half-life of 36 days for antibodies against pertussis toxin and 40 days for filamentous haemaglutinin was reported in a study from 1990.[43] For anti-diphtheria toxin antibodies, estimates of 35 days and 31 days were reported in a study in 1949.[44] Longer half-lives have been reported for tetanus toxin (49-50 days)[45] and measles antibodies (46, 48, and 53 days).[46] Due to the age of most studies which report half-lives of passively-acquired antibodies, only approximate estimates are provided, with no corresponding confidence intervals and no accounting for repeated measures.

Reliable estimates of the rates of decay of maternal antibodies are essential for modelling the impact of programmes of vaccination in pregnancy.

In addition, understanding how the age of a child at the time of their first vaccination influences their antibody response to vaccination independent of the effect of maternal antibody interference is an important consideration. Antibody responses to polysaccharides do not occur in infants less than 2 years of age and, in general, young infants have lower antibody responses to vaccination than older children or adults.[47] However, the age of infants when commencing vaccination programmes varies by only a few weeks or a month from country to country and this small amount of variation in that age infants are first vaccinated may have little effect of antibody responses. Of necessity, vaccine manufacturers test their new vaccines under development according to the immunisation schedule that is already in place in the country of interest. The effect of varying the timing of vaccination schedules is rarely investigated in the majority of vaccine clinical trials. In addition, when infants are enrolled into vaccine clinical trials, their age at enrolment generally varies by only a few weeks, thus within any single clinical trial it is rarely ever possible to examine the impact of starting immunisation programmes at different ages. The effect of variation in the age at which vaccination commences for infant remains largely unclear.

Antibody correlates of protection

Although maternal antibodies may affect immunogenicity of infant vaccines, it is unclear whether this would result in reduced protection against disease. In order to understand the clinical implications of maternal antibody interference it is necessary to understand the antibody levels that result in protection against infection or disease. If protective levels of antibody are still induced by vaccination, even in children who have reduced responses due to high levels of maternal antibody, then there may be limited or no negative impact of maternal immunisation on the protection afforded by vaccination against clinical endpoints.

For some vaccine antigens, antibody concentrations that are indicative of protection are well established. Yet for others, these are unknown. For tetanus antibodies, concentrations above 0.1 IU/ml measured by an in vivo neutralization assay or 0.15 IU/mL by ELISA are widely accepted as being protective, however data in humans underpinning these levels are scarce and cases of tetanus have been documented in those with antibody levels higher than these thresholds.[9,48] Similarly, for diphtheria, an antibody level higher than 0.1 IU/ml is thought to provide basic immunity however variation exists.[49] There is no widely accepted threshold for anti-pertussis toxin antibodies.

Computing correlates of protection

Ideally, the concentration of antibody that is sufficient to protect an infant against infection (the correlate of protection) could be determined from large vaccine clinical efficacy studies if all participants in the study had antibody levels assessed post-vaccination and were followed up for the full study period. The disease outcomes for each participant could then be modelled as a function of their individual antibody response to vaccination. However, in practice in large efficacy trials of new vaccines, logistical and financial constraints mean that generally only a small subset of children have blood samples taken and antibody concentrations measured. For example, in early efficacy trials of pneumococcal vaccines between 190 (1.4%) and 445 (15%) of children enrolled in the pneumococcal vaccine arm of the trials had antibody levels assayed.[50] More recently, in the development of the Sanofi Pasteur chimeric yellow fever dengue

tetravalent vaccine two phase 3 trials were conducted in which 1325 of 6778 (20%) and 1331 of 13268 (10%) dengue vaccine recipients in the two trials had blood sample taken.[51,52] If clinical outcomes are hard to measure due to tests that lack sensitivity to the specific pathogen, or other difficulties in diagnosis, and blood samples are only taken on a small proportion of participants, then the number of participants with blood samples who also experience the primary clinical outcome will be very small. The individual participant level data from such small subsets in these situations is not very informative in estimating protective antibody concentrations. In such situations the overall trial-level estimates of the proportion with disease and the proportions with antibody below a threshold level are used to define a correlate of protection.[50,53,54] A pooled correlate of protection against invasive pneumococcal disease of 0.35 μg/mL was derived from a meta-analysis of three pneumococcal vaccine efficacy trials[50] in which the trial-specific serum IgG correlates of protection were estimated to be 0.2, 0.68 and 1.0 μg/mL respectively.[55-57] For Group C meningococcal conjugate vaccine, the original correlate of protection came from a 1969 prospective observational study in military recruits that showed the proportion of recruits with pre-exposure serum bactericidal antibody titres > 1:4 was very low (5.6%) in those who went on to develop meningococcal disease, but high (82%) in those were remained well.[58] This threshold was then validated in a UK postlicensure study in 2003 using the same methodology as was used to establish the correlate of protection for pneumococcal vaccines.[59] It has not been established that this method produces robust or unbiased estimates of protective antibody thresholds and may be more suited to validation studies than for derivation of initial thresholds.

The overall estimated correlate of protection for pneumococcal vaccine of 0.35 μg/mL was calculated by combining antibody levels across all serotypes in the vaccine, however serotype-specific antibody levels that protect against disease may vary, as has been seen in some post-vaccine impact studies.[60] The pooled correlate of protection against invasive pneumococcal disease provides a basis for licensure of new vaccines, however may not be appropriate for assessing serotype-specific vaccine impact. Additionally, correlates of protection for other end points of clinical importance such as acute otitis media, non-bacteraemic pneumonia, and nasopharyngeal carriage have not been established. For new

vaccines, such as typhoid conjugate vaccines, there are no widely accepted correlates of protection.

Summary

Infant immunisation programmes are cost-effective public health interventions which prevent millions of cases of disease and death globally. Vaccine policies evolve over time as new vaccines are licensed or as the epidemiology of disease changes. As vaccination during pregnancy becomes more common, the optimisation of immunogenic infant immunisation schedules will require an understanding of the interaction between maternal prenatal and infant immunisation programmes. For many vaccine antigens, there are limited or no reliable data on the impact of maternal antibody on the response to infant vaccination, the optimal age when vaccination should commence, and the level of antibody that is sufficient to protect against disease. In addition, current estimates of parameters such as antibody half-lives that are needed for inclusion in mathematical modelling of immunisation programmes, are not robust. Furthermore, for many vaccines, correlates of protection have not been established. Current methods to compute such correlates of protection have not been validated and thus may not give accurate results. Where possible, vaccines are licensed based on their evaluation according to agreed correlates of protection. If calculated correlates are not correct, future vaccines may fail to meet licensure criteria even though they are highly protective, or conversely, ineffective vaccines may be licensed and implemented for use.

Filling these gaps in our understanding of infant vaccines has the potential to improve the way infants are vaccinated and provide improved protection against disease.

3.2 OBJECTIVES OF THIS THESIS

The specific objectives of the thesis are:

A. Determine the rate of decay of maternal antibodies to common vaccine antigens;

B. Characterise the magnitude and extent of maternal antibody interference on the immunogenicity of infant vaccines;
C. Determine if the age at which infants are vaccinated affects the magnitude of their antibody response to vaccination independent of the effect of maternal antibody;
D. Determine if current methods to calculate correlates of protection for infant vaccines are fit for purpose;
E. Determine the level of anti-capsular antibody that is protective against acquisition of nasopharyngeal carriage of pneumococcal serotypes in infants.

3.3 INDIVIDUAL PARTICIPANT DATA

Data are hard to obtain

Obtaining data specifically to inform the various issues described above would be costly, time consuming, and in some situations, not feasible. Studies to inform our understanding of the interactions between maternal antibody and infant vaccine responses, require enrolment and vaccination of women during pregnancy, and the vaccination and follow up of their infants. Such studies of mother-infant pairs are often, of necessity, small and thus non-significant findings may be due to statistical underpowering rather than a true lack of effect. Studies to determine the effect of the age at first vaccination would be hard to finance as the need for such a study would be hard to demonstrate. Studies to estimate correlates of protection in large efficacy trials are almost impossible due to the logistical difficulty of obtaining blood samples on all participants in very large trials of thousands or tens of thousands of infants.

For these reasons, alternative sources of data are necessary.

Re-analysis of trial data from multiple clinical trials offers an alternative

The results I present in this thesis are based on either simulated data or individual level data from a number of industry clinical trials of vaccines in infants.

I submitted requests for access to fully anonymised individual participant data from a large number of studies through https://clinicalstudydatarequest.com on two separate occasions. Applications submitted through the website were reviewed by an external panel and after being accepted a data-sharing agreement was put in place between the University of Oxford and the company that owned the data (in this case, GlaxoSmithKline). Once the data-sharing agreement was signed, datasets were anonymised and documentation prepared by the company. Due to the scale of my data requests, datasets were gradually made available over a three year period. The order in which trial datasets were made available was agreed between myself and the company, with trials of diphtheria, tetanus, pertussis, pneumococcal, and meningococcal vaccines prioritised. Not all studies are included in the final thesis as analysis of the final datasets from MMR and rotavirus vaccine trials continues.

Datasets were not sent to Oxford. Instead, data were accessed through an online portal (SAS® Clinical Trial Data Transparency Portal). After logging in to the external server, online versions of SAS and R were available to conduct the analyses. The online version of SAS that was available using this system did not have full functionality and crashed constantly. One of the learning opportunities afforded by this doctorate was, therefore, the opportunity to learn to code in R. Most of the analyses presented herein were conducted using R.[61]

Once data access was given, each study was processed and combined into larger datasets for analysis. Data from clinical trials generally consist of multiple separate datasets; for demographic data, serological data, vaccine administration data, etc. A program was written for each trial to combine all relevant datasets into one trial dataset, ensuring that all fields needed for the analysis were included. A dataset for each trial was saved on the online server and these were then combined into one large multi-study dataset. I was the only person with access to the raw data. I did all data processing and conducted all the analyses.

All figures and tables are, therefore, my work unless otherwise indicated. My supervisors reviewed my outputs, discussed my plans and methods, and gave advice on all aspects of the process.

A full list of all 49 clinical trials included in the thesis is provided in the Appendix, along with an indication of which chapters in the thesis include data from each study. The 49 studies represented 30 cohorts of children. The purposes of the trials varied and they assessed the immunogenicity of different vaccines using different study designs therefore not all studies are included in all analyses as the relevant antigen or time point may not have been measured. As some studies were conducted in multiple countries which differ in their vaccination programmes and disease epidemiology, a 'country cohort' variable was created for use in all analyses, which comprised the country name and a number which was unique to each country within each study. All studies throughout the thesis are referred to and displayed by country cohort.

The 49 trials included in the thesis include 48 clinical trials provided by GSK and one additional clinical trial of a pneumococcal conjugate vaccine conducted in Nepal. Data from this study were kindly made available to me by Professor Andrew Pollard, and Professor Shrijana Shrestha from the Patan Academy of Health Sciences, Kathmandu, Nepal.

No ethics approval was required for any of the work included in the thesis as these results are from re-analysis of anonymised data from previously published studies, or use simulated data.

As part of the agreement to share data the company required notice of any publications or presentations of findings within one-week of the publication date. They did not review or provide feedback on any publications or presentations and were not involved in the decision to submit results for publication.

There were many advantages in accessing large amounts of data from industry clinical trials for this work. The main advantage was that the amount of data available was extensive, allowing for analyses to be conducted that were novel in their size and scope, with no limitations due to sample size, and no limitations due to the need to use published estimates such as are included in aggregate data

meta-analyses. In addition, all serology data were processed in one central laboratory thereby minimising the effect of inter-laboratory variation on assay values, increasing the robustness of the findings. There were also limitations associated with the use of these data. Results from any in-house assays used in the included clinical trials may differ to in-house assays used by a different company. Therefore extrapolating my results to vaccines and assays from other manufacturers needs to be done with caution. In addition, clinical trials from a single company only address questions of relevance to that company's vaccines. The available GSK clinical trials of pneumococcal vaccines in general measured the 10 serotypes included in the GSK pneumococcal conjugate vaccine. Additional data on the 3 extra serotypes included in the Pfizer pneumococcal conjugate vaccine would have added further insight but was unfortunately not available.

4 PREVALENCE AND DECAY OF MATERNAL ANTIBODIES

The work contained in this chapter is published in;

> Voysey M, Sadarangani M, Pollard AJ, Fanshawe T. *Prevalence and decay of maternal pneumococcal and meningococcal antibodies: a meta-analysis of type-specific decay rates.* Vaccine 2017. Oct 13;35(43):5850-7. https://doi.org/10.1016/j.vaccine.2017.09.002
>
> *This article was published under the terms of the Creative Commons Attribution License (CC BY). All text, analyses, tables, and figures were produced by Merryn Voysey. Co-authors have given permission to include the material in this thesis.*

This work was included in an oral presentation at the International Neonatal and Maternal and Immunisation Symposium (INMIS), Brussels, September 2017.

> *Prevalence and decay of maternal pneumococcal and meningococcal antibodies in 8022 infants: a meta-analysis of type-specific decay rates.*

4.1 BACKGROUND

At the time of their first vaccination, some infants already have antibodies against vaccine antigens owing to the placental transfer of antibody during pregnancy. Almost all antibody is transferred across the placenta in the third trimester of pregnancy and if still present at the time of vaccination, this passively-acquired antibody may interfere with the infant's ability to mount a robust immune response to vaccination.[6] The source of the antibody in the mother during pregnancy can be either a recent symptomatic or asymptomatic infection, the residual antibody from previous vaccinations over her life course, or due to receipt of vaccine during the current pregnancy. The concentration of antibody in infant sera at the time of their first vaccination depends on the serum antibody concentration in the mother, placental function, the gestational age at birth, age at vaccination, and the rate of antibody decay.[30,62] In addition, differences may exist between species of bacteria or between serotypes in the rate of transfer of antibody across the placenta, and the rate of decay of antibody between birth and the first vaccination, all of which will affect the residual concentration of antibody circulating at the time of vaccination.

The half-life of passively-acquired maternal pneumococcal antibodies has been estimated as approximately 35 days. This estimate comes from a combined analysis of antibody against two serotypes of pneumococcus in one study in Bangladesh.[63] A shorter half-life of 20 days for groups A and C meningococcal antibodies has been estimated in one study in the Gambia.[64] These estimates from studies conducted more than two decades ago have not been confirmed in other studies and the variability in these half-lives is unknown. It is also unclear whether they apply to children in different geographic regions or whether antibodies against different polysaccharides decay at different rates.

In this chapter the variation in the prevalence of maternal antibodies in infants from different countries is explored. The seroprevalence of maternal antibodies is summarised for many different antigens as serology data were available for many antigens. Half-lives of maternal antibodies are also calculated. For the estimation of half-lives, data at 2 time points were needed from children who had not received the vaccine antigen of interest and suitable data was available

only for pneumococcal and meningococcal vaccines so analyses are restricted to these antigens.

4.2 METHODS

Seroprevalence of maternal antibodies

For the assessment of the seroprevalence of maternal antibodies, studies were included in which blood samples were taken prior to receipt of the first dose of vaccine and the concentration of IgG was measured (Supplementary Table). Infant seropositivity to maternal antibody at the first visit (baseline) was defined as an antibody concentration above the lower limit of quantification (LLQ) at baseline, prior to receipt of any vaccination.

Half-lives of maternal antibodies against pneumococcal and meningococcal polysaccharides

For the computation of antibody half-lives, data were included from a subset of studies in which antibody concentrations were measured at two time points and some children were allocated to unvaccinated control groups. The first time point was prior to receipt of any vaccine (at study enrolment), and the second time point was one month after the third dose of the receipt of the routine infant vaccines (at age ~ 5-7 months). Only studies of pneumococcal and meningococcal conjugate vaccines fulfilled the criteria of having an unvaccinated control group. Studies of vaccines included in routine infant immunisation schedules, such as diphtheria, tetanus, and pertussis vaccines, are given to all children in randomised controlled trials as it would be unethical to have an unvaccinated control group for these antigens. Therefore, half-lives for these antigens could not be determined from the available data.

Children were included in the analysis as follows:

Pneumococcal vaccine studies:

- Studies of PCV7 versus no PCV: Antibody concentrations from children in the 'no PCV' control group were included.

- Studies of PCV7 versus either PCV10 or PCV11: Antibody concentrations for pneumococcal serotypes 1, 5 and 7F (non-PCV7 serotypes) were included from children in the PCV7 group.

Meningococcal vaccine studies:

- Studies of group C meningococcal (MenC) vaccine versus no MenC vaccine: Antibody concentrations from children in the 'no MenC' control group were included
- Studies comparing multivalent meningococcal vaccines (AC or ACWY) versus MenC vaccine: Antibody concentrations for group A were included from children who received MenC vaccine. No data were available for capsular groups W and Y.

Samples from some children were excluded from the analysis of half-lives. Firstly, children were excluded from the analysis if their initial antibody concentration was below the limit of quantification of the assay as these results cannot decay in a measurable way and give half-life estimates which are undefined. Secondly, children were excluded if their antibody concentration increased between the first and second time points, as such results give negative half-lives and are likely due to exposure to the antigen or unreported vaccinations between visits.

Thirdly, children were excluded if their antibody concentrations exhibited an implausibly slow rate of decay during the time between samples as these results were thought to be influenced by nasopharyngeal carriage or unreported vaccinations. An arbitrary cut-off of a half-life of 80 days was used to make such exclusions as this is more than twice the half-life reported previously for pneumococcal antibodies and for many other antigens.[43,44,63-65] Infants with antibody below the LLQ at the second time point were retained in the analysis and these values were substituted with half the lower limit for analysis purposes. The rate of decay calculated for these infants is therefore an approximation.

Half-life estimation

Antibody concentrations were log_2-transformed and assumed to decay log-linearly. For each individual, the rate of decay per day was computed as the difference between the log_2-antibody concentrations at the two time points divided by the number of days between the two samples.

All individual serotype-specific pneumococcal antibody decay rates from participants in all country cohorts were combined in a single mixed effects model with child-specific random intercepts. The model included fixed effects for country cohort and serotype. The serotype-specific decay rates were obtained from the model coefficients and the overall decay rate computed as a linear combination of the serotype-specific fixed effects. Decay rates were converted into half-lives by taking the reciprocal of the absolute value of the decay rate.

Heterogeneity in decay rates across country cohorts and across serotypes was assessed using an F-test (type III p value).

These models were fitted using SAS PROC MIXED. The code is as follows;

```
proc mixed data = dataset empirical noclprint;
class country_cohort unique_pid serotype/ref=first;
model log2diff_days = serotype country_cohort/s ddfm=kr noint; *where
log2diff_days is the individual serotype-specific decay rate;
random intercept/subject=unique_pid; *participant specific random
intercepts;
lsmeans serotype/cl ; *generate means for each serotype;
lsmestimate serotype 'average across serotypes'= 1 1 1 1 1 1 1 1 1
1/divisor=10 cl;
ods output lsmeans=lsmeans lsmestimates=estimates; *This line saves the
least-squares means for each serotype and the overall mean generated using
the lsmestimate statement as separate datasets;
run;

*****compute half-lives from parameter estimates***;
*Serotype specific half-lives;
data half_lives; set lsmeans;
half_life=1/abs(Estimate);
LCL=1/abs(lower);
UCL=1/abs(upper);
run;

*overall half-life;
data overall; set estimates;
half_life=1/abs(Estimate);
LCL=1/abs(lower);
UCL=1/abs(upper);
```

```
run;

proc print data = half_lives; var label half_life lcl ucl; run;
proc print data = overall; var label half_life lcl ucl; run;
```

If the antibody decay over time is a linear process when measured on a log-scale, then it should make no difference at what age samples are taken for analysis. Thus if the first blood sample is taken from a 6 week old child or from a 10-week old child, the estimate of the antibody decay rate should be consistent. This assumption that passive antibody decays in a log-linear (exponential) fashion was tested in a separate model by including the age of the child at the time of the first vaccination.

Analysis of groups A and C meningococcal antibodies was similar but models contained no terms for capsular group or participant as each participant only contributed once to the analysis. Due to the limited data available for these antibodies, the two capsular groups were analysed separately.

Sensitivity analyses were undertaken to examine factors which influenced the half-life estimates.

Firstly, to examine the influence of including small studies which only contributed to the analysis of a limited number of serotypes, a sensitivity analysis was conducted for pneumococcal serotype-specific half-lives, which included only studies which measured the majority of serotypes, i.e. the serotypes contained in the PCV7 vaccine.

Secondly, to examine the influence of the initial antibody level at the first time point, sensitivity analyses were undertaken adjusting for this variable.

Thirdly, the overall half-live of pneumococcal antibodies across all serotypes was computed with each serotype-specific estimate weighted according to the proportion of participants whose antibody levels dropped below the lower limit of detection of the assay at the second time point and thus had half-life estimates which were approximations.

4.3 RESULTS

Seroprevalence of maternal antibodies

There were 29 cohorts of children who had antibody concentrations assessed prior to the first dose of any vaccine (Supplementary Table). Children ranged in age from 4 weeks to 24 weeks at the time of their first vaccination and were from a diverse group of countries in Europe, Africa, Latin America, and South and East Asia (Figure 1).

Maternal antibodies against diphtheria, tetanus and polio

The seroprevalence of maternal antibodies against diphtheria and tetanus toxin is displayed in

Figure 2. For both diphtheria and tetanus antibodies there was a large degree of variation in the proportion of children with detectable levels of antibody at the time of their first vaccination. In two studies in Finland and one in France a large majority of children had diphtheria maternal antibodies (63% in Finland, 87% in France), in two Polish studies approximately half the children had diphtheria maternal antibodies (56% and 52%), and in Chile, The Czech Republic, and Korea, seroprevalence was very low, between 5 and 7%. Similar patterns were seen for tetanus antibodies with the lowest proportions of children with maternal antibodies in Chile and Korea with 20% and 12% seroprevalence respectively (

Figure 2).

Figure 1 Age at enrolment for children included in the analysis of maternal antibody seroprevalence

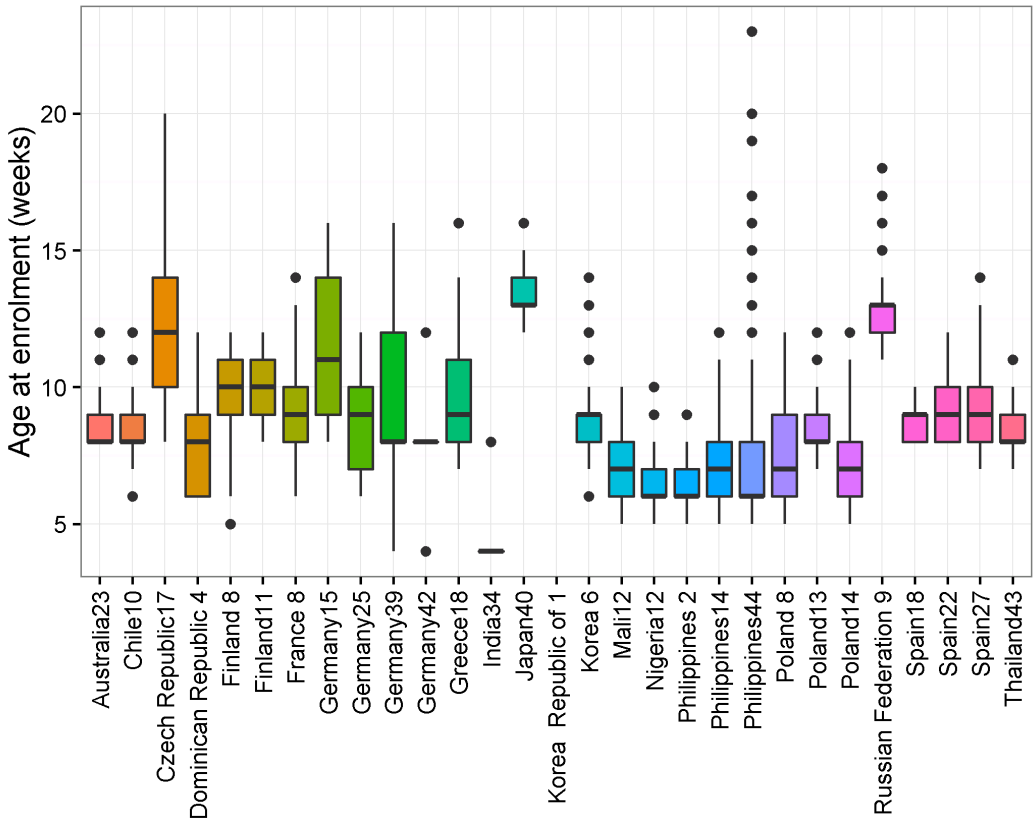
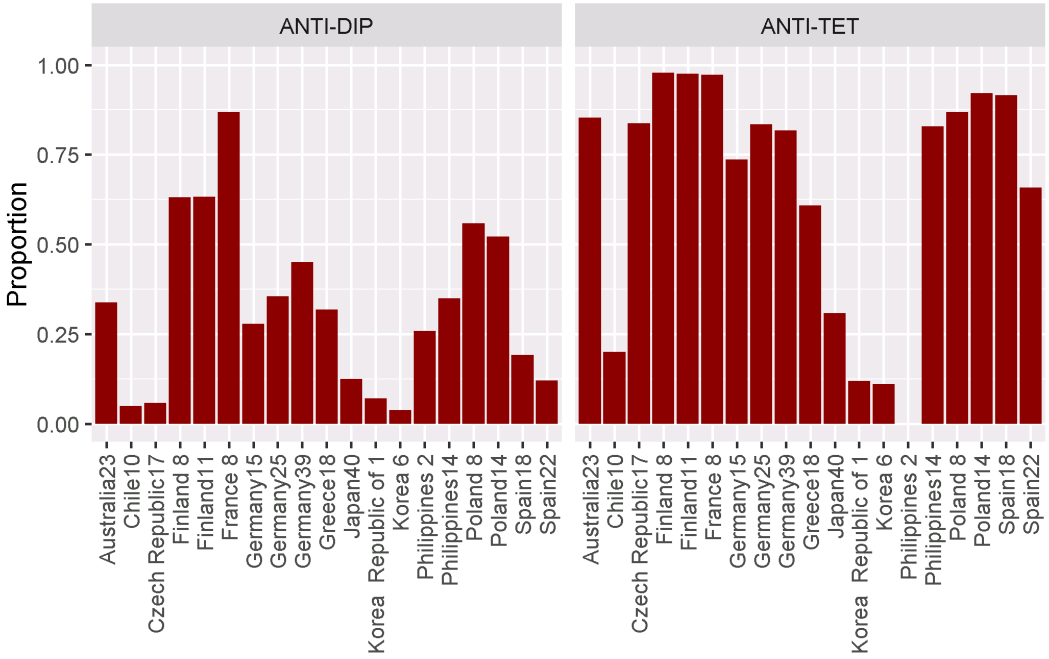


Figure 2 Proportion of infants with detectable levels of maternal anti-diphtheria or anti-tetanus antibodies prior to their first vaccination

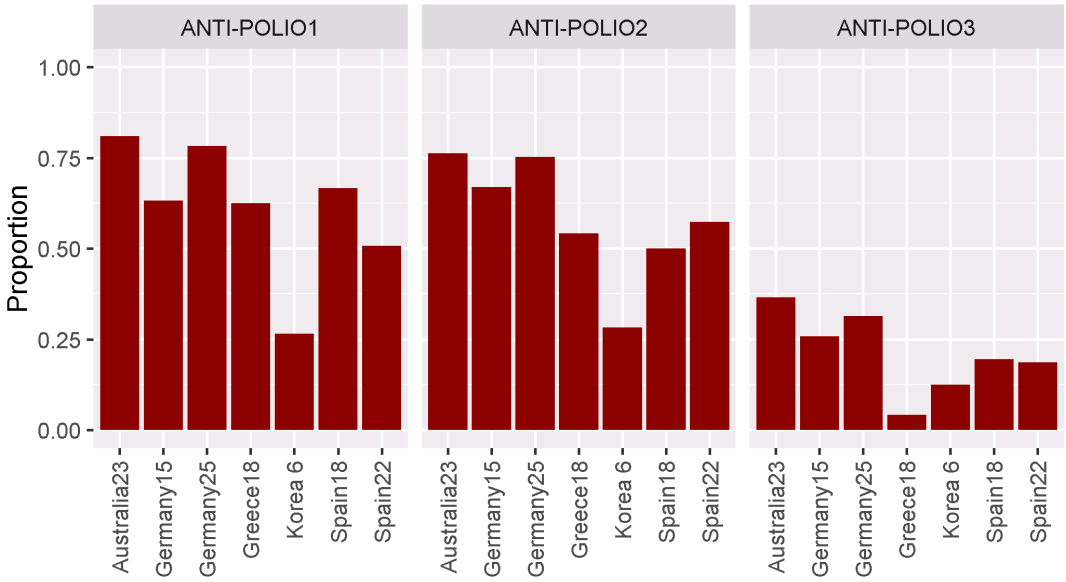


Missing bars indicate assay was not conducted

With the exception of the Philippines, none of these countries are known to have had maternal tetanus vaccination programmes in place at the time of the study.

Fewer studies measured antibodies to the three types of poliovirus. Anti-polio antibodies were high for types 1 and 2 polio in all countries except Korea which had 26% and 28% for types 1 and 2 respectively. Type 3 antibodies were less prevalent (Figure 3).

Figure 3 Proportion of infants with detectable levels of maternal anti-polio antibodies prior to their first vaccination

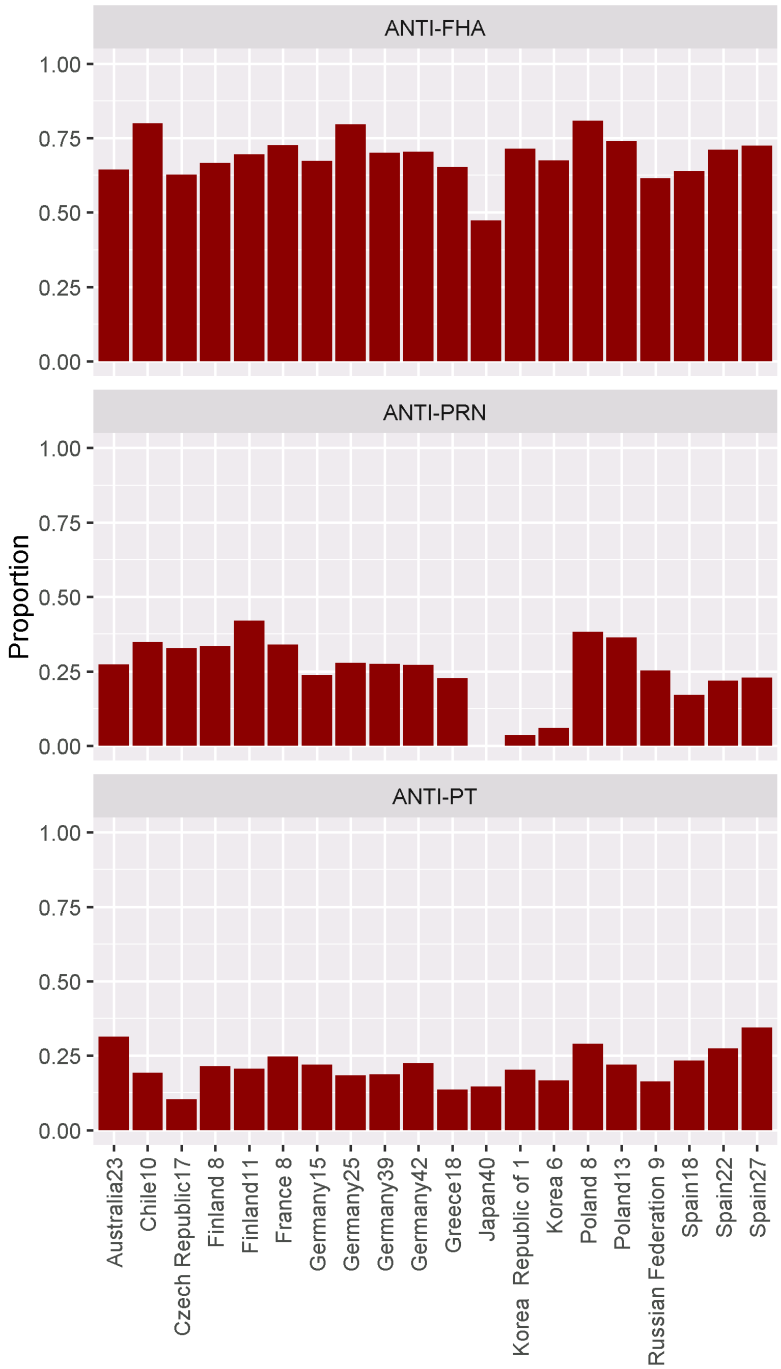


Missing bars indicate assay was not conducted

Maternal antibodies against pertussis antigens

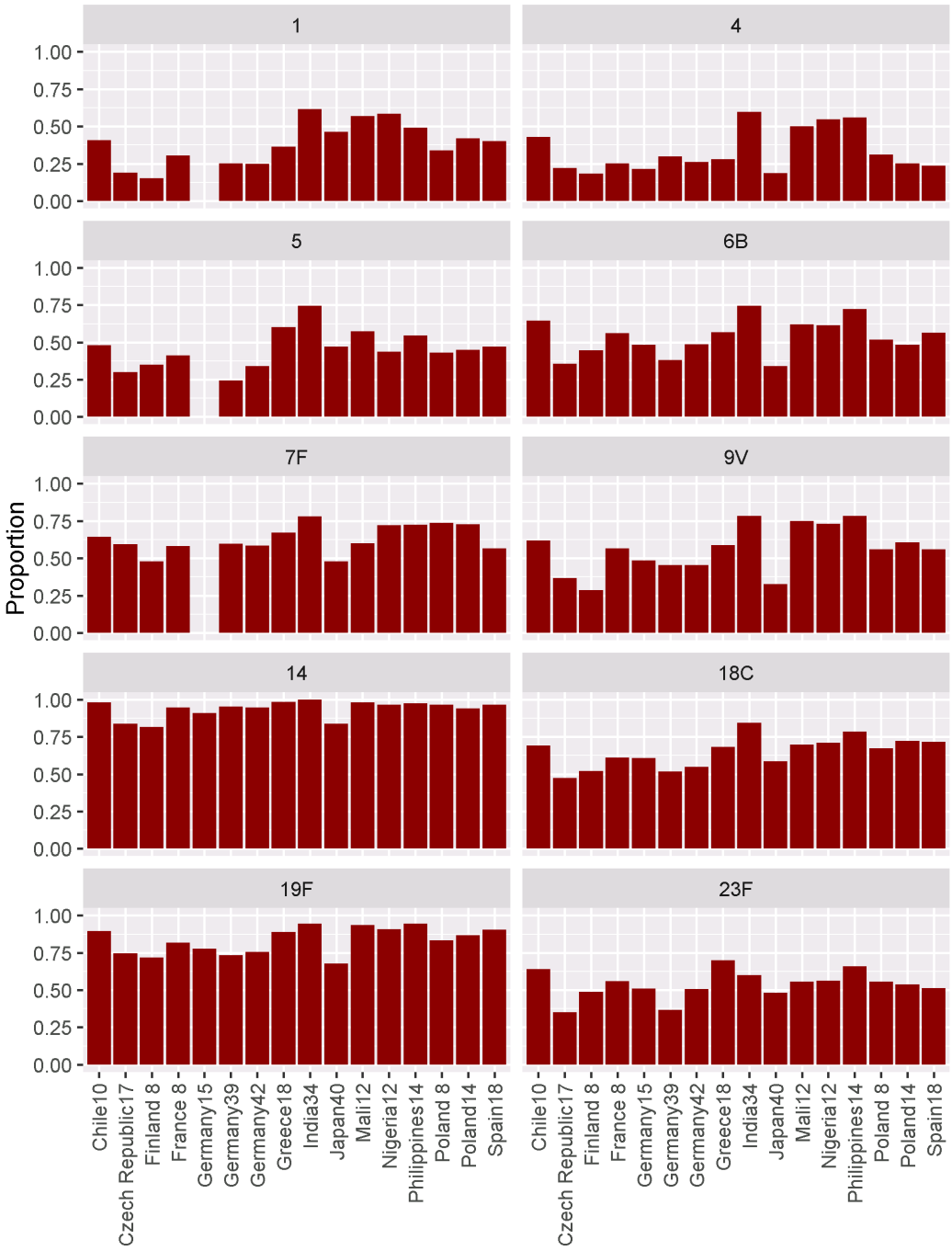
In contrast to diphtheria and tetanus antibodies, maternal antibodies against the three antigens in the acellular pertussis vaccine were consistent across countries, with high seroprevalence for filamentous haemagglutinin (FHA), and low seroprevalence for pertactin (PRN) and pertussis toxoid (PT) antibodies regardless of the region in which the study was conducted (Figure 4).

