Factors influencing vaccine responses in the first year of life

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ABSTRACT

Immunisation is the most cost-effective life-saving medical intervention and is estimated to save at least 2.5 million lives each year. However, there is substantial variation between individuals in the immune response to immunisations. This has consequences for both protective efficacy and duration of protection. My PhD research project focused on evaluating factors that influence antibody responses to routine immunisations given in infancy.

The first section of my thesis focuses on intrinsic, perinatal and external factors that might influence responses to immunisation in infancy. Within this, I investigated the effect of maternal immunisation during pregnancy. Antenatal diphtheria-tetanus-acellular pertussis (dTpa) was associated with significantly reduced infant antibody responses to both specific (diphtheria and pertussis) and heterologous (polio and pneumococcal) vaccines. In contrast, maternal influenza immunisation had minimal effect on infant vaccine responses. I also investigated the effect of early-life factors, namely sex, delivery mode, breastfeeding and antibiotic exposure on antibody responses to routine immunisations given in the first year of life. While there were some differences between males and females in antibody responses to routine immunisations in the first year of life, delivery mode, breastfeeding and antibiotic exposure did not exert a substantial influence.

The second section of my thesis focuses on the 'non-specific' or immunomodulatory effects of the Bacillus Calmette-Guérin (BCG) vaccine, one of the most widely used vaccines worldwide, and specifically, how it influences vaccine responses to heterologous (routine) immunisations. It comprises a systematic review of studies that investigated the effect of previous or co-administered BCG on heterologous vaccine responses in infants and adults, and an original study in which I investigated the effect of BCG immunisation at birth on antibody responses to routine infant vaccines. In this study, although not statistically significant, antibody responses in BCG-

vaccinated infants were consistently higher against diphtheria, tetanus, pneumococcal, measles and mumps antigens, but lower against *Haemophilus influenzae* type b compared to BCG-naïve infants. These findings add to the evidence that BCG immunisation at birth has broad heterologous effects on the infant immune system.

The third section of my thesis focuses on the correlation between the response to different vaccines, for which there was previously no data available. I found that correlation between antibody responses to similar antigens in the same vaccine (such as different serotypes of a bacterium or virus), as well as responses to antigens conjugated to similar carrier proteins are strong. In contrast, correlation between responses to other vaccines are weak and there is a negative correlation between responses to measles-mumps-rubella vaccine antigens and non-live vaccine antigens. There was also a weak correlation between antibody responses to vaccines of the same type (e.g. polysaccharide or toxoid vaccines). This has important clinical implications as it means the current practice of measuring antibody responses to one or a few vaccine antigens does not offer a reliable surrogate marker of responses to unrelated vaccines.

Recognising factors which might influence vaccine antibody responses, is important for the design of vaccine studies and decisions on vaccine schedules. It also offers ways to optimise vaccine immunogenicity and effectiveness, as well as the possibility to individualise immunisation. The complex interplay between different components of the immune system in vaccine responses is not yet fully understood, and future studies should focus on additional factors, including the innate and cellular immune system.

DECLARATION

This is to certify that:

(i) The thesis comprises only my original work towards the PhD degree
(ii) due acknowledgement has been made in the text to all other material used
(iii) the thesis is fewer than 100,000 words in length, exclusive of tables, bibliography and appendices.

Petra Zimmermann

PREFACE

Contributions

The material presented in this thesis is my original work, although many people contributed to the final published manuscripts in Chapters 3, 4, 5 and 6. The nature of each individual contributions is declared and acknowledged in detail below.

My supervisors Prof Nigel Curtis, Prof Roy Robins-Browne, Dr Kirsten P Perrett and A/Prof Nicole Ritz helped with the conception, development, analysis and interpretation of all studies included in the thesis. Prof Julie Bines, the chair of my advisory committee, provided feedback about study conduct during regular review meetings. Prof Susan Donath was also part of my advisory committee and provided continuous statistical support.

The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) was led by Prof Nigel Curtis together with the project's other investigators (Prof Katie Allen, Dr Dan Casalaz, Dr Tobias Kollmann, Prof Helder Nakaya, Prof Anne-Louise Ponsonby, Prof Roy Robins-Browne, Prof Frank Shann, Prof Mike South and A/Prof Peter Vuillermin), as well as other members of the team (Ms Veronica Abruzzo, Ms Rhian Bonnici, Ms Hannah Elborough, Dr Bridget Freyne, Ms Kaya Gardiner, Ms Susie Germano and Ms Clare Morrison). The study described in Chapter 4 was done under the ethical approval from the Royal Children's Hospital (RCH) Human Research Ethics Committee (HREC) for MIS BAIR. When I decided to use the collected blood samples from MIS BAIR for other sub-studies, I submitted new study protocols for ethics amendments with the RCH HREC. Dr Nicole L Messina helped with the selection of the blood samples. Susie Germano and Rhian Bonnici helped with data entry and the processing of blood samples. Kaya Gardiner was responsible for project co-ordination and Veronica Abruzzo for data management.

I did the literature review included in this chapter 1 and wrote the manuscript, which was critically reviewed and revised by Prof Nigel Curtis, who also helped with the design of the figures included

in the published paper. I also did the systematic literature review included in chapter 4 and wrote the manuscript for the published paper, which was reviewed and revised by Prof Nigel Curtis.

Together with Dr Kirsten P Perrett, A/Prof Nicole Ritz (University of Basel) and Prof Nigel Curtis, I was responsible for the study design of the studies included in chapter 2, 3, 4 and 5. Prof Andrew Pollard (University of Oxford) also provided helpful advice. Dr Fiona van der Klis (National Institute of Public Health and the Environment, Bilthoven) was responsible for the analyses of the blood samples included in these studies. I selected the participants to be included in the studies with the assistance of Dr Nicole Messina, and did the statistical analyses, under the supervision of Prof Susan Donath and Prof Nigel Curtis. I designed the figures and tables, and drafted the manuscripts for the published papers, which were revised by all co-authors. For each chapter, journal reviewers and journal editors provided suggestions to the manuscript prior to publication.

I wrote chapter 6 in its entirety, which I revised after critical discussion with and advice from Prof Nigel Curtis.

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I owe debt of gratitude to Dr Bridget Freyne for all her work with MIS BAIR. I am indebted to Dr Fiona van der Klis who kindly agreed to analyse our blood samples despite the competing demands from researchers worldwide to run their samples on her unique platform, and for sharing her expertise and experience in vaccine research. I also thank Dr Guy Berbers for sharing his expertise on pneumococcal vaccine responses. I am grateful for the support on the statistical analyses from Prof Susan Donath of the Clinical Epidemiology and Biostatistics Unit, Murdoch Children's Research Institute (MCRI) who was also a valuable member of my PhD Advisory Committee. I thank Dr Nicole L Messina, post-doctoral fellow, for her help with the blood sample selection and Susie Germano, senior research assistant, and Rhian Bonnici, junior research assistant, who helped with data entry and the processing of the blood samples. I am grateful for Kaya Gardiner and Veronica Abruzzo for their project co-ordination and data management. Prof

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ABBREVIATIONS

BCG	Bacillus Calmette-Guérin vaccine
CI	Confidence interval
dTpa	Diphtheria-tetanus-acellular pertussis vaccine
DTPa-IPV-Hib	Diphtheria-tetanus-acellular pertussis-inactivated polio-*Haemophilus influenzae* type b vaccine
FHA	Filamentous haemagglutinin
GMC	Geometric mean concentration (of antibody)
GMR	Geometric mean ratio (of antibody)
HepA	Hepatitis A
HepB	Hepatitis B
Hib	*Haemophilus influenzae* type b
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
IPV	Inactivated polio vaccine
IQR	Interquartile range
MCV	Measles-containing vaccine
MIS BAIR	The Melbourne Infant Study: BCG for Allergy and Infection Reduction
MenC	Meningococcus type C
MMR	Measles-mumps-rubella vaccine
ORV	Oral rotavirus vaccine
PCV	Pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
Pn	Pneumococcal serotype
PPV	Pneumococcal polysaccharide vaccine

PRN	Pertactin
PT	Pertussis toxin
TCV	Tetanus-containing vaccine
Th	T helper
TIV	Trivalent inactivated influenza vaccine

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Publications included in thesis

1) **Zimmermann P,** Curtis N. Factors that influence the immune response to immunisation. *Clinical Microbiology Reviews.* 2019. PMID: 30867162.

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3) **Zimmermann P,** Donath S, Perrett KP, Messina NL, Ritz N, Netea MG, Flanagan KL, Van der Klis F, Curtis N. The influence of neonatal Bacillus Calmette-Guérin (BCG) immunisation on heterologous vaccine responses in infants in a randomised controlled trial. *Vaccine*. 2019. PMID: 31153688.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Variation in vaccine responses

After hygiene, immunisation has been one of the major advances for human health. It is the most cost-effective life-saving medical intervention and is estimated to save at least 2.5 million lives each year.[1,2] Protection induced by immunisations is mediated through a complex interplay between the innate, humoral and cell-mediated immune system with T cell immunity and antibody production by B cells playing an important role.[3,4] There are many different methods to quantify the immune response to vaccines, however, the most frequently used method is the measurement of specific antibody concentrations.

There is substantial variation between individuals in the immune response to immunisations. For example, for trivalent inactivated influenza (TIV) and hepatitis B (HepB) vaccine, antibody responses vary more than 100-fold and some individuals do not produce measurable antibodies.[5,6] This has consequences for both protective efficacy and duration of protection. Worryingly, a significant number of vaccine-preventable infections occur in vaccinated individuals.[7] The reasons behind these vaccine-failures remain unclear.

Intrinsic host factors, perinatal factors, as well as extrinsic host factors might all influence vaccine responses. Understanding these factors when designing vaccine studies and deciding on vaccine schedules offers ways to improve vaccine immunogenicity and effectiveness and the possibility to individualise immunisation.

1.1.2 The influence of maternal immunisation during pregnancy on infant vaccine responses

The neonatal period is a vulnerable time for being affected by infectious diseases. Immunisation during pregnancy helps to protect infants during the period before they complete their primary

immunisations and has been proven to be safe and beneficial.[8-11] The transfer of maternal antibodies to the foetus is an active process but not all immunoglobulins are transferred to the same extent. Of the multiple factors that influence the transfer of immunoglobulin G (IgG) across the placenta, maternal antibody concentration is an important one. Infants whose mothers receive diphtheria, tetanus and acellular pertussis (dTpa) immunisation during pregnancy have higher antibody concentrations to these antigens at birth compared to infants whose mothers do not receive the vaccine.[11-17] The strategy of immunising pregnant women with a pertussis-containing vaccine has been shown to be highly effective in preventing severe pertussis in infants.[18-20] Nevertheless, despite the benefits of antenatal immunisation, there are also potential disadvantages. Pre-existing maternal antibodies can diminish antibody responses following infant immunisation, an effect which is called 'blunting'. While a large number of studies have investigated the association between maternal antibody concentrations and infant antibody production,[21] the effect of maternal immunisation on infant vaccine responses has been less extensively investigated.[12-16,22-24] Additionally, previous studies have investigated only the influence of dTpa and not influenza immunisation during pregnancy.[12-16] Furthermore, the majority of studies investigated antibody concentrations only to the specific vaccine antigens included in the maternal immunisation, and there is scarce data available on heterologous vaccine responses, particularly pneumococcal responses.

1.1.3 The influence of sex on vaccine responses

A large body of literature of literature indicates that biological sex influences immune responses.[25] This includes the humoral response to vaccination. There are several studies which show that females have higher antibody responses to HepA,[26-39] HepB,[30-33,40-66] rabies,[67-69] and TIV[70-80] immunisation, while males have higher antibody responses to diphtheria,[81-84] pneumococcal polysaccharide (PPV)[69,85-90] and tetanus[91-93] immunisation. Even though, overall, females have a tendency for higher antibody responses, they may have faster waning of antibodies, which has

been shown for antibodies after HepA[37] and PPV[86,90] immunisation. Studies investigating sex differences in responses to live-vaccines report less consistent findings with some studies reporting higher antibody responses to measles,[94] mumps,[95] and rubella[96] immunisation in females, and other studies reporting transiently higher antibody responses to rubella[97] and measles immunisation in males[98] or no sex differences in antibody responses.[99,100]

To date, almost all the studies that investigated the effect of sex on vaccine responses were done in adults. However, one meta-analysis summarised findings from vaccine trials in children under three years of age and found that vaccine antibody and memory B cells responses are "consistently higher or equivalent" in girls.[101]

1.1.4 The influence of breastfeeding on vaccine responses

Breastfeeding has been shown to be beneficial in preventing many communicable and non-communicable diseases.[102] However, despite intensive research into the positive health effects of breastfeeding and its immunomodulatory, anti-inflammatory and antimicrobial effects, the underlying mechanisms are still not fully understood. One of the beneficial effects of breastfeeding is the transfer of maternal antibodies to the infant.[103] As with trans-placental transfer of maternal antibodies, antibodies passed on through breastmilk can also interfere with infant antibody responses following immunisation. To date, most studies investigating the influence of breastfeeding on vaccine responses have focused on oral vaccines. One recent meta-analysis summarising studies which have investigated the effect of breast-feeding on antibody response to oral rotavirus immunisation (ORV) reported reduced antibody production in breastfed infants in all of the included studies.[104] More recent studies, which were not included in the mentioned meta-analysis, also show that higher levels of rotavirus-specific IgA in breast milk leads to lower antibody responses in infants after ORV.[105]

There is less data available about the influence of breastfeeding on responses to parenteral immunisation. However, in contrast to lower antibody responses to oral vaccines observed in breastfed infants, it has been shown that after parenteral immunisation breastfed infants have higher antibody levels to diphtheria[106] and Hib[107,108] antigens in serum and higher antibody levels to tetanus and diphtheria antigens in saliva.[106]

1.1.5 The influence of delivery mode on vaccine responses

Delivery mode has been shown to strongly influence the intestinal microbiota: infants born vaginally are predominantly colonised by microbes resembling the maternal vaginal and intestinal microbiota, whereas infants born by Caesarean Section are predominantly colonised with the maternal skin microbiota.[109-111] Growing evidence shows that the composition of the intestinal microbiota in infants plays and important role in the development and regulation of the immune system, especially in the early-life 'critical window' during which the microbiota and the immune response develop concurrently.[112] Delivery mode might also, through changes in the microbiota, influence vaccine responses. Currently, there are no studies that have investigated the association between delivery mode and vaccine responses.

1.1.6 The influence of antibiotic exposure on vaccine responses

Antibiotics are amongst the commonly used drugs, especially in infants and children. Even before birth, over 40% of infants are already exposed to antibiotics as a result of group B streptococcal (GBS) or surgical (Caesarean) prophylaxis.[113] Additionally, it is estimated that more than two-thirds of children receive antibiotics before reaching the age of two years.[114] There is increasing evidence that antibiotic administration in early-life leads to adverse health outcomes later in life. Antibiotic exposure *in utero* and during infancy has been associated with an increased risk of obesity,[115] allergies,[116] asthma,[116] inflammatory bowel disease[117] and juvenile idiopathic arthritis.[118] There is evidence from animal studies that antibiotic administration might influence vaccine responses. A

study in mice shows that administration of clarithromycin or doxycycline leads to lower antibody responses after immunisation with HepB, tetanus, or PPV, while administration of ampicillin leads to higher antibody responses after immunisation with live attenuated *Salmonella* Typhi vaccine.[119] A study in calves shows that administration of tetracycline leads to lower antibody responses after immunisation with *Brucella abortus* vaccine.[120] To date, there are no studies which have investigated the influence of antibiotic administration on vaccine responses in humans.

1.1.7 Immunomodulatory effects of Bacillus Calmette-Guérin vaccine and its influence on heterologous vaccine responses

Bacillus Calmette-Guérin vaccine (BCG) is one of the most widely used vaccines worldwide. In addition to providing protection from tuberculosis, it has 'non-specific' (heterologous) immunomodulatory effects.[121] BCG reduces all-cause infant mortality in high-mortality settings,[122] decreases hospitalisation rates due to respiratory infections and sepsis,[123,124] is used in the treatment of bladder cancer,[125] might decrease the incidence of eczema[126] and other allergies,[127] and is proposed to be beneficial in the treatment of autoimmune diseases.[128,129]

It is likely that through its immunomodulatory effects BCG also influences responses to heterologous immunisations. Several studies have investigated the influence of BCG immunisation on humoral response to other vaccines but, to date, these have not been systematically reviewed.

1.1.8 Correlation of vaccine responses

In clinical practice, e.g. when investigating for primary immunodeficiency, measuring antibody responses to diphtheria, tetanus and pneumococcal vaccine antigens is common practice.[130] When the immunisation status of an individual is unknown, antibody responses to tetanus is often used as a surrogate marker.[131] This strategy makes the assumption that there is a correlation within an individual between the response to different vaccines.

One study compared the antibody response to different serotypes within the conjugate pneumococcal vaccine and showed moderate correlation between antibody concentrations.[132] However, currently, no study has investigated the correlation in antibody response between different vaccines. It is therefore unknown whether there is any consistency within individuals in the magnitude of antibody responses to all vaccines or to vaccines of a certain type (e.g. subunit, live or attenuated vaccines).

1.2 HYPOTHESIS AND AIMS

Hypothesis 1: Sex, delivery mode, breastfeeding and exposure to antibiotics influences the immune response to routine vaccines in infants.

Aim 1**:** To compare antibody levels to routine vaccines at 7 months (22 antigens) and 13 months (26 antigens) of age (4-6 weeks after immunisation) between:

- male and female infants
- infants born vaginally and those born by Caesarean section
- infants who were breastfed and those who only received formula milk
- infants who received antibiotics before their 6-weeks immunisation and those who did not receive antibiotics.

Timing	**Antibody levels**
7 months	Diphtheria, tetanus, pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN), Hib, polio type 1-3, 13 pneumococcal serotypes
13 months	As above plus meningococcus C (MenC), measles, mumps, rubella

Hypothesis 2**:** Maternal immunisation during pregnancy influences infant antibody responses to routine immunisation in the first year of life.

Aim 2**:** To compare antibody levels to routine scheduled vaccines at 7 months (DTPa, inactivated polio vaccine (IPV), Hib, pneumococcal conjugate vaccine (PCV)) and 13 months (DTPa, IPV, Hib, PCV, MenC, MMR) of age (4-6 weeks after immunisation) between infants whose mother did or did not receive dTpa or TIV immunisation during pregnancy.

***Hypothesis 3*:** BCG immunisation has immunomodulatory effects that influences the antibody responses to routine vaccines.

***Aim 3*:** To compare antibody levels to routine scheduled vaccines at 7 months (DTPa, IPV, Hib, PCV) and 13 months (DTPa, IPV, Hib, PCV, MenC, MMR) of age (4-6 weeks after immunisation) between infants who did or did not receive BCG at birth.

***Hypothesis 4*:** There is a correlation of vaccine antibody responses to different routine vaccines or between vaccines of a certain type.

***Aim 4*:** To investigate the correlation between antibody responses between different routine vaccines or between vaccines of a certain type at 7 and 13 months of age (4-6 weeks after routine immunisation).

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REVIEW

Factors That Influence the Immune Response to Vaccination

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SUMMARY There is substantial variation between individuals in the immune response to vaccination. In this review, we provide an overview of the plethora of studies that have investigated factors that influence humoral and cellular vaccine responses in humans. These include intrinsic host factors (such as age, sex, genetics, and comorbidities), perinatal factors (such as gestational age, birth weight, feeding method, and maternal factors), and extrinsic factors (such as preexisting immunity, microbiota, infections, and antibiotics). Further, environmental factors (such as geographic location, season, family size, and toxins), behavioral factors (such as smoking, alcohol consumption, exercise, and sleep), and nutritional factors (such as body mass index, micronutrients, and enteropathy) also influence how individuals respond to vaccines. Moreover, vaccine factors (such as vaccine type, product, adjuvant, and dose) and administration factors (schedule, site, route, time of vaccination, and coadministered vaccines and other drugs) are also important. An understanding of all these factors and their impacts in the design of vaccine studies and decisions on vaccination schedules offers ways to improve vaccine immunogenicity and efficacy.

KEYWORDS antibodies, cellular, cytokines, humoral, immunization, immunoglobulin

INTRODUCTION

Vaccination is the most cost-effective life-saving medical intervention and is estimated to save at least 2.5 million lives each year (1, 2). Protection induced by vaccinations is mediated through a complex interplay between innate, humoral, and cell-mediated immunity (3, 4). Methods to quantify vaccine responses include measuring geometric mean antibody titers (GMTs), seroconversion rates (SCRs), seroprotection rates (SPRs), functional antibodies (by flow cytometric opsonophagocytosis assays), antibody avidity, B and T cell activation, lymphoproliferation, and cytokine responses. There is substantial variation between individuals in the immune response to vaccination, in both quantity and quality. For example, the antibody responses to yellow fever (YF) vaccination vary >10-fold between individuals (5), those to 7- and 13-valent conjugated pneumococcal (PCV7 and PCV13) and *Haemophilus influenzae* type b (Hib) vaccination up to 40-fold (6), and those to trivalent inactivated influenza vaccine (TIV) (7) and hepatitis B (HepB) vaccination >100-fold (6, 8). Similarly, cytokine responses to *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination vary up to 10-fold (9). Other examples of differences in the quality of vaccine responses include a lower avidity of antibodies (10) or strength of cell-mediated immune responses (11) in neonates. These variations in vaccine responses have consequences for both protective efficacy and the duration of protection. Worryingly, a significant proportion of vaccine-preventable infections occur in vaccinated individuals (12). It is estimated that large numbers of vaccinated children are unprotected due to vaccine ineffectiveness, including 77 million from tuberculosis (TB) (following BCG vaccination), 19 million from measles, 18 million from poliomyelitis (following vaccination with inactivated polio vaccine [IPV]), and 10 million from pertussis and from pneumococcus (13).

In this review, we provide a general overview of factors that influence the immune response to vaccination (Fig. 1). A greater understanding of these factors offers opportunities to improve vaccine immunogenicity and efficacy.

FACTORS INFLUENCING VACCINE RESPONSES

Intrinsic Host Factors

Age. Age is an important factor that influences vaccine responses, especially in the extreme ages of life. Infants should receive immunizations as early as possible to minimize the time that they are susceptible to infections. However, neonates have a

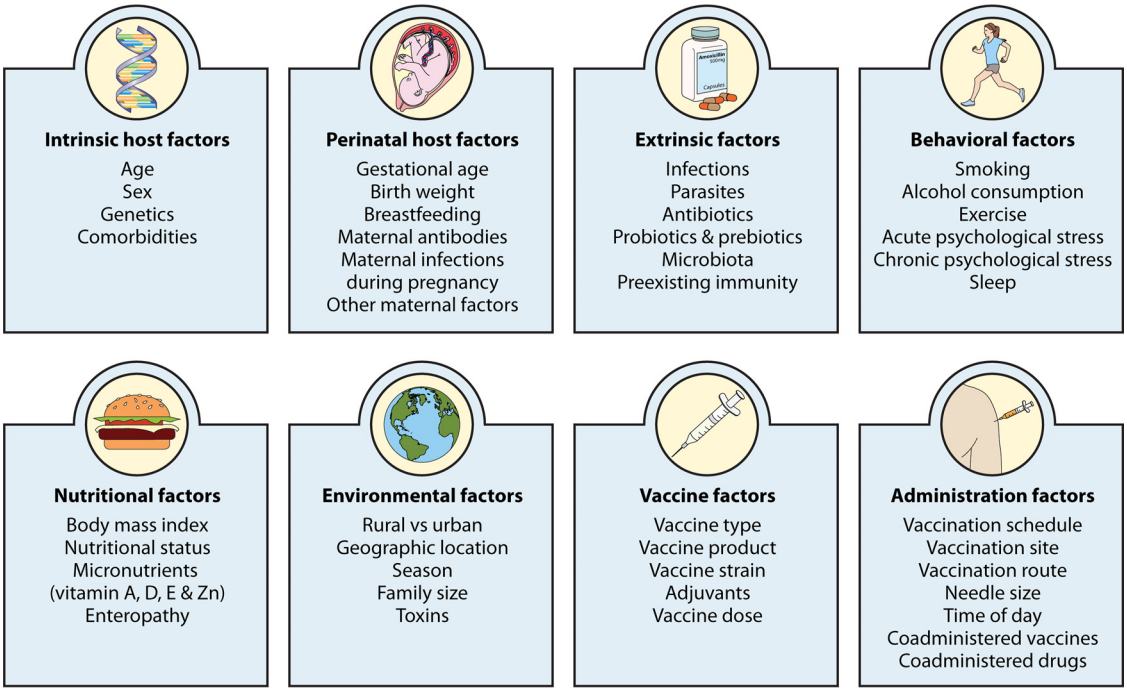


FIG 1 Factors that influence the immune response to vaccination.

lower level of antibody production and, moreover, passively acquired maternal antibodies interfere with vaccine responses (14; P. Zimmermann, K. Perrett, N. Messina, S. Donath, N. Ritz, F. R. M. van der Klis, and N. Curtis, submitted for publication). Additionally, cell-mediated immune responses are less strong, and the response to T-independent polysaccharide antigens is poor (11). Studies in the 1950s sought to establish the optimal age to start vaccination (15, 16). These studies showed, for example, that oral polio vaccine (OPV) given during the first week of life leads to adequate serum antibody responses in only 30% to 70% of infants but that, when it is given after 4 to 8 weeks of age, it leads to adequate responses in nearly all infants (15). Similarly, the diphtheria-tetanus-pertussis (DTP) vaccine is less effective when the first dose is given within the first week of life than when it is given at 6 months of age (16) (Table 1). Results of studies investigating the immunogenicity of BCG given at different ages are conflicting, with some studies showing better immunogenicity when the vaccine is given after the age of 2 months than when it is given at birth (17) and others reporting lower immunogenicity when the vaccine is given at 4 months than when it is given at birth (18). HepB vaccine given in the first year of life leads to lower long-term antibody responses than those obtained when it is given later in childhood: only 40% of adolescents who were HepB vaccinated at birth had antibody levels of $>$10 mIU/ml at the age of 15 years (19), and after a booster dose, antibodies rose above this protective level in only half of individuals (19, 20). However, importantly, in contrast to antibody levels, the number of memory B cells does not decrease over time (21), and hence booster doses of this vaccine are not necessary.

The most-studied vaccine in relation to the effect of age on vaccine responses, by far, is the measles vaccine (10, 22–48). A meta-analysis of 20 studies shows that the proportion of infants seroconverting after one dose of measles vaccination increases from 50% at 4 months of age to 85% at 8 months and that GMTs are lower in children who receive their first dose before 9 months of age than in those who receive their first dose at an older age (49). Additionally, antibodies wane significantly more quickly in infants who receive the first dose of measles vaccine before the age of 9 months (49), and antibodies have significantly lower avidity when the first dose of measles vaccine is given before the age of 6 months than when it is given at 9 to 12 months of age (10).

TABLE 1 Results with regard to age from studies investigating intrinsic host factors that influence vaccine responses[a]

	Vaccine: measurement [reference(s)]		
Type of response	**Effective when given at birth**	**Lower vaccine responses in neonates and young infants**	**Lower vaccine responses in elderly people**
Humoral responses	aP: GMTs (52, 53) HepB: SPRs (55) OPV: SPRs (15)	Diphtheria: SPRs (16) OPV: SPRs (15) Tetanus: SPRs (16) wP: SPRs (16)	Diphtheria: SPRs (60) HepA: GMTs (61, 63, 65, 142), SCRs (63, 76), SPRs (61, 62, 65, 142) HepB: GMTs (61, 69, 71, 73, 75, 77), SCRs (70, 71, 75, 80, 110), SPRs (61, 62, 66–69, 72–75, 77–79, 81) PPV23: GMTs (82–84) TBE: GMTs (88) Tetanus: GMTs (88), SPRs (60, 89) TIV: GMTs (87)
Cellular responses	BCG: Th1, Th2, Treg responses (18)	BCG: CD4 T cell response (17)	TIV: Th cell and cytotoxic T cell responses (86), lymphoproliferative responses (87)
Cytokine responses	BCG: IL-6 and IL-17 production (18)		TIV: IL-2 (85), IL-10 (87), and IFN-γ (87) production

[a]AGGs, agglutinogens; aP, acellular pertussis; BCG, bacillus Calmette-Guérin; CD, cluster of differentiation; FHA, filamentous hemagglutinin; GMTs, geometric mean antibody titers; HepA, hepatitis A; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; HLA, human leukocyte antigen; HSV, herpes simplex virus; MHC, major histocompatibility complex; IFN, interferon; IL, interleukin; IPV, inactivated polio vaccine; MCV, meningococcal conjugate vaccine; MPV, meningococcal polysaccharide vaccine; OCV, oral cholera vaccine; OPV, oral polio vaccine; ORV, oral rotavirus vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; PRN, pertactin; PRR, pattern recognition receptor; PT, pertussis toxin; SCRs, seroconversion rates; SPRs, seroprotection rates; TBE, tick-borne encephalitis; TGF, transforming growth factor; Th, T helper; TIV, trivalent inactivated influenza vaccine; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, T regulatory cell; wP, whole-cell pertussis.

A recent large study summarizing randomized controlled trials (RCTs) shows that children who receive their first measles vaccine before the age of 12 months have lower SCRs and geometric mean concentrations (GMCs) than those of children who receive their first measles vaccine after the age of 15 months (50). Lower antibody concentrations persist after the second dose of measles vaccine (49, 50). In contrast, age at first measles vaccination does not influence cellular responses: no differences in *in vitro* T cell proliferation in response to measles virus were observed after starting vaccination at 4, 6, 9, or 12 months (30, 37, 38, 51).

Despite the fact that many vaccines have lower immunogenicity in neonates, it has been shown that for acellular pertussis (aP) (52, 53), BCG (54), HepB (55), and OPV (15) vaccines, vaccination can be effective in the neonatal age group when adjuvants and formulations are adapted to the function of the early immune system (56) (Table 1). The early neonatal immune system shows suboptimal interaction between antigen-presenting cells and T cells, leading to impairment of CD4 and CD8 T cell function and a polarization toward T helper type 2 (Th2) cells (57) and toward induction of memory B cells rather than antibody-secreting plasma cells (58, 59). T cells largely remain unaffected by passively acquired maternal antibodies.

In addition to those in early life, vaccine responses are also diminished in the elderly, who also have more rapid waning of antibodies. Elderly people have lower antibody responses to diphtheria (60), hepatitis A (HepA) (61–65), HepB (61, 62, 66–81), pneumococcal polysaccharide vaccine (PPV23) (82–84), TIV (85–87), tick-borne encephalitis (TBE) (88), and tetanus (60, 88, 89) vaccination (Table 1). After vaccination with TIV, elderly people also have lower cellular vaccine responses (85–87, 90). In contrast, one study reports higher antibody responses to TIV vaccination in elderly people, with a higher avidity of antibodies and slower antibody dissociation (91). Aging is accompanied by a shift toward anti-inflammatory interleukin-10 (IL-10), which is associated with a decline in CD8 T cells responsible for clearing influenza virus. Hence, adjuvants in TIV that stimulate inflammatory cytokines and suppress the IL-10 response would potentially enhance protection in this age group (92).

Notably, the influence of age on vaccine responses is not seen just at the extremes of age. For example, antibody responses to TIV are higher in children of 2 to 4 years of

age than in younger children (93), and GMTs after meningococcal conjugate vaccine (MCV-C) vaccination are higher in children over the age of 10 years than in younger children (94). The findings of studies that investigated the influence of age on vaccine responses are summarized in Table 1.

Sex. The findings across studies and meta-analyses investigating the effect of sex on vaccine responses are largely consistent (Table 2). Females have higher antibody responses to dengue (95), HepA (61–65, 96–104), HepB (61, 62, 66–78, 80, 100, 101, 105–117), Hib (118), IPV (119), rabies (120–122), smallpox (123), and TIV (124–134) vaccination, while males have higher antibody responses to diphtheria (135–138), MCV-A (139), PCV7 (140), PPV23 (83, 122, 140–144), and tetanus (88, 89, 119) vaccination. Additionally, females also have higher cellular responses to a herpes simplex virus (HSV) vaccine that is not currently used (145). Only a few studies report opposite findings, with higher antibody responses to diphtheria (60, 146), PCV13 (118), and tetanus (60, 147) vaccination in females, higher antibody responses to TIV (91) vaccination in males, or no difference between sexes in antibody responses to rabies (148) vaccination (Table 2).

Notably, even though females have an overall tendency to have higher antibody responses, faster waning of antibodies has been shown following HepA (103) and PPV23 (83, 144) vaccination.

Studies investigating sex differences in response to the measles-mumps-rubella (MMR) vaccine report less consistent findings. Some studies report higher GMTs or SPRs after measles (149), mumps (150), and rubella (151) vaccination and higher cytokine responses after measles (152) vaccination in females, while other studies report transiently higher GMTs and lymphoproliferative responses after rubella (153) vaccination and higher SCRs after measles (154) vaccination in males or no sex differences in GMTs after mumps (155) and rubella (156) vaccination or cytokine responses after rubella (156) vaccination (Table 2).

Reported sex differences in antibody responses to YF vaccine vary depending on the vaccine used (157–159). Interestingly, 3 to 10 days after YF vaccination, expression of 660 genes changes in women, while only 67 genes are expressed differently in men (160). Many of these differentially expressed genes are involved in the early innate immune response (160).

Genetics. Different ethnic groups living in the same location have varied responses to vaccination (64, 89, 161–166) and decline of antibodies (89), indicating a genetic influence on vaccine responses. Studies of twins estimate the degree of heritability to be 36 to 90% for humoral responses (167–173) and 39 to 90% for cellular responses, depending on the specific vaccine (167, 169) (Table 3).

One mechanism identified with variations in humoral (150, 174–190) and cellular (145, 150, 186, 191) vaccine responses is polymorphism in major histocompatibility complex (MHC) genes (summarized in Table 3). Further genetic factors are polymorphisms in pattern recognition receptors (PRRs), such as Toll-like receptor (TLR) or RIG-like receptor (RLR) genes (192–195) (Table 3). In addition, many single-nucleotide polymorphisms (SNPs) in other genes, for example, those coding for cytokines or cytokine, viral, or vitamin receptors, are also associated with variations in vaccine responses (182, 190, 192, 193, 196–211) (Table 3). Further, different expression levels of genes also influence vaccine responses. For example, individuals with increased expression of genes involved in early interferon signaling, antigen processing, and antigen presentation have higher antibody responses to TIV (194, 212).

Blood group antigens are important receptors or coreceptors for microorganisms. Additionally, they can also modify the innate immune response to infection (213). It is therefore likely that blood group antigens influence responses to vaccination. It has been suggested that a cholera subunit-killed oral vaccine is less efficient in individuals with blood group O (214, 215). In contrast, the oral cholera vaccine (OCV) has been reported to induce higher antibody concentrations in individuals with blood group O (216). This has not, however, been a consistent finding (217).

Comorbidities. Numerous studies have investigated vaccine responses in children with celiac disease (CD) (218–233) (Table 4). Most found that children with CD have

TABLE 2 Results with regard to sex from studies investigating intrinsic host factors that influence vaccine responses

Type of response	Vaccine and age group: measurement [reference(s)]			
	Higher vaccine responses in females	**Higher vaccine responses in males**	**No difference in vaccine responses between females and males**	**Inconsistent findings in vaccine responses between females and males**
Humoral responses	Dengue Adults: GMTs (95) Diphtheria Adults: SPRs (60), GMTs (146) HepA Children: GMTs (63, 142), SCRs (63), SPRs (142) Adults: GMTs (63, 65, 96–101, 142), SCRs (63, 101, 106), SPRs (65, 101, 103, 142) Adults with chronic liver disease: GMTs (102), SCRs (102) HIV-positive adults: SPRs (104) Elderly: GMTs (61), SPRs (61, 62) HepB Infants: GMTs (116) Children: GMTs (114, 115, 117) Adolescents: GMTs (105), SCRs (105) Adults: GMTs (69–71, 73, 76, 80, 100, 101, 107, 111), SCRs (70, 71, 76, 80, 101), SPRs (67–69, 72–75, 77, 78, 101, 107, 110, 111, 113) Adults on hemodialysis: SCRs (112) Diabetic adults: GMTs (108, 109), SPRs (108) Elderly: GMTs (61), SPRs (61) Hib Infants: GMTs (118) IPV Adults: SPRs (119) Measles Adolescents: GMTs (149) Mumps Children: GMTs (150) PCV13 Infants: GMTs (118) Rabies Children: SCRs (122) Adults: GMTs (120, 121) Rubella Children: SPRs (151) Adults: SPRs (151) Smallpox Adults: GMTs (123) Tetanus Adults: GMTs (60), SPRs (60), titers (147) TIV Adults: GMTs (90, 124, 126, 127, 129, 131, 133, 134), SPRs (90, 129, 133, 134) Elderly: GMTs (125, 130, 132), SCRs (130), SPRs (128)	Diphtheria Adolescents: titers (137) Adults: titers (135) Elderly: GMTs (138) MCV-A Adolescents: GMTs (139) Measles Infants: SCRs (154) PCV7 Elderly: GMTs (140) PPV23 Children: SCRs (122) Adult alcoholics: GMTs (142) Elderly: GMTs (83, 140, 141, 143, 144) Rubella Adolescents: GMTs (153) Tetanus Children: SPRs (89) Adults: GMTs (88), SPRs (89, 119) TIV Adults: GMTs (91)	Mumps Children: GMTs (155) Rabies Children: GMTs (148) Adults: GMTs (148) Rubella Children: GMTs (156)	YF Adults: GMTs (158, 159), SCRs (159), SPRs (157, 158)
Cellular responses	HSV Adults: CD4 T cell responses (145)	Rubella Adolescents: lymphoproliferative responses (153)		
Cytokine responses	Measles Children: IL-6 (152), IFN-γ (152), and TNF-α (152) production		Rubella Children: IL-10 (156) and IFN-γ (156) production	

TABLE 3 Results with regard to genetics from studies investigating intrinsic host factors that influence vaccine responses

Type of response	Vaccine (estimated degree of heritability in vaccine responses [%]) [reference(s)]	Variation in vaccine response and vaccine: MHC genes for which polymorphisms have been identified to be associated with variation in vaccine response [reference(s)]	Variation in vaccine response and vaccine: PRR genes for which polymorphisms have been identified to be associated with variation in vaccine response [reference(s)]	Variation in vaccine response and vaccine: proteins for which single-nucleotide polymorphisms in genes are associated with variations in vaccine responses [reference(s)]
Humoral responses	aP (53–65) (169) Diphtheria (49) (169) HepA (36) (170) HepB (61–91) (169–171) Hib (51) (172) Measles (90) (167, 168) Mumps (39) (168) OPV (60) (169) Rubella (46) (168) Tetanus (44) (169) Varicella (45) (173)	Higher antibody production HepB: HLA-DQB1*05 (178), HLA-DQB1*0602 (178), HLA-DQB1*0603 (178), HLA-DRB1*0102 (178), HLA-DRB1*0103 (178), HLA-DRB1*02 (176), HLA-DRB1*1301 (177, 178), HLA-DRB1*15 (178), HLA-DR7; DQ2, DR4; DQ3, DR15; DQ6, DR3; DQ2 (55) Rubella: HLA-A*02-C*03-B*15-AAAACGGGGC-DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*04 (190), A*02-C*03-B*15-DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*04 (190), HLA-DPB1, rs2064479 (188) TIV: HLA-DPB1*0401 (187), HLA-DQB1*0603 (186), HLA-DRB1*040 (187) Lower antibody production HepB: C4ADel (181), C4AQ0 (179–181), HLA-B8, SC01, DR3 (235), HLA-DPB*0201 (180), HLA-DRA, rs5000563 (182), HLA-DRB1*07(01) (174, 176, 177, 180, 181), HLA-DQA1*0201 (180), HLA-DQB1*0102 (178), HLA-DQB1*02(01) (174, 175, 179, 180), HLA-DQB1*0604 (178), HLA-DRB1*03 (177, 181), HLA-DRB1*1301 (181), HLA-DRB1*1302 (178), HLA-DR3 (extended B8, SC01) (183, 184), HLA-DR7 (extended B44, FC31) (184) Measles: HLA-DPA1*0201 (185), HLA-DRB1*03 (185) Mumps: HLA-DQB1*0303 (150), HLA-A*29-Cw*16-B*44 (569) Rubella: DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*04 (190), DRB1*15/16-DQB1*06-DPB1*03 (569), DRB1*04-DQB1*03-DPB1*03 (569) TIV: HLA-DRB1*07 (186)	Higher antibody production MCV-C: TRL3 (193) Lower antibody production Measles: TLR3 (192)	Variations in antibody production Diphtheria: IL-10 (196) HepB: FOXP1 (182), IL-4 (197), IL-4R (197), IL-10 promotor (198), IL-12B (196), TNF-α (196) TIV: IL-28B (199) Measles: CD46 (200, 201), CD209 (200), IL-2 (202), IL-6 (192), IL-7R (203), IL-10 (202, 203), IL-12RB (202–204), SLAM (201), TNF-α (192) MCV-C: CD44 (193) Mumps: IL-10RA, IL-12RB1 (150) PCV7/PPV23: IL-1β (196), IL-4 (205), IL-4R (196, 205), IL-10 (196), IL-12RB1 (196), IL-13 (196, 205) Rubella: CD209/DC-SIGN (206), IL-6 (206), leukocyte specific transcript 1 (190), lymphotoxin alpha (190, 207), MOG (206), PVR (206), PVRL2 (206), RARB (206), TNF-α (190) Smallpox: IL-18 (208) Tetanus: IL-4R (196)
Cellular responses		Higher lymphoproliferative responses Measles: HLA-A*26-Cw*12-B*28 (569), DRB1*03-DQB1*02-DPB1*04 (569), DRB1*04-DQB1*03-DPB1*03 (569) Mumps: HLA-B*1302 (150), HLA-B*3701 (150), HLA-B*3801 (150), HLA-DQA1*0101 (150), HLA-DQA1*0105 (150), HLA-DQB1*0201 (150), HLA-DRB1*0101 (150), HLA-DRB1*0701 (150), HLA-DRB1*1001 (150), HLA-A*26-Cw*12-B28 (569), DRB1*03-DQB1*02-DPB1*04 (569) Rubella: DRB1-DQA1-DQB1-DPA1-DPB1*13-*01-*06-*01-*03, DRB1-DQA1-DQB1-DPA1-DPB1*03-*05-*02-*01-*04 (191), DRB1*04-DQB1*03-DPB1*03 (569), DRB1*04-DQB1*03-DPB1*03 (569) Lower lymphoproliferative responses HepB: HLA-Bw54-DR4-DRw53-SQw4 (570, 571) Mumps: HLA-DQA1*0401 (150), HLA-DQA1*0501 (150), HLA-DRB1*0301 (150), HLA-DRB1*0801 (150), HLA-DRB1*1201 (150), HLA-DRB1*1302 (150)	Lower lymphoproliferative responses Measles: TLR3 (192)	Variations in lymphoproliferative responses Measles: IL-2 (202), IL-10 (202), IL-12RB (202) Mumps: IL-10RA, IL-12RB1 (150)

(Continued on next page)

TABLE 3 (Continued)

Type of response	Vaccine (estimated degree of heritability in vaccine responses [%]) [reference(s)]	Variation in vaccine response and vaccine: MHC genes for which polymorphisms have been identified to be associated with variation in vaccine response [reference(s)]	Variation in vaccine response and vaccine: PRR genes for which polymorphisms have been identified to be associated with variation in vaccine response [reference(s)]	Variation in vaccine response and vaccine: proteins for which single-nucleotide polymorphisms in genes are associated with variations in vaccine responses [reference(s)]
Cytokine responses	BCG (39–46) (169) Measles (90) (167) Pertussis (53–65) (169) Tetanus (64) (169)	Higher IFN-γ production Rubella: A*02-C*07-B*07 (191), A*03-C*04-B*35 (191), DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*03 (191) Higher IL-2 production Rubella: DRB1*04-DQA1*03- DQB1*03-DPA1*01-DPB1*02 (191), DRB1*04-DQA1*03-DQB1*03-DPA1*01-DPB1*04 (191) Higher IL-4 production Rubella: DRB1*15-DQA1*01-DQB1*06- DPA1*01-DPB1*02 (191), DRB1*07-DQA1*02-DQB1*02-DPA1*02-DPB1*11 (191) Higher IL-5 production Rubella: A*02-C*07-B*08 (191) Higher IL-6 production Rubella: A*24-C*07-B*07 (191) Higher IL-10 production Rubella: DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*02 (191) Higher IL-12p40 production Rubella: A*03-C*03-B*40 (191), A*03-C*07-B*07 (191), DRB1*07- DQA1*02-DQB1*03-DPA1*01-DPB1*04 (191) Higher TNF-α production Rubella: A*01-C*07-B*08-DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*04 (190), A*29-C*16-B*44 (191) Lower TNF-α production Rubella: DRB1*13-DQA1*01-DQB1*06-DPA1*01-DPB1*03 (191) Lower IL-2 production Rubella: DRB1*13-DQA1*01- DQB1*06-DPA1*01-DPB1*03 (191) Lower IL-5 production Rubella: DRB1*11-DQA1*05-DQB1*03-DPA1*01-DPB1*02 (191) Lower IL-6 production Rubella: DRB1*03-DQA1*05-DQB1*02- DPA1*01-DPB1*04 (191), AAACGGGGC-DRB1*01-DQA1*01-DQB1*05-DPA1*01-DPB1*04 (190) Lower IL-10 production Rubella: DRB1*03-DQA1*05-DQB1*02-DPA1*01-DPB1*04 (191) Lower IL-12p40 production Rubella: A*02-C*03-B*40 (191)	Higher granulocyte-macrophage colony-stimulating factor (GM-CSF) production Rubella: TLR3 (194) Higher IL-2 production BCG: TLR1, TLR6 (195) Higher IL-4 production Measles: TLR4 (192) Higher IFN-γ production BCG: TLR1, TLR6 (195) Measles: TLR5 (192), TLR6 (192) Rubella: RIG-I (194) Lower IL-10 production Rubella: RIG-I (194) Lower IFN-γ production Rubella: TLR3 (194) Lower TNF-α production Rubella: TLR3 (194) Variations in GM-CSF production Rubella: RIG-I (194), TLR3, TLR4 (194) Variations in IL-2 production Rubella: TLR3 (194), RIG-I (194) Variations in IL-2 production Rubella: RIG-I (194) Variations in TNF-α production Rubella: RIG-I (194)	Variations in GM-CSF production Rubella: CASP8 (194), RARB (194), TRIM5 (194), TRIM22 (194) Variations in IL-2 production Rubella: RARA (194), RARB (194), TRIM5 (194), TRIM22 (194) Variations in IL-4 production TIV: IL-28B (199) Variations in IL-6 production Measles: CD46 (200) Rubella: ADAR (210), PVR (210), TRIM22 (194) Variations in IL-10 production Measles: SLAM (200) Rubella: RARB (194), RXRA (194) Variations in IFN-α production Measles: CD46 (200, 201) Variations in IFN-γ production Rubella: ACO1 (211), butyrophilin (210), IL-10RB/IFNAR1 (210), PTPRD (211), RARB (194), RARG (194), TRIM22 (194) Measles: IL-2 (203), IL-7R (203), IL-10 (203), IL-12RB (204), SLAM (200), DC-SIGN promotor (209) Variations in TNF-α production Measles: CD46 (200) Rubella: TRIM5 (194), VDR (194)

TABLE 4 Results with regard to comorbidities from studies investigating intrinsic host factors that influence vaccine responses

	Variation in vaccine response, vaccine, age group, measurement [reference(s)]			
Type of response	**Celiac disease**	**Diabetes mellitus**	**Chronic renal failure requiring hemodialysis**	**Chronic liver failure**
Humoral responses	Lower antibody production HepA Children: SPRs (225) HepB Infants: SPRs (221) Children: SPRs (219, 220, 222–226, 228, 231, 233) Adults: GMTs (227), SPRs (218, 227) No difference in antibody production HepA Children: GMTs (229), SPRs (229) HepB Children: GMTs (219, 232), SPRs (232) Adults: GMTs (232), SPRs (232) Hib Children: SPRs (219) Measles Children: GMTs (232), SPRs (232) Adults: GMTs (232), SPRs (232) Rubella Children: SPRs (219) Tetanus Children: SPRs (219) TIV Children: GMTs (230), SPRs (230)	Lower antibody production HepB Children: GMTs (232, 238), SPRs (232, 237–239) Adults: GMTs (232, 243–245), SPRs (242–245) Measles Children: SPRs (241) Monovalent influenza vaccine Adults: SCRs (90), SPRs (90) PPV23 Children: SPRs (240) Rubella Children: GMTs (241) No difference in antibody production Diphtheria Children: GMTs (240, 241) Hib Children: GMTs (240) HepB Children: GMTs (572), SPRs (232, 572) Adults: GMTs (572), SPRs (232, 572) Elderly: SPRs (248) Measles Children: GMTs (232, 241), SPRs (232) Adults: GMTs (232), SPRs (232) Rubella Children: SPRs (241) PCV7 Children: GMTs (240) Pertussis Children: SPRs (241) Tetanus Children: GMTs (240, 241)	Lower antibody production Diphtheria Adults: SPRs (250) HepB Adults: SPRs (252) IPV type 2 Children: GMTs (260) Tetanus Adults: GMTs (249), SPRs (249, 250) TIV Adults: GMTs (473), SPRs (473) No difference in antibody production IPV type 1 Children: GMTs (260), SPRs (260) IPV type 2 Children: SPRs (260) IPV type 3 Children: GMTs (260), SPRs (260) Faster weaning of antibodies Diphtheria Adults: (254) HepB Adults: (252, 255) Tetanus Adults: (251)	Higher antibody production Diphtheria Children: GMTs (260) IPV type 1 Children: GMTs (260) IPV type 3 Children: GMTs (260) Lower antibody production HepA Children: GMTs (265) Adults: GMTs (102, 261, 262), SPRs (263, 264) HepB Adults: GMTs, SPRs (242) Children: GMTs (265), SPRs (263, 265) No difference in antibody production Diphtheria Children: SPRs (260) HepA Children: SPRs (265) Adults: SPRs (102, 263) IPV type 1 Children: SPRs (260) IPV type 2 Children: GMTs (260), SPRs (260) IPV type 3 Children: SPRs (260) Tetanus Children: GMTs (260), SPRs (260)
Cytokine responses	Lower cytokine production Tetanus Children: IL-4 (219, 573)	No difference in cytokine responses TIV Children: IL-2 (247), granzyme B (247)		

lower antibody responses to HepB vaccination (218–228, 231–233), with more rapid waning of antibodies (227). A meta-analysis shows that, in retrospective studies, the SCR to HepB vaccination in children with CD is 54%, and that in prospective studies is 66% (compared to 95% in healthy individuals) (234). It has been postulated that the presence of HLA-DQ2 or -DQ8, which confers a genetic predisposition to CD, may be the driver of the lower responses to HepB vaccination in CD patients. However, the presence of HLA-DQ2 is associated with higher antibody responses to HepB vaccination

(55). Nevertheless, HLA-DR3, -DR4, and -DR7, which can also be found in patients with CD (229) and patients with diabetes mellitus (DM), are associated with lower responses to HepB vaccination (183, 184, 235). Children with CD and DM have lower SPRs after HepB vaccination than those of children that have DM only (228), suggesting that gluten might be a factor in decreasing HepB vaccine efficacy, and some studies suggest that the response rate to HepB vaccination correlates with gluten intake (220, 223). Children with CD who are on a strict gluten-free diet have no difference in SPRs to HepB vaccination compared to those of healthy children (222, 226). Apart from lower antibody responses to HepB vaccination, one study also showed that CD patients are less responsive to HepA vaccination (225). However, another study did not find a difference in antibody responses to HepA vaccination in CD patients compared to healthy controls (229). There is also no difference in antibody responses to tetanus (219), measles (236), rubella (219), Hib (219), and TIV (230) vaccination between children with CD and healthy children.