Figure 4 Proportion of infants with detectable levels of maternal anti-filamentous haemagglutinin, anti-pertactin or anti-pertussis antibodies prior to their first vaccination



Missing bars indicate assay was not conducted

Figure 5 Proportion of infants with detectable levels of maternal anti-pneumococcal antibodies prior to their first vaccination, by serotype

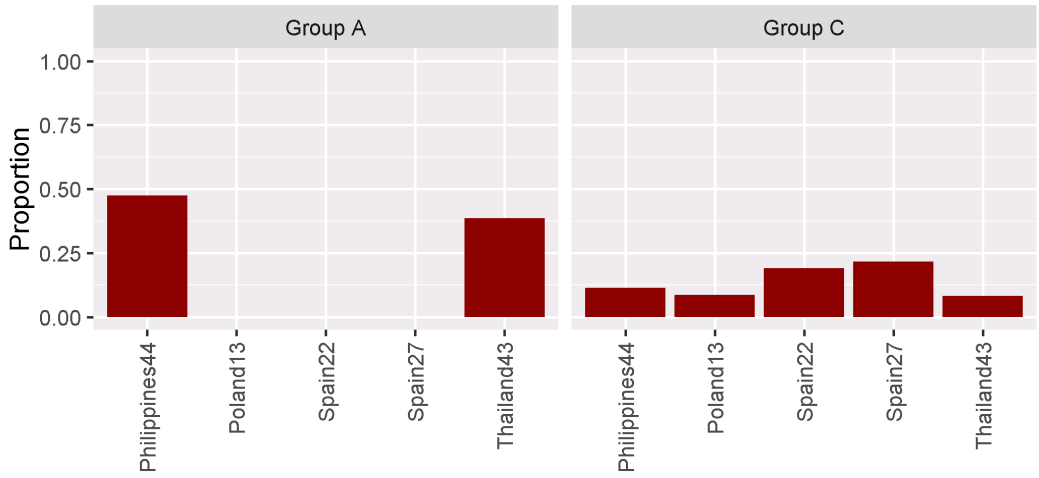


Missing bars indicate assay was not conducted

The seroprevalence of maternal pneumococcal antibodies in infants was highest for serotypes 14, and 19F (92% and 80% respectively) and lowest for serotypes 4 and 1 (30% and 34% respectively) (Figure 5). Overall across serotypes, seroprevalence was highest in studies from Mali, Nigeria, India, and The Philippines, (all >65%) and lowest in The Czech Republic and Finland (both <45%) (Figure 5).

Meningococcal antibodies were measured in studies of children aged 6-12 weeks at the time of the first vaccination. 374/2889 (13%) infants had detectable levels of group C and 604/1403 (43%) had group A meningococcal antibodies prior to vaccination (Figure 6).

Figure 6 Proportion of infants with detectable levels of maternal anti-meningococcal antibodies prior to their first vaccination, by capsular group



Missing bars indicate assay was not conducted

Half-lives of maternal antibodies

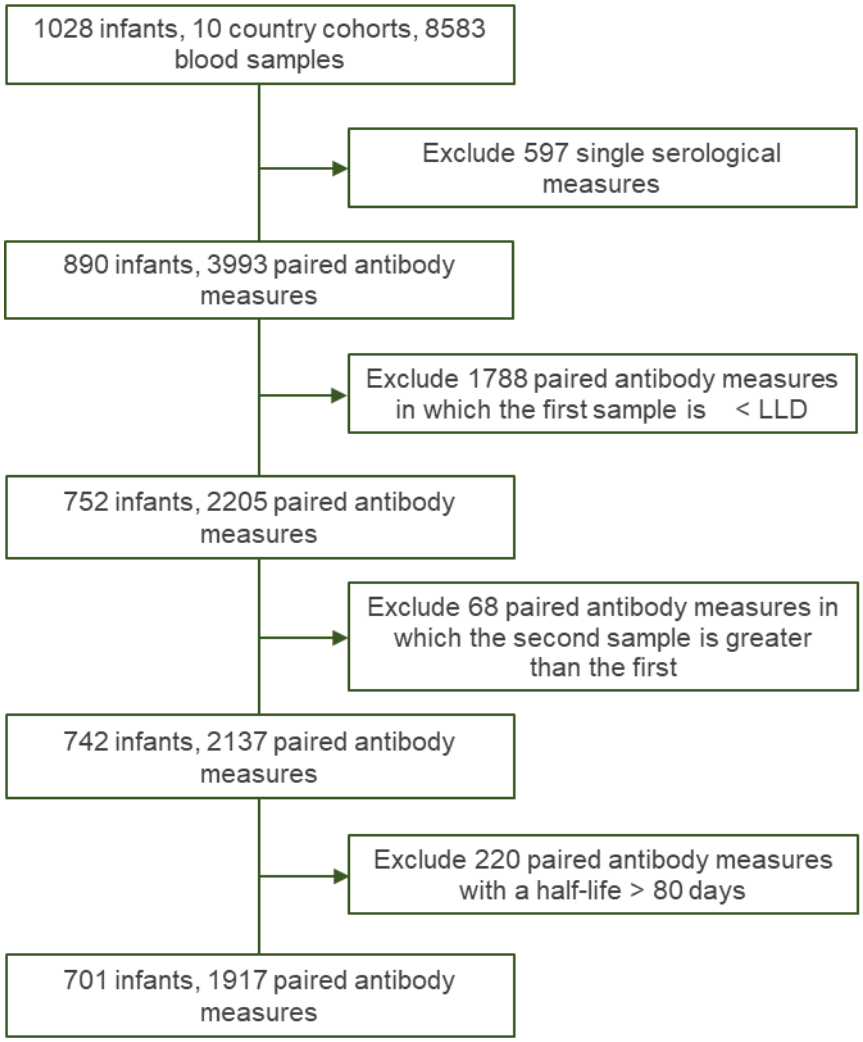
Pneumococcal antibody half-lives

In studies of PCVs there were 10 out of 16 country cohorts in which a second blood sample was taken from infants 3-5 months after the first. Of these, 701 infants had 1917 pairs of pneumococcal antibody measures available for the analysis of half-lives (Figure 7). Antibody levels at the time of the first blood sample (approximately 2-3 months of age) were assumed to be maternal in origin. However, an antibody response to carriage in some infants may have occurred and this source of antibody cannot be ruled out. Antibody in response to carriage is likely to decay at a dissimilar rate to maternal antibody, leading to the potential for bias. However, carriage in infants at this age is rare and therefore assumed to have only a small potential impact on findings.

For each serotype, between 3 and 9 country cohorts had sufficient data to be included in the meta-analysis. The full dataset of all paired blood samples is displayed in Figure 8. Serotype-specific half-lives were computed for each country cohort and are displayed in Figure 8. Half-lives for each country cohort ranged from 36 days for serotype 7F in the Philippines 14 cohort and 55 days for serotype 1 in the Poland 14 cohort. Serotype 4 had the least number of samples available for analysis (N=86 paired samples from 3 country cohorts) and serotype 7F had the most (N=409 paired samples from 10 country cohorts) (Figure 9).

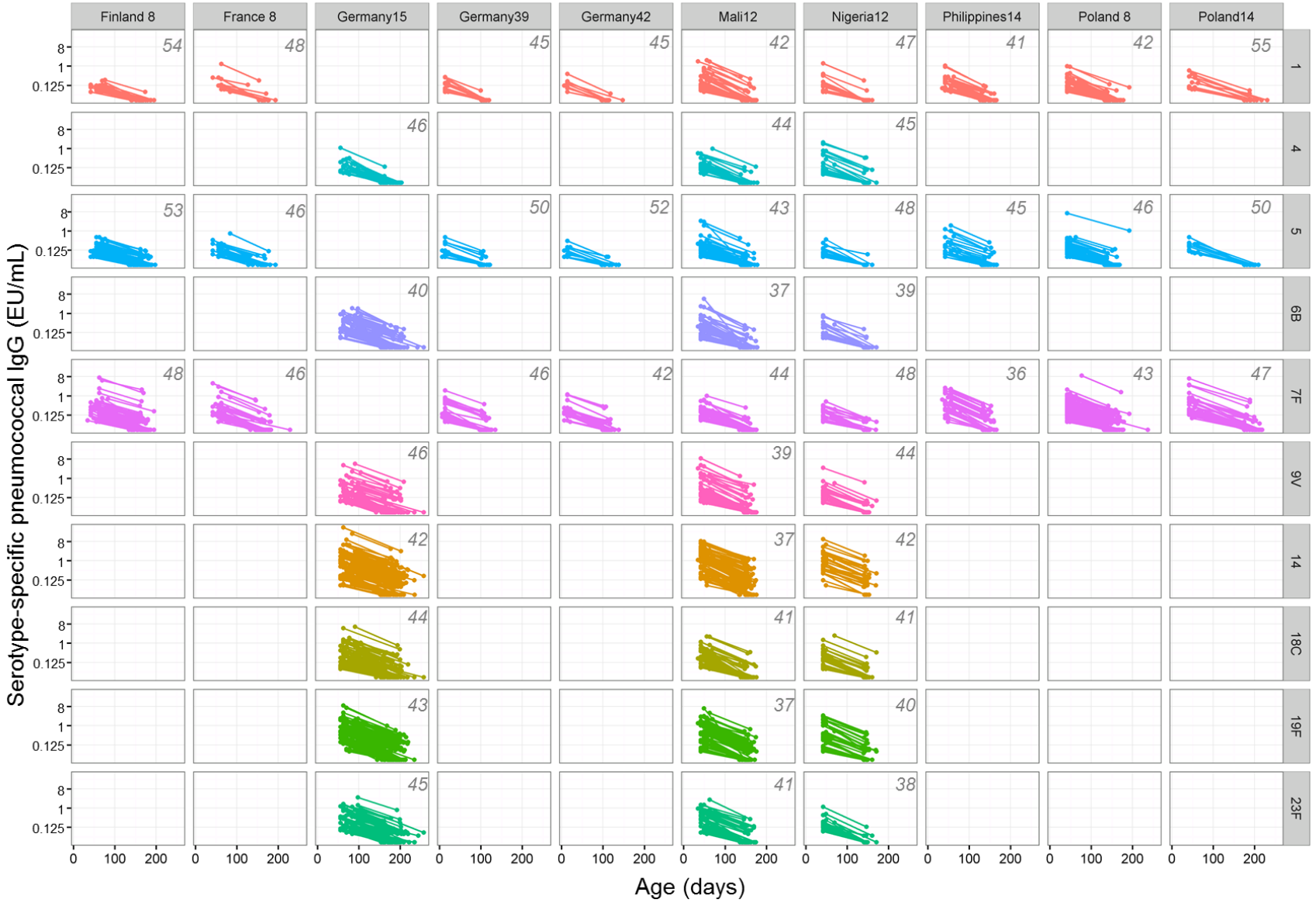
There was statistically significant variation in half-life estimates between country cohorts and between serotypes (both $p<0.0001$). Half-life estimates ranged from 38.7 days (95%CI 36.6–41.0) for serotype 6B, to 48.3 days (95%CI 46.7–50.2) for serotype 5. The overall estimate across serotypes was 42.6 days (95%CI 41.5–43.7) (Figure 8).

Figure 7 Flow chart of available data and reasons for exclusions from half-life estimations for pneumococcal immunoglobulin G concentrations



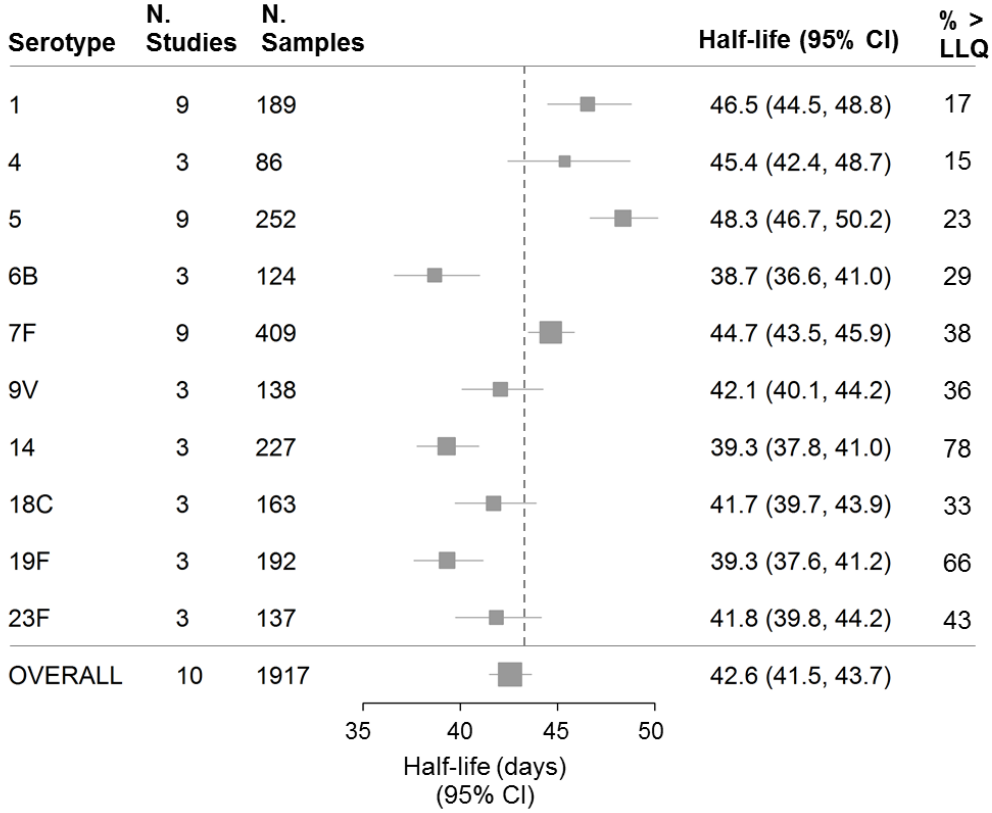
LLQ: Lower limit of assay quantification

Figure 8 Serotype-specific pneumococcal antibody concentrations and estimated antibody half-lives



Top right hand corner of each cell contains the serotype-specific half-life for that country cohort

Figure 9 Individual participant data meta-analyses from mixed effects model of serotype-specific pneumococcal antibody half-lives



Heterogeneity across serotypes and country cohorts: F-test both p<0.0001

% > LLQ: Percent of samples above the lower limit of quantification at the second time point. When weighted according to these percentages, the overall estimate was 41.5 days (95%CI 40.38-42.69).

Sensitivity analyses of half-lives of maternal pneumococcal antibodies

The first sensitivity analysis using only a subgroup of trials that measured PCV7 serotypes, had similar results with statistically significant heterogeneity between serotypes and country cohorts (both p<0.0001).

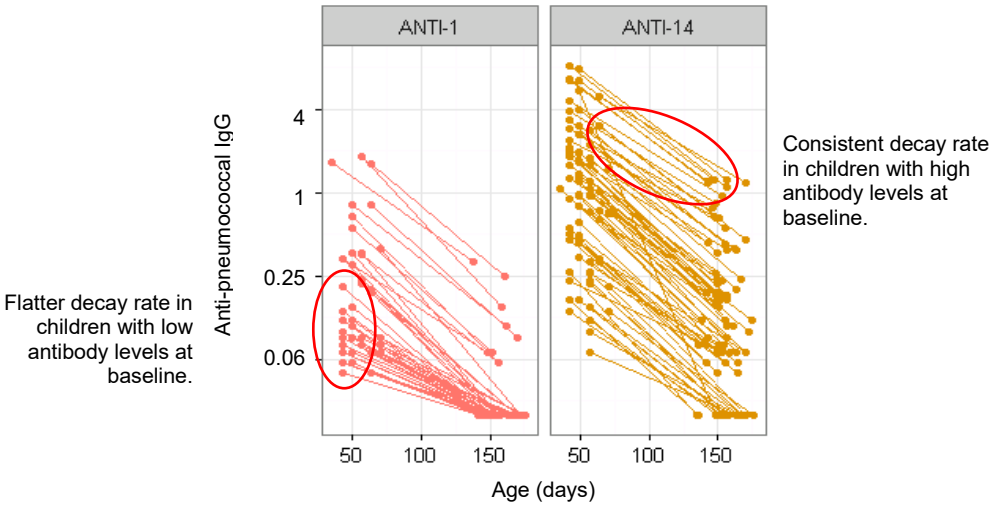
The timing of blood samples in the trials, with long gaps between the first and second blood samples, meant many samples were below the LLQ at the second time point. For these samples a value half the LLQ was substituted and the time at which the antibody concentration fell below the LLQ is unknown. The half-life is therefore inflated owing to these two assumptions. This can be seen in Figure 9 where the serotypes with high numbers of samples above the LLQ at

the second time point were also the serotypes with lower estimated half-lives. Thus serotypes 14 and 19F have 78% and 66% of samples above the LLQ at the second time-point and half-lives of 39.3 days. In contrast serotypes 1 and 4 had only 17% and 15% of samples above the LLQ at the second time point and half-lives of 46.5 and 45.4 days. When the overall half-life was estimated and weighted according to the proportion of samples above the LLQ, this reduced the half-life to 41.5 days (95%CI 40.4-42.7) by placing more weight on serotypes 14 and 19F and less weight on serotypes 1 and 4.

A second sensitivity analysis was undertaken to examine the effect of adjusting for baseline antibody levels. Children with baseline antibody levels at the first time point that were close to the LLQ had a flatter trajectory of decay as their second blood sample was below the LLQ and therefore substituted with half the lower limit (Figure 10).

A sensitivity analysis to assess the assumption of log-linear decay was conducted which showed that the age of the child was not significantly associated with decay rates (p=0.103). This confirmed the assumption of exponential decay and the validity of the model.

Figure 10 Anti-pneumococcal antibody in Mali for serotypes 1 and 14



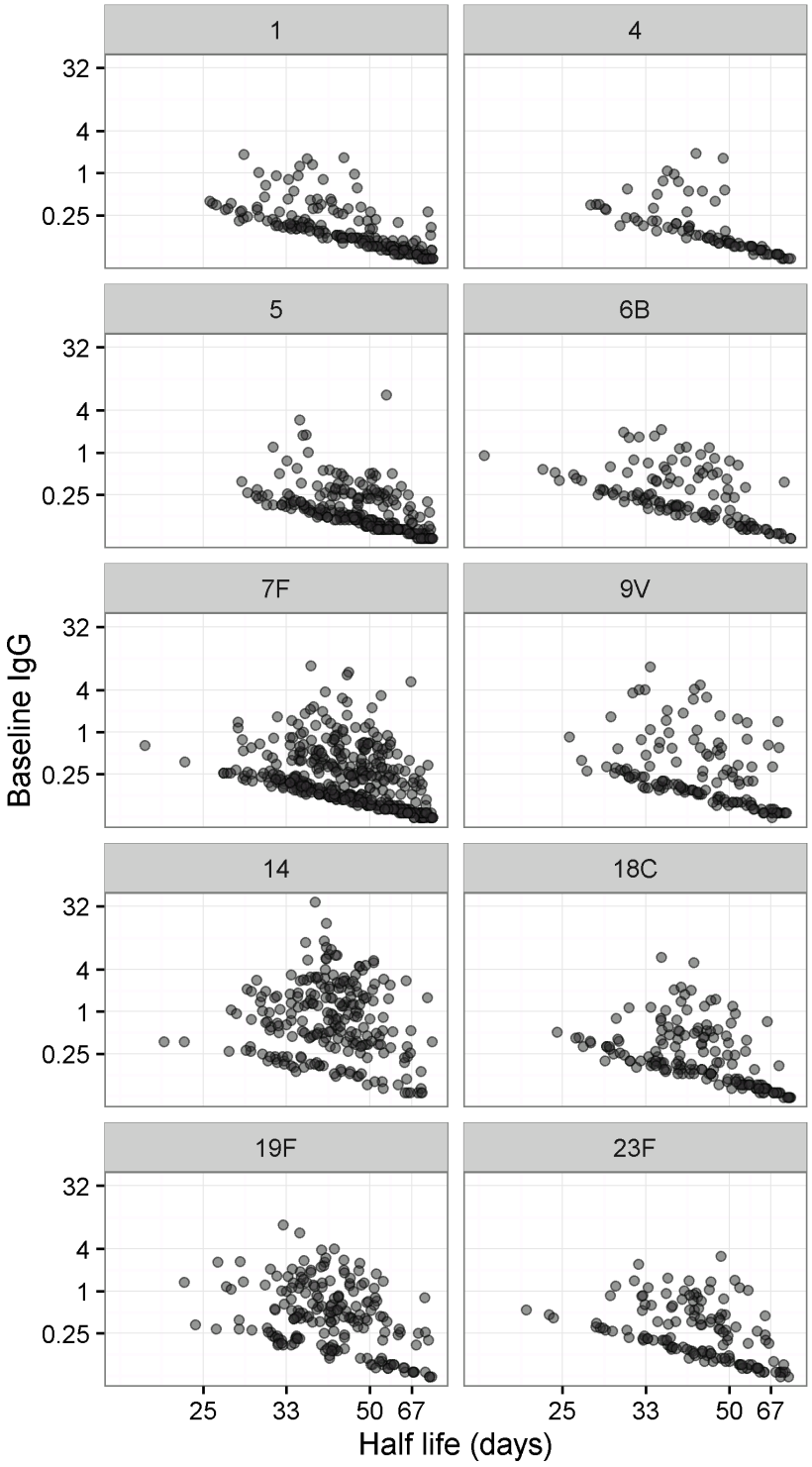
In addition, applying an arbitrary threshold excluding those with half-lives greater than 80 days, meant that if, for example, there were 100 days between

samples, then a child with antibody measured at the lower limit at the first time point (0.05) and below the LLQ at the second time point (substituted with 0.025) would always be excluded as their half-life was > 80 days. Children with low antibody levels to start with, could therefore only be included if there was a short period of time between samples. This truncation of the data at the LLQ can be seen in Figure 11 which plots the baseline antibody levels against the calculated half-life for each included individual and has a clear line of truncation. In general, those with larger half-lives had lower starting antibody levels thus a relationship exists between the baseline IgG and the half-life which is an artefact of the data rather than a biological mechanism. The effect of this relationship was explored by adjusting for baseline values in the overall model and the results are presented in Table 2.

Table 2 Sensitivity analysis of pneumococcal half-lives adjusting for baseline

Serotype	Unadjusted half-life estimates (95%CI)	Adjusted half-life for baseline IgG (95%CI)	Geometric mean IgG at baseline (95%CI)
1	46.54 (44.50, 48.78)	43.10 (41.68, 44.61)	0.15 (0.13, 0.17)
4	45.36 (42.44, 48.71)	42.50 (40.50, 44.70)	0.15 (0.13, 0.18)
5	48.35 (46.67, 50.16)	44.75 (43.30, 46.29)	0.15 (0.13, 0.16)
6B	38.68 (36.61, 41.00)	38.45 (36.77, 40.30)	0.25 (0.21, 0.29)
7F	44.65 (43.51, 45.85)	43.75 (42.75, 44.80)	0.22 (0.20, 0.24)
9V	42.07 (40.10, 44.24)	42.37 (40.61, 44.30)	0.26 (0.21, 0.31)
14	39.30 (37.78, 40.95)	45.52 (43.56, 47.65)	0.75 (0.63, 0.88)
18C	41.72 (39.74, 43.90)	41.40 (39.77, 43.18)	0.22 (0.19, 0.26)
19F	39.32 (37.63, 41.17)	42.47 (40.86, 44.21)	0.44 (0.38, 0.52)
23F	41.85 (39.77, 44.16)	41.91 (40.13, 43.86)	0.25 (0.22, 0.30)
OVERALL	**42.56 (41.51, 43.66)**	**42.54 (41.69, 43.42)**	

Figure 11 Bias due to truncation of antibody below the lower limit of quantification



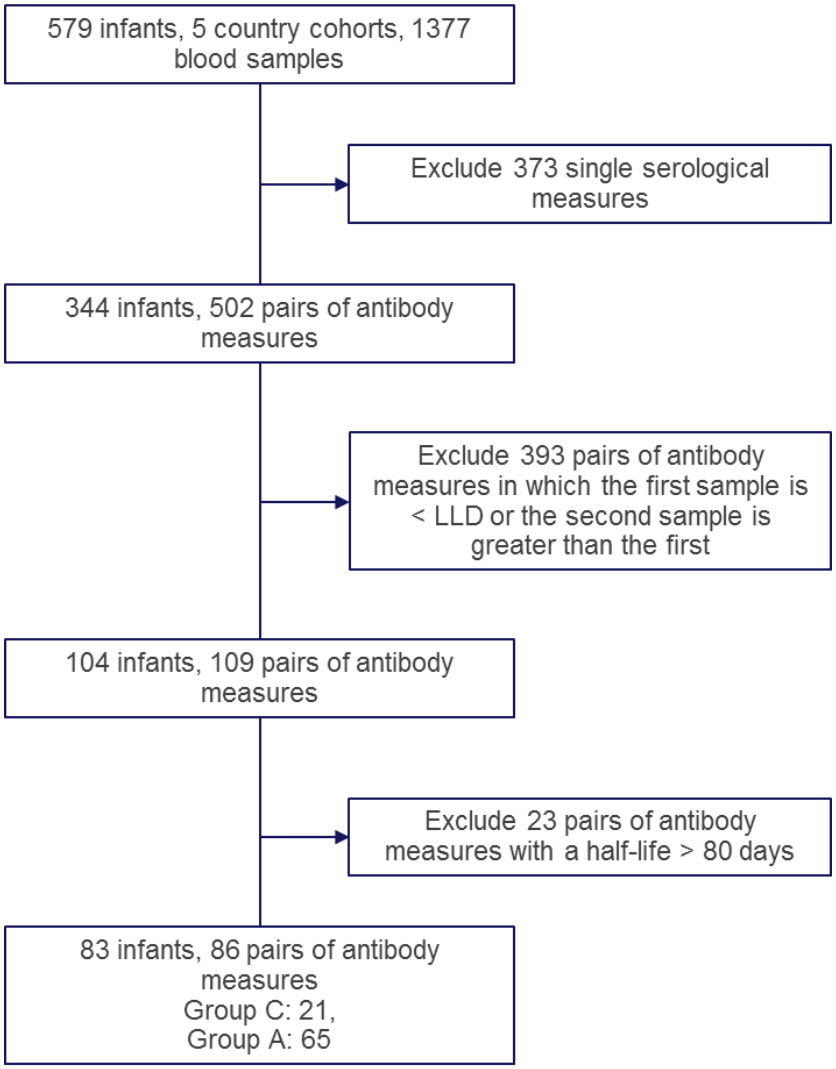
For serotypes with high antibody levels at baseline, adjusting for baseline values had the effect of inflating the half-life. Thus for serotype 14 and 19F the adjusted estimates were higher than the unadjusted estimates. For serotypes with the lowest baseline antibody levels (serotypes 1, 4 and 5) the adjusted estimates were deflated and were lower than unadjusted estimates (Table 2). The results of adjusted analyses appeared in some cases to be counter intuitive. For example, for serotype 4 in which the three country-specific estimates were 44, 45, and 46 days, the adjusted overall estimate was only 42.5 days.

Meningococcal antibody half-lives

For the estimation of the half-lives of group A and C maternal meningococcal antibody, 502 pairs of samples from 344 infants were available (group A: 206 infants; group C: 296 infants) (Figure 12).

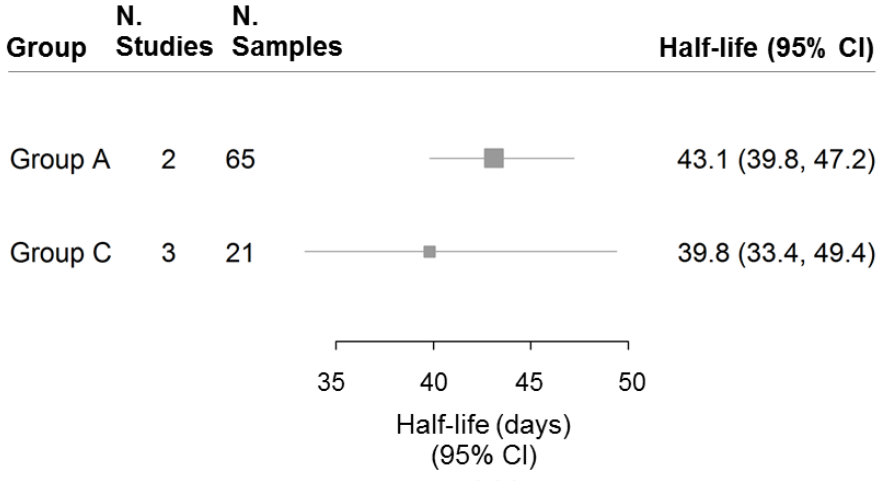
Half-lives were 43.1 days (95% CI 39.8–47.2) and 39.8 days (95% CI 33.4–49.4) for groups A and C respectively (Figure 13).

Figure 12 Flow chart of available data and reasons for exclusions from half-life estimations for meningococcal immunoglobulin G concentrations



LLQ: Lower limit of assay quantification

Figure 13 Individual participant data meta-analyses of capsular group-specific meningococcal antibody half-lives



4.4 DISCUSSION

These analyses reveal that for almost all antigens, maternal antibodies are present in some infants at the time of vaccination and that the proportion of children with such antibodies varies by country and by antigen. For pathogens such as diphtheria, which no longer circulate widely in many communities, maternal antibodies in infants likely indicate the extent of vaccination against diphtheria in these mothers over their life course. Maternal antibody seroprevalence in infants may therefore be a correlate of the level of vaccination against diphtheria in the community. Deaths due to diphtheria have been virtually eliminated in most high-income countries but the disease remains endemic in low-income countries.[66,67] Of note however, deaths due to diphtheria do still occasionally occur in high-income countries; one unvaccinated 6-year old boy died in Spain in 2015, the first occurrence since 1986, another child died in Belgium soon after, and a 2011 death due to diphtheria was reported in Australia.[66,68]

The seroprevalence of maternal tetanus antibodies is also an indicator of maternal vaccination history, however some countries such as the Philippines, vaccinate mothers during pregnancy with a tetanus toxoid vaccine.[3] This increases maternal seroprevalence of tetanus antibodies and protects infants against neonatal tetanus infection.

In contrast to diphtheria and tetanus, seroprevalence of maternal antibodies against the three pertussis antigens were similar across countries. This is likely due to the continuing circulation of pertussis world-wide resulting in high rates of exposure in adults and a subsequent antibody response of limited duration. The antibody response to both pertussis vaccination and to infection is short-lived and does not result in prolonged immunity.[69] This results in roughly 5-year cyclical pattern in disease incidence in most countries. Of note, the seroprevalence of FHA antibodies was far higher than seroprevalence of pertussis toxin antibodies in this analysis. Whilst antibodies against pertussis toxin are unique to infection with *B pertussis*, FHA antibodies can be induced by other species of *Bordetella,* resulting in increased seroprevalence of these antibodies.[70]

In these data substantial proportions of infants had antibodies to many vaccine serotypes of pneumococcus. Conversely, antibodies against capsular groups A and C meningococcal polysaccharides were less common, particularly for group C which was only present in 13% of infants in the five countries contained in this analysis. Passively acquired maternal antibody has been shown to adversely affect the magnitude of the immune response to vaccination with pneumococcal conjugate vaccine,[34,35] and increase the occurrence of otitis media in infants under 6 months of age.[71]

The proportion of young infants with maternal anti-pneumococcal antibody differed between serotypes, with serotype 14 pneumococcal antibodies being present in almost all infants, and very high proportions of infants with serotype 19F antibodies.

Antibody concentrations in infants are highly correlated with the concentration of serum antibody in mothers during pregnancy. However, antibody may also be transferred across the placenta during pregnancy at different rates. A higher rate of transplacental transmission for serotype 14 than for other serotypes has been reported in a study of 15 mother-infant pairs in Brazil[72] however this is not consistently seen.[73] In the larger study by Gupta *et al* of HIV uninfected mother-infant pairs in Bangladesh, the geometric mean cord:maternal antibody ratio was similar across all serotypes with confidence intervals overlapping for all. Similarly, in studies of meningococcal vaccines in mother-infant pairs, similar rates of transplacental transfer have been observed for groups A and C meningococcal antibodies in both unvaccinated and vaccinated mothers.[64,74] Differences in seroprevalence of antibodies between serotypes or between capsular groups in this analysis are therefore more likely to be due to serotype-specific differences in the magnitude of the antibody responses to nasopharyngeal carriage in mothers, and variation in the serotypes that circulate in any given community.