Children with DM have lower antibody responses to HepB (232, 237–239) vaccination, and possibly PPV23 (240), rubella (241), and measles (241) vaccination, but not to diphtheria (240, 241), Hib (240), PCV7 (240), pertussis (241), or tetanus (240, 241) vaccination. Adults with DM also have lower antibody responses to HepB (242–245) vaccination. Adding selenium to the HepB vaccine can increase antibody responses in patients with DM (246). It is unclear whether DM influences the antibody response to TIV vaccination. Some studies report lower responses after vaccination with a monovalent influenza vaccine in adults (90), while studies of elderly people with type II DM show no difference in antibody or cytokine responses to TIV vaccination (247, 248) (Table 4).

Adults with chronic renal failure who are on hemodialysis have lower antibody responses to diphtheria (249–251), HepB (252–255), and tetanus (249–251) vaccination, with faster waning of antibodies (252–255), especially when they suffer from additional diabetes mellitus (256–259). Children on hemodialysis have lower antibody responses to poliovirus type 2 (260). SPR after HepB vaccination is inversely associated with glomerular filtration rate (253). Factors responsible for lower vaccine responses in patients with chronic renal failure include malnutrition, uremia, and a generalized immunosuppressive state. Patients with chronic renal failure benefit from an increased vaccination dose (259).

Adults with chronic liver disease have lower GMTs after HepA vaccination (102, 261, 262) (with similar SPRs [102, 262] or lower SPRs [263, 264]) and lower GMTs and SPRs after HepB vaccination (242) than those of healthy adults. Children with chronic liver disease also have lower GMTs (265) and SPRs (263, 265) after HepB vaccination (265), with no difference in SCRs. However, in contrast, they have higher antibody responses to IPV and diphtheria (260) vaccination (Table 4).

Perinatal Host Factors

Gestational age. Preterm infants are at increased risk of infections, including vaccine-preventable infections. Differences in the immune system which render preterm infants less responsive to vaccination include diminished pathogen recognition by dendritic cells and macrophages and diminished T cell activity (especially Th1 activity) and diminished B cell interaction with T cells (266). Variations in vaccine responses between term and preterm infants depend on the specific vaccine. In comparison to those of term infants, preterm infants have significantly lower antibody levels after the first dose of DTP (267). Significantly lower GMTs also persist after primary immunization with three doses for diphtheria (268), HepB (268–270), Hib (269, 271–275), PCV7 (276, 277), poliovirus type 3 (269), and pertussis (267, 268, 278–280) vaccines. However, preterm infants reach sufficient SPRs for most vaccines (Table 5), with the notable exception of responses to HepB (269), Hib (269, 271–275), pertussis (279), and poliovirus type 3 (281) vaccines. In addition to having lower GMTs and SPRs, preterm infants can also have quicker waning of antibodies, as shown for antibodies produced in response to MCV-C (282) vaccination.

TABLE 5 Results with regard to infant factors from studies investigating perinatal factors that influence vaccine responses

	Timing of response, vaccine response, vaccine, age, measurement [reference(s)]		
Type of response	**Gestational age**	**Birth weight**	**Feeding**
Humoral responses	**After primary immunization with 3 doses (*2 doses)** Higher antibody production in preterm infants than in term infants PCV7 <37 weeks: GMTs (277) for serotypes 19F, 9V, and 4 Lower antibody production in preterm infants than in term infants Diphtheria 31–35 weeks: GMTs (268) <31 weeks: GMTs (268) HepB 24–36 weeks: GMTs (269), SPRs (269) <31 weeks: GMTs (268) <35 weeks: GMTs (270) Hib 24–36 weeks: GMTs (269, 271, 272), SPRs (269, 271, 272) <32 weeks: GMTs (271, 273), SPRs (271, 273) 23–32 weeks: GMTs (274),* SPRs (274)* <30 weeks: GMTs (275), SPRs (275) PCV7 <37 weeks: GMTs (276) IPV type 3 24–36 weeks: GMTs (269) <29 weeks: SPRs (281) Pertussis AGGs 2 and 3 <37 weeks: GMTs (278) aP 28–34 weeks: GMTs (267) FHA 25–35 weeks: GMTs (279), SPRs (279) PCT 31–35 weeks: GMTs (268) <31 weeks: GMTs (268) PT <32 weeks: GMTs (280) 25–35 weeks: GMTs (279), SPRs (279) <31 weeks: GMTs (268) No difference in antibody production between preterm and term infants Diphtheria 24–36 weeks: GMTs, SPRs (269) <32 weeks: GMTs (280) 28–34 weeks: SPRs (267) <37 weeks: GMTs, SPRs (278) 25–35 weeks: SPRs (268) Hib <29 weeks: GMTs (281), SPRs (281) MCV-C <32 weeks: GMTs (273), SPRs (273) 32–36 weeks: GMTs (282) OPV <36 weeks: SPRs (574) IPV type 1 24–36 weeks: GMTs, SPRs (269) <29 weeks: GMTs (281), SPRs (281) IPV type 2 24–36 weeks: GMTs, SPRs (269) <29 weeks: GMTs (281), SPRs (281) IPV type 3 24–36 weeks: SPRs (269) <29 weeks: GMTs (281) Pertussis AGGs 2 and 3 <37 weeks: SPRs (278) aP 28–34 weeks: SPRs (267) FHA 24–36 weeks: GMTs, SPRs (269) <32 weeks: GMTs (280) <37 weeks: GMTs, SPRs (278) PCT 25–35 weeks: SPRs (268)	**After primary immunization with 3 doses** Lower antibody production in VLBW infants than in LBW infants Pertussis (PT): GMTs (285), SPRs (285) Hib: SPRs (285) IPV type 3: GMTs (285) Lower antibody production in LBW infants than in infants with a normal birth weight HepB: SPRs (286) Hib: SPRs (272) No difference in antibody production between VLBW and LBW infants Diphtheria: GMTs (285), SPRs (285) HepB: GMTs (285), SPRs (285) IPV type 1: GMTs (285), SPRs (285) IPV type 2: GMTs (285), SPRs (285) IPV type 3: SPRs (285) Pertussis FHA: GMTs (285), SPRs (285) PRN: GMTs (285), SPRs (285) PT: GMTs (285), SPRs (285) Tetanus: GMTs (285), SPRs (285) No difference in antibody production between VLBW and LBW/normal-birth-weight infants Pertussis FHA: GMTs (279) PT: GMTs (279) No difference in antibody production between LBW and normal-birth-weight infants PCV7: GMTs (277), SPRs (277) Measles: SCRs (287) **After a booster at 18–24 months** Lower antibody production in VLBW infants than in LBW infants Diphtheria: GMTs (285) HepB: GMTs (285), SPRs (285) No difference in antibody production between VLBW and LBW infants Diphtheria: SPRs (285) IPV type 1: GMTs (285), SPRs (285) IPV type 2: GMTs (285), SPRs (285) IPV type 3: SPRs (285) Pertussis FHA: GMTs (285), SPRs (285) PRN: GMTs (285), SPRs (285) PT: GMTs (285), SPRs (285) Tetanus: GMTs (285), SPRs (285)	Higher antibody production in breastfed than in formula-fed infants Diphtheria: IgA % standard plasma in saliva (292), IgG % standard plasma in serum (292) Hib: IgA median antibody titer in serum (291), IgM median antibody titer in serum (291), IgG median antibody titer in serum (291), IgG GMTs in serum (290) OPV: IgA % standard plasma in saliva (292), IgG % standard plasma in serum (289, 292), IgM % standard plasma in stool (292) Tetanus: IgA % standard plasma in saliva (292), IgM % standard plasma in stool (292) Lower responses in breastfed infants than in formula-fed infants OPV: SCRs (295)

(Continued on next page)

TABLE 5 (Continued)

Type of response	Timing of response, vaccine response, vaccine, age, measurement [reference(s)]		
	Gestational age	Birth weight	Feeding
	PRN 24–36 weeks: GMTs, SPRs (269) <32 weeks: GMTs (280) PT 24–36 weeks: GMTs (269), SPRs (269) <37 weeks: GMTs, SPRs (278) Tetanus 24–36 weeks: GMTs (269), SPRs (269) <29 weeks: GMTs (281), SPRs (281) <32 weeks: GMTs (280) 28–34 weeks: SPRs (267) <37 weeks: GMTs, SPRs (278)		

Preterm infants have lower vaccine responses not only after primary immunization but also after booster doses. At 2 to 3 years of age, they have lower antibody responses to HepB (270, 283), Hib (283), and poliovirus type 3 (283) vaccination, and at the age of 5 years, they have lower responses to HepB (283) and pertussis (284) vaccination, as well as reduced lymphoproliferation and cytokine responses in the latter case.

Birth weight. One month after the third dose of DTaP-Hib-IPV-HepB vaccination given at 2, 4, and 6 months, very-low-birth-weight (VLBW; less than 1.5 kg) infants have significantly lower GMTs to aP and poliovirus type 3 and lower SPRs to Hib than infants with a low birth weight (LBW; 1.5 to 2 kg), while both groups have similar GMTs and SPRs after diphtheria, tetanus, and HepB (285) vaccination. However, 1 month after a booster dose given at the age of 18 to 24 months, the SPR to HepB in VLBW infants is lower than that in LBW infants, and there is a trend toward lower GMTs for all other vaccine components (285) (Table 5). Similarly, 1 month after completing a 3-dose HepB schedule given at 0, 1, and 6 months, LBW infants have lower GMTs to HepB vaccine than infants with a normal birth weight (286). A decreasing birth weight is associated with a progressive reduction in antibody responses to Hib (272) vaccination. However, there is no difference in GMTs after a 3-dose schedule of PCV7 vaccine between LBW infants and infants with a normal weight (277) and no correlation between birth weight and SCRs after measles vaccination (287).

In one study, the antibody response to *Salmonella enterica* serovar Typhi vaccination (but not rabies vaccination) in adults was reported to be related to birth weight (288).

Breastfeeding. After routine vaccination, compared to formula-fed infants, breastfed infants have higher serum IgG levels after diphtheria (289), Hib (290, 291), and OPV (289, 292) vaccination, higher salivary IgA levels after tetanus, diphtheria, and OPV vaccination (289), and higher stool IgM levels after tetanus and OPV vaccination (289) (Table 5).

While one meta-analysis summarizing studies on the effect of breastfeeding on the antibody response to oral rotavirus vaccine (ORV) reports reduced SCRs in breastfed infants in all included studies (293), another meta-analysis reports decreased responses in only 3 of 16 studies (294). However, in all the included studies, only one dose of ORV was given, and infants with mixed feeding were sometimes classified as breastfed and sometimes as nonbreastfed. More recent studies show that higher levels of rotavirus-specific IgA in breast milk lead to lower SCRs in infants after ORV (a 2-fold increase is associated with a 22% decrease in SCR) (295). IgA levels in breast milk depend on geographic location: Indian women have higher levels than those in Korean and Vietnamese women and American women (296). Additionally, the neutralizing activity of IgA in breast milk from Indian women is higher than that in breast milk from American women (296). Three studies investigating withholding breastfeeding for an hour before and after ORV showed no effect on rotavirus-specific IgA SCRs in infants (297–299).

Maternal antibodies. Preexisting maternal antibodies inhibit infant antibody responses to vaccination (14) (Table 6). After primary immunization, a 2-fold higher

TABLE 6 Results with regard to maternal factors from studies investigating perinatal factors that influence vaccine responses

Type of response	Vaccine response and vaccine: measurement [reference(s)]			
	Maternal antibodies associated with lower infant responses to vaccination	Maternal high body mass index	Maternal malnutrition	Antenatal steroids
Humoral responses	Lower antibody production HepA: GMTs (305, 306) Diphtheria: GMTs (14) Measles: SCRs (49) OPV: titers (575) ORV: SCRs (295, 296, 307–310) Pertussis PRN: GMTs (14) IPV: GMTs (14) Tetanus: GMTs (14)		Lower antibody production OPV: SPRs (326) ORV: SPRs (326)	Lower antibody production Tetanus: GMTs (280)
Cytokine responses		Lower cytokine production BCG: IL-2 (325)		

maternal antibody level results in approximately one-fourth lower postvaccination GMTs in infants (28% to IPV, 24% to diphtheria, 22% to pertactin, and 13% to tetanus). Maternal antibodies also influence infant vaccine responses after booster doses in the second year of life (14).

A meta-analysis summarizing 16 studies investigating the influence of maternal antibodies on responses to measles vaccination shows that the presence of maternal antibodies decreases SCRs, on average, by 33.2% (49). However, maternal antibodies to measles virus in infants decrease quickly, and by the age of 5 months, only 12% of infants have protective levels. Maternal age is the only factor associated with maternal measles antibody levels (300, 301), while maternal weight has no influence (300). Measles-vaccinated women have significantly lower GMTs than those of women with naturally acquired immunity (301, 302).

Maternal antibodies against HepA in infants remain high during the first 6 months but then drop quickly, with only approximately 37% of infants having protective titers by the age of 12 months (303, 304). When the HepA vaccine is given to infants within the first 6 months of life, GMTs in those without maternal antibodies are significantly higher at the age of 12 to 15 months than those in infants with maternal antibodies. However, there is no difference in SPRs (305, 306). For ORV, transplacental transfer of maternal antibodies (307–309) and transfer of antibodies through breast milk (295, 296, 310) lead to decreased infant vaccine responses, in a dose-dependent matter.

The amount of maternal antibodies transferred to infants depends on several factors, including maternal antibody levels, IgG subclass, gestational age, and placental characteristics (311). The transfer of maternal antibodies can be optimized by maternal vaccination during pregnancy. This strategy is currently recommended for vaccination against tetanus, pertussis, and influenza. However, appropriate timing of vaccination during pregnancy is important. For example, Tdap given during the second trimester of pregnancy leads to significantly higher neonatal antibodies than those obtained when it is given during the third trimester (312).

Maternal infections during pregnancy. Most studies do not find an association between maternal infection with filariae, hookworm, malaria parasites, *Mansonella perstans*, or *Schistosoma mansoni* during pregnancy and infant antibody responses to diphtheria (313, 314), HepB (313–315), Hib (314), pertussis (314), and tetanus (313, 316) vaccination. However, one study shows that maternal infection with malaria parasites, filariae, or hookworm is independently associated with lower infant antibody responses to Hib vaccination (313), while another study shows that maternal infection with *S. mansoni* is associated with lower infant antibody responses to HepB vaccination (317). Maternal malaria during pregnancy is associated with lower infant SPRs after measles vaccination (318). The presence of multiple maternal infections is associated with lower

infant antibody responses to Hib, diphtheria, and tetanus (313) vaccination. In contrast, maternal infection with *Strongyloides* during pregnancy is associated with higher antibody responses to Hib, HepB, and pertussis toxin (PT) (314) vaccination.

Maternal infection with *M. perstans* during pregnancy is associated with higher infant IL-10 responses after BCG and tetanus vaccination, but there are no differences in gamma interferon (IFN-γ), IL-5, and IL-13 (319) production. Maternal infection with filariae (320), *Trypanosoma cruzi* (321), or *S. mansoni* (320) during pregnancy is associated with lower IFN-γ production in children after BCG vaccination, while other helminth infections show no effect on infant responses to BCG vaccination (319, 322, 323). Empiric treatment of mothers with albendazole or praziquantel during pregnancy does not influence infant vaccine responses to BCG (324), diphtheria (314), HepB (314), Hib (314), measles (324), pertussis (314), or tetanus (324) vaccination (Table 7).

Other maternal factors. In infants, cytokine responses to BCG vaccination correlate inversely with maternal body mass index (BMI) (325). On the other hand, maternal malnutrition is associated with lower infant antibody responses to OPV and ORV vaccination, while it does not influence responses to parenteral vaccines (326).

It has been suggested that low maternal education status is associated with higher infant GMTs to tetanus vaccination (327) and lower infant IFN-γ and IL-5 production after tetanus vaccination (319), while a lower maternal socioeconomic status is associated with lower infant IL-10 production after tetanus vaccination (319) (Table 8). However, this might be confounded by other factors, such as malnourishment and nonadherence to vaccination schedules.

Maternal tetanus immunization during pregnancy is associated with higher infant antibody responses to tetanus vaccination, while the presence of a maternal BCG scar is associated with lower infant IL-5 (319), IL-10 (323), and IL-13 (319) responses after BCG vaccination (319).

Infants who are exposed to HIV during pregnancy but remain uninfected have lower IFN-γ, IL-5, and IL-13 responses after tetanus and BCG vaccination (319). Antenatal, but not postnatal, steroids decrease infant antibody production in response to tetanus vaccination (280) (Table 6).

Extrinsic Factors

Infections. Most studies show no difference in antibody responses to MMR vaccination between children with diarrhea (287, 328), fever (287, 328), or afebrile upper respiratory tract infections (URTI) (329–332) and healthy children. However, studies have reported lower SCRs after measles (330) and varicella (331) vaccination for children with URTI (without differences in GMTs), lower GMTs after Hib vaccination in children with fever (333), and lower GMTs and SCRs for children with diarrhea after OPV vaccination (334, 335). Concurrent infection with nonpoliovirus enteroviruses at the time of vaccination with OPV reduces SCRs against poliovirus type 1 but not type 2 or 3 (334) (Table 9).

Children who are infected with Epstein-Barr virus (EBV) have reduced antibody responses to meningococcal polysaccharide (MPV) and measles vaccination. However, infants who are infected with both EBV and cytomegalovirus (CMV) have responses similar to those of uninfected infants, suggesting that the effects of EBV infection might be countered by CMV infection (336). Children who are infected with CMV also have higher GMTs after vaccination with measles vaccine (337), while results from studies investigating vaccine responses in CMV-seropositive adults are conflicting. Studies report higher antibody responses to TIV vaccination in young adults (GMTs) (338) and elderly people (SCRs) (248) who are seropositive for CMV than in seronegative individuals, while other studies show reduced antibody responses (SCRs) (339, 340), lower B and T cell activation (341), and switched memory B cells (339) in response to TIV vaccination in CMV-seropositive individuals (Table 9).

Adults who are infected with HIV have lower SPRs after vaccination with HepA vaccine. A lower viral load and higher CD4 cell count are predictors of a higher response to vaccination (104). Pregnant women who are infected with HIV have lower cytokine

TABLE 7 Results with regard to maternal infections from studies investigating perinatal factors that influence vaccine responses

Type of response	Vaccine response and vaccine: measurement [reference(s)]							
	Filariasis	**Helminths**	**Hookworm**	**Malaria**	***Mansonella perstans***	**Schistosomiasis**	**Strongyloidiasis**	***Trypanosoma cruzi***
Humoral responses	Lower antibody production Hib: GMTs (313) No difference in antibody production Diphtheria: GMTs (313) HepB: GMTs (313) Tetanus: GMTs (313)		Lower antibody production Hib: GMTs (313) No difference in antibody production aP: GMTs (314) Diphtheria: GMTs (313, 314) HepB: GMTs (313, 314) Hib: GMTs (314) Tetanus: GMTs (313)	Lower antibody production Hib: GMTs (313) Measles: SPRs (318) No difference in antibody production Diphtheria: GMTs (313) HepB: GMTs (313) Tetanus: GMTs (313, 316)	No difference in antibody production aP: GMTs (314) Diphtheria: GMTs (314) HepB: GMTs (314) Hib: GMTs (314)	Lower antibody production HepB: GMTs (317) No difference in antibody production aP: GMTs (314) Diphtheria: GMTs (313, 314) HepB: GMTs (313–315) Hib: GMTs (314) Tetanus: GMTs (313)	Higher antibody production Hib: GMTs (314) HepB: GMTs (314) PT: GMTs (314)	
Cytokine responses	Lower cytokine production BCG: IFN-γ (320)	No difference in cytokine production BCG (319, 322, 323)			Higher cytokine production BCG: IL-10 (319) Tetanus: IL-10 (319) Lower cytokine production BCG: IL-5 (323)	Lower cytokine production BCG: IFN-γ (320)		Lower cytokine production BCG: IFN-γ (321)

TABLE 8 Results with regard to other maternal factors from studies investigating perinatal factors that influence vaccine responses

Type of response	Vaccine response and vaccine: measurement [reference(s)]			
	Low maternal education status	Low maternal socioeconomic status	Maternal treatment with albendazole or praziquantel during pregnancy	Maternal vaccination
Humoral responses	Higher antibody production Tetanus: GMTs (327)		No difference in antibody production aP: GMTs (314) Diphtheria: GMTs (314) HepB: GMTs (314) Hib: GMTs (314)	Maternal tetanus vaccination during pregnancy Higher antibody production (GMTs) to tetanus in infants (319)
Cytokine responses	Lower cytokine production Tetanus: IFN-γ (319), IL-5 (319)	Lower cytokine production Tetanus: IFN-γ (319)		Presence of maternal BCG scar associated with lower production in infants IL-5 (319) IL-10 (323) IL-13 (319)

responses to BCG (319, 323) and tetanus (319) vaccination. Infants who are infected with HIV have lower cytokine responses after vaccination with BCG (319, 323), measles (337), and tetanus (319) vaccines and lower antibody responses after vaccination with measles vaccine (318). Children who are infected with HIV have quicker waning of antibodies after vaccination with HepA vaccine (342).

Individuals who are chronically infected with either hepatitis B or hepatitis C virus have lower antibody responses to HepA vaccination (102, 263, 343, 344).

Parasites. Infants who are symptomatically infected with malaria parasites have lower antibody responses to Hib (333), measles (318), MCV-C (345), *Salmonella* Typhi (intradermal), and tetanus (345–347) vaccination and lower cytokine responses (IFN-γ, IL-5, and IL-13) after vaccination with tetanus (319) and BCG (319, 323) vaccines (Table 10). Children with asymptomatic malaria have lower GMTs after MCV-C (94, 348) and aP (349) vaccination but no difference in antibody responses to tetanus vaccination (350, 351). Malaria prophylaxis given to children prior to vaccination has no influence on GMTs or SCRs after diphtheria (352–355), IPV (355), measles (352, 353, 356), OCV (357), OPV (353), pertussis (352), *Salmonella* Typhi (353, 357), or tetanus (352–355, 358, 359) vaccination. Malaria prophylaxis also has no influence on the size of the tuberculin skin reaction after BCG vaccination (353). However, GMTs after vaccination with MCV-A and MCV-C are (transiently) higher in children who are on malaria prophylaxis (348, 353).

Individuals who are infected with *Onchocerca volvulus* produce fewer antibodies and have less proliferation of mononuclear cells and lower IFN-γ production (360) after tetanus vaccination, and individuals who are infected with *S. mansoni* produce less IFN-γ (361) after tetanus vaccination. In children and adults with *Ascaris lumbricoides* infection, antibody and cytokine responses (IFN-γ and IL-2) to OCV are diminished but can be partially restored by albendazole treatment prior to vaccination (362, 363). Childhood helminth infections are not associated with cytokine responses after BCG vaccination. Empiric quarterly albendazole treatment during the first 5 years of age leads to less IFN-γ and IL-13 production after vaccination with BCG (323). In contrast, albendazole treatment in individuals with proven helminth infection leads to more T cell proliferation (364) and IFN-γ (364, 365) and IL-12 (365) production after BCG vaccination but to less transforming growth factor beta (TGF-β) and concanavalin A production (365) (Table 11). Infants who are congenitally infected with *Trypanosoma cruzi* have stronger *in vitro* IFN-γ responses after vaccination with diphtheria, HepB, and tetanus vaccines (321) (Table 10).

Antibiotics, probiotics, and prebiotics. There are no studies of humans that have investigated the effects of antibiotics on vaccine responses. However, a study in mice showed that administration of clarithromycin or doxycycline leads to lower antibody responses after vaccination with a HepB, tetanus, or PPV23 vaccine, while administra-

TABLE 9 Results with regard to symptoms or infections from studies investigating extrinsic factors that influence vaccine responses

Type of response	Vaccine response, vaccine, age group, measurement [reference(s)]						
	Diarrhea	**Fever**	**URTI**	**CMV infection**	**EBV infection**	**Hepatitis B virus infection**	**Hepatitis C virus infection**
Humoral responses	Lower antibody production OPV Infants: GMTs (335) Children: SCRs (334) No difference in antibody production Measles Infants: SCRs (287) Children: GMTs (328), SCRs (328)	Lower antibody production Hib Children: GMTs (333) No difference in antibody production Measles Infants: SCRs (287)	Lower antibody production Measles Infants: SCRs (330) Varicella Children: SPRs (331) No difference in antibody production Measles Infants: SCRs (287) Children: GMTs (329, 331), SCRs (329, 331, 332) Mumps Children: GMTs (329, 331), SCRs (329, 331) Rubella Children: GMTs (329, 331), SCRs (329, 331, 332) Varicella Children: GMTs (331)	Higher antibody production Influenza Adolescents: GMTs (338) Elderly: SCRs (248) Lower antibody production Measles Infants: GMTs (337) Influenza Adults: SCRs (339, 340)	Lower antibody production Measles Infants: median IgM and IgG levels (336) MPV Infants: median IgM and IgG levels (336)	Lower antibody production HepA Adults: SPRs (263) HepB Adults: SPRs (263)	Lower antibody production HepA Adults: GMTs (343), SPRs (102, 263, 343) HIV-coinfected adults: SPRs (344) HepB Adults: SPRs (263)
Cellular responses				Lower cellular responses TIV Adults: switched memory B cells (339) Adults: B and T cell activation (341)			

TABLE 10 Results with regard to parasitic infections from studies investigating extrinsic factors that influence vaccine responses

	Vaccine response, vaccine, age group, measurement [reference(s)]					
Type of response	**HIV infection**	***Ascaris lumbricoides***	**Malaria**	***Onchocerca volvulus***	***Schistosoma mansoni***	***Trypanosoma cruzi***
Humoral responses	Lower antibody production HepA Adults: SPRs (104) Measles Infants: SPRs (318)	Lower antibody production OCV Children: GMTs (363), SCRs (363)	Lower antibody production Hib Children: GMTs (333) MCV-C Children: GMTs (94, 345, 348) Measles Infants: SPRs (318) PT Infants: GMTs (349) *Salmonella* Typhi Children: GMTs (345, 346) Tetanus Children: GMTs (346, 347) No difference in antibody production Tetanus Children: GMTs (351)	Lower antibody production Tetanus Adults: GMTs (360)		
Cellular responses				Lower cellular responses Tetanus Adults: proliferation of mononuclear cells (360)		
Cytokine responses	Lower cytokine production BCG Infants: IFN-γ (319, 323), IL-5 (319, 323), IL-10 (319), IL-13 (319, 323) Women: IFN-γ (319, 323), IL-5 (319), IL-13 (319, 323) Measles Infants: IFN-γ (337) Tetanus Infants: IFN-γ (319), IL-5 (319), IL-13 (320) Women: IFN-γ (319), IL-5 (319), IL-13 (319)	Lower cytokine production OCV Adults: IFN-γ (362), IL-2 production (362)	Lower cytokine production BCG Women: IFN-γ (319, 323), IL-5 (319), IL-13 (319) Tetanus Women: IFN-γ (319), IL-5 (319), IL-13 (319)	Lower cytokine production Tetanus Adults: IFN-γ (360)	Lower cytokine production Tetanus Adults: IFN-γ (361)	Higher cytokine production Diphtheria Women: IFN-γ (321) HepB Women: IFN-γ (321) Tetanus Women: IFN-γ (321)

TABLE 11 Results with regard to antiparasitic treatment from studies investigating extrinsic factors that influence vaccine responses

	Vaccine response, vaccine, age group, measurement [reference(s)]				
Type of response	Responses after treatment with albendazole	Responses on prophylaxis with amodiaquine	Responses on prophylaxis with atovaquone-proguanil	Responses on prophylaxis with chloroquine	Responses on prophylaxis with sulfadoxine-pyrimethamine
Humoral responses	Higher antibody production OCV, *A. lumbricoides* Children: GMTs (363)	No difference in antibody production Diphtheria Infants: median titers (355) Children: GMTs (352), SCRs (352) IPV Infants: median titers (355) Measles Children: GMTs (352), SCRs (352), SPRs (356) Pertussis Children: GMTs (352), SCRs (352) Tetanus Infants: median titers (355) Children: GMTs (352), SCRs (352), titers (358, 359)	No difference in antibody production OCV Children: GMTs (357) *Salmonella* Typhi Children: GMTs (357)	Higher antibody production MCV-A Children: GMTs (348, 353) MCV-C Children: GMTs (348, 353) No difference in antibody production Diphtheria Children: GMTs (353) Measles Children: GMTs (353) OPV Children: GMTs (353) *Salmonella* Typhi Children: GMTs (353) Tetanus Children: GMTs (352, 353), SCRs (352)	No difference in antibody production Diphtheria Infants: SCRs (354) Measles Infants: SCRs (354) Tetanus Infants: SCRs (354)
Cellular responses	Higher cellular response BCG, any helminth Adults: T cell proliferation (364)			No difference in cellular response BCG Children: tuberculin skin test (353)	
Cytokine responses	Higher cytokine production BCG, any helminth Adults: IFN-γ (364, 365), IL-2 (365), IL-12 (365) OCV, *A. lumbricoides* Adults: IL-2 (362) Lower cytokine production BCG, any helminth Adults: concanavalin A (365), TGF-β (365) BCG, empiric treatment Female adults: IFN-γ (323), IL-13 (323)				

TABLE 12 Results with regard to microbiota from studies investigating extrinsic factors that influence vaccine responses

	Bacterium/bacterial group, age group, affected vaccine response (reference)		
Type of response	Higher relative abundance in intestinal microbiota associated with higher vaccine responses	Higher relative abundance in intestinal microbiota associated with lower vaccine responses	Higher relative abundance in nasopharyngeal microbiota associated with higher vaccine responses
Humoral responses	Phylum *Actinobacteria* Infants: OPV (385), HepB (385) Phylum *Firmicutes* Infants: ORV (386)	Phylum *Proteobacteria* Infants: OPV (385) Phylum *Bacteroidetes* Infants: ORV (386)	*Bacteroides ovatus* Adults: LAIV (390) *Lactobacillus helveticus* Adults: LAIV (390) *Prevotella melaninogenica* Adults: LAIV (390) *Streptococcus infantis* Adults: LAIV (390) *Veillonella dispar* Adults: LAIV (390)
Cellular responses	Phylum *Actinobacteria* Infants: BCG (385), OPV (385), tetanus (385) Phylum *Firmicutes* Adults: *Salmonella* Typhi (387)	Phylum *Proteobacteria* Infants: BCG (385), HepB (385), OPV (385), tetanus (385)	

tion of ampicillin leads to higher antibody responses after vaccination with a live attenuated *Salmonella* Typhi vaccine (366). A study in calves showed that concurrent administration of oxytetracycline leads to lower antibody responses to subcutaneous vaccination with *Brucella abortus* vaccine (367).

The effects of probiotics on vaccine responses are detailed in a recent systematic review (368). A beneficial effect of probiotics has been reported for responses to diphtheria (369), HepA (370), Hib (371), IPV (372), OCV (373), ORV (374), OPV (375), and TIV (376–380) vaccination. However, the choice of probiotic strains and dose as well as the duration and timing of administration varied largely between studies.

Studies investigating the effects of nutritional supplements containing a mix of probiotics (*Lactobacillus paracasei* [381] or *Bifidobacterium longum* bv. *infantis* [341, 382]) and prebiotics (fructo-oligosaccharides [381] or gluco-oligosaccharide [341, 382]) show no effect of the supplements on antibody responses to TIV (381–383) or PPV23 (381, 383) vaccination or on B or T cell responses (341) to TIV vaccination.

Microbiota. There is an association between the intestinal microbiota and vaccine responses (384). In infants, a higher relative abundance of actinobacteria (especially the species *B. longum*) has been associated with either higher antibody or cellular vaccine responses to BCG (385), HepB (385), IPV (372), OPV (385), and tetanus (385) vaccination. In contrast, a higher relative abundance of proteobacteria (especially *Pseudomonadales*) has been associated with lower responses to these vaccines (385). Additionally, a higher relative abundance of *Firmicutes* bacteria (families *Lachnospiraceae* and *Ruminococcaceae*) has been associated with higher humoral responses to ORV (386) in infants and cellular responses to *Salmonella* Typhi (387) in adults. Additionally, in infants, one study found a higher relative abundance of *Bacteroidetes* organisms to be associated with lower humoral responses to ORV (386), while another study did not find any association between the composition of the intestinal microbiota and responses to ORV (388) (Table 12). Children with small bowel bacterial overgrowth have reduced antibody responses to OCV (389).

The one study which investigated the influence of the respiratory microbiota on vaccine responses shows a positive association between the presence of *Bacteroides ovatus*, *Lactobacillus helveticus*, *Prevotella melaninogenica*, *Streptococcus infantis*, and *Veillonella dispar* in the nasopharynx and influenza virus-specific H1 IgA levels in nasal washings after vaccination with live attenuated influenza virus (LAIV) (390).

Preexisting immunity. Individuals with higher prevaccination tetanus antibody titers have higher SPRs after booster vaccination (60), while the antibody response to diphtheria is not affected by prevaccination titers (391). Similarly, the antibody response to TIV is positively associated with prevaccination titers (93). Elderly people who have low antibody titers against influenza virus before vaccination might not seroconvert after one dose of TIV (392). Further, priming with an antigen different from that included in the TIV vaccine leads to lower SCRs and GMTs (393, 394) than those obtained after priming with similar antigens. Further evidence that preexisting immunity influences vaccine responses comes from studies showing that individuals who have antibodies against flaviviruses have higher antibody responses to dengue fever vaccination (95). Previous exposure to Hib-like environmental bacteria increases antibody responses to Hib vaccination (327). In contrast, there is evidence that lower cytokine production after BCG vaccination can be explained partly by previous exposure to other mycobacteria (395–397). However, this is not the case in all studies (398). The evidence for antibody responses to HepB vaccination is also conflicting, with one study showing higher antibody responses in those who are anti-HBc positive (242) and another showing lower responses (72). For OCV, SCRs have been observed to be lower for individuals with preexisting immunity (399).

Behavioral Factors

Smoking. Smoking leads to lower antibody responses to HepB vaccination in some (66, 68, 69, 74, 75, 78, 80, 400) but not all studies (81, 100, 401, 402) and to increased antibody responses to YF vaccination (157). Smoking has no influence on GMTs after human papillomavirus (HPV) vaccination, but it increases the risk of having low-avidity antibodies after vaccination (403). Smokers have higher SCRs after vaccination with LAIV, but waning of antibodies is quicker in smokers than in nonsmokers after vaccination with LAIV and TIV (404) (Table 13).

Alcohol consumption. Alcohol intake has been found not to influence antibody responses to HepA (405) or HepB (68, 69, 81, 402, 406) vaccination but might influence responses to pneumococcus vaccination (142). After vaccination with PPV23, alcoholics have lower SCRs to serotypes 3, 4, 7F, 8, and 19F (however, this is significant only for serotypes 3 and 19F) (142) (Table 13).

Exercise. A small study showed that antibody responses to tetanus vaccination were higher in runners vaccinated after they completed a marathon than in a nonrunner control group (407). However, a larger study found no differences in antibody responses to diphtheria, tetanus, and PPV23 vaccines given to triathletes after they completed a race compared to the responses in triathletes vaccinated when they had not been exercising or in vaccinated sedentary controls (408). A further study reports higher GMTs to TIV (only to A/Panama [H3N2]) in females who used an ergometer in the 45 min before vaccination (409), and one study showed that active participants over the age of 62 years (defined as more than 20 min of vigorous exercise three or more times per week) had higher antibody responses to TIV than those of sedentary participants (410), while other studies with interventions to increase physical activity in adults do not show an association between increasing exercise and antibody responses to TIV (411) or PPV23 (412) vaccination (Table 13).

One study showed that eccentric exercise of the arm before administration of TIV leads to increased interferon gamma production (with the increase in cytokine production correlating with the increase in arm circumference) in males and antibody production in females (124). The increase in antibody response to TIV vaccination through exercise varies among different vaccine strains, with low-immunogenicity strains being particularly responsive to augmentation through exercise (127). However, a follow-up study could not confirm an association between eccentric exercise of the arm and the humoral or cellular vaccine response to TIV (413) (Table 13).

Acute psychological stress. In elderly people, positive mood on the day of vaccination is associated with higher antibody responses to TIV (414). In contrast, mental stress before vaccination leads to higher GMTs after TIV vaccination in females (only to

TABLE 13 Results from studies investigating behavioral factors that influence vaccine responses

	Vaccine response, vaccine, age group, task or characteristic, measurement [reference(s)]					
Type of response	**Smoking**	**Alcohol consumption**	**Exercise**	**Acute psychological stress**	**Chronic psychological stress**	**Sleep deprivation**
Humoral responses	Higher antibody production LAIV Adults: SPRs (404) YF Adults: SPRs (157) Lower antibody production HepB Adults: GMTs (66, 68, 69, 74, 75, 78, 80, 400), SCRs (66, 68, 69, 74, 75, 78, 80, 400), SPRs (66, 68, 69, 74, 75, 78) Quicker weaning of antibodies LAIV Adults: SPRs (404) TIV Adults: SPRs (404) Lower avidity of antibodies HPV Female adults (403) No difference in antibody production HepA Adults: SPRs (405) HepB Adolescents: SPRs (401) Adults: GMTs (81, 100) Pregnant women: SCRs (402) HPV Female adults: GMTs (403)	Lower antibody production PPV23 Adults: GMTs (142), SPRs (142) No difference in antibody production HepA Adults: SPRs (405) HepB Adults: GMTs (69, 81, 406), SCRs (406), SPRs (68, 69, 406) Pregnant women: SCRs (402)	Higher antibody production Tetanus Male adults, run: % standard plasma (407) TIV Female adults, eccentric arm exercise: GMTs (124) Female adults, ergometer: GMTs (409) Elderly, vigorous exercise: GMTs (410) No difference in antibody production Diphtheria Adult males, triathlon: GMTs (408) Tetanus Adult males, triathlon: GMTs (408) TIV Adult males, eccentric arm exercise: GMTs (413) Adults, increased exercise: GMTs (411) PPV23 Adult males, triathlon: GMTs (408), increased exercise (412)	Higher antibody production MCV-A Male adults, mental task: GMTs (139) TIV Female adults, mental task: GMTs (409) Elderly, good mood on day of vaccination: GMTs (414)	Higher antibody production HepB Adolescents, chronic stress: GMTs (435) Lower antibody production HepB Adolescents, stressful life events: SCRs (431) Adolescents, chronic stress: GMTs (432, 433), SPRs (430) MCV-C Adolescents, perceived stress: GMTs (434) MPV-C Adolescents, stressful life events: GMTs (423) PPV23 Children, kindergarten entry (441): GMTs Elderly, chronic stress: GMTs (420) Rubella Adults, low self-esteem: GMTs (438) TIV Adolescents, stressful life events: GMTs (423) Adolescents, loneliness: GMTs (424) Adults, perceived stress: GMTs (411, 421, 422) Elderly, chronic stress: GMTs (419), SCRs (416–418) Elderly, perceived stress: GMTs (410) No difference in antibody production HepB Adolescents, chronic stress: GMTs (437), SPRs (437), titers (436, 437) TIV Adults, chronic stress: GMTs (425, 426) MPV-A Adolescents, stressful life events: GMTs (423)	Lower antibody production HepA Adults, night after vaccination: GMTs (443) Adult males, night after vaccination: GMTs HepB Adults, 7 nights around vaccination: GMTs (442), SPRs (442) TIV Adult males: GMTs 7 nights after vaccination (445) No difference in antibody production TIV Elderly: GMTs (414) Adults with obstructive sleep apnea: GMTs (446), SCRs (446)

(Continued on next page)

TABLE 13 (Continued)

	Vaccine response, vaccine, age group, task or characteristic, measurement [reference(s)]					
Type of response	**Smoking**	**Alcohol consumption**	**Exercise**	**Acute psychological stress**	**Chronic psychological stress**	**Sleep deprivation**
Cellular responses					Lower cellular responses HepB Adolescents, stressful life events: blastogenic response (431) TIV Adults, chronic stress: specific CD8 T cells (425)	Lower cellular responses HepA Specific Th cells (444)
Cytokine responses			Higher cytokine production TIV Males, eccentric arm exercise: IFN-γ (124) No difference in cytokine production TIV Adult males, eccentric arm exercise: IFN-γ (413)		Higher cytokine production TIV Elderly, chronic stress: IL-6 production (419) Lower cytokine production TIV Elderly, chronic stress: IL-1β (416, 417) Elderly, chronic stress: IL-2 (416, 417) Elderly, perceived stress: IL-2 (410) No difference in cytokine production TIV Adults, chronic stress: IFN-γ (426) Adults, chronic stress: IL-4 (426) Elderly, chronic stress: IL-6 (416)	

A/Panama [H3N2]) (409) and to higher GMTs to MCV-A in males (139). Individuals who have greater blood pressure reactions (with evidence of delayed diastolic blood pressure recovery) toward the end of a mental arithmetic stress task that causes aversive stimuli have higher SCRs after vaccination with MPV-A (415) and TIV (to A/Panama [H3N2] and B/Shandong) (Table 13).

Chronic psychological stress. Many studies report (mostly negative) associations between chronic psychological stress and vaccine responses. For example, elderly people who suffer from stress by being caregivers of relatives with dementia have lower antibody responses (416–419), less IL-1β and IL-2 production (410, 416, 417), and more IL-6 production (419) after vaccination with TIV, as well as lower antibody responses after vaccination with PPV23 (420). In contrast, greater optimism in elderly people is associated with higher antibody responses (411) and more IL-10 production (410) after vaccination with TIV. Adults with high perceived stress, stressful life events, or loneliness also have lower antibody (410, 421–424) and cellular (425) responses after vaccination with TIV. This is not the case for adults who care for patients with multiple sclerosis (426). Responses to influenza A/H1N1 and B viruses appear to be more sensitive to the influence of psychological stress than those to A/H3N2 viruses (427). Having better social support or being married is associated with higher antibody responses to HepB (417) and TIV (423, 428) vaccination, while bereavement is associated with lower antibody responses to TIV (428) (however, one study reports lower antibody responses to TIV vaccination in patients with better social support [429]). Mindfulness-based stress reduction exercises do not influence antibody responses to TIV vaccination (411).

Young adults with stressful life events, chronic stress, and a low level of psychological well-being can have lower antibody responses to HepB (430–433) and MCV-C (434) vaccination. However, in contrast, one study shows higher antibody responses to HepB vaccination in young adults with chronic stress (435), and some studies do not find any associations between psychological stress and antibody responses to HepB vaccination (436, 437). Antibody responses might also be influenced by coping mechanisms. For example, individuals who accept the reality of stressful situations have higher antibody responses to HepB vaccination, while individuals who cope by substance use (430) or have high trait negative affect (436) have lower responses. High internalizing behavior, neuroticism, and low self-esteem are associated with lower antibody responses to rubella vaccination (438), while children with internalizing mood symptoms (depressive or anxious symptoms) have higher antibody responses to MCV-4 (439). One study found no association between coping mechanisms and antibody responses to HepB vaccination (432).

Observed negative/conflict behaviors in an interaction task between parents and children predicts lower antibody responses to serotype C after MCV-4 vaccination in children (440). Children who have higher salivary cortisol levels after starting kindergarten have lower antibody responses to PPV23 (441) (Table 13).

Sleep. Short sleep duration, but not subjective sleep quality, in the week around vaccination is associated with lower antibody responses to HepB vaccination (442). Deliberate sleep deprivation during the night after HepA vaccination is associated with lower antibody levels (443, 444) and specific Th cell responses (444), while high sleep slow-wave activity is associated with higher antibody responses (444). Deliberate sleep deprivation 1 week after vaccination with TIV is also associated with lower antibody responses (445), while no association is found between sleep duration (414) or obstructive sleep apnea (446) and antibody responses to TIV vaccination (Table 13).

Nutritional Factors

Body mass index. Many studies in adults show that an increase in body mass index (BMI) is inversely correlated with antibody responses to HepA (61, 447) and HepB (61, 66, 68, 77, 78, 80, 100, 259, 401, 402, 448–454) vaccination. After vaccination with TIV, an increase in BMI is initially correlated with higher antibody responses. However, 12 months after vaccination, a higher BMI is associated with a greater decline in

antibodies, and obese individuals also have fewer specific CD8 T cells and less IFN-γ production (449) (Table 14).

Nutritional status. Many vaccine responses are not influenced by malnourishment. However, malnourished children are reported to have lower antibody responses to HepB (455), MPVS (456), measles (318, 457–459), OPV (335), pertussis (460), *Salmonella* Typhi (461), and tetanus (462) vaccination. Additionally, malnourished children have smaller tuberculin skin reactions after BCG vaccination (463–465) (Table 14).

Micronutrients (vitamins A, D, and E and zinc). Vitamin D plays an important role in innate, humoral, and cellular immune responses (466). However, its role in influencing vaccine responses is not clear (466). Most studies reporting on the effects of vitamin D on vaccine responses have investigated responses to TIV. The results of these studies are conflicting (Table 14). In adults, there is no association between vitamin D levels or administration of vitamin D and antibody responses to TIV (467–469). In children, some studies find no association between vitamin D levels or administration of vitamin D and antibody responses to TIV (470, 471), while others show a trend toward decreased SPRs against TIV in children with vitamin D deficiency (472). In contrast, after vaccination with LAIV, higher antibody responses against two influenza B virus strains were observed in children with low vitamin D levels, while there was no association between vitamin D levels and GMTs against the A strain of the LAIV vaccine (471). In adult dialysis patients, parenteral calcitriol treatment leads to higher antibody responses to TIV (473). Additionally, there is also a clear association between vitamin D deficiency in hemodialysis patients and diminished antibody responses to HepB vaccine (474). In children with idiopathic nephrotic syndrome, there is no correlation between vitamin D levels and antibody responses to PPV23 (475). In asplenic patients vaccinated against Hib, MCV-C, and PCV7, there is no correlation between vitamin D levels and vaccine antibody levels (476). Adults receiving vitamin D supplementation before a booster dose of tetanus have higher antibody levels against tetanus (477). Infants receiving supplementation with vitamins A and D have stronger tuberculin skin reactions (478) and lower IFN-γ responses after BCG vaccination.

Studies investigating coadministration of vitamin A and measles vaccine also report conflicting results. Vitamin A given simultaneously with a measles vaccine at 6 months of age leads to lower SCRs (154), but only in infants with maternal antibodies. Vitamin A given simultaneously with measles vaccination at 9 months of age does not lead to a difference in GMTs and SCRs at the age of 12 months (479) but does lead to higher GMTs at the ages of 18 months (35) and 6 to 8 years (480), especially in boys. Vitamin A given simultaneously with measles vaccination at 9 months of age to malnourished infants leads to significantly higher GMTs (479). In children with and without vitamin A deficiency, there is no difference in antibody responses to diphtheria and tetanus vaccination (481). In elderly people who are not vitamin deficient, there is no association between levels of vitamin A, vitamin E, or zinc and antibody responses to TIV (482). Polymorphisms of vitamin A and D receptor are associated with lower antibody responses to HepB vaccination (483) and with variations in cytokine responses after rubella and measles vaccination (194, 484).