In this investigation, maternal pneumococcal antibody decayed exponentially with an average half-life of 42.6 days, however, variation between serotypes was statistically significant. The half-lives for serotypes 6B, 14, and 19F maternal antibodies were 3 to 4 days shorter, whilst half-lives for serotypes 1, 4, 5, and 7F were between 2 and 6 days longer than the overall average. Previous studies

have assumed the same rate of decay for passively-acquired antibody. Shahid et al in a study in 55 Bangladeshi mother-infant pairs estimated a half-life for maternal pneumococcal antibodies of about 35 days based on serotypes 19F and 6B.[63] Antibodies against these two serotypes had similar half-lives of 39 days in our analysis thus the reason for the shorter overall half-life reported in that study may be due to the choice of serotypes against which antibodies were measured. In constrast O'Dempsey et al describe a half-life of 20-30 days for antibodies against 6 serotypes of pneumococcus however they present no details of the methods used.[65]

The half-lives estimated for group A and C meningococcal antibodies of 43 and 40 days are comparable to the estimates for pneumococcal antibodies thus it is possible that passively-acquired anti-capsular antibodies for both species decay similarly. O'Dempsey et al describe a much lower half-life of 20 days for groups A and C maternal meningococcal antibodies.[64] It is unclear what factors may contribute to the much lower estimate in the O'Dempsey study, as the calculations are not described and the half-life is described as 'approximate'. However, of note is that mothers in that study were vaccinated in pregnancy with a conjugate vaccine, whereas in our study, no maternal vaccination programmes were in place.

Differences in distribution of IgG subclasses[75], Fc structure/Fc receptor binding[76], and glycosylation[77] of maternal antibodies against the different polysaccharides could potentially explain the variation in half-lives observed in the current study. However, of note is that longer half-life estimates were observed for the serotypes and capsular groups with a higher proportion of data below the LLQ at the second time point. Thus bias due to the LLQ is a contributing factor to the variation observed.

The trajectory of decay in these analyses of passively-acquired antibodies differs from the pattern of decay for antibodies induced by vaccination in infants. Vaccine-induced antibody decays rapidly in the first year after vaccination, after which time the rate of decay slows considerably as is not log-linear across all time points.[69]

Maternal prenatal vaccination programmes are currently recommended in many countries to protect infants against neonatal pertussis and tetanus. Other

vaccines aimed at protecting infants through prenatal immunisation are currently in development, such as those for RSV and group B streptococcus, and may be followed by introduction of infant programmes as well. For pneumococcal disease, currently licensed infant conjugate vaccines are effective, but cover at most, 13 serotypes. Immunisation during pregnancy with the 23-valent polysaccharide vaccine, which is immunogenic in adults but not infants, may extend the number of serotypes against which infants are protected in the first months of life.

Implementation of a prenatal maternal immunisation programme covering the same antigens as an infant immunisation programme, gives rise to the potential for reduced vaccine responses in infants owing to higher levels of maternal passively-acquired antibody. Implementing complementary prenatal and infant programmes against the same antigens requires an understanding of the complex interplay between the influence of passive antibody on infant responses, and its rate of clearance from the body, in order to time such vaccination programmes effectively. Accurate estimates of antibody half-lives are therefore required when planning such interventions.

Strengths, limitations, and sensitivity analyses

The strength of this analysis lies in the large number of paired sera available from a wide range of countries and multiple antigens and serotypes. Samples which may skew estimates upwards due to the influence of carriage on antibody responses were excluded. Carriage of meningococcus is rare in young infants, however the influence of carriage cannot be ruled out entirely. Antibody half-lives estimated in this investigation are from populations with no routine programmes of immunisation in pregnancy for pneumococcal or meningococcal diseases, thus passively-acquired antibody is due to community-acquired nasopharyngeal carriage or disease rather than immunisation and may differ from decay rates for passive antibodies induced by maternal vaccinations.

The trials from which these data derive were not conducted with the purpose of estimating half-lives and therefore some assumptions were made in the analysis which may have introduced bias.

When making exclusions from the analysis of half-lives for those whose antibody declined, we only included samples which gave a half-life of less than 80 days. The largest half-life was 926 days. 5% of paired samples had a half-life of 95.8 days or higher, and 10% of paired samples had a half-life of at least 80.8 days. The median half-life was 45.7 days (IQR 37 – 61 days).

This cut point retained 90% of the data from those whose antibody declined. It is not possible to confirm whether these excluded results were due to carriage events, nor whether the data included in the analysis is entirely free of bias from carriage, therefore exclusions due to long half-lives are of necessity arbitrary exclusions. This may bias results either upwards or downwards depending on how close to the 'truth' such exclusions were, resulting in half-lives a few days longer or shorter than reality.

The long time gap between blood samples is not ideal for the calculation of half-lives as the time delay allows for many participants to have antibody that has decayed below the LLQ by the time of the second sample. The substitution of half the LLQ for values below this level is an approximation which overestimates the half-life in these samples by at least 1 day as seen in the weighted sensitivity analysis which gave an overall half-life estimate that was one days shorter (41.5 days) than the main estimate (42.6 days). The sensitivity analysis used weights based on the proportion of samples above the LLQ at the second time point and thus places more emphasis on the half-lives estimated from serotypes which were less affected by this source of bias but does not remove it altogether. Every serotype has some samples below the LLQ at the second time point and so any estimate from these data will be at least slightly inflated. The weighted estimate of 41.5 days is likely closer to the truth, however a difference of 1 day is of little material importance.

An alternative analysis would be to exclude samples below the LLQ however further bias may be introduced by such restrictions as a very large proportion of samples would be removed, including those with fast decay rates.

There was a clear relationship between baseline antibody level and the antibody half-life that was due to the truncation of the data at the lower limit of the assay and this was further explored in sensitivity analyses. Estimates from baseline adjusted models represent the half-life for each serotype if all serotypes had the

same average baseline antibody levels. Thus serotypes with high baseline antibody concentrations, such as serotype 14 which was measured in three countries and had half-life estimates of 42, 37 and 42 days, had an unadjusted half-life of 39.3 days overall but an adjusted half-life of 45.5 days. The adjusted estimate was therefore counter-intuitive as it was higher than all individual country-specific estimates. The unadjusted estimate is an average of the 3 country-specific estimates (weighted by the proportion of data in each study) whereas the adjusted estimate was the average half-life if serotype 14 had baseline antibody similar to the average baseline antibody level. Adjustment for baseline values therefore did not remove the bias due to the truncation of data at the LLQ and instead produced non-intuitive results for individual serotypes even though the overall half-life remained the same.

4.5 CONCLUSION

Substantial proportions of infants in a wide variety of countries have maternally-derived antibodies to many serotypes of pneumococcus at the age when a vaccine programme might normally commence, however few infants have maternally acquired antibodies to meningococcus. Maternal antibodies to diphtheria, tetanus and polio vary substantially between countries, reflecting the variation in vaccination histories of mothers in different regions. In contrast, maternal antibodies to the three antigens in the pertussis vaccine varied little between countries, an indication of the widespread circulation of this infection world-wide.

Maternally-derived passive antibody decays exponentially. Passively-acquired antibody decays with a half-life of 6 weeks, in line with previously described half-lives for antibodies against polysaccharide and protein antigens. Small but significant differences exist between serotypes or capsular groups, however these amount to differences of only a few days. These estimates are useful for modelling the impact of vaccination programmes which utilise a combination of both maternal and infant strategies, and consideration of schedules with a delayed start.

5 THE INFLUENCE OF MATERNALLY-DERIVED ANTIBODY AND INFANT AGE AT VACCINATION ON INFANT VACCINE RESPONSES

The work contained in this chapter is published in;

> Voysey M, Kelly DF, Fanshawe TR, Sadarangani M, O'Brien KL, Perera R, Pollard AJ. *The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses: An Individual Participant Meta-analysis.* JAMA Pediatr. 2017; 171(7): 637-646. doi:10.1001/jamapediatrics.2017.0638.
>
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>
> *All text, analyses, tables, and figures were produced by Merryn Voysey. Co-authors have given permission to include the material in this thesis.*

This work was also presented in the following oral presentations:

1. *The impact of maternal antibody on infant vaccine responses: an analysis of immunity in 7630 children.* Oral poster presentation, European Society of Paediatric Infectious Diseases, Madrid, May 2017
2. *The impact of age at vaccination on infant vaccine responses: an analysis of immunity in 7630 children.* Oral poster presentation (plenary session) European Society of Paediatric Infectious Diseases, Madrid, May 2017.
3. *The Influence of Maternally Derived Antibody and Infant Age at Vaccination on Infant Vaccine Responses.* Invited speaker, ERC TransMID workshop on serology, Antwerp, May 2018.

5.1 BACKGROUND

Two key factors which can affect the immunogenicity of infant vaccines are a) the residual concentration of maternal placentally-transferred antigen-specific antibody at the time of immunisation, and b) the age at which immunisations are given.

Maternal antibody is transferred to an infant via the placenta during the third trimester of pregnancy and is important in providing protection against infection during the first months of life.[6,78,79] The amount of antibody transferred from mother to infant depends primarily on the gestational age at birth, placental function, and serum antibody concentration in the mother.[30,62] Following birth, the concentration of maternally derived antibody declines over weeks to months while infant immune competence gradually increases.[6]

High concentrations of maternally acquired antibody have been shown to inhibit the immune response to infant vaccination against some antigens.[29-36] However, due to the small size of most studies, there is inconsistent evidence on the extent of this inhibition and a lack of consensus regarding which antigens or vaccines are impacted. Additionally, the magnitude of the infant antibody response to vaccination is affected by the age of the infant, with older infants generally responding better to vaccines than younger infants.[6,80,81] Since transplacentally acquired antibody decays over time, the interference with vaccine responses reduces with age and observed age effects on vaccine responses may therefore be due to reduced maternal antibody interference. Previous studies have not been able to separate the influence of these interrelated components on the infant immune response leading to the observation that, "*multivariate analyses on a large number of infants are required to identify the main determinants of vaccine antibody responses*."[82]

Reduced response to infant vaccination is of importance in the context of prenatal immunisation programmes. Currently, vaccination with a pertussis-containing vaccine in the second or third trimester of pregnancy is recommended in many countries.[83-87] Maternal immunisation with diphtheria-tetanus-pertussis-polio vaccine during pregnancy effectively protects infants against pertussis disease in the first few months of life.[21,22] However, subsequent

diminished responses to infant vaccination may leave children more susceptible to disease in later infancy and increase transmission rates to unvaccinated cohorts.[36]

In this investigation serology data were combined from multiple randomised controlled trials to create a large cohort of infants vaccinated at various ages under different schedules, from whom sera were measured both pre- and one-month post-vaccination (Supplementary Table). The effects of pre-existing antibody and age at vaccination on antibody responses to priming and booster vaccines were calculated. From these results, the relationship between maternal antibody decay and maternal antibody interference was modelled to determine what magnitude of delay in the first infant vaccination might offset the maternal inhibition of infant antibody responses. Whether maternal antibody inhibition was less pronounced in schedules with a wider spacing of doses was also determined.

5.2 METHODS

Outcomes

The outcomes for analysis were antigen-specific immunoglobulin G (IgG) concentrations measured at one-month after the third priming dose (aged ~5-7 months), and pre- and one month post- booster vaccination.

Analysis

Antibody concentrations were $\log_2$-transformed and analysed using mixed effects models for each antigen and time point separately. Infants were included if antibody concentrations were available at both the pre-vaccination baseline visit (where antibody was assumed to be maternal in origin) and the one-month post-vaccination timepoint. Children who received a placebo or no vaccine were excluded. Maternal antibody was included in models as a continuous covariate (model 1), and for comparison, sensitivity analyses were conducted with maternal antibody as a binary variable (antibody classified as above the lower limit of assay detection or not) (model 2). Mixed effects models contained country-cohort-level random intercepts, and fixed effects for age at first

vaccination (months). Additional parameters known to influence antibody responses (e.g. sex, vaccine received, and the scheduled spacing of doses) were included as fixed effects in models to account for their influence as 'nuisance' parameters.

The anti-log of the coefficients from models, and their 95% CIs, are presented as geometric mean ratios (GMR). These are interpreted as the relative increase (fold-rise) in antibody response that is associated with a one-unit change in the given covariate. For age at vaccination, the GMR is the relative increase in antibody response for a one-month older child. For $\log_2$-transformed maternal antibody concentrations, the GMR is the relative increase in antibody response associated with a doubling in maternal antibody concentration.

In a secondary analysis, the effect of maternal antibody to related carrier proteins on antibody responses to pneumococcal and Hib vaccines was conducted using a similar model to model 1 above. Maternal antibody to the homologous antigen (pneumococcus or Hib) was included in the model along with the maternal antibody concentration for the carrier proteins (diphtheria and tetanus) to assess the effect of pre-existing antibody to related carrier proteins whilst simultaneously adjusting for the effect of pre-existing antibody to the homologous antigen.

Subgroup analyses were conducted for subsets of trials in which vaccines were administered at 2, 4, and 6 months, or 2, 3, and 4 months of age. These models were unadjusted for the schedule of administration as all children in the subset received the same vaccine schedule.

In each model, fixed effects were removed if a factor had only one level, or if there were too few levels for model stability (e.g. if all included studies administered the same vaccine type or the same schedule).

Effect of delaying first vaccination

Output from mixed effect models described above were used to model the effect of delaying vaccination. The antibody half-life was used to estimate the extent of decay in maternal antibody concentrations per week delay, and the subsequent reduction in maternal antibody interference that would result was determined

from the model assessing maternal antibody interference. The impact of reduced maternal antibody interference was combined with the effect of increased responses due to being older when first immunised to estimate the total benefit in increased responses which could be expected with each week of delay in vaccination.

The number of weeks delay in infant vaccination required to offset the interference due to increased infant levels of maternal antibody was therefore calculated as follows;

$$w = \frac{-\hat{\beta}_1 \cdot \mathrm{X}_1}{(-\hat{\beta}_1 \cdot p) + \hat{\beta}_2}$$

where;

w = the number of weeks delay in the start of an infant immunisation programme that would offset the effect of maternal antibody interference due to higher levels of maternal antibody (e.g. due to the introduction of a maternal prenatal vaccination programme)

X_1 = $\log_2$-increase in infant levels of maternal antibody due to prenatal vaccination (hypothetical values ranging from no increase to a 20-fold increase)

$\hat{\beta}_1$ = $\log_2$-estimate of the effect of maternal antibody on infant vaccine responses (per 2-fold increase) from model 1, (Table 5).

$\hat{\beta}_2$ = $\log_2$-estimate of the effect of age per week, scaled from model 1, Table 5 by dividing the coefficient by 4.333 (weeks per month).

p = one week expressed as a proportion of the antibody half-life (7/d where d=half-life in days)

Thus to estimate the number of weeks (w) delay in vaccination required to offset e.g. the effect of a 5-fold increase in pertussis antibody concentration in infants due to a prenatal vaccination programme, we calculate:

$X_1 = \log_2(5)$,

$\hat{\beta}_1 = \log_2(0.886) = -0.1746$, (from Table 5)

$\hat{\beta}_2 = \log_2(1.150)/4.333 = 0.0465$, (from Table 5)

$p = 7/36 = 0.1944$, (using estimated half-life of pertussis: 36 days)

$$w = \frac{0.1746 \cdot log_2(5)}{\left(0.1746 \cdot \left(\frac{7}{36}\right)\right) + 0.0465} = 5.04\ weeks$$

As antibody half-lives for diphtheria, tetanus, and pertussis toxoids are not well characterised in the published literature, we calculated estimates based on half-lives of 7 days longer and 7 days shorter to give a range of estimates. For pneumococcal serotypes, the half-life estimates computed in Chapter 4 were used.

A list of all assays and assay thresholds is included in Table 3.

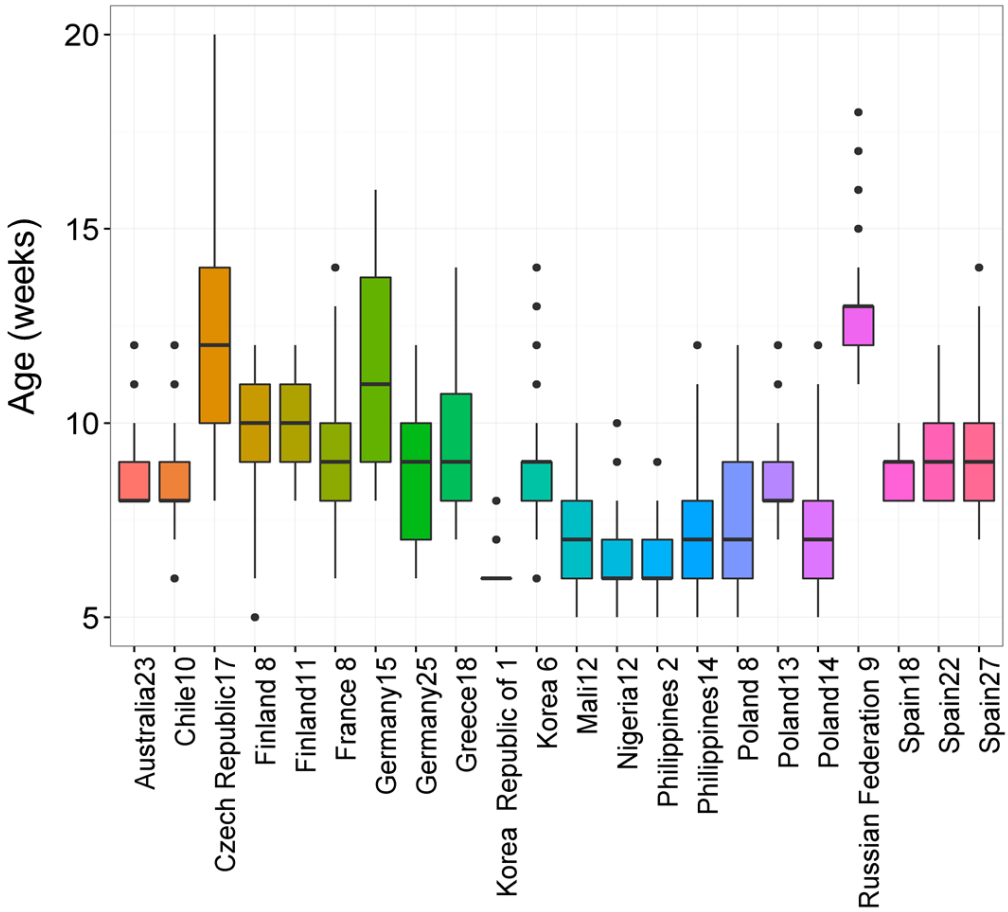
Table 3 Laboratory assays

Antigen	*Assay Method*	*Assay Unit*	*Assay lower limit of detection*
Diphtheria toxoid	ELISA	IU/mL	0.1
Tetanus toxoid	ELISA	IU/mL	0.1
Pertussis toxoid	ELISA	EL.U/mL	5
Filamentous haemagglutinin	ELISA	EL.U/mL	5
Pertactin	ELISA	EL.U/mL	5
Polio neutralising antibody	Micro-neutralisation test	Dilution for 50% neutralisation	8
Polyribosylribitol phosphate	ELISA	µg/mL	0.15
Hepatitis b surface antigen	ELISA	mIU/mL	10
Pneumococcal serotypes	ELISA	µg/mL	0.05
Group C meningococcus	ELISA	µg/mL	0.30

5.3 RESULTS

Antibody data were available for 21 antigens from 19 child cohorts (7630 infants) from 32 studies in 17 countries (Supplementary Table). Children from different studies varied in age at the time of enrolment resulting in a total age range of 5 to 20 weeks (Figure 14). Children received vaccines according to one of four schedules including: 6, 10, 14 weeks; 2, 3, 4 months; 2, 4, 6 months, or 3, 4, 5 months. Studies were conducted in Europe (Spain, France, Germany, Greece, Poland, Czech Republic, Finland), Africa (Mali, Nigeria), Latin America (Chile, Nicaragua, Argentina, Dominican Republic), East Asia (The Philippines, Korea), Russia, and Australia.

Figure 14 Age in weeks at study enrolment for each country cohort



The geometric mean antibody concentrations at one month after infant priming doses are displayed for diphtheria, tetanus, pertussis toxoid, and PRP for each country cohort in Table 4 according to whether or not an infant had detectable levels of maternal antibody at the time of vaccination. The geometric mean antibody levels post-vaccination in children who had pre-existing maternal antibody, was lower than in those who did not have pre-existing antibody in many cohorts. For tetanus 10/16 cohorts had non-overlapping confidence intervals for those who did and did not have maternal antibody at the time of their first vaccination. Diphtheria and pertussis toxoid results were similar with 11/17 and 9/17 cohorts having non-overlapping confidence intervals respectively. For PRP antibodies the pattern was different with no significant differences between those with and without pre-existing antibodies for any cohort.

Table 4 Antibody concentration at one-month post priming, in those who did and did not have detectable levels of maternal antibody prior to immunisation

Country cohort	Tetanus (IU/mL)		Diphtheria (IU/mL)		Pertussis toxoid (EL.U/mL)		PRP (µg/mL)	
	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies
	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)
Korea Republic of 1	6.7 (5.6, 8.0)	3.3 (1.8, 6.0)	3.5 (2.9, 4.2)	3.9 (1.7, 8.6)	75.2 (65.3, 86.6)	63.1 (47.7, 83.5)		
Philippines 2			1.2 (1.17, 1.3)	0.6 (0.5, 0.8)				
Korea 6	9.1 (8.6, 9.7)	4.3 (3.6, 5.2)	3.3 (3.1, 3.6)	2.9 (1.9, 4.3)	60.2 (56.6, 64.0)	53.5 (45.8, 62.6)		
Finland 8	3.4 (1.9, 6.2)	2.2 (2.1, 2.3)	2.1 (1.9, 2.3)	0.8 (0.7, 0.9)	61.6 (58.2, 65.3)	44.4 (39.6, 49.9)	2.2 (1.8, 2.6)	1.6 (1.3, 1.9)
France 8	4.7 (2.6, 8.5)	1.7 (1.5, 2.0)	3.6 (2.2, 5.8)	0.9 (0.7, 1.0)	41.0 (35.6, 47.2)	37.1 (28.0, 49.1)	2.0 (1.3, 3.0)	2.4 (1.6, 3.6)
Poland 8	2.9 (2.2, 3.8)	1.8 (1.7, 2)	2.1 (1.9, 2.4)	0.9 (0.8, 1.1)	51.3 (47.3, 55.7)	29.6 (25.8, 33.8)	2.1 (1.7, 2.6)	1.7 (1.4, 2.1)
Russian Federation 9					76.0 (70.9, 81.4)	66.6 (56.4, 78.7)		
Chile10	8.0 (7.1, 9.0)	4.6 (3.3, 6.4)	4.7 (4.2, 5.3)	3.7 (2.2, 6.3)	84.1 (73.5, 96.2)	67.4 (50.5, 90.0)	15.0 (11.3, 19.9)	9.9 (7.2, 13.5)
Finland11	2.7 (1.6, 4.3)	1.5 (1.5, 1.6)	1.1 (0.9, 1.2)	0.4 (0.4, 0.5)	63 (59.5, 66.8)	44.8 (40.5, 49.5)		
Poland13					40.4 (37.9, 43.1)	31.1 (27.7, 34.9)	2.0 (1.6, 2.4)	2.1 (1.8, 2.6)
Philippines14	8.5 (6.8, 10.7)	4.1 (3.6, 4.7)	1.6 (1.3, 1.8)	1.63 (1.3, 2.1)			25.2 (20.0, 31.8)	28.2 (21.6, 36.8)
Poland14	3.5 (2.4, 5.2)	3.3 (2.9, 3.7)	1.7 (1.5, 2.0)	1.2 (1.0, 1.4)			8.8 (7.2, 10.7)	9.5 (7.7, 11.8)
Germany15	2.9 (2.4, 3.4)	1.3 (1.2, 1.4)	2.5 (2.2, 2.7)	1.0 (0.9, 1.2)	50.5 (46.6, 54.7)	38.6 (33.3, 44.8)	1.9 (1.6, 2.3)	2.0 (1.5, 2.7)
Czech Republic17	4.6 (3.9, 5.4)	1.8 (1.7, 2.0)	3.2 (3.0, 3.4)	1.4 (1.0, 2.1)	42.4 (40.1, 44.8)	28.6 (24.2, 33.9)	3.5 (2.9, 4.1)	2.8 (2.4, 3.4)
Greece18	9.8 (7.1, 13.5)	6.1 (4.8, 7.9)	4.7 (3.3, 6.8)	1.2 (0.4, 3.4)	54.2 (41.3, 71.2)	50.6 (1.5, 1680)	9.0 (3.1, 26.0)	8.8 (2.0, 39.1)

Country cohort	Tetanus (IU/mL)		Diphtheria (IU/mL)		Pertussis toxoid (EL.U/mL)		PRP (µg/mL)	
	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies	No maternal antibodies	Maternal antibodies
	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)	GMC (95%CI)
Spain18	10.4 (5.3, 20.7)	7.5 (6.4, 8.8)	3.6 (3.0, 4.4)	1.4 (0.5, 3.9)	48.7 (39.0, 60.7)	31.9 (18.7, 54.3)	7.4 (4.8, 11.5)	6.1 (3.0, 12.8)
Spain22	4.0 (3.6, 4.4)	1.6 (1.5, 1.7)	6.4 (6.0, 6.9)	3.2 (2.5, 4.0)	58.5 (55.0, 62.2)	47.4 (43.0, 52.3)	4.7 (4.0, 5.5)	4.1 (3.2, 5.2)
Australia23	3.4 (2.8, 4.1)	2.1 (1.83, 2.2)	2.2 (2.0, 2.5)	0.9 (0.8, 1.1)	65.8 (60, 72.1)	61.1 (53.4, 70.0)		
Germany25	5.6 (4.7, 6.8)	2.1 (1.9, 2.3)	1.8 (1.6, 2.0)	0.6 (0.5, 0.7)	68.7 (64.5, 73.2)	46.1 (40.0, 53.1)	2.6 (2.1, 3.2)	2.4 (1.9, 3.1)
Spain27					64.2 (60.2, 68.4)	49.4 (45.5, 53.8)		

GMC = Geometric mean concentration; CI=confidence interval; IU: International units; EL.U: ELISA units. Blue highlighted estimates have non-overlapping confidence intervals. Not all trials included in the study are shown here – only trials with antibodies measured for the displayed antigens.

Effect of pre-existing homologous antibody on responses to a priming series of immunisations

In mixed effects models, with pre-existing antibody in the infant modelled as a continuous covariate (model 1), maternal antibody inhibited the response to a priming series of vaccinations for 20 of the 21 measured antigens – the exception being serotype 7F pneumococcal polysaccharide (Figure 15). A similar result was obtained with pre-existing antibody modelled as a binary variable with reduced antibody response seen for 18 of the 21 antigens assessed – the exceptions being antibody responses to serotype 7F and 4 pneumococcal polysaccharides, and *Haemophilus influenzae* type b polysaccharide capsule (polyribosylribitol phosphate - PRP) (Table 5). The largest effects were observed for inactivated polio vaccine responses where 2-fold higher pre-existing antibody resulted in 20–28% lower post-vaccination antibody (GMRs ranging from 0.72 to 0.80) (Figure 15, Table 5). When pre-existing antibody was modelled as a binary variable, infant seropositivity to polio vaccines was associated with a 36–60% decrease in post-vaccine responses (Table 5).

For acellular pertussis antigens (pertussis toxoid: PT, filamentous hemagglutinin: FHA, and pertactin: PRN), 2-fold higher pre-existing antibody was associated with 11% lower post-vaccination antibody for PT and FHA (GMRs both 0.89), and 22% lower PRN antibody (GMR 0.78, 95%CI 0.77–0.80). For tetanus and diphtheria these estimates were 13% and 24% respectively (Figure 15, Table 5).

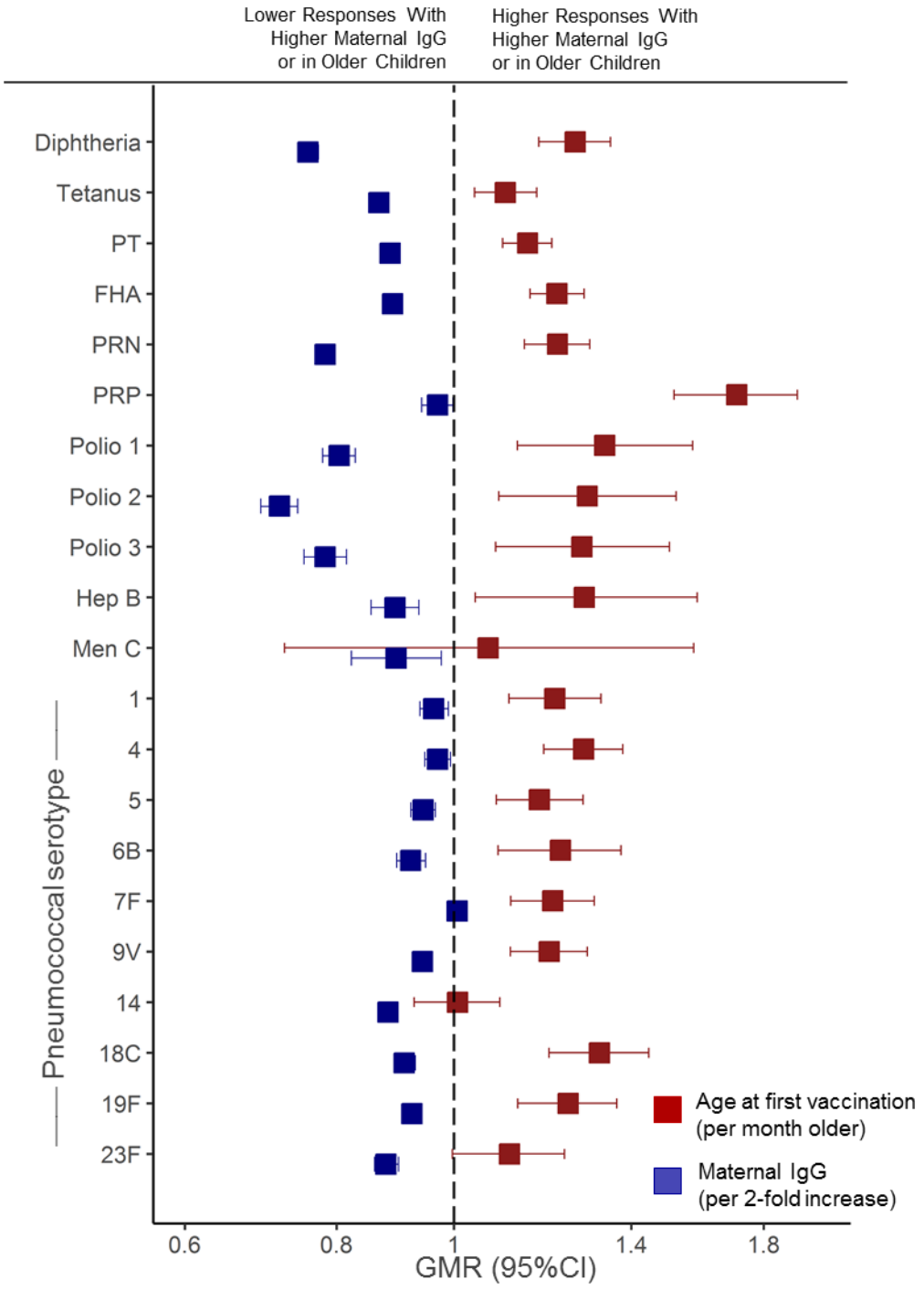
In the binary analysis, infants who were seropositive for pertussis antigens had 23% lower post-vaccination PT antibody, 27% lower FHA antibody, and 50% lower PRN antibody. For both tetanus and diphtheria, seropositive infants had 53% lower post-vaccine antibody (Table 5).

The effect of age at first vaccination on response to priming immunisations

For antibodies against 18 out of the 21 antigens studied, children who were older when initially immunised had between 10% and 71% higher post-vaccination antibody per additional month of age (Figure 15). These effects remained after

adjusting for pre-existing antibody. The largest effect was seen for PRP antibody where responses were 71% higher per month (GMR 1.71, 95%CI 1.52–1.92). No effect of age at first vaccination was seen for capsular group C meningococcal antibodies, however this may be due to the trials included for the Men C analysis having only enrolled children within a narrow age range of 8-12 weeks. Serotypes 14 and 23F pneumococcal antibodies were also non-significant. (Figure 15, Table 5: model 1).

Figure 15 Influence of age at first vaccination and pre-existing antibody concentration prior to vaccination, on antibody concentration post-3rd priming dose (age ~5-7 months).



GMR: Geometric mean ratio; IgG: immunoglobulin G; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate; Men C: group C meningococcus; Hepatitis B; Data are displayed in Table 5.

Table 5 Influence of age at first vaccination and pre-existing antibody concentration prior to vaccination, on antibody concentration post-3rd priming dose (age 5~7 months)

		Model 1						Model 2 (sensitivity analysis)					
		Age at first vaccination (per month older)			IgG pre-vaccination (per 2-fold increase)			Age at first vaccination (per month older)			IgG pre-vaccination (> LLD)		
Antigen	**N**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**
Diphtheria	4897	1.259	1.175	1.346	0.758	0.744	0.773	1.285	1.198	1.374	0.472	0.445	0.500
Tetanus	4188	1.102	1.040	1.170	0.867	0.856	0.879	1.156	1.092	1.224	0.469	0.441	0.499
Pertussis toxoid	4941	1.150	1.097	1.204	0.886	0.872	0.901	1.152	1.100	1.207	0.771	0.741	0.801
FHA	4952	1.216	1.155	1.280	0.890	0.879	0.900	1.255	1.191	1.321	0.734	0.704	0.764
PRN	4778	1.217	1.143	1.294	0.783	0.770	0.797	1.222	1.149	1.299	0.498	0.474	0.522
PRP	3369	1.710	1.519	1.919	0.969	0.940	0.998	1.719	1.527	1.928	0.832	0.849	1.025
Polio type 1	1699	1.331	1.128	1.573	0.804	0.779	0.829	1.440	1.215	1.709	0.568	0.498	0.648
Polio type 2	1699	1.288	1.089	1.525	0.718	0.693	0.743	1.395	1.171	1.662	0.400	0.351	0.457
Polio type 3	1699	1.275	1.082	1.505	0.783	0.752	0.815	1.321	1.115	1.566	0.636	0.552	0.733
Hepatitis B	1180	1.281	1.041	1.587	0.894	0.854	0.935	1.286	1.045	1.592	0.521	0.407	0.665
Group C Meningococcal IgG	1082	1.067	0.725	1.576	0.896	0.823	0.976	1.077	0.732	1.592	0.760	0.591	0.978
Pneumococcal serotype-specific IgG													
1	2387	1.211	1.110	1.322	0.962	0.937	0.989	1.217	1.116	1.328	0.924	0.857	0.995
4	3057	1.279	1.186	1.378	0.969	0.946	0.993	1.281	1.188	1.380	0.934	0.874	1.001
5	2387	1.176	1.084	1.278	0.943	0.921	0.965	1.193	1.100	1.297	0.926	0.868	0.989
6B	3057	1.224	1.087	1.373	0.921	0.897	0.947	1.239	1.100	1.390	0.787	0.716	0.868

		Model 1						Model 2 (sensitivity analysis)					
		Age at first vaccination (per month older)			IgG pre-vaccination (per 2-fold increase)			Age at first vaccination (per month older)			IgG pre-vaccination (> LLD)		
Antigen	**N**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**	**GMR**	**LCL**	**UCL**
7F	2387	1.206	1.114	1.305	1.006	0.989	1.024	1.206	1.115	1.306	1.028	0.965	1.098
9V	3057	1.198	1.113	1.288	0.942	0.925	0.959	1.214	1.127	1.305	0.892	0.839	0.950
14	3057	1.007	0.927	1.091	0.882	0.869	0.896	1.070	0.982	1.162	0.747	0.659	0.847
18C	3057	1.318	1.198	1.447	0.910	0.892	0.929	1.367	1.241	1.500	0.811	0.748	0.879
19F	3057	1.242	1.129	1.362	0.923	0.906	0.940	1.287	1.168	1.411	0.839	0.758	0.928
23F	3057	1.111	0.997	1.233	0.879	0.860	0.900	1.141	1.022	1.268	0.734	0.673	0.803

GMR: Geometric mean ratio; IgG: immunoglobulin G; LLD: lower limit of assay detection; LCL: lower limit of 95% confidence interval; UCL: Upper limit of 95% confidence interval; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate; Men C: capsular group C meningococcus; Hepatitis B; N=number of children with sera included in the analysis.

For age at first vaccination, GMRs greater than 1.0 indicate children who are older at the time of their first vaccination have higher antibody concentrations post-3rd dose. For maternal antibody, GMRs greater than 1.0 indicate that children with higher antibody have higher antibody concentrations post-3rd dose.

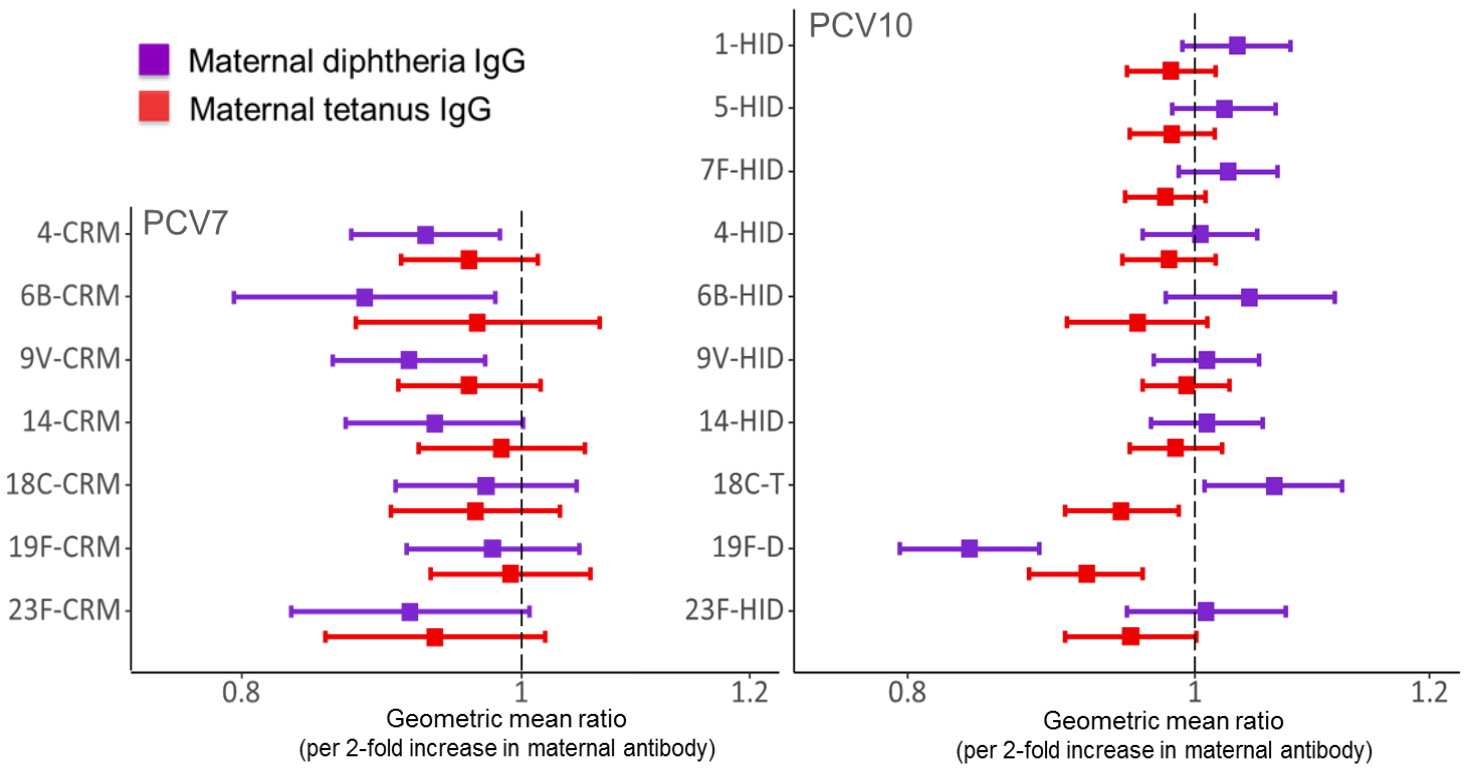
Numbers in blue are confidence intervals that do not contain 1.0.

Effect of pre-existing antibody to carrier proteins on responses to a priming series of immunisations with protein-polysaccharide conjugate vaccines

In an analysis of a subset of participants who received pneumococcal vaccines (PCV7 N=466, PCV10 N=1457), the effect of pre-existing antibody to related carrier proteins (diphtheria and tetanus) was assessed simultaneously whilst also adjusting for pre-existing pneumococcal antibodies. For PCV7 vaccine, conjugated to diphtheria cross-reacting material (CRM), pre-existing diphtheria antibodies were associated with reduced responses to serotypes 4, 6B, and 9V pneumococcal polysaccharides (GMRs 0.93, 0.88, 0.91), whereas pre-existing tetanus antibodies had no effect. In contrast, in PCV10 vaccine recipients, pre-existing diphtheria antibodies were associated with reduced responses to serotype 19F antibodies only (the only serotype conjugated to diphtheria protein) (GMR 0.84, 95%CI 0.80–0.89), whereas pre-existing tetanus antibodies affected both serotype 18C and 19F pneumococcal antibodies (18C conjugated to tetanus protein) (GMR 0.94, 95%CI 0.91–0.99; and 0.92, 95%CI 0.88–0.96 respectively) (Figure 16,Table 6).

Similarly, in recipients of Hib vaccine conjugated to tetanus toxoid, a small degree of inhibition of PRP responses was associated with pre-existing PRP antibodies (GMR: 0.96; 95% CI 0.93–0.99), and pre-existing tetanus antibodies (GMR: 0.97; 95% CI: 0.94–0.996). Pre-existing diphtheria antibodies had no influence on PRP responses (data not shown).

Figure 16 The influence of maternal diphtheria and tetanus antibodies on pneumococcal antibody concentration post-3rd priming dose (age ~5-7 months)



Data are displayed in Table 6. CRM: diphtheria cross reacting material; HID-Non typeable Haemophilus influenzae protein D; D: Diphtheria toxoid; T-Tetanus toxoid; GMR: Geometric mean ratio per 2-fold increase in pre-existing antibody concentration.

Estimates are from mixed effects models for each serotype with country-cohort-level random intercepts and adjusted for sex and age at first vaccination. The dependent variable is the log2-transformed serotype-specific pneumococcal antibody measured one-month post-priming, and the independent variables are the maternal antibody to the same serotype of pneumococcus, maternal diphtheria IgG, and maternal tetanus IgG.

Table 6 Influence of pre-existing pneumococcal, diphtheria and tetanus antibody concentrations prior to vaccination, on pneumococcal antibody concentration post-3rd priming dose (age ~5-7 months)

Number PCV7 recipients	Serotype–	Pre-existing pneumococcal antibodies			Pre-existing diphtheria antibodies			Pre-existing tetanus antibodies		
N	carrier protein	GMR	LCL	UCL	GMR	LCL	UCL	GMR	LCL	UCL
464	4–CRM	0.909	0.858	0.962	0.926	0.873	0.983	0.959	0.908	1.013
463	6B–CRM	0.887	0.824	0.955	0.882	0.795	0.979	0.965	0.876	1.064
465	9V–CRM	0.905	0.864	0.947	0.914	0.860	0.971	0.959	0.906	1.015
466	14–CRM	0.854	0.819	0.891	0.933	0.869	1.001	0.984	0.921	1.052
465	18C–CRM	0.941	0.893	0.993	0.972	0.904	1.045	0.964	0.901	1.031
466	19F–CRM	0.915	0.876	0.956	0.977	0.912	1.047	0.991	0.930	1.056
465	23F–CRM	0.833	0.780	0.889	0.915	0.832	1.006	0.933	0.855	1.019
Number PCV10 recipients										
1451	1–HID	0.974	0.933	1.016	1.033	0.990	1.077	0.981	0.948	1.016
1454	4–HID	1.035	0.988	1.084	1.004	0.960	1.049	0.980	0.945	1.016
1456	5–HID	0.941	0.907	0.975	1.023	0.982	1.064	0.982	0.950	1.015
1450	6B–HID	0.942	0.901	0.985	1.043	0.977	1.114	0.956	0.905	1.009
1459	7F–HID	1.014	0.988	1.040	1.026	0.987	1.066	0.977	0.947	1.008
1451	9V–HID	0.955	0.926	0.986	1.009	0.968	1.051	0.993	0.960	1.027
1454	14–HID	0.916	0.895	0.938	1.009	0.966	1.054	0.985	0.950	1.021
1457	18C–T	0.913	0.883	0.945	1.063	1.007	1.121	0.944	0.904	0.987
1457	19F–D	0.945	0.916	0.975	0.839	0.795	0.886	0.919	0.879	0.960
1451	23F - HID	0.903	0.869	0.938	1.008	0.948	1.073	0.951	0.904	1.001

CRM: diphtheria cross reacting material; HID-Non typeable Haemophilus influenzae protein D; D: Diphtheria toxoid; T-Tetanus toxoid; GMR: Geometric mean ratio per 2-fold increase in pre-existing antibody concentration; LCL: lower limit of 95% confidence interval; UCL: Upper limit of 95% confidence interval.

Each line represents output from a single mixed effects model with country-cohort-level random intercepts and adjusted for sex and age at first vaccination. The dependent variable is the log2-transformed serotype-specific pneumococcal antibody measured one-month post-priming, and the independent variables are the three measures of maternal antibody to the same serotype of pneumococcus, and diphtheria and tetanus antigens.

Numbers in blue are confidence intervals that do not contain 1.0.

Scheduled spacing of doses

Twelve studies were conducted using a 2, 4, 6 month schedule, and 3 studies used a 2, 3, 4 month schedule. When models were fitted within these subgroups separately, the effect of maternal antibody in terms of proportionate reduction in post-vaccination responses was similar for both schedules (Figure 17).

Persistence of antibody at pre-booster time points (age 12–24 months)

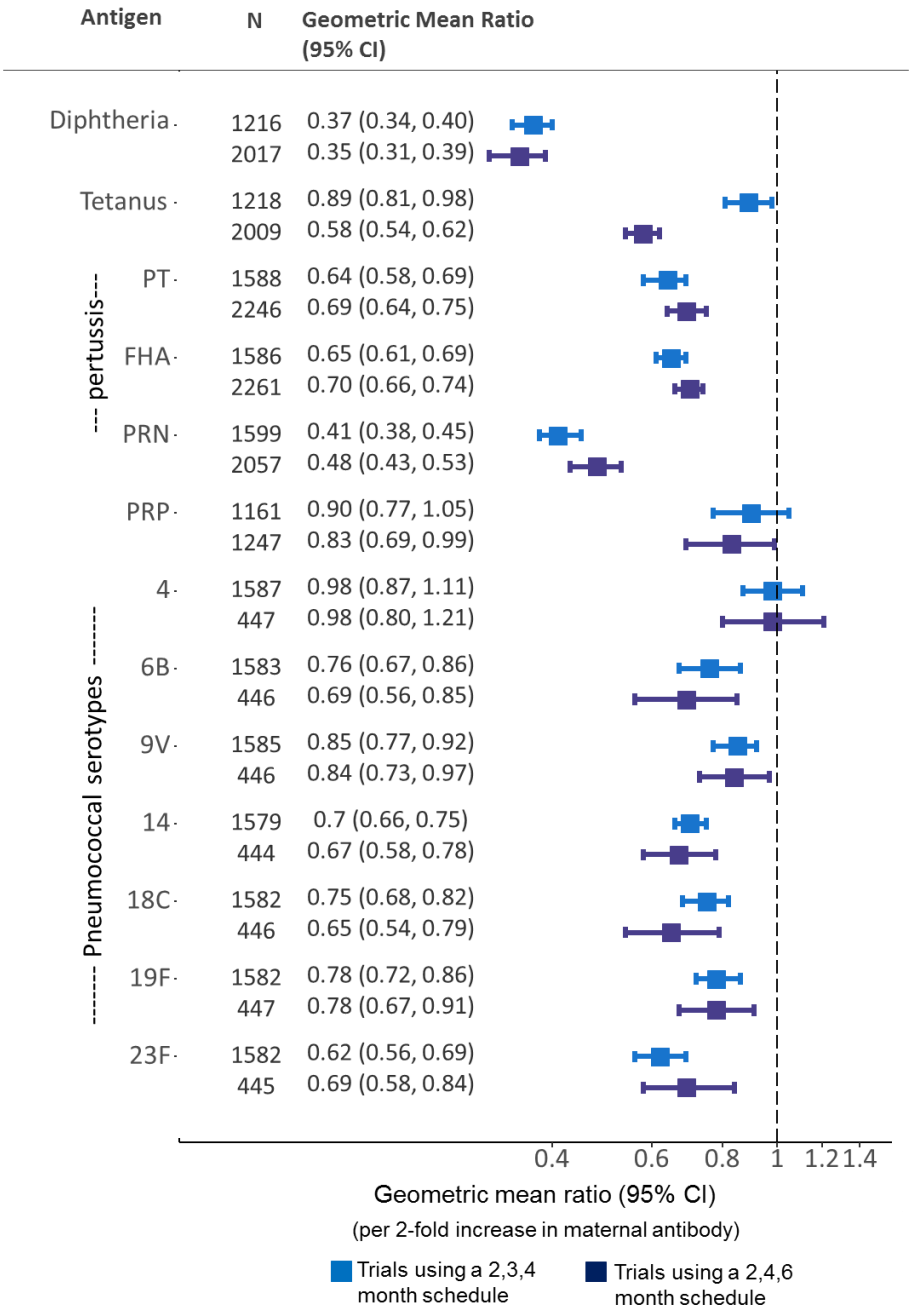
A subset of 12 trials administered booster vaccinations between 12 and 24 months of age. At pre-booster time points lower responses associated with pre-existing maternal antibody at the time of the first vaccination, were still evident for 16 of the 20 antigens. Age at first vaccination also had a significant effect on antibody levels prior to booster vaccination for all antigens except tetanus and serotype 23F pneumococcal antibodies (Figure 18 and Table 7)

Antibody concentration after booster vaccines

The concentration of maternal antibody prior to the first vaccination was associated with reduced responses to booster doses for diphtheria, pertussis antigens, inactivated polio and 5/10 (50%) serotypes of pneumococcus (Figure 19 and Table 8).

For diphtheria, pertussis antigens PT and FHA, inactivated polio and 5/10 (50%) serotypes of pneumococcus, the age at first vaccination had a persistent positive effect on post-booster antibody levels. The largest differences were observed for diphtheria and pneumococcal serotypes 5 and 18C, where antibody levels were between 28% and 32% higher for each month of delay in the initial vaccination. In contrast, the age at booster vaccination was associated with higher antibody only for tetanus and PRP antibodies (9% and 15% increase per month respectively), and associated with lower antibody post-booster for polio (5–6% lower per month) (Figure 19 and Table 8).

Figure 17 Effect of pre-existing antibody at the time of vaccination, on post-3rd dose antibody concentrations (age ~5–7 months), under 2, 3, 4 month schedules and 2, 4, 6 month schedules

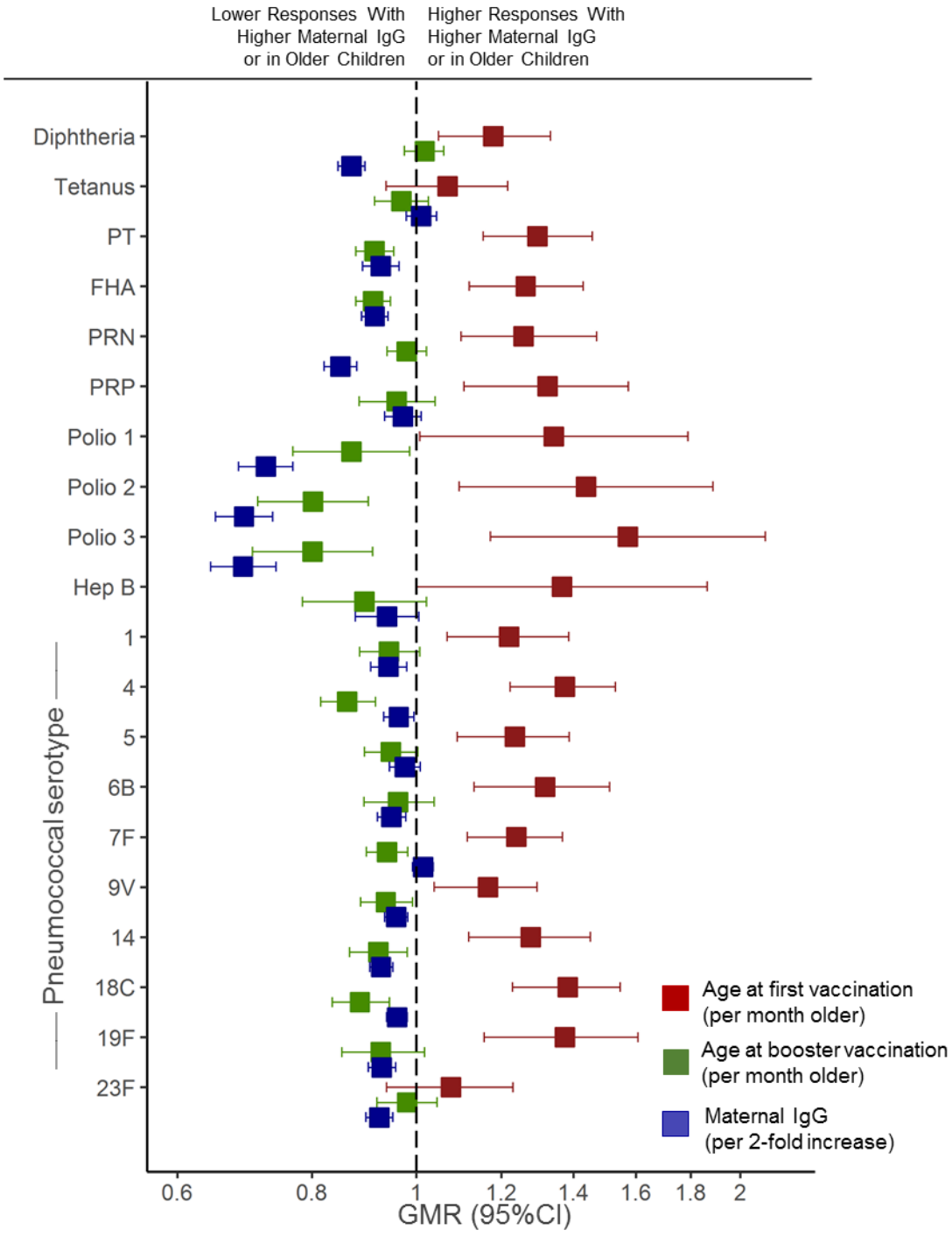


GMR: Geometric mean ratio (per 2-fold increase in pre-existing maternal antibody); PT: pertussis toxoid; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate; 4,6B, 9V, 14, 18C, 19F, 23F: serotypes of pneumococcus.

GMRs less than 1.0 indicate that children with higher antibody prior to vaccination have lower antibody concentrations post-3rd dose.

Data are from Model 1 which includes maternal antibody in the model as a continuous variable and were unadjusted for schedule of administration.

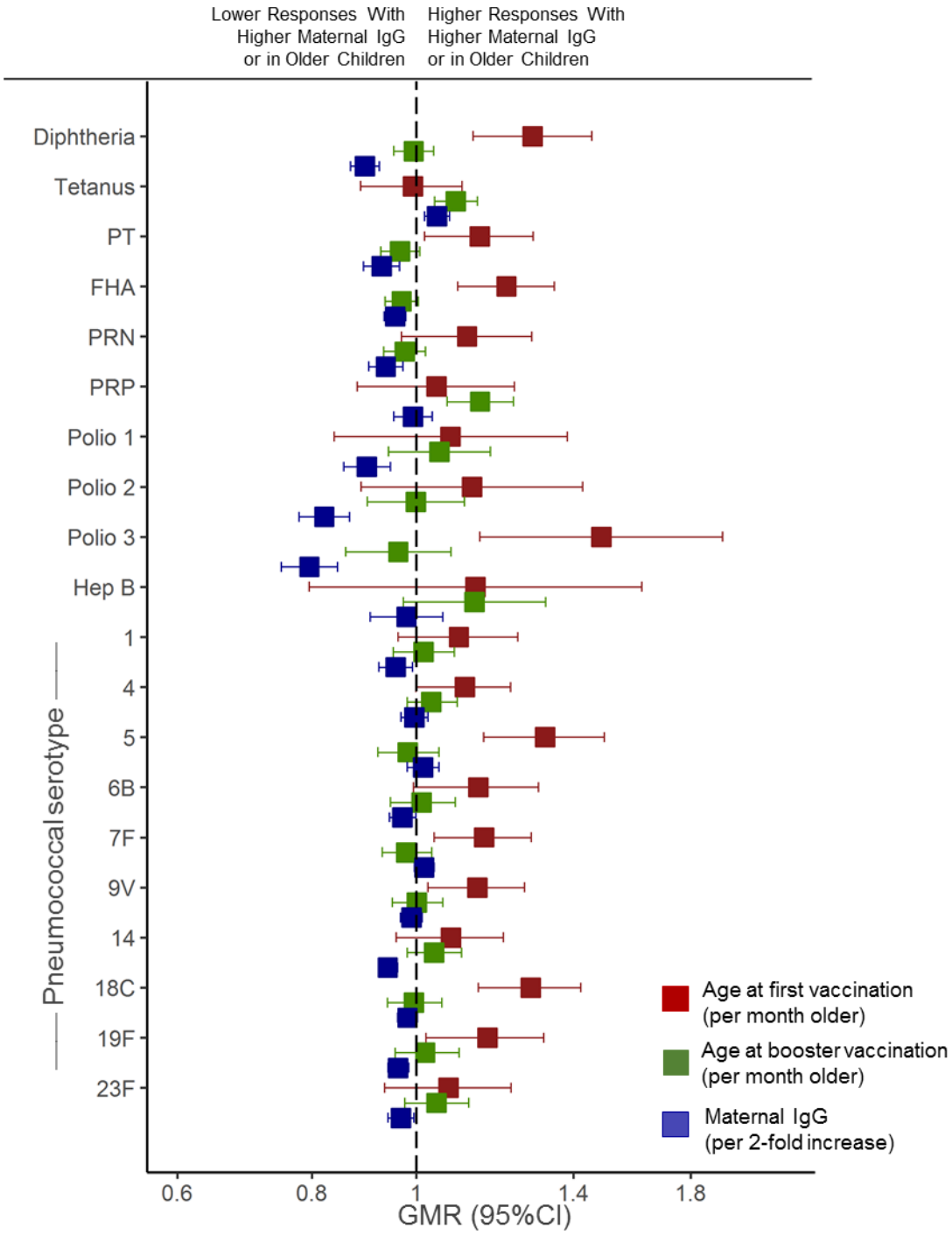
Figure 18 Influence of age at first vaccination, pre-existing antibody at first vaccination, and age at booster, on persistence of antibody to pre-booster time point (age ~12-24 months)



GMR: Geometric mean ratio; IgG: immunoglobulin G; PT: pertussis toxoid; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate; Hep B: hepatitis B;

For maternal antibody GMRs less than 1.0 indicate that children with higher antibody prior to vaccination have lower antibody concentrations prior to the booster dose. For age effects, GMRs greater than 1.0 indicate higher antibody concentrations in older children. These data are from models with maternal antibody as a continuous variable.

Figure 19 Influence of age at first vaccination, age at booster vaccination, and pre-existing antibody at first vaccination, on antibody concentrations post-booster (age ~12–24 months)



GMR: Geometric mean ratio; IgG: immunoglobulin G; PT: pertussis toxoid; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate; Hep B: hepatitis B.

For maternal antibody GMRs less than 1.0 indicate that children with higher antibody prior to first vaccination have lower antibody concentrations post booster. For age effects, GMRs greater than 1.0 indicate higher antibody post-booster in those who were older at the time of priming or booster vaccination. Data are from model 1 with maternal antibody as a continuous variable (Table 8)

Table 7 Influence of age at first vaccination, pre-existing antibody at first vaccination, and age at booster, on persistence of antibody to pre-booster time point (age ~12-24 months)

	Age at first vaccination (per month older)			IgG pre-vaccination (per 2-fold increase - model 1)			Age at booster dose (per month older)		
	GMR	LCL	UCL	GMR	LCL	UCL	GMR	LCL	UCL
Diphtheria	1.179	1.048	1.333	0.871	0.846	0.896	1.018	0.974	1.061
Tetanus	1.069	0.937	1.216	1.011	0.979	1.044	0.969	0.914	1.027
Pertussis toxoid	1.296	1.154	1.457	0.927	0.892	0.964	0.914	0.879	0.953
FHA	1.264	1.120	1.429	0.915	0.89	0.941	0.912	0.879	0.946
PRN	1.258	1.101	1.470	0.850	0.821	0.881	0.980	0.940	1.022
PRP	1.324	1.108	1.575	0.972	0.935	1.011	0.959	0.885	1.041
Polio type 1	1.342	1.007	1.788	0.725	0.684	0.768	0.870	0.768	0.986
Polio type 2	1.438	1.096	1.886	0.692	0.651	0.736	0.802	0.712	0.903
Polio type 3	1.366	1.001	1.863	0.939	0.877	1.006	0.895	0.784	1.022
Hepatitis B	1.572	1.171	2.110	0.690	0.644	0.740	0.801	0.704	0.911
Pneumococcal serotype-specific IgG									
1	1.220	1.068	1.385	0.942	0.907	0.980	0.944	0.886	1.007
4	1.375	1.222	1.530	0.963	0.933	0.995	0.862	0.815	0.916
5	1.235	1.092	1.388	0.976	0.944	1.008	0.947	0.895	1.003
6B	1.317	1.132	1.513	0.948	0.920	0.977	0.962	0.894	1.039
7F	1.239	1.115	1.367	1.015	0.992	1.037	0.939	0.898	0.982
9V	1.166	1.039	1.295	0.958	0.934	0.982	0.937	0.888	0.992
14	1.278	1.119	1.451	0.927	0.905	0.951	0.922	0.867	0.981

18C	1.383	1.228	1.547	0.960	0.939	0.981	0.886	0.835	0.944
19F	1.374	1.157	1.607	0.929	0.902	0.957	0.927	0.853	1.017
23F	1.077	0.938	1.230	0.924	0.898	0.951	0.981	0.920	1.046

GMR: Geometric mean ratio; LCL: lower limit of 95% confidence interval; UCL: Upper limit of 95% confidence interval; IgG: immunoglobulin G; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate.

For maternal antibody GMRs less than 1.0 indicate that children with higher antibody prior to vaccination have lower antibody concentrations prior to the booster dose. For age effects, GMRs greater than 1.0 indicate higher antibody concentrations in older children. For Polio antibodies, only two studies were available therefore a fixed effect for country cohort rather than a random effect was used.

Numbers in blue are confidence intervals that do not contain 1.0.

Table 8 Influence of age at first vaccination, age at booster vaccination, and pre-existing antibody at first vaccination, on antibody concentrations post-booster (age ~12-24 months)

	Age at first vaccination (per month older)			IgG pre-vaccination (per 2-fold increase – model 1)			Age at booster dose (per month older)		
	GMR	LCL	UCL	GMR	LCL	UCL	GMR	LCL	UCL
Diphtheria	1.282	1.130	1.456	0.896	0.868	0.924	0.995	0.953	1.038
Tetanus	0.993	0.887	1.103	1.045	1.017	1.074	1.088	1.040	1.140
Pertussis toxoid	1.146	1.018	1.285	0.928	0.894	0.965	0.966	0.927	1.008
FHA	1.213	1.092	1.343	0.956	0.934	0.978	0.969	0.936	1.005
PRN	1.115	0.969	1.280	0.937	0.903	0.972	0.975	0.932	1.020
PRP	1.044	0.882	1.234	0.993	0.953	1.035	1.146	1.068	1.231
Polio type 1	1.100	1.006	1.202	0.903	0.887	0.920	0.946	0.910	0.983
Polio type 2	1.117	1.024	1.218	0.899	0.882	0.917	0.941	0.906	0.977
Polio type 3	1.164	1.059	1.280	0.895	0.876	0.915	0.938	0.900	0.977
Hepatitis B	1.079	0.981	1.188	0.984	0.964	1.005	0.967	0.929	1.008
Pneumococcal serotype-specific IgG									
1	1.095	0.962	1.242	0.957	0.923	0.992	1.015	0.952	1.085
4	1.109	0.999	1.224	0.996	0.968	1.026	1.032	0.981	1.091
5	1.317	1.155	1.495	1.014	0.981	1.049	0.982	0.921	1.050
6B	1.142	0.994	1.299	0.971	0.944	0.999	1.012	0.946	1.087
7F	1.156	1.039	1.279	1.017	0.997	1.039	0.979	0.930	1.033
9V	1.140	1.026	1.261	0.990	0.967	1.013	1.001	0.950	1.059
14	1.077	0.958	1.204	0.941	0.922	0.960	1.039	0.981	1.102

	Age at first vaccination (per month older)			IgG pre-vaccination (per 2-fold increase – model 1)			Age at booster dose (per month older)		
	GMR	LCL	UCL	GMR	LCL	UCL	GMR	LCL	UCL
18C	1.277	1.142	1.421	0.981	0.961	1.003	0.995	0.941	1.056
19F	1.165	1.021	1.313	0.962	0.941	0.984	1.019	0.956	1.096
23F	1.072	0.935	1.224	0.968	0.942	0.995	1.044	0.976	1.119

GMR: Geometric mean ratio; IgG: immunoglobulin G; PT: pertussis toxoid; FHA: Filamentous haemagglutinin; PRN: pertactin; PRP: polyribosylribitol phosphate.

For maternal antibody GMRs less than 1.0 indicate that children with higher antibody prior to vaccination have lower antibody concentrations post-3rd dose. For age effects, GMRs greater than 1.0 indicate higher antibody concentrations post-booster in those who were older at the time of priming or booster vaccination. Numbers in blue are confidence intervals that do not contain 1.0.

Overall effect of delaying vaccination

These analyses show that a delay in the age at which an infant is first immunised will result in a combined beneficial effect on immunogenicity due to:

1) a reduction in maternal antibody interference, and

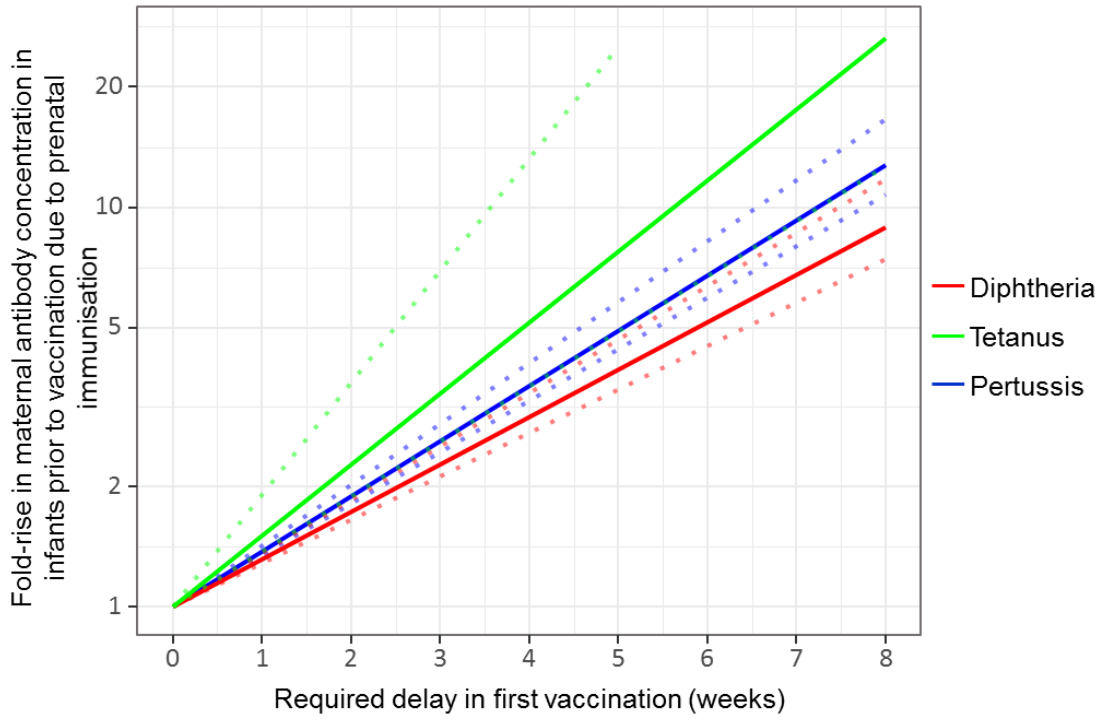
2) an increase in infant age.

The total benefit of a delay in the first dose of infant immunisation can therefore be calculated from the estimates in Table 5 as described in the methods in section 5.2.

For acellular pertussis, assuming a half-life of maternal antibodies of 36 days, infant PT antibody responses will be 1.06 fold higher for every week of delay in the first infant dose of vaccine. Delaying the start of infant immunisation by four weeks, and therefore having a 3-4-5 month schedule rather than a 2-3-4 month schedule, would offset a 3.4-fold increase in maternal pertussis antibodies when measured at the time of first immunisation. A maternal immunisation programme may conceivably increase the levels of PT antibody in the infant at the time of their first vaccination by approximately 2- to 5-fold and thus a one-month delay would be sufficient to offset the interference caused by the maternal prenatal immunisation programme (Figure 20).

Maternal prenatal programmes would also affect diphtheria and tetanus responses. A four-week delay in commencing infant immunisation would offset a 3.0-fold increase in diphtheria antibodies and a 5.1-fold increase in tetanus antibodies (Figure 20).