Enteropathy. Enteropathy is defined as intestinal inflammation not necessarily associated with diarrhea. It occurs more frequently in individuals exposed to poor sanitary conditions. It is often proposed that enteropathy is one of the mechanisms behind the lower effectiveness of oral vaccines in developing countries. However, only one study has investigated the effect of enteropathy on vaccine responses. That study found that infants with enteropathy have lower SPRs against OPV and ORV but not against parenteral vaccines (326).

Environmental Factors

Rural versus urban environment. Children living in rural areas have higher antibody responses to tetanus (351) and an experimental malaria vaccine (485, 486). They have a Th1-skewed response (IL-5 production) to tetanus and TIV vaccination, while children living in semiurban areas have a Th2-skewed response (IFN-γ production) (351, 487).

TABLE 14 Results from studies investigating nutritional factors that influence vaccine responses

	Vaccine response, vaccine, age group, measurement [reference(s)]			
Type of response	**Higher body mass index**	**Malnourishment**	**Low vitamin D levels**	**Enteropathy**
Humoral responses	Higher antibody production TIV Adults: GMTs (449) HepB Adults: GMTs (101) Lower antibody production HepA Adults: GMTs (447), SCRs (447) Elderly: GMTs (61), SPRs (61) HepB Children: GMTs (448) Adolescents: SPRs (401), titers (453) Adults: GMTs (77, 100, 449), SCRs (80, 450), SPRs (66, 68, 77, 78, 450, 454) Women: SPRs (451) Elderly: GMTs (61), SPRs (61) Pregnant women: SCRs (402) Dialysis patients: SPRs (259) TIV Adults: GMTs (449) No difference in antibody production HepA (61, 447) Adults: SPRs (405)	Lower antibody production HepB Children: GMTs (455), SPRs (455) MPVs A and C Children: GMTs (456) Measles Children: GMTs (457, 458), SCRs (458, 459), SPRs (318) OPV Infants: GMTs (335) Pertussis (PT) Infants: GMTs (460) *Salmonella* Typhi Children: GMTs (461), SPRs (461) Tetanus Children: GMTs (462) No difference in antibody production Diphtheria Infants: GMTs (335) Children: GMTs (462, 576), SPRs (576) HepB Infants: GMTs (162) Children: GMTs (577), SPRs (577) Hib Infants: GMTs (162) Measles Infants: GMTs (335) Children: GMTs (576, 578), SCRs (287, 578–580), SPRs (576) MCV-C Children: GMTs (94) MPVs A and C Children: GMTs (576), SPRs (576) OPV Children: GMTs (576), SPRs (576) PPV23 Children: SPRs (122) Rabies Children: SPRs (122) *Salmonella* Typhi Children: GMTs (576), SPRs (576) Smallpox Children: GMTs (578), SCRs (578) Tetanus Infants: GMTs (335) Children: GMTs (457, 462, 576, 581), SPRs (576), titers (359)	Higher antibody production LAIV B strain Children: GMTs (471) Lower antibody responses TIV Children: SPRs (472) No difference in antibody production HepB Adults with chronic kidney disease: GMTs (474), SPRs (474) Hib Asplenic adults: GMTs (476) LAIV A strain Children: GMTs (471) MCV-C Asplenic adults: GMTs (476) PCV7 Asplenic adults: GMTs (476) PPV23 Children with nephrotic syndrome: GMTs (475) TIV Children: GMTs (470, 471), SCRs (470), SPRs (470) Adults: SCRs (467), SPRs (467) HIV-infected adults: SCRs (469), SPRs (469)	Lower antibody production OPV Infants: SPRs (326) ORV Infants: SPRs (326) No difference in antibody production Diphtheria Infants: SPRs (326) Hib Infants: SPRs (326) Measles Infants: SPRs (326) Tetanus Infants: SPRs (326)
Cellular responses	Lower cellular responses TIV Adults: specific CD8 T cells (449)	Lower cellular response BCG Children: size of tuberculin skin reaction (463–465) No difference in cellular responses Tetanus Children: lymphoproliferation (582)		
Cytokine responses	Lower cytokine production TIV Adults: IFN-γ (449)			

In contrast, humoral and cellular responses to TIV are lower in children living in rural areas than in those living in semiurban areas (487). Similarly, antibody responses to HepB vaccination (161, 486, 488) and cytokine responses after vaccination with BCG (323) are lower in children and adults living in rural areas.

Geographic location. Children living in developing countries have higher antibody responses to Hib (489), diphtheria (489), PCV7 (490), and pertussis (489) vaccination but lower antibody responses to measles (491–493), OCV (494), OPV (495, 496), and *Salmonella* Typhi (497) vaccination. Adults in developing countries have lower antibody responses to HepB vaccination (498). Children living in developed countries have a Th2-skewed response to BCG vaccination, with more IFN-γ production (396, 398, 499, 500), while children in developing countries have a Th1-skewed response (499).

Season. There is an association between month of administration and antibody responses to pertussis (460), PPV23 (146), rabies (146), and *Salmonella* Typhi (146) vaccination but no association between month of vaccine administration and antibody responses to diphtheria (146), HepB (146), and tetanus (146) vaccination (Table 15). African infants born in the wet season have stronger CD154 vaccine responses to BCG than those of infants born in the dry season (325). In adolescents, antibody responses (GMTs) to HepB vaccination are lower in individuals who receive their first and second doses in summer than in those who receive them in winter. However, after the third dose, no statistically significant difference in GMTs is found. It has been suggested that these variations in vaccine responses may be explained through differences in exposure to UV irradiation (501). Nevertheless, exposing healthy volunteers to UVB on five consecutive days before HepB vaccination does not lead to a difference in GMTs or cellular vaccine responses (in a lymphocyte stimulation test) between UVB- and non-UVB-exposed individuals (502).

Family size. A larger number of people living in the household is associated with superior antibody responses to Hib vaccine (327).

Toxins. One study compared vaccine responses in lead-exposed children with metabolic impairment to those in healthy children and did not find a difference in antibody responses to tetanus (503). Another study showed that early-life arsenic exposure leads to decreased antibody levels to mumps virus after vaccination with MMR (504). It has also been suggested that pre- and postnatal (breast milk) exposure to polychlorinated biphenyls and dioxins leads to lower antibody responses to measles and mumps viruses (505).

Vaccine Factors

Vaccine type, product, and strain. Immune responses vary widely with different vaccine types and products. For example, live vaccines usually induce high vaccine responses leading to life-long protection, often after just one dose, while inactivated, subunit, or toxoid vaccines usually require several doses, including booster doses, to achieve similar protection. Responses to different subunit vaccines also vary. Polysaccharide vaccines induce a T cell-independent vaccine response which does not induce immune memory and is therefore short-lived. Such vaccines are not protective in children under 2 years of age and do not decrease nasopharyngeal carriage. In contrast, polysaccharide-protein-conjugated vaccines have superior immunogenicity, protect infants under 2 years of age, and reduce nasopharyngeal carriage. The responses to similar vaccines against the same disease can also vary according to specific product or strain. This has been shown, for example, for BCG (323, 506, 507), HepB (107, 508), TIV (86), PCV7 (490), rabies (509), and measles (49) vaccines. However, detailed discussion of this topic is beyond the scope of this review.

Adjuvants. Adjuvants are added to vaccines to elicit stronger immune responses. There is great variation in the magnitude and quality of vaccine responses depending on which adjuvants are added to a vaccine (510). For example, the new adjuvant MF59 increases antibody affinity and cross-protection against TIV (511, 512), and compared to those obtained with the standard HepB vaccine, a vaccine containing the new adjuvant AS04 induces higher SPRs and GMTs (513).

TABLE 15 Results from studies investigating environmental factors that influence vaccine responses

	Vaccine response, vaccine, age group, measurement [reference(s)]						
Type of response	**Living in rural areas**	**Living in developing countries**	**Season**	**Larger family size**	**Arsenic exposure**	**Lead exposure**	**Polychlorinated biphenyls and dioxins exposure**
Humoral responses	Higher antibody production Tetanus Children: GMTs (351) Malaria (PfAMA1, PfRh5) Children: GMTs (485) Lower antibody production HepB Adolescents: SPRs (161) Children: SPRs (486, 488) TIV Children: GMTs (487)	Higher antibody production Hib Infants: GMTs (489), SPRs (489) Diphtheria Infants: GMTs (489) PCV7 Children: GMTs (490) Pertussis (FHA) Infants: GMTs (489) Pertussis (PT) Infants: GMTs (489) Tetanus Infants: GMTs (489) Lower antibody production HepB Adults: SPRs (498) Measles Children: GMTs (491–493), SCRs (491–493) OCV Children: GMTs (494) OPV Children: SCRs (495, 496) *Salmonella* Typhi: SCRs (497), SPRs (497) No difference in antibody production Diphtheria Infants: SPRs (489) Pertussis (FHA) Infants: SPRs (489) Pertussis (PT) Infants: SPRs (489) Tetanus Infants: SPRs (489)	Association between month of administration and antibody production Pertussis (PT) Infants: GMTs (460) PPV23 Children: GMTs (146) Rabies Children: GMTs (146) Adults: GMTs (146) *Salmonella* Typhi Adults: GMTs (146) No association between month of administration and antibody production Diphtheria Infants: GMTs (146) HepB Infants: GMTs (146) Tetanus Infants: GMTs (146)	Higher antibody production Hib Infants: GMTs (327)	Lower antibody levels Mumps Infants: GMTs (504)	No difference in antibody production Tetanus Children: titers (503)	Lower antibody levels Measles Children: GMTs (505) Mumps Children: GMTs (505)
Cellular responses	Differences in cellular responses Tetanus Skewed towards Th1 (351) TIV Skewed towards Th1 (487)	Lower cellular response BCG Smaller tuberculin skin reaction (396), skewed towards Th1 (499)	Higher cellular response BCG CD154 (325)				
Cytokine responses	Higher cytokine production Tetanus IL-5 (351) TIV IL-5 (487) Lower cytokine production Tetanus IFN-γ (351) TIV IFN-γ (487)	Lower cytokine production BCG IFN-γ (396, 398, 500)					

Vaccine dose. A study investigating IPV showed that there is a linear relationship between the dose of administered antigen and antibody responses (514). With ORV, the inhibitory effect of transplacental or breast milk-transferred maternal antibodies can be overcome by increasing the vaccine dose (309).

In adolescents receiving HepB vaccination, 10 μg instead of the more commonly used 20-μg dose is sufficient to induce seroprotection (454). However, it leads to lower GMTs (71, 515) and SCRs (71). In patients with end-stage kidney disease, 80 μg of HepB vaccine increases the likelihood of persistent protective antibodies (259).

For the Hib vaccine, it has been shown that even one-eighth the usually given dose leads to adequate antibody titers associated with long-term immunity (516).

For TIV, it has been shown for elderly people that higher doses of antigens are associated with higher antibody responses (130, 517), while in previously immunized participants of 18 to 49 years of age, half the usual vaccine dose leads to comparable SCRs and GMTs, especially in females (126).

Administration Factors

Vaccination schedule. A further factor influencing vaccine responses is the spacing of vaccine doses. Schedules that have longer intervals between vaccine doses usually lead to higher immune responses. For example, antibody responses to aP are significantly higher after a 2-month to 4- to 6-month schedule than after an accelerated 2-month to 3- to 4-month schedule (349). Similarly, antibody responses to DTP vaccination are higher after a 3-month to 5- to 9-month schedule than after an accelerated 2-month to 3- to 4-month schedule (518). For HepB vaccination, increased time between the first and second doses or the second and third doses also correlates with increasing GMTs and SPRs (117, 401). However, one study showed lower GMTs after HepB vaccination on a 0-month to 12- to 24-month schedule than after a 0-month to 1- to 6-month schedule (448), and similarly, another study also showed that individuals who have an interval of more than 1 month between the first and second doses have lower SPRs (79).

In contrast, GMTs after HepA vaccination are higher after a 0-month to 1- to 12-month schedule than after a 0-month to 6- to 12-month schedule (99) or a 0-month to 1- to 2-month schedule (64).

In infants receiving two doses of measles, mumps, rubella, and varicella virus vaccination (MMRV), GMTs are higher to all components of the vaccine when the doses are given 12 months apart instead of 4 weeks apart (519). When influenza vaccination is given to children below the age of 24 months, there is no difference in antibody responses if one priming dose is given in spring followed by the second dose in fall or if both doses are given in fall (520).

Vaccination site. In adults, HepB vaccination given in the buttock area leads to lower responses than those obtained when it is given in the upper arm (521–524). However, this is not the case for infants (525). In children receiving Hib vaccination, there is also no difference in GMTs between children who receive the vaccination in the vastus lateralis and those who receive it in the deltoid muscle (526). Similarly, there is no difference in GMTs after HepB vaccination in infants who receive the vaccine in the anterolateral thigh compared to those who receive it in the ventrogluteal region (8).

Vaccination route. Intramuscular administration of TIV leads to higher antibody responses than those obtained by subcutaneous administration (125). However, there is no difference in antibody responses to diphtheria (137), PPV23 (143), or tetanus (137) vaccination after intramuscular or subcutaneous administration. For HepB and rabies vaccines, a lower dose given intradermally induces an antibody response similar to that induced by a larger intramuscular dose (148, 527–529). Intramuscular vaccination relies on a T cell-mediated response, while intradermal vaccination activates a dendritic cell-mediated response, which requires lower antigen doses (530). Individuals who do not respond to intramuscular HepB vaccination might respond to intradermal administration (531, 532). While 5 μg given intradermally leads to higher SCRs than those obtained with 40 μg given intramuscularly (533), 2 μg given intradermally leads to

lower SPRs (74) and GMTs (75) than those obtained with 20 μg given intramuscularly (75). There is no difference in time required to seroconvert or time to peak antibody titer (75).

Needle size. The size of the needle used to administer vaccines has not been found to influence vaccine responses (534, 535).

Time of day. Studies investigating diurnal variations in antibody responses to TIV in adults show conflicting results. One study showed that individuals receiving the vaccine in the morning have significantly higher antibody responses to the H1N1 A strain and a trend toward higher responses to the B strain, while the time of vaccine administration has no influence on responses to the H3N2 A strain (536). A second study indicated that diurnal variations in antibody responses after TIV are strain specific (537). Additionally, a third study showed higher GMTs to TIV after morning (compared to afternoon) vaccination, but only in men (538). The same has been shown for GMTs after HepA vaccination, but again only in men (538).

Coadministered vaccines. A vast number of studies have investigated the influence of concurrent administration of live vaccines on vaccine responses. Two meta-analyses summarized RCTs comparing vaccine responses in infants receiving either one dose of MMRV or MMR and V as separate vaccines (539, 540). The first meta-analysis, which included 10 RCTs, did not find any difference in GMTs to any of the vaccine components (540). However, the second meta-analysis, which included 24 RCTs, showed that after a single vaccination in children of 9 to 24 months of age, GMTs against mumps and varicella viruses are comparable between infants receiving MMRV and those receiving MMR and V (MMR+V) or MMR. In contrast, individuals receiving MMRV achieve significantly higher GMTs to measles virus than those in individuals receiving MMR+V or MMR. GMTs to rubella virus are reduced in those receiving MMRV compared to those in individuals receiving MMR+V or MMR (539). MMRV also leads to lower SCRs than those with MMR+V or MMR, but this is likely confounded by lower virus antigen doses in early vaccines (539). In investigations of immune responses after two vaccine doses, similar GMTs to most vaccine viruses are observed (541–545). However, there is a tendency toward higher varicella (541, 542, 544, 545) and mumps (541, 543–545) GMTs and lower measles (542–544) and rubella (541, 544, 545) GMTs in groups receiving MMRV as a second dose than in those receiving MMR+V. Concurrent administration of LAIV with MMR+V does not influence GMTs or SPRs against any of the vaccine components (546). YF vaccine given simultaneously with the MMR vaccine leads to lower SCRs than those obtained when the vaccines are given 1 month apart (61% versus 71% for mumps, 90% versus 97% for rubella, and 70% versus 87% for YF), while SCRs for measles are not influenced by simultaneous vaccination (547). One study suggests that a live attenuated Japanese encephalitis (JE) vaccine given 30 days before a YF vaccine might interfere with SCRs to YF vaccination (548). However, a subsequent study did not find a difference in SCRs to YF vaccine whether JE was given 30 days before, simultaneously with, or after the vaccine. For JE, even though there were also no significant differences in SCRs between individuals given the two vaccinations at the same time and those receiving them 30 days apart, GMTs were significantly higher in participants who receive JE 30 days before YF rather than the other way around or at the same time (549).

Studies show that simultaneous administration of nonlive vaccines with MMR or V vaccine does not influence the response to any of the vaccine components. This includes 7-valent conjugated pneumococcal (Pn) (550–552), 10-valent Pn-Hib (553), meningococcal C (MenC) (554), DTaP-HBV-IPV-Hib (555), and HepA (551, 552) vaccines.

An additional dose of acellular pertussis (aP) vaccine at birth might lead to subsequently lower HepB (52, 53), Hib (52, 53), and diphtheria (556) GMTs.

Studies investigating the influence of coadministering BCG vaccine show that it leads to significantly higher levels of antibodies against HepB, OPV, PCV7, and TIV but not against DTaP-Hib or *Salmonella* Typhi (557).

There are several studies which show that concomitant administration of OPV reduces vaccine responses to ORV (558, 559). Infants who receive the two vaccines

concomitantly are less likely to seroconvert than those who receive both vaccines in a staggered manner (558). The interference is greater after the first dose of OPV, presumably because the first dose is associated with the greatest intestinal replication of vaccine poliovirus strains (559). However, when the vaccines are given concomitantly after the age of 3 months, neither OPV nor ORV influences vaccine responses (560).

There is less interference reported with coadministration of nonlive vaccines. Coadministration of PCV7 vaccine with either acellular pertussis or whole-cell pertussis vaccine has no effect on the immunogenicity of the PCV7 vaccine, expect for that against serotype 14, for which the specific antibody response is lower when the vaccine is given with the acellular pertussis vaccine (490). One RCT showed that when DPT-IPV and Hib are given together as a single dose, the antibody response to tetanus is significantly lower than that obtained when DPT-IPV is given at an injection site different from that used for Hib or given 6 months apart (561). A Cochrane review summarizing studies that compared the immunogenicities of the combined DTP-HBV-Hib vaccine and the DTP-HBV and Hib vaccines given at separate sites did not find conclusive results, as only one study reported significantly lower immunological responses to Hib and tetanus when the vaccine components were given in a combined vaccine (562).

Coadministered drugs. Several studies have investigated the effect of concurrent administration of paracetamol around the time of vaccination. A review summarizing 13 RCTs which investigated the influence of prophylactic antipyretic administration after vaccination shows that infants receiving paracetamol after each of 3 doses of routine vaccination have lower GMTs to all Pn serotypes, Hib, diphtheria, tetanus, and pertactin but not to pertussis toxin, filamentous hemagglutinin (FHA), HepB, or IPV. After a booster dose, lower GMTs persisted in the prophylactic paracetamol group for all Pn serotypes, diphtheria, and tetanus (563). The one study that investigated the effect of ibuprofen administration around the time of vaccination shows that GMTs to FHA and tetanus are diminished after primary administration but not after a booster dose, while responses to conjugated Pn vaccine are not affected (564). Further, a recent large study in elderly individuals showed that the intake of statins diminishes GMTs to TIV (565). Similarly, preterm infants who receive dexamethasone for chronic lung disease have significantly lower GMTs against Hib after basic immunization with 3 doses (566).

CONCLUDING REMARKS

This review summarizes many important (and often unrecognized or underappreciated) factors that might affect how individuals respond to vaccines (Fig. 1). There is solid evidence that intrinsic factors, such as genetics, sex, age at time of vaccination, and comorbidities, as well as vaccine-related factors, such as choice of vaccine products, adjuvants, and vaccination schedule, strongly influence vaccine responses. Good evidence also exists for the interaction between maternal antibodies and vaccine responses in infants. In contrast, the available data on the influence of other perinatal factors, such as birth weight or feeding method, or on the influence of infections, antibiotics, the microbiota, and nutrition are less robust. For smoking, alcohol consumption, psychological stress, and exercise, the data from different studies are inconsistent. Many studies report differences in vaccine responses depending on geographic region. However, many other factors, such as preexisting immunity, nutritional status, and other behavioral factors, as well as genetics and the microbiota, might confound this observation. The potential for confounding also exists for many of the other factors discussed in this review, as the response to vaccination is complex, likely involving the interplay of multiple different factors. It is therefore important not to overinterpret findings from single studies.

There are many different methods for quantifying vaccine responses (Fig. 2). However, most studies investigating vaccine responses quantify antibodies. The antibody concentration defined as a protective correlate varies depending on whether it relates to protection against colonization, mucosal disease, or invasive disease. However,

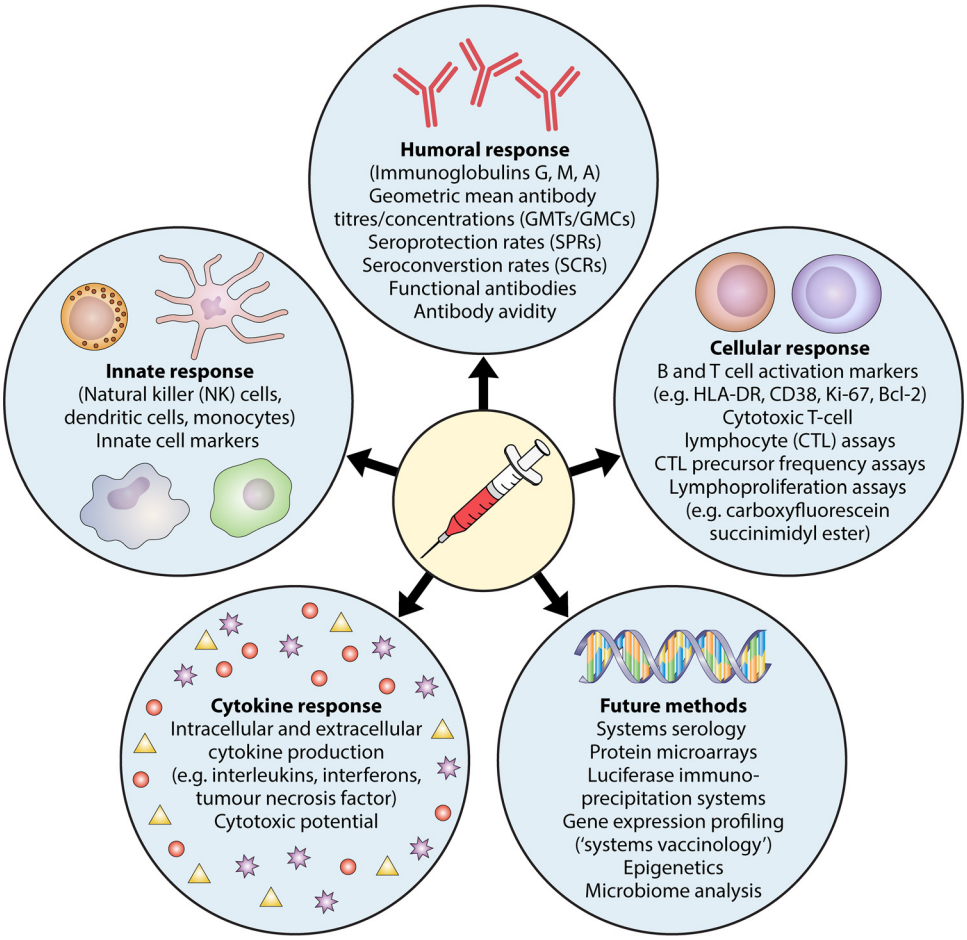


FIG 2 Methods for quantifying vaccine responses.

vaccine responses are far more complex than just measurable antibody concentrations. Other parts of the immune system, such as innate, cellular, and cytokine responses, also play a crucial role in vaccine efficacy. The complex interplay between these different components of the immune system in vaccine responses is not fully understood, and surrogate markers of vaccine-induced protection are imperfect. As most vaccines induce very high antibody responses, small differences in antibody concentrations between groups of individuals may not be clinically significant in terms of protective efficacy, may be relevant only in individuals with poor responses, or may impact only the duration of protection. Moreover, the quality of antibody response is an important consideration, as only a subset of total detectable antibody can neutralize pathogens. SPRs or GMCs do not take this into account and are therefore imperfect correlates of protection. Further, the level of antibody response *in vitro* does not necessarily correlate with health outcomes. For example, seroconversion does not mean full protection against a disease, and nonseroconversion is not necessarily associated with susceptibility (567). Moreover, antibody levels wane with time, but seronegative individuals can still be protected through other immune mechanisms, as shown, for example, after HepB vaccination (568).

This review provides an overview of the current evidence for factors that might influence vaccine responses and identifies factors that require further investigation. Important topics for future studies include the influences of the microbiota (intestinal and respiratory), concurrent infections, and antibiotics on vaccine responses. Further important lines of investigation include the association between preexisting immunity and vaccine responses, as well as the influence of behavioral factors. Understanding

these interactions in more depth will open new avenues for improving vaccine immunogenicity and effectiveness as well as designing vaccine schedules that optimize the benefits of vaccination.

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CHAPTER 2. THE EFFECT OF MATERNAL IMMUNSATION DURING PREGNANCY ON INFANT VACCINE RESPONSES

2.1 INTRODUCTION

This chapter contains the published version of a study that investigated the effect of dTpa and TIV immunisation during pregnancy on specific and heterologous infant antibody responses to routine immunisations given in the first year of life.

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The Effect of Maternal Immunisation During Pregnancy on Infant Vaccine Responses

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ABSTRACT

Introduction: Immunisation during pregnancy to protect infants against tetanus, pertussis and influenza is recommended in many countries. However, maternal antibodies can interfere with infant vaccine responses. We investigated the effect of antenatal diphtheria-tetanus-acellular pertussis (dTpa) and trivalent inactivated influenza (TIV) immunisation on specific and heterologous antibody responses to routine immunisations given in the first year of life.

Methods: In total, 471 healthy infants were included. At 7 and 13 months of age, antibodies to the primary course of routine vaccines given at 6 weeks, 4 and 6 months of age (pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (type 1, 2, 3), *Haemophilus influenzae* type b (Hib), pneumococcus (serotype 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)) were measured, and at 13 months of age, antibodies to the 12-month routine vaccines (Hib, meningococcus C, measles, mumps and rubella). The seroprotection rates for each vaccine and the geometric mean concentrations (GMC) of antibodies were compared between infants whose mothers did or did not receive dTpa or TIV immunisation during pregnancy.

Results: A total of 369 infants were included in the final analysis. Maternal dTpa immunisation was associated with reduced antibody responses to both specific (diphtheria and pertussis) and heterologous (polio and pneumococcus) vaccine antigens. This effect was stronger for persistence of antibodies at 13 months of age than it was at 7 months of age. At 7 months of age, adjusted average antibody concentrations were significantly lower for diphtheria, pertussis (PT, FHA, PRN) and polio type 2, and at 13 months of age, for diphtheria, pertussis (PT, FHA, PRN), polio type 1–3 and pneumococcal serotypes 1, 4, 5, 6A, 6B, 7F, 18C and 23F. Additionally, at 13 months of age, seroprotection rates for diphtheria, PT, pneumococcal serotype 1, 6A and 6B were significantly lower in infants after maternal dTpa immunisation. In contrast, for Hib, in infants with maternal dTpa immunisation, the adjusted average antibody concentration and the seroprotection rate were higher, particularly at 7 months of age. Maternal TIV immunisation had minimal effect on infant vaccine responses.

Conclusion: Whilst maternal immunisation protects infants in the first few months of life, it might interfere with both specific and heterologous (unrelated) vaccines responses in infants.

Research in context: Evidence before this study: Maternal immunisation during pregnancy helps to protect infants during the period before they complete their primary immunisations. It has been proven to be safe and beneficial. However, pre-existing maternal antibodies can influence antibody responses following infant immunisation, an effect called 'blunting'. Previous studies have investigated the influence of dTpa but not influenza immunisation during pregnancy on infant vaccine responses. The majority of studies investigated antibody concentrations only to the specific vaccine antigens included in the maternal immunisation, and there is scarce data available on heterologous vaccine responses, particularly pneumococcal responses.

Abbreviations: BCG, Bacillus Calmette-Guérin vaccine; CI, confidence interval; dTpa, diphtheria-tetanus-acellular pertussis vaccine; FHA, filamentous haemagglutinin; FIM, fimbriae; GMC, geometric mean antibody concentration; GMR, geometric mean antibody ratio; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IgG, immunoglobulin G; IPV, inactivated polio vaccine; MenC, meningococcus type C; MIS BAIR, Melbourne Infant Study: BCG for Allergy and Infection Reduction; MMR, measles-mumps-rubella vaccine; PCV13, 13-valent conjugate pneumococcal vaccine; PT, pertussis toxin; PRN, pertactin; TCV, tetanus-containing vaccine; TIV, trivalent inactivated influenza vaccine.

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Added value of this study: In this study, we have shown that maternal dTpa immunisation during pregnancy is associated with reduced antibody responses to both specific (diphtheria and pertussis) and heterologous (polio and pneumococcus) vaccine antigens. This effect is stronger for persistence of antibodies at 13 months of age than after primary immunisation at 7 months of age. In contrast, for Hib, in infants with maternal dTpa immunisation, antibody concentrations are higher, particularly at 7 months of age. Maternal TIV immunisation has minimal effect on infant vaccine responses.
Implications of all the available evidence: Whilst maternal immunisation protects infants in the first few months of life, it might interfere with both specific and heterologous (unrelated) vaccines responses in infants. As most vaccines induce very high antibody responses, small differences in antibody concentrations may not be of clinical significance. However, since maternal immunisation during pregnancy also influences seroprotection rates, strategies, such as additional booster doses in the second year of life, particularly for pertussis and pneumococcus, might need to be considered to address this.

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1. Introduction

Immunisation during pregnancy aims to protect infants during the period before they complete their primary immunisations. Antenatal immunisation against tetanus has been recommended in certain settings for decades and is estimated to have reduced the burden of neonatal tetanus by 96% [1]. Recently, many countries have reported a steep rise in pertussis cases, with high rates of disease in infants younger than 2 months of age, who are too young to have been vaccinated. This age group has the highest rates of pertussis-related hospitalisations and deaths. For this reason, maternal pertussis immunisation has been recommended in many countries since 2012 [2,3]. Similarly, because of the increased risk of severe influenza in pregnant women, as well as adverse outcomes for infants, influenza immunisation is also routinely recommended during pregnancy [4].

The transfer of maternal immunoglobulin G (IgG) to the foetus is an active process but not all IgG antibodies are transported to the same extent. This is reflected by the fact that, towards the end of pregnancy, IgG antibody concentrations against polysaccharide antigens are similar in the foetus and mother, whereas those against proteins are higher in the foetus [5]. Of the multiple factors that influence the transfer of IgG across the placenta, maternal antibody concentration is an important one. Infants whose mothers receive diphtheria, tetanus and acellular pertussis (dTpa) immunisation during pregnancy have higher antibody concentrations to these antigens at birth compared to those whose mothers do not receive the vaccine [6–12]. Following birth, maternal antibodies in infants wane with a half-life of approximately four to six weeks [13]. Higher antibody concentrations at birth are associated with a longer duration of protection [14].

Despite the benefits of maternal immunisation, there are also potential disadvantages. Pre-existing maternal antibodies influence antibody responses following infant immunisation, an effect called 'blunting'. A recent meta-analysis showed that for immunisation with inactivated polio vaccine (IPV), a two-fold higher maternal antibody concentration is associated with up to 28% lower post-immunisation antibodies in infants [13]. For acellular pertussis, tetanus and diphtheria, these estimates are 11%, 13% and 24%, respectively [13]. Apart from the type of vaccine, blunting of infant antibody production is also influenced by the age of infants at immunisation, the effect being stronger in younger infants [13]. Nevertheless, for acellular pertussis, diphtheria and IPV, the influence of maternal antibodies has been reported to be still evident after booster immunisation at 12 to 24 months of age [13].

Whilst a large number of studies have investigated the association between maternal antibody concentrations and infant antibody production [13], the effect of maternal immunisation on infant vaccine responses has been less extensively investigated [6–10,15–17]. Previous studies have investigated the influence of dTpa but not influenza immunisation during pregnancy [6–10]. The results of these studies show that antibody concentrations against diphtheria, tetanus and pertussis, as well as against hepatitis B (HepB), polio and Hib, are higher at birth in infants whose mothers received dTpa during pregnancy [6–10]. Notably, the majority of studies investigated antibody concentrations only to the specific vaccine antigens included in the maternal immunisation, and there is scarce data available on heterologous vaccine responses, particularly pneumococcal responses.

In this study, we investigated the effect of dTpa and influenza immunisation during pregnancy on specific and heterologous antibody responses to routine infant immunisations given in the first year of life.

2. Methods

2.1. Study Design and Population

Participants were a subset of infants from the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR). This randomised controlled trial comprises 1272 healthy infants whose mothers were recruited antenatally from 2013 to 2016 to investigate whether Bacillus Calmette-Guérin (BCG) immunisation at birth protects against childhood infection, allergy and asthma. Inclusion criteria were: >32 week gestation, birth weight > 1500 g and absence of symptoms or signs of illness. Three-monthly parent questionnaires were used to prospectively collect demographic and other data.

From participants whose parent/guardian provided consent, blood samples were obtained during study visits at 7 and 13 months of age, designed to be 4 weeks after the administration of routine scheduled immunisations. Only participants who had a blood taken between 28 ± 14 days after their 6-month and 12-month routine vaccines, respectively, were included in the final analysis (with the exception of the subset of infants who had their blood analysed at 13 months of age for persistence of antibodies to their primary course of vaccines ending at 6 months). Participants of twins were excluded, because they were not considered as being independent.

2.2. Infant Immunisation

All infants received routine immunisations according to the Australian National Immunisation Program: at birth: intramuscular HepB vaccine (H-B-Vax II Paediatric® (*bioCSL*)); at 6 weeks, 4 and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa)-HepB-inactivated polio (IPV)-*Haemophilus influenzae* type b (Hib) vaccine (Infanrix® hexa (*GlaxoSmithKline*)), intramuscular 13-valent conjugate pneumococcal vaccine (PCV13) (conjugated to CRM197, a diphtheria toxin) (Prevenar13® (*Wyeth*)), and oral rotavirus vaccine (RotaTeq® (*Merck*)); at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (Priorix® (*GlaxoSmithKline*)) or (M-M-R®II (*Seqirus*)) and intramuscular combined Hib and meningococcal C vaccine (conjugated to tetanus toxin) (Menitorix® (*GlaxoSmithKline*)). Approximately half of the infants were randomised to receive intradermal BCG-Denmark (*Statens Serum Institute, Copenhagen*) shortly after birth as part of the MIS BAIR. None of the infants received an influenza vaccine. Vaccine records were obtained from the

National Australian Immunisation register and/or individual immunisation records.

2.3. Maternal Immunisation

Maternal immunisation during pregnancy was self-reported. Participants' mothers received dTpa (Boostrix® (*GlaxoSmithKline*)) and/or a trivalent inactivated influenza vaccine (TIV), or neither during pregnancy.

2.4. Blood Collection

Blood samples were collected at study visits approximately 4 weeks after the scheduled 6-month routine vaccines ('7 months') and the scheduled 12-month routine vaccines ('13 months') in tubes containing sodium-heparin (S-monovette® (*Sarstedt*)). Plasma was stored at −80 °C until analysis.

2.5. Antibody Assay

Blood samples were analysed at the National Institute for Health and Environment, in Bilthoven, the Netherlands. IgG antibodies against 26 vaccine antigens (diphtheria, tetanus, pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), meningococcus type C (MenC), measles, mumps and rubella) were measured in using fluorescent bead-based multiplex immune-assays (Luminex xMAP technology) [18–23]. In all assays, an international or in-house reference, controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

2.6. Statistical Analysis

The proportion of infants with an antibody concentration above the standard protective correlate value for each vaccine (seroprotection rate) [21,24,25] was calculated in each group of infants, together with 95% confidence intervals (CI) using the Clopper-Pearson method. For pertussis no clear correlate of protections exists. However, an antibody concentration against pertussis toxin of >25 IU/mL is generally considered as protective and was therefore used in this study, although there is no consensus on this. We did not calculate seroprotection rates of FHA and PRN for which no accepted protective correlate value exists. Proportions were compared using Fisher's exact test and 95% CIs for differences in proportions estimated. In each group, the geometric mean concentration (GMC) for each vaccine antibody (immunoglobulin G) was calculated. Geometric mean antibody ratios (GMR) with 95% confidence interval (CI) were derived from the anti-logged coefficient using a linear regression with log-concentrations as outcome and maternal immunisation status (dTpa or TIV) as covariate. Effect modification was assessed using the following pre-specified potential

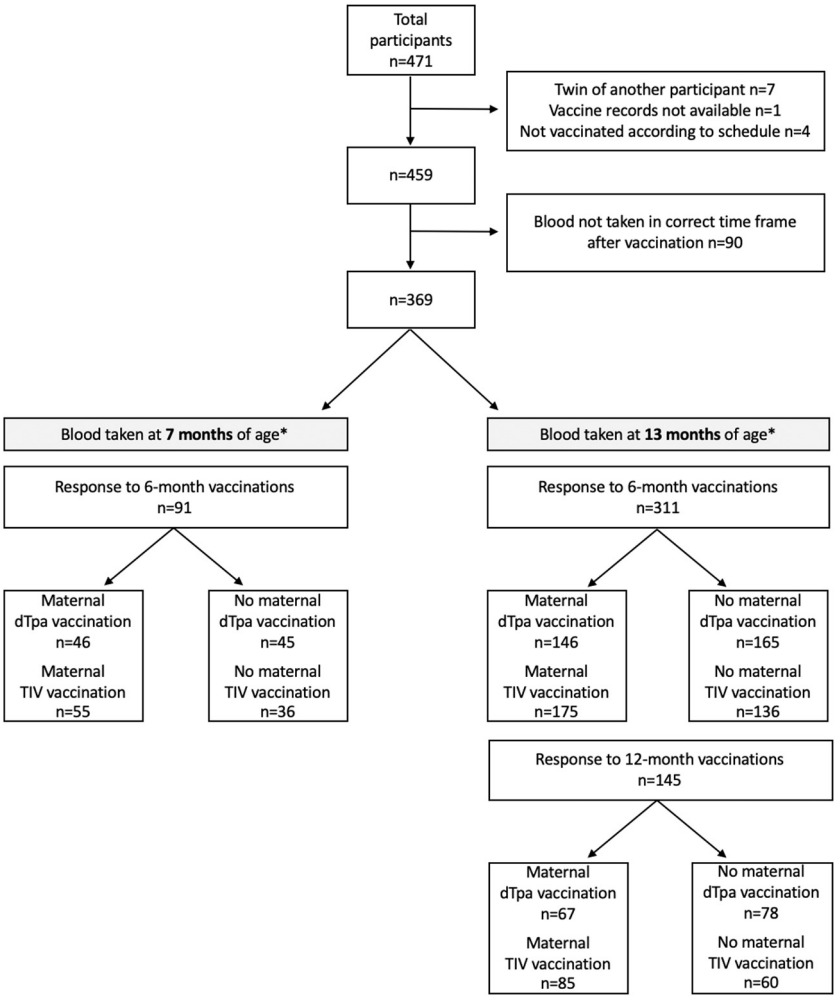


Fig. 1. Selection of participants.
* 33 participants had their blood analysed at 7 and 13 months of age dTpa = diphtheria-tetanus-acellular pertussis vaccine TIV = trivalent inactivated influenza vaccine.

confounding factors: maternal age, gestational age, delivery mode, sex, birth weight, age at first DTPa-HepB-IPV-Hib/PCV13 immunisation, infant BCG immunisation status, MMR/Hib-MenC immunisation status (for responses at 13 months of age), age at sampling and time between 6-month or 12-month immunisation and blood sampling. The GMR for each vaccine antigen was adjusted for those factors that had an effect on the vaccine antigen responses (detailed in each table). As Menitorix includes a tetanus toxoid and Hib component, infants who had received this vaccine before blood sampling at 13 months of age were excluded from the analysis of antibody persistence against these two vaccine antigens. A 5%-significance level was used. All statistical analyses were done using R version 3.4.3.

2.7. Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation (38124A)). MIS BAIR is registered with the Australia & New Zealand Clinical Trials Registry (1051228) and the U.S. National Institutes of Health (NCT01906853).

3. Results

Of the 471 potentially eligible participants, seven were excluded because they were a twin of another participant, four because they were not vaccinated according to the vaccine schedule and one because vaccine records were not available. Of the remaining 459 participants, 368 had blood taken in the predefined time frame (28 ± 14 days after their 6-month and 12-month vaccines, respectively (Fig. 1)). At 7 months of age, 91 (45 with and 46 without maternal dTpa immunisation, 55 with and 36 without maternal TIV immunisation) were included in the final analysis. At 13 months of age, 311 (146 with and 165 without maternal dTpa immunisation, 175 with and 136 without maternal TIV immunisation) were included for measurement of persistence of antibodies against the primary course of vaccines ending at 6-months and 145 (67 with and 78 without maternal dTpa immunisation, 85 with and 60 without maternal TIV immunisation) for responses to the 12-months vaccines. The number of participants who were included at both time points (n = 33) was too small to allow longitudinal analysis. Baseline characteristics are summarised in Table 1 and Supplementary Table 1.

A comparison of the GMCs for each of the vaccine antigens in infants with and without maternal dTpa immunisation is shown in Supplementary Figs. 1 and 2, and in infants with and without maternal TIV immunisations is shown in Supplementary Figs. 3 and 4.

3.1. The effect of Maternal dTpa Immunisation on Antibody Concentrations at 7 Months of Age

Adjusted GMRs at 7 months of age were less than one (indicating lower average antibody concentrations in the group with maternal dTpa immunisation) for all vaccine antigens except tetanus, Hib and pneumococcal serotype 3 and 4. These differences reached statistical significance for diphtheria, pertussis (PT, FHA and PRN), polio type 2 and Hib.

At 7 months of age, the large majority of infants had antibody concentrations above the standard protective correlate value (the seroprotection rate was above 95% for 16 of the 22 antigens). Low seroprotection rates were observed for Hib and pneumococcal serotype 4 (Table 2 and Fig. 2).

At 7 months of age, seroprotection rates were generally lower for infants with maternal dTpa immunisation, with the largest difference observed for pneumococcal serotype 4 and PT. In contrast, the seroprotection rate was higher for Hib in infants with maternal dTpa immunisation. None of these differences reached statistical significance (Table 3 and Fig. 3).

Table 1
Characteristics of participants whose mothers did or did not receive dTpa during pregnancy.

	To primary course of vaccines ending at 6 months of age						To 12-month vaccines		
	At 7 months of age n (%) or median (IQR)			At 13 months of age n (%) or median (IQR)			At 13 months of age n (%) or median (IQR)		
	Maternal dTpa (n = 46)	No maternal dTpa (n = 45)	p-Value	Maternal dTpa (n = 146)	No maternal dTpa (n = 165)	p-Value	Maternal dTpa (n = 67)	No maternal dTpa (n = 78)	p-Value
Maternal age at birth (y)	33.6 (32.1–35.1)	33.8 (32.3–36.2)	0.57	32.8 (30.5–36.4)	33.3 (30.1–46.5)	0.97	33.4 (31.1–36.5)	33.5 (30.5–38.6)	0.52
Gestational age (w)	40.1 (39.0–40.6)	39.1 (38.5–40.3)	0.06	39.3 (38.3–40.3)	39.3 (38.4–40.4)	0.71	39.2 (38.4–40.4)	39.2 (38.4–40.4)	0.81
Caesarean section	12 (26)	23 (51)	0.10	55 (38)	62 (38)	0.99	26 (39)	28 (36)	0.81
Sex (male)	20 (43)	23 (51)	0.66	66 (45)	86 (52)	0.47	30 (45)	42 (54)	0.53
Birth weight (kg)	3.48 (3.15–3.65)	3.48 (3.06–3.76)	0.65	3.46 (3.14–3.75)	3.43 (3.10–3.76)	0.49	3.42 (3.09–3.67)	3.36 (3.14–3.67)	0.55
BCG-vaccinated	18 (39)	27 (60)	0.25	85 (58)	84 (51)	0.48	38 (57)	38 (49)	0.59
MMR/Hib-MenC-vaccinated	–	–	–	101 (69)	110 (67)	0.84	–	–	–
Age at routine immunisation (d)									
- 6-w vaccines	44 (42–47)[a]	45 (43–48)	0.29	45 (43–47)	47 (44–52)	0.06	44 (43–47)	46 (43–50)	0.43
- 4-m vaccines	123 (115–127)	123 (119–127)	0.83	125 (122–130)	125 (120–134)	0.16	125 (122–130)	124 (119–132)	0.90
- 6-m vaccines	187 (180–192)	187 (182–195)	0.23	191 (186–200)	191 (184–212)	0.44	191 (186–198)	191 (184–212)	0.90
- 12-m vaccines	–	–	–	–	–	–	377 (369–383)	375 (369–384)	0.60
Interval between (d)									
- 6-m vaccines to 7-m blood sample	29 (22–36)	28 (21–39)	0.74	–	–	–	–	–	–
- 6-w vaccines to 13-m blood sample	–	–	–	213 (197–224)	204 (186–225)	0.95	–	–	–
- 12-m vaccines to 13-m blood sample	–	–	–	–	–	–	29 (23–36)	28 (21–36)	0.46
Age at sampling (d)	216 (207–225)	223 (208–228)	0.20	402 (390–418)	400 (388–415)	0.41	403 (398–415)	406 (396–414)	1.00

p-values were determined by t-test for continuous variables and Pearson's Chi-squared test for categorical variables.
d = days, dTpa = diphtheria-tetanus-acellular pertussis vaccine, m = month, w = week, y = years.
[a] Age for one participant not available.

Table 2
Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) at 7 and 13 months of age in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Bold denotes results with p-value < 0.05.