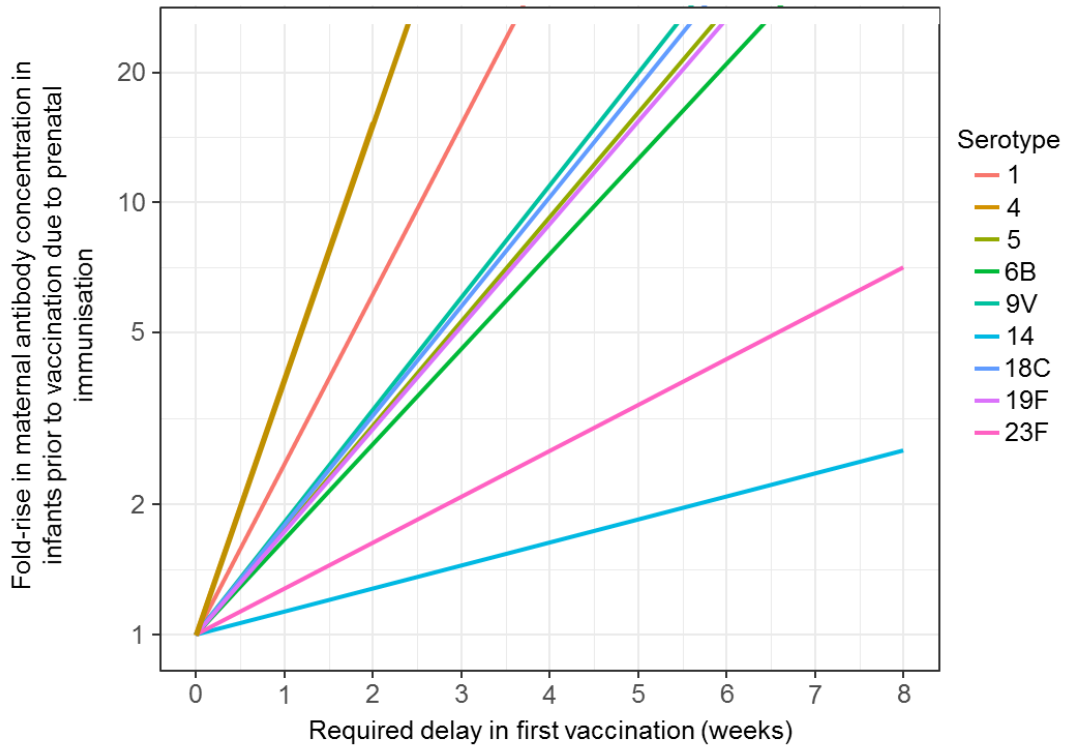
Figure 20 Number of weeks delay required to offset the effect of increased infant concentrations of maternal antibody against diphtheria, tetanus or pertussis



Solid lines represent average number of weeks delay required to offset the effect of increased concentrations of maternal antibody. Dotted lines are estimates using a 7 day higher or 7 day lower estimate of the half-life of maternal antibodies.

For maternal pneumococcal antibodies the half-life of 42.5 days from Chapter 4 was used for calculations shown in Figure 21. There was no significant maternal antibody interference for serotype 7F (see Table 5) and therefore this was not included in calculations of delay. For all other serotypes except 14 and 23F, the interference caused my maternal antibodies was of small magnitude and therefore a 4-week delay in first infant immunisation offsets more than a 7.5-fold increase in maternal antibody. For serotypes 14 and 23F the effect of maternal antibody interference was greater with GMRs of 0.88 for both serotypes and longer periods required to offset large increases in maternal antibody (Figure 21).

Figure 21 Number of weeks delay required to offset the effect of increased infant concentrations of maternal pneumococcal antibody



5.4 DISCUSSION

This analysis of the sera of over 7600 infants from 17 countries surmounts the problem of insufficient statistical power inherent in previous attempts to investigate maternal antibody interference on infant vaccine immunogenicity, which have led to inconsistent and/or non-significant findings. This analysis comprehensively models the effects of maternal antibody inhibition and infant age at vaccination on the majority of vaccine antigens contained in current global infant immunisation programmes and reveals that, for almost all antigens, transplacental antibody inhibits the antibody response to priming vaccinations and these effects are not diminished by administration of a booster dose. These analyses further reveal the benefit of being older when first immunised, an association which remains after adjusting for waning maternal antibody.

Strikingly, and in contrast to previous reports,[34] the effects of maternal antibody and the age when first immunised, are not only seen in response to a priming series of vaccines but continue to affect antibody responses to booster vaccinations at 12–24 months of age for many antigens. This suggests the importance of the quality of the immune response to the first dose of antigen regardless of subsequent doses.

The influence of maternal antibody on measles vaccine immunogenicity has been widely studied to determine the optimal age for vaccination. Younger children (~6 months) have higher maternal antibody concentrations and lower antibody responses to measles vaccine than that of older children (~9-12 months), but also produce lower neutralizing antibody responses in the absence of maternally-derived measles antibodies.[88] In contrast, there is little consensus in the medical literature regarding the potential for, extent of, or clinical implications of maternal antibody interference for vaccines received in early infancy. O'Brien et al reported maternal antibody interference for priming doses of PCV7 in infants,[34] and Englund et al reported inhibition for whole cell pertussis vaccine, however, observed no association between maternal antibody to pertussis toxoid and infant responses to 13 different acellular pertussis vaccines, in a combined, yet unadjusted, analysis.[32] Other studies are limited in size or incorporate only historical controls–methods prone to bias. In a small study of 36 infants[89], Jones et al showed that high levels of maternal antibody

inhibited responses to pneumococcal conjugate vaccine as well as to tetanus toxoid but no effect was seen against acellular pertussis toxoid nor *Haemophilus influenzae* type b (Hib), and other small studies have failed to find significant effects.[37,39,90]

This analysis reveals that not only are infant responses inhibited by maternal antibody to the same antigen, but responses to conjugate vaccines in infancy are also affected by antibody to related carrier proteins.

2,4,6 and 2,3,4 month schedules

Schedules with a wider spacing of doses deliver the second and third doses of vaccine after a longer interval, allowing for greater decay of maternal antibody prior to administration. Surprisingly, in this analyses, substantial inhibition was associated with pre-existing antibody for both 2,4,6 month, and 2,3,4 month schedules for all antigens except tetanus. The interference due to maternal antibody seems to be predominantly mediated through the impact on response to the first dose of vaccine.

Policy implications

In settings where prenatal immunisation programmes are well established with high coverage, maternal antibody provides protection against pertussis to most infants in the first few months of life.[21] In such settings, a delay in administration of the first vaccination would increase responses to infant vaccination if vaccine coverage is high, and the current schedule already includes an early first dose (≤ 2 months). The magnitude of the expected total increase in vaccine antibody due to a delay in vaccination was estimated by combining model parameters with the published half-life of maternal antibody. The magnitude of the increase in maternal antibody concentrations in infants due to maternal prenatal immunisation programmes has not been well established, but estimates from a small randomised trial and other non-randomised studies suggest that for pertussis, between 2- and 5-fold higher antibody concentrations may be induced.[37,91,92] These models show that the inhibitory effect of a 2- to 5-fold increase in maternal antibody concentrations in infants can be offset by a delay

in vaccination of between 2.2 and 5 weeks, time periods which are short enough to make delayed-start vaccination a feasible option.

Since current prenatal immunisation programmes use diphtheria and tetanus-containing vaccines, a delay in immunisation may also improve responses to other vaccines containing these carrier proteins such as the 13-valent pneumococcal vaccine.

Decisions regarding the timing of infant vaccinations need to consider the length of time during which direct protection afforded by prenatal immunisation is maintained in infants. There are currently few data on the length of protection afforded by maternal vaccination programmes, however, VE for children up to 3 months of age appears similar to that for children up to 2 months of age in the UK.[21] Infant immunisation programmes need to consider the level of disease risk in early infancy, and the level of coverage of prenatal programmes, as well as balance the complex interaction between the direct protection afforded the infant early in life by maternal vaccination, against the increased risk of disease later in infancy as a result of reduced immunogenicity, and the increase risk of disease in the population due to reduced indirect effects. This tradeoff is minimised if the vaccine schedule can be improved to increase infant responses to vaccination.

Limitations

The studies included herein were conducted in the absence of prenatal vaccination programmes. The effects of antibodies induced by natural exposure and those induced by vaccination during pregnancy may differ. In addition, pre-existing antibody in the infant may be due to exposure of the infant to circulating pathogens such as pneumococcal or pertussis antigens in the first few months of life rather than being entirely due to maternal antibody transmission. These models may, therefore, underestimate the effect of maternal antibody interference for widely circulating pathogens.

Conclusion

Older infants mount greater antibody responses to vaccination and maternal transplacental antibodies inhibit infant responses. These effects are observed for

both priming and booster vaccine doses, and occur for almost all antigens present in global immunisation programmes. Antibody responses to conjugate vaccines are not only affected by interference from pre-existing antibody to the polysaccharide antigen, but also from antibody associated with carrier proteins. Prenatal immunisation programmes containing multi-component vaccines, therefore, have the potential to interfere with immunogenicity of current immunisation programmes. However, the clinical relevance in terms of potential reductions in disease due to the maternal antibody interference on infant vaccine immunogenicity is not known.

Decisions regarding the design of infant immunisation schedules are increasingly complex due to increased numbers of vaccine antigens, prenatal immunisation programs, and the addition of booster doses of vaccines through childhood and adolescence. These data on key factors that influence the immune response will help in the planning of immunisation schedules and provide important parameters for evaluating their cost-effectiveness.

6 THRESHOLD ANTIBODY LEVELS OF PROTECTION: AN ASSESSMENT OF METHODOLOGICAL BIAS

The work contained in this chapter is published in;

Voysey M, Sadarangani M, Pollard AJ, Fanshawe TR. *Computing threshold antibody levels of protection in vaccine clinical trials: An assessment of methodological bias.* PLoS One. 2018 Sep 7;13(9):e0202517.

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6.1 INTRODUCTION

The process of developing, testing, and licensing a new vaccine necessitates an assessment of the vaccine's ability to prevent disease in the population of interest. This can be done by conducting clinical trials in which participants are randomised to receive the new vaccine or a placebo/control vaccine, and are subsequently followed up for a period of time to determine disease outcomes. These methods have been used to assess efficacy of many vaccines including the 7-valent pneumococcal vaccine,[55-57] rotavirus vaccine,[93,94] typhoid,[95,96] cholera,[97] and many others. If clinical outcomes in such trials are hard to measure due to tests that lack sensitivity or other difficulties in diagnosis (e.g. blood culture positive typhoid fever, invasive pneumococcal disease), then outcomes may occur in a relatively small proportion of participants. Such trials can require tens of thousands of participants and involve high costs, and a substantial number of years investment. If an immunological parameter ('correlate of protection') can be identified that is associated with protection against disease, such as circulating antibody, neutralising antibody, or a measure of cellular immunity, then the process of testing a new vaccine can be accelerated. Smaller trials of hundreds rather than thousands of participants can then be conducted, in which immunological measures are assessed rather than clinical disease endpoints.

The 10-valent and 13-valent pneumococcal conjugate vaccines were licensed using immunogenicity trials alone. No clinical efficacy data were available for the vaccines however clinical efficacy trials had been conducted for the 7-valent vaccine and these informed the derivation of an overall threshold (across all vaccine serotypes) of 0.35 μg/mL that was used in licensing the newer vaccines.[53] There were three pneumococcal conjugate vaccine efficacy trials used to assess the antibody correlate of protection, which when combined included 65 834 children. There were, however, only 61 cases of vaccine-type invasive pneumococcal disease (0.09%), and 1863 (2.8%) children had blood samples taken for antibody measures.[50] Such low proportions mean that the children sampled for immunogenicity and the children with disease were essentially different groups of children and no statistical models can be fit to these data to link the antibody responses to disease outcomes on an individual participant level. A post-licensure study was used to refine the previous overall estimate of

the threshold correlate of protection for the 13-valent vaccine, giving a higher estimate of 0.59 μg/mL (95%CI 0.35, 2.45) for PCV7 serotypes, and 0.98 (95%CI 0.77, 1.25) μg/mL for PCV13 serotypes. This was done in the UK based on a large dataset of 716 serotyped cases on invasive disease. The larger sample size also allowed for estimates to be made on a serotype-specific level and these showed substantial variation.[60] Group C meningococcal conjugate vaccines were also licensed in the UK based on serological assessment of efficacy without clinical efficacy studies of the conjugate vaccine.[98] The correlate of protection used for licensing had been previously established based on observational studies in military recruits[58] and subsequently validated for the conjugate vaccine using postlicensure surveillance data.[59] After introduction of these vaccines into the routine infant immunisation programme there were substantial decreases in disease incidence, confirming the clinical efficacy of these vaccines.

For other vaccines such as those for typhoid, rotavirus, and ebolavirus, there is no established correlate of protection and no thresholds that can be used to predict efficacy of a new vaccine. For these vaccines it is therefore still necessary to conduct large field studies to establish efficacy.

To determine the level of antibody that is sufficient to protect against disease, one needs data on disease events as well as antibody measures in those vaccinated and a way of correlating these two entities. When regression models cannot be applied, an alternative method is often used in which the proportion of participants with antibody below a threshold in each randomised group in the immunogenicity subset is compared to the proportions with disease in each group in the larger trial.

In this paper the model commonly used to define a threshold cutpoint of the correlate of protection is examined in detail. Simulated scenarios based on both high and low efficacy vaccines, with varying relationships between the immunological measure and the likelihood of disease, explore the performance of the model in each setting.

The purpose of this simulation study was to assess the settings in which the method is fit for purpose and to demonstrate situations where the method should be avoided.

6.2 METHODS

Definitions

The term 'correlate of protection' is used here to describe a measureable immunological parameter induced by vaccination that is related to protection against a specific clinical endpoint. The term 'threshold of protection' is used to describe a cutpoint on the correlate of protection that is used to test and compare vaccines, and above which a certain degree of protection is thought to exist. This can be absolute (100%) or relative (< 100%) protection and is affected by the rate at which the level of the immunological parameter wanes post-vaccination. Thresholds for correlates of protection are derived herein for antibody measured at 4-6 weeks after vaccination only, as this is the most common and relevant time point for vaccine clinical trials. Thresholds of protection for other time points, such as the time at which exposure occurs, are also of interest to researchers, and are far more difficult to define and measure.

Calculating a threshold of protection

The method for calculating an immunological threshold for protection in this simulation study is that which was used to establish thresholds for pneumococcal and group C meningococcal vaccines.[50,53,59,60]

Firstly, the observed vaccine efficacy ($VE.obs$) is calculated from an efficacy trial or post-licensure study as,

$$VE.obs = 1 - \frac{\text{Attack rate in vaccine group}}{\text{Attack rate in control group}}$$

where the attack rate is the proportion of participants with clinical disease.

The threshold (t) is the calculated from a smaller immunogenicity substudy within the main trial, assuming:

$$VE.ims = 1 - \frac{\text{Proportion with } Ab < t \text{ in vaccine group}}{\text{Proportion with } Ab < t \text{ in control group}}$$

where *VE.ims* is the vaccine efficacy defined using an immunological parameter alone from the immunogenicity subset participants, and *Ab* is the immunological parameter (usually measured one-month after vaccination). Different values for *t* are trialled until a value is found for which *VE.obs* will match *VE.ims* and this value of *t* is then retained as the threshold.[53,54] In practice, one rounds estimates of VE to the nearest 2 or 3 decimal places and this affects whether a true match is found. With more decimal places, it becomes less likely for the two numbers to be equal. For our simulations data were not rounded. Instead, the value of *t* at which *VE.obs* was closest to *VE.ims* was retained as the threshold as this made it more likely that a single solution for *t* could be found.

The above method for calculating thresholds, includes clinical disease and levels of the immunological parameter as the only factors to be considered in the assessment of protection against disease. The difference between vaccine and control groups is therefore assumed to be entirely mediated through the immunological parameter. Values of the immunological parameter above the threshold value are assumed to induce sterilizing immunity. This is a simplifying assumption as in reality, immunity is not mediated through a single immunological parameter such as circulating serum IgG, but also relies on other factors such as cellular immune function, and mucosal immunity. In addition, assumptions about sterilizing immunity may not be realistic for some immunological parameters.

Population data

Individual participant data were simulated for a theoretical population of N=400,000 people eligible for inclusion in a vaccine efficacy study, equally divided between vaccine and control groups. For each person an antibody level (*Ab*) for a hypothetical parameter assumed to be associated with protection against a suitable clinical endpoint (disease, infection, hospitalisation etc.), was simulated according to each person's assignment to vaccine or control. An arbitrary value of 1 μg/mL was set as the threshold antibody level at which the greatest protection against disease was reached (*t**). Three vaccine scenarios were simulated; a) a vaccine that induces an antibody response which in most people is higher than the control group, however the antibody distributions

overlap slightly (STD), b) a vaccine that induces a very high antibody response which for all people is higher than the true threshold of protection (HIGH), and c) a vaccine with low efficacy and a large proportion of non-responders (LOW) (Table 9 and Figure 22).

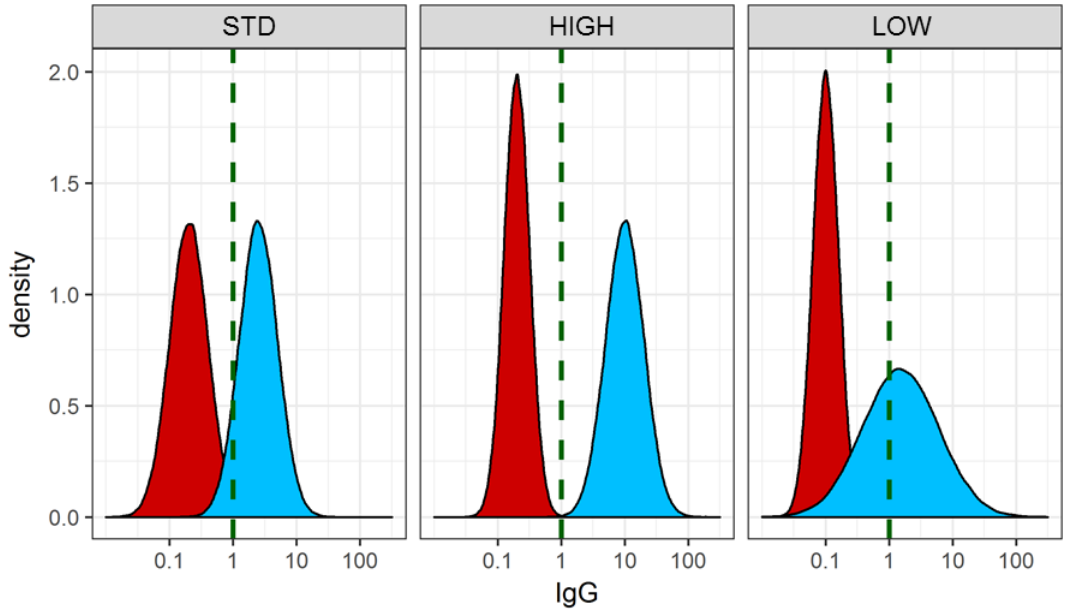
Table 9 Parameters for simulations of antibody distributions in vaccinated and control populations for three scenarios

Scenario	*Vaccine antibody distribution* *(blue)*	*Control antibody distribution* *(red)*
STD: Vaccination produced a good antibody response however vaccine and control distributions still overlap a small amount	GM=2.5 μg/mL, μ=0.398, σ=0.3	GM=0.2 μg/mL, μ= -0.699, σ=0.3
HIGH: Vaccine induces very high antibody response. Vaccine and control group antibody distributions are non-overlapping and all vaccinated participants have antibody above the threshold.	GM=10 μg/mL, μ=1.0, σ=0.3	GM=0.2 μg/mL, μ= -0.699, σ=0.2
LOW: A large proportion of the vaccine group are non-responders. Vaccine and control distributions overlap substantially. Low vaccine efficacy.	GM=1.5 μg/mL, μ=0.176, σ=0.6	GM=0.1 μg/mL, μ= -1.0, σ=0.2

GM= geometric mean, μ=mean (on $\log_{10}$ scale), σ=standard deviation of μ; Antibody concentrations are simulated to be log-normally distributed, using a log of base 10.

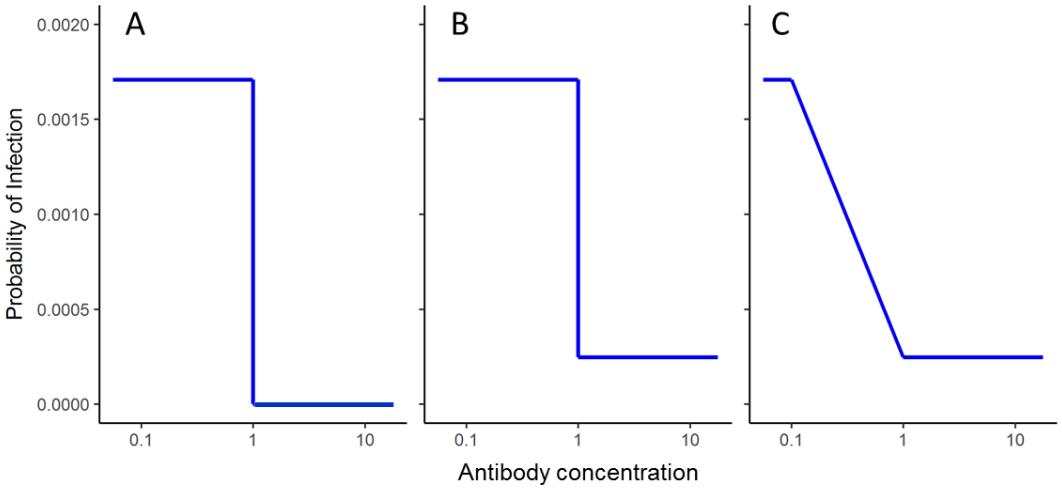
Each person's probability of disease (p) was determined according to their simulated antibody level, and the underlying relationship between disease and antibody as shown in Figure 23. Instances of disease were simulated from independent Bernoulli(p) distributions.

Figure 22 Simulated antibody distributions in vaccinated and control populations for three scenarios shown in Table 9



red = control group, blue = vaccinated group. Green dashed line = antibody threshold that gives greatest level of protection.

Figure 23 Three types of simulated relationships between antibody concentration and the probability of infection



A: Step function with antibody levels higher than the 1 mcg/mL threshold produced sterilizing immunity; B: Step function with antibody levels higher than the 1 mcg/mL threshold not producing sterilizing immunity; C: non-step function and non-sterilizing immunity.

Three different relationships between antibody levels and disease probability were simulated. Firstly, the relationship between antibody and the probability of infection followed a step function and antibody levels higher than the threshold produced sterilizing immunity such that no disease cases were observed above this level (Figure 23A). This scenario aligns with the assumptions of the model and tests the performance of the model when all assumptions hold true. The second and third simulated scenarios test the performance of the method if the assumptions do not hold – that is, if the relationship between antibody and disease is not a step function or if sterilising immunity is not achieved (Figure 23B & C). Thus, in the second scenario, the relationship between disease probability and antibody level was simulated as a step-function with antibody levels above the step threshold reducing disease probability substantially but not absolutely (Figure 23B). This represents a relative rather than an absolute threshold. In the third scenario a relative rather than absolute threshold was also investigated with the probability of disease simulated to decrease gradually as antibody levels increased, a situation likely to be closer to the true relationship between and antibody and disease than a step function (Figure 23C).

Trial level data

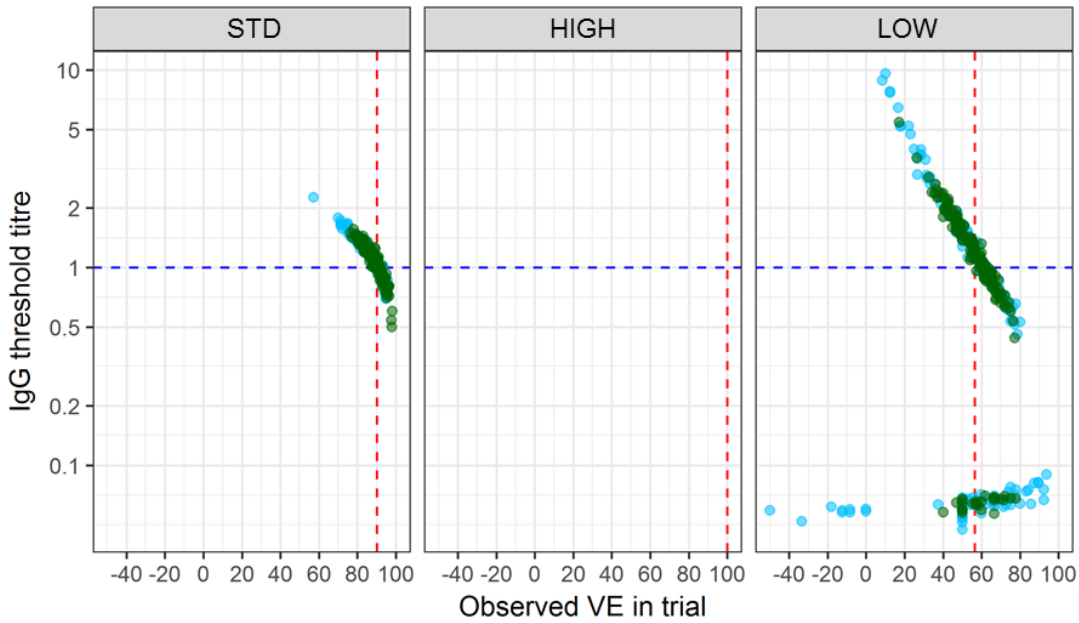
From the simulated population data, samples were repeatedly drawn to simulate enrolment of participants into separate trials. In total, 2700 trials were simulated with 150 trials for each of 18 scenarios (Table 10). Trials were either of size 20,000 or 60,000 people and subsets of 1000 participants in each trial were randomly selected for inclusion in a simulated immunogenicity substudy. For the selected immunogenicity substudy participants, their antibody level was considered known (*Ab*), and for the non-selected participants, their antibody level was considered unknown (*Ab*=NULL). From each simulated trial population, *VE.obs* and *VE.ims* were computed and used to estimate t by the method described previously.

Table 10 Simulated scenarios and results

Relationship between Ab and disease	*Vaccine scenario*	*True VE*	*Trial size*	*Immuno substudy size*	*Number of trials simulated*	*Range of values for VE.Obs in 150 trials*	*threshold (t) (μg/mL) median [range]*	*Number of trials with multiple values of t*
Sterilizing immunity – Figure 23A	Fig 22 STD	90.1%	20000	1000	150	57–100%	1.04 [0.69, 2.27]	1
		90.1%	60000	1000	150	76–98%	1.07 [0.50, 1.57]	1
	Fig 22 HIGH	100%	20000	1000	150	100–100%	-	
		100%	60000	1000	150	100–100%	-	
	Fig 22 LOW	56.4%	20000	1000	150	-50%–94%	0.89 [0.05, 9.58]	24
		56.4%	60000	1000	150	17–78%	1.11 [0.06, 5.43]	12
Step function threshold – Figure 23B	Fig 22 STD	79.0%	20000	1000	150	25–96%	1.47 [0.73, 3.74]	4
		79.0%	60000	1000	150	47–94%	1.45 [0.90, 2.59]	0
	Fig 22 HIGH	85.8%	20000	1000	150	50 – 96%	4.93 [2.97, 10.53]	0
		85.8%	60000	1000	150	61 – 98%	4.83 [2.35, 8.05]	1
	Fig 22 LOW	47.0%	20000	1000	150	-50–95%	1.29 [0.05, 12.9]	15
		47.0%	60000	1000	150	-3–69%	1.59 [0.04, 14.7]	7
Graduated threshold – Figure 23C	Fig 22 STD	80.6%	20000	1000	150	13 – 94%	1.43 [0.77, 5.9]	5
		80.6%	60000	1000	150	57 - 93%	1.37 [0.92, 2.2]	3
	Fig 22 HIGH	81.4%	20000	1000	150	22 – 94%	5.43 [3.09, 16.8]	6
		81.4%	60000	1000	150	48 – 97%	5.40 [2.62, 10.3]	2
	Fig 22 LOW	71.7%	20000	1000	150	- 25 – 95%	0.31 [0.05, 7.2]	41
		71.7%	60000	1000	150	38 – 91%	0.64 [0.06, 2.4]	5
Total number of trials simulated					*2700*			

The first set of results are presented in Figure 24 for the scenario in which the relationship between antibody and disease probability is a step function and antibody levels higher than the threshold are fully protective (Illustrated in Figure 23A). In Figure 24 (and similar figures following) each dot represents one trial, thus one estimate of *VE.obs* plotted against the calculated threshold of protection (*t*) from the immunogenicity substudy embedded within the hypothetical trial. The horizontal dotted line represents the true threshold (*t**) and the vertical dotted line indicates the true underlying *VE*. There was a decreasing relationship between *VE.obs* and *t*. Trials with estimates of *VE.obs* close to the true value, also had values of *t* close to the true value. However, trials with estimates of *VE.obs* that were lower than the true value, overestimated *t* and vice versa.

Figure 24 Estimates of the threshold of protection in 150 trials of size 20,000 (light blue) or 60,000 (dark green) using three scenarios in Figure 22, and assuming that antibody levels above the true threshold produce sterilizing immunity in all people.



Blue dotted line = true threshold of protection (t) of 1 μg/mL, red dotted line = true vaccine efficacy. No estimates of the threshold could be obtained for the middle panel as all observed VE were 100%. Multiple values for t were obtained in 36 trials in the right hand panel, producing two distinct distributions. Simulation parameters shown in Table 10.*

For the first scenario in which the simulated vaccine had good efficacy (scenario illustrated in Figure 22 STD & Figure 23A), estimates of *VE.obs* from simulated 'small' trials of 20,000 participants covered a wider range of values than *VE.obs* estimates from 'large' trials of 60,000 participants (Range: trials of 20,000, 57% to 100%; trials of 60,000, 76% to 98%). (Figure 24 STD). The median threshold was 1.04 (range: 0.69, 2.3) μg/mL for small trials and 1.07 (range: 0.05, 1.6) μg/mL for large trials.

In 28 small trials *VE.obs* was 100% and therefore a threshold could not be computed. In such situations, any value of antibody that is lower than the lowest value in the vaccine arm (i.e. all the antibody levels for the participants in the control arm) gave *VE.ims* estimates of 100% and fulfil the equations of the model. There are, therefore, almost as many valid values of *t* in each trial as there are participants in the control arm and no single solution can be obtained.

Of the remaining 122 trials, a single threshold was obtained in 121 trials, however there were two solutions to the equations for one trial and thus two equally valid thresholds. This occurred when *VE.obs* was 62.5%. With a threshold of 2.012 μg/mL the numbers of participants with antibody lower than the threshold in the vaccine and control groups were 184/500 and 500/500 respectively, giving *VE.ims* of 62.6%. At a threshold of 2.022 μg/mL the numbers were 188/500 and 500/500 giving *VE.ims* of 62.4%. Both thresholds give values of *VE.ims* that are equidistant from the *VE.obs* of 62.5% and thus both are equally valid solutions to the equation. In this example there is little material difference between the two thresholds obtained and the average of the two values could be retained as the final estimate. However, duplicates could also occur at very different values of *t*. In another example (Figure 24 LOW), when *VE.obs* was 60.0% and the threshold of 0.059 μg/mL was tested, the numbers below the threshold were 2/500 and 5/500 for vaccine and control groups respectively, giving a *VE.ims* of 60.0%. At a high value of *t* of 1.06 μg/mL, the numbers below the threshold were 200/500 and 500/500, which also gives a *VE.ims* values of 60.0%. The difference between a threshold of 0.059 μg/mL and 1.06 μg/mL would not be considered an immaterial difference and so taking the average of the two is not a suitable way to resolve the problem of multiple solutions to the equations.

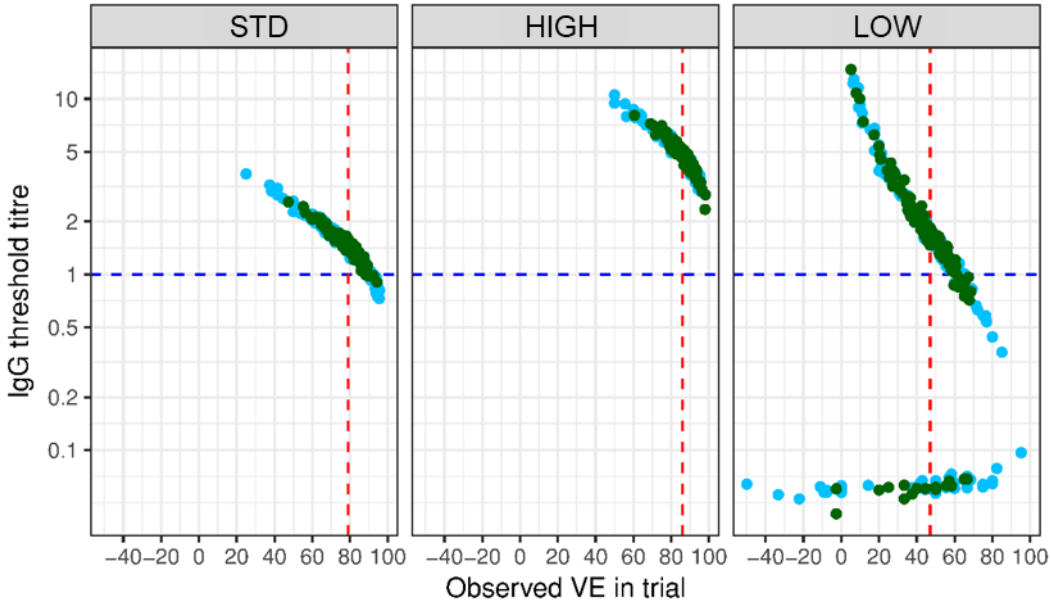
When the vaccine had high efficacy no cases of disease were observed in the vaccine arm thus each trial had *VE.obs* of 100% and no threshold could be obtained (Figure 24 HIGH).

For the low efficacy vaccine the median thresholds were 0.89 (range: 0.05, 9.6) and 1.11 (range: 0.06, 5.4) μg/mL in small and large trials respectively. 16% and 8% of small and large trials respectively had more than one valid solution to the equations thus multiple estimates of *t* (Figure 24 LOW).

The second set of results are presented for the scenario in which the relationship between antibody and disease probability is a step function but antibody levels higher than the threshold are not absolutely protective (illustrated in Figure 23B).