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	Maternal dTpa (n = 46)	No maternal dTpa (n = 45)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value	Maternal dTpa (n = 146)	No maternal dTpa (n = 165)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
	GMC (95% CI)	GMC (95% CI)					GMC (95% CI)	GMC (95% CI)				
Diphtheria[1]	**0.31 (0.25, 0.39)**	**0.51 (0.41, 0.63)**	**0.61 (0.45, 0.82)**	**<0.01**	**0.68 (0.50, 0.93)**[b,f,h]	**0.02**	**0.06 (0.05, 0.07)**	**0.11 (0.09, 0.12)**	**0.54 (0.43, 0.67)**	**<0.01**	**0.57 (0.46, 0.70)**[g,h,i,j]	**<0.01**
Tetanus[1]	1.22 (0.98, 1.51)	1.01 (0.83, 1.23)	1.21 (0.91, 1.62)	0.19	1.24 (0.93, 1.64)[d]	0.14	0.45 (0.35, 0.58)[3]	0.44 (0.34, 0.56)[3]	1.03 (0.72, 1.46)[3]	0.89[3]	0.98 (0.70, 1.37)[3,j]	0.89[3]
PT[1]	**49.69 (40.50, 69.96)**	**99.60 (79.34, 125.02)**	**0.50 (0.37, 0.67)**	**<0.01**	**0.53 (0.39, 0.72)**[a]	**<0.01**	**12.21 (10.45, 14.26)**	**24.15 (20.79, 28.06)**	**0.51 (0.41, 0.63)**	**<0.01**	**0.53 (0.43, 0.66)**[g,h,i,j]	**<0.01**
FHA[1]	**49.01 (38.93, 61.70)**	**83.36 (65.77, 105.65)**	**0.59 (0.42, 0.81)**	**<0.01**	**0.68 (0.49, 0.93)**[b,d,g]	**0.02**	**14.14 (12.04, 16.62)**	**25.06 (21.90, 28.68)**	**0.56 (0.46, 0.70)**	**<0.01**	**0.57 (0.47, 0.70)**[d,g,i]	**<0.01**
PRN[1]	**48.31 (37.36, 62.47)**	**76.12 (56.23, 103.03)**	**0.63 (0.43, 0.94)**	**0.02**	**0.67 (0.45, 0.98)**[b,e]	**0.04**	**8.94 (7.31, 10.95)**	**15.85 (13.30, 18.88)**	**0.56 (0.43, 0.74)**	**<0.01**	**0.59 (0.45, 0.76)**[d,i]	**<0.01**
Hib[2]	0.62 (0.39, 0.97)	0.42 (0.26, 0.68)	1.46 (0.76, 2.79)	0.26	**2.01 (1.08, 3.77)**[b,d,g,h]	**0.03**	1.32 (0.78, 2.24)[3]	1.03 (0.64, 1.66)[3]	1.28 (0.63, 2.60)[3]	0.49[3]	1.36 (0.70, 2.64)[3,c,h]	0.36[3]
Polio type 1[1]	29.42 (20.75, 41.71)	40.87 (31.43, 53.14)	0.72 (0.47, 1.11)	0.13	0.78 (0.50, 1.21)[b,e]	0.26	**9.25 (7.70, 11.10)**	**13.28 (11.17, 15.78)**	**0.70 (0.54, 0.89)**	**<0.01**	**0.71 (0.56, 0.91)**[g,i,j]	**<0.01**
Polio type 2[1]	**48.59 (33.71, 70.04)**	**82.10 (64.97, 103.76)**	**0.59 (0.38, 0.91)**	**0.02**	**0.59 (0.39, 0.90)**[a,i]	**0.02**	**16.44 (13.48, 20.04)**	**27.66 (22.75, 33.62)**	**0.59 (0.45, 0.78)**	**<0.01**	**0.61 (0.46, 0.80)**[g,i]	**<0.01**
Polio type 3[1]	21.00 (14.79, 29.81)	21.89 (15.94, 30.06)	0.96 (0.60, 1.53)	0.86	0.95 (0.60, 1.49)[i]	0.81	**6.87 (5.50, 8.59)**	**11.35 (9.41, 13.69)**	**0.61 (0.45, 0.81)**	**<0.01**	**0.61 (0.46, 0.81)**[d,g,i,j]	**<0.01**
Pn 1[2]	3.83 (2.78, 5.29)	4.99 (3.76, 6.63)	0.77 (0.50, 1.17)	0.22	0.89 (0.60, 1.33)[f,h,i]	0.56	**1.03 (0.89, 1.19)**	**1.31 (1.15, 1.49)**	**0.79 (0.65, 0.96)**	**0.02**	**0.80 (0.67, 0.97)**[i]	**0.02**
Pn 3[2]	1.43 (1.15, 1.78)	1.44 (1.20, 1.73)	0.99 (0.75, 1.31)	0.94	1.06 (0.80, 1.39)[g]	0.70	0.504 (0.44, 0.57)	0.58 (0.51, 0.66)	0.87 (0.72, 1.04)	0.11	0.87 (0.73, 1.03)[i]	0.11
Pn 4[2]	0.53 (0.40, 0.70)	0.50 (0.41, 0.62)	1.04 (0.73, 1.48)	0.81	1.12 (0.80, 1.57)[h]	0.49	**0.19 (0.18, 0.22)**	**0.24 (0.21, 0.27)**	**0.82 (0.70, 0.95)**	**<0.01**	**0.83 (0.72, 0.96)**[b,f,g,i]	**0.01**
Pn 5[2]	2.81 (2.10, 3.77)	3.56 (2.77, 4.56)	0.79 (0.54, 1.16)	0.22	0.91 (0.63, 1.30)[f,h,i]	0.59	**0.80 (0.68, 0.93)**	**1.05 (0.91, 1.20)**	**0.76 (0.62, 0.93)**	**<0.01**	**0.78 (0.64, 0.95)**[h,i,j]	**0.01**
Pn 6A[2]	**3.98 (2.99, 5.28)**	**5.85 (4.73, 7.25)**	**0.68 (0.48, 0.97)**	**0.03**	0.80 (0.57, 1.12)[f,g,h]	0.19	**0.82 (0.68, 0.97)**	**1.20 (1.05, 1.38)**	**0.68 (0.54, 0.85)**	**<0.01**	**0.70 (0.57, 0.86)**[h,i]	**<0.01**
Pn 6B[2]	1.51 (0.98, 2.32)	2.44 (1.53, 3.89)	0.62 (0.33, 1.16)	0.13	0.77 (0.41, 1.45)[c,h]	0.41	**0.42 (0.35, 0.51)**	**0.62 (0.53, 0.74)**	**0.68 (0.52, 0.87)**	**<0.01**	**0.69 (0.54, 0.88)**[i]	**<0.01**
Pn 7F[2]	5.37 (4.14, 6.96)	7.03 (5.80, 8.51)	0.76 (0.55, 1.05)	0.10	0.80 (0.58, 1.10)[h]	0.17	**1.69 (1.49, 1.91)**	**2.06 (1.83, 2.32)**	**0.82 (0.69, 0.97)**	**0.02**	**0.83 (0.70, 0.98)**[a,i,j]	**0.03**
Pn 9V[2]	2.15 (1.62, 2.85)	2.82 (2.28, 3.49)	0.76 (0.54, 1.08)	0.12	0.92 (0.66, 1.27)[f,g,h,i]	0.61	0.55 (0.48, 0.64)	0.64 (0.56, 0.73)	0.86 (0.71, 1.04)	0.12	0.88 (0.73, 1.06)[h,i,j]	0.17
Pn 14[2]	2.47 (1.72, 3.54)	2.89 (1.96, 4.26)	0.85 (0.51, 1.44)	0.55	0.85 (0.51, 1.44)	0.55	1.02 (0.86, 1.21)	1.08 (0.93, 1.25)	0.94 (0.75, 1.18)	0.62	0.94 (0.75, 1.18)[g,h,i,j]	0.59
Pn 18C[2]	2.80 (2.09, 3.76)	3.09 (2.36, 4.04)	0.91 (0.61, 1.34)	0.63	0.97 (0.66, 1.42)[h]	0.86	**0.58 (0.51, 0.66)**	**0.73 (0.64, 0.83)**	**0.79 (0.66, 0.95)**	**0.01**	**0.81 (0.68, 0.97)**[h,i,j]	**0.02**
Pn 19A[2]	1.45 (1.10, 1.91)	2.04 (1.54, 2.71)	0.71 (0.48, 1.05)	0.09	0.76 (0.52, 1.13)[b]	0.18	0.45 (0.36, 0.57)	0.47 (0.39, 0.56)	0.97 (0.72, 1.29)	0.81	1.00 (0.75, 1.33)[i,j]	0.99
Pn 19F[2]	**9.62 (7.26, 12.76)**	**13.87 (10.97, 17.55)**	**0.69 (0.48, 1.00)**	**0.05**	0.74 (0.52, 1.05)[h]	0.09	2.97 (2.45, 3.61)	3.19 (2.76, 3.70)	0.93 (0.73, 1.18)	0.56	0.96 (0.76, 1.21)[h,i,j]	0.73
Pn 23F[2]	2.11 (1.51, 2.95)	2.59 (1.84, 3.65)	0.81 (0.51, 1.31)	0.39	0.94 (0.59, 1.50)[f,h]	0.78	**0.48 (0.39, 0.58)**	**0.65 (0.54, 0.79)**	**0.73 (0.56, 0.96)**	**0.03**	**0.72 (0.55, 0.94)**[d,e]	**0.01**

Vaccine antigen	Antibodies to 12-month vaccines measured at 13 months of age					
	Maternal dTpa (n = 69)	No maternal dTpa (n = 79)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
	GMC (95% CI)	GMC (95% CI)				
Measles[1]	3.50 (2.84, 4.30)	2.67 (2.01, 3.54)	1.31 (0.92, 1.87)	0.14	1.27 (0.90, 1.81)[d]	0.18
Mumps[1]	62.27 (44.46, 87.21)	57.66 (42.96, 75.90)	1.09 (0.71, 1.68)	0.69	1.02 (0.72, 1.45)[a,h,i]	0.89
Rubella[1]	58.51 (41.39, 82.70)	50.48 (36.71, 69.40)	1.16 (0.73, 1.85)	0.53	1.03 (0.73, 1.47)[h,i]	0.85
MenC[2]	19.40 (15.91, 23.64)	16.49 (13.69, 19.87)	1.18 (0.90, 1.54)	0.24	1.08 (0.79, 1.49)[a,d,i]	0.63
Hib[2]	19.11 (13.22, 27.63)	12.93 (9.02, 18.54)	1.48 (0.88, 2.47)	0.13	1.47 (0.90, 2.41)[c,i]	0.12
Tetanus[1]	1.09 (0.80, 1.49)	0.93 (0.72, 1.20)	1.17 (0.79, 1.73)	0.43	1.17 (0.79, 1.73)	0.43

CI = confidence interval, dTpa = diphtheria-tetanus-acellular pertussis vaccine, FHA = filamentous haemagglutinin, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PRN = pertactin, PT = pertussis toxin.

[1] IU/mL
[2] μg/mL
[3] only participants who have not had Hib-MenC
[a] maternal influenza immunisation during pregnancy
[b] gestational age
[c] delivery mode
[d] sex
[e] birth weight
[f] BCG immunisation
[g] age at first DTPa-HepB-IPV-Hib / PCV13 immunisation
[h] age at bleeding
[i] time between immunisation and bleeding
[j] MMR/Hib-MenC immunisation

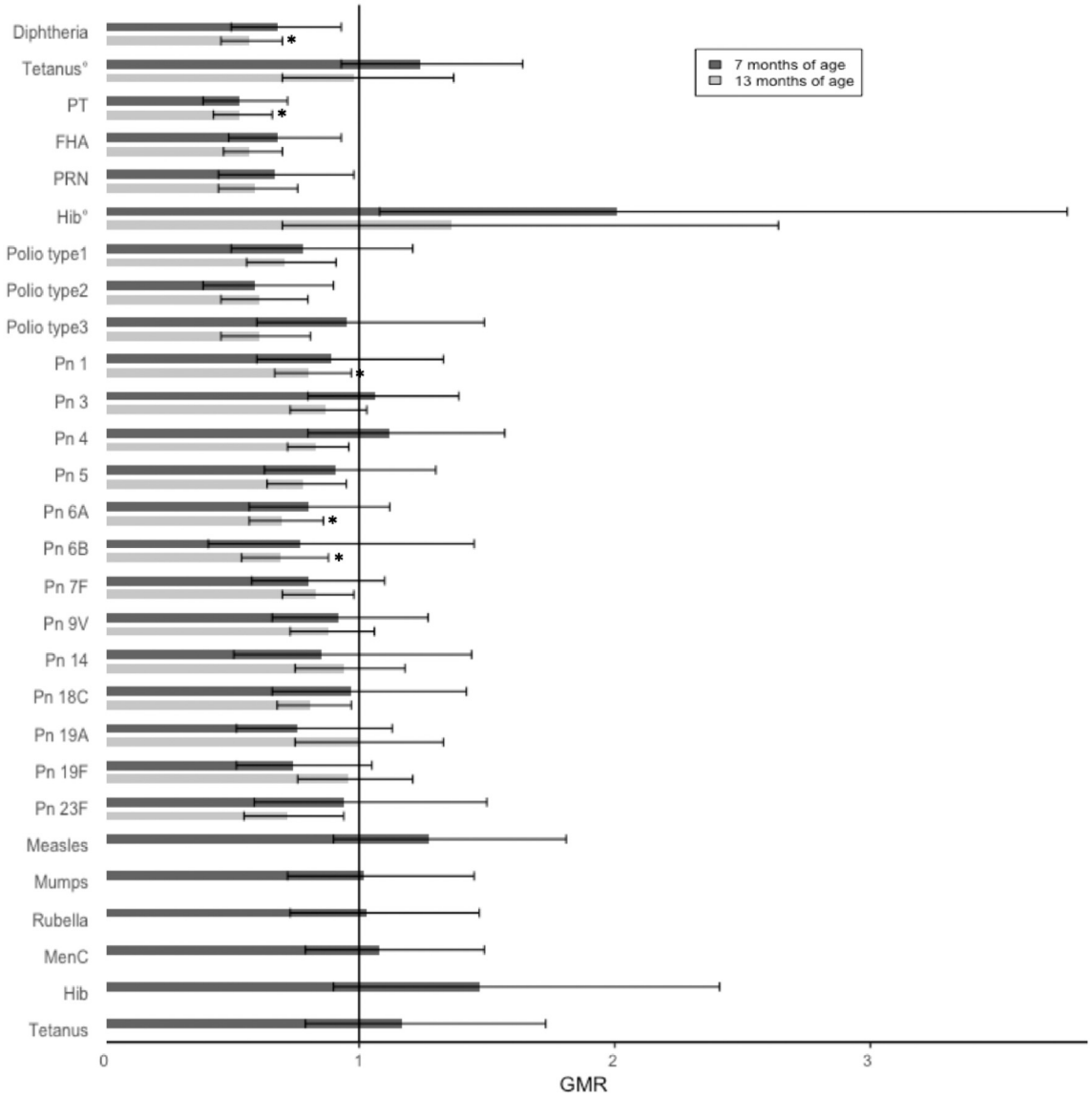


Fig. 2. Geometric mean antibody ratios (GMRs) with 95% CI in participants with or without maternal dTpa immunisation in pregnancy at 7 and 13 months of age.
° participants who have not yet received Hib-MenC, * $p < 0.05$.

3.2. The Effect of Maternal dTpa Immunisation on Antibody Concentrations at 13 Months of Age to the Primary Course of Vaccines

At 13 months of age, adjusted GMRs were below one (indicating lower antibody concentrations in the group with maternal dTpa immunisation) for all antigens, except tetanus. The differences reached statistical significance for diphtheria, pertussis (PT, FHA and PRN), polio type 1–3, pneumococcal serotype 1, 4, 5, 6A, 6B, 7F, 18C and 23F (Table 2 and Fig. 2).

At 13 months of age, infants with maternal dTpa immunisation had lower seroprotection rates for the large majority of vaccine antigens. These differences reached statistical significance for diphtheria, PT, pneumococcal serotype 1, 6A and 6B (Table 3 and Fig. 3).

3.3. The Effect of Maternal dTpa Immunisation on Antibody Concentrations at 13 Months of Age to the 12-Month Vaccines

Following the 12-month immunisations, GMRs were above one for measles, mumps and rubella, MenC and Hib. Seroprotection rates were higher in infants with maternal dTpa immunisation for measles, mumps and rubella and, but these findings did not reach statistical significance (Tables 2–3 and Figs. 2–3).

3.4. The Effect of Maternal TIV Immunisation on Antibody Concentrations at 7 Months of Age

Adjusted GMRs at 7 months of age were greater than one (indicating higher average antibody concentrations in the group with TIV immunisation). However, this only reached statistical significance for pneumococcal serotype 1 and 19F. In contrast, the adjusted GMR for PT was statistically significantly less than one (Supplementary Table 2).

Maternal TIV immunisation did not influence seroprotection rates at 7 months of age in any consistent direction and differences in seroprotection rates were smaller than those observed between infants whose mothers did or did not receive dTpa immunisation (Supplementary Table 3).

Table 3
Seroprotection rates at 7 and 13 months of age in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Bold denotes results with p-value < 0.05.

Vaccine antigen	Protective correlate	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
		Maternal dTpa (n = 46)	No maternal dTpa (n = 45)	Difference % (95% CI)	p-value	Maternal dTpa (n = 146)	No maternal dTpa (n = 165)	Difference % (95% CI)	p-value
		% (n); (95% CI)	% (n); (95% CI)			% (n); (95% CI)	% (n); (95% CI)		
Diphtheria	0.1 IU/mL[a]	95.7 (44); (85.2, 99.5)	97.8 (44); (88.2, 99.9)	-2.1 (-12.7, 7.8)	1	**96.6 (141); (92.2, 98.9)**	**100 (165); (97.8, 100)**	**-3.4 (-7.8, -1.1)**	**0.02**
Tetanus	0.1 IU/mL[b]	100 (46); (92.3, 100)	100 (45); (92.1, 100[b]	0 (-7.8, 7.9)	-	100 (79); (95.4, 100)[b]	100 (87); (95.8, 100)[b]	0 (-4.7, 4.3)[2]	-
PT	25 IU/mL	84.8 (39); (71.1, 94.7)	95.6 (43); (84.9, 99.5)	-10.8 (-24.6, 1.8)	0.16	**18.5 (27); (12.6, 25.8)**	**41.2 (68); (33.6, 49.1)**	**-22.6 (-32.3, 12.7)**	**<0.01**
Hib	0.15 μg/mL	82.6 (38); (68.6, 92.2)	71.1 (32); (55.7, 83.6)	11.5 (-6.0, 28.9)	0.22	79.7 (63); (69.2, 88.0)[b]	74.7 (65); (64.3, 83.4)[b]	5.0 (-8.0, 17.8)[2]	0.47[b]
Polio type 1	0.23 IU/mL	100 (46); (92.3, 100)	100 (45); (92.1, 100)	0 (-7.8, 7.9)	-	100 (146); (97.6, 100)	100 (165); (97.8, 100)	0 (-2.6, 2.3)	-
Polio type 2	0.29 IU/mL	100 (46); (92.3, 100)	100 (45); (92.1, 100)	0 (-7.8, 7.9)	-	99.3 (145); (96.2, 100)	100 (165); (97.8, 100)	-0.7 (-3.8, 1.6)	0.47
Polio type 3	0.12 IU/mL	100 (46); (92.3, 100)	100 (45); (92.1, 100)	0 (-7.8, 7.9)	-	100 (146); (97.6, 100)	100 (165); (97.8, 100)	0 (-2.6, 2.3)	-
Pn 1	0.35 μg/mL	95.7 (44); (85.2, 99.5)	97.8 (44); (88.2, 99.9)	-2.1 (-12.7, 7.8)	1	**89.0 (130); (82.8, 93.6)**	**97.6 (161); (93.9, 99.3)**	**-8.5 (-14.9, 3.3)**	**<0.01**
Pn 3	0.35 μg/mL	97.8 (45); (88.5, 99.9)	100 (45); (92.1, 100)	-2.2 (-11.4, 5.9)	1	67.8 (99); (59.6, 75.3)	71.5 (118; (64.0, 78.3)	-3.7 (-14.0, 6.5)	0.54
Pn 4	0.35 μg/mL	63.0 (29); (47.5, 76.8)	75.6 (34); (60.4, 87.1)	-12.5 (-30.8, 6.7)	0.26	19.2 (28); (13.1, 26.5)	27.3 (45); (20.6, 34.7)	-8.1 (-17.4, 1.4)	0.11
Pn 5	0.35 μg/mL	95.7 (44); (85.2, 99.5)	100 (45); (92.1, 100)	-4.3 (-14.6, 3.8)	0.49	84.9 (124); (78.1, 90.3)	89.1 (147); (83.3, 93.4)	-4.2 (-12.0, 3.3)	0.31
Pn 6A	0.35 μg/mL	95.7 (44); (85.2, 99.5)	100 (45); (92.1, 100)	-4.3 (-14.6, 3.8)	0.49	**80.1 (117); (72.7, 86.3)**	**92.1 (152); (86.9, 95.7)**	**-12.0 (-20.0, 4.4)**	**<0.01**
Pn 6B	0.35 μg/mL	87.0 (40); (73.7, 95.1)	91.1 (41); (78.8, 97.5)	-4.2 (-18.3, 9.7)	0.74	**57.5 (84); (49.1, 65.7)**	**70.3 (116); (62.7, 77.2)**	**-12.8 (-23.3, 2.1)**	**0.02**
Pn 7F	0.35 μg/mL	100 (46); (92.3, 100)	100 (45); (92.1, 100)	0 (-7.8, 7.9)	-	99.3 (145); (96.2, 100)	99.4 (164); (96.7, 100)	-0.1 (-3.2, 2.7)	1
Pn 9V	0.35 μg/mL	95.7 (44); (85.2, 99.5)	100 (45); (92.1, 100)	-4.3 (-14.6, 3.8)	0.49	73.3 (107); (65.3, 80.3)	78.2 (129); (71.1, 84.2)	-4.9 (-14.5, 4.6)	0.35
Pn 14	0.35 μg/mL	93.5 (43); (82.1, 98.6)	88.9 (40); (75.9, 96.3)	4.6 (-8,1, 18.1)	0.49	84.2 (123); (77.3, 89.7)	88.5 (146); (82.6, 92.9)	-4.2 (-12.2, 3.4)	0.32
Pn 18C	0.35 μg/mL	95.7 (44); (85.2, 99.5)	95.6 (43); (85.9, 99.5)	0 (-10.8, 11.2)	1	72.6 (106); (64.6, 79.7)	80.6 (133); (73.7, 86.3)	-8.0 (-17.6, 1.4)	0.11
Pn 19A	0.35 μg/mL	89.1 (41); (76.4, 96.4)	97.8 (44); (88.2, 99.9)	-8.6 (-21.3, 2.1)	0.20	47.3 (69); (38.9, 55.7)	52.1 (86); (44.2, 59.9)	-4.9 (-15.9, 6.3)	0.43
Pn 19F	0.35 μg/mL	100 (46); (92.3, 100)	100 (45); (92.1, 100)	0 (-7.8, 7.9)	-	98.6 (144); (95.1, 99.8)	100 (165); (97.8, 100)	-1.4 (-4.9, 0.9)	0.22
Pn 23F	0.35 μg/mL	93.5 (43); (82.1, 98.6)	93.3 (42); (81.7, 98.6)	0.1 (-11.9, 12.4)	1	56.2 (82); (47.7, 64.4)	66.1 (109); (58.3, 73.2)	-9.9 (-20.6, 1.0)	0.08

Vaccine antigen	Protective correlate	Antibodies to 12-month vaccines measured at 13 months of age			
		Maternal dTpa (n = 67)	No maternal dTpa (n = 78)	Difference % (95% CI)	p-value
		% (n); (95% CI)	% (n); (95% CI)		
Measles	0.12 IU/mL	100 (67); (94.6, 100)	96.2 (75); (89.2, 99.2)	3.8 (-1.7, 10.7)	0.25
Mumps	45 IU/mL	64.2 (43); (51.5, 75.5)	59.0 (46); (47.3, 70.0)	5.2 (-10.7, 20.7)	0.61
Rubella	10 IU/mL	88.1 (59); (77.8, 94.7)	85.9 (67); (76.2, 92.7)	2.2 (-9.5, 13.4)	0.83
MenC	2 μg/mL	100 (67); (94.6, 100)	98.7 (77); (93.1, 100)	1.3 (-4.2, 6.9)	1
Hib	0.15 μg/mL	98.5 (66); (92.0, 100)	98.7 (77); (93.1, 100)	-0.2 (-6.9, 5.6)	1
Tetanus	0.01 IU/mL	100 (67); (94.6, 100)	100 (78); (95.4, 100)	0 (-5.5, 4.7)	-

CI = confidence interval, dTpa = diphtheria-tetanus-acellular pertussis vaccine, Hib = *H. influenzae* type b, Pn = pneumococcus serotype, PT = pertussis toxin, MenC = meningococcus C

[a] 0.01 IU/mL at 13 months of age

[b] only participants who have not had Hib-MenC

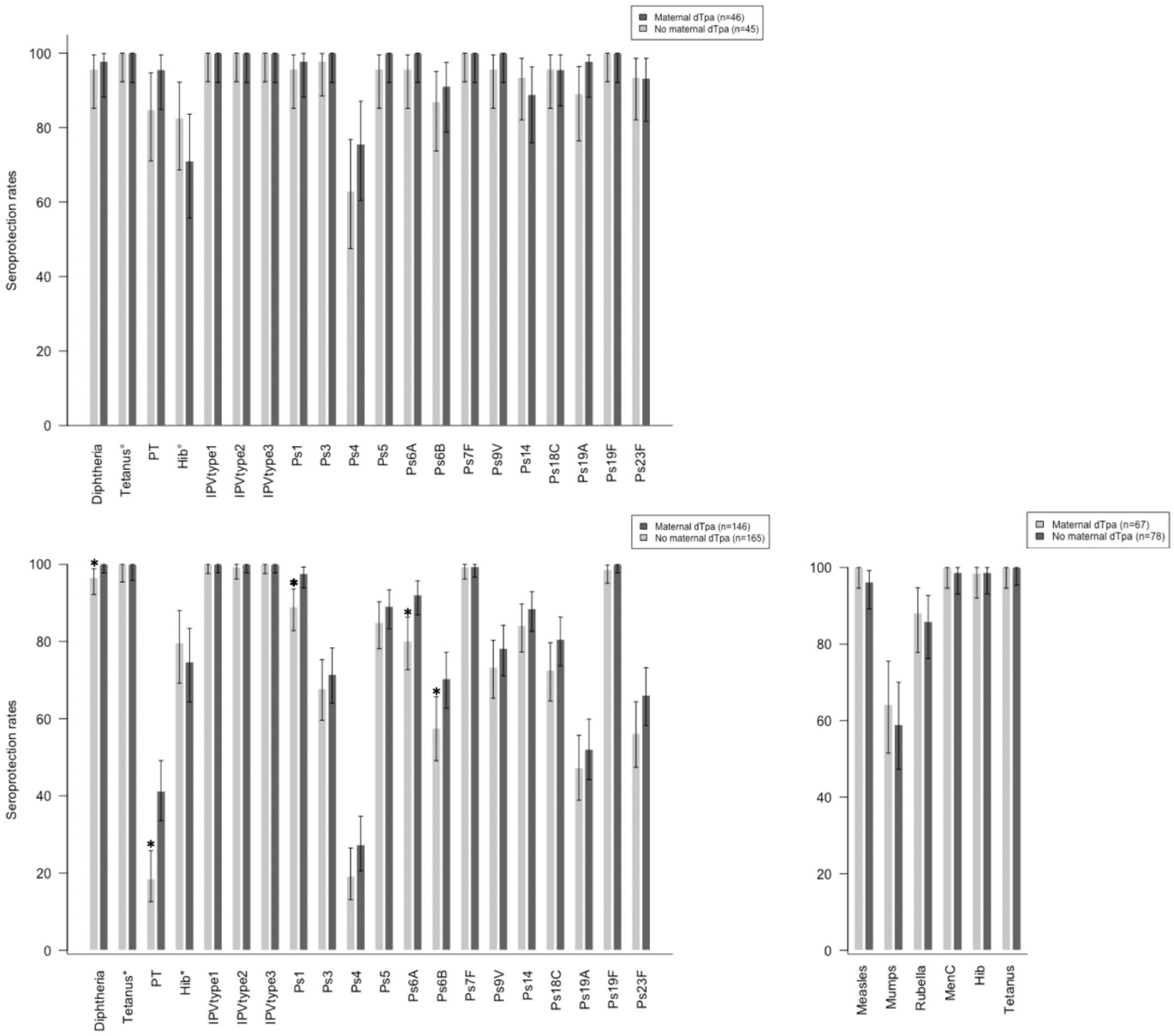


Fig. 3. Seroprotection rates with 95% CI in participants with or without maternal dTpa immunisation in pregnancy at 7 and 13 months of age.
° participants who have not yet received Hib-MenC, * $p < 0.05$.

3.5. The Effect of Maternal TIV Immunisation on Antibody Concentrations at 13 Months of Age (to the Primary Course of Vaccines and to the 12-Month Vaccines)

There was no consistent effect of maternal TIV immunisation on GMRs or seroprotection rates at 13 months of age (Supplementary Tables 2 and 3).

4. Discussion

Maternal immunisation has been proven to be safe and beneficial, and is now widely recommended [11,26–28]. For pertussis, for example, maternal immunisation leads to higher infant antibody concentrations at birth and during the first few months of life, which is highly effective in preventing severe pertussis in infants. The vaccine effectiveness against hospitalisation is estimated to be 91–94% and for preventing infection or mild disease 69–90% [29–31].

Our study investigated the effect of maternal dTpa immunisation on infant antibody responses to a large number of specific and heterologous vaccines, including measurement of the persistence of antibodies at 13 months of age. It is the first study to investigate the influence of maternal influenza immunisation on heterologous infant vaccine responses. Our results show that antenatal maternal dTpa immunisation strongly influences infant antibody responses at both 7 and 13 months of age and is associated with both reduced specific (diphtheria and pertussis) and heterologous (polio and pneumococcal) vaccine responses, but higher Hib responses at 7 months of age. In contrast, maternal TIV immunisation during pregnancy has no consistent effect on infant vaccine responses.

The findings of our study in relation to the primary course of vaccines are consistent with previous studies. To date, four studies have compared antibody concentrations one month after completion of primary immunisations between infants whose mother did or did not receive dTpa during pregnancy. These report higher GMCs for tetanus [7, 10,32] and Hib [10,32] in infants with maternal dTpa immunisation

during pregnancy, but lower GMCs for diphtheria [7,10,16,32], pertussis (FHA, fimbriae (FIM), PRN and PT) [7,10,16], HepB [10] and polio [10]. Two additional studies report higher GMCs for tetanus [8,33] and Hib [33], and lower GMCs for diphtheria [8] and pertussis (PRN, PT, FHA, FIM) [8,33], in infants whose mother received a pertussis-containing vaccine during pregnancy compared with those whose mother received a tetanus-containing vaccine (TCV) only.

Our findings in relation to persistence of antibodies before a booster-dose are also consistent with two studies which found higher GMCs for tetanus [10,16] and Hib [10], and lower GMCs for diphtheria [16], pertussis (FHA, FIM, PRN and PT) [10,16] and polio [10], in infants whose mothers received dTpa during pregnancy. Additionally, one larger study found higher GMCs for tetanus and Hib, and lower GMCs for pertussis (FHA, FIM, PRN and PT), in infants whose mother received a pertussis-containing vaccine during pregnancy compared with those whose mother received a TCV only [33].

Two previous studies have compared pneumococcal vaccine responses between infants whose mother did and did not receive dTpa during pregnancy. Both reported significantly lower GMCs against pneumococcal serotype 1, 3, 4, 5, 6A, 7F, 9V, 14 and 19A and lower seroprotection rates for serotype 3, 5 and 9V one month after primary immunisation [32,34]. The second study also measured vaccine responses 2.5 months after a booster dose of pneumococcal immunisation at 12 months of age and reported that GMCs remained lower in infants whose mother received a vaccine during pregnancy for serotype 1 and 4, without a difference in seroprotection rates [34].

One proposed mechanism for the inhibition of infant antibody production after maternal immunisation is that maternal antibodies bind to vaccine epitopes and mask them from infant B lymphocytes, leading to attenuation of responses to these antigens. Hence, the ratio between maternal antibody concentrations and dose of vaccine antigen might influence the degree of inhibition [35,36]. Since the carrier protein CRM197 in PCV13 is a modified diphtheria toxin, maternal antibodies against diphtheria might also mitigate responses to pneumococcal conjugate immunisation, which might explains the lower infant pneumococcal vaccine responses. Hib is conjugated to tetanus toxin as a carrier protein and responses to both tetanus and Hib are consistently (although not always significantly) higher in infants whose mother received dTpa during pregnancy. An unexpected finding in our study, which cannot be explained by a carrier protein, is that infants whose mothers received dTpa immunisation during pregnancy also had lower antibody responses to IPV.

Previously, it has been suggested that because of the short half-life of maternal antibodies (e.g. pertussis-specific IgGs are undetectable by the age of 2 months in infants of unvaccinated mothers) [37], maternal antibody interference of infant antibody production is only relevant during the first few months of life. However, in our study the effect of maternal dTpa immunisation during pregnancy (both reduced GMCs and seroprotection rates) was stronger at 13 months of age than at 7 months of age. Furthermore, three studies that compared vaccine responses in infants whose mothers received dTpa during pregnancy after booster immunisations given in the second year of age reported consistently (although not statistically significant) lower antibody concentrations for diphtheria [7,10,38], pertussis (FHA, FIM, PRN and PT) [7,10,38], Hib [10], HepB [10] and polio [10]. This suggests that the effect of maternal immunisation during pregnancy persists even after booster doses.

In our study, the lowest seroprotection rates were observed for pertussis (although the protection correlate value is debated) and pneumococcus. For pertussis, the main aim is to protect infants during the highest risk period occurring during the first few months of life and hence, the shift of the disease to a later period, as a result of maternal pertussis immunisation [39], might by an acceptable trade-off. However, the low seroprotection rates against pneumococcus at the age of 13 months are a concern, as this is a vulnerable age group for invasive pneumococcal disease.

Priming also occurs in infants after maternal vaccination. As most vaccines induce very high antibody responses, small differences in antibody concentrations may not be of clinical significance in terms of protective efficacy or may only impact the duration of protection. However, seroprotection rates are defined as correlates of clinical significance. Therefore, the differences in seroprotection rates found in our study might influence clinical outcomes. Nevertheless, protection induced by vaccines may not relate only to antibody concentration, but also to the quality of antibodies and cellular and cytokine responses. For example, for pertussis, there is no clear correlation between antibody concentration and protection rates. Nevertheless, countries that implement maternal immunisation might consider adapting their infant immunisation schedule to a later start (starting at 3 months of age with a 2-dose primary schedule results in higher antibody responses than a 3-dose schedule started at 2 months of age) [40] or including additional booster doses (e.g. pneumococcal vaccine in the second year of life to ensure protection during the at-risk period up to 5 years of age). Of note, new vaccines which interfere less with maternal antibodies are in development: animal studies show that co-injection of antigen and antigen-specific IgM increases antibody responses in the presence of inhibitory IgGs [41].

The strengths of our study include the low variation in background characteristics between groups, the detailed demographic and clinical data available from participants allowing for adjustment for potential confounding factors, the homogeneity in the vaccines given and the broad range of antibodies measured. The limitations of the study include the non-random allocation of maternal immunisations during pregnancy, the fact that maternal vaccines were given at different time points during pregnancy, that not all infants had antibody response measured exactly 4 weeks after immunisation and that not all infants had received their 12-month immunisation by the time blood samples were taken at 13 months, meaning a smaller number of participants available for assessment of responses to the 12-month vaccines, and that the number of infants included at both time points was too low to allow longitudal analysis. In addition, in the interpretation of our results, the lack of consensus on the protective correlate value for PT and the use of multiple significance testing need to be taken into consideration.

In conclusion, whilst maternal immunisation protects infant in the first few months of life, it might increase the risk of disease in older infants as a result of blunting of responses to both related (specific) and unrelated (heterologous) vaccines. Since the risk of hospitalisation and death is less in older infants, this might be an acceptable trade-off. Strategies, such as additional booster doses in the second year of life, particularly for pertussis and pneumococcus, should be considered to address this.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eclinm.2019.06.010.

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2.3 ADDITIONAL INFORMATION

Table 2.1 Characteristics of participants whose mothers did or did not receive TIV during pregnancy

	To primary course of vaccines ending at 6 months of age						To 12-month vaccines		
	At 7 months of age n (%) or median (IQR)			At 13 months of age n (%) or median (IQR)			At 13 months of age n (%) or median (IQR)		
	Maternal TIV (n=55)	No maternal TIV (n=36)	p-value	Maternal TIV (n=175)	No maternal TIV (n=136)	p-value	Maternal TIV (n=88)	No maternal TIV (n=60)	p-value
Maternal age at birth (y)	33.1 (32.1-35.5)	34.4 (32.6-35.9)	0.75	33.5 (30.9-37.8)	32-4 (29.6-36.0)	0.36	34.5 (31.2-37.6)	32.7 (30.3-36.5)	0.12
Gestational age (w)	40 (38.8-40.6)	39.3 (38.5-40.4)	0.42	39.2 (38.4-40.3)	39.3 (38.3-40.3)	0.79	39.3 (38.5-40.4)	39.1 (38.1-40.2)	0.21
Caesarean section	19 (35)	16 (44)	0.53	69 (39)	48 (35)	0.61	30 (34)	24 (40)	0.62
Sex (male)	26 (47)	17 (47)	1.00	88 (50)	64 (47)	0.74	39 (44)	34 (57)	0.39
Birth weight (kg)	3.49 (3.06-3.66)	3.46 (3.24-3.78)	0.67	3.41 (3.14-3.73)	3.52 (3.09-3.83)	0.96	3.37 (3.14-3.38)	3.43 (2.94-3.75)	0.81
BCG-vaccinated	24 (44)	21 (58)	0.43	93 (53)	76 (56)	0.79	42 (49)	34 (57)	0.55
MMR/MenC-Hib-vaccinated	-	-	-	114 (65)	96 (71)	0.65	-	-	-
Age at routine immunisation (d)									
- 6-w vaccines	45 (43-48)[1]	44 (43-47)	0.98	45 (43-49)	46 (44-52)	0.85	45 (43-48)	45 (43-49)	0.49
- 4-m vaccines	123 (118-126)	125 (115-128)	0.91	124 (120-129)	127 (121-133)	0.03	123 (121-128)	126 (119-132)	0.25
- 6-m vaccines	186 (182-193)	190 (178-193)	0.71	190 (184-201)	191 (186-208)	0.13	183 (184-201)	191 (186-207)	0.09
- 12-m vaccines	-	-	-	-	-	-	375 (339-384)	377 (371-383)	0.93
Interval between (d)									
- 6-m vaccines to 7-m blood sample	28 (21-36)	30 (22-38)	0.67	-	-	-	-	-	-
- 6-w vaccines to 13-m blood sample	-	-	-	209 (191-224)	209 (190-224)	0.50	-	-	-
- 12-m vaccines to 13-m blood sample	-	-	-	-	-	-	29 (24-36)	27 (21-35)	0.06
Age at sampling (d)	216 (208-226)	222 (207-228)	0.55	400 (386-418)	404 (392-416)	0.43	406 (395-417)	406 (397-413)	0.23

[1] age for one participant not available

p-values were determined by t-test for continuous variables and Pearson's Chi-squared test for categorical variables

d = days, m = month, TIV = trivalent inactivated influenza vaccine, w = week, y = years

Table 2.2 Seroprotection rates at 7 at 13 months of age in infants whose mothers did or did not receive TIV immunisation during pregnancy

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Maternal TIV** (n=55) **% (n); (95% CI)**	**No maternal TIV** (n=36) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**	**Maternal TIV** (n=175) **% (n); (95% CI)**	**No maternal TIV** (n=136) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**
Diphtheria	0.1 IU/mL[1]	98.2 (54); (90.3, 100)	94.4 (34); (81.3, 99.3)	3.8 (-4.9, 16.6)	0.56	97.7 (171); (94.3, 99.4)	99.3 (135); (96.0, 100)	-1.6 (-5.1, 2.0)	0.39
Tetanus	0.1 IU/mL[1]	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	100 (90); (96.0, 100)[2]	100 (76); (95.3, 100)[2]	0 (-4.1, 4.8)[2]	-
PT	25 IU/mL	87.3 (48); (75.5, 94.7)	94.4 (34); (81.3, 99.3)	-7.2 (-19.6, 6.9)	0.31	27.4 (48); (21.0, 34.7)	34.6 (47); (26.6, 43.2)	-7.1 (-17.5, 3.2)	0.21
Hib	0.15 µg/mL	80.0 (44); (67.0, 89.6)	72.2 (26); (54.8, 85.8)	7.8 (-9.7, 26.6)	0.62	74.4 (67); (64.1, 83.1)[2]	78.9 (60); (68.1, 87.5)[2]	-4.5 (-17.2, 8.7)[2]	0.71[2]
Polio type 1	0.23 IU/mL	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	100 (175); (97.9, 100)	100 (136); (97.3, 100)	0 (-2.2, 2.8)	-
Polio type 2	0.29 IU/mL	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	99.4 (174); (96.9, 100)	100 (136); (97.3, 100)	-0.6 (-3.2, 2.2)	1
Polio type 3	0.12 IU/mL	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	100 (175); (97.9, 100)	100 (136); (97.3, 100)	0 (-2.2, 2.8)	-
Pn 1	0.35 µg/mL	98.2 (54); (90.3, 100)	94.4 (34); (81.3, 99.3)	3.7 (-4.9, 16.6)	0.56	94.3 (165); (89.7, 97.2)	92.6 (126); (86.9, 96.4)	1.6 (-4.0, 7.9)	0.64
Pn 3	0.35 µg/mL	98.2 (54); (90.3, 100)	100 (36); (90.3, 100)	-1.8 (-9.7, 8.0)	1	68.0 (119); (60.5, 74.8)	72.1 (98); (63.7, 79.4)	-4.1 (-14.1, 6.3)	0.46
Pn 4	0.35 µg/mL	69.1 (38); (55.2, 80.9)	69.4 (25); (51.9, 84.7)	0.4 (-19.0, 19.5)	1	21.7 (38); (15.8, 28.6)	25.7 (35); (18.6, 33.9)	-4.0 (-13.8, 5.4)	0.42
Pn 5	0.35 µg/mL	96.4 (53); (87.5, 100)	100 (36); (90.3, 100)	-3.6 (-12.4, 6.2)	0.52	87.4 (153); (81.6, 91.2)	86.8 (118); (79.9, 91.2)	0.7 (-6.8, 8.6)	0.87
Pn 6A	0.35 µg/mL	98.2 (54); (90.3, 100)	97.2 (35); (85.5, 100)	1.0 (-7.3, 12.6)	1	84.0 (147); (77.7, 89.1)	89.7 (122); (83.3, 94.3)	-5.7 (-13.2, 2.1)	0.18
Pn 6B	0.35 µg/mL	90.9 (50); (80.0, 97.0)	86.1 (31); (70.5, 95.3)	4.8 (-8.5, 20.8)	0.51	61.7 (108); (54.1, 68.9)	67.6 (92); (59.1, 75.4)	-5.9 (-16.4, 4.9)	0.29
Pn 7F	0.35 µg/mL	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	98.9 (173); (95.9, 99.9)	100 (136); (97.3, 100)	-1.1 (-4.1, 1.6)	0.51
Pn 9V	0.35 µg/mL	98.2 (54); (90.3, 100)	97.2 (35); (85.5, 100)	1 (-7.3, 12.6)	1	74.9 (131); (67.8, 81.0)	77.2 (105); (69.2, 84.0)	-2.3 (-11.8, 7.4)	0.69
Pn 14	0.35 µg/mL	89.1 (49); (77.8, 95.9)	94.4 (34); (81.3, 99.3)	-5.4 (-17.4, 8.5)	0.47	84.6 (148); (78.4, 89.6)	89.0 (121); (82.5, 93.7)	-4.4 (-11.9, 3.5)	0.32
Pn 18C	0.35 µg/mL	94.5 (52); (84.9, 98.9)	97.2 (35); (85.5, 100)	-2.7 (-12.6, 9.3)	1	76.0 (133); (69.0, 82.1)	77.9 (106); (70.0, 84.6)	-1.9 (-11.2, 7.7)	0.79
Pn 19A	0.35 µg/mL	94.5 (52); (84.9, 98.9)	91.7 (33); (77.5, 98.2)	2.9 (-8.1, 17.1)	0.68	50.9 (89); (43.2, 58.5)	49.5 (66); (39.9, 57.2)	2.3 (-8.8, 13.4)	0.73
Pn 19F	0.35 µg/mL	100 (55); (93.5, 100)	100 (36); (90.3, 100)	0 (-6.6, 9.7)	-	100 (175); (97.9, 100)	98.5 (134); (94.8, 99.8)	1.5 (-0.7, 5.2)	0.19
Pn 23F	0.35 µg/mL	96.4 (53); (87.5, 100)	88.9 (32); (74.9, 96.9)	7.5 (-3.3, 22.2)	0.21	60.6 (106); (52.9, 67.9)	62.5 (85); (54.8, 70.6)	-1.9 (-12.7, 9.0)	0.81
						Antibodies to 12-month vaccines measured at 13 months of age			
						Maternal TIV (n=85) **% (n)**	**No maternal TIV** (n=60) **% (n)**	**Difference % (95% CI)**	**p-value**
Measles	0.12 IU/mL					97.6 (83); (91.8, 99.7)	98.3 (59); (91.1, 100)	0.6 (-6.5, 6.8)	1
Mumps	45 IU/mL					68.2 (58); (57.2, 77.9)	51.7 (31); (38.4, 64.8)	16.6 (0.4, 32.2)	0.06
Rubella	10 IU/mL					88.2 (75); (79.4, 94.2)	85.0 (51); (73.4, 92.9)	3.2 (-7.9, 15.7)	0.62
MenC	2 µg/mL					98.8 (84); (93.6, 100)	100 (60); (94.0, 100)	-1.2 (-6.4, 4.9)	1
Hib	0.15 µg/mL					98.8 (84); (93.6, 100)	98.3 (59); (91.1, 100)	0.5 (-4.9, 7.8)	1
Tetanus	0.01 IU/mL					100 (85); (95.8, 100)	100 (60); (94.0, 100)	0 (-4.4, 6.1)	-

[1] 0.01 IU/mL at 13 months of age [2] only participants who have not had Hib-MenC

TIV = trivalent inactivated influenza vaccine, CI = confidence interval, PT = pertussis toxin, Hib = *H. influenzae* type b, Pn = pneumococcus serotype, MenC = meningococcus C

Table 2.3 Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) at 7 and 13 months of age in infants whose mothers did or did not receive TIV during pregnancy. Bold denotes results with p-value < 0.05.