For the scenario in which the simulated vaccine had good efficacy, the median thresholds were 1.47 μg/mL (range: 0.73 – 3.74) and 1.45 μg/mL (range: 0.90 – 2.59) for trials of size 20,000 and 60,000 respectively (Figure 25 STD, Table 10). When the vaccine produced high antibody levels that did not overlap with the distribution of antibody in the control group (Figure 22 HIGH). The median threshold was more than 5 times larger than the true value (Median 4.93 μg/mL, range: 2.97 – 10.53; and median 4.83 μg/mL, range: 2.35 – 8.05, for small and large trials respectively) (Figure 25 HIGH). When the vaccine had poor efficacy median thresholds were 1.29 μg/mL (range: 0.05 – 12.9) and 1.59 μg/mL (range: 0.04 – 14.7) for the small and large trials respectively (Figure 25 LOW). In 22 of the simulated trials for the scenario displayed in Figure 25 LOW there were multiple solutions to the equations and thus some trials had very low threshold values as well as a threshold value similar to the main distribution of estimates of *t*.

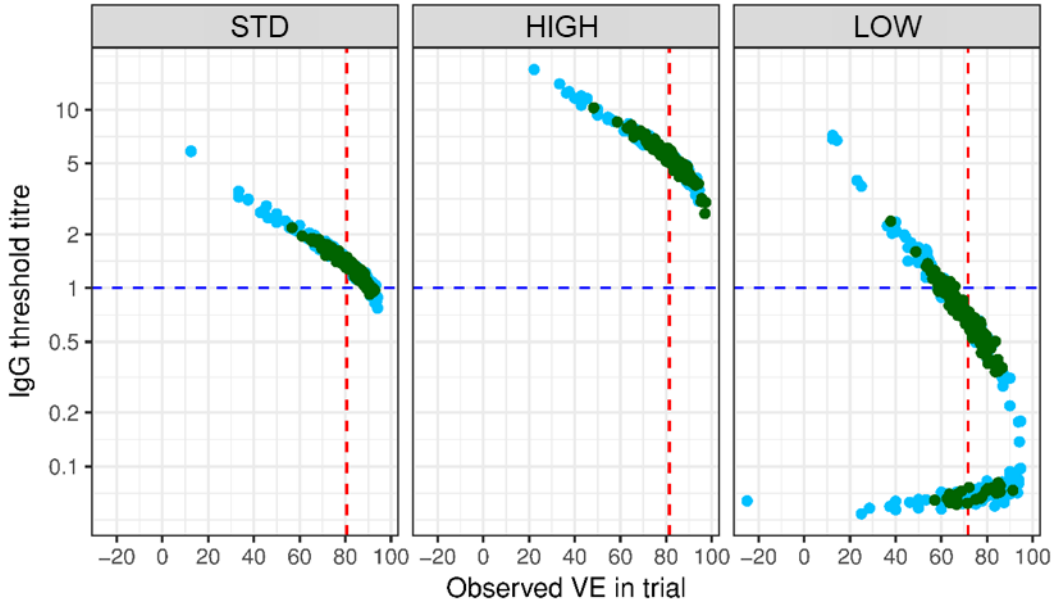
Figure 25 Estimates of the threshold of protection in 150 trials of size 20,000 (light blue) or size 60,000 (dark green) using three scenarios in Figure 22, assuming a step function in the relationship between antibody and disease and that antibody above the threshold is not fully protective in all people.



Blue dotted line = true threshold of protection (t) of 1 μg/mL, red dotted line = true vaccine efficacy. Multiple values for t were obtained in 22 trials in the right hand panel, producing two distinct distributions. Simulation parameters shown in Table 10.*

When a more realistic relationship between antibody and disease was used for simulations which incorporated a gradual decline in disease probability with increasing antibody levels, (Figure 23C) the pattern of threshold estimates was similar to that in which there was a hard step function (Figure 26 STD & HIGH). In Figure 26 LOW which displays the scenario in which there are many non-responders, threshold estimates were lower than the true value in the majority of cases.

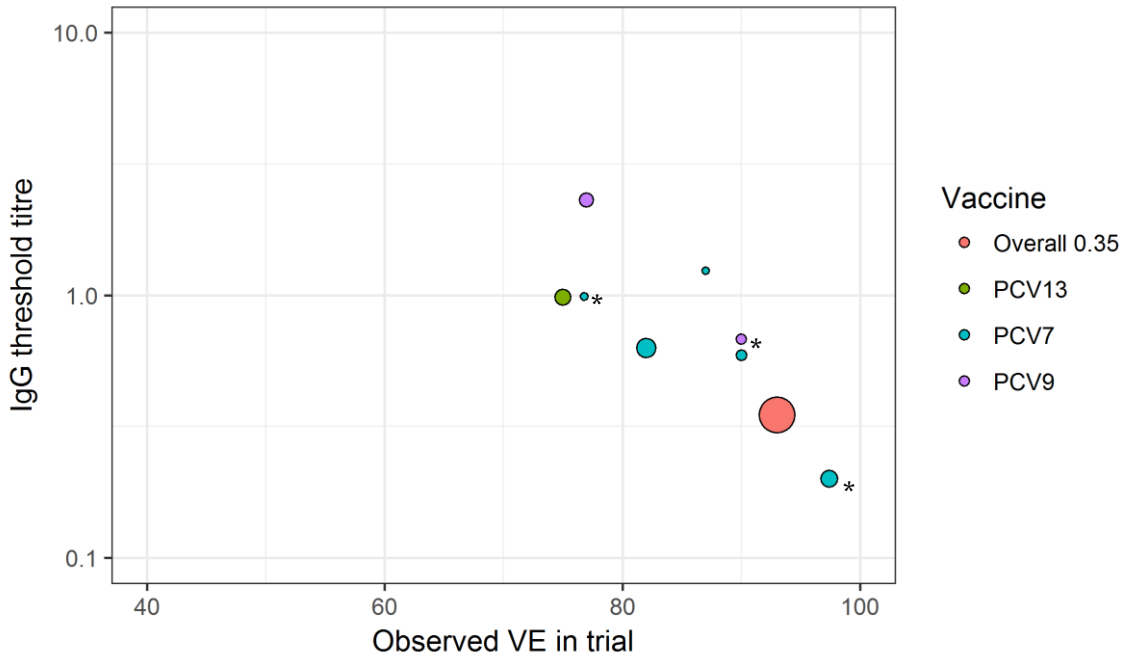
Figure 26 Estimates of the threshold of protection in 150 trials using three scenarios in Figure 22, assuming a graduated threshold (non-step function) in the relationship between antibody and disease, and that antibody above the threshold is not fully protective in all people



Light blue dots = trials of size 20,000; dark green dots = trials of size 60,000; Blue dotted line = true threshold of protection (t) of 1 mcg/m;, red dotted line = true vaccine efficacy. Multiple values for t were obtained in 46 trials in the right hand panel, producing two distinct distributions. Simulation parameters shown in Table 9 & Table 10.*

Published estimates of overall pneumococcal conjugate vaccine correlates defined from vaccine efficacy studies using the methods described herein are displayed in Figure 27. A clear relationship between the estimated thresholds of protection and the observed vaccine efficacy estimates can be seen in the published studies that mimics the relationship seen in the simulated data. Higher values of *VE.obs* produce lower threshold values and the reverse is also true. The similarity between published and simulated estimates suggest that the simulations provide a true depiction of the findings of current research.

Figure 27 Published estimates of overall anti-capsular IgG thresholds of protection against invasive pneumococcal disease from vaccine efficacy trials and indirect cohort studies



Estimates of anti-capsular IgG thresholds of protection against invasive pneumococcal disease from individual studies of PCV7, PCV9, and PCV13 from refs [50,55-57,60,99,100]. Overall: the threshold of 0.35 µg/mL derived from three studies combined (*)[55-57], and used for vaccine licensure. The size of each circle is proportionate to the inverse of the standard error of the observed vaccine efficacy estimate.

6.4 DISCUSSION

Calculating thresholds of protection by matching vaccine efficacy estimates from studies measuring clinical disease and embeded substudies with immunological endpoints, produces estimates which can be highly inflated. Our simulations show that when a highly efficacious vaccine induces a distribution of antibody values that is higher than the control group, and higher than a true level of protection, the model will either produce substantially inflated threshold estimates, or if the observed VE is 100%, then no threshold at all. This could lead to the situation whereby a true threshold that is fully protective might exist at e.g. 1 μg/ml, but the threshold computed based on a trial of a highly efficacious vaccine is greatly inflated (e.g. 5 μg/mL). Such an over-inflated threshold estimate would have the opposite outcome to that desired and prevent other vaccines being licensed. For example, a new, highly efficacious vaccine that induced on average 3 mcg/mL of antibody at one-month post vaccination would be considered not sufficiently immunogenic for licensure under this paradigm.

For both pneumococcal and meningococcal polysaccharide-protein conjugate vaccines the defined and accepted thresholds of protection have been shown to have limitations. Clear instances of disease occurring in the presence of high antibody levels have been observed[101] as well as serotype-specific differences in protective levels for pneumococcal vaccines.[60,102] However, this method has been used to establish licensure for more than one vaccine that has subsequently proven to be effective and thus defining the original threshold was a useful process which led to successful vaccine development.[59,103-105]

In the case of pneumococcal conjugate vaccines, the trials used to estimate the accepted threshold of 0.35 μg/mL observed high efficacy values.[55-57] These simulations show that when *VE.obs* is high the threshold value computed is low and maybe lower than the true threshold. It is possible therefore, that the threshold of 0.35 μg/mL for pneumococcal vaccines is an underestimate of the true value.

An assumption that a threshold of absolute protection does exist is more reasonable for some vaccines than for others. For some (in particular polysaccharide capsular conjugate) vaccines a true threshold of absolute

protection may exist. However, for other organisms the relationship between antibody and infection may be different due to different underlying immunological processes, such as for live attenuated viral vaccines or intracellular pathogens. This can be observed for infections with *Salmonella* Typhi in which an increasing level of anti-Vi IgG is associated with increasing protection against disease, yet challenge studies and seroefficacy estimates reveal that there is no observable threshold of Vi-IgG whereby protection becomes absolute.[106,107] For pathogens in which data suggest absolute thresholds are implausible, our simulations reveal that attempting to calculate thresholds of protection will lead to inflated values in many scenarios, particularly when the vaccine induces a good antibody response. It is not surprising that biased estimates of thresholds result from situations in which the antibody response is non-sterilising as this violates the assumption underpinning the model. The violation of the assumptions that lie beneath the model can result in either inflated or deflated thresholds and the effect of the violation of assumptions may depend on the degree to which they are violated, how much higher the antibody response in the vaccinated cohort is relative to the true threshold, and the vaccine efficacy.

The challenge for vaccine development for pathogens for which an absolute threshold is not plausible, is to find a method to compare and test the protective efficacy of new vaccines without requiring large field studies.

In these situations, a relative rather than absolute correlate of protection needs to be the focus. For example, Vi-polysaccharide typhoid vaccines have an estimated 2-year efficacy of 59% (95%CI 45–69%) against typhoid fever [108], and typically elicit antibody responses 6 weeks after vaccination of between 300 and 500 EU/mL when measured using the VaccZyme ELISA kit.[109] The Vi-tetanus toxoid typhoid conjugate vaccine induces higher antibody levels in the range of 1000 to 1500 EU/mL in an endemic setting[109], and although effectiveness in the field has not yet been demonstrated, trials are underway, and a seroefficacy study has suggested the vaccine may be 85% protection.[107] Rather than seek to determine an absolute threshold of protection when such a thing may not exist, future vaccines can be compared with licensed vaccines with known efficacy by comparing the proportion of participants above a median value. Since the variation in a proportion is maximised at 50%, this value (equivalent to the

median) may be the most useful and the most conservative cutpoint to use for clinical trial comparisons. It does not matter to the clinical trial results that this cutpoint does not equate to a level of absolute protection. Thus for Vi polysaccharide typhoid vaccines, if the proportion of participants with Vi-IgG above 400 EU/mL is similar in both arms of a trial then vaccines would be expected to be similarly effective in the field and it does not matter than the value of 400 EU/mL is not a protective threshold. For conjugate vaccines, a value of 400 EU/mL is too low as almost all participants will achieve this level and thus a value of 1250 EU/mL would be more appropriate for comparisons. Comparisons of vaccines in which 99% of participants have antibody above a certain cutpoint in both arms of the trial do not provide enough information to discriminate between the vaccines and indicate that the cutpoint used for comparisons is too low. Comparisons of the proportions of participants above a value expected to be similar to the median antibody level in the control arm of the trial, are similar to a comparison of geometric means, a method which also has benefits.

For all pathogens, it is important to determine whether an absolute threshold of protection is likely to exist. This can be achieved by examining the relationship between an immunological parameter and the probability of disease when modelled appropriately using logistic or generalised additive models, or other appropriate probabilistic models. This has been done successfully for pneumococcal carriage studies.[102] However, these models require suitable numbers of events to appropriately characterise the shape of the relationship and cannot therefore be used when modelling rare events such as blood culture positive enteric fever or invasive bacterial diseases. In these situations, models based on serological methods for detecting disease events show greater promise.

6.5 CONCLUSIONS

Current methods for determining a threshold of protection for use in vaccine development produce inflated thresholds in situations where a vaccine induces high levels of antibody, or when the threshold is not indicative of sterilizing immunity. Absolute thresholds of protection are unlikely to exist for many

pathogens and in these situations we recommend using alternative statistical methods, such as modelling of serologically defined infections to suitably characterise the shape of the relationship between an immunological parameter and the probability of infection.

7 SEROTYPE-SPECIFIC CORRELATES OF PROTECTION FOR PNEUMOCOCCAL CARRIAGE

The work contained in this chapter is published in;

> Voysey M, Fanshawe TR, Kelly DF, O'Brien KL, Kandasamy R, Shrestha S, Thorson S, Hinds J, Pollard AJ. *Serotype-specific correlates of protection for pneumococcal carriage: an analysis of immunity in 19 countries*. Clinical Infectious Diseases. Volume 66, Issue 6, 5 March 2018, Pages 913–920, https://doi.org/10.1093/cid/cix895.

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All text, analyses, tables, and figures were produced by Merryn Voysey. Co-authors have given permission to include the material in this thesis.

7.1 INTRODUCTION

Vaccination with pneumococcal conjugate vaccines (PCV) induces IgG antibody production which peaks approximately one month after vaccination and wanes thereafter in the absence of serotype-specific exposures[82]. The concentration of circulating serum antibody that is sufficient to protect an infant against invasive disease during the post-vaccination period is not well defined. The level of antibody required to protect against mucosal colonisation is also unclear.

In large efficacy trials with clinical endpoints, only a small proportion of children will have antibody concentrations assessed after vaccination, precluding analyses which directly link the antibody concentration in an individual with the disease outcome for that participant. In such situations the overall trial-level estimates of the proportion with disease and the proportions with antibody below a threshold level can be used to define an antibody level associated with protection which is referred to as a correlate of protection.[50,53,54] A pooled correlate of protection against invasive pneumococcal disease (IPD) of 0.35 μg/mL was derived from a meta-analysis of three efficacy trials[50] in which the trial-specific serum IgG correlates of protection were estimated to be 0.2, 0.68 and 1.0 μg/mL respectively.[55-57] Although these correlates have provided a means to establish licensure of additional products, they cannot predict protection for the individual, as evidenced by a case report of vaccine failure in a 9-month old child, 10 days after a measured antibody concentration of 4.98 μg/mL.[101] Studies of serotype specific post-vaccine impact paired with immunogenicity data in the same population have resulted in inferences about serotype-level correlates of protection some of which are higher and some of which are lower than the pooled estimate.[22]

Current correlates of protection refer only to protection against IPD, and not to other endpoints of clinical importance such as acute otitis media, non-bacteraemic pneumonia, nor to nasopharyngeal carriage. The effector molecule for protection against mucosal colonisation is unlikely to be circulating serum IgG, but instead may be IgG or IgA that is produced by mucosally associated B-cells, or IgG which leaks from circulation to mucosa. Nevertheless, circulating serum IgG concentrations can serve as a proxy for the level of immune response to pneumococcal conjugate vaccines which in turn provides protection from

carriage. Since nasopharyngeal colonisation is a necessary precondition for development of pneumococcal disease and is the mechanism for transmission of pneumococcal infection, vaccine efficacy against this endpoint is key to reducing transmission, inducing herd immunity, and thereby delivering maximum health benefits of PCV.[110,111]

The aim of this study was to calculate the concentration of serotype-specific serum IgG which is associated with protection against nasopharyngeal colonisation in the individual.

7.2 METHODS

Data

Data are from trials in which pneumococcal conjugate vaccines were administered to infants. A full list of all trials is provided in the Supplementary Table.

Serotype-specific pneumococcal IgG concentrations from children within each country and each study ('country cohorts') were analysed separately. For each participant, antibody concentrations were compared at the one-month post-priming visit and the booster visit. Any increase in antibody at the latter time-point was considered evidence for natural boosting due to pneumococcal carriage acquisition ('seroincidence').

Randomised Trial in Nepal

Immunogenicity data were also available from an open-label randomised controlled trial of 10-valent PCV (PCV10) in Nepal (authors own data). [112]

To determine if any increase in antibody between two time points is a good proxy measure for nasopharyngeal colonisation acquisition, antibody data from the Nepal RCT were paired with data from a cross-sectional carriage study of 600 healthy children (aged 6 weeks to 24 months) conducted during the same period and the same study centre where the immunogenicity study was carried out.[113] High correlation between the proportions of children with an increase in antibody levels after the booster dose, and the proportions of community

children with positive nasopharyngeal swabs was considered supportive evidence for the use of seroincidence as a marker of nasopharyngeal carriage acquisition.

Use of carriage seroincidence to define antibody thresholds of protection

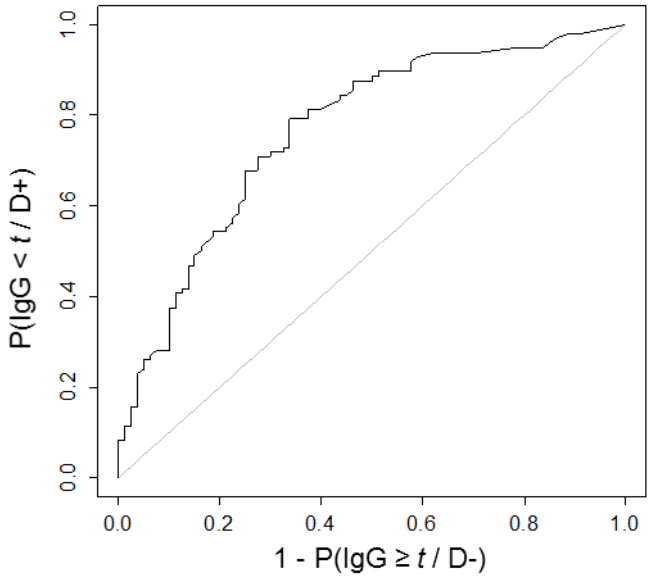
Correlates of protection against seroincidence were derived for antibody measured at one-month post priming, for each serotype and country cohort separately, using two different methods as follows.

Firstly, correlates of protection were derived using receiver-operator characteristic (ROC) curves. For each potential threshold antibody level (t,) a 2 x 2 table was computed which tabulated the proportion of children with antibody above or below threshold t who did and did not have seroincidence (a,b,c,d, Table 11). These proportions can be analysed in a similar way to the analysis of sensitivity and specificity in diagnostic test studies, with the sensitivity (a) plotted against 1-specificity (1-d) in an ROC curve (Figure 28). From each ROC curve, the antibody level which maximised the sum of $a + d$ was retained as the correlate of protection with the greatest discrimination.

Table 11 Example of 2 x 2 table used to compute ROC curves

	Carriage seroincidence D+	*No carriage seroincidence D-*
IgG < t	*a*	*b*
IgG ≥ t	*c*	*d*

Figure 28 Example ROC curve



Study-specific correlates of protection for each serotype were combined across studies by computing a weighted average. Studies with larger numbers of seroincidence events had smoother ROC curves and presumably a more accurate estimation of the correlate of protection. This was taken into account when combining across countries with each correlate of protection weighted according to the square root of the number of children with a rise in antibody in each study.

The second method for derivation of correlates of protection used generalised additive models (GAM) for binary data. In a similar analysis to a logistic regression model, the generalised additive model for binary data analyses the log-odds of the outcome, however relaxes the required linear relationship between the outcome (seroincidence) and the predictor variable (IgG). Instead of a linear relationship, the model fits a smooth function thus allowing the functional form of the relationship to be any shape that suits the data.

A GAM was applied to each study and serotype separately with additional adjustments in the model for age at vaccination and sex. The probability of seroincidence at each level of antibody in the dataset was predicted from the models. For each set of predicted probabilities, a smooth spline was applied and the point at which the smoothed probability function fell below 0.05 was taken as the threshold of protection.

The arbitrary cutoff of 0.05 applied to the probability functions was appropriate for this analysis as nasopharyngeal carriage of pneumococcus is a common event and therefore the predicted probability of carriage was much greater than 0.05 in most cases. For serotype 1 however, carriage probabilities were low and the 0.05 cutpoint could not be reasonably applied.

These methods are appropriate for high incidence settings and such methods could not be transferred to analysis of rare diseases.

These models were fitted using the gam function from the mgcv package in R.

```
gam(antibody_increase ~ s(log10IgG) + s(age) + sex + s(country_cohort, bs= "re"), family=binomial, data = data)
```

The probability of seroincidence was derived from GAM models at the lower limit of detection of the assay (negligible antibody levels) and at the level of antibody equivalent to the ROC correlate of protection. From these two probabilities, the estimated protective efficacy of antibody at the level of the ROC correlate of protection was computed as follows;

$$PE = 1 - \left[\frac{\text{Probability of carriage at ROC correlate of protection antibody}}{\text{Probability of carriage at assay lower limit of detection}}\right] \times 100\%$$

Linear mixed effects models were used to compute combined estimates across all serotypes. For the calculation of the average difference in antibody thresholds between low or lower middle income countries (L/LMIC) and high or upper middle income countries (H/UMIC), a weighted mixed effects model was used with independent random intercepts for country cohort and serotype. Low/High income classification was included as a fixed effect and data were weighted according to the square root of the number of 'events' (number of children with a rise in antibody) in each country cohort.

From each ROC curve, the antibody level which maximised the sum of sensitivity + specificity was retained as the antibody level most able to discriminate between

those with and without presumed carriage acquisition in the post-vaccination period.[114,115]

Protective thresholds from ROC curves were compared across income strata (high/upper middle income countries (H/UMIC) vs low/lower middle income countries (L/LMIC)) using mixed effects models. In addition, the proportion of infants with maternal antibody prior to vaccination was compared. Infants with antibody against pneumococcal serotypes prior to vaccination are likely indicative of mothers who had been susceptible, exposed and infected (likely with asymptomatic carriage) within a time period which enabled transplacental transfer of antibody to occur. The seroprevalence of type-specific pneumococcal antibodies in infants aged ~8 weeks is therefore interpreted as a surrogate measure of the force of infection and its association with calculated ROC antibody thresholds was investigated.

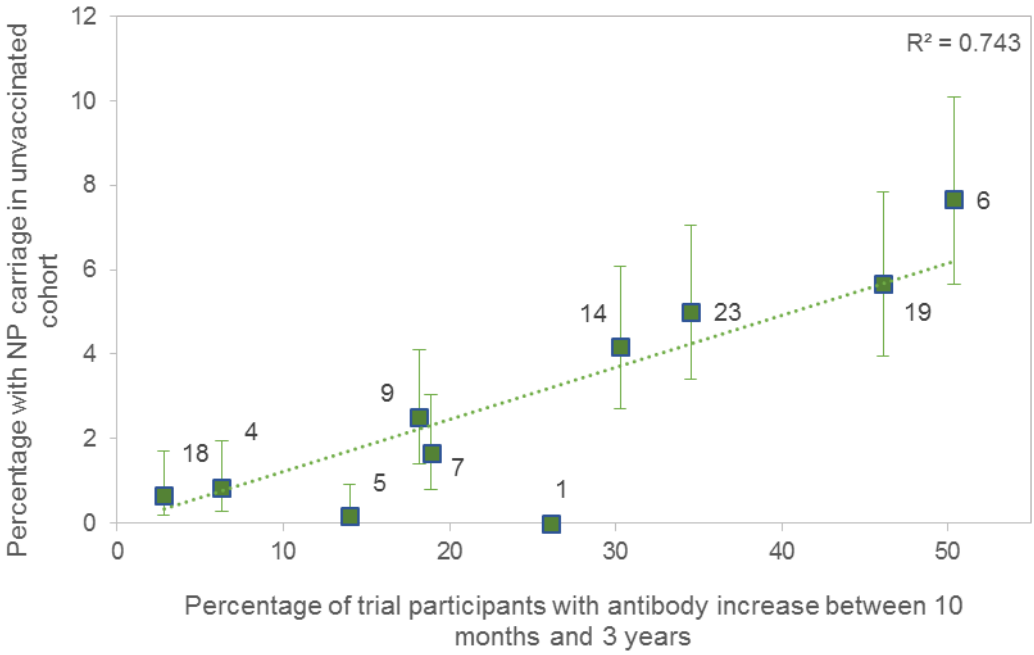
Sensitivity analyses were undertaken using different definitions of seroincidence to explore the effect of misclassification.

7.3 RESULTS

Antibody increase and circulating serotypes in the community

There was a strong relationship (R^2=0.743) between seroincidence in the immunogenicity trial participants in Nepal, and nasopharyngeal carriage prevalence among children in the same community as the trial participants. Because antibodies to some serotypes are cross-reactive to other serotypes within the same serogroup (vaccine-related types), and because multiple serotypes within a serogroup were circulating in the community carriage study, it was not possible to know which serotype might be responsible for an observed antibody increase among the immunogenicity participants. For this reason, serotype data from nasopharyngeal carriage observations were combined into serogroups. With the exception of serotypes 1 and 5, confidence intervals for nasopharyngeal carriage included the line of best fit (Figure 29).

Figure 29 The percentage of participants in a randomised PCV immunogenicity trial in Nepal with an increase in vaccine serotype IgG between 10 months and 3 years of age, and serogroup-specific nasopharyngeal carriage prevalence in a cohort of healthy unvaccinated children from the same community



The dotted line represents the line of best fit from linear regression with intercept set at 0. There was no carriage of serotype 1 detected. 95% confidence intervals use the binomial exact method.

Serotype-specific antibody thresholds of protection against seroincidence

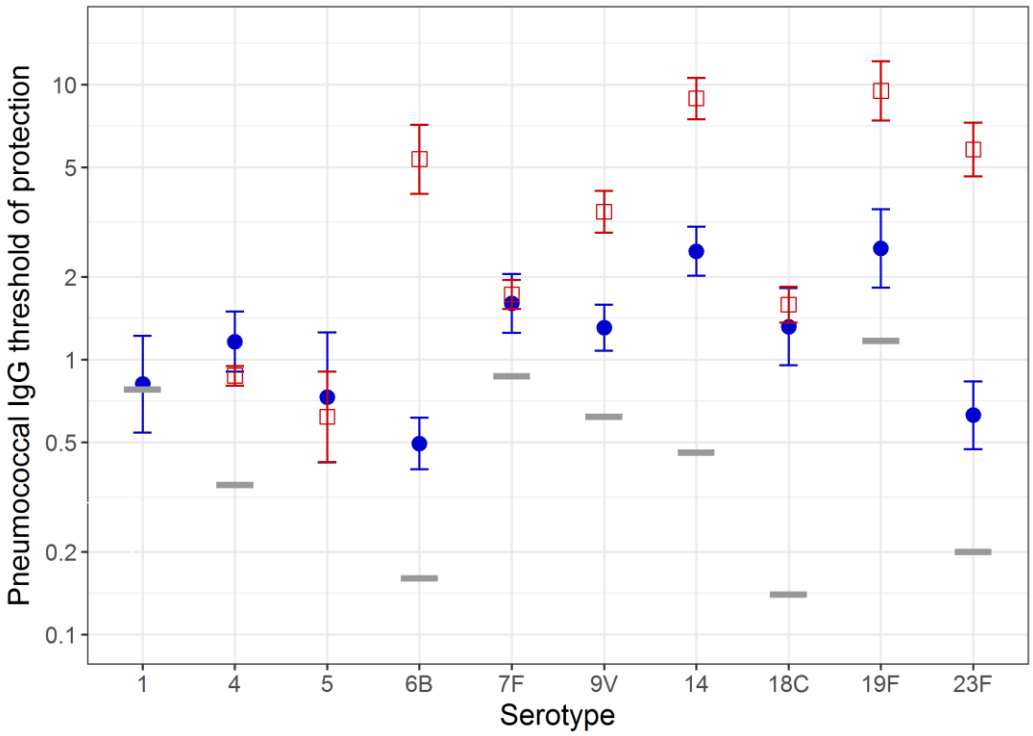
Pneumococcal IgG serum concentrations were available from 29 studies (19 countries, and 5224 children). Studies were conducted in Europe (Denmark, The Czech Republic, Finland, France, Germany, The Netherlands, Poland, Slovakia, Spain, Sweden), Asia (India, Japan, Malaysia, Nepal, Philippines, and Singapore), Africa (Mali, Nigeria), and Chile. Children received either PCV7, PCV10, or PCV11 according to different priming schedules: 6,10,14 weeks of age; 2,3,4 months; 3,4,5 months; or 2,4,6 months of age (Supplementary Table).

Figure 30 displays the serotype-specific thresholds of protection against carriage derived from ROC curves (blue circles) and from GAM models (red squares). Serotype-specific thresholds against IPD from Andrews et al[60] are also displayed for comparisons, and were in general, lower than thresholds for carriage.

Pneumococcal antibody thresholds of protection derived from ROC curves varied by serotype. The lowest threshold was 0.50 μg/mL and the highest was 2.54 μg/mL, for pneumococcal serotypes 6B and 19F antibodies respectively (Figure 30, Table 12).

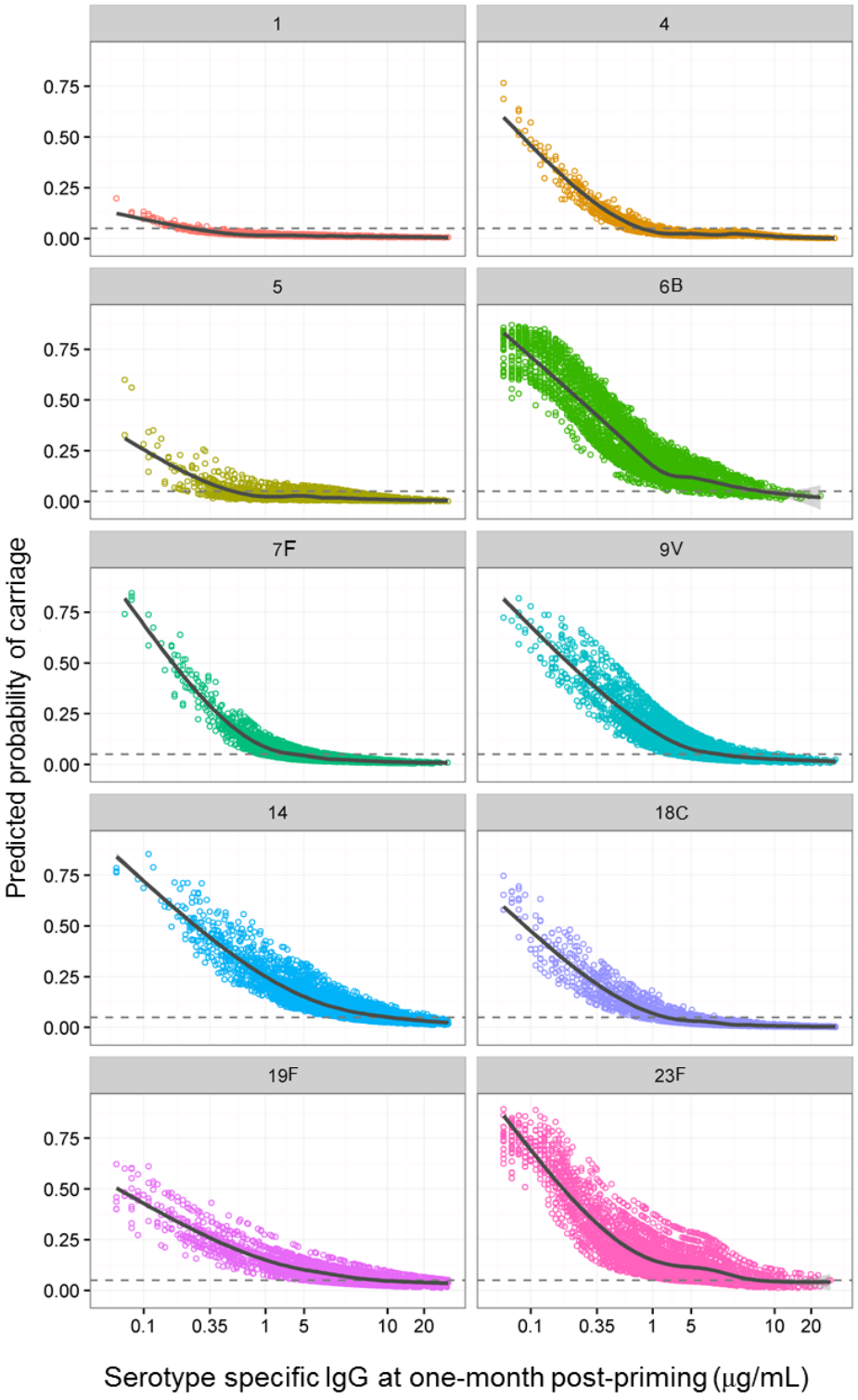
The probability of seroincidence from GAM models declined as antibody increased, until a point where the probability became negligible and curves flattened out (Figure 31). For serotype 1 the probability of seroincidence was low for all levels of post-primary antibody thus the point at which the probability became negligible could not be defined (Figure 31). For serotypes 4, 5, 7F and 18C, the post-primary antibody thresholds from both ROC and GAM methods were essentially the same, thus the probability of seroincidence is negligible for antibody concentrations above these levels. For the remaining serotypes (6B, 9V, 14, 19F and 23F) antibody concentrations of at least 3.45 μg/mL were required to minimise seroincidence to the 5% level. However, substantial protection against seroincidence was available with antibody at levels of 0.50 to 2.54 μg/mL as defined by ROC curves, and protective efficacy estimates at these levels was between 62% and 87% (Table 12).

Figure 30 Serotype-specific pneumococcal antibody correlates of protection against seroincidence in infancy



Blue filled circles represent serotype specific correlates of protection against seroincidence (defined as any rise in antibody between the one-month post-priming vaccine dose and the booster dose) from ROC curves and their 95% confidence intervals. Red open squares represent thresholds of protection from GAM models. For reference, grey bars are antibody correlates of protection for IPD from Andrews et al[60] (not available for serotype 5). Data are also presented in Table 12. ROC: Receiver operator characteristic; GAM: generalised additive models; IPD invasive pneumococcal disease

Figure 31 The relationship between the serotype-specific probability of seroincidence estimated from generalised additive models, and the antibody concentration measured one-month after administration of 2 or 3 priming doses of pneumococcal conjugate vaccine



Each dot represents one participant. Horizontal dashed lines at a probability of 0.05.

Table 12 Serotype-specific pneumococcal antibody correlates of protection against seroincidence in infancy

Serotype	*Protective antibody concentration (ROC) (µg/mL)*	*LCL (µg/mL)*	*UCL (µg/mL)*	*Antibody level equivalent to 5% seroincidence probability (GAM)* (µg/mL)*	*LCL (µg/mL)*	*UCL (µg/mL)*	*Probability† of seroincidence at antibody level from ROC curves (column 2)*	*PE of ROC antibody threshold compared to antibody at the LLD*
1	0.81	0.54	1.22	N/A	N/A	N/A	N/A	N/A
4	1.16	0.90	1.49	0.87	0.81	0.95	5%	>90%
5	0.73	0.42	1.26	0.62	0.42	0.90	5%	>90%
6B	0.50	0.40	0.61	5.35	4.01	7.14	33.1%	62%
7F	1.60	1.25	2.05	1.73	1.53	1.95	5%	>90%
9V	1.31	1.08	1.58	3.45	2.90	4.11	12.4%	86%
14	2.48	2.02	3.04	8.90	7.49	10.58	15.0%	84%
18C	1.32	0.95	1.82	1.58	1.37	1.84	5%	>90%
19F	2.54	1.83	3.53	9.48	7.39	12.15	9.9%	87%
23F	0.63	0.47	0.83	5.81	4.64	7.28	21.7%	74%

Data are displayed in Figure 30. PE: protective efficacy, ROC: receiver operator characteristic curve method for derivation of correlates of protection, GAM: generalised linear model method for derivation of thresholds of protection, LLD: lower limit of assay detection, LCL: lower 95% confidence limit, UCL: upper 95% confidence limit

Protective efficacy is calculated as 1-(pr(ROC)/pr(LLD))x 100%.

pr(ROC) is the probability of seroincidence predicted from GAM model at a level of antibody equivalent to the ROC correlate of protection.

pr(LLD) is the probability of seroincidence predicted from GAM model at the lower limit of detection of the assay (0.05 µg/ml)

**Antibody level at which the probability of seroincidence is less than 5%*

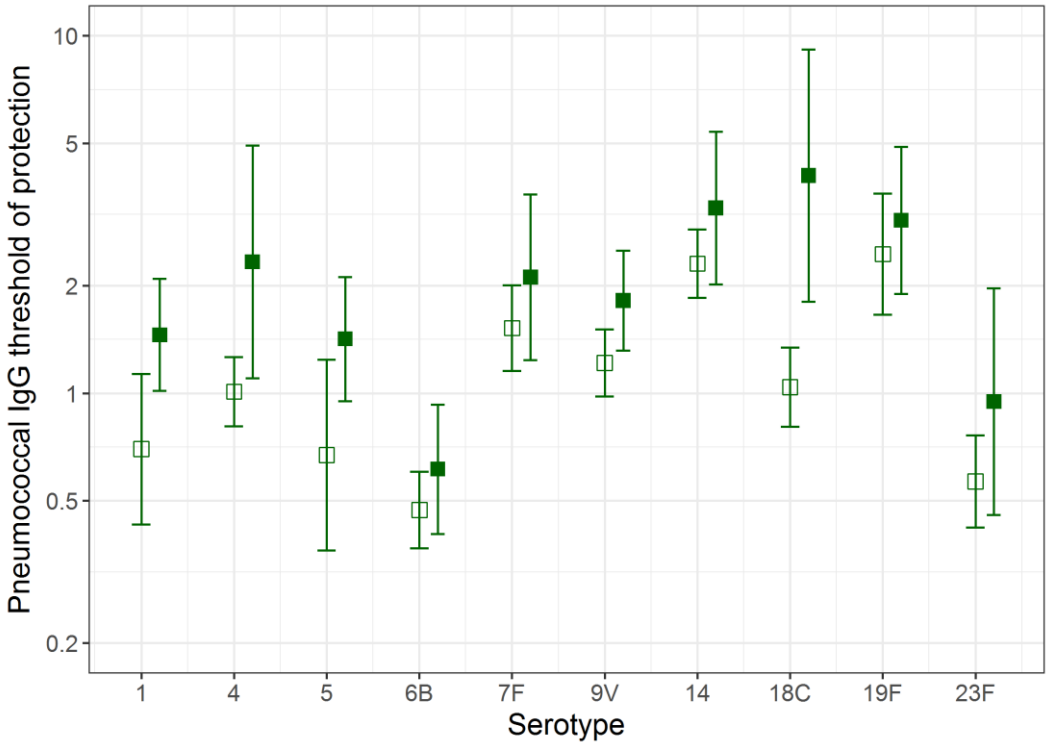
†Predicted probability from GAM model

For serotypes 4, 5, 7F and 18C ROC and GAM thresholds had substantially overlapping confidence intervals. The probability of seroincidence is considered to be the same (5%) for both methods.

High and low income countries

Overall across all serotypes, thresholds derived from ROC curves were twice as high in L/LMICs, than in H/UMICs (GMR 2.15 95% CI 1.46–3.17, p=0.0024) although for individual serotypes confidence intervals overlapped (Figure 32). Seroprevalence of maternal antibodies in infants prior to vaccination was also higher in L/LMICs (p=0.0006), and there was a significant relationship between the proportion of children with maternal antibody (force of infection) and the estimated correlate of protection for the same serotypes (p=0.0347) (Figure 33).

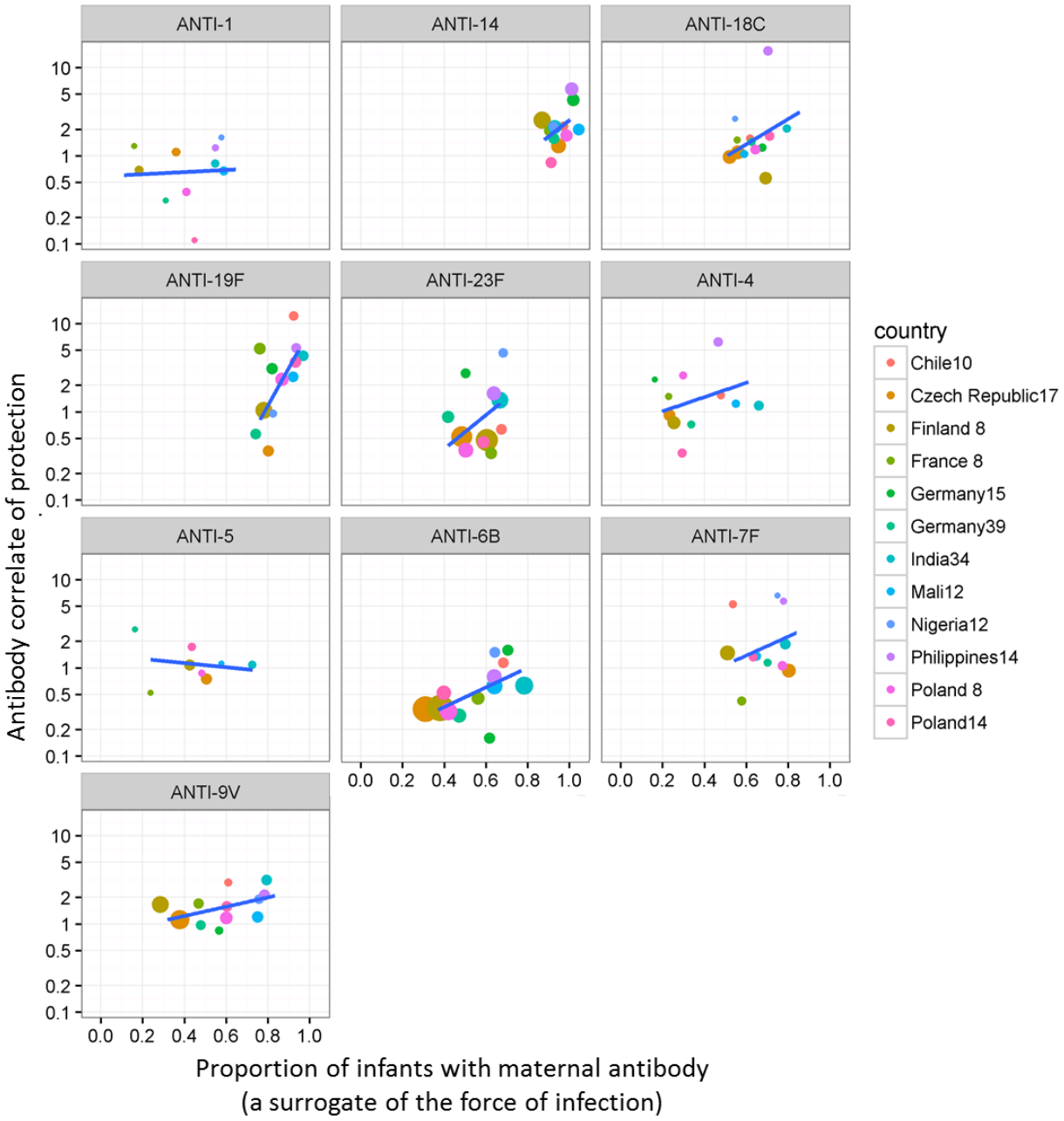
Figure 32 Serotype-specific pneumococcal antibody correlates of protection in infancy stratified by income status of country setting



Bars represent serotype specific correlates of protection and their 95% confidence intervals. Filled squares: Low or lower middle income countries (L/LMIC): India, Mali, Nepal, Nigeria, The Philippines; Open squares: High or upper middle income countries (H/UMIC): Chile, Czech Republic, Denmark, Finland, France, Germany, Japan, The Netherlands, Norway, Poland, Singapore, Slovakia, Spain, Sweden.

L/LMIC vs H/UMIC: GMR 2.15, 95%CI (1.46–3.17), p=0.0024

Figure 33 The relationship between the proportion of infants with maternal antibody prior to vaccination (a surrogate of the force of infection) and the estimated antibody concentration associated with protection in the same study



Each dot represents a single country cohort with size relative to the number of children with antibody which increased (seroincidence). Blue lines are linear regression lines of best fit. The overall relationship between the proportion of infants with maternal antibody and correlates of protection was statistically significant when combined across all serotypes and all countries in a linear model with country-specific and serotype-specific random effects p=0.0347.

Sensitivity analysis

Sensitivity analyses were conducted using different definitions of seroincidence to explore the effect of misclassification. Some participants may have had an antibody response to carriage which had decayed by the time of the booster dose resulting in misclassification of an unknown proportion of children as not having had any carriage acquisition. A different definition of seroincidence – an increase in antibody between priming and booster vaccines, or a decrease in antibody of at most *x*% from one-month post-priming values – was investigated to determine if it would alter the correlates of protection derived from the data. Decreases of 5%, 10%, 15% and 20% were used. Correlates of protection derived using these different thresholds were similar to those derived using the original definition. The degree of difference from the original correlates ranged from a decrease of 0.39 μg/mL for serotype 5 to an increase of 0.23 μg/mL for serotype 14. The average difference was an overall decrease in the computed thresholds for protection of 0.064 μg/mL.

Table 13 Sensitivity analyses of correlates calculated using different definitions of seroincidence

Serotype	Overall correlate of protection (μg/mL)	Correlates of protection derived using varying definition of seroincidence*				Maximal difference from original value
		5%	10%	15%	20%	
1	0.81	0.97	0.95	0.95	0.66	-0.16
4	1.16	1.11	1.14	1.16	1.17	+0.05
5	0.73	0.88	0.94	1.12	0.86	-0.39
6B	0.50	0.57	0.55	0.57	0.55	-0.07
7F	1.60	1.60	1.59	1.61	1.65	-0.05
9V	1.31	1.41	1.44	1.40	1.45	-0.14
14	2.48	2.36	2.25	2.39	2.40	+0.23
18C	1.32	1.43	1.50	1.43	1.45	-0.18
19F	2.54	2.51	2.56	2.32	2.43	+0.22
23F	0.63	0.73	0.75	0.78	0.69	-0.15
						Average: -0.064

** Seroincidence defined as any increase in antibody between priming and booster vaccines, or a decrease in antibody of at most x% from one-month post-priming values. Decreases of 5%, 10%, 15% and 20% were used.*

7.4 DISCUSSION

This analysis of infant immunogenicity data from 5224 infants in 29 studies in 19 countries, estimates pneumococcal serotype-specific antibody concentrations associated with protection against an increase in antibody following the primary vaccination series, which we infer as a proxy for nasopharyngeal carriage. It reveals that the correlate for carriage protection is much higher than the current pooled correlate of vaccine serotype IPD efficacy (0.35 μg/mL), used as the basis for licensing new vaccines. All calculated antibody concentrations in our study of carriage seroincidence were higher than serotype-specific correlates of protection previously derived for IPD.[22] This is consistent with mucosal immunity to pneumococcus being more likely to be dependent on IgA rather than IgG, as has been demonstrated in mice, and observations that IgA levels are typically lower than and proportional to systemic IgG levels.[116] Alternatively this may indicate that higher blood levels of IgG correlate with local production of IgG at the mucosa or that high levels of circulating IgG is required to directly raise the mucosal IgG concentration.

Relationship between seroincidence and circulating serotypes detected in nasopharyngeal swabs

The serogroup-specific prevalence of nasopharyngeal carriage in unvaccinated healthy children in a cross-sectional carriage study in Nepal was closely associated with the proportions of children who had antibody increases in the PCV immunogenicity trial. The two studies were conducted in the same period and location, providing useful comparative data and compelling, indirect, evidence of the validity of using serology to infer colonisation. For serotypes 1 and 5, the frequency of seroincidence was not closely associated with nasopharyngeal carriage prevalence in the community. No positive swabs were obtained for serotype 1, and only one serotype 5 colonisation episode was detected among 600 children tested, yet large proportions of children in the immunogenicity trial had antibody increases by 3 years of age for these serotypes. These serotypes appear to circulate more widely than is indicated by cross-sectional carriage studies, consistent with data showing a short half-life of

colonisation for serotypes 1 and 5, and rapid transmission.[117] Serotypes 1 and 5 are associated with the majority of invasive disease among young children in Nepal,[113,118] therefore serological methods to detect natural boosting of antibody may be better at assessing rates of colonisation for these serotypes than nasopharyngeal swabs, which appear to under-detect true prevalence.

Protective antibody thresholds

Substantial variation exists in the level of antibody associated with protection against carriage. Thresholds defined using ROC curves represent the antibody level which best discriminates between those who are protected and those who are not.

Using ROC curves, the lowest correlates of protection (0.50 μg/mL and 0.63 μg/mL) were observed for serotypes 6B and 23F respectively, and the highest were 2.54 μg/mL and 2.48 μg/mL for serotypes 19F and 14. Andrews et al. calculated correlates of protection using IPD cases in UK children and found similar patterns of differences between serotypes, with higher levels of antibody required for protection against 19F and lower levels for 6B, and 23F.[22] These findings are consistent with previous evidence suggesting more IgG is required to kill serotype 19F than 6B in vitro.[119]

GAM thresholds can be interpreted as the level of antibody at which the probability of seroincidence becomes negligible (<5%). For serotypes 4, 5, 7F, and 18C, the ROC and GAM thresholds were similar, thus ROC correlates are sufficient to provide individual protection. For serotypes 6B, 9V, 14, 19F and 23F, much higher levels of antibody were consistent with a probability of seroincidence of 5% than those obtained from ROC curves, thus the ROC correlates for these serotypes indicate less than complete protection for the individual.

These antibody concentrations show similarities with thresholds derived for other mucosal endpoints. Our estimate of 0.50 μg/mL for serotype 6B derived using ROC methods, had a protective efficacy of 62%. This is very similar to the predicted vaccine efficacy (VE) of ≥ 65% against acute otitis media (AOM) at 0.5 μg/mL reported by Jokinen et al.[120] Thus an antibody concentration of 0.5 μg/mL more than halves the probability of 6B carriage and the majority of protection

occurs with induction of this small amount of antibody. The induction of an addition 4.85 μg/mL of antibody will reduce the probability of 6B carriage to less than 5% (PE >90%) however such large amounts may not be required to see clinical benefit and population effects.

In contrast, VE against serotype 23F AOM was negligible in Jokinen et al at 0.65 μg/mL with wide confidence intervals[120] whereas in our analysis protective efficacy against colonisation was 74% at this antibody level. Millar et al investigated 17 cases of serotype 23F carriage acquisition after vaccination with PCV7 and report no carriers achieving serotype 23F antibody concentrations greater than 4 μg/mL before acquisition. This threshold value for total protection is slightly lower than the estimate from GAM analysis of 5.81 μg/mL (95%CI 4.64–7.28) possibly due to the small number of children in whom carriage was detected.[121]

The probability of seroincidence predicted from GAM models for serotypes 9V, 14, 19F and 23F was between 10% and 22% for antibody at the level of the ROC thresholds. Thus these levels of antibody provide substantial protection even though not representing levels which provide absolute protection for an individual.

High and low income countries

Antibody concentrations associated with protection against seroincidence calculated from studies conducted in L/LMICs were on average twice as high as those in more wealthy countries. Carriage of pneumococci increases with factors such as household size, exposure to smoke (including from cooking fires), and underlying illnesses.[122-124] Such factors are also related to socioeconomic status. Higher rates of nasopharyngeal colonisation in low income settings have been observed from carriage studies in young children[124] and in rural settings compared with urban.[123] Higher rates of colonisation in the community (i.e. higher force of infection) would increase the probability that such colonisation occurs during pregnancy, resulting in higher prevalence of maternally-derived pneumococcal antibodies in early infancy prior to vaccination. Our analysis shows the seroprevalence of maternal antibodies in infants (a surrogate for the force of infection) is higher in L/LMICs, and is significantly related to the

antibody concentration required for protection against seroincidence for the same serotypes. The higher level of antibody needed for protection in L/LMICs may relate to a higher infectious load delivered at each nasopharyngeal exposure event, requiring higher levels of antibody for individual protection. In addition, the variation across serotypes in both force of infection and antibody levels required for protection indicates biological variation in characteristics of the bacteria resulting in varying abilities of serotypes of pneumococci to colonise the nasopharynx.

Limitations and strengths

Deriving robust thresholds of protection from any single study is problematic due to the large degree of variation between studies in both VE and estimated correlates of protection.[22,50,55,57] The main strength of this analysis is therefore the uniquely large dataset of paired immunogenicity data in infants from a large number of studies. Data are from randomised controlled trials across a diverse range of countries, yet all results were assayed in one laboratory, removing the influence of inter-laboratory variation.

The use of seroincidence as a proxy for carriage improves the detection of acquisition events as the data are not limited by the timing and frequency of nasopharyngeal swabs but instead create a historical record of the acquisition events experienced during the period between the post primary visit and the booster vaccination. This improves the differential misclassification that affects cross-sectional carriage studies, however cannot remove it entirely. Misclassification of true carriage events in our data can be due to (1) rises and then falls in antibody between blood draws resulting in an overall observed decline, or (2) rises in antibody from transient exposure but not a true carriage event.

Even in those with an overall observed decline in antibody level, it is likely that high antibody levels at the second time point are relatively more likely to be the result of undetected carriage in the intervening period than those with low antibody at the second time point. This would be less likely to affect those with lower post primary responses as a carriage event in those participants would results in an overall observed rise in antibody between the two time points.

Misclassification would therefore happen more often in those with high antibody to start with, resulting in an overall underestimation of the proportions with carriage. Underestimation and misclassification may introduce bias into the results. The effect of such potential biases on these findings is hard to quantify but could be further explored in the future using modelling studies.

There were varying lengths of time between priming and vaccine booster doses across studies; studies with a longer interval have a reduced likelihood of detecting seroincidence and would underestimate the correlates of protection.

7.5 CONCLUSION

The variation between serotypes in antibody levels which are associated with an inferred protection against carriage in this investigation support previous observations that combined correlates of protection for all serotypes are inaccurate for some serotypes.[125]

Consideration should be given to the use of serotype-specific thresholds of protection in clinical trials to demonstrate induction of antibody levels associated with high protection against colonisation to support vaccine development, dosing schedule optimization, and the assessment of vaccine impact.

In settings with high force of infection, often low-income settings, higher levels of antibody are required to interrupt transmission. These findings are important for global vaccination policy especially with regard to assessment of alternative schedules aimed at reducing transmission to gain indirect effects (herd immunity) of the vaccine and maximizing the impact of the vaccine.

8 CONCLUSION

8.1 INTRODUCTION

Routine infant vaccination programmes have changed substantially in recent years with the addition of multiple new vaccines into infant schedules. Additional booster doses have been added when required and priming doses removed when possible. In addition to changes in routine infant schedules, in the UK in 2012 a maternal prenatal vaccination programme against pertussis commenced to combat the rise in incidence of this disease. With each change in a vaccination programme comes the possibility of unintended effects as vaccines interact in ways that are unexpected. Conducting clinical trials to assess every potential interaction between different infant vaccines, different schedules, or to assess the interplay between infant and maternal programmes, would substantially delay the implementation of important life-saving vaccination programmes. It is incumbent upon the research community therefore to use information that is already to hand to provide these answers without recourse to additional expensive and lengthy clinical trials. The availability of data from decades-worth of industry clinical trials of infant vaccines conducted in a wide range of geographic locations, is a vast and previously untapped resource which I have used in this thesis to answer important questions of relevance to decision-makers. In total, data from 49 trials conducted in 27 countries contributed to these analyses.

In Chapters 4 and 5 of this thesis I have investigated the prevalence, decay, and influence of maternal antibodies on infant vaccine responses and modelled the number of weeks delay in an infant immunisation programme that would offset the inhibitory effect of maternal antibody from a prenatal vaccination programme. The relevance of the inhibitory effect of maternal antibody depends on the level of antibody needed to prevent disease. It is possible that infant vaccines are less immunogenic in the presence of a maternal prenatal programme covering the same antigens, yet remain protective. The level of antibody that protects against important diseases varies by antigen, by disease, and are not established for some important clinical endpoints. In Chapter 7 I

have computed the level of antibody induced by pneumococcal conjugate vaccines that is sufficient to prevent acquisition of nasopharyngeal carriage in infants. These analyses use novel analyses of serological data to estimate protective antibody levels. Current statistical methods used to compute thresholds may not be fit for purpose and the performance of such methods is systematically evaluated in Chapter 6.

8.2 SUMMARY OF FINDINGS

In the absence of maternal prenatal immunisation programmes substantial proportions of children worldwide have maternal antibody present at the time of their first vaccination. The presence of maternal diphtheria or tetanus antibodies in infants varies substantially between countries, reflecting differences in vaccination histories of pregnant women. For maternal pertussis or pneumococcal antibodies differences between countries are less pronounced reflecting the widespread circulation of these pathogens. Overall, maternal pneumococcal antibodies decayed with a half-life of 42.5 days. Groups A and C meningococcal antibodies decayed at similar rates of 43 and 40 days respectively.

Maternal antibody inhibited the response to a priming series of infant vaccinations for almost all antigens included in global immunisation programmes. The interference due to the maternal antibody present at the time of the first vaccination also influenced responses to booster doses for some antigens. A wider spacing of doses for infant vaccines did not mitigate these effects, highlighting the importance of the primary immune response at the first exposure to a pathogen in infancy. The age at the first vaccination had a significant positive impact on infant immune response to primary doses of vaccine that was independent of the concentration of maternal antibody present at the time of vaccination. These two effects, which work in opposite directions, have never previously been adequately separated and no independent estimates existed prior to this work. The published manuscript based on these models of maternal antibody was discussed at the World Health Organisation Strategic Advisory Group of Experts, Working Group on Pneumococcal Conjugate Vaccines in 2017, revealing the importance and current relevance of this topic.

Understanding the level of antibody induced by the vaccine that would protect against disease allows for vaccines to be licensed without conducting large clinical efficacy trials. Such threshold levels of antibody have been used to successfully licence new vaccines such as the 10- and 13-valent pneumococcal vaccines, based on immunogenicity alone. However, the methods used to compute the threshold applied to such immunogenicity trials is inherently flawed, as revealed by the simulations in Chapter 6. In situations where vaccines have high efficacy, thresholds will either be highly inflated or not-estimable and alternative methods are needed. The use of regression models based on serological definitions of infection as shown in Chapter 7, offers and alternative way forward.

In Chapter 7 I use methods based on ROC curves and generalised additive models, to compute protective antibody thresholds for pneumococcal carriage. These analyses use a serological definition of carriage rather than culture from nasopharyngeal swabs. Carriage is presumed to have been established if an infant had a rise in antibody levels between their one-month post-priming study visit (at age ~5 – 7 months) and the booster dose, as normally antibody during this period would decay rapidly. This serological definition was highly correlated with results from nasopharyngeal swabs in a cross-sectional study, confirming the validity of the method.

Threshold antibody levels indicative of protection using serological methods varied between serotypes and were higher than serotype-specific thresholds previously established for invasive disease. It was also seen that more antibody was needed to establish protection against carriage in low/lower-middle income countries than in high/upper middle income countries.

8.3 FURTHER WORK

Whilst many interesting questions were able to be investigated for this doctorate, there remain a number of outstanding areas requiring further research which I intend to pursue in my post-doctoral work.

Half-lives of maternal antibodies for diphtheria, tetanus, and pertussis

With the data available for this doctorate, I was able to characterise the half-lives of pneumococcal and meningococcal maternal antibodies. However, there remain other antigens, such as diphtheria, tetanus, and pertussis, for which maternal antibody half-lives remain poorly characterised, and these are important antigens that are included in maternal immunisation programmes. Half-lives for these antigens were unable to be calculated in this doctorate as data is required from children unvaccinated against the antigen of interest in order to measure maternal antibody levels. Since this doctorate used data from clinical trials of infant vaccines, there were no data from children who were unvaccinated against standard infant vaccines as such a trial would be unethical.

In order to estimate half-lives of diphtheria, tetanus, and pertussis maternal antibodies, two blood samples from infants who have not yet received their first vaccine are needed. These data can be obtained from studies of mother-infant pairs in which a cord blood sample was taken as well as a blood sample from the infant prior to their first vaccination at 2 months of age. There are multiple such studies and the principal investigators of these studies and I have formed a collaborative group to do this work.[40,90,126-128]

Correlates of protection against nasopharyngeal carriage for additional serotypes

The correlates of protection against nasopharyngeal carriage of pneumococcus in this thesis covered 10 serotypes of pneumococcus as the data were obtained from clinical trials of the 10-valent pneumococcal vaccine. There is interest in knowing the equivalent correlates of protection for the additional serotypes contained in the 13-valent Pfizer vaccine, in particular for serotype 3 which has not been shown to be effective in the UK.[129-131] This could be done by repeating the project using data from studies of PCV13.

Wider use of seroepidemiological methods to estimate vaccine efficacy

The use of serological data to define the infection status of an individual is a powerful analysis that can be further utilised in many areas. In this thesis I used

seroepidemiological methods to define pneumococcal carriage infections and estimate correlates of protection. In an additional paper not included in the thesis, I used similar seroepidemiological methods to define typhoid infection status and estimate the vaccine efficacy of a typhoid conjugate vaccine using data from an immunogenicity study.[107] A commentary on this paper notes the attractive aspect of such methods is that they pave the way for future typhoid conjugate vaccines to be evaluated in endemic settings at a fraction of the recruitment required for traditional typhoid efficacy trials.[132] In addition, seroepidemiological methods may be useful to assess vaccine efficacy of new pneumococcal protein vaccines.

My analyses utilised data from previously conducted studies that were conveniently available. The timing of blood sampling was, therefore, not done to optimise the estimation of serologically defined infections. Going forward, if trials to estimate vaccine seroefficacy are to be conducted for candidate conjugate vaccines or new pneumococcal protein vaccines, then the design of such trials needs to be carefully considered. Information on how to conduct such studies, addressing the time points that are optimal for blood sampling, the number of samples needed, and the methods used to compute vaccine efficacy, would be useful to the wider research community. I plan to conduct a simulation study that simulates serological data from clinical trials in order to determine the optimal time points for blood sampling in order to inform clinical trialists, vaccine developers, and funders planning such studies.

Wider use of seroepidemiological methods in the meta-analysis of vaccine trials

Serological methods are not just useful in the assessment of vaccine efficacy of new vaccines, but could be used to inform vaccine choice for the comparison of currently licensed vaccines. For licensed pneumococcal conjugate vaccines, there is currently little evidence to show whether one vaccine is better than the other therefore both vaccines (PCV10 and PCV13) are widely used and both are considered efficacious. In cost-effectiveness models both vaccines are assumed to have the same efficacy as the older PCV7 vaccine.[133,134] This is unlikely to be

correct, however more accurate estimates do not exist for use in cost-effectiveness modelling.

Seroepidemiological methods applied to clinical trial data can be used to meta-analyse previous head-to-head studies of PCV7, PCV10, and PCV13 to determine the relative efficacy of each and plans for this work are underway.

8.4 CONCLUSION

These data on key factors that influence the infant immune response, provide valuable insights for the planning of infant immunisation schedules. Estimates of half-lives of maternal antibody decay provide important information that can be combined with estimates of the interference associated with maternal antibody to use for modelling the impact of maternal vaccination programmes. The observation that older infants respond better to vaccination than younger infants, independent of maternal antibody interference, is key when making decisions about the age of administration of the first dose of vaccine. Data on the level of pneumococcal antibody that protects against nasopharyngeal carriage provide a way of easily comparing vaccine effectiveness against these serotypes. In addition, thresholds of protection were derived using novel methods less subject to the methodological biases inherent in previous methods and these can be used to compute thresholds that could be applied to other vaccines.

The design of effective vaccination schedules that protect populations from disease is increasingly complex. This body of work provides key data useful to global vaccine policy-makers for the planning and implementation of cost-effective programmes that save millions of lives.

9 APPENDIX

Supplementary Table: List of studies included in analysis for each chapter

Cohort	Study Number (Prime + boost)	Country	N Infants	Age at enrolment (weeks), mean, [min, max]	Study vaccine schedule	Ch 4	Ch 5	Ch 7	Ref
1	101197	Korea	84	6.3 [6, 10]	2, 4, 6 months	Ch 4	Ch 5		[135]
2	101222 + 104065	Philippines	725	6.4 [5, 9]	6, 10, 14 weeks	Ch 4	Ch 5		[136]
3	101223 + 106602	Nicaragua, Argentina, Chile	580	8.4 [8, 10] 6.9 [6, 10] 8.6 [8, 12]	2, 4, 6 months		Ch 5		[137]
4	104489	Dominican Republic	288	7.8 [6, 12]	2, 4, 6 months	Ch 4			[138]
6	104871	Korea	450	8.8 [6, 14]	2, 4, 6 months	Ch 4			[139]
7	105539	Denmark, Norway, Sweden, Slovakia	84, 68, 120, 60	12.8 [9, 16] 11.6 [8, 16] 12.3 [8, 15] 11.4 [8, 16]	2, 3, 4 + 11 months			Ch 7	[140]
8	105553 + 107046	Poland, Finland,	302, 522,	7.5 [5, 12] 10.0 [5, 12]	2, 3, 4 + 12-18 months	Ch 4	Ch 5	Ch 7	[141,142]

		France	76	9.2 [6, 14]					
9	105910 + 110478	Russia	413	12.9 [11, 18]	3, 4, 5 months	Ch 4	Ch 5		143,144
10	106208 + 110031	Chile	83	8.3 [6, 12]	2, 4, 6 + 18-21 months	Ch 4	Ch 5	Ch 7	145
11	106786 + 111344	Finland	443	10.0 [8, 12]	2, 3, 4 months	Ch 4	Ch 5		146,147
12	110521 + 113166 + 113199	Mali, Nigeria	140, 69	7.4 [5, 10] 6.3 [5, 9]	6, 10, 14 weeks + 15-21 months	Ch 4	Ch 5	Ch 7	148,149
13	112157 + 113978	Poland	420	8.7 [7, 12]	2, 3, 4 months	Ch 4	Ch 5		150
14	107007 + 109509	Poland, Philippines	191, 185	7.4 [6, 12] 7.5 [5, 12]	2, 4, 6 months, or 6,10,14 weeks, + 12-18 months	Ch 4	Ch 5	Ch 7	151,152
15	217744/078 + 217744/081	Germany	137	11.6 [8, 16]	3, 4, 5 + 12-23 months	Ch 4	Ch 5	Ch 7	153
16	107005 + 109507	Germany, Spain, Poland	128, 194, 317	10.1 [6, 17] 7.8 [6, 12] 7.4 [6, 16]	2, 4, 6 + 11-18 months			Ch 7	141,142, 154
17	107017 + 107137	Czech Rep	410	12.3 [8, 20]	3, 4, 5 + 12-15 months	Ch 4	Ch 5	Ch 7	155
18	107737	Spain, Greece	70	8.8 [8, 10] 9.8 [7, 16]	2, 4, 6 months	Ch 4	Ch 5		156
20	110142 + 111053	Netherlands	469	7.5 [6, 12]	2, 3, 4 + 11-12 months			Ch 7	157,158

22	217744/076	Spain	467	9.1 [8, 12]	2, 4, 6 months	Ch 4	Ch 5		159
23	217744/077	Australia	278	8.6 [8, 12]	2, 4, 6 months	Ch 4	Ch 5		160
25	217744/086 + 217744/095	Germany	453	8.7 [6, 12]	2, 4, 6 months	Ch 4	Ch 5		161,162
27	217744/097	Spain	462	9.1 [7, 14]	2, 4, 6 months	Ch 4	Ch 5		163,164
34	111188 + 112909	India	195	1 month [1, 2 months]	6, 10, 14 weeks + 9-18 or 15-18 months	Ch 4		Ch 7	165,166
35	111654 + 113266	Singapore	224	7.25 [5, 11]	2, 3, 5 + 18-21 months			Ch 7	167
39	105554 + 106623	Germany	108	2.2 months [1, 4 months]	2, 3, 4, + 11 months	Ch 4		Ch 7	168
40	112640	Japan	229	13.5 [12, 16]	3, 4, 5 + 18 months	Ch 4		Ch 7	169
41	107058	Finland	138	10.8 [9, 12]	3, 4, 5 + 12 months			Ch 7	170
42	103488	Germany	677	2.1 months [1, 2 months]	2, 3, 4 months	Ch 4		Ch 7	171
43	100480	Thailand	850	8.5 [7, 11]	2,4,6 months	Ch 4			172
44	100478	Philippines	735	7.1 [5, 23]	6,10,14 weeks	Ch 4			173
	OVG 2009/03	Nepal	191	6.1 [6, 9]	6, 10, 14 weeks or 6,14 weeks + 9 months			Ch 7	112
TOTAL									
31 cohorts	**49 studies**								

In addition to the papers and URLs cited above, further details for each study (with the exception of OVG 2009/03), including full clinical reports can be found at https://www.gsk-clinicalstudyregister.com.