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**Maternal TIV (n=55) GMC (95% CI)**	**No maternal TIV (n=36) GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**	**Maternal TIV (n=175) GMC (95% CI)**	**No maternal TIV (n=136) GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Diphtheria[1]	0.42 (0.34, 0.51)	0.37 (0.28, 0.49)	1.13 (0.81, 1.57)	0.48	1.31 (0.96, 1.78)[b,c,g,j]	0.08	0.07 (0.06, 0.09)	0.09 (0.07, 0.10)	0.86 (0.68, 1.08)	0.19	1.00 (0.81, 1.23)[b,h,i,j,k]	0.97
Tetanus[1]	1.19 (0.98, 1.46)	0.99 (0.81, 1.22)	1.20 (0.90, 1.62)	0.22	1.20 (0.90, 1.62)	0.22	0.39 (0.31, 0.49)[3]	0.52 (0.40, 0.67)[3]	0.75 (0.53, 1.07)[3]	0.12[3]	0.84 (0.60, 1.18)[3,i]	0.32[3]
PT[1]	**58.59 (47.63, 72.07)**	**92.14 (70.73, 120.03)**	**0.64 (0.46, 0.88)**	**<0.01**	**0.72 (0.53, 0.98)[b]**	**0.04**	16.28 (13.96, 18.98)	19.28 (16.26, 22.87)	0.84 (0.67, 1.06)	0.15	0.96 (0.77, 1.19)[b,h,i,j,k]	0.71
FHA[1]	62.08 (49.38, 78.04)	66.34 (50.71, 86.79)	0.94 (0.66, 1.33)	0.71	1.06 (0.76, 1.48) [b,c,h]	0.72	18.59 (15.96, 21.65)	19.92 (17.10, 23.22)	0.93 (0.75, 1.16)	0.53	1.05 (0.85, 1.29)[b,e,h,k]	0.66
PRN[1]	59.99 (46.68, 77.09)	61.25 (43.33, 86.58)	0.98 (0.65, 1.48)	0.92	1.06 (0.71, 1.58) [b,c,f]	0.79	11.14 (9.25, 13.43)	13.49 (11.07, 16.46)	0.83 (0.63, 1.09)	0.17	0.92 (0.71, 1.20)[b,e,k]	0.55
Hib[2]	0.55 (0.36, 0.85)	0.46 (0.27, 0.77)	1.21 (0.62, 2.37)	0.57	1.33 (0.70, 2.51)[c,h,j]	0.38	0.91 (0.57, 1.46)[3]	1.55 (0.91, 2.64)[3]	0.59 (0.29, 1.19)[3]	0.14[3]	0.73 (0.37, 1.43)[3,d,j]	0.36[3]
Polio type 1[1]	33.74 (25.39, 44.83)	35.98 (25.24, 51.31)	0.94 (0.60, 1.47)	0.78	0.97 (0.62, 1.50)[c,f]	0.88	11.23 (9.44, 13.35)	11.17 (9.26, 13.48)	1.00 (0.78, 1.30)	0.97	1.07 (0.83, 1.38)[b,i,h,k]	0.59
Polio type 2[1]	54.13 (39.59, 74.03)	79.37 (59.42, 106.02)	0.68 (0.44, 1.07)	0.09	0.71 (0.46, 1.10) [b,k]	0.12	21.12 (17.26, 25.84)	22.38 (18.40, 27.24)	0.94 (0.71, 1.26)	0.79	1.05 (0.79, 1.39)[b,h,k]	0.73
Polio type 3[1]	23.05 (16.79, 31.83)	19.19 (13.75, 26.77)	1.20 (0.75, 1.93)	0.45	1.18 (0.74, 1.88)[k]	0.48	9.22 (7.56, 11.25)	8.66 (6.96, 10.77)	1.06 (0.79, 1.43)	0.68	1.19 (0.89, 1.59)[b,e,i,h,k]	0.24
Pn 1[2]	4.95 (3.78, 6.47)	3.61 (2.52, 5.15)	1.37 (0.89, 2.11)	0.15	**1.50 (1.01, 2.22)[g,j,k]**	**0.04**	1.14 (1.01, 1.28)	1.20 (1.02, 1.42)	0.95 (0.78, 1.16)	0.61	0.99 (0.82, 1.20)[b,d,k]	0.95
Pn 3[2]	1.43 (1.19, 1.73)	1.44 (1.15, 1.80)	0.99 (0.75, 1.33)	0.97	1.02 (0.77, 1.35)[h]	0.91	0.52 (0.46, 0.58)	0.57 (0.50, 0.66)	0.91 (0.75, 1.09)	0.29	0.94 (0.79, 1.12)[k]	0.48
Pn 4[2]	0.55 (0.43, 0.71)	0.46 (0.37, 0.58)	1.20 (0.84, 1.72)	0.30	1.25 (0.89, 1.75)[j]	0.20	0.21 (0.19, 0.23)	0.22 (0.20, 0.25)	0.94 (0.81, 1.09)	0.42	1.00 (0.86, 1.16)[b,c,g,h,k]	0.98
Pn 5[2]	3.39 (2.62, 4.39)	2.83 (2.12, 3.77)	1.20 (0.81, 1.77)	0.36	1.32 (0.92, 1.88)[g,j,k]	0.14	0.86 (0.75, 0.99)	1.00 (0.85, 1.17)	0.87 (0.70, 1.06)	0.16	0.93 (0.76, 1.14)[b,i,j,k]	0.48
Pn 6A[2]	4.98 (3.94, 6.30)	4.57 (3.42, 6.10)	1.09 (0.75, 1.58)	0.64	1.27 (0.90, 1.77) [b,g,h,j]	0.17	0.94 (0.81, 1.09)	1.09 (0.92, 1.29)	0.86 (0.69, 1.08)	0.19	0.95 (0.77, 1.17)[b,j,k]	0.64
Pn 6B[2]	2.13 (1.41, 3.22)	1.63 (0.99, 2.69)	1.31 (0.69, 2.49)	0.41	1.45 (0.78, 2.70)[f,j]	0.24	0.47 (0.40, 0.56)	0.59 (0.48, 0.72)	0.81 (0.62, 1.05)	0.11	0.91 (0.70, 1.17)[a,b,k]	0.44
Pn 7F[2]	6.48 (5.09, 8.25)	5.63 (4.68, 6.78)	1.15 (0.83, 1.60)	0.40	1.24 (0.90, 1.71) [b,j]	0.19	1.75 (1.56, 1.95)	2.06 (1.79, 2.36)	0.85 (0.71, 1.01)	0.07	0.89 (0.75, 1.06)[b,j,k]	0.19
Pn 9V[2]	2.67 (2.11, 3.38)	2.17 (1.65, 2.84)	1.23 (0.86, 1.76)	0.25	1.38 (1.00, 1.90)[g,h,j,k]	0.05	0.58 (0.51, 0.65)	0.63 (0.54, 0.74)	0.91 (0.75, 1.11)	0.36	0.96 (0.80, 1.16)[i,j,k]	0.69
Pn 14[2]	2.62 (1.81, 3.79)	2.74 (1.91, 3.92)	0.96 (0.56, 1.64)	0.88	0.96 (0.56, 1.64)	0.88	1.02 (0.88, 1.18)	1.09 (0.91, 1.30)	0.94 (0.74, 1.18)	0.57	0.97 (0.78, 1.22)[h,i,j,k]	0.80
Pn 18C[2]	3.08 (2.34, 4.06)	2.74 (2.07, 3.61)	1.13 (0.75, 1.68)	0.56	1.16 (0.79, 1.71)[j]	0.45	0.63 (0.56, 0.70)	0.69 (0.59, 0.80)	0.91 (0.76, 1.10)	0.35	0.98 (0.82, 1.18)[a,b,i,j,k]	0.86
Pn 19A[2]	1.94 (1.50, 2.51)	1.43 (1.05, 1.96)	1.35 (0.91, 2.02)	0.14	1.50 (1.01, 2.22) [b,c]	0.05	0.47 (0.36, 0.57)	0.46 (0.36, 0.57)	1.02 (0.76, 1.37)	0.88	1.06 (0.80, 1.42)[i,k]	0.68
Pn 19F[2]	12.83 (10.27, 16.04)	9.79 (7.08, 13.55)	1.31 (0.90, 1.91)	0.16	**1.45 (1.01, 2.08)** [b,j]	**0.04**	2.88 (2.48, 3.34)	3.38 (2.78, 4.11)	0.85 (0.67, 1.09)	0.20	0.87 (0.69, 1.11)[i,j,k]	0.26
Pn 23F[2]	2.59 (1.96, 3.43)	2.00 (1.30, 3.06)	1.30 (0.80, 2.10)	0.29	1.42 (0.89, 2.27)[g,j]	0.14	0.52 (0.44, 0.62)	0.62 (0.50, 0.78)	0.84 (0.63, 1.10)	0.20	0.88 (0.67, 1.16)[b,e,f]	0.38
							Antibodies to 12-month vaccines measured at 13 months of age					
							Maternal TIV (n=85) GMC (95% CI)	**No maternal TIV (n=60) GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Measles[1]							2.96 (2.29, 3.82)	3.12 (2.45, 3.98)	0.94 (0.66, 1.37)	0.78	0.90 (0.63, 1.30)[d]	0.59
Mumps[1]							**74.85 (56.60, 98.99)**	**42.87 (30.79, 59.69)**	**1.75 (1.14, 2.68)**	**0.01**	1.34 (0.94, 1.92)[i,j]	0.11
Rubella[1]							60.31 (44.52, 81.68)	46.26 (32.08, 66.72)	1.30 (0.81, 2.09)	0.27	0.94 (0.66, 1.35)[i,j]	0.75
MenC[2]							16.05 (13.33, 19.32)	20.55 (16.94, 24.91)	0.78 (0.60, 1.02)	0.07	0.79 (0.60, 1.03)[d,j]	0.08
Hib[2]							16.31 (11.82, 22.51)	14.39 (9.34, 22.18)	1.13 (0.67, 1.91)	0.64	1.10 (0.65, 1.84)[b,j]	0.73
Tetanus[1]							0.91 (0.71, 1.18)[3]	1.15 (0.84, 1.57) [3]	0.80 (0.53, 1.18)	0.26	0.80 (0.53, 1.18)	0.26

[1] IU/ml
[2] µg/ml
[3] only participants who have not had Hib-MenC

[a] maternal age
[b] maternal dTpa immunisation during pregnancy
[c] gestational age
[d] delivery mode
[e] sex
[f] birth weight
[g] BCG immunisation
[h] age at first DTPa-HepB-IPV-Hib / PCV13 immunisation
[i] MMR/MenC-Hib immunisation
[j] age at bleeding
[k] time between immunisation and bleeding

CI = confidence interval, FHA = filamentous haemagglutinin, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PRN = pertactin, PT = pertussis toxin, TIV = trivalent inactivated influenza vaccine

Figure 2.1 GMCs of antibodies against DTPa-IPV-Hib at 7 months of age in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

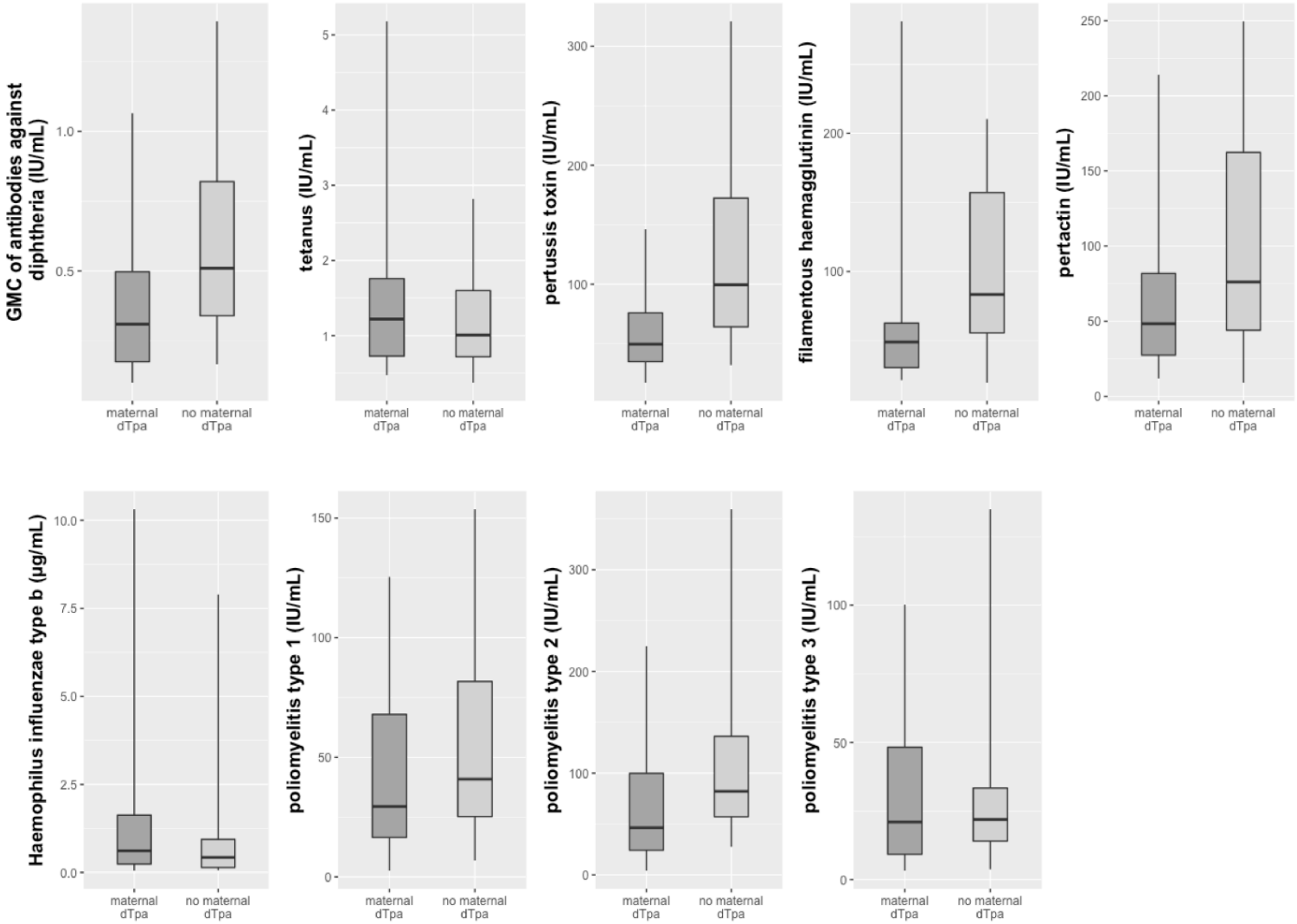


Figure 2.2 GMCs of antibodies against pneumococcus at 7 months of age in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

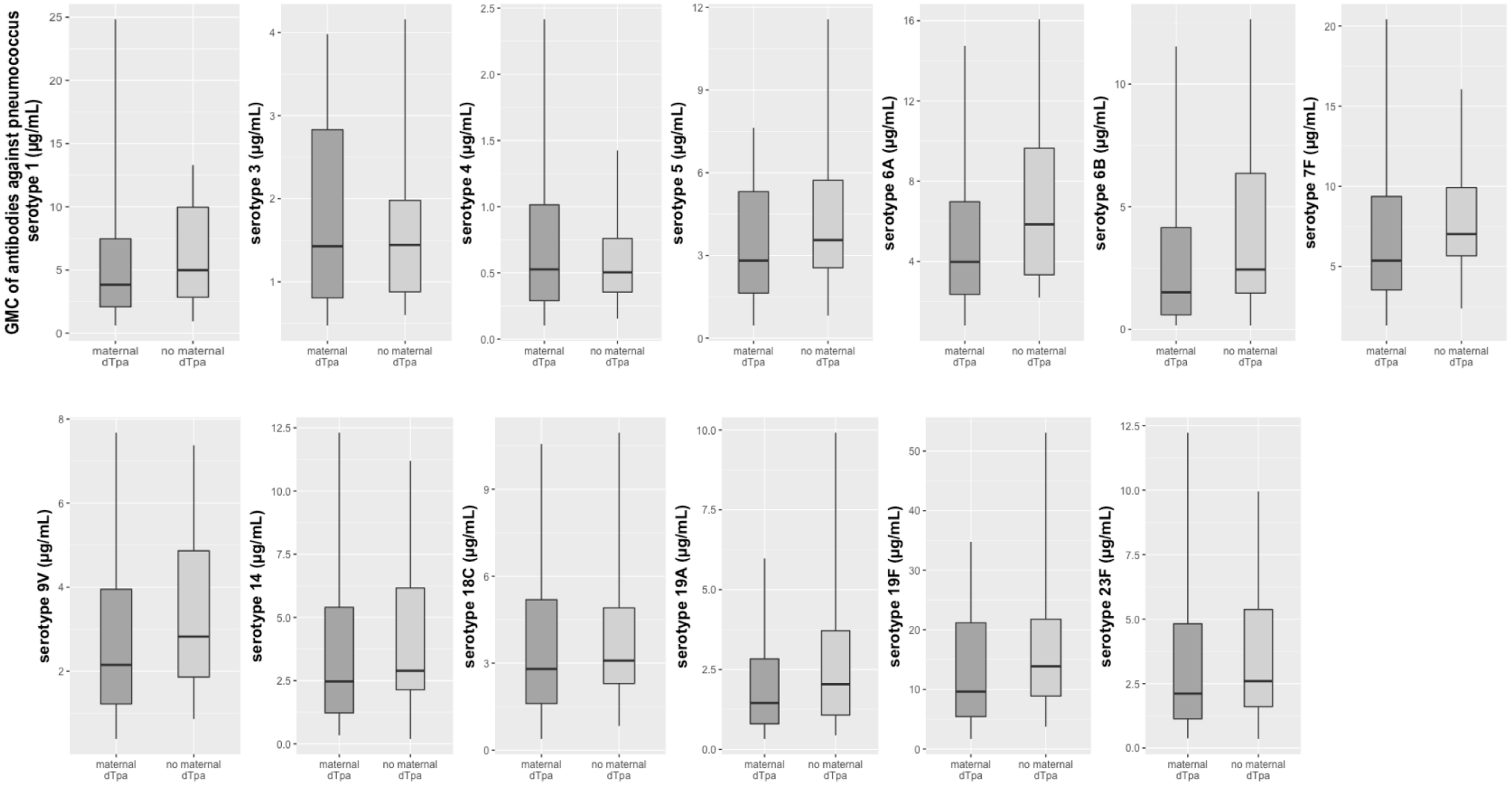
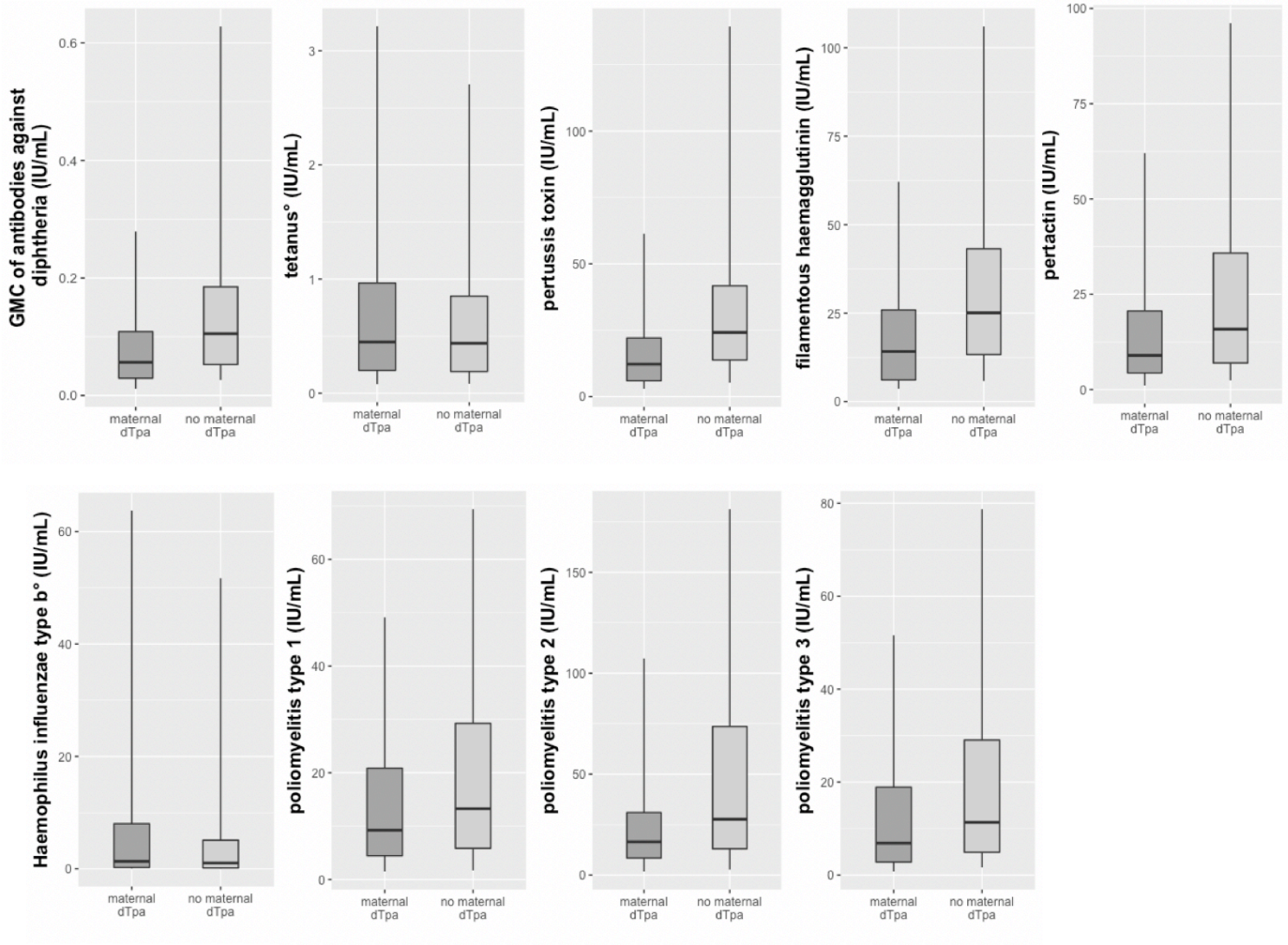


Figure 2.3 GMCs of antibodies against DTPa-IPV-Hib at 13 months of age (persistence of antibodies after 6-months immunisations) in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.



° only participants who have not had Hib-MenC

Figure 2.4 GMCs of antibodies against pneumococcus at 13 months of age (persistence of antibodies after 6-months immunisations) in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

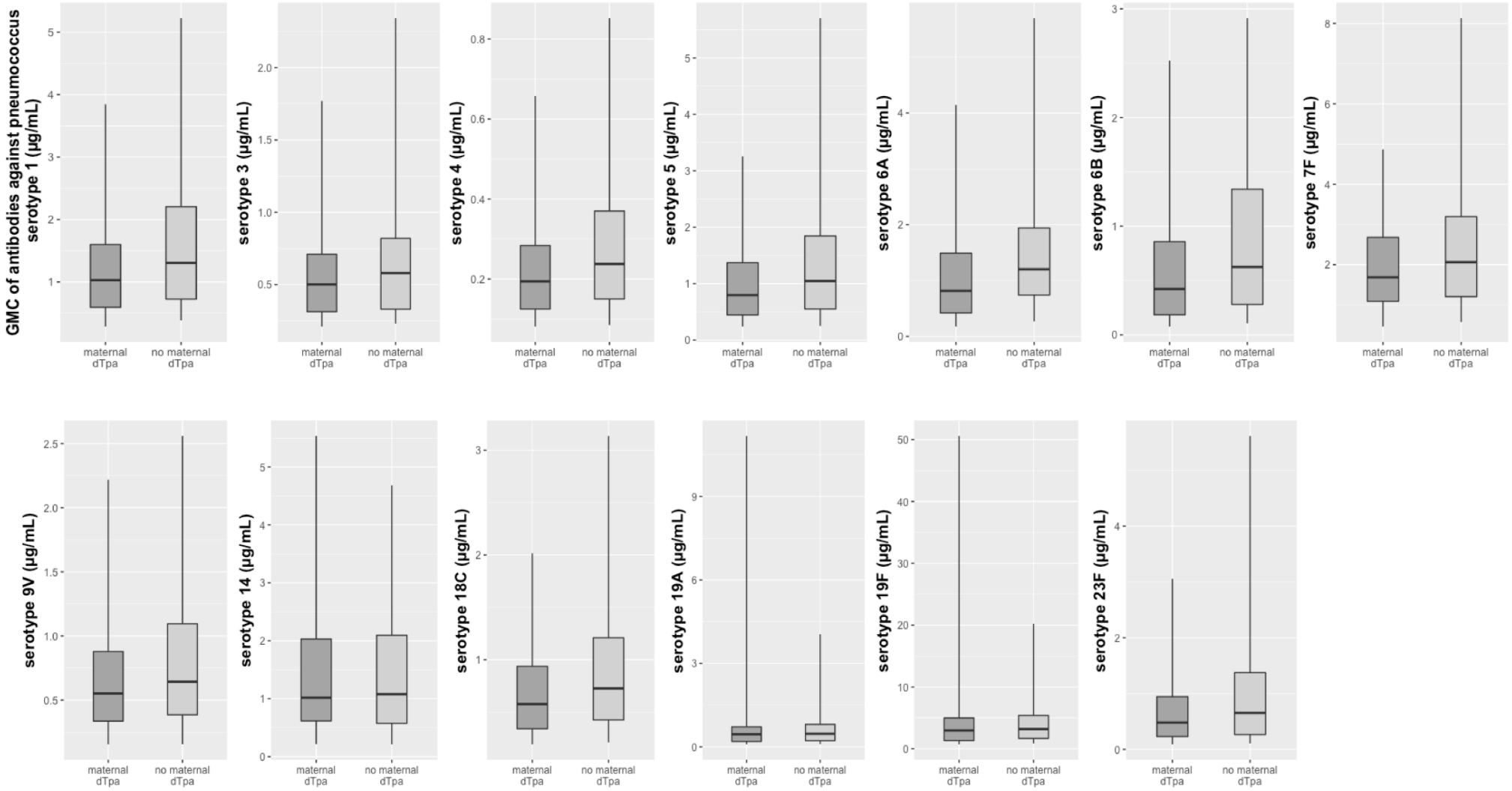


Figure 2.5 GMCs of antibodies against MMR, MenC and Hib at 13 months of age (antibodies against 12-months vaccines) in infants whose mothers did or did not receive dTpa immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

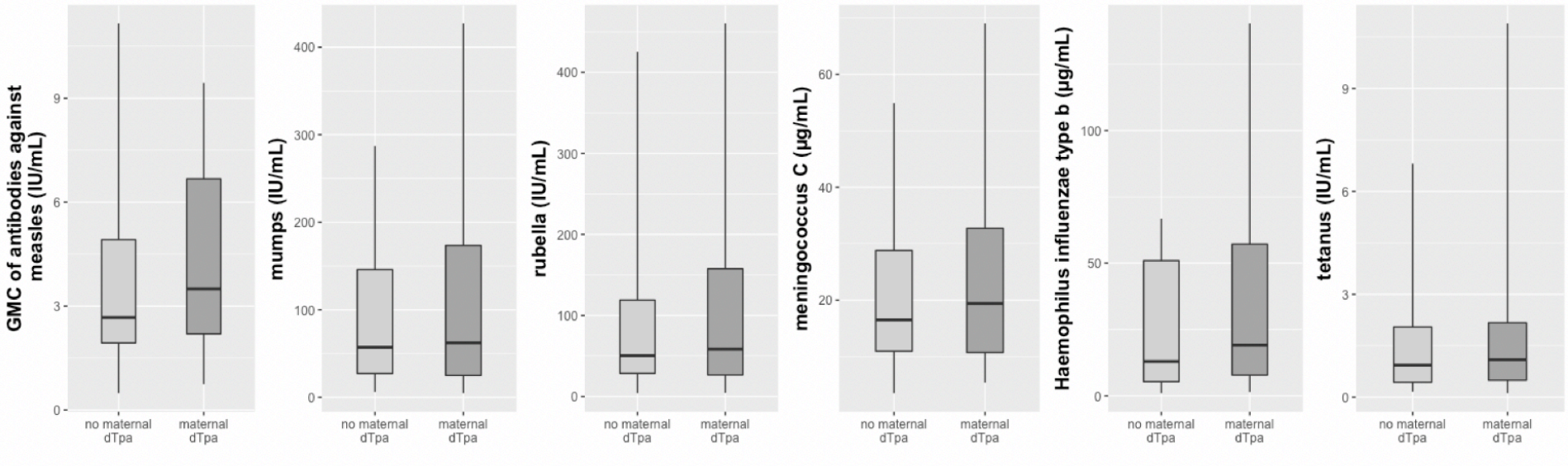


Figure 2.6 GMCs of antibodies against DTPa-IPV-Hib at 7 months of age in infants whose mothers did or did not receive TIV immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

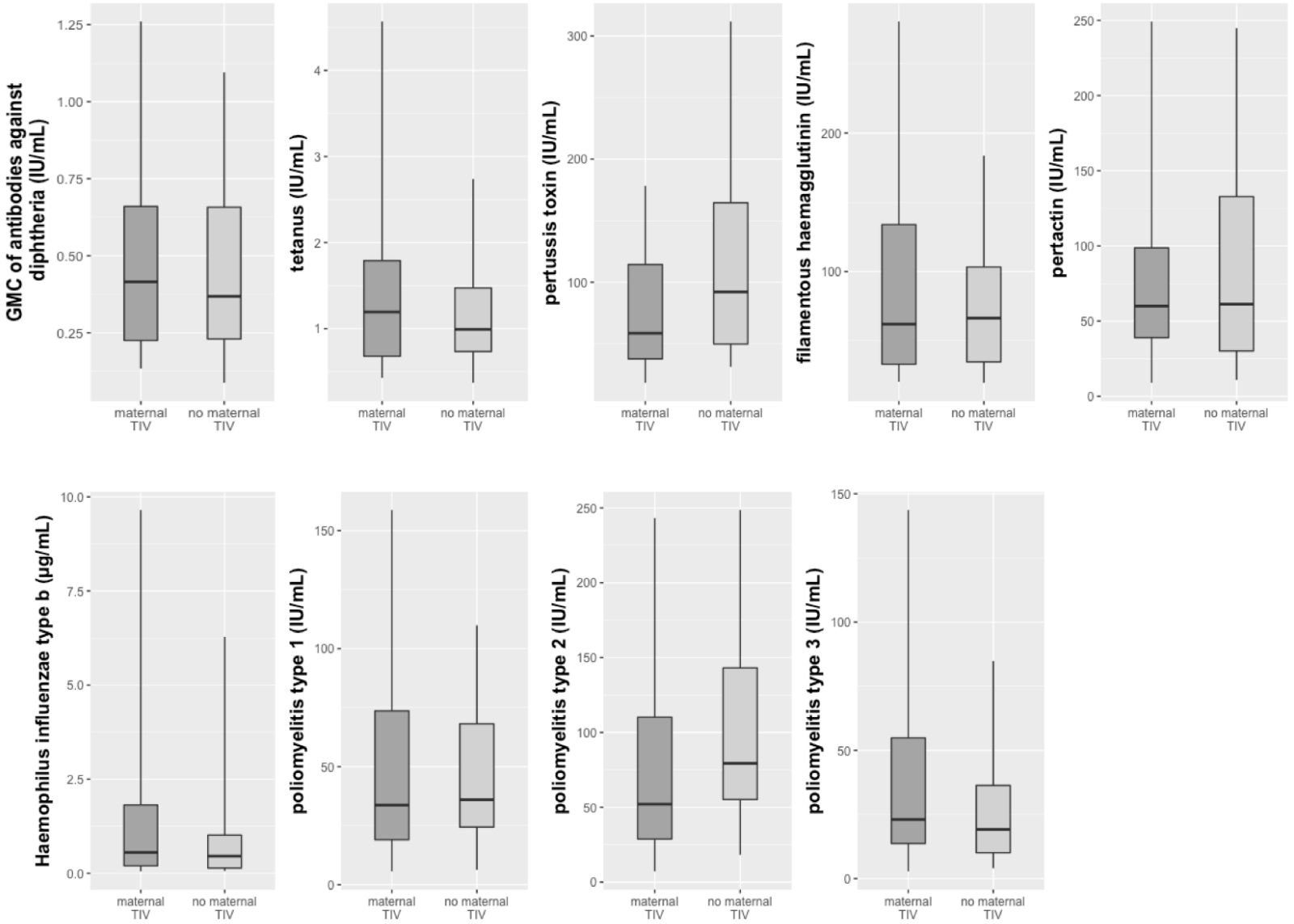


Figure 2.7 GMCs of antibodies against pneumococcus at 7 months of age in infants whose mothers did or did not receive TIV immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

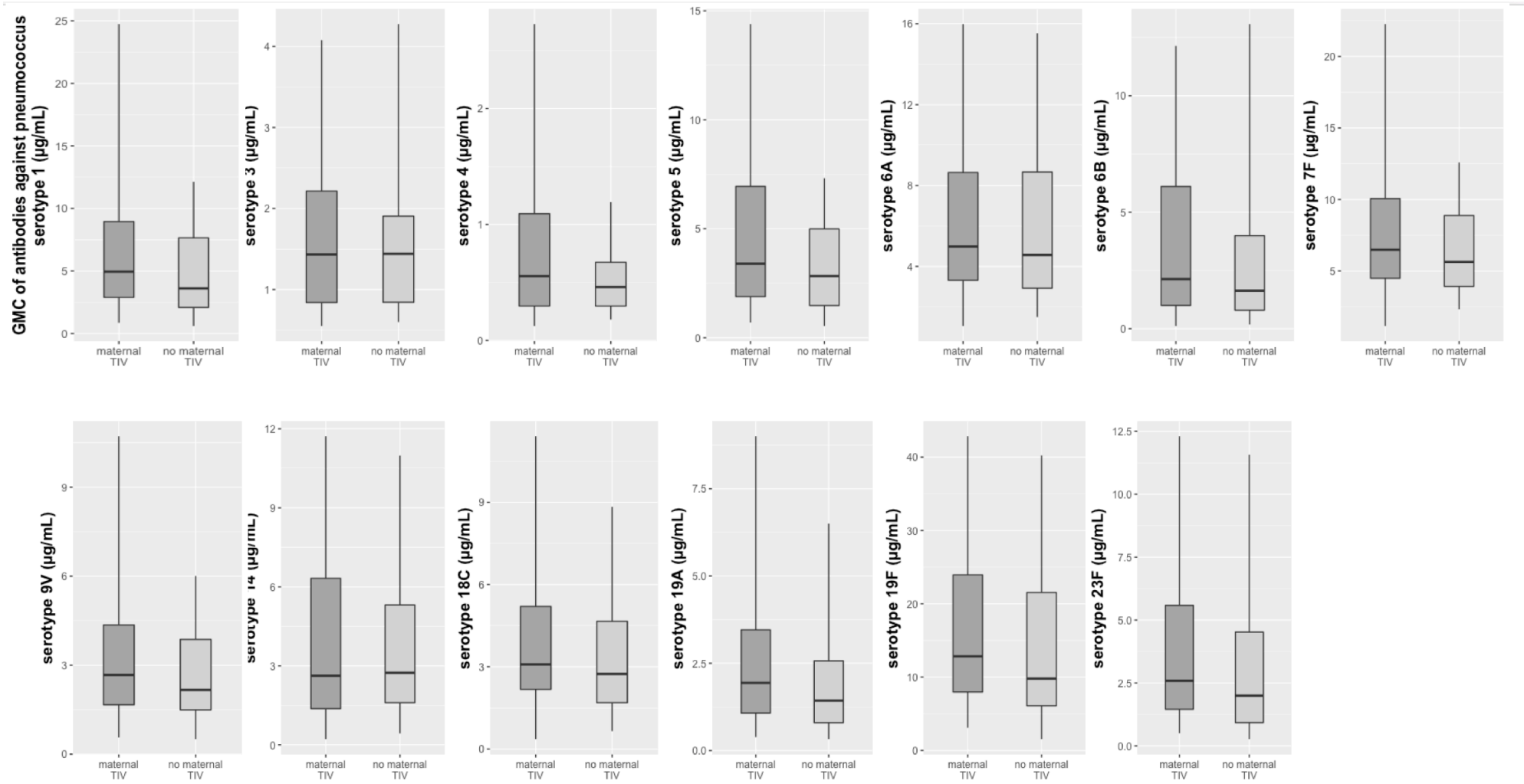
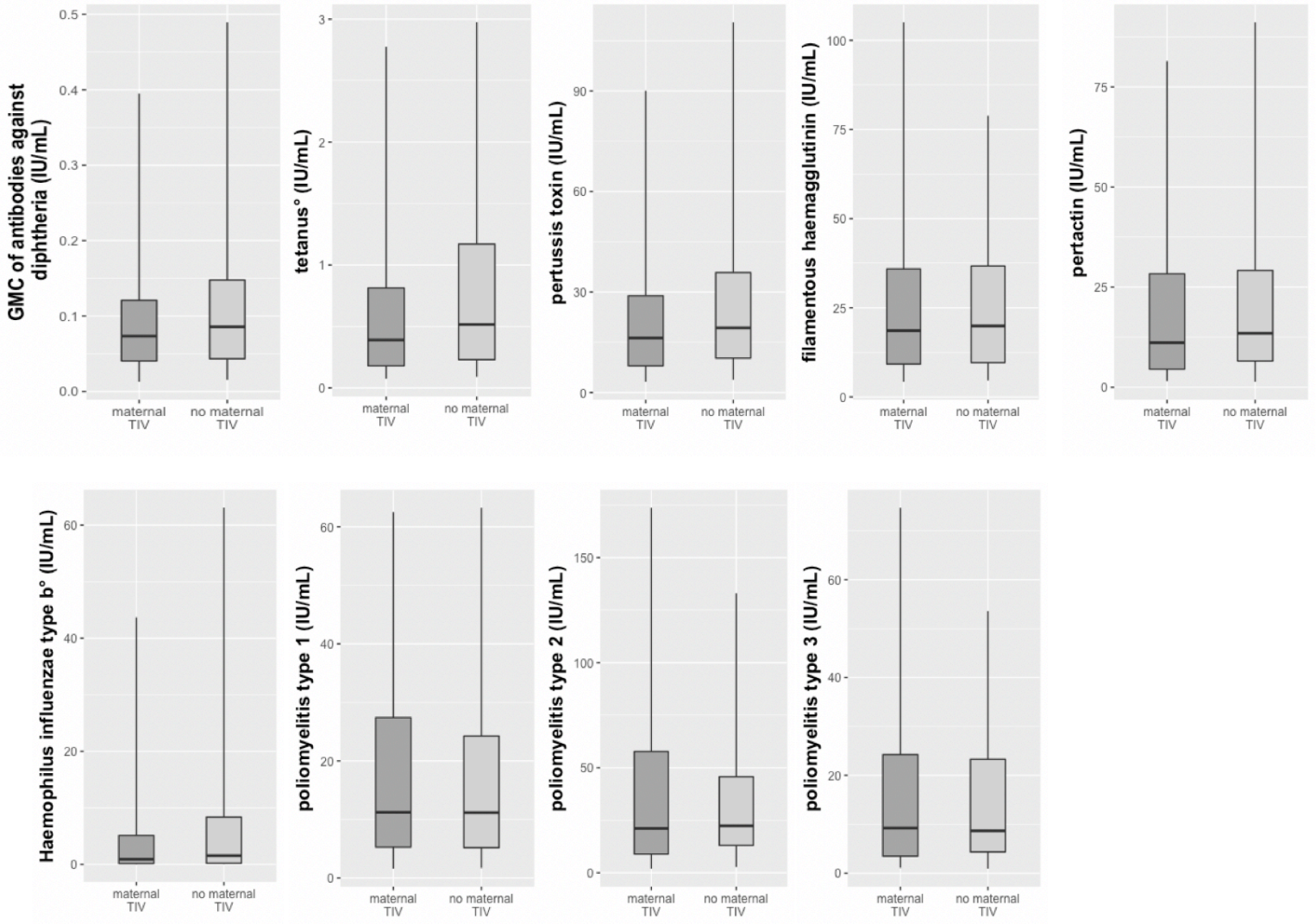


Figure 2.8 GMCs of antibodies against DTPa-IPV-Hib at 13 months of age (persistence of antibodies after 6-months immunisations) in infants whose mothers did or did not receive TIV immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.



° only participants who have not had Hib-MenC

Figure 2.9 GMCs of antibodies against pneumococcus at 13 months of age (persistence of antibodies after 6-months immunisations) in infants whose mothers did or did not receive TIV immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

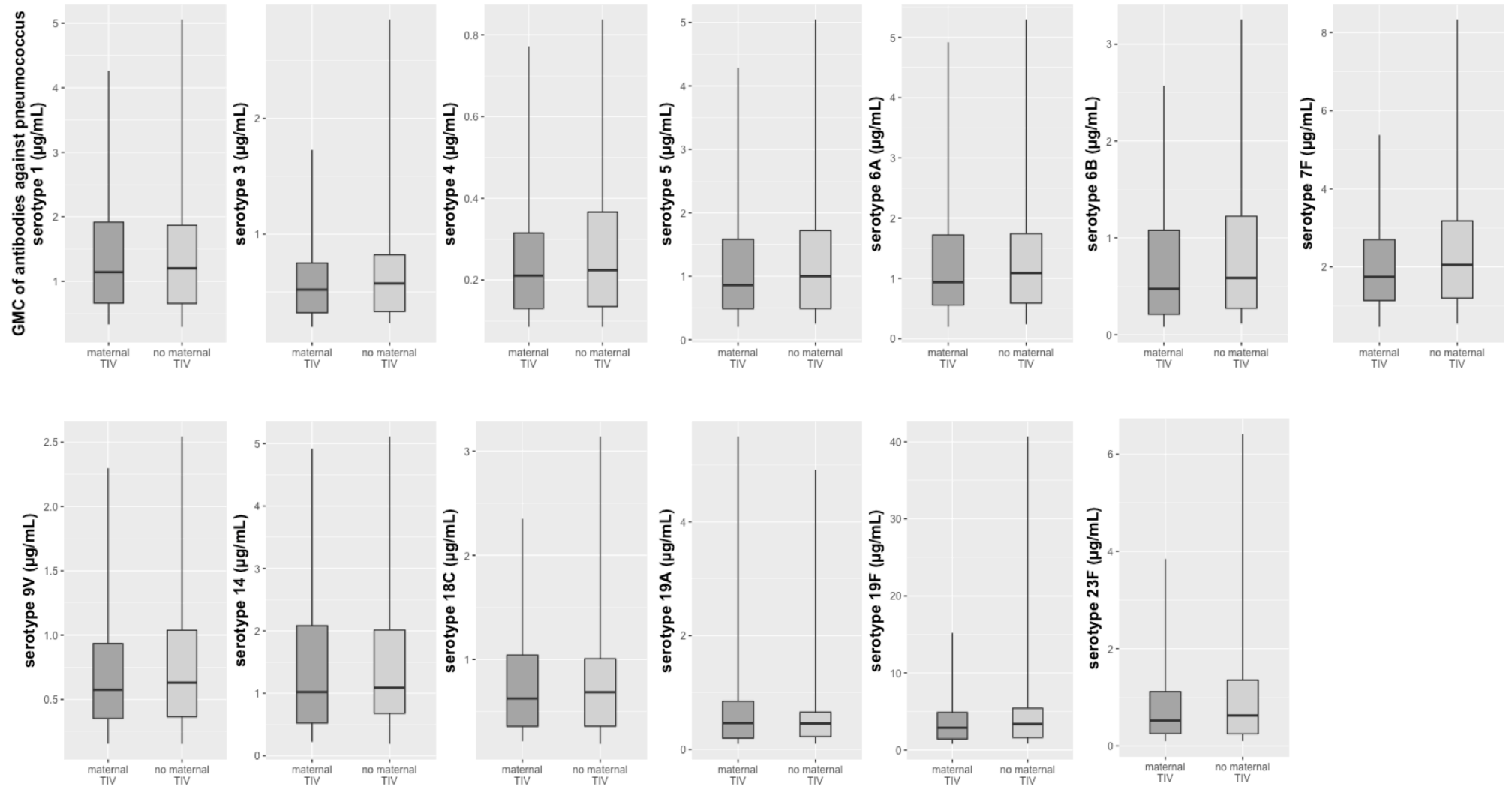
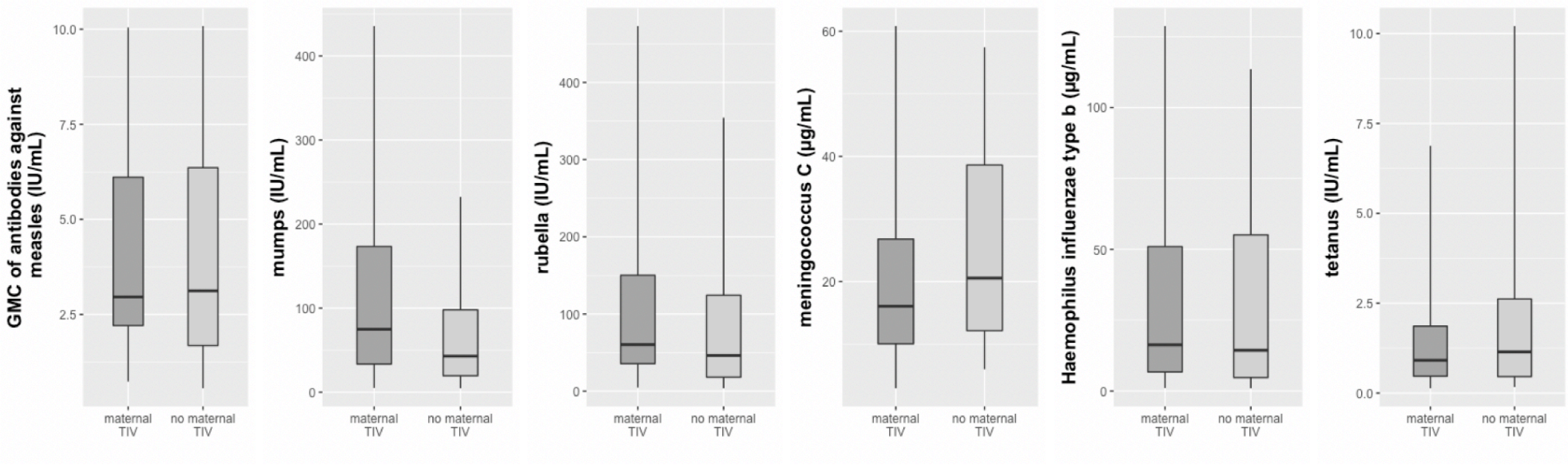


Figure 2.10 GMCs of antibodies against MMR, MenC and Hib at 13 months of age (antibodies against 12-months vaccines) in infants whose mothers did or did not receive TIV immunisation during pregnancy. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.



CHAPTER 3. THE INFLUENCE OF EARLY-LIFE FACTORS ON ANTIBODY RESPONSES TO ROUTINE IMMUNISATIONS DURING THE FIRST YEAR OF LIFE

3.1 INTRODUCTION

This chapter contains the published version of a study that investigated the effect of early-life factors, namely sex, delivery mode, breastfeeding and antibiotic exposure, on antibody responses to routine immunisations administered during the first year of life.

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REGULAR ARTICLE

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

Biological sex influences antibody responses to routine vaccinations in the first year of life

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Abstract

Aim: We investigated the effect of early-life factors, namely sex, delivery mode, feeding method and antibiotic exposure, on antibody responses to routine vaccinations administered during the first year of life.

Methods: One and seven months after the primary course of routine vaccines and 1 month after routine vaccines at 12 months of age, antibodies against 26 vaccine antigens were measured in 398 healthy infants. The geometric mean concentration (GMC) of antibodies (adjusted for effect modifiers with multiple linear regression) and the seroprotection rate for each vaccine were compared for each early-life factor.

Results: Sex had an influence on GMCs. Antibody concentrations were significantly lower at 7 months of age in females for tetanus and filamentous haemagglutinin and at 13 months of age for pertactin. In contrast, at 13 months of age, antibody concentrations were significantly higher in females for polio type 3, pneumococcal serotype 6A and measles. Sex did not have an influence on seroprotection rates. Delivery mode, feeding method and antibiotic exposure did not exert a substantial influence on vaccine antibody concentrations.

Conclusion: There is a difference between males and females in the humoral response to routine vaccinations in the first year of life.

KEYWORDS

antibodies, humoral, immunisation, immunoglobulin, infant, titre

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; DTPa, diphtheria-tetanus-acellular pertussis vaccine; FHA, filamentous haemagglutinin; GMC, geometric mean concentration; GMR, geometric mean ratio; HepA, hepatitis A; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; Ig, immunoglobulin; IPV, inactivated polio vaccine; IQR, interquartile range; MenC, meningococcus C; MIS BAIR, The Melbourne Infant Study: BCG for Allergy and Infection Reduction; ORV, oral rotavirus vaccine; PCV13, 13-valent conjugate pneumococcal vaccine; Pn, pneumococcal serotype; PRN, pertactin; PT, pertussis toxin.

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1 | INTRODUCTION

Vaccination is one of the most cost-effective, life-saving medical interventions. However, there is substantial variation between individuals in the magnitude of the immune response to vaccination, which might have implications for both protective efficacy and duration of protection. At the age of 6 months, for example, antibody responses to pneumococcal and *Haemophilus influenzae* type b (Hib) vaccines may vary up to 40-fold.[1]

A number of intrinsic host, perinatal and extrinsic factors are likely to contribute to the variation in vaccine responses.[2] One of the intrinsic factors that has been most investigated is biological sex. But studies to date have almost exclusively been in adults. Other intrinsic factors include polymorphisms in genes encoding major histocompatibility complexes or pattern recognition receptors, such as Toll-like or RIG-like receptors.[2] Perinatal factors that might influence vaccine responses include gestational age, birthweight [3,4] and feeding method (formula vs breastfed). Potential extrinsic factors include exposure to probiotics and antibiotics. While there is evidence for a beneficial effect of probiotics on vaccine responses,[2] to date, only one study has investigated the effect of antibiotics on vaccine responses in humans. This study reported that administration of antibiotics before vaccination did not alter serum immunoglobulin (Ig) A concentrations to oral rotavirus vaccine (ORV) in adults.[5]

Determining the influence of intrinsic and extrinsic factors on vaccine antibody responses has implications for optimising vaccine recommendations and individualising vaccine schedules.

In this study, we investigated the effect of biological sex, mode of delivery, feeding method and antibiotic exposure on antibody responses to routine vaccinations given in the first year of life.

Key notes

- There are substantial differences between individuals in the immune response to vaccination.
- In this study, we found that there are differences between males and females in antibody responses to routine vaccinations in the first year of life.
- In contrast, delivery mode, feeding method and antibiotic exposure did not affect antibody responses.

2 | METHODS

2.1 | Study design and population

Participants were a subset of 471 healthy infants from The Melbourne Infant Study: Bacille Calmette-Guérin (BCG) for Allergy and Infection Reduction (MIS BAIR) (http://misbair.org). In this trial, infants were recruited at birth from 2013 to 2016 and randomised to receive neonatal BCG vaccination or no intervention to investigate whether BCG protects from childhood allergies. Inclusion criteria included the following: >32 weeks' gestation and birthweight >1500 g. Details about feeding and antibiotic exposure were prospectively collected by parent questionnaire.

From the subset of participants whose parent/guardian provided consent, blood samples were collected at study visits at 7 and/or 13 months of age (designed to be 4 weeks after the administration of routine scheduled vaccinations). Only participants who had a blood taken 28 ± 14 days after their 6-month and/or 12-month routine vaccinations were included in the analysis of immediate post-vaccination responses. For those in whom persistence of antibodies to their 6-month vaccines was measured, blood was taken 7 months ± 23 days after their 6-month vaccinations.

2.2 | Infant vaccination

All infants received routine vaccinations according to the Australian National Immunisation Program: at birth: intramuscular hepatitis B (HepB) vaccine (H-B-Vax II Paediatric®; bioCSL); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa)-HepB-inactivated polio (IPV)-Hib vaccine (Infanrix® Hexa; GlaxoSmithKline), intramuscular conjugate pneumococcal vaccine (PCV13) (Prevenar13®; Wyeth) and ORV (RotaTeq®; Merck); and at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (Priorix®; GlaxoSmithKline) and intramuscular combined Hib and conjugated meningococcal C vaccine (Menitorix®; GlaxoSmithKline). Approximately half of the infants received intradermal BCG-Denmark (Statens Serum Institut, Copenhagen) shortly after birth as part of the MIS BAIR. Vaccine records were obtained from individual vaccination records and/or the Australian Immunisation Register.

2.3 | Blood collection and antibody assay

Following collection (S-monovette®; Sarstedt), plasma was stored at −80°C until analysis at the National Institute for Health and Environment, in Bilthoven, the Netherlands. IgG antibodies against 26 vaccine antigens (diphtheria, tetanus, pertussis [pertussis toxin [PT], filamentous haemagglutinin [FHA], pertactin [PRN]]), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), meningococcus type C (MenC), measles, mumps and rubella were measured using fluorescent bead-based multiplex immune assays (Luminex xMAP technology). In all assays, international or in-house reference controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories).

2.4 | Categorisation of participants

Infants were categorised by biological sex (male or female), delivery mode (vaginal vs caesarean section), feeding method and antibiotic

exposure using information collected from hospital records and parent questionnaires. Feeding method was categorised as follows: (a) infants who did or did not receive formula before leaving their birth hospital; (b) infants who were or were not still receiving breastmilk at 1 month of age; and (c) infants who were or were not still receiving breastmilk at 6 months of age. Antibiotic exposure was categorised as follows: (a) infants whose mother did or did not receive antibiotics during delivery and (b) infants who did or did not receive systemic antibiotics before their first routine vaccinations scheduled at the age of 6 weeks (assessed individually).

2.5 | Statistical analysis

In each group, the proportion of infants with an antibody concentration above the standard protective correlate value (seroprotection rate) was calculated for each vaccine.[6-8] The Clopper-Pearson method was used to estimate the 95% confidence intervals (CIs) of the seroprotection rates. The rates were compared between groups using Fisher's exact test and the 95% confidence interval (CI) for differences in proportions estimated. For FHA and PRN, no standard protective correlate value exists. In each group, the geometric mean concentration (GMC) for each vaccine antibody was calculated. The geometric mean ratio (GMR) with 95% confidence interval (CI) was obtained as the anti-logged coefficient from a linear regression with log-concentrations as outcome and early-life factors (biological sex, delivery mode, feeding choice and antibiotic exposure) as covariates (univariate regression). Effect modification was assessed using multiple linear regression with the following pre-specified factors: maternal age, maternal diphtheria-tetanus-pertussis (dTpa, Boostrix®; GlaxoSmithKline) and trivalent influenza vaccination in pregnancy, birthweight, gestational age, infant BCG and MMR vaccination status, age at first DTPa-HepB-IPV-Hib/ PCV13 vaccination, age at sampling and time interval between 6-month or 12-month vaccination and blood sampling. The GMR for each vaccine antigen was adjusted for those factors that had an effect on vaccine antigen response. As the Hib-MenC vaccine includes a tetanus toxoid (as carrier protein) and a Hib component, infants who had received this vaccine before blood sampling at 13 months of age were excluded from the analysis of persistence of antibodies against these two vaccine antigens. A 5% significance level was used. All statistical analyses were done using R version 3.4.3.

2.6 | Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation, 38124A).

3 | RESULTS

Of the 471 participants, seven were excluded because they were a twin of another participant, five because extended parental consent to use the blood samples was not available, four because they were not vaccinated according to the routine schedule and one because vaccine records were not available (Figure 1). Of the remaining 454 participants, 365 had blood taken in the predefined time frame. At 7 months of age, 91 were included in the final analysis. At 13 months of age, 307 were included for measurement of persistence of antibodies against the primary course of vaccines ending at 6 months and 141 for antibody responses to the 12-month vaccines. The background characteristics of the 365 included participants are summarised in Table 1. The median gestational age was 39.3 weeks (interquartile range [IQR] 38.4-40.4) and the median birthweight 3.45 kg (IQR 3.13-3.76).

Of the 365 infants, 179 (49%) were male, 137 (37%) were born by caesarean section, and 100 (28%) had received formula milk before discharge from their birth hospital. At 1 month of age, 302 (83%) infants were still receiving breastmilk, and at 6 months of age, this number was 263 (72%). In total, 70 (19%) mothers received antibiotics during delivery: 36 (51%) because they were colonised with group B streptococcus, 27 (39%) because of prolonged rupture of membranes, 5 (7%) because of caesarean section delivery and for 2 (3%) the reason was not specified. The administered antibiotics were benzylpenicillin (n = 30), amoxycillin (n = 14), ampicillin (n = 11), cefazolin (n = 5), cefotaxime (n = 1), metronidazole (n = 1) and not specified (n = 11).

Antibiotics were administered to 30 (8%) infants before their first routine vaccination schedule at 6 weeks of life. These infants received only one course of antibiotics each. The majority (n = 22, 73%) received antibiotics within the first week of life (intravenous benzylpenicillin and gentamicin [n = 18], intravenous benzylpenicillin, gentamicin and vancomycin [n = 1], intravenous antibiotics not documented [n = 1], oral cephalexin [n = 1] and oral flucloxacillin [n = 1]). The duration of administration varied between 2 and 7 days (mean 3, median 3). Of the remaining eight infants, four were admitted to hospital for intravenous antibiotics (azithromycin [n = 1] and antibiotics not documented [n = 3]) between 2 and 5 weeks of age (mean 3.5, median 3.5). The duration of administration was less than 5 days (n = 2 infants) and between 5 and 10 days (n = 2 infants). The infant who received intravenous azithromycin was continued on oral azithromycin for 3 days. Additionally, two infants were continued on oral antibiotics (not documented), one for 5 days and one for more than 10 days. There were four infants who received oral antibiotics after the first week of life (age range 2-4, mean 3.8, median 4 weeks) (amoxicillin [n = 2], cephalexin [n = 2]). The duration of administration was 5-10 days for all.

3.1 | Effect of sex on vaccine responses

At 7 months of age, females had a lower seroprotection rate against Hib compared with male infants (66% [95% CI 51.6%-79.6%] vs 86% [95% CI 72.1%-94.7%]; $P = .05$). However, overall there were no statistically significant differences between males and females in seroprotection rates at 7 or 13 months of age (Table 2a and Figure 2).

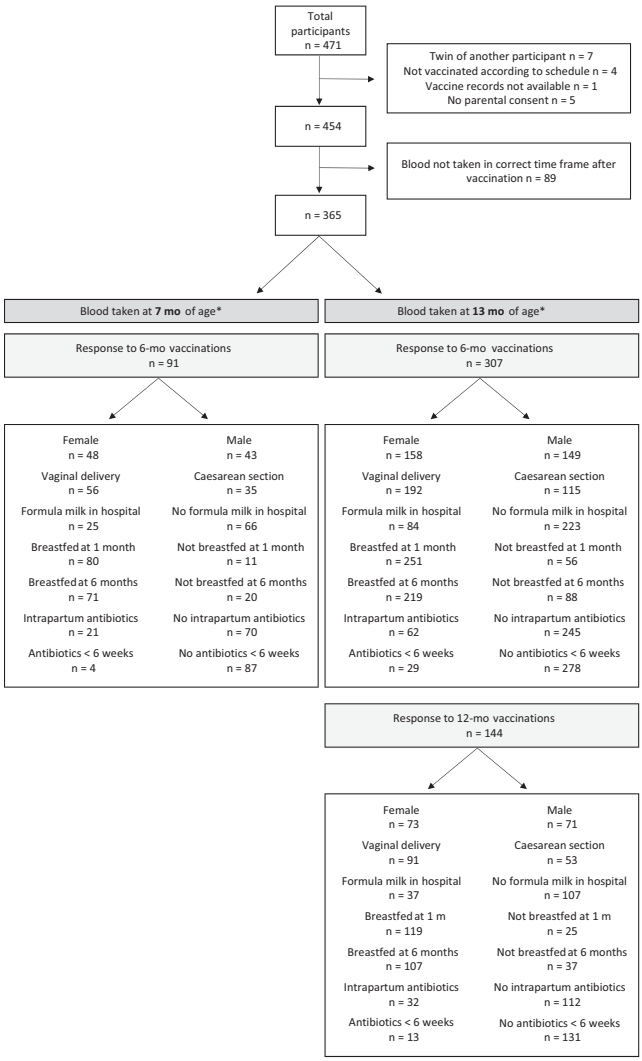


FIGURE 1 Selection of participants. *33 participants had their blood analysed at both 7 and 13 mo of age

After adjustment for pre-specified factors, the female:male GMR was below one (indicating lower average antibody concentrations) in females for tetanus and FHA (Table 2b and Figure 2) at 7 months of age and for PRN at 13 months of age. These findings were statistically significant. At 13 months of age, adjusted average antibody concentrations were statistically significantly higher in females for polio type 3, pneumococcus serotype 6A and measles (Table 2b and Figure 2).

3.2 | No effect of delivery mode on vaccine responses

At 7 months of age, seroprotection rates were lower in infants born vaginally than in those born by caesarean section for the majority of vaccine antigens. However, none of these differences reached statistical significance. At 13 months of age, there were no differences in seroprotection rates between infants born vaginally or by caesarean section (Table S1a and Figure S1). At 13 months of age, infants born vaginally had a higher average antibody concentration against Hib (Table S1b and Figure S1).

3.3 | No effect of feeding method on vaccine responses

At 7 and 13 months of age, there were no statistically significant differences in seroprotection rates or adjusted average antibody concentrations between infants who received or did not receive formula milk in first few days of life or between infants who were still receiving breastmilk at 1 or 6 months of age and those who were not (Tables S2a-S4b and Figures S2-S4). The only exception was a statistically significant higher adjusted average antibody concentration (ie, a GMR greater than one) against pneumococcus serotype 7F at 7 months of age in infants who did not receive formula milk during the first few days of life compared to those who did (Table S2b and Figure S2), and higher antibody concentration for pneumococcus serotype 19F and MenC at 13 months of age in infants who were still receiving breast at 6 months of age (Tables S4b and Figure S4).

3.4 | Minimal effect of intrapartum or infant antibiotic exposure on vaccine responses

At 7 and 13 months of age, there were no differences in seroprotection rates between infants whose mothers did or did not receive antibiotics during delivery (Table S5a and Figure S5). Infants whose mother received intrapartum antibiotics had a significantly higher adjusted average antibody concentration against PRN at 7 and 13 months of age (Tables S5b and Figure S5). Antibiotic exposure before their first routine vaccination scheduled at 6 weeks of age did not influence seroprotection rates or average antibody concentrations (Table S6a,b and Figure S6).

4 | DISCUSSION

This study is the first to investigate the effect of multiple different early-life factors on antibody responses to routine vaccinations in infancy. We found that infant biological sex had an impact on responses but delivery mode, feeding method, intrapartum antibiotics and antibiotic exposure before the first routine immunisation did not.

Our finding that females had lower antibody responses against tetanus and higher responses against polio (type 3) is consistent with previous studies in adults.[9-11] Previous studies have also reported female adults to have higher antibody responses to dengue, HepA, HepB, rabies, smallpox and trivalent influenza vaccines, while male adults have been reported to have higher responses to diphtheria and conjugated meningococcus A vaccines.[2] The only studies which have previously been done in infants reported higher antibody responses in females to HepB, Hib and PCV13.[2] Aside from a higher average antibody concentration in females against one pneumococcal serotype (6A), we did not find sex differences in the response to pneumococcal vaccination. This contrasts with studies in adults, in

TABLE 1 Characteristics of study participants

	Total cohort	Samples at 7 mo of age for antibodies	Samples at 13 mo of age for persistence of antibodies	Samples at 13 mo of age for antibodies
		to primary course of vaccines ending at 6 mo of age		to 12-mo vaccines
	(n = 365) n (%) or median (IQR)	(n = 91) n (%) or median (IQR)	(n = 307) n (% or IQR)	(n = 144) n (% or IQR)
Sex (male)	179 (49)	43 (47)	149 (49)	71 (49)
Gestational age (wk)	39.3 (38.4-40.4)	39.5 (38.6-40.5)	39.3 (38.4-40.3)	39.2 (38.4-40.4)
Birthweight (kg)	3.45 (3.13-3.76)	3.48 (3.10-3.78)	3.45 (3.13-3.76)	3.41 (3.13-3.67)
Caesarean section	136 (37)	35 (38)	115 (37)	53 (37)
Received formula milk in hospital in first week of life	100 (27)	25 (27)	84 (27)	37 (26)
Still receiving breast at 1 mo of age	302 (83)	80 (88)	251 (82)	119 (83)
Still receiving breast at 6 mo of age	263 (72)	71 (78)	219 (71)	107 (74)
Antibiotics during admission for delivery				
Intrapartum antibiotics	70 (19)	21 (23)	62 (20)	32 (22)
Antibiotics before first vaccination	30 (8)	4 (4)	29 (9)	13 (9)
BCG-vaccinated	198 (54)	45 (49)	166 (54)	76 (53)
Maternal dTpa vaccination in pregnancy	174 (48)	46 (51)	146 (48)	67 (47)
Maternal influenza vaccination in pregnancy	208 (57)	55 (60)	171 (56)	84 (58)
Age at routine vaccination (d)				
6-wk vaccines	45 (43-494)[a]	45 (43-48)[a]	45 (43-49)	45 (43-48)
4-mo vaccines	125 (120-131)	123 (117-127)	125 (121-132)	124 (120-131)
6-mo vaccines	190 (184-202)	187 (181-193)	191 (185-204)	190 (185-201)
12-mo vaccines	376 (369-383)	–	–	376 (369-383)
Interval between (d)				
6-mo vaccines and 7-mo blood sample	–	28 (21-37)	–	–
6-mo vaccines and 13-mo blood sample	–	–	209 (190-224)	–
12-mo vaccines and 13-mo blood sample	–	–	–	28 (22-36)
Age at blood sampling (d)	–	218 (208-227)	400 (389-418)	406 (397-414)

Abbreviations: BCG, Bacille Calmette-Guérin; dTpa, diphtheria-tetanus-acellular pertussis vaccine.
[a]Age for one participant not available.

TABLE 2 (a) Seroprotection rates at 7 and 13 mo of age in female and male infants. (b) Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) at 7 and 13 mo of age in female and male infants

(a)

Vaccine antigen	Protective correlate	Antibodies to primary course of vaccines ending at 6 mo of age measured at 7 mo of age				Antibodies to primary course of vaccines ending at 6 mo of age measured at 13 mo of age			
		Female (n = 48)	Male (n = 43)	Difference	Two-sided	Female (n = 158)	Male (n = 149)	Difference	Two-sided
		% (n); (95% CI)	% (n); (95% CI)	% (95% CI)	*P*-value	% (n); (95% CI)	% (n); (95% CI)	% (95% CI)	*P*-value
Diphtheria	0.1 IU/mL[a]	97.9 (47); (88.9, 99.9)	95.3 (41); (84.1, 99.4)	2.6 (−6.9, 13.7)	.60	98.7 (156); (95.5, 99.8)	98.0 (146); (94.2, 99.6)	0.7 (−2.7, 4.6)	.68
Tetanus	0.1 IU/mL[a]	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5, 8.3)	–	100 (85); (96.8, 100)[b]	100 (78); (95.4, 100)[b]	0 (−4.3, 4.7)[b]	–
PT	25 IU/mL	89.6 (43); (77.3, 96.5)	90.7 (39); (77.9, 97.4)	−1.1 (−14.4, 12.7)	1	28.5 (45); (21.6, 36.2)	31.5 (47); (24.2, 39.7)	−3.1 (−13.3, 7.2)	.62
Hib	0.15 µg/mL	66.7 (32); (51.6, 79.6)	86.0 (37); (72.1, 94.7)	−19.4 (−36.0, −1.7)	.05	80.0 (68) (69.9, 87.9)[b]	73.1 (57); (61.8, 82.5)[b]	6.9 (−6.1, 20.1)[b]	.36[b]
Polio type 1	0.23 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5, 8.3)	–	100 (158); (97.7, 100)	100 (149); (97.6, 100)	0 (−2.4, 2.5)	–
Polio type 2	0.29 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5, 8.3)	1	100 (158); (97.7, 100)	99.3 (148); (96.3, 100)	0.7 (−1.7, 3.7)	.49
Polio type 3	0.12 IU/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5; 8.3)	–	100 (158); (97.7, 100)	100 (149); (97.6, 100)	0 (−2.4, 2.5)	–
Pn 1	0.35 µg/mL	95.8 (46); (85.7, 99.5)	97.7 (42); (87.7, 99.9)	−1.8 (−12.1, 8.4)	1	92.4 (146); (87.1, 96.0)	95.3 (142); (90.6, 98.1)	−2.9 (−8.7, 2.7)	.35
Pn 3	0.35 µg/mL	97.9 (47); (88.9, 99.9)	100 (43); (91.8, 100)	−2.1 (−11.0, 6.3)	1	70.3 (111); (62.5, 77.3)	69.1 (103); (61.0, 76.4)	1.1 (−9.1, 11.4)	.90
Pn 4	0.35 µg/mL	66.7 (32); (51.6, 79.6)	72.1 (31); (56.3, 84.7)	−5.4 (−24.0, 13.8)	.65	24.1 (38); (17.6, 31.5)	21.5 (32); (15.2, 28.9)	2.6 (−6.9, 12.0)	.68
Pn 5	0.35 µg/mL	95.8 (46); (86.7, 99.5)	100 (43); (85.6, 99.5)	−4.2 (−14.1, 4.3)	.50	89.2 (141); (83.3, 93.6)	84.6 (126); (77.7, 90.0)	4.7 (−2.9, 12.5)	.24
Pn 6A	0.35 µg/mL	97.9 (47); (88.9, 99.9)	97.7 (42); (87.7, 99.9)	0.2 (−8.9, 10.3)	1	88.0 (139); (81.9, 92.6)	85.2 (127); (78.5, 90.5)	2.7 (−5.0, 10.6)	.50
Pn 6B	0.35 µg/mL	83.3 (40); (69.8, 92.5)	95.3 (41); (84.2, 99.4)	−12.0 (−25.8, 1.1)	.10	68.4 (108); (60.5, 75.5)	59.7 (89); (51.4, 67.7)	8.6 (−2.1, 19.2)	.12
Pn 7F	0.35 µg/mL	100 (48); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5, 8.3)	–	99.4 (157); (96.5, 100)	99.3 (148); (96.3, 100)	0 (−2.9, 3.1)	1
Pn 9V	0.35 µg/mL	97.9 (47); (88.9, 99.9)	97.7 (42); (87.7, 99.9)	0.2 (−8.9, 10.2)	1	79.1 (125); (71.9, 85.2)	72.5 (108); (64.6, 79.5)	6.6 (−3.0, 16.2)	.18
Pn 14	0.35 µg/mL	91.7 (44); (80.0, 97.7)	90.7 (39); (77.8, 97.4)	1.0 (−11.8, 14.6)	1	84.8 (134); (78.2, 90.0)	87.9 (131); (81.2, 92.3)	−3.1 (−10.9, 4.7)	.51
Pn 18C	0.35 µg/mL	95.8 (46); (85.7, 99.5)	95.3 (41); (84.2, 99.4)	0.5 (−10.0, 11.9)	1	78.5 (124); (71.2, 84.6)	75.2 (112); (67.4, 81.9)	3.3 (−6.2, 12.8)	.50
Pn 19A	0.35 µg/mL	93.8 (45); (82.8, 98.7)	93.0 (40); (80.9, 98.5)	0.7 (−11, 13.3)	1	47.5 (75); (39.5, 55.6)	52.3 (78); (44.0, 60.6)	−4.9 (−15.9, 6.3)	.42
Pn 19F	0.35 µg/mL	100 (46); (92.6, 100)	100 (43); (91.8, 100)	0 (−7.5; 8.3)	–	98.7 (156); (95.5, 99.8)	100 (149); (97.6, 100)	−1.3 (−4.5, 1.3)	.50
Pn 23F	0.35 µg/mL	91.7 (44); (80.0, 97.7)	95 (41); (84.2, 99.4)	−3.7 (−15.7, 8.3)	.68	62.7 (99); (54.6, 70.2)	59.7 (89); (51.4, 67.7)	2.9 (−8.0, 13.8)	.64

Vaccine antigen	Protective correlate	Antibodies to 12-mo vaccines measured 13 mo of age			
		Female (n = 73)	Male (n = 71)	Difference	Two-sided
		% (n); (95% CI)	% (n); (95% CI)	% (95% CI)	*P*-value
Measles	0.12 IU/mL	100 (73); (95.1, 100)	95.8 (68); (88.1, 99.1)	4.2 (−0.9, 11.7)	.12
Mumps	45 IU/mL	58.9 (43); (46.8, 70.3)	62.0 (44); (49.7, 73.2)	−3.1 (−18.8, 12.9)	.74
Rubella	10 IU/mL	91.8 (67); (83.0, 96.9)	81.7 (58); (70.7, 89.9)	10.1 (−1.1, 21.8)	.09
MenC	2 µg/mL	100 (73); (95.1, 100)	98.6 (70); (92.4, 100)	1.4 (−3.7, 7.6)	.49
Hib	0.15 µg/mL	98.6 (72); (92.6, 100)	98.6 (70); (92.5, 100)	0 (−6.1, 6.4)	1
Tetanus	0.01 IU/mL	100 (73); (95.1, 100)	100 (71); (94.3, 100)	0 (−5.0, 5.1)	–

(Continues)

TABLE 2 (Continued)

(b)

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 mo of age measured at 7 mo of age						Antibodies to primary course of vaccines ending at 6 mo of age measured at 13 mo of age					
	Female (n = 48) GMC (95% CI)	Male (n = 43) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided *P*-value	Adjusted[e] GMR (95% CI)	Two-sided *P*-value	Female (n = 158) GMC (95% CI)	Male (n = 149) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided *P*-value	Adjusted[f] GMR (95% CI)	Two-sided *P*-value
Diphtheria[c]	0.38 (0.30, 0.48)	0.41 (0.33, 0.52)	0.92 (0.67, 1.27)	.61	0.98 (0.72, 1.32)	.88	0.08 (0.07, 0.09)	0.08 (0.06, 0.09)	1.05 (0.83, 1.32)	.69	1.12 (0.91, 1.38)	.29
Tetanus[c]	0.98 (0.82, 1.18)	1.27 (1.01, 1.60)	0.77 (0.58, 1.03)	.08	**0.72 (0.54, 0.97)**	**.03**	0.41 (0.32, 0.52)[b]	0.48 (0.37, 0.61)[b]	0.85 (0.60, 1.22)[b]	.38[b]	0.78 (0.56, 1.10)[b]	.16
PT[c]	63.68 (51.21, 79.18)	77.99 (60.10, 101.22)	0.82 (0.58, 1.13)	.23	0.85 (0.62, 1.17)	.31	16.67 (14.26, 19.47)	18.19 (15.37, 21.54)	0.92 (0.73, 1.15)	.45	0.98 (0.80, 1.21)	.87
FHA[c]	**52.41 (42.42, 64.76)**	**79.28 (60.59, 103.73)**	**0.66 (0.47, 0.92)**	**.02**	**0.71 (0.51, 0.98)**	**.04**	**16.96 (14.50, 19.83)**	**21.17 (18.28, 24.52)**	**0.80 (0.65, 0.99)**	**.04**	0.85 (0.69, 1.04)	.11
PRN[c]	54.00 (42.26, 69.00)	68.66 (49.36, 95.50)	0.79 (0.53, 1.17)	.24	0.80 (0.53, 1.21)	.29	**10.13 (8.39, 12.24)**	**14.24 (11.73, 17.28)**	**0.71 (0.54, 0.93)**	**.02**	**0.73 (0.57, 0.95)**	**.02**
Hib[d]	0.40 (0.25, 0.64)	0.68 (0.44, 1.06)	0.58 (0.30, 1.11)	.10	0.66 (0.35, 1.26)	.21	1.5 (0.80, 2.51)[b]	0.82 (0.51, 1.33)[b]	1.82 (0.90, 3.70)[b]	.09[b]	1.51 (0.88, 2.61)[b]	.14[b]
Polio type 1[c]	33.75 (24.34, 46.81)	35.59 (26.50, 47.80)	0.95 (0.61, 1.47)	.81	1.04 (0.66, 1.65)	.86	11.07 (9.28, 13.19)	11.32 (9.41, 13.61)	0.98 (0.76, 1.26)	.86	1.03 (0.80, 1.32)	.82
Polio type 2[c]	65.36 (49.45, 86.40)	60.43 (42.14, 86.66)	1.08 (0.69, 1.69)	.73	1.14 (0.73, 1.78)	.57	22.73 (18.74, 27.57)	20.46 (16.54, 25.31)	1.11 (0.83, 1.48)	.47	1.18 (0.89, 1.56)	.25
Polio type 3[c]	23.43 (17.03, 32.24)	19.41 (13.69, 27.52)	1.21 (0.76, 1.92)	.42	1.24 (0.76, 2.01)	.38	10.17 (8.29, 12.48)	7.71 (6.25, 9,53)	1.32 (0.98, 1.77)	.06	**1.39 (1.04, 1.84)**	**.03**
Pn 1[d]	3.97 (2.86, 5.50)	4.87 (3.70, 6.40)	0.82 (0.53, 1.25)	.34	0.89 (0.59, 1.32)	.55	1.18 (1.03, 1.35)	1.15 (1.00, 1.33)	1.03 (0.85, 1.25)	.78	1.06 (0.87, 1.28)	.58
Pn 3[d]	1.37 (1.15, 1.64)	1.52 (1.21, 1.90)	0.90 (0.68, 1.20)	.48	0.91 (0.68, 1.21)	.51	0.57 (0.50, 0.65)	0.50 (0.44, 0.57)	1.14 (0.95, 1.37)	.15	1.17 (0.98, 1.39)	.09
Pn 4[d]	0.53 (0.41, 0.69)	0.50 (0.40, 0.63)	1.06 (0.75, 1.51)	.73	1.09 (0.77, 1.54)	.61	0.22 (0.20, 0.25)	0.21 (0.19, 0.23)	1.05 (0.90, 1.22)	.52	1.07 (0.92, 1.24)	.39
Pn 5[d]	2.91 (2.17, 3.89)	3.46 (2.71, 4.43)	0.84 (0.57, 1.23)	.36	0.92 (0.63, 1.33)	.65	0.96 (0.83, 1.12)	0.88 (0.76, 1.02)	1.09 (0.89, 1.34)	.41	1.14 (0.93, 1.39)	.22
Pn 6A[d]	4.82 (3.66, 6.34)	4.81 (3.81, 6.08)	1.00 (0.70, 1.44)	1.00	1.01 (0.72, 1.42)	.97	1.08 (0.93, 1.25)	0.93 (0.78, 1.10)	1.16 (0.93, 1.45)	.19	**1.24 (1.01, 1.53)**	**.04**
Pn 6B[d]	1.64 (1.04, 2.59)	2.27 (1.46, 3.54)	0.72 (0.38, 1.36)	.31	0.72 (0.38, 1.34)	.29	0.56 (0.47, 0.68)	0.47 (0.39, 0.57)	1.20 (0.93, 1.56)	.17	1.25 (0.97, 1.61)	.08
Pn 7F[d]	5.72 (4.51, 7.27)	6.62 (5.31, 8.26)	0.86 (0.63, 1.20)	.38	0.85 (0.62, 1.15)	.29	1.93 (1.70, 2.18)	1.83 (1.61, 2.07)	1.06 (0.89, 1.26)	.55	1.09 (0.93, 1.30)	.29
Pn 9V[d]	2.40 (1.83, 3.16)	2.52 (2.01, 3.16)	0.95 (0.67, 1.36)	.79	0.99 (0.71, 1.37)	.94	0.63 (0.55, 0.72)	0.57 (0.49, 0.65)	1.12 (0.92, 1.36)	.26	1.16 (0.96, 1.40)	.12
Pn 14[d]	2.54 (1.82, 3.56)	2.81 (1.86, 4.26)	0.90 (0.54, 1.53)	.70	0.88 (0.52, 1.51)	.65	1.09 (0.93, 1.29)	1.00 (0.85, 1.18)	1.09 (0.87, 1.37)	.46	1.13 (0.90, 1.43)	.28

(Continues)

TABLE 2 (Continued)

(b)

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 mo of age measured at 7 mo of age						Antibodies to primary course of vaccines ending at 6 mo of age measured at 13 mo of age					
	Female (n = 48) GMC (95% CI)	Male (n = 43) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided *P*-value	Adjusted[e] GMR (95% CI)	Two-sided *P*-value	Female (n = 158) GMC (95% CI)	Male (n = 149) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided *P*-value	Adjusted[f] GMR (95% CI)	Two-sided *P*-value
Pn 18C[d]	2.68 (1.96, 3.65)	3.27 (2.59, 4.13)	0.82 (0.55, 1.21)	.31	0.83 (0.56, 1.22)	.35	0.69 (0.60, 0.78)	0.62 (0.54, 0.71)	1.11 (0.92, 1.34)	.28	1.16 (0.97, 1.40)	.10
Pn 19A[d]	1.77 (1.34, 2.34)	1.67 (1.25, 2.22)	1.06 (0.71, 1.58)	.77	1.14 (0.76, 1.69)	.52	0.48 (0.38, 0.60)	0.45 (0.37, 0.54)	1.06 (0.79, 1.42)	.70	1.10 (0.83, 1.47)	.51
Pn 19F[d]	10.20 (7.71, 13.50)	13.22 (10.40, 16.79)	0.77 (0.53, 1.11)	.17	0.80 (0.56, 1.15)	.22	3.21 (2.72, 3.79)	3.00 (2.52, 3.58)	1.07 (0.84, 1.36)	.58	1.11 (0.87, 1.40)	.40
Pn 23F[d]	2.27 (1.64, 3.15)	2.41 (1.69, 3.44)	0.94 (0.58, 1.52)	.81	0.96 (0.60, 1.52)	.85	0.63 (0.52, 0.77)	0.50 (0.41, 0.61)	1.26 (0.96, 1.66)	.10	1.27 (0.97, 1.68)	.08

Vaccine antigen	Antibodies to 12-mo vaccines measured 13 mo of age					
	Female (n = 73) GMC (95% CI)	Male (n = 71) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided *P*-value	Adjusted[e] GMR (95% CI)	Two-sided *P*-value
Measles[c]	**3.66 (3.05-4.40)**	**2.49 (1.83-3.41)**	**1.47 (1.03, 2.10)**	**.03**	**1.45 (1.01, 2.09)**	**.04**
Mumps[c]	58.93 (43.31, 80.20)	57.40 (42.07, 78.32)	1.03 (0.67, 1.58)	.90	0.94 (0.65, 1.35)	.73
Rubella[c]	60.78 (44.83, 82.40)	45.04 (31.74, 63.92)	1.35 (0.85, 2.14)	.20	1.25 (0.86, 1.81)	.25
MenC[d]	19.47 (16.47, 23.00)	15.89 (12.84, 19.66)	1.23 (0.94, 1.60)	.14	1.27 (0.97, 1.65)	.08
Hib[d]	18.21 (13.08, 25.35)	13.01 (8.74, 19.38)	1.40 (0.84, 2.33)	.20	1.53 (0.93, 2.53)	.10
Tetanus[c]	0.83 (0.64, 1.08)	1.19 (0.88, 1.60)	0.70 (0.47, 1.04)	.07	0.73 (0.49, 1.08)	.12

Bold denotes results with *P*-value < .05.

Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; FHA, filamentous haemagglutinin; GMC, geometric mean antibody concentration; GMR, geometric mean antibody ratio; Hib, *Haemophilus influenzae* type b; MenC, meningococcus C; MMR, measles-mumps-rubella; Pn, pneumococcus serotype; PRN, pertactin; PT, pertussis toxin.

[a]0.01 IU/mL at 13 mo of age.

[b]Includes only participants who have not had Hib-MenC.

[c]IU/mL.

[d]µg/mL.

[e]Adjusted for maternal dTpa (diphtheria-tetanus-aceulluar pertussis) and trivalent influenza vaccine during pregnancy, gestational age, BCG vaccination status, age at first DTPa-HepB-IPV-Hib/ PCV13 vaccination, age at sampling and time between vaccination and sampling using multiple linear regression.

[f]Adjusted for maternal dTpa (diphtheria-tetanus-aceulluar pertussis) and trivalent influenza vaccine during pregnancy, gestational age, BCG and MMR vaccination status, age at first DTPa-HepB-IPV-Hib/ PCV13 vaccination, age at sampling and time between vaccination and sampling using multiple linear regression.

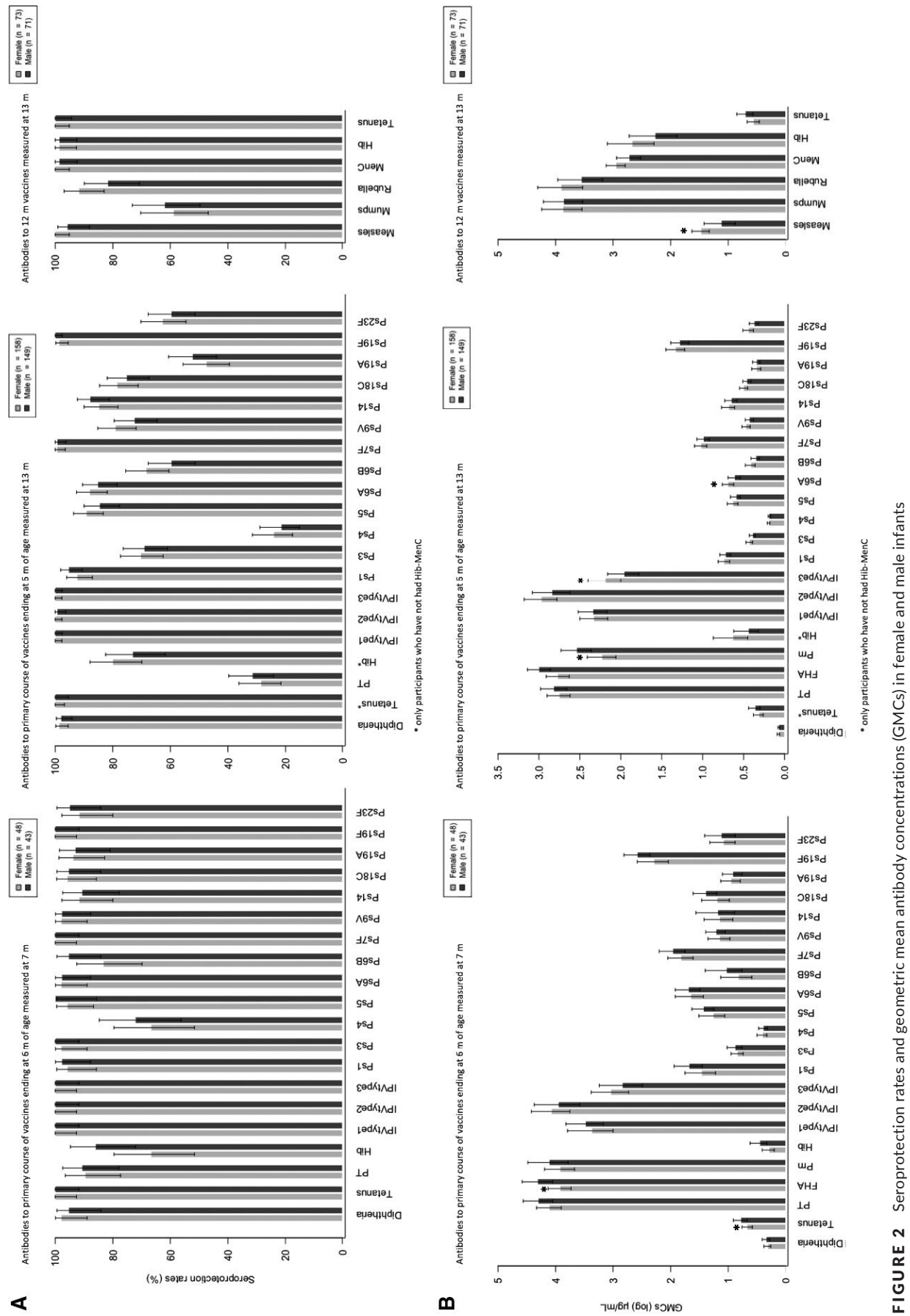


FIGURE 2 Seroprotection rates and geometric mean antibody concentrations (GMCs) in female and male infants

which males have generally been reported to have higher antibody responses to pneumococcal vaccines.[2]

In our study, we found that female infants had higher antibody levels against measles at 13 months of age than corresponding males. Previous studies investigating sex differences in responses to MMR vaccination report inconsistent findings. Some studies found higher antibody responses to measles, mumps and rubella vaccination in females, while others found transiently lower GMCs to rubella and lower seroconversion rates to measles vaccination in females.[2] Other studies did not find any sex differences in antibody responses to mumps and rubella, rubella vaccination. Notably, even though females are often reported to have higher antibody responses, faster waning of antibodies has been observed for antibodies against HepA and pneumococcus in females.[2]

One mechanism through which sex can affect vaccine responses is through hormones: oestradiol, progesterone and testosterone all influence the function of immune cells.[12] However, as sex differences in vaccine responses also occur before puberty and after menopause, other factors might also be involved. Many genes for proteins involved in the immune responses are encoded on the X chromosome, and mutations and polymorphisms of X-linked genes therefore have a greater impact in males.[13] A further mechanism through which sex differences in vaccine responses might occur is through epigenetic programming, which is also influenced by sex hormones.[12]

The microbiota, especially the intestinal microbiota, plays a crucial role in the development and regulation of the immune system, and its composition affects how individuals respond to vaccinations.[14] Sex hormones play a key role in bacterial-host interactions,[15] and sex differences in the microbiota might therefore influence immune responses.[16,17] As delivery mode and feeding method strongly affect the composition of the infant microbiota,[18] these factors might be expected to influence vaccine responses. However, in our study, we did not observe any significant impact of delivery mode or feeding method on vaccines responses.

Maternal antibodies may inhibit infant antibody responses to some vaccines.[19] Although the majority of maternal antibody transfer is through the placenta, breastmilk also contains antibodies that might interfere with immune responses. Small studies have reported that after routine vaccination, breastfed infants have higher serum IgG levels to diphtheria,[20] Hib[21,22] and oral polio virus (OPV)[20,23] vaccination, and higher salivary IgA levels to tetanus, diphtheria and OPV vaccination.[20] For ORV, reduced seroconversion rates were observed in breastfed infants compared with formula-fed infants[24,25]; however, this was not confirmed in subsequent studies.[26] Infants with mixed feeding are sometimes classified as breastfed and sometimes as non-breastfed[26] and IgA levels, as well as the neutralising activity of IgA in breastmilk, may depend on geographic location.[27] These factors could explain some of the inconsistency in results from different studies.

The strengths of our study include the ability to adjust for a large number of possible confounding factors, the standardisation in the vaccines given and the wide range of measured antibody responses. The limitations of the study are the sample size and risk of type-2 error. Furthermore, we did not analyse antibody responses to the HepB vaccine, as this antigen was not included in our multiplex assay and we had insufficient sample volume for alternative assays. We also did not evaluate the effect of antibiotics that might have been transferred through breastmilk on infant vaccine responses.

As most vaccines induce antibody concentrations above the protective threshold in the majority of infants, small differences in antibody concentrations between groups of individuals are unlikely to be clinically significant. However, differences in antibody concentrations might be relevant for the duration of protection. Nonetheless, when developing new vaccines and designing vaccine schedules, recognising factors which might influence vaccine antibody responses offers ways to optimise vaccine immunogenicity and efficacy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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3.3 ADDITIONAL INFORMATION

Table 3.1 Seroprotection rates at 7 and 13 months of age in infants born vaginally or by Caesarean section

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Vaginal delivery** (n=56) **% (n); (95% CI)**	**Caesarean section** (n=35) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Vaginal delivery** (n=192) **% (n); (95% CI)**	**Caesarean section** (n=115) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	94.6 (53); (85.1, 98.8)	100 (35); (90.0, 100)	-5.4 (-14.6, 4.8)	0.28	97.9 (188); (94.8, 99.4)	99.1 (114); (95.3, 100)	-1.2 (-4.5, 2.8)	0.65
Tetanus	0.1 IU/mL[1]	100 (56); (93.6, 100)	100 (35); (90.0, 100)	0 (-6.5, 10)	-	100 (101); (96.4, 100)[2]	100 (62); (94.2, 100)[2]	0 (-3.7, 5.9[2]	-
PT	25 IU/mL	85.7 (48); (73.8, 93.6)	97.1 (34); (85.1, 100)	-11.4 (-23.4, 1.7)	0.15	29.2 (56); (22.8, 36.1)	31.3 (36); (22.9, 40.6)	-2.1 (-13.0, 8.2)	0.70
Hib	0.15 µg/mL	73.2 (41); (59.7, 84.2)	80 (28); (63.1, 91.6)	-6.8 (-23.6, 12.2)	0.62	84.2 (85); (75.6, 90.7)[2]	64.5 (40); (51.3, 76.3)[2]	19.6 (6.1, 33.7)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (56); (93.6, 100)	100 (35); (90.0, 100)	0 (-6.5, 10)	-	100 (192); (98.1, 100)	100 (115); (96.8, 100)	0 (-2.0, 3.2)	-
Polio type 2	0.29 IU/mL	100 (56); (93.6, 100)	100 (35); (90.0, 100)	0 (-6.5, 10)	-	99.5 (191); (97.1, 100)	100 (115) ; (96.8, 100)	-0.5 (-2.9, 2.7)	1
Polio type 3	0.12 IU/mL	100 (56); (93.6, 100)	100 (35); (90.0, 100)	0 (-6.5, 10)	-	100 (187); (98.1, 100)	100 (115) ; (96.8, 100)	0 (-2.0, 3.2)	-
Pn 1	0.35 µg/mL	94.6 (53); (85.1, 98.9)	100 (35); (90.0, 100)	-5.4 (-14.6, 4.8)	0.28	95.3 (183); (91.3, 97.8)	91.3 (105); (84.6, 96.8)	4 (-1.5, 11)	0.22
Pn 3	0.35 µg/mL	98.2 (55); (90.4, 100)	100 (35); (90.0, 100)	-1.8 (-9.5, 8.3)	1	68.8 (132); (61.7, 75.2)	71.3 (82); (62.1, 79.4)	-2.6 (-12.8, 8.3)	0.70
Pn 4	0.35 µg/mL	67.9 (38); (54.0, 79.7)	71.4 (25); (53.7, 85.4)	-3.6 (-22.0, 16.5)	0.82	22.9 (44); (17.1, 29.5)	22.6 (22.6); (15.2, 31.3)	-0.3 (-9.8, 9.7)	1
Pn 5	0.35 µg/mL	98.2 (55); (90.4, 100)	97.1 (34); (85.1, 100)	1.1 (-7.0, 13.0)	1	88.5 (170); (83.2, 92.7)	84.3 (97); (76.4, 90.5)	4.2 (-3.4, 12.9)	0.30
Pn 6A	0.35 µg/mL	96.4 (54); (87.7, 99.6)	100 (35); (90.0, 100)	-3.6 (-12.1, 6.6)	0.52	89.1 (171); (83.8, 93.1)	82.6 (95); (74.4, 89.0)	4.2 (-1.4, 15.3)	0.12
Pn 6B	0.35 µg/mL	83.9 (47); (71.7, 92.4)	97.1 (34); (85.1, 100)	-13.2 (-26, 0.1)	0.08	64.6 (124); (57.4, 71.3)	63.5 (73); (54.0, 72.3)	1.11 (-9.8, 12.3)	0.90
Pn 7F	0.35 µg/mL	100 (53); (93.6, 100)	100 (35), (90.0, 100)	0 (-6.5, 10)	-	100 (192); (98.1, 100)	98.3 (113); (98, 100)	1.74 (-0.3, 6.1)	0.14
Pn 9V	0.35 µg/mL	96.4 (54); (87.7, 99.6)	100 (35); (90.0, 100)	-3.6 (-12.1, 6.6)	0.52	78.6 (151); (72.2, 84.2)	71.3 (82); (62.1, 79.4)	7.3 (-2.5, 17.8)	0.17
Pn 14	0.35 µg/mL	91.1 (51); (80.4, 97.0)	91.4 (32); (76.9, 98.2)	-0.4 (-12.4, 14.6)	1	85.4 (164); (79.6, 90.1)	87.8 (101); (80.4, 93.2)	-2.4 (-10.0, 6.0)	0.61
Pn 18C	0.35 µg/mL	92.9 (52); (82.7, 98.0)	100 (35); (90.0, 100)	-7.1 (-17, 3.1)	0.29	78.1 (150); (71.6, 84.8)	74.8 (86); (65.8, 82.4)	3.3 (-6.2, 13.5)	0.57
Pn 19A	0.35 µg/mL	89.3 (50); (78.1, 96.0)	100 (35); (90.0, 100)	-10.7 (-21.5, 3.1)	0.08	50.5 (97); (43.2, 57.8)	48.7 (56); (39.3, 58.2)	1.8 (-9.7, 13.3)	0.81
Pn 19F	0.35 µg/mL	100 (56); (93.6, 100)	100 (35); (90.0, 100)	0 (-6.4, 10)	-	99.0 (190); (96.3, 99.9)	100 (115); (96.8, 100)	-1.0 (-3.7, 2.2)	0.53
Pn 23F	0.35 µg/mL	92.9 (52); (82.7; 98.0)	94.2 (33); (80.8, 99.3)	-1.4 (-12.4, 12.3)	1	61.5 (118); (54.2, 68.4)	60.9 (70); (51.3, 69.8)	0.6 (-10.5, 11.9)	1
						Antibodies to 12-month vaccines measured 13 months of age			
						Vaginal delivery (n=91) **% (n); (95% CI)**	**Caesarean section** (n=53) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					96.7 (88); (90.7, 99.3)	100 (53); (93.3, 100)	-3.3 (-9.2, 3.6)	0.12
Mumps	45 IU/mL					65.9 (60); (55.3, 75.6)	50.9 (27); (36.8, 64.9)	15.0 (-1.6, 31.2)	0.74
Rubella	10 IU/mL					87.9 (80); (79.4, 93.8)	84.9 (45); (72.4, 93.3)	3.0 (-8.1, 16.2)	0.09
MenC	2 µg/mL					100 (91); (96.0, 100)	98.1 (52), (90.0, 100)	1.9 (-2.2, 10.0)	0.49
Hib	0.15 µg/mL					98.9 (90); (94.0, 100)	98.1 (52); (90.0, 100)	0.8 (-4.3, 9.0)	1
Tetanus	0.01 IU/mL					100 (91); (96.0, 100)	100 (53); (93.3, 100)	0 (-4.1, 6.8)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.2 GMCs and GMRs at 7 and 13 months of age in infants born vaginally or by Caesarean section

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**Vaginal delivery** (n=56) **GMC (95% CI)**	**Caesarean section** (n=35) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**	**Vaginal delivery** (n=192) **GMC (95% CI)**	**Caesarean section** (n=115) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted** GMR (95% CI)**	**Two-sided p-value**
Diphtheria[1]	0.36 (0.29, 0.45)	0.46 (0.36, 0.57)	0.79 (0.57, 1.10)	0.17	0.89 (0.65, 1.23)	0.49	0.08 (0.07, 0.09)	0.08 (0.06, 0.10)	0.97 (0.77, 1.23)	0.82	1.02 (0.81, 1.27)	0.89
Tetanus[1]	1.12 (0.93, 1.32)	1.11 (0.86, 1.44)	0.99 (0.74, 1.35)	0.99	0.88 (0.65, 1.19)	0.40	0.46 (0.36, 0.58)[3]	0.41 (0.31, 0.55)[3]	1.12 (0.77, 1.61)[3]	0.55[3]	1.07 (0.75, 1.54)[3]	0.69[3]
PT[1]	63.11 (50.69, 78.58)	82.87 (64.07, 107.19)	0.76 (0.54, 1.07)	0.11	0.90 (0.64, 1.26)	0.52	17.68 (15.43, 20.25)	16.92 (13.77, 20.78)	1.04 (0.83, 1.32)	0.72	1.10 (0.87, 1.38)	0.42
FHA[1]	58.30 (47.14, 72.10)	73.50 (54.72, 98.71)	0.79 (0.56, 1.13)	0.19	0.87 (0.61, 1.23)	0.41	18.57 (16.37, 21.06)	19.43 (15.93, 23.69)	0.96 (0.77, 1.19)	0.69	1.03 (0.82, 1.28)	0.82
PRN[1]	59.27 (45.42, 77.33)	62.49 (45.56, 85.72)	0.95 (0.63, 1.43)	0.80	1.13 (0.73, 1.75)	0.58	11.48 (9.69, 13.61)	12.78 (10.17, 16.06)	0.90 (0.68, 1.19)	0.45	0.86 (0.66, 1.14)	0.30
Hib[2]	0.53 (0.34, 0.81)	0.49 (0.29, 0.82)	1.07 (0.55, 2.11)	0.84	0.90 (0.46, 1.76)	0.75	**1.49 (0.97, 2.30)**[3]	**0.71 (0.39, 1.30)**[3]	**2.10 (1.02, 4.33)**[3]	**0.04**[3]	**1.89 (1.07, 3.36)**[3]	**0.03**[3]
Polio type 1[1]	34.21 (24.98, 46.84)	35.26 (26.68, 46.61)	0.97 (0.62, 1.52)	0.89	1.09 (0.67, 1.77)	0.73	10.43 (8.91, 12.19)	12.58 (10.14, 15.61)	0.83 (0.64, 1.08)	0.16	0.88 (0.68, 1.16)	0.36
Polio type 2[1]	64.53 (47.27, 88.09)	60.59 (44.67, 82.19)	1.06 (0.67, 1.68)	0.79	1.20 (0.75, 1.93)	0.45	21.51 (18.12, 25.53)	21.76 (16.86, 28.07)	0.99 (0.74, 1.33)	0.94	1.03 (0.76, 1.39)	0.85
Polio type 3[1]	22.28 (16.16, 30.72)	20.15 (14.41, 28.17)	1.11 (0.68, 1.79)	0.68	1.12 (0.67, 1.88)	0.66	8.86 (7.40, 10.62)	8.95 (6.94, 11.53)	0.99 (0.73, 1.34)	0.95	0.98 (0.72, 1.33)	0.90
Pn 1[2]	4.06 (2.99, 5.52)	4.91 (3.75, 6.42)	0.83 (0.53, 1.28)	0.39	0.85 (0.56, 1.31)	0.46	1.25 (1.10, 1.43)	1.04 (0.90, 1.19)	1.21 (0.99, 1.48)	0.06	1.23 (1.00, 1.51)	0.05
Pn 3[2]	1.39 (1.14, 1.70)	1.51 (1.25, 1.82)	0.92 (0.69, 1.23)	0.57	0.90 (0.66, 1.23)	0.52	0.56 (0.50, 0.64)	0.50 (0.44, 0.56)	1.14 (0.94, 1.37)	0.18	1.16 (0.96, 1.41)	0.12
Pn 4[2]	0.49 (0.38, 0.62)	0.55 (0.42, 0.72)	0.89 (0.62, 1.28)	0.53	0.86 (0.60, 1.24)	0.42	0.22 (0.20, 0.24)	0.21 (0.18, 0.23)	1.07 (0.91, 1.25)	0.43	1.05 (0.89, 1.23)	0.55
Pn 5[2]	3.01 (2.33, 3.89)	3.41 (2.56, 4.55)	0.88 (0.60, 1.31)	0.53	0.94 (0.63, 1.40)	0.76	0.96 (0.84, 1.10)	0.85 (0.73, 1.00)	1.13 (0.91, 1.40)	0.27	1.17 (0.94, 1.46)	0.15
Pn 6A[2]	4.47 (3.48, 5.74)	5.42 (4.23, 6.95)	0.83 (0.57, 1.19)	0.30	0.90 (0.63, 1.30)	0.58	1.04 (0.89, 1.20)	0.95 (0.80, 1.13)	1.09 (0.87, 1.38)	0.46	1.15 (0.91, 1.44)	0.23
Pn 6B[2]	1.54 (1.00, 2.37)	2.72 (1.75, 4.22)	0.57 (0.30, 1.07)	0.08	0.59 (0.30, 1.13)	0.11	0.54 (0.46, 0.65)	0.48 (0.39, 0.58)	1.14 (0.87, 1.50)	0.34	1.18 (0.90, 1.56)	0.23
Pn 7F[2]	6.16 (4.95, 7.67)	6.08 (4.76, 7.78)	1.01 (0.73, 1.41)	0.94	1.07 (0.77, 1.45)	0.69	1.96 (1.76, 2.18)	1.75 (1.51, 2.03)	1.12 (0.93, 1.34)	0.22	1.17 (0.98, 1.40)	0.09
Pn 9V[2]	2.30 (1.79, 2.95)	2.74 (2.18, 3.45)	0.84 (0.58, 1.20)	0.33	0.89 (0.63, 1.25)	0.50	0.61 (0.54, 0.69)	0.58 (0.49, 0.68)	1.07 (0.87, 1.30)	0.54	1.11 (0.90, 1.36)	0.32
Pn 14[2]	2.53 (1.79, 3.57)	2.91 (1.92, 4.39)	0.87 (0.51, 1.49)	0.61	0.96 (0.54, 1.70)	0.90	1.00 (0.86, 1.17)	1.12 (0.94, 1.34)	0.89 (0.71, 1.13)	0.35	0.93 (0.73, 1.19)	0.56
Pn 18C[2]	2.68 (2.04, 3.53)	3.40 (2.61, 4.43)	0.79 (0.53, 1.18)	0.24	0.78 (0.51, 1.18)	0.24	0.69 (0.60, 0.78)	0.60 (0.52, 0.69)	1.15 (0.95, 1.40)	0.16	1.22 (1.00, 1.48)	0.05
Pn 19A[2]	1.62 (1.25, 2.10)	1.89 (1.38, 2.60)	0.85 (0.57, 1.28)	0.44	0.97 (0.64, 1.48)	0.90	0.47 (0.39, 0.57)	0.45 (0.35, 0.57)	1.06 (0.78, 1.44)	0.69	1.12 (0.82, 1.54)	0.48
Pn 19F[2]	11.38 (8.82, 14.68)	11.77 (9.00, 15.41)	0.97 (0.66, 1.41)	0.86	1.06 (0.72, 1.54)	0.78	3.19 (2.74, 3.72)	2.96 (2.43, 3.61)	1.08 (0.84, 1.38)	0.56	1.12 (0.86, 1.44)	0.40
Pn 23F[2]	2.10 (1.51, 2.94)	2.77 (2.02, 3.79)	0.76 (0.47, 1.24)	0.27	0.83 (0.51, 1.35)	0.45	0.59 (0.49, 0.70)	0.53 (0.42, 0.67)	1.10 (0.83, 1.47)	0.50	1.05 (0.78, 1.42)	0.72
							Antibodies to 12-month vaccines measured 13 months of age					
							Vaginal delivery (n=91) **GMC (95% CI)**	**Caesarean section** (n=53) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**
Measles[1]							3.03 (2.35, 3.92)	3.03 (2.42, 3.79)	1.00 (0.69, 1.45)	1.00	1.03 (0.69, 1.52)	0.90
Mumps[1]							64.97 (48.81, 86.49)	48.11 (34.69, 66.74)	1.35 (0.86, 2.11)	0.19	1.07 (0.72, 1.58)	0.75
Rubella[1]							57.83 (43.24, 77.33)	44.31 (30.18, 65.07)	1.36 (0.84, 2.30)	0.22	1.07 (0.71, 1.60)	0.75
MenC[2]							18.66 (15.64, 22.27)	15.94 (12.96, 19.60)	1.17 (0.88, 1.55)	0.26	1.31 (0.99, 1.75)	0.06
Hib[2]							15.55 (11.29, 21.41)	15.23 (9.77, 23.73)	1.02 (0.60, 1.74)	0.94	0.97 (0.56, 1.67)	0.90
Tetanus[1]							1.06 (0.82, 1.37)	0.89 (0.65, 1.22)	1.19 (0.79, 1.79)	0.41	1.20 (0.78, 1.85)	0.41