10 REFERENCES

1. World Health Organisation. WHO recommendations for routine immunization - summary tables. http://www.who.int/immunization/policy/immunization_tables/en/ (accessed 28 June 2017.
2. Salisbury D, Ramsey M, Noakes K. Immunisation against infectious disease ('the Green Book'). London: Department of Health. 2007, Updated September 2014.
3. World Health Organisation. WHO vaccine-preventable diseases: monitoring system. 2017 global summary. http://apps.who.int/immunization_monitoring/globalsummary/schedules (accessed 28 June 2017.
4. MacLennan R, Schofield FD, Pittman M, Hardegree MC, Barile MF. Immunization against neonatal tetanus in New Guinea: Antitoxin response of pregnant women to adjuvant and plain toxoids. *Bull WHO* 1965; **32**(5): 683-97.
5. Rahman M, Chen LC, Chakraborty J, et al. Use of tetanus toxoid for the prevention of neonatal tetanus. 1. Reduction of neonatal mortality by immunization of non-pregnant and pregnant women in rural Bangladesh. *Bull WHO* 1982; **60**(2): 261-7.
6. Siegrist CA. Neonatal and early life vaccinology. *Vaccine* 2001; **19**(25-26): 3331.
7. Schofield F, Tucker V. Neonatal Tetanus in New Guinea: Effect of Active Immunization in Pregnancy. *BMJ*; **2**(5255): 785-9.
8. European Centre for Disease Prevention and Control. Vaccine schedules in all countries of the European Union. http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx (accessed June 1, 2017.
9. World Health Organization. Tetanus vaccines: WHO position paper. *Weekly Epidemiological Record*; **6**(92): 53-76.
10. Edwards KM, Decker M. Pertussis Vaccines. Philadelphia: Saunders; 2013.
11. World Health Organization. Pertussis vaccines: WHO position paper. *Weekly Epidemiological Record* 2015; **35**(90): 433-60.
12. World Health Organization. WHO SAGE pertussis working group. Background paper. . *http://wwwwhoint/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_webpdf?ua=1*.
13. Guimarães LM, da Costa Carneiro ELN, Carvalho-Costa FA. Increasing incidence of pertussis in Brazil: a retrospective study using surveillance data. *BMC Infect Dis* 2015; **15**(1): 442.
14. Solano R, Gómez-Barroso D, Simón F, et al. Retrospective space-time cluster analysis of whooping cough re-emergence in Barcelona, Spain, 2000-2011. *Geospatial Health* 2014; **8**(2): 455-61.
15. Vizzotti C, Juarez MV, Bergel E, et al. Impact of a maternal immunization program against pertussis in a developing country. *Vaccine* 2016; **34**(50): 6223-8.
16. Choi YH, Campbell H, Amirthalingam G, van Hoek AJ, Miller E. Investigating the pertussis resurgence in England and Wales, and options for future control. *BMC Med* 2016; **14**(1): 121.
17. Mooi FR, Van Der Maas NAT, De Melker HE. Pertussis resurgence: waning immunity and pathogen adaptation – two sides of the same coin. *Epidem and Infect* 2013; **142**(4): 685-94.

18. Sealey KL, Belcher T, Preston A. Bordetella pertussis epidemiology and evolution in the light of pertussis resurgence. *Infect Genet Evol* 2016; **40**: 136-43.
19. Burns DL, Meade BD, Messionnier NE. Pertussis Resurgence: Perspectives From the Working Group Meeting on Pertussis on the Causes, Possible Paths Forward, and Gaps in Our Knowledge. *J Infect Dis* 2014; **209**(suppl_1): S32-S5.
20. UK Department of Health. Pregnant women to be offered whooping cough vaccination. http://www.dh.gov.uk/health/2012/09/whooping-cough/ (accessed 28 Sept 2012.
21. Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. *Lancet* 2014; **384**(9953): 1521-8.
22. Dabrera G, Amirthalingam G, Andrews N, et al. A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012–2013. *Clin Infect Dis* 2014: ciu821.
23. Amirthalingam G, Campbell H, Ribeiro S, et al. Sustained Effectiveness of the Maternal Pertussis Immunization Program in England 3 Years Following Introduction. *Clin Infect Dis* 2016; **63**(suppl 4): S236-S43.
24. Baxter R, Bartlett J, Fireman B, Lewis E, Klein NP. Effectiveness of Vaccination During Pregnancy to Prevent Infant Pertussis. *Pediatrics* 2017; **139**(5).
25. Bellido-Blasco J, Guiral-Rodrigo S, Miguez-Santiyan A, Salazar-Cifre A, Gonzalez-Moran F. A case-control study to assess the effectiveness of pertussis vaccination during pregnancy on newborns, Valencian community, Spain, 1 March 2015 to 29 February 2016. *Euro Surveill* 2017; **22**(22).
26. Vaccine Knowledge Project. Pertussis (whooping cough) vaccine in pregnancy. http://vk.ovg.ox.ac.uk/pertussis-vaccine-in-pregnancy (accessed 28 June 2017.
27. Giersing BK, Karron RA, Vekemans J, Kaslow DC, Moorthy VS. Meeting report: WHO consultation on Respiratory Syncytial Virus (RSV) vaccine development, Geneva, 25–26 April 2016. *Vaccine* 2017.
28. Kobayashi M, Schrag SJ, Alderson MR, et al. WHO consultation on group B Streptococcus vaccine development: Report from a meeting held on 27–28 April 2016. *Vaccine* 2016.
29. Moon S-S, Groome MJ, Velasquez DE, et al. Prevaccination Rotavirus Serum IgG and IgA Are Associated With Lower Immunogenicity of Live, Oral Human Rotavirus Vaccine in South African Infants. *Clin Infect Dis* 2016; **62**(2): 157-65.
30. Szenborn L, Tischer A, Pejcz J, Rudkowski Z, Wojcik M. Passive acquired immunity against measles in infants born to naturally infected and vaccinated mothers. *Med Sci Mon* 2003; **9**(12): Cr541-6.
31. Bjorkholm B, Granstrom M, Taranger J, Wahl M, Hagberg L. Influence of high titers of maternal antibody on the serologic response of infants to diphtheria vaccination at three, five and twelve months of age. *Ped Infect Dis J* 1995; **14**(10): 846-9.
32. Englund JA, Anderson EL, Reed GF, et al. The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids. *Pediatrics* 1995; **96**(3): 580-4.
33. Sarvas H, Kurikka S, Seppälä IJ, Mäkelä PH, Mäkelä O. Maternal antibodies partly inhibit an active antibody response to routine tetanus toxoid immunization in infants. *J Infect Dis* 1992; **165**(5): 977-9.

34. O'Brien KL, Moïsi J, Moulton LH, et al. Predictors of pneumococcal conjugate vaccine immunogenicity among infants and toddlers in an American Indian PnCRM7 efficacy trial. 007; **196**(1): 104-14.
35. Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. *Vaccine* 2014; **32**(8): 996-1002.
36. Ladhani SN, Andrews NJ, Southern J, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. *Clin Infect Dis* 2015: civ695.
37. Hoang HT, Leuridan E, Maertens K, et al. Pertussis vaccination during pregnancy in Vietnam: Results of a randomized controlled trial. *Vaccine* 2016; **34**(1): 151-9.
38. Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 Acellular Pertussis Vaccines: Overview and Serologic Response. *Pediatrics* 1995; **96**(3): 548-57.
39. Hardy-Fairbanks AJ, Pan SJ, Decker MD, et al. Immune responses in infants whose mothers received Tdap vaccine during pregnancy. *Ped Infect Dis J* 2013; **32**(11): 1257-60.
40. Maertens K, Tran TMP, Hens N, Van Damme P, Leuridan E. Effect of prepregnancy pertussis vaccination in young infants. *J Infect Dis* 2017; **215**(12): 1855-61.
41. Maertens K, Burbidge P, Van Damme P, Goldblatt D, Leuridan E. Pneumococcal Immune Response in Infants Whose Mothers Received Tetanus, Diphtheria and Acellular Pertussis Vaccination During Pregnancy. *Pediatr Inf Dis J* 2017; **36**(12): 1186-92.
42. Madhi SA, Koen A, Cutland CL, et al. Antibody Kinetics and Response to Routine Vaccinations in Infants Born to Women Who Received an Investigational Trivalent Group B Streptococcus Polysaccharide CRM197-Conjugate Vaccine During Pregnancy. *Clin Infect Dis* 2017; **65**(11): 1897-904.
43. Van Savage J, Decker MD, Edwards KM, Sell SH, Karzon DT. Natural history of pertussis antibody in the infant and effect on vaccine response. *J Infect Dis* 1990; **161**(3): 487-92.
44. Barr M, Glenny AT, Randall KJ. Concentration of diphtheria antitoxin in cord blood and rate of loss in babies. *Lancet* 1949; **2**(6573): 324-6.
45. Sarvas H, Seppala I, Kurikka S, Siegberg R, Makela O. Half-life of the maternal IgG1 allotype in infants. *J Clin Immunol* 1993; **13**(2): 145-51.
46. Black FL, Berman LL, Borgono JM, et al. Geographic variation in infant loss of maternal measles antibody and in prevalence of rubella antibody. *Am J Epidemiol* 1986; **124**(3): 442-52.
47. Siegrist CA. The Challenges of Vaccine Responses in Early Life: Selected Examples. *J Compar Pathol* 2007; **137**: S4-S9.
48. Borrow R, Balmer P, Roper MH. The immunological basis for immunization series Module 3: Tetanus: World Health Organization; 2007.
49. Scheifele DW, Ochnio JJ. The immunological basis for immunization series Module 2: Diphtheria: World Health Organization; 2009.
50. Siber GR, Chang I, Baker S, et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine* 2007; **25**(19): 3816-26.

51. Hadinegoro SR, Arredondo-García JL, Capeding MR, et al. Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease. *New Engl J Med* 2015; **373**(13): 1195-206.
52. Halstead SB, Russell PK. Protective and immunological behavior of chimeric yellow fever dengue vaccine. *Vaccine* 2016; **34**(14): 1643-7.
53. Jodar L, Butler J, Carlone G, et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine* 2003; **21**(23): 3265-72.
54. World Health Organization. Correlates of vaccine-induced protection: methods and implications. 2013. http://www.who.int/iris/handle/10665/84288WHO/IVB/13.01).
55. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Ped Infect Dis J* 2000; **19**(3): 187-95.
56. Klugman KP, Madhi SA, Huebner RE, et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003; **349**(14): 1341-8.
57. O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* 2003; **362**(9381): 355-61.
58. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus: I. The role of humoral antibodies. *J Exp Med* 1969; **129**(6): 1307-26.
59. Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. *Clin Diag Lab Immunol* 2003; **10**(5): 780-6.
60. Andrews NJ, Waight PA, Burbidge P, et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. *Lancet Infect Dis*2014; **14**(9): 839-46.
61. R Core Team. R: A language and environment for statistical computing. 2015. https://www.R-project.org/.
62. Le Doare K, Taylor S, Allen L, et al. Placental transfer of anti-group B Streptococcus immunoglobulin G antibody subclasses from HIV-infected and uninfected women to their uninfected infants. *AIDS (London, England)* 2016; **30**(3): 471-5.
63. Shahid NS, Steinhoff MC, Hoque SS, Begum T, Thompson C, Siber GR. Serum, breast milk, and infant antibody after maternal immunisation with pneumococcal vaccine. *Lancet* 1995; **346**(8985): 1252-7.
64. O'Dempsey TJ, McArdle T, Ceesay SJ, et al. Meningococcal antibody titres in infants of women immunised with meningococcal polysaccharide vaccine during pregnancy. *Arch Dis Child Fetal Neonatal Ed* 1996; **74**(1): F43-6.
65. O'Dempsey TJ, McArdle T, Ceesay SJ, et al. Immunization with a pneumococcal capsular polysaccharide vaccine during pregnancy. *Vaccine* 1996; **14**(10): 963-70.
66. Adler NR, Mahony A, Friedman ND. Diphtheria: forgotten, but not gone. *Intern Med J* 2013; **43**(2): 206-10.
67. Swain R, Behera C, Arava SK, Kundu N. Sudden death of a child due to respiratory diphtheria. *Med Legal J* 2016; **84**(2): 90-3.
68. Vargha D, Greene JA. Grey-market medicines: diphtheria antitoxin and the decay of biomedical infrastructure. *Lancet* 2017; **389**(10080): 1690-1.

69. Voysey M, Kandasamy R, Yu LM, et al. The predicted persistence and kinetics of antibody decline 9years after pre-school booster vaccination in UK children. *Vaccine* 2016; **34**(35): 4221-8.
70. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to Bordetella pertussis and other Bordetella subspecies. *Clin Microbiol Rev* 2005; **18**(2): 326-82.
71. Daly KA, Scott Giebink G, Lindgren BR, et al. Maternal immunization with pneumococcal 9-valent conjugate vaccine and early infant otitis media. *Vaccine* 2014; **32**(51): 6948-55.
72. Carvalho BT, Carneiro-Sampaio MM, Sole D, Naspitz C, Leiva LE, Sorensen RU. Transplacental transmission of serotype-specific pneumococcal antibodies in a Brazilian population. *Clin Diagn Lab Immunol* 1999; **6**(1): 50-4.
73. Gupta A, Mathad JS, Yang WT, et al. Maternal pneumococcal capsular IgG antibodies and transplacental transfer are low in South Asian HIV-infected mother-infant pairs. *Vaccine* 2014; **32**(13): 1466-72.
74. McCormick JB, Gusmao HH, Nakamura S, et al. Antibody response to serogroup A and C meningococcal polysaccharide vaccines in infants born of mothers vaccinated during pregnancy. *J Clin Invest* 1980; **65**(5): 1141-4.
75. Mankarious S, Lee M, Fischer S, et al. The half-lives of IgG subclasses and specific antibodies in patients with primary immunodeficiency who are receiving intravenously administered immunoglobulin. *J Lab Clin Med* 1988; **112**(5): 634-40.
76. Zhu Q, McLellan JS, Kallewaard NL, et al. A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants. *Sci Trans Med* 2017; **9**(388).
77. Liu L. Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins. *Protein & cell* 2017.
78. Kampmann B, Jones CE. Factors influencing innate immunity and vaccine responses in infancy. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* 2015; **370**(1671).
79. Lindsey B, Jones C, Kampmann B. Bridging the gap: maternal immunisation as a means to reduce neonatal deaths from infectious diseases. *Pathogens and Global Health* 2012; **106**(3): 137-8.
80. Kayhty H, Karanko V, Peltola H, Sarna S, Makela PH. Serum antibodies to capsular polysaccharide vaccine of group A Neissera meningitidis followed for three years in infants and children. *J Infect Dis* 1980; **142**(6): 861-8.
81. Osborn JJ, Dancis J, Julia JF. Studies of the immunology of the newborn infant I. Age and antibody production. *Pediatrics* 1952; **9**(6): 736-44.
82. Siegrist CA. Vaccine Immunology. *Vaccine* 2008; **5**: 1725 (http://www.who.int/immunization/documents/Elsevier_Vaccine_immunology.pdf).
83. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) in pregnant women--Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR* 2013; **62**(7): 131.
84. Davies S, Bennett V, Ridge K. Temporary programme of pertussis (whooping cough) vaccination of pregnant women. *Department of Health London* *https://wwwgovuk/government/uploads/system/uploads/attachment_data/file/212947/CMO-Pertussis-27-09-2012-FINALpdf* 2012: 1-10.
85. Fallo AA, Neyro SE, Manonelles GV, et al. Prevalence of Pertussis Antibodies in Maternal Blood, Cord Serum, and Infants From Mothers With and Those Without Tdap Booster Vaccination During Pregnancy in Argentina. *J Ped Infect Dis* 2016.

86. Abu Raya B, Bamberger E, Almog M, Peri R, Srugo I, Kessel A. Immunization of pregnant women against pertussis: The effect of timing on antibody avidity. *Vaccine* 2015; **33**(16): 1948-52.
87. Walls T, Graham P, Petousis-Harris H, Hill L, Austin N. Infant outcomes after exposure to Tdap vaccine in pregnancy: an observational study. *BMJ Open* 2016; **6**(1).
88. Gans H, DeHovitz R, Forghani B, Beeler J, Maldonado Y, Arvin AM. Measles and mumps vaccination as a model to investigate the developing immune system: passive and active immunity during the first year of life. *Vaccine* 2003; **21**(24): 3398-405.
89. Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. Specific antibodies against vaccine-preventable infections: a mother–infant cohort study. *BMJ Open* 2013; **3**(4): e002473.
90. Munoz FM, Bond NH, Maccato M, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (tdap) immunization during pregnancy in mothers and infants: A randomized clinical trial. *JAMA* 2014; **311**(17): 1760-9.
91. Eberhardt CS, Blanchard-Rohner G, Lemaitre B, et al. Maternal Immunization Earlier in Pregnancy Maximizes Antibody Transfer and Expected Infant Seropositivity Against Pertussis. *Clin Infect Dis* 2016; **62**(7): 829-36.
92. Leuridan E, Hens N, Peeters N, de Witte L, Van der Meeren O, Van Damme P. Effect of a prepregnancy pertussis booster dose on maternal antibody titers in young infants. *Ped Infect Dis J* 2011; **30**(7): 608-10.
93. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010; **376**(9741): 606-14.
94. Joensuu J, Koskenniemi E, Pang X-L, Vesikari T. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. *The Lancet* 1997; **350**(9086): 1205-9.
95. Khan MI, Soofi SB, Ochiai RL, et al. Effectiveness of Vi capsular polysaccharide typhoid vaccine among children: a cluster randomized trial in Karachi, Pakistan. *Vaccine* 2012; **30**(36): 5389-95.
96. Sur D, Ochiai RL, Bhattacharya SK, et al. A cluster-randomized effectiveness trial of Vi typhoid vaccine in India. *N Engl J Med* 2009; **361**(4): 335-44.
97. Richie E, Punjabi NH, Sidharta Y, et al. Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. *Vaccine* 2000; **18**(22): 2399-410.
98. Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. *Infect and Imm* 2001; **69**(3): 1568-73.
99. Saaka M, Okoko BJ, Kohberger RC, et al. Immunogenicity and serotype-specific efficacy of a 9-valent pneumococcal conjugate vaccine (PCV-9) determined during an efficacy trial in The Gambia. *Vaccine* 2008; **26**(29-30): 3719-26.
100. Cutts FT, Zaman SMA, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005; **365**(9465): 1139-46.
101. O'Brien KL, Moisi J, Romero-Steiner S, et al. Pneumococcal antibodies in a child with type 14 pneumococcal conjugate vaccine failure. *Vaccine* 2009; **27**(12): 1863-8.

102. Dagan R, Juergens C, Trammel J, et al. Modeling pneumococcal nasopharyngeal acquisition as a function of anticapsular serum antibody concentrations after pneumococcal conjugate vaccine administration. *Vaccine* 2016; **34**(36): 4313-20.
103. Balmer P, Borrow R, Miller E. Impact of meningococcal C conjugate vaccine in the UK. *J Med Microbiol* 2002; **51**(9): 717-22.
104. Ramsay ME, Andrews N, Kaczmarski EB, Miller E. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. *Lancet* 2001; **357**(9251): 195-6.
105. Andrews N, Waight PA, Borrow R, et al. Using the indirect cohort design to estimate the effectiveness of the seven valent pneumococcal conjugate vaccine in England and Wales. *PLoS One* 2011; **6**(12): e28435.
106. Jin C, Gibani MM, Moore M, et al. Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of Salmonella Typhi: a phase IIb observer-participant-blinded, randomised controlled trial. *Lancet*.
107. Voysey M, Pollard AJ. Sero-efficacy of Vi-polysaccharide tetanus-toxoid typhoid conjugate vaccine (Typbar-TCV). *Clin Infect Dis* 2018.
108. Anwar E, Goldberg E, Fraser A, Acosta CJ, Paul M, Leibovici L. Vaccines for preventing typhoid fever. *Cochrane Database Syst Rev* 2014; (1): CD001261.
109. Mohan VK, Varanasi V, Singh A, et al. Safety and immunogenicity of a Vi polysaccharide-tetanus toxoid conjugate vaccine (Typbar-TCV) in healthy infants, children, and adults in typhoid endemic areas: a multicenter, 2-cohort, open-label, double-blind, randomized controlled phase 3 study. *Clin Infect Dis* 2015; **61**(3): 393-402.
110. Kayhty H, Auranen K, Nohynek H, Dagan R, Makela H. Nasopharyngeal colonization: a target for pneumococcal vaccination. *Expert Rev Vaccines* 2006; **5**(5): 651-67.
111. Trotter CL, McVernon J, Ramsay ME, et al. Optimising the use of conjugate vaccines to prevent disease caused by *Haemophilus influenzae* type b, Neisseria meningitidis and Streptococcus pneumoniae. *Vaccine* 2008; **26**(35): 4434-45.
112. Hamaluba M, Kandasamy R, Upreti SR, et al. Comparison of two-dose priming plus 9-month booster with a standard three-dose priming schedule for a ten-valent pneumococcal conjugate vaccine in Nepalese infants: a randomised, controlled, open-label, non-inferiority trial. *Lancet Infect Dis* 2015; **15**(4): 405-14.
113. Kandasamy R, Gurung M, Thapa A, et al. Multi-serotype pneumococcal nasopharyngeal carriage prevalence in vaccine naive Nepalese children, assessed using molecular serotyping. *PLoS One* 2015; **10**(2): e0114286.
114. Yin J, Tian L. Optimal linear combinations of multiple diagnostic biomarkers based on Youden index. *Stats in Med* 2014; **33**(8): 1426-40.
115. Youden WJ. Index for rating diagnostic tests. *Cancer* 1950; **3**(1): 32-5.
116. Lynch JM, Briles DE, Metzger DW. Increased protection against pneumococcal disease by mucosal administration of conjugate vaccine plus interleukin-12. *Infect and Immunity* 2003; **71**(8): 4780-8.
117. Sleeman KL, Griffiths D, Shackley F, et al. Capsular serotype-specific attack rates and duration of carriage of Streptococcus pneumoniae in a population of children. *J Infect Dis* 2006; **194**(5): 682-8.
118. Kelly DF, Thorson S, Maskey M, et al. The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal. *Int J Infect Dis* 2011; **15**(1): e17-23.

119. Ekström N, Väkeväinen M, Verho J, Kilpi T, Käyhty H, the Fin OMSG. Functional Antibodies Elicited by Two Heptavalent Pneumococcal Conjugate Vaccines in the Finnish Otitis Media Vaccine Trial. *Infect and Imm* 2007; **75**(4): 1794-800.
120. Jokinen JT, Åhman H, Kilpi TM, Makela PH, Kayhty MH. Concentration of Antipneumococcal Antibodies as a Serological Correlate of Protection: An Application to Acute Otitis Media. *J Infect Dis* 2004; **190**(3): 545-50.
121. Millar EV, O'Brien KL, Bronsdon MA, et al. Anticapsular serum antibody concentration and protection against pneumococcal colonization among children vaccinated with 7-valent pneumococcal conjugate vaccine. *Clin Infect Dis* 2007; **44**(9): 1173-9.
122. Davidson M, Parkinson AJ, Bulkow LR, Fitzgerald MA, Peters HV, Parks DJ. The epidemiology of invasive pneumococcal disease in Alaska, 1986-1990--ethnic differences and opportunities for prevention. *J Infect Dis* 1994; **170**(2): 368-76.
123. Hanieh S, Hamaluba M, Kelly DF, et al. Streptococcus pneumoniae carriage prevalence in Nepal: evaluation of a method for delayed transport of samples from remote regions and implications for vaccine implementation. *PLoS One* 2014; **9**(6): e98739.
124. Mackenzie GA, Leach AJ, Carapetis JR, Fisher J, Morris PS. Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC Infect Dis* 2010; **10**: 304.
125. Goldblatt D, Hussain M, Andrews N, et al. Antibody responses to nasopharyngeal carriage of Streptococcus pneumoniae in adults: a longitudinal household study. *J Infect Dis* 2005; **192**(3): 387-93.
126. Maertens K, Cabore RN, Huygen K, Hens N, Van Damme P, Leuridan E. Pertussis vaccination during pregnancy in Belgium: Results of a prospective controlled cohort study. *Vaccine* 2016; **34**(1): 142-50.
127. Maertens K, Hoang TT, Nguyen TD, et al. The Effect of Maternal Pertussis Immunization on Infant Vaccine Responses to a Booster Pertussis-Containing Vaccine in Vietnam. *Clin Infect Dis* 2016; **63**(suppl 4): S197-S204.
128. Wanlapakorn N, Thongmee T, Vichaiwattana P, Leuridan E, Vongpunsawad S, Poovorawan Y. Antibodies to Bordetella pertussis antigens in maternal and cord blood pairs: a Thai cohort study. *PeerJ* 2017; **5**: e4043.
129. Ladhani SN, Collins S, Djennad A, et al. Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000–17: a prospective national observational cohort study. *Lancet Infect Dis* 2018; **18**(4): 441-51.
130. Miller E, Andrews NJ, Waight PA, Slack MPE, George RC. Effectiveness of the new serotypes in the 13-valent pneumococcal conjugate vaccine. *Vaccine* 2011; **29**(49): 9127-31.
131. Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MPE, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. *Lancet Infect Dis* 2015; **15**(5): 535-43.
132. Klugman KP. Usefulness of the serial measurement of Vi antibodies. *Clin Infect Dis* 2018.
133. Earnshaw SR, McDade CL, Zanotti G, Farkouh RA, Strutton D. Cost-effectiveness of 2+ 1 dosing of 13-valent and 10-valent pneumococcal conjugate vaccines in Canada. *BMC Infect Dis* 2012; **12**(1): 101.

134. Klok RM, Lindkvist R-M, Ekelund M, Farkouh RA, Strutton DR. Cost-effectiveness of a 10-versus 13-valent pneumococcal conjugate vaccine in Denmark and Sweden. *Clin Ther* 2013; **35**(2): 119-34.
135. Kang JH, Kim JH, Lee JH, Lee SY, Hong YJ, Kim CH. The immunogenicity and safety of three-component DTaP vaccine in Korean infants. *Korean J Ped* 2007; **50**(4): 355-62.
136. Gatchalian S, Reyes M, Bernal N, et al. A new DTPw-HBV/Hib vaccine is immunogenic and safe when administered according to the EPI (Expanded Programme for Immunization) schedule and following hepatitis B vaccination at birth. *Hum Vaccines* 2005; **1**(5): 198-203.
137. Espinoza F, Tregnaghi M, Gentile A, et al. Primary and booster vaccination in Latin American children with a DTPw-HBV/Hib combination: a randomized controlled trial. *BMC Infect Dis* 2010; **10**(1): 297.
138. GlaxoSmithKline. Study 104489: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/965801af-10cf-40d4-9a74-1bf766e6ea4b (accessed April 20, 2018.
139. GlaxoSmithKline. Study 104871: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/20036.pdf (accessed April 20, 2018.
140. Silfverdal SA, Hogh B, Bergsaker MR, et al. Immunogenicity of a 2-dose priming and booster vaccination with the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine. *Ped Infect Dis J* 2009; **28**(10): e276-e82.
141. Knuf M, Szenborn L, Moro M, et al. Immunogenicity of routinely used childhood vaccines when coadministered with the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV). *Ped Infect Dis J* 2009; **28**(4): S97-S108.
142. Vesikari T, Wysocki J, Chevallier B, et al. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. *Ped Infect Dis J* 2009; **28**(4): S66-S76.
143. GlaxoSmithKline. Study 105910: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/20071.pdf (accessed April 23, 2018.
144. GlaxoSmithKline. Study 110478: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/20253.pdf (accessed April 23, 2018.
145. Lagos R, Muñoz A, Levine M, et al. Safety and immunogenicity of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) in Chilean children. *Hum Vacc* 2011; **7**(5): 511-22.
146. GlaxoSmithKline. Study 106786: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/f7d85be9-4b9c-48eb-ae65-8b9d57f87be9 (accessed April 23, 2018.
147. GlaxoSmithKline. Study 111344: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/f2e73675-1db1-4ef6-8fd2-594c52592led (accessed April 23, 2018.
148. Dicko A, Odusanya OO, Diallo AI, et al. Primary vaccination with the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) in infants in Mali and Nigeria: a randomized controlled trial. *BMC Pub Health* 2011; **11**(1): 882.

149. Odusanya OO, Kuyinu YA, Kehinde OA, et al. Immunogenicity, safety and reactogenicity of the 10-valent pneumococcal non-typeable haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Nigerian Infants: a randomised trial. *Nigerian Postgrad Med J* 2013; **20**(4): 272-81.
150. Szenborn L, Czajka H, Brzostek J, et al. A randomized, controlled trial to assess the immunogenicity and safety of a heptavalent diphtheria, tetanus, pertussis, hepatitis B, poliomyelitis, hib and meningococcal serogroup C combination vaccine administered at 2, 3, 4 and 12–18 months of age. *Ped Infect Dis J* 2013; **32**(7): 777-85.
151. Bermal N, Szenborn L, Chrobot A, et al. The 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) coadministered with DTPw-HBV/Hib and poliovirus vaccines: assessment of immunogenicity. *Ped Infect Dis J* 2009; **28**(4): S89-S96.
152. Bermal N, Szenborn L, Alberto E, et al. Safety and immunogenicity of a booster dose of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine coadministered with DTPw-HBV/Hib and poliovirus vaccines. *Ped Infect Dis J* 2011; **30**(1): 69-72.
153. Tichmann-Schumann I, Soemantri P, Behre U, et al. Immunogenicity and reactogenicity of four doses of diphtheria-tetanus-three-component acellular pertussis-hepatitis B-inactivated polio virus-*Haemophilus influenzae* type b vaccine coadministered with 7-valent pneumococcal conjugate vaccine. *Ped Infect Dis J* 2005; **24**(1): 70-7.
154. Wysocki J, Tejedor JC, Grunert D, et al. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) when coadministered with different Neisseria meningitidis serogroup C conjugate vaccines. *Ped Infect Dis J* 2009; **28**(4): S77-S88.
155. Prymula R, Siegrist C-A, Chlibek R, et al. Effect of prophylactic paracetamol administration at time of vaccination on febrile reactions and antibody responses in children: two open-label, randomised controlled trials. *Lancet* 2009; **374**(9698): 1339-50.
156. Omeñaca F, Merino JM, Tejedor J-C, et al. Immunization of preterm infants with 10-valent pneumococcal conjugate vaccine. *Pediatrics* 2011: peds. 2010-1184.
157. Van den Bergh MR, Spijkerman J, François N, et al. Immunogenicity, safety, and reactogenicity of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine and DTPa-IPV-Hib when coadministered as a 3-dose primary vaccination schedule in The Netherlands: a randomized controlled trial. *Ped Infect Dis J* 2011; **30**(9): e170-e8.
158. Van Den Bergh MR, Spijkerman J, François N, et al. Immunogenicity, safety and reactogenicity of a booster dose of the 10-valent pneumococcal Nontypeable H. influenzae Protein D conjugate vaccine coadministered with DTPa-IPV-Hib in Dutch children. *Ped Inf Dis J* 2016; **35**(7): e206-e19.
159. Tejedor JC, Omeñaca F, García-Sicilia J, et al. Immunogenicity and reactogenicity of a three-dose primary vaccination course with a combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-*Haemophilus influenzae* type b vaccine coadministered with a meningococcal C conjugate vaccine. *Ped Infect Dis J* 2004; **23**(12): 1109-15.
160. Nolan T, Lambert S, Roberton D, Marshall H, Richmond P, Streeton C. DTPa-HBV-IPV vaccine for primary vaccination of infants. *J Paediatr Child Health* 2007; **43**(9): 587-92.
161. Tichmann I, Grunert D, Habash S, et al. Persistence of antibodies in children primed with two different hexavalent diphtheria, tetanus, acellular pertussis,

hepatitis B, inactivated poliovirus and *Haemophilus influenzae* type B vaccines and evaluation of booster vaccination. *Hum Vacc* 2006; **2**(6): 249-54.
162. Tichmann I, Preidel H, Grunert D, et al. Comparison of the immunogenicity and reactogenicity of two commercially available hexavalent vaccines administered as a primary vaccination course at 2, 4 and 6 months of age. *Vaccine* 2005; **23**(25): 3272-9.
163. Tejedor JC, Moro M, Ruiz-Contreras J, et al. Immunogenicity and Reactogenicity of Primary Immunization With a Novel Combined *Haemophilus influenzae* Type b and Neisseria meningitidis Serogroup C-Tetanus Toxoid Conjugate Vaccine Coadministered With a Diphtheria–Tetanus–Acellular Pertussis–Hepatitis B–Inactivated Poliovirus Vaccine at 2, 4 and 6 Months. *Ped Infect Dis J* 2007; **26**(1): 1-7.
164. Tejedor JC, Moro M, Ruiz-Contreras J, et al. Immunogenicity and reactogenicity of primary immunization with a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-*Haemophilus influenzae* type B vaccine coadministered with two doses of a meningococcal C-tetanus toxoid conjugate vaccine. *Ped Infect Dis J* 2006; **25**(8): 713-20.
165. Lalwani S, Chatterjee S, Chhatwal J, et al. Randomized, open-label study of the impact of age on booster responses to the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine in children in India. *Clin Vacc Immunol* 2014; **21**(9): 1292-300.
166. Lalwani S, Chatterjee S, Chhatwal J, et al. Immunogenicity, safety and reactogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) when co-administered with the DTPw-HBV/Hib vaccine in Indian infants: a single-blind, randomized, controlled study. *Hum Vacc Immunother* 2012; **8**(5): 612-22.
167. Lim FS, Koh MT, Tan KK, et al. A randomised trial to evaluate the immunogenicity, reactogenicity, and safety of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) co-administered with routine childhood vaccines in Singapore and Malaysia. *BMC Infect Dis* 2014; **14**(1): 530.
168. Knuf M, Pankow-Culot H, Grunert D, et al. Induction of immunologic memory following primary vaccination with the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine in infants. *Ped Infect Dis J* 2012; **31**(1): e31-e6.
169. Iwata S, Kawamura N, Kuroki H, et al. Immunogenicity and safety of the 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) co-administered with DTPa vaccine in Japanese children: A randomized, controlled study. *Hum Vacc Immuno* 2015; **11**(4): 826-37.
170. Vesikari T, Karvonen A, Korhonen T, et al. Immunogenicity of 10-valent pneumococcal nontypeable Haemophilus Influenzae Protein D Conjugate Vaccine when administered as catch-up vaccination to children 7 months to 5 years of age. *Ped Inf Dis J* 2011; **30**(8): e130-e41.
171. GlaxoSmithKline. Study 103488: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/583f26a3-0b31-4c0f-b9ff-101e1e738b1b (accessed May 10, 2018.
172. Kerdpanich A, Warachit B, Kosuwon P, et al. Primary vaccination with a new heptavalent DTPw–HBV/Hib–Neisseria meningitidis serogroups A and C combined vaccine is well tolerated. *Int J Infect Dis* 2008; **12**(1): 88-97.

173. GlaxoSmithKline. Study 100478: Scientific Results Summary. https://www.gsk-clinicalstudyregister.com/files2/72a65ae0-3f69-4173-815d-6b474b4a92a5 (accessed May 10. 2018.