[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Table 3.3 Seroprotection rates at 7 and 13 months of age in infants who did or did not receive formula milk before discharge from birth hospital

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**No formula milk** (n=66) **% (n); (95% CI)**	**Formula milk** (n=25) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**No formula milk** (n=223) **% (n); (95% CI)**	**Formula milk** (n=84) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	95.5 (63); (87.3, 99.1)	100 (25); (86.3, 100)	2.3 (-12.5, 9.1)	0.60	97.8 (218); (94.8, 99.3)	100 (84); (95.7, 100)	-2.24 (-5.2, 2.1)	0.68
Tetanus	0.1 IU/mL[1]	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.6, 13.4)	-	100 (114); (96.8, 100)[2]	100 (49); (92.7, 100)[2]	0 (-3.3, 7.3)[2]	-
PT	25 IU/mL	87.9 (58); (77.5, 94.6)	96.0 (24); (79.6, 99.9)	-8.1 (-19.2, 8.4)	1	28.7 (64); (22.9, 35.1)	33.3 (28); (23.4, 44.5)	-4.6 (-16.7, 6.5)	0.62
Hib	0.15 µg/mL	74.2 (49); (62.0, 84.2)	80.0 (20); (59.3, 93.2)	-5.8 (-22.2, 15.7)	0.05	76.3 (87); (67.4, 83.8)[2]	77.6 (38); (63.4, 88.2)[2]	-1.2 (14.2, 14.0)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.6, 13.4)	-	100 (223); (98.4, 100)	100 (84); (95.7, 100)	0 (-1.7, 4.3)	-
Polio type 2	0.29 IU/mL	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.6, 13.4)	-	100 (223); (98.4, 100)	100 (84); (95.7, 100)	-0.4 (-2.5, 3.9)	0.49
Polio type 3	0.12 IU/mL	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.4, 17.0)	-	100 (211); (98.3, 100)	100 (84); (95.7, 100)	0 (-1.7, 4.3)	-
Pn 1	0.35 µg/mL	95.5 (63); (87.3, 99.1)	100 (25); (86.3, 100)	-4.5 (-12.6, 9.1)	1	93.7 (209); (89.7, 96.6)	94.0 (79); (86.7, 98.0)	-0.3 (-5.7, 7.3)	0.35
Pn 3	0.35 µg/mL	98.4 (65); (91.2, 100)	100 (25); (86.3, 100)	-1.5 (-0.9, 12.0)	1	68.6 (153); (62.1, 74.6)	72.6 (61); (61.8, 81.8)	-4.0 (-14.7, 7.9)	0.90
Pn 4	0.35 µg/mL	74.2 (49); (62.0, 84.2)	56.0 (14); (34.9, 76.6)	18.2 (-2.9, 39.9)	0.65	22.9 (51); (17.5, 28.9)	22.6 (19); (14.2, 33.0)	0.3 (-11-0, 10.0)	0.68
Pn 5	0.35 µg/mL	98.5 (65); (91.8, 100)	96.0 (24); (79.6, 99.9)	2.5 (-4.9, 18.3)	0.50	86.5 (193); (81.4, 90.7)	88.1 (74); (79.2, 94.1)	-1.5 (-9.0, 7.9)	0.24
Pn 6A	0.35 µg/mL	96.7 (64); (89.5, 99.6)	100 (25); (86.3, 100)	-3.3 (-10.5, 10.5)	1	86.1 (192); (80.9, 90.4)	88.1 (74); (79.2, 94.1)	-2.0 (-9.5, 7.5)	0.51
Pn 6B	0.35 µg/mL	87.9 (58); (77.5, 94.6)	92.0 (23) (74.0, 99.0)	-4.1 (-16.25, 14.0)	0.10	65.0 (145); (58.4, 71.3)	61.9 (52); (50.7, 72.3)	3.1 (-8.6, 15.4)	0.12
Pn 7F	0.35 µg/mL	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.6, 13.4)	-	100 (223); (98.4, 100)	97.6 (82); (91.7, 99.7)	2.4 (-0.7, 8.3)	1
Pn 9V	0.35 µg/mL	96.7 (64); (89.5, 99.6)	100 (25); (86.3, 100)	-3.0 (-10.5, 10.5)	1	77.1 (172); (71.1, 82.5)	72.6 (61); (61.8, 81.8)	4.5 (-5.9, 16.1)	0.18
Pn 14	0.35 µg/mL	90.9 (60); (81.3, 96.6)	92.0 (23); (73.4, 99.0)	-1.1 (-12.5, 16.8)	1	85.2 (190); (79.9, 89.6)	89.3 (75); (80.6, 95.0)	-4.1 (-11.5, 5.2)	0.51
Pn 18C	0.35 µg/mL	95.5 (63); (87.3, 99.1)	96.0 (24); (79.6, 99.9)	-5.5 (-9.5, 15.5)	1	77.1 (172); (71.1, 82.5)	76.2 (64); (65.7, 84.8)	0.9 (-9.0, 12.3)	0.50
Pn 19A	0.35 µg/mL	92.4 (61); (83.2, 97.5)	96.0 (24); (79.6, 99.9)	-3.6 (-18, 8.5)	1	50.7 (113); (43.9, 57.4)	47.6 (40); (36.6, 58.8)	3.1 (-9.4, 15.4)	0.42
Pn 19F	0.35 µg/mL	100 (66); (94.6, 100)	100 (25); (86.3, 100)	0 (-5.4, 17.0)	-	99.1 (221); (96.8, 99.9)	100 (84); (95.7, 100)	-0.9 (-3.2, 3.5)	0.50
Pn 23F	0.35 µg/mL	92.4 (61); (83.2, 97.5)	96.0 (24); (79.6, 99.9)	-3.6 (-13.6, 12.6)	0.68	63.2 (141); (56.6, 69.6)	56.0 (47); (44.7, 66.8)	7.3 (-4.8, 19.6)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						No formula milk (n=107) **% (n); (95% CI)**	**Formula milk** (n=37) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					98.1 (105); (93.4, 99.8)	97.3 (36); (85.8, 99.9)	0.8 (-4.4, 12.1)	0.12
Mumps	45 IU/mL					61.7 (66); (51.8, 70.1)	56.8 (21); (39.5, 72.9)	4.9 (-7.1, 27.9)	0.74
Rubella	10 IU/mL					86.9 (93); (79.0, 92.7)	86.5 (32); (71.2, 95.5)	0.4 (-10.7, 15.9)	0.09
MenC	2 µg/mL					99.1 (106); (94.9, 100)	100 (37); (90.5, 100)	-0.9 (-5.1, 8.6)	0.49
Hib	0.15 µg/mL					99.1 (106); (94.9, 100)	97.3 (36); (85.8, 99.9)	1.8 (-2.9, 13.0)	1
Tetanus	0.01 IU/mL					100 (107); (96.6, 100)	100 (37); (90.1, 100)	0 (-3.4, 9.5)	

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.4 GMCs and GMRs at 7 and 13 months of age in infants who did or did not receive formula milk before discharge from hospital

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**No formula milk** (n=66) **GMC (95% CI)**	**Formula milk** (n=25) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**	**No formula milk** (n=223) **GMC (95% CI)**	**Formula milk** (n=84) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted** GMR (95% CI)**	**Two-sided p-value**
Diphtheria[1]	0.39 (0.32, 0.47)	0.42 (0.31, 0.56)	0.93 (0.65, 1.34)	0.71	1.12 (0.79, 1.60)	0.52	**0.07 (0.06, 0.08)**	**0.10 (0.08, 0.12)**	**0.73 (0.56, 0.94)**	**0.01**	0.82 (0.64, 1.04)	0.10
Tetanus[1]	1.09 (0.91, 1.29)	1.17 (0.89, 1.53)	0.93 (0.67, 1.28)	0.64	0.84 (0.60, 1.17)	0.30	0.45 (0.37, 0.55)[3]	0.42 (0.29, 0.60)[3]	1.07 (0.72, 1.57)[3]	0.74[3]	0.93 (0.63, 1.35)[3]	0.69[3]
PT[1]	64.16 (52.67, 78.15)	88.47 (64.69, 121.00)	0.73 (0.50, 1.05)	0.09	0.84 (0.58, 1.21)	0.34	16.89 (14.77, 19.33)	18.78 (15.10, 23.36)	0.90 (0.70, 1.16)	0.42	1.00 (0.79, 1.28)	0.98
FHA[1]	**56.82 (47.28, 68.28)**	**86.30 (58.45, 127.42)**	**0.66 (0.45, 0.96)**	**0.03**	0.73 (0.49, 1.06)	0.10	17.86 (15.81, 20.19)	21.90 (17.51, 27.38)	0.82 (0.64, 1.04)	0.10	0.91 (0.72, 1.15)	0.42
PRN[1]	56.69 (44.42, 72.34)	71.79 (50.14, 102.77)	0.79 (0.50, 1.24)	0.30	0.82 (0.50, 1.33)	0.41	11.38 (9.71, 13.34)	13.61 (8.67, 15.21)	0.84 (0.62, 1.13)	0.25	0.82 (0.61, 1.11)	0.20
Hib[2]	0.51 (0.35, 0.75)	0.51 (0.26, 1.00)	1.01 (0.49, 2.11)	0.97	1.05 (0.50, 2.21)	0.90	1.19 (0.78, 1.82)[3]	0.98 (0.50, 1.91)[3]	1.22 (0.56, 2.65)[3]	0.61[3]	1.01 (0.55, 1.88)	0.96[3]
Polio type 1[1]	34.37 (26.42, 44.71)	35.37 (23.37, 53.20)	0.97 (0.60, 1.59)	0.92	1.05 (0.62, 1.80)	0.85	11.38 (9.74, 13.29)	10.70 (8.63, 13.27)	1.06 (0.80, 1.41)	0.67	1.24 (0.93, 1.65)	0.14
Polio type 2[1]	67.95 (52.79, 87.48)	51.54 (32.19, 82.52)	1.32 (0.80, 2.16)	0.27	1.43 (0.85, 2.41)	0.18	22.11 (18.68, 26.17)	20.30 (15.46, 26.66)	1.09 (0.79, 1.50)	0.60	1.23 (0.89, 1.70)	0.21
Polio type 3[1]	23.51 (18.00, 30.71)	16.80 (10.36, 27.24)	1.40 (0.83, 2.35)	0.20	1.40 (0.79, 2.46)	0.24	9.16 (7.67, 10.94)	8.23 (6.33, 10.69)	1.11 (0.80, 1.55)	0.52	1.28 (0.92, 1.78)	0.14
Pn 1[2]	4.33 (3.36, 5.58)	4.47 (2.95, 6.77)	0.97 (0.60, 1.56)	0.90	1.20 (0.75, 1.92)	0.45	1.18 (1.05, 1.33)	1.14 (0.69, 1.35)	1.03 (0.83, 1.29)	0.76	1.03 (0.82, 1.28)	0.80
Pn 3[2]	1.40 (1.20, 1.64)	1.54 (1.11, 2.12)	0.91 (0.66, 1.25)	0.56	0.97 (0.69, 1.36)	0.85	0.52 (0.47, 0.58)	0.58 (0.48, 0.69)	0.91 (0.74, 1.12)	0.37	0.89 (0.72, 1.09)	0.25
Pn 4[2]	0.53 (0.43, 0.66)	0.47 (0.34, 0.65)	1.14 (0.77, 1.69)	0.51	1.22 (0.82, 1.82)	0.33	0.22 (0.20, 0.24)	0.21 (0.18, 0.24)	1.03 (0.87, 1.22)	0.72	1.01 (0.85, 1.20)	0.89
Pn 5[2]	3.01 (2.42, 3.74)	3.59 (2.39, 5.39)	0.84 (0.55, 1.29)	0.42	1.01 (0.65, 1.56)	0.97	0.90 (0.80, 1.02)	0.97 (0.79, 1.18)	0.93 (0.74, 1.18)	0.57	0.97 (0.77, 1.22)	0.79
Pn 6A[2]	4.81 (3.90, 5.94)	4.81 (3.32, 6.98)	1.00 (0.67, 1.50)	1.00	1.24 (0.84, 1.85)	0.28	1.00 (0.87, 1.15)	1.00 (0.83, 1.23)	1.00 (0.78, 1.29)	1.00	1.06 (0.83, 1.36)	0.62
Pn 6B[2]	1.99 (1.37, 2.89)	1.73 (0.93, 3.22)	1.15 (0.57, 2.33)	0.70	1.62 (0.78, 3.38)	0.19	0.54 (0.46, 0.63)	0.47 (0.38, 0.58)	1.14 (0.85, 1.54)	0.37	1.25 (0.93, 1.63)	0.14
Pn 7F[2]	6.39 (5.34, 7.66)	5.49 (3.82, 7.88)	1.16 (0.81, 1.67)	0.40	**1.44 (1.02, 2.05)**	**0.04**	1.89 (1.71, 2.09)	1.84 (1.55, 2.19)	1.03 (0.84, 1.25)	0.80	1.06 (0.87, 1.29)	0.56
Pn 9V[2]	2.42 (1.97, 2.97)	2.57 (1.80, 3.67)	0.94 (0.63, 1.39)	0.75	1.19 (0.81, 1.74)	0.38	0.58 (0.52, 0.66)	0.64 (0.53, 0.77)	0.91 (0.74, 1.14)	0.44	0.94 (0.76, 1.17)	0.59
Pn 14[2]	2.68 (1.97, 3.63)	2.65 (1.55, 4.53)	1.01 (0.56, 1.82)	0.97	1.14 (0.61, 2.14)	0.68	0.98 (0.85, 1.13)	1.24 (1.03, 1.49)	0.80 (0.62, 1.03)	0.08	0.83 (0.64, 1.08)	0.17
Pn 18C[2]	2.98 (2.35, 3.77)	2.84 (1.97, 4.10)	1.05 (0.67, 1.63)	0.84	1.19 (0.75, 1.90)	0.46	0.65 (0.58, 0.72)	0.66 (0.55, 0.79)	0.98 (0.80, 1.21)	0.87	1.03 (0.83, 1.27)	0.82
Pn 19A[2]	1.70 (1.37, 2.11)	1.77 (1.12, 2.80)	0.96 (0.62, 1.50)	0.86	1.15 (0.72, 1.84)	0.54	0.48 (0.40, 0.57)	0.43 (0.33, 0.56)	1.10 (0.79, 1.53)	0.58	0.99 (0.71, 1.38)	0.94
Pn 19F[2]	11.35 (9.15, 14.08)	12.03 (8.21. 17.62)	0.94 (0.62, 1.43)	0.78	1.18 (0.77, 1.79)	0.44	3.23 (2.78, 3.75)	2.80 (2.31, 3.40)	1.15 (0.88, 1.51)	0.30	1.17 (0.89, 1.54)	0.26
Pn 23F[2]	2.24 (1.67, 2.99)	2.63 (1.73, 3.98)	0.85 (0.50, 1.45)	0.55	1.02 (0.59, 1.75)	0.95	0.58 (0.49, 0.68)	0.54 (0.41, 0.70)	1.07 (0.78, 1.46)	0.67	1.06 (0.77, 1.45)	0.72

	Antibodies to 12-month vaccines measured 13 months of age					
	No formula milk (n=107) **GMC (95% CI)**	**Formula milk** (n=37) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**
Measles[1]	3.02 (2.43, 3.75)	3.08 (2.23, 4.25)	0.98 (0.65, 1.48)	0.92	0.93 (0.61, 1.43)	0.74
Mumps[1]	61.11 (47.59, 78.46)	50.46 (32.25, 78.95)	1.21 (0.74, 1.99)	0.45	1.02 (0.66, 1.57)	0.94
Rubella[1]	51.32 (40.06, 65.74)	55.79 (31.87, 97.67)	0.92 (0.54, 1.56)	0.76	0.84 (0.54, 1.30)	0.43
MenC[2]	17.81 (15.05, 21.08)	17.04 (13.93, 20.83)	1.05 (0.77, 1.42)	0.78	1.12 (0.82, 1.54)	0.47
Hib[2]	17.20 (12.92, 22.90)	11.27 (6.36, 19.95)	1.53 (0.85, 2.74)	0.16	1.36 (0.75, 2.45)	0.31
Tetanus[1]	1.02 (0.81, 1.28)	0.92 (0.63, 1.36)	1.10 (0.70, 1.73)	0.67	1.09 (0.68, 1.76)	0.71

[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Table 3.5 Seroprotection rates at 7 and 13 months of age in infants who were or were not still receiving breast milk at the age of 1 month

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Breastfed** (n=80) **% (n); (95% CI)**	**Not Breastfed** (n=11) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Breastfed** (n=251) **% (n); (95% CI)**	**Not Breastfed** (n=56) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	96.3 (77); (89.4, 99.2)	100 (11); (71.5, 100)	-3.8 (-10.5, 22.5)	0.60	98.4 (247); (96.0, 99.6)	98.2 (55); (90.4, 100)	0.2 (-2.7, 7.9)	0.68
Tetanus	0.1 IU/mL[1]	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	100 (132); (97.2, 100)[2]	100 (31); (88.8, 100)[2]	0 (-2.8, 11.1)[2]	-
PT	25 IU/mL	88.8 (71); (79.7, 94.7)	100 (11); (71.5, 100)	-11.3 (-20.1, 15.3)	1	30.7 (77); (25.0, 36.8)	26.8 (15); (15.8, 40.3)	3.9 (-10.0, 15.6)	0.62
Hib	0.15 µg/mL	72.3 (58); (61.4, 81.9)	100 (11); (71.5, 100)	-27.5 (-38.2, 0.5)	0.05	79.5 (105); (71.7, 86.0)[2]	64.5 (20); (45.4, 80.8)[2]	15 (-1.4, 33.9)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	100 (251); (98.5, 100)	100 (56); (93.6, 100)	0 (-1.5, 6.4)	-
Polio type 2	0.29 IU/mL	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	99.6 (250); (97.4, 100)	100 (56); (93.6, 100)	-0.4 (-2.2, 5.0)	0.49
Polio type 3	0.12 IU/mL	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	100 (251); (98.5, 100)	100 (56); (93.6, 100)	0 (-1.5, 6.4)	-
Pn 1	0.35 µg/mL	96.3 (77); (89.4, 99.2)	100 (11); (71.5, 100)	-3.8 (-10.5, 22.5)	1	93.2 (234); (89.4, 96.0)	96.4 (54); (87.7, 99.6)	-3.2 (-8.0, 5.6)	0.35
Pn 3	0.35 µg/mL	98.8 (79); (93.2, 100)	100 (11); (71.5, 100)	-1.3 (-6.8, 24.9)	1	67.3 (169); (61.1, 73.1)	80.4 (45); (67.6, 89.8)	-13.0 (-23.5, 0.3)	0.90
Pn 4	0.35 µg/mL	70.0 (56); (38.8, 79.7)	63.6 (7); (30.8, 89.1)	6.4 (-18.0, 36.6)	0.65	22.7 (57); (17.7, 28.4)	23.2 (13); (13.0, 36.4)	-0.5 (-14.0, 10.3)	0.68
Pn 5	0.35 µg/mL	98.8 (79); (93.2, 100)	90.9 (10); (74.0, 99.9)	-7.8 (-0.8, 36.7)	0.50	86.5 (217); (81.6, 90.4)	89.3 (50); (78.1, 96.0)	-2.8 (-10.4, 8.5)	0.24
Pn 6A	0.35 µg/mL	97.5 (78); (89.8, 99.6)	100 (11); (71.5, 100)	-2.5 (-8.7, 23.7)	1	85.7 (215); (80.7, 89.7)	91.1 (51); (81.0, 95.4)	-5.4 (-12.6, 5.5)	0.51
Pn 6B	0.35 µg/mL	87.5 (70); (78.2, 93.8)	100 (11); (71.5, 100)	-12.5 (-21.6, 14.1)	0.10	64.1 (161); (57.9, 70.1)	64.3 (36); (50.4, 76.7)	-0.1 (-13.1, 14.2)	0.12
Pn 7F	0.35 µg/mL	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	99.2 (249); (97.2, 99.9)	100 (56); (93.6, 100)	-0.8 (-28.6, 5.7)	1
Pn 9V	0.35 µg/mL	97.5 (78); (91.3, 99.7)	100 (11); (71.5, 100)	-2.5 (-8.7, 23.7)	1	76.9 (193); (79.8, 89.8)	71.4 (40); (57.8, 82.7)	5.5 (6.2, 19.3)	0.18
Pn 14	0.35 µg/mL	91.3 (73); (82.8, 96.4)	90.9 (10); (58.7, 99.8)	0.3 (-11.3, 29.7)	1	85.3 (214); (80.3, 89.4)	91.1 (51); (80.4, 97.0)	-5.8 (-13.1, 5.2)	0.51
Pn 18C	0.35 µg/mL	95.0 (76); (87.7, 98.6)	100 (11); (71.5, 100)	-5.0 (-12.2, 21.3)	1	76.9 (193); (71.2, 81.2)	76.8 (43); (63.4, 87)	0.1 (-10.7, 13.6)	0.50
Pn 19A	0.35 µg/mL	92.5 (74); (84.4, 97.2)	100 (11); (71.5, 100)	-7.5 (-15.5, 18.0)	1	48.2 (121); (41.9, 54.6)	57.1 (32); (43.2, 70.3)	-8.9 (-22.6, 5.5)	0.42
Pn 19F	0.35 µg/mL	100 (80); (95.5, 100)	100 (11); (71.5, 100)	0 (-4.6, 26.1)	-	99.2 (249); (97.2, 99.9)	100 (56); (93.6, 100)	-0.8 (-2.9, 5.7)	0.50
Pn 23F	0.35 µg/mL	92.5 (74); (84.4, 97.2)	100 (11); (71.5, 100)	-7.5 (-15.5, 18.0)	0.68	62.2 (156); (55.8, 68.2)	57.1 (32); (43.2, 70.3)	5.0 (-8.7, 19.3)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						Breastfed (n=119) **% (n); (95% CI)**	**Not Breastfed** (n=25) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					97.5 (116); (92.8, 99.5)	100 (25); (86.3, 100)	-2.5 (-7.2, 10.9)	0.12
Mumps	45 IU/mL					61.3 (73); (52.0, 70.1)	56.0 (14); (34.9, 75.6)	5.3 (-14.5, 26.3)	0.74
Rubella	10 IU/mL					86.6 (103); (79.1, 92.1)	88.0 (22); (68.8, 97.5)	-1.45 (-12.5, 17.3)	0.09
MenC	2 µg/mL					99.2 (118); (95.4, 100)	100 (25); (86.3, 100)	-0.8 (-4.6, 24.6)	0.49
Hib	0.15 µg/mL					99.2 (118); (95.4, 100)	96 (24); (79.6, 99.9)	3.2 (-1.6, 18.8)	1
Tetanus	0.01 IU/mL					100 (119); (96.9, 100)	100 (25); (86.3, 100)	0 (-3.1, 13.4)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.6 GMCs and GMRs at 7 and 13 months of age in infants who were or were not still receiving breast milk at the age of 1 month

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	Breastfed (n=80) GMC (95% CI)	Not Breastfed (n=11) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted* GMR (95% CI)	Two-sided p-value	Breastfed (n=251) GMC (95% CI)	Not Breastfed (n=56) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted** GMR (95% CI)	Two-sided p-value
Diphtheria[1]	0.38 (0.32, 0.45)	0.53 (0.22, 0.83)	0.73 (0.44, 1.19)	0.20	0.82 (0.52, 1.30)	0.39	0.07 (0.07, 0.08)	0.09 (0.07, 0.13)	0.79 (0.59, 1.06)	0.11	0.8 (0.63, 1.08)	0.17
Tetanus[1]	1.09 (0.94, 1.27)	1.26 (0.74, 2.12)	0.84 (0.58, 1.20)	0.53	0.98 (0.63, 1.52)	0.92	0.44 (0.36, 0.53)[3]	0.45 (0.28, 0.72)[3]	1.03 (0.66, 1.62)[3]	0.89[3]	1.04 (0.68, 1.59)[3]	0.86[3]
PT[1]	68.91 (57.34, 82.82)	79.23 (53.47, 117.39)	0.87 (0.54, 1.41)	0.57	0.95 (0.72, 1.25)	0.57	17.48 (15.37, 19.98)	16.97 (13.26, 21.72)	1.03 (0.77, 1.38)	0.84	1.05 (0.80, 1.38)	0.72
FHA[1]	61.37 (51.13, 73.66)	83.90 (48.37, 145.52)	0.72 (0.44, 1.18)	0.19	1.11 (0.85, 1.44)	0.46	18.39 (16.32, 20.72)	21.29 (16.49, 27.50)	0.86 (0.65, 1.14)	0.30	0.90 (0.69, 1.18)	0.46
PRN[1]	62.25 (49.98, 77.54)	49.07 (29.60, 81.36)	1.24 (0.53, 1.21)	0.50	0.89 (0.63, 1.24)	0.48	12.11 (10.44, 14.05)	11.27 (8.00, 15.87)	1.07 (0.76, 1.53)	0.69	1.13 (0.81, 1.58)	0.48
Hib[2]	0.46 (0.33, 0.65)	1.10 (0.39, 3.16)	0.55 (0.21, 1.43)	0.21	0.54 (0.27, 1.07)	0.08	1.19 (0.81, 1.76)[3]	0.86 (0.35, 2.16)[3]	0.72 (0.29, 1.78)[3]	0.48[3]	1.85 (0.94, 3.64)	0.08[3]
Polio type 1[1]	33.78 (26.54, 43.00)	41.30 (25.68, 66.42)	0.79 (0.40, 1.59)	0.51	0.84 (0.61, 1.17)	0.30	11.43 (9.89, 13.22)	10.14 (7.89, 13.03)	1.13 (0.81, 1.57)	0.47	1.18 (0.86, 1.64)	0.30
Polio type 2[1]	63.97 (50.47, 81.10)	56.22 (27.54, 114.78)	1.10 (0.56, 1.79)	0.78	0.75 (0.52, 1.08)	0.12	22.55 (19.27,26.40)	17.81 (12.65, 25.08)	1.27 (0.88, 1.83)	0.21	1.33 (0.93, 1.91)	0.12
Polio type 3[1]	21.63 (16.88, 27.71)	20.09 (9.18, 43.97)	1.00 (0.48, 2.02)	0.99	1.12 (0.87, 1.44)	0.37	9.05 (7.69, 10.64)	8.24 (5.78, 11.74)	1.10 (0.75, 1.61)	0.63	1.11 (0.77, 1.61)	0.58
Pn 1[2]	4.20 (3.32, 5.31)	5.80 (3.55, 9.45)	0.92 (0.50, 1.70)	0.79	1.13 (0.90, 1.42)	0.31	1.14 (1.03, 1.27)	1.29 (1.00, 1.67)	0.88 (0.69, 1.14)	0.33	0.89 (0.70, 1.15)	0.37
Pn 3[2]	**1.37 (1.17, 1.59)**	**2.07 (1.63, 2.63)**	0.71 (0.46, 1.09)	0.12	1.09 (0.90, 1.33)	0.36	0.52 (0.47, 0.58)	0.61 (0.50, 0.75)	0.85 (0.68, 1.08)	0.19	0.89 (0.71, 1.12)	0.31
Pn 4[2]	0.50 (0.42, 0.61)	0.61 (0.35, 1.06)	0.97 (0.57, 1.64)	0.90	0.98 (0.75, 1.27)	0.86	0.21 (0.19, 0.23)	0.23 (0.19, 0.28)	0.92 (0.75, 1.11)	0.36	0.91 (0.75, 1.11)	0.36
Pn 5[2]	3.09 (2.52, 3.79)	3.69 (1.96, 6.98)	1.05 (0.59, 1.85)	0.88	0.96 (0.69, 1.34)	0.82	0.92 (0.82, 1.03)	0.92 (0.70, 1.21)	1.00 (0.76, 1.31)	1.00	1.02 (0.79, 1.33)	0.86
Pn 6A[2]	4.67 (3.83, 5.69)	6.02 (4.13, 8.76)	0.93 (0.55, 1.55)	0.77	1.24 (0.80, 1.93)	0.33	1.00 (0.89, 1.14)	0.99 (0.75, 1.30)	1.01 (0.76, 1.36)	0.93	1.04 (0.79, 1.37)	0.76
Pn 6B[2]	1.79 (1.27, 2.52)	3.16 (1.47, 6.80)	0.72 (0.28, 1.86)	0.49	1.32 (0.58, 2.97)	0.50	0.51 (0.44, 0.59)	0.53 (0.39, 0.72)	0.98 (0.70, 1.37)	0.89	1.04 (0.75, 1.45)	0.82
Pn 7F[2]	6.02 (5.06, 7.19)	6.89 (4.29, 11.06)	1.01 (0.63, 1.61)	0.98	1.30 (0.88, 1.93)	0.19	1.85 (1.68, 2.03)	2.02 (1.61, 2.55)	0.91 (0.73, 1.14)	0.42	0.92 (0.74, 1.15)	0.48
Pn 9V[2]	2.41 (1.99, 2.91)	2.84 (1.68, 4.81)	1.08 (0.66, 1.78)	0.75	1.29 (0.78, 1.81)	0.42	0.59 (0.53, 0.66)	0.63 (0.48, 0.82)	0.95 (0.73, 1.22)	0.66	0.97 (0.76, 1.24)	0.81
Pn 14[2]	2.76 (2.08, 3.67)	2.07 (1.02, 4.17)	1.47 (0.65, 3.31)	0.35	1.84 (0.92, 3.68)	0.09	1.01 (0.89, 1.15)	1.21 (0.92, 1.58)	0.84 (0.62, 1.13)	0.25	0.83 (0.62, 1.12)	0.23
Pn 18C[2]	2.88 (2.32, 3.58)	3.41 (2.18, 5.32)	0.95 (0.52, 1.75)	0.88	1.15 (0.68, 1.94)	0.59	0.65 (0.59, 0.72)	0.66 (0.52, 0.84)	0.98 (0.77, 1.25)	0.88	1.00 (0.79, 1.26)	0.98
Pn 19A[2]	1.66 (1.35, 2.05)	2.20 (1.12, 4.32)	0.84 (0.46, 1.53)	0.56	1.04 (0.63, 1.73)	0.87	0.44 (0.38, 0.52)	0.56 (0.39, 0.80)	0.79 (0.54, 1.16)	0.24	0.82 (0.56, 1.19)	0.30
Pn 19F[2]	11.10 (9.08, 13.58)	15.15 (9.41, 24.41)	0.85 (0.50, 1.47)	0.57	1.15 (0.72, 1.84)	0.56	2.96 (2.89, 5.07)	3.83 (2.89, 5.07)	0.77 (0.57, 1.06)	0.10	0.78 (0.57, 1.06)	0.11
Pn 23F[2]	2.21 (1.71, 2.86)	3.46 (1.82, 6.58)	0.80 (0.40, 1.03)	0.52	1.35 (0.76, 2.40)	0.31	0.55 (0.48, 0.64)	0.62 (0.43, 0.90)	0.89 (0.62, 1.28)	0.53	0.92 (0.64, 1.32)	0.65
							Antibodies to 12-month vaccines measured 13 months of age					
							Breastfed (n=119) GMC (95% CI)	Not Breastfed (n=25) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted* GMR (95% CI)	Two-sided p-value
Measles[1]							2.93 (2.38, 3.62)	3.55 (2.64, 4.78)	0.83 (0.51, 1.33)	0.43	0.91 (0.56, 1.49)	0.71
Mumps[1]							59.29 (46.88, 74.98)	53.14 (29.56, 95.51)	1.12 (0.63, 1.98)	0.71	1.17 (0.72, 1.92)	0.52
Rubella[1]							48.60 (38.06, 62.07)	75.22 (39.04, 144.93)	0.64 (0.35, 1.19)	0.16	0.66 (0.40, 1.08)	0.10
MenC[2]							18.24 (15.67, 21.23)	14.89 (11.17, 19.85)	1.22 (0.86, 1.75)	0.26	1.29 (0.90, 1.85)	0.16
Hib[2]							**17.41 (13.49, 22.48)**	**8.67 (3.68, 20.45)**	**2.01 (1.03, 3.92)**	**0.04**	1.85 (0.95, 3.62)	0.07
Tetanus[1]							1.03 (0.83, 1.28)	0.83 (0.51, 1.38)	0.81 (0.48, 1.36)	0.43	1.26 (0.74, 2.16)	0.39

[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Table 3.7 Seroprotection rates at 7 and 13 months of age in infants who were or were not still receiving breast milk at the age of 6 months

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Breastfed** (n=71) **% (n); (95% CI)**	**Not Breastfed** (n=20) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Breastfed** (n=219) **% (n); (95% CI)**	**Not Breastfed** (n=88) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	95.8 (68); (88.1, 99.1)	100 (20); (83.2, 100)	-4.2 (-11.8, 12.2)	0.60	98.2 (215); (95.4, 99.5)	98.9 (87); (93.8, 100)	-0.7 (-3.7, 4.4)	0.68
Tetanus	0.1 IU/mL[1]	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	100 (112); (96.8, 100)[2]	100 (51); (93.0, 100)[2]	0 (-3.3, 7.0)[2]	-
PT	25 IU/mL	88.7 (63); (79.0, 95.0)	95.0 (19); (75.1, 99.9)	-6.3 (-17.1, 13.2)	1	32.4 (71); (26.3, 39.1)	23.9 (21); (15.4, 34.1)	8.6 (-2.9, 18.8)	0.62
Hib	0.15 µg/mL	76.1 (54); (64.5, 85.4)	75.0 (15); (50.9, 91.3)	1.1 (-17.2, 24.9)	0.05	76.8 (86); (62.9, 84.2)[2]	76.5 (39); (62.5, 87.2)[2]	0.3 (12.8, 15.4)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	100 (219); (98.3, 100)	100 (88); (95.6, 100)	0 (-1.8, 4.4)	-
Polio type 2	0.29 IU/mL	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	99.5 (218); (97.5, 100)	100 (88); (95.6, 100)	-0.5 (-2.5, 3.7)	0.49
Polio type 3	0.12 IU/mL	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	100 (219); (98.3, 100)	100 (88); (95.6, 100)	0 (-1.8, 4.4)	-
Pn 1	0.35 µg/mL	95.8 (68); (88.1, 99.1)	100 (20); (83.2, 100)	-4.2 (-11.8, 12.2)	1	94.1 (206); (90.1, 96.8)	93.2 (82); (85.7, 97.5)	0.9 (-4.5, 8.6)	0.35
Pn 3	0.35 µg/mL	98.6 (70); (92.4, 100)	100 (20); (83.2, 100)	-1.4 (-7.6, 14.9)	1	67.6 (148); (60.9, 73.7)	75.0 (66); (64.6, 83.6)	-7.4 (-17.8, 4.2)	0.90
Pn 4	0.35 µg/mL	73.2 (52); (61.4, 83.1)	55.0 (11); (31.5, 76.9)	18.2 (-4.3, 41.5)	0.65	23.3 (51); (17.9, 29.5)	21.6 (19); (13.5, 31.6)	1.7 (-9.2, 11.3)	0.68
Pn 5	0.35 µg/mL	98.6 (70); (92.4, 100)	100 (20); (83.2, 100)	3.6 (-3.6, 22.4)	0.50	86.8 (190); (81.5, 90.9)	87.5 (77); (78.7, 94.6)	-0.7 (-8.3, 8.6)	0.24
Pn 6A	0.35 µg/mL	97.2 (69); (90.2, 99.7)	100 (20); (83.2, 100)	-2.8 (-9.8, 13.6)	1	85.4 (187); (80.0, 89.8)	89.7 (79); (81.5, 95.2)	-4.4 (-11.7, 4.6)	0.51
Pn 6B	0.35 µg/mL	88.7 (63); (79.0, 95.0)	90.0 (18) (68.3, 98.8)	-1.3 (-13.7, 19.8)	0.1	63.5 (139); (56.7, 69.9)	65.9 (58); (55.0, 75.7)	-2.4 (-13.7, 9.7)	0.12
Pn 7F	0.35 µg/mL	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	100 (219); (98.3, 100)	97.7 (86); (92.0, 99.7)	2.3 (-0.5, 7.9)	1
Pn 9V	0.35 µg/mL	97.2 (69); (90.2, 99.7)	100 (20); (83.2, 100)	-2.8 (-9.8, 13.6)	1	78.1 (171); (72.0, 83.4)	70.5 (62); (59.8, 79.7)	7.6 (-2.8, 19.1)	0.18
Pn 14	0.35 µg/mL	91.5 (65); (82.5, 96.8)	90 (18; (68.3, 98.8)	1.5 (-10.2, 22.4)	1	85.3 (180); (79.8, 89.8)	88.3 (68); (79.0, 94.5)	-3 (-10.8, 7.0)	0.57
Pn 18C	0.35 µg/mL	94.4 (67); (86.2, 98.4)	100 (20); (83.2, 100)	-5.6 (-13.7, 10.8)	1	77.6 (170); (71.5, 83.0)	75.0 (66); (64.6, 83.6)	2.6 (-7.3, 13.8)	0.50
Pn 19A	0.35 µg/mL	91.5 (65); (82.5, 96.8)	100 (20); (83.2, 100)	-8.5 (-17.3, 8.1)	1	46.6 (102); (39.9, 53.4)	58.0 (51); (47.0, 68.4)	-11.4 (-23.3, 1.0)	0.42
Pn 19F	0.35 µg/mL	100 (71); (94.9, 100)	100 (20); (83.2, 100)	0 (-5.2, 16.3)	-	99.1 (217); (96.7, 99.9)	100 (88); (95.6, 100)	-0.9 (-3.3, 3.3)	0.50
Pn 23F	0.35 µg/mL	94.4 (67); (86.2, 98.4)	90.0 (18); (68.3, 98.8)	4.4 (-6.6, 25.0)	0.68	62.6 (137); (55.8, 69.0)	58.0 (51); (47.0, 68.4)	4.6 (-7.3, 16.8)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						Breastfed (n=107) **% (n); (95% CI)**	**Not Breastfed** (n=37) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					98.1 (105); (93.4, 99.8)	97.3 (36); (85.8, 99.9)	0.8 (-4.4, 12.1)	0.11
Mumps	45 IU/mL					60.7 (65); (50.8, 70.0)	59.5 (22); (42.1, 75.2)	1.3 (-16.1, 19.8)	0.74
Rubella	10 IU/mL					85.0 (91); (76.9, 91.2)	91.9 (34); (78.1, 98.3)	-6.8 (-16.9, 7.4)	0.09
MenC	2 µg/mL					100 (107); (96.6, 100)	97.3 (36); (85.8, 99.9)	2.7 (-0.8, 13.9)	0.49
Hib	0.15 µg/mL					99.1 (106); (94.9, 100)	97.3 (36); (85.8, 99.9)	1.8 (-2.9, 13.0)	1
Tetanus	0.01 IU/mL					100 (107); (96.6, 100)	100 (37); (90.5, 100)	0 (-3.4, 9.4)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.8 GMCs and GMRs at 7 and 13 months of age in infants who were or were not still receiving breast milk at the age of 6 months

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**Breastfed** (n=71) **GMC (95% CI)**	**Not Breastfed** (n=20) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**	**Breastfed** (n=219) **GMC (95% CI)**	**Not Breastfed** (n=88) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted** GMR (95% CI)**	**Two-sided p-value**
Diphtheria[1]	0.40 (0.33, 0.48)	0.39 (0.28, 0.55)	1.02 (0.69, 1.51)	0.90	0.97 (0.67, 1.39)	0.85	0.07 (0.06, 0.08)	0.09 (0.07, 0.11)	0.85 (0.66, 1.10)	0.22	0.90 (0.71, 1.13)	0.37
Tetanus[1]	1.06 (0.91, 1.25)	1.28 (0.89, 1.85)	0.83 (0.58, 1.18)	0.30	0.87 (0.62, 1.23)	0.42	0.43 (0.35, 0.53)[3]	0.46 (0.32, 0.65)[3]	1.06 (0.72, 1.56)[3]	0.76[3]	1.02 (0.71, 1.46)[3]	0.92[3]
PT[1]	69.87 (57.48, 84.92)	70.84 (50.47, 99.43)	0.99 (0.66, 1.48)	0.95	0.95 (0.65, 1.39)	0.81	17.98 (15.68, 20.61)	16.00 (12.97, 19.74)	1.12 (0.87, 1.45)	0.36	1.19 (0.94, 1.50)	0.15
FHA[1]	63.55 (52.36, 77.13)	64.40 (43.11, 96.20)	0.99 (0.65, 1.50)	0.95	0.90 (0.61, 1.33)	0.59	18.86 (16.61, 21.43)	18.94 (15.42, 23.27)	1.00 (0.79, 1.26)	0.97	1.06 (0.85, 1.34)	0.60
PRN[1]	62.60 (49.35, 79.42)	53.54 (36.91, 77.67)	1.17 (0.72, 1.90)	0.52	1.13 (0.69, 1.85)	0.62	11.93 (10.16, 14.02)	12.00 (9.26, 15.54)	0.99 (0.74, 1.34)	0.97	1.02 (0.80, 1.42)	0.66[3]
Hib[2]	0.50 (0.35, 0.72)	0.56 (0.25, 1.25)	0.89 (0.40, 1.96)	0.76	0.77 (0.36, 1.63)	0.49	1.09 (0.72, 1.67)[3]	1.19 (0.61, 2.32)[3]	1.09 (0.51, 2.34)[3]	0.83[3]	1.31 (0.74, 2.34)	0.36
Polio type 1[1]	34.05 (26.13, 44.35)	36.68 (25.77, 52.22)	0.93 (0.55, 1.57)	0.78	0.81 (0.47, 1.39)	0.44	11.16 (9.56, 13.02)	11.26 (9.00, 14.08)	0.99 (0.75, 1.31)	0.95	1.06 (0.80, 1.40)	0.68
Polio type 2[1]	64.74 (49.78, 84.18)	57.14 (37.84, 86.28)	1.13 (0.66, 1.94)	0.64	1.03 (0.60, 1.76)	0.90	22.30 (18.84, 26.38)	19.96 (15.20, 26.22)	1.12 (0.81, 1.53)	0.49	1.21 (0.88, 1.65)	0.24
Polio type 3[1]	21.44 (16.39, 28.05)	21.42 (13.03, 35.24)	1.00 (0.57, 1.76)	1.00	0.90 (0.50, 1.60)	0.71	9.16 (7.70, 10.88)	8.27 (6.23, 10.98)	1.11 (0.80, 1.53)	0.54	1.15 (0.84, 1.59)	0.38
Pn 1[2]	4.50 (3.53, 5.74)	3.93 (2.45, 6.31)	1.14 (0.68, 1.92)	0.61	1.12 (0.70, 1.81)	0.63	1.15 (1.03, 1.29)	1.21 (1.00, 1.46)	0.96 (0.77, 1.18)	0.68	0.97 (0.78, 1.20)	0.75
Pn 3[2]	1.38 (1.18, 1.62)	1.65 (1.21, 2.26)	0.83 (0.59, 1.17)	0.29	0.81 (0.57, 1.14)	0.21	0.53 (0.48, 0.60)	0.55 (0.47, 0.64)	0.97 (0.79, 1.18)	0.75	1.00 (0.82, 1.22)	0.98
Pn 4[2]	0.52 (0.43, 0.63)	0.50 (0.32, 0.78)	1.03 (0.67, 1.57)	0.89	1.02 (0.68, 1.54)	0.91	0.21 (0.19, 0.23)	0.22 (0.19, 0.25)	0.97 (0.82, 1.15)	0.72	0.97 (0.82, 1.15)	0.75
Pn 5[2]	3.35 (2.74, 4.08)	2.57 (1.51, 4.39)	1.30 (0.82, 2.06)	0.26	1.27 (0.82, 1.98)	0.28	0.93 (0.82, 1.05)	0.90 (0.73, 1.11)	1.03 (0.81, 1.30)	0.80	1.05 (0.84, 1.32)	0.67
Pn 6A[2]	4.95 (4.02, 6.09)	4.37 (3.00, 6.37)	1.13 (0.73, 1.75)	0.57	1.13 (0.75, 1.69)	0.56	1.01 (0.88, 1.15)	0.99 (0.81, 1.22)	1.01 (0.79, 1.30)	0.92	1.03 (0.81, 1.30)	0.81
Pn 6B[2]	1.93 (1.34, 2.78)	1.87 (0.96, 3.64)	1.03 (0.48, 2.21)	0.94	1.07 (0.50, 2.25)	0.87	0.51 (0.44, 0.60)	0.53 (0.42, 0.66)	0.97 (0.73, 1.30)	0.85	1.02 (0.77, 1.36)	0.87
Pn 7F[2]	6.40 (5.34, 7.67)	5.27 (3.59, 7.72)	1.22 (0.82, 1.79)	0.32	1.26 (0.88, 1.81)	0.21	1.90 (1.72, 2.10)	1.83 (1.53, 2.19)	1.04 (0.86, 1.26)	0.69	1.05 (0.87, 1.27)	0.62
Pn 9V[2]	2.53 (1.42, 3.51)	2.98 (2.22, 4.00)	1.13 (0.74, 1.73)	0.56	1.14 (0.77, 1.67)	0.52	0.60 (0.54, 0.67)	0.59 (0.48, 0.72)	1.03 (0.83, 1.28)	0.81	1.04 (0.84, 1.28)	0.73
Pn 14[2]	2.98 (2.22, 4.00)	1.79 (1.01, 3.17)	1.67 (0.89, 3.10)	0.11	1.77 (0.95, 3.31)	0.07	1.03 (0.89, 1.18)	1.09 (0.89, 1.34)	0.98 (0.75, 1.28)	0.90	0.92 (0.71, 1.19)	0.54
Pn 18C[2]	2.95 (2.34, 3.73)	2.90 (2.03, 4.16)	1.02 (0.63, 1.63)	0.95	1.03 (0.64, 1.66)	0.90	0.66 (0.59, 0.74)	0.63 (0.52, 0.75)	1.06 (0.86, 1.30)	0.59	1.07 (0.87, 1.31)	0.51
Pn 19A[2]	1.77 (1.42, 2.21)	1.55 (0.97, 2.48)	1.14 (0.71, 1.84)	0.58	1.06 (0.66, 1.69)	0.82	0.43 (0.36, 0.51)	0.56 (0.43, 0.74)	0.76 (0.55, 1.05)	0.09	0.75 (0.55, 1.04)	0.08
Pn 19F[2]	11.87 (9.64, 14.60)	10.41 (6.73, 16.09)	1.14 (0.73, 1.78)	0.56	1.12 (0.73, 1.72)	0.59	2.89 (2.51, 3.32)	3.73 (2.96, 4.71)	0.77 (0.59, 1.01)	0.06	**0.76 (0.58, 0.99)**	**0.04**
Pn 23F[2]	2.40 (1.84, 3.13)	2.14 (1.23, 3.70)	1.12 (0.63, 1.99)	0.69	1.13 (0.65, 1.95)	0.67	0.57 (0.49, 0.67)	0.55 (0.42, 0.71)	1.06 (0.77 ,1.43)	0.75	1.08 (0.80, 1.47)	0.62
							Antibodies to 12-month vaccines measured 13 months of age					
							Breastfed (n=107) **GMC (95% CI)**	**Not Breastfed** (n=37) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**
Measles[1]							3.09 (2.56, 3.74)	2.86 (1.82, 4.49)	1.08 (0.71, 1.64)	0.71	1.15 (0.74, 1.77)	0.53
Mumps[1]							58.53 (45.27, 75.69)	57.14 (37.77, 86.46)	1.02 (0.62, 1.68)	0.92	1.17 (0.76, 1.80)	0.48
Rubella[1]							46.00 (35.22, 60.08)	76.54 (48.77, 120.15)	0.60 (0.36, 1.01)	0.06	0.66 (0.43, 1.03)	0.07
MenC[2]							**19.32 (16.52, 22.60)**	**13.46 (10.45, 17.35)**	**1.44 (1.06, 1.94)**	**0.02**	**1.53 (1.12, 2.11)**	**<0.01**
Hib[2]							17.25 (13.16, 22.63)	11.17 (5.93, 21.01)	1.55 (0.86, 2.77)	0.14	1.51 (1.11, 2.06)	0.33
Tetanus[1]							1.08 (0.85, 1.36)	0.79 (0.54, 1.16)	1.10 (0.70, 1.73)	0.67	1.40 (0.87, 2.24)	0.16

[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation age at sampling and time between immunisation and sampling

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Table 3.9 Seroprotection rates at 7 and 13 months of age in infants whose mothers did or did not received antibiotics during delivery

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Intrapartum antibiotics** (n=21) **% (n); (95% CI)**	**No intrapartum antibiotics** (n=70) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Intrapartum antibiotics** (n=62) **% (n); (95% CI)**	**No intrapartum antibiotics** (n=245) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	100 (21); (83.9, 100)	95.7 (67); (88.0, 99.1)	4.3 (-11.5.4, 11.9)	0.6	98.4 (61); (91.3, 100)	98.4 (241); (95.9, 99.5)	0 (-7.0, 2.9)	0.68
Tetanus	0.1 IU/mL[1]	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	100 (30); (88.4, 100)[2]	100 (133); (97.3, 100)[2]	0 (-11.4, 2.8)[2]	-
PT	25 IU/mL	90.5 (19); (69.6, 98.8)	90.0 (63); (80.5, 95.9)	0.5 (-19.8, 12.5)	1	32.3 (20); (20.9, 45.3)	29.4 (72); (23.8, 35.6)	2.9 (-9.2, 16.4)	0.62
Hib	0.15 µg/mL	71.4 (15); (47.8, 88.7)	77.1 (54); (65.6, 86.3)	-5.7 (-29.1, 13.2)	0.05	83.3 (25); (65.3; 94.4)[2]	75.2 (100); (67.0; 82.3)[2]	8.1 (-10.0, 20.9)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	100 (62); (94.2, 100)	100 (245); (98.5, 100)	0 (-11.1, 1.4)	-
Polio type 2	0.29 IU/mL	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	98.4 (61); (91.3, 100)	100 (245); (98.5, 100)	-1.6 (-8.6, 0)	0.49
Polio type 3	0.12 IU/mL	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	100 (62); (94.2, 100)	100 (245); (98.5, 100)	0 (-5.9, 1.5)	-
Pn 1	0.35 µg/mL	100 (21); (83.9, 100)	95.7 (67); (88.0, 99.1)	4.3 (-11.5 11.9)	1	95.2 (59); (86.5, 99.0)	93.5 (229); (89.6, 96.2)	1.7 (-7.1, 6.8)	0.35
Pn 3	0.35 µg/mL	100 (21); (83.9, 100)	98.6 (69); (92.3, 100)	1.4 (-14.2, 7.7)	1	74.2 (46); (61.5, 84.5)	68.6 (168); (62.4, 74.3)	5.6 (-7.6, 16.9)	0.90
Pn 4	0.35 µg/mL	76.2 (16); (52.8, 91.8)	67.1 (47); (54.8, 77.9)	9.1 (-14.7, 27.4)	0.65	25.8 (16); (15.6, 38.5)	22.0 (54); (17.0, 27.8)	3.8 (-7.2, 16.8)	0.68
Pn 5	0.35 µg/mL	100 (21); (83.9, 100)	97.1 (68); (90.1, 99.7)	2.9 (-12.9, 9.9)	1	91.9 (57); (82.2, 97.3)	85.7 (210); (81.6, 90.1)	6.2 (-3.9, 13.2)	0.24
Pn 6A	0.35 µg/mL	100 (21); (83.9, 100)	97.1 (68); (90.1, 99.7)	2.9 (-12.9, 9.9)	1	90.3 (56); (80.1, 96.4)	85.7 (210); (81.6, 90.1)	4.6 (-5.9, 12.0)	0.51
Pn 6B	0.35 µg/mL	95.2 (20); (76.2, 100)	87.1 (61); (77.0, 93.9)	9.1 (-10.8, 19.3)	0.09	67.7 (42); (54.7, 79.1)	63.3 (155); (56.9, 69.3)	4.5 (-9.2, 16.7)	0.12
Pn 7F	0.35 µg/mL	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	100 (62); (94.2, 100)	99.2 (243), (97.1, 99.9)	0.8 (-5.0, 2.9)	1
Pn 9V	0.35 µg/mL	100 (21); (83.9, 100)	97.1 (68); (90.1, 99.7)	2.9 (-12.9, 9.9)	1	83.9 (52); (72.3, 92.0)	73.9 (181); (69.8, 80.3)	10.0 (-2.1, 19.4)	0.18
Pn 14	0.35 µg/mL	95.2 (20); (76.2, 100)	90.0 (63); (80.5, 95.9)	5.2 (-13.4, 15.8)	1	88.7 (55); (70.2, 96.4)	85.7 (210); (81.6, 90.1)	3.0 (-7.9, 10.7)	0.51
Pn 18C	0.35 µg/mL	100 (21); (83.9, 100)	94.3 (66); (86.9, 98.4)	5.7 (-12.9, 9.9)	1	83.9 (52); (72.3, 92.0)	75.1 (184); (69.2, 80.4)	8.8 (-3.4, 18.2)	0.50
Pn 19A	0.35 µg/mL	100 (21); (83.9, 100)	91.4 (64); (82.3, 96.8)	8.6 (-7.3, 17.5)	1	46.8 (29), (34.0, 59.9)	50.6 (121); (44.2, 57.0)	-3.8 (-17.4, 10.0)	0.42
Pn 19F	0.35 µg/mL	100 (21); (83.9, 100)	100 (70); (94.9, 100)	0 (-15.6, 5.3)	-	98.4 (61); (91.3, 100)	99.6 (244); (97.7, 100)	-1.2 (-8.2, 0.1)	0.50
Pn 23F	0.35 µg/mL	100 (21); (83.9, 100)	91.4 (64); (82.3, 96.8)	8.6 (-7.3, 17.5)	0.68	66.1 (41), (53.0, 77.7)	60.0 (147), (53.6, 66.2)	6.1 (-7.7, 18.5)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						Intrapartum antibiotics (n=32) **% (n); (95% CI)**	**No intrapartum antibiotics** (n=112) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					100 (32); (89.1, 100)	97.3 (109); (92.4, 99.4)	2.7 (-8.2, 7.7)	0.12
Mumps	45 IU/mL					84.4 (27); (67.2, 94.7)	87.5 (98); (79.9, 93.0)	-3.1 (-20.2, 8.6)	0.09
Rubella	10 IU/mL					53.1 (17); (34.7, 70.9)	62.5 (70); (52.9, 71.5)	-9.4 (-28.4, 9.3)	0.74
MenC	2 µg/mL					100 (32); (89.1, 100)	99.1 (111); (95.1, 100)	-9.4 (-9.9, 4.9)	0.49
Hib	0.15 µg/mL					100 (32); (89.1, 100)	98.2 (110); (93.7, 99.8)	1.8 (-9.0, 6.3)	1
Tetanus	0.01 IU/mL					100 (32); (89.1, 100)	100 (112); (96.8, 100)	0 (-10.8, 3.3)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.10 GMCs and GMRs at 7 and 13 months of age in infants whose mothers did or did not received antibiotics during delivery

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	Intrapartum antibiotics (n=21) GMC (95% CI)	No intrapartum antibiotics (n=70) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted* GMR (95% CI)	Two-sided p-value	Intrapartum antibiotics (n=62) GMC (95% CI)	No intrapartum antibiotics (n=245) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted** GMR (95% CI)	Two-sided p-value
Diphtheria[1]	0.49 (0.36, 0.68)	0.37 (0.31, 0.45)	1.33 (0.91, 1.95)	0.14	1.27 (0.89, 1.82)	0.19	0.08 (0.06, 0.10)	0.08 (0.07, 0.09)	1.33 (0.91, 1.94)	0.14	1.10 (0.85, 1.43)	0.46
Tetanus[1]	1.15 (0.91, 1.46)	1.10 (0.92, 1.31)	1.05 (0.75, 1.49)	0.77	1.16 (0.82, 1.64)	0.40	0.53 (0.22, 0.85)[3]	0.42 (0.35, 0.51)[3]	1.25 (0.79, 1.98)[3]	0.33[3]	1.35 (0.88, 2.07)[3]	0.17[3]
PT[1]	78.15 (55.17, 110.72)	67.83 (55.89, 82.32)	1.15 (0.78, 1.71)	0.48	1.06 (0.72, 1.55)	0.76	17.90 (13.83, 23.16)	17.26 (15.19, 19.62)	1.12 (0.76, 1.63)	0.57	1.09 (0.84, 1.42)	0.53
FHA[1]	59.70 (44.06, 80.89)	64.99 (52.86, 79.91)	0.92 (0.61, 1.38)	0.68	0.85 (0.57, 1.25)	0.39	18.16 (14.45, 22.81)	19.08 (16.87, 21.57)	1.25 (0.87, 1.78)	0.22	0.99 (0.76, 1.27)	0.91
PRN[1]	**95.00 (70.77, 127.54)**	**52.83 (41.61, 67.07)**	**1.80 (1.13, 2.85)**	**0.01**	**1.97 (1.23, 3.16)**	**<0.01**	15.08 (11.50, 19.78)	11.27 (9.64, 13.17)	1.49 (0.95, 2.33)	0.09	**1.46 (1.06, 2.01)**	**0.02**
Hib[2]	0.56 (0.25, 1.25)	0.50 (0.35, 0.72)	1.11 (0.51, 2.42)	0.79	0.90 (0.42, 1.92)	0.78	1.70 (0.71, 4.03)[3]	1.02 (0.69, 1.51)[3]	1.66 (0.66, 4.13)[3]	0.28[3]	1.33 (0.66, 2.66)[3]	0.42[3]
Polio type 1[1]	34.05 (19.51, 59.43)	34.78 (27.42, 44.09)	0.98 (0.58, 1.65)	0.94	0.96 (0.56, 1.67)	0.89	11.39 (8.57, 15.13)	11.14 (9.66, 12.84)	0.80 (0.53, 1.22)	0.30	1.06 (0.78, 1.45)	0.70
Polio type 2[1]	60.83 (39.31, 94.12)	63.64 (49.02, 82.64)	0.96 (0.56, 1.62)	0.87	0.90 (0.53, 1.54)	0.69	24.54 (17.56, 34.29)	20.91 (17.85, 24.50)	0.71 (0.44, 1.14)	0.16	1.20 (0.85, 1.70)	0.30
Polio type 3[1]	16.88 (10.70, 26.65)	23.03 (17.54, 30.24)	0.73 (0.42, 1.27)	0.27	0.77 (0.43, 1.38)	0.38	8.27 (5.83, 11.74)	9.06 (7.70, 10.66)	0.78 (0.48, 1.27)	0.31	0.95 (0.66, 1.36)	0.78
Pn 1[2]	5.63 (3.49, 9.08)	4.05 (3.18, 5.15)	1.39 (0.84, 2.30)	0.20	1.26 (0.78, 2.03)	0.34	1.29 (1.04, 1.58)	1.14 (1.02, 1.27)	1.00 (0.73, 1.39)	0.99	1.17 (0.92, 1.49)	0.19
Pn 3[2]	1.55 (1.13, 2.11)	1.40 (1.20, 1.65)	1.10 (0.79, 1.54)	0.57	1.03 (0.73, 1.46)	0.86	0.56 (0.47, 0.68)	0.53 (0.48, 0.59)	0.99 (0.73, 1.33)	0.93	1.08 (0.87, 1.35)	0.48
Pn 4[2]	0.58 (0.43, 0.78)	0.50 (0.40, 0.62)	1.16 (0.76, 1.75)	0.49	1.02 (0.68, 1.54)	0.91	0.22 (0.20, 0.28)	0.21 (0.19, 0.23)	0.90 (0.70, 1.16)	0.43	1.16 (0.96, 1.39)	0.12
Pn 5[2]	3.76 (2.45, 5.78)	3.00 (2.41, 3.72)	1.26 (0.80, 1.97)	0.32	1.16 (0.74, 1.81)	0.50	1.01 (0.83, 1.22)	0.90 (0.80, 1.02)	1.03 (0.73, 1.45)	0.89	1.15 (0.89, 1.48)	0.27
Pn 6A[2]	5.84 (4.25, 8.02)	4.54 (3.67, 5.63)	1.28 (0.84, 1.96)	0.24	1.17 (0.78, 1.76)	0.43	1.05 (0.83, 1.32)	0.99 (0.87, 1.13)	0.92 (0.63, 1.33)	0.65	1.09 (0.84, 1.42)	0.53
Pn 6B[2]	2.59 (1.56, 4.28)	1.75 (1.19, 2.57)	1.48 (0.70, 3.12)	0.30	1.37 (0.65, 2.90)	0.40	0.54 (0.41, 0.73)	0.51 (0.44, 0.59)	1.03 (0.67, 1.60)	0.87	1.07 (0.78, 1.47)	0.67
Pn 7F[2]	6.77 (4.92, 7.20)	5.95 (4.92, 7.20)	1.14 (0.77, 1.67)	0.51	1.05 (0.73, 1.52)	0.78	2.03 (1.72, 2.39)	1.84 (1.66, 2.04)	1.03 (0.77, 1.38)	0.83	1.13 (0.91, 1.39)	0.27
Pn 9V[2]	2.96 (2.04, 4.31)	2.32 (1.90, 2.85)	1.27 (0.84, 1.93)	0.25	1.16 (0.79, 1.71)	0.45	0.67 (0.56, 0.80)	0.58 (0.52, 0.65)	1.05 (0.76, 1.45)	0.78	1.18 (0.93, 1.50)	0.17
Pn 14[2]	3.24 (2.05, 5.12)	2.52 (1.84, 3.44)	1.29 (0.69, 2.39)	0.42	1.18 (0.62, 2.23)	0.61	1.12 (0.88, 1.42)	1.03 (0.90, 1.17)	1.08 (0.74, 1.58)	0.68	1.13 (0.85, 1.50)	0.41
Pn 18C[2]	3.24 (2.32, 4.51)	2.86 (2.25, 3.62)	1.13 (0.71, 1.81)	0.60	1.03 (0.64, 1.65)	0.91	0.71 (0.59, 0.87)	0.64 (0.57, 0.71)	0.99 (0.72, 1.35)	0.94	1.16 (0.92, 1.45)	0.21
Pn 19A[2]	1.82 (1.21, 2.73)	1.69 (1.34, 2.13)	1.08 (0.67, 1.72)	0.76	1.05 (0.66, 1.69)	0.83	0.52 (0.36, 0.75)	0.45 (0.38, 0.53)	0.96 (0.59, 1.57)	0.87	1.24 (0.87, 1.78)	0.24
Pn 19F[2]	14.42 (10.19, 20.42)	10.78 (8.67, 13.40)	1.34 (0.87, 2.07)	0.19	1.31 (0.85, 1.99)	0.22	3.48 (2.63, 4.60)	3.02 (2.64, 3.45)	1.03 (0.69, 1.53)	0.90	1.21 (0.90, 1.62)	0.21
Pn 23F[2]	2.91 (1.87, 4.53)	2.19 (1.65, 2.90)	1.33 (0.76, 2.33)	0.32	1.24 (0.72, 2.16)	0.43	0.59 (0.44, 0.80)	0.56 (0.48, 0.65)	0.79 (0.50, 1.24)	0.30	1.04 (0.74, 1.47)	0.83

Vaccine antigen	Antibodies to 12-month vaccines measured 13 months of age					
	Intrapartum antibiotics (n=32) GMC (95% CI)	No intrapartum antibiotics (n=112) GMC (95% CI)	Unadjusted GMR (95% CI)	Two-sided p-value	Adjusted* GMR (95% CI)	Two-sided p-value
Measles[1]	3.66 (2.89, 4.64)	2.87 (2.30, 3.59)	0.69 (0.38, 1.27)	0.23	1.33 (0.86, 2.07)	0.20
Mumps[1]	47.61 (30.54, 74.23)	61.60 (47.99, 79.07)	0.57 (0.27, 1.18)	0.13	0.82 (0.53, 1.27)	0.37
Rubella[1]	**42.71 (25.43, 71.73)**	**55.59 (42.89, 72.05)**	**0.43 (0.20, 0.92)**	**0.03**	0.75 (0.47, 1.18)	0.21
MenC[2]	20.46 (14.89, 28.13)	16.87 (14.54, 19.57)	1.12 (0.64, 1.95)	0.70	1.23 (0.89, 1.70)	0.21
Hib[2]	14.73 (8.40, 25.83)	15.63 (11.67, 20.95)	1.03 (0.44, 2.44)	0.95	1.13 (0.62, 2.09)	0.68
Tetanus[1]	1.06 (0.69, 1.62)	0.97 (0.78, 1.22)	1.09 (0.68, 1.75)	0.72	1.16 (0.72, 1.89)	0.54

[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Table 3.11 Seroprotection rates at 7 and 13 months of age in infants who did or did not received antibiotics before their 6-weeks immunisation

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**Antibiotics** (n=4) **% (n); (95% CI)**	**No antibiotics** (n=87) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**	**Antibiotics** (n=29) **% (n); (95% CI)**	**No antibiotics** (n=278) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Diphtheria	0.1 IU/mL[1]	100 (4); (39.8, 100)	96.6 (84); (90.3, 99.3)	3.4 (-46.0, 9.7)	0.60	100 (29); (88.1, 100)	98.2 (273); (95.9, 99.4)	1.8 (-10.0, 4.1)	0.68
Tetanus	0.1 IU/mL[1]	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (16); (79.4, 100)[2]	100 (147); (97.5, 100)[2]	0 (-19.5, 2.6)[2]	-
PT	25 IU/mL	100 (4); (39.8, 100)	89.7 (78); (81.3, 95.2)	10.5 (-39.3, 18.6)	1	31.0 (9); (15.2, 50.8)	29.9 (83); (24.5, 35.6)	1.2 (-13.9, 20.2)	0.62
Hib	0.15 µg/mL	100 (4); (39.8, 100)	74.7 (65); (64.3, 83.4)	25.3 (-24.8, 35.4)	0.05	81.3 (13); (54.4; 96.0)[2]	76.2 (112); (68.5, 82.8)[2]	5.1 (-20.1, 19.9)[2]	0.36[2]
Polio type 1	0.23 IU/mL	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (29); (88.1, 100)	100 (278); (98.7, 100)	0 (-11.7, 1.4)	-
Polio type 2	0.29 IU/mL	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (29); (88.1, 100)	99.6 (277); (98.0; 100)	0.4 (-11.74, 2.0)	0.49
Polio type 3	0.12 IU/mL	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (29); (88.1, 100)	100 (278); (98.7, 100)	0 (-11.7, 1.4)	-
Pn 1	0.35 µg/mL	100 (4); (39.8, 100)	96.6 (84); (90.3, 99.3)	3.4 (-46.0, 9.7)	1	93.1 (27); (77.2, 99.2)	93.9 (261); (90.4, 96.4)	-0.8 (-16.0, 5.5)	0.35
Pn 3	0.35 µg/mL	100 (4); (39.8, 100)	98.9 (86); (93.8, 100)	1.1 (-48.2, 6.3)	1	79.3 (23); (58.9, 90.4)	68.7 (191); (62.9, 74.1)	10.6 (-7.9, 23.1)	0.90
Pn 4	0.35 µg/mL	75.0 (3); (19.4, 99.4)	69.0 (60); (58.1, 78.5)	6.0 (-40.4, 30.1)	0.65	17.2 (5); (5.5, 33.7)	23.4 (65); (18.5, 28.8)	-6.1 (-17.4, 11.8)	0.68
Pn 5	0.35 µg/mL	100 (4); (39.8, 100)	97.7 (85); (91.9, 99.7)	2.3 (-47.1, 8.0)	1	93.1 (27); (77.2, 99.2)	86.3 (240); (81.7, 90.1)	6.8 (-8.7, 13.9)	0.24
Pn 6A	0.35 µg/mL	100 (4); (39.8, 100)	97.7 (85); (91.9, 99.7)	2.3 (-47.1, 8.0)	1	82.8 (24); (64.2, 94.2)	87.1 (242); (82.5, 90.8)	-4.3 (-22.0, 6.5)	0.51
Pn 6B	0.35 µg/mL	75.0 (3); (19.4, 99.4)	89.7 (78); (81.3, 95.2)	-14.7 (-60.3, 7.7)	0.10	65.5 (19); (45.7, 82.1)	64.0 (178); (58.1, 69.7)	1.5 (-17.6, 17.3)	0.12
Pn 7F	0.35 µg/mL	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (29); (88.1, 100)	99.3 (276), (97.4, 99.9)	0.7 (-11.0, 2.6)	1
Pn 9V	0.35 µg/mL	100 (4); (39.8, 100)	97.7 (85); (91.9, 99.7)	2.3 (-47.1, 8.0)	1	82.8 (24); (64.2, 94.2)	75.2 (209); (69.7, 80.1)	7.6 (-10.4, 18.9)	0.18
Pn 14	0.35 µg/mL	100 (4); (39.8, 100)	90.8 (79); (82.7, 95.9)	9.2 (-40.4, 17.2)	1	86.2 (25); (68.3, 96.1)	86.3 (240); (81.7, 90.1)	-0.1 (-17.3, 9.5)	0.51
Pn 18C	0.35 µg/mL	75.0 (3); (19.4, 99.4)	96.6 (84); (90.3, 99.3)	-21.6 (-66.9, 0.3)	0.28	72.4 (21), (52.8, 87.3)	77.3 (215); (72.0, 82.1)	-4.9 (-23.7, 9.2)	0.66
Pn 19A	0.35 µg/mL	75.0 (3); (19.4, 99.4)	94.3 (82); (87.1, 98.1)	-19.3 (-64.7, 2.4)	0.24	51.7 (15), (32.5, 70.6)	49.6 (138); (43.6, 55.7)	2.0 (-16.3, 20.1)	0.42
Pn 19F	0.35 µg/mL	100 (4); (39.8, 100)	100 (87); (95.8, 100)	0 (-49.3, 4.3)	-	100 (29); (88.1, 100)	99.3 (276); (97.4, 99.9)	0.7 (-11, 2.6)	0.50
Pn 23F	0.35 µg/mL	100 (4); (39.8, 100)	93.1 (81); (85.6, 97.4)	6.9 (-42.6, 14.3)	0.68	51.7 (15), (32.5, 70.6)	62.2 (173), (56.2, 68.0)	-10.5 (-28.8, 7.5)	0.64
						Antibodies to 12-month vaccines measured 13 months of age			
						Antibiotics (n=13) **% (n); (95% CI)**	**No antibiotics** (n=131) **% (n); (95% CI)**	**Difference % (95% CI)**	**Two-sided p-value**
Measles	0.12 IU/mL					100 (13); (75.3, 100)	97.7 (128); (93.5, 99.5)	2.3 (-20.7, 6.5)	0.12
Mumps	45 IU/mL					46.2 (6); (19.2, 74.9)	61.8 (81); (52.9, 70.2)	-15.7 (-40.4, 10.7)	0.74
Rubella	10 IU/mL					76.9 (10); (46.2, 95.0)	87.8 (115); (80.9, 92.9)	-10.9 (-81.8, 8.5)	0.09
MenC	2 µg/mL					100 (13); (75.3, 100)	98.5 (129); (94.6, 99.8)	0.8 (-21.4, 5.4)	0.49
Hib	0.15 µg/mL					100 (13); (75.3, 100)	98.5 (128); (94.5, 99.8)	1.5 (-20.2, 5.5)	1
Tetanus	0.01 IU/mL					100 (13); (75.3, 100)	100 (131); (97.2, 100)	0 (-22.9, 2.9)	-

[1] 0.01 IU/mL at 13 months of age [2] includes only participants who have not had Hib-MenC

CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 3.12 GMCs and GMRs at 7 and 13 months of age in infants who did or did not received antibiotics before their 6-weeks immunisation

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	Antibiotics (n=4) **GMC (95% CI)**	**No antibiotics** (n=87) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**	**Antibiotics** (n=29) **GMC (95% CI)**	**No antibiotics** (n=278) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted** GMR (95% CI)**	**Two-sided p-value**
Diphtheria[1]	0.17 (0.07, 2.55)	0.40 (0.34, 0.47)	1.04 (0.47, 2.29)	0.92	0.91 (0.44, 1.89)	0.80	0.10 (0.07, 0.16)	0.08 (0.07, 0.08)	1.36 (0.92, 2.01)	0.12	1.30 (0.91, 1.86)	0.15
Tetanus[1]	1.44 (0.84, 2.46)	1.10 (0.94, 1.27)	1.31 (0.65, 2.66)	0.45	1.34 (0.67, 2.70)	0.40	0.42 (0.20, 0.87)[3]	0.44 (0.37, 0.53)[3]	0.96 (0.53, 1.74)[3]	0.88[3]	1.06 (0.60, 1.85)[3]	0.85[3]
PT[1]	82.19 (17.30, 390.41)	69.57 (58,71, 82.44)	1.18 (0.52, 2.67)	0.69	1.20 (0.56, 2.57)	0.63	18.99 (12.76, 28.26)	17.23 (15.29, 19.42)	1.10 (0.74, 1.63)	0.63	1.03 (0.72, 1.48)	0.87
FHA[1]	74.62 (14.35, 388.01)	63.27 (53.15, 75.32)	1.18 (0.51, 2.73)	0.70	1.15 (0.52, 2.51)	0.73	23.54 (16.90, 32.79)	18.46 (16.47, 20.69)	1.28 (0.88, 1.84)	0.19	1.18 (0.83, 1.68)	0.35
PRN[1]	80.66 (10.03, 648.31)	59.69 (48.75, 73.09)	1.35 (0.51, 3.60)	0.54	1.33 (0.49, 3.58)	0.57	16.22 (10.20, 25.77)	11.58 (10.04, 13.35)	1.40 (0.88, 2.23)	0.15	1.37 (0.88, 2.14)	0.16
Hib[2]	1.14 (0.17, 7.71)	0.49 (0.35, 0.69)	2.31 (0.47, 11.38)	0.30	1.86 (0.35, 1.26)	0.42	0.97 (0.28, 3.34)[3]	1.14 (0.78, 1.66)[3]	0.85 (0.26, 2.08)[3]	0.79[3]	0.96 (0.39, 2.37)[3]	0.93[3]
Polio type 1[1]	31.38 (1.99, 495.32)	34.77 (27.98, 43.19)	0.90 (0.31, 2.62)	0.85	0.83 (0.28, 2.49)	0.74	8.57 (5.54, 13.27)	11.59 (10.07, 13.13)	0.75 (0.48, 1.15)	0.18	0.72 (0.47, 1.10)	0.12
Polio type 2[1]	43.69 (1.68, 1134.3)	64.05 (51.63, 79.47)	0.68 (0.23, 2.00)	0.48	0.76 (0.26, 2.24)	0.62	14.61 (9.74, 21.93)	22.50 (19.34, 26.18)	0.65 (0.40, 1.06)	0.08	0.63 (0.39, 1.01)	0.06
Polio type 3[1]	15.87 (1.23, 204.86)	21.73 (17.20, 27.46)	0.73 (0.22, 2.27)	0.58	0.75 (0.23, 2.40)	0.62	6.68 (4.33, 10.32)	9.16 (7.84, 10.71)	0.73 (0.44, 1.20)	0.31	0.76 (0.47, 1.24)	0.27
Pn 1[2]	6.93 (1.41, 33.92)	4.28 (3.44, 5.32)	1.62 (0.57, 4.57)	0.36	1.32 (0.51, 3.47)	0.56	1.16 (0.78, 1.72)	1.17 (1.06, 1.29)	0.99 (0.71, 1.38)	0.95	0.99 (0.71, 1.38)	0.97
Pn 3[2]	1.65 (0.30, 9.00)	1.42 (1.24, 1.64)	1.16 (0.58, 2.30)	0.67	1.06 (0.53, 2.14)	0.87	0.54 (0.39, 0.75)	0.54 (0.49, 0.59)	1.00 (0.74, 1.37)	0.99	1.01 (0.74, 1.37)	0.95
Pn 4[2]	0.66 (0.21, 2.06)	0.51 (0.43, 0.61)	1.29 (0.55, 3.03)	0.56	1.19 (0.52, 2.71)	0.68	0.20 (0.15, 0.25)	0.22 (0.20, 0.23)	0.91 (0.70, 1.18)	0.48	0.93 (0.71, 1.19)	0.53
Pn 5[2]	6.62 (1.81, 24.21)	3.05 (2.52, 3.70)	2.17 (0.87, 5.44)	0.10	1.75 (0.72, 4.25)	0.21	0.93 (0.65, 1.33)	0.92 (0.82, 1.03)	1.01 (0.71, 1.44)	0.96	0.98 (0.69, 1.39)	0.90
Pn 6A[2]	4.16 (0.77, 22.60)	4.85 (4.04, 5.82)	0.86 (0.36, 2.07)	0.73	0.73 (0.32, 1.65)	0.44	0.90 (0.58, 1.40)	1.01 (0.90, 1.14)	0.89 (0.61, 1.30)	0.55	0.85 (0.59, 1.23)	0.39
Pn 6B[2]	2.19 (0.03, 163.57)	1.90 (1.39, 2.60)	1.15 (0.25, 5.39)	0.86	0.93 (2.04, 4.19)	0.92	0.52 (0.31, 0.88)	0.52 (0.45, 0.59)	1.01 (0.64, 1.58)	0.97	0.97 (0.62, 1.50)	0.88
Pn 7F[2]	8.85 (2.69, 29.18)	6.03 (5.11, 7.11)	1.47 (0.67, 3.23)	0.34	1.36 (0.65, 2.84)	0.40	1.89 (1.33, 2.69)	1.88 (1.72, 2.05)	1.01 (0.75, 1.36)	0.95	0.97 (0.73, 1.30)	0.85
Pn 9V[2]	3.50 (1.18, 10.40)	2.42 (2.02, 2.90)	1.45 (0.61, 3.42)	0.39	1.17 (0.53, 2.56)	0.69	0.63 (0.46, 0.85)	0.60 (0.54, 0.66)	1.05 (0.75, 1.47)	0.77	1.03 (0.74, 1.44)	0.84
Pn 14[2]	3.71 (0.42, 32.35)	2.63 (2.01, 3.43)	1.41 (0.39, 5.04)	0.59	1.28 (0.35, 4.64)	0.70	1.11 (0.66, 1.88)	1.04 (0.93, 1.17)	1.07 (0.72, 1.58)	0.75	1.05 (0.71, 1.56)	0.79
Pn 18C[2]	2.87 (0.29, 28.05)	2.94 (2.42, 3.58)	0.98 (0.38, 2.55)	0.96	0.87 (0.34, 2.28)	0.78	0.64 (0.44, 0.92)	0.65 (0.59, 0.72)	0.97 (0.71, 1.35)	0.88	0.95 (0.69, 1.30)	0.74
Pn 19A[2]	4.01 (0.27, 58,78)	1.65 (1.37, 2.00)	2.43 (0.94, 6.28)	0.07	2.04 (0.80, 5.22)	0.14	0.45 (0.28, 0.72)	0.45 (0.40, 0.54)	0.96 (0.58, 1.59)	0.88	0.98 (0.59, 1.60)	0.92
Pn 19F[2]	20.83 (2.54, 171.12)	11.22 (9.34, 13.48)	1.86 (0.76, 4.54)	0.17	1.60 (0.68, 3.76)	0.28	3.11 (2.01, 4.81)	3.11 (2.74, 3.52)	1.00 (0.66, 1.51)	1.00	0.98 (0.65, 1.48)	0.94
Pn 23F[2]	6.71 (0.80, 56.00)	2.23 (1.76, 2.82)	3.02 (0.97, 9.37)	0.06	2.40 (0.81, 7.13)	0.11	0.44 (0.26, 0.73)	0.58 (0.50, 0.67)	0.75 (0.46, 1.21)	0.24	0.72 (0.45, 1.16)	0.18

Vaccine antigen	Antibodies to 12-month vaccines measured 13 months of age					
	Antibiotics (n=13) **GMC (95% CI)**	**No antibiotics** (n=131) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**Two-sided p-value**	**Adjusted* GMR (95% CI)**	**Two-sided p-value**
Measles[1]	2.00 (1.08, 3.72)	3.16 (2.61, 3.82)	0.63 (0.34, 1.18)	0.15	0.65 (0.34, 1.26)	0.20
Mumps[1]	36.46 (18.28, 72.72)	60.93 (48.48, 76.58)	0.60 (0.28, 1.27)	0.18	1.28 (0.66, 2.48)	0.47
Rubella[1]	**21.73 (11.24, 42.02)**	**57.22 (44.95, 72.84)**	**0.38 (0.17, 0.84)**	**0.02**	0.73 (0.37, 1.44)	0.36
MenC[2]	20.17 (13.26, 30.68)	17.37 (15.06, 20.05)	1.16 (0.73, 1.86)	0.53	0.98 (0.61, 1.60)	0.95
Hib[2]	14.33 (4.53, 45.34)	15.54 (11.94, 20.24)	0.92 (0.38, 2.26)	0.86	0.95 (0.38, 2.38)	0.92
Tetanus[1]	1.40 (0.62, 3.15)	0.96 (0.78, 1.18)	1.46 (0.74, 2.89)	0.28	1.30 (0.63, 2.69)	0.48

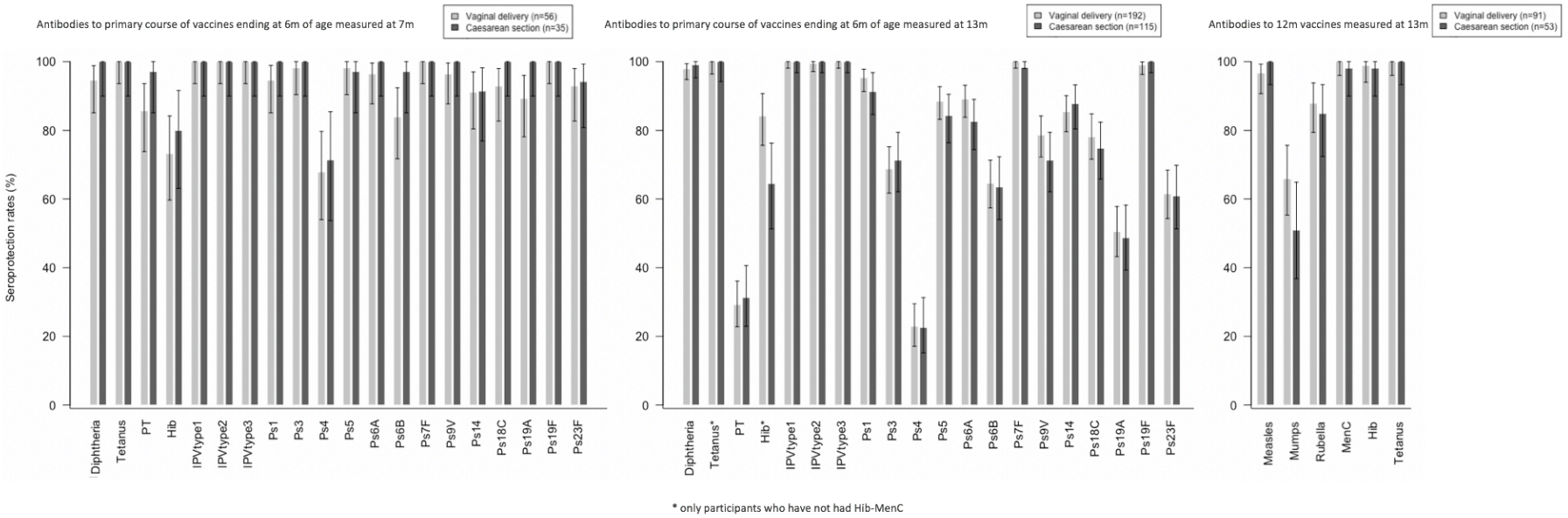
[1] IU/ml [2] µg/ml [3] includes only participants who have not had Hib-MenC

*adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

**adjusted for maternal dTpa (diphtheria-tetanus-acellular pertussis) and trivalent influenza vaccine during pregnancy, gestational age, sex, BCG and MMR immunisation status, age at first DTPa-HepB-IPV-Hib / PCV13 immunisation, age at sampling and time between immunisation and sampling using multiple linear regression

BCG = Bacillus Calmette-Guérin, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MMR = measles-mumps-rubella, MenC = meningococcus C, Pn = pneumococcal serotype, PRN = pertactin, PT = pertussis toxin

Figure 3.1 Seroprotection rates and geometric mean antibody concentrations (GMCs) in infants born vaginally or by Caesarean section



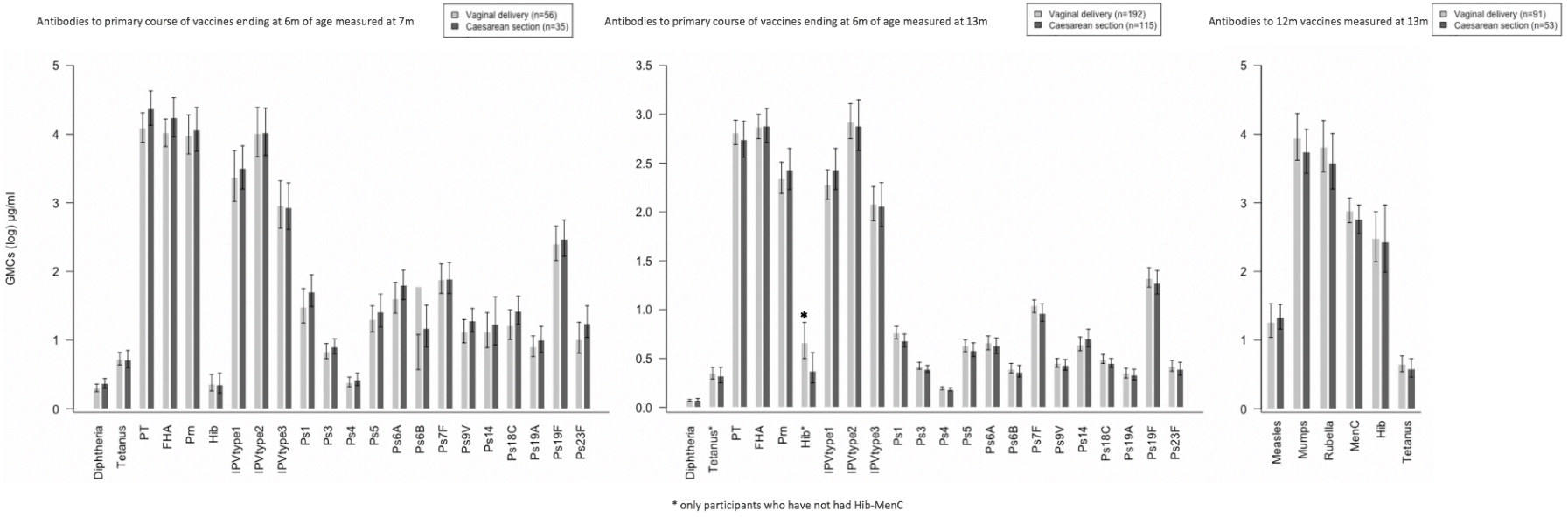


Figure 3.2 Seroprotection rates and GMCs infants who did or did not receive formula milk before discharge from birth

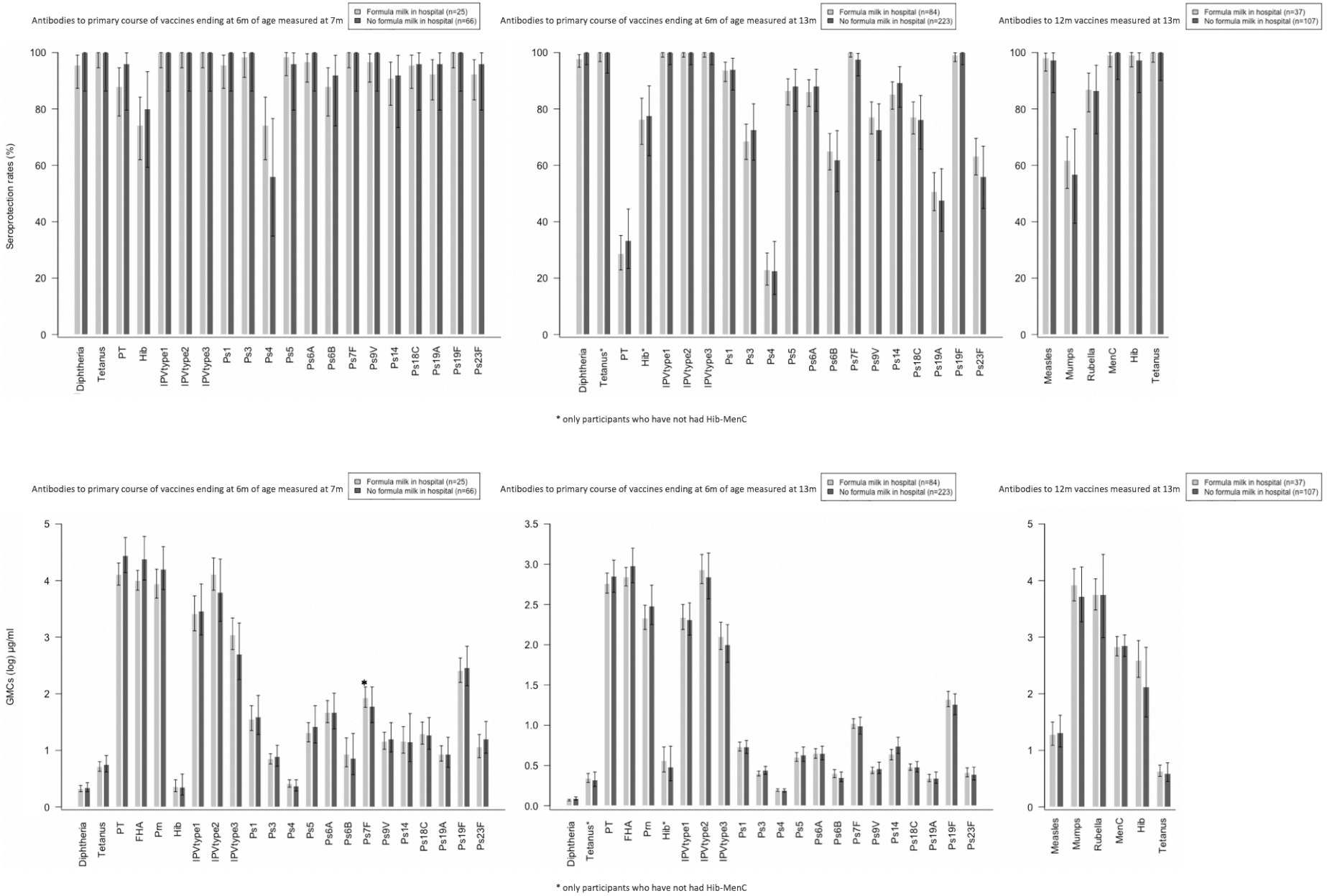
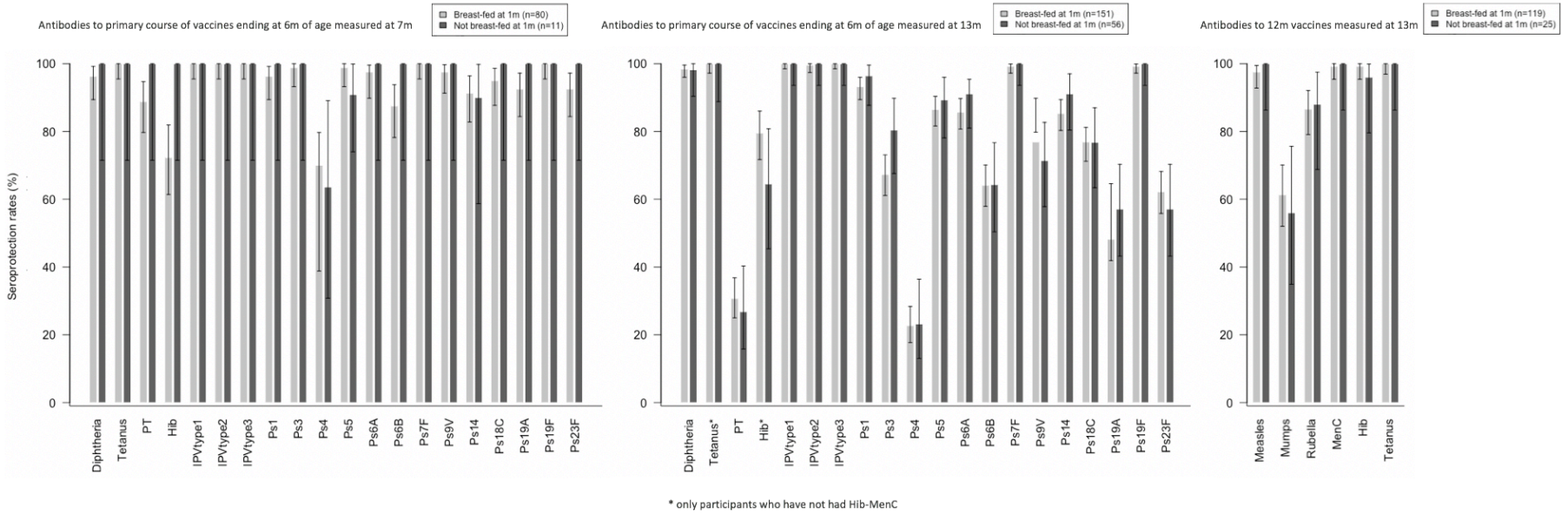
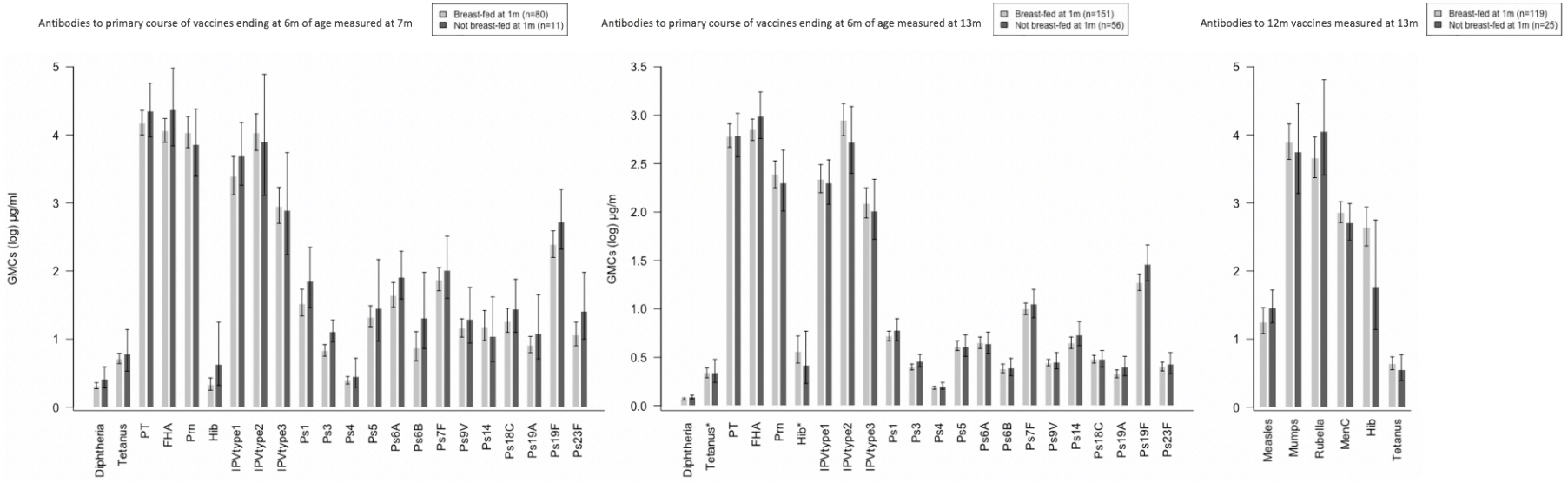


Figure 3.3 Seroprotection rates and GMCs in infants who were or were not still breastfed at 1 month of age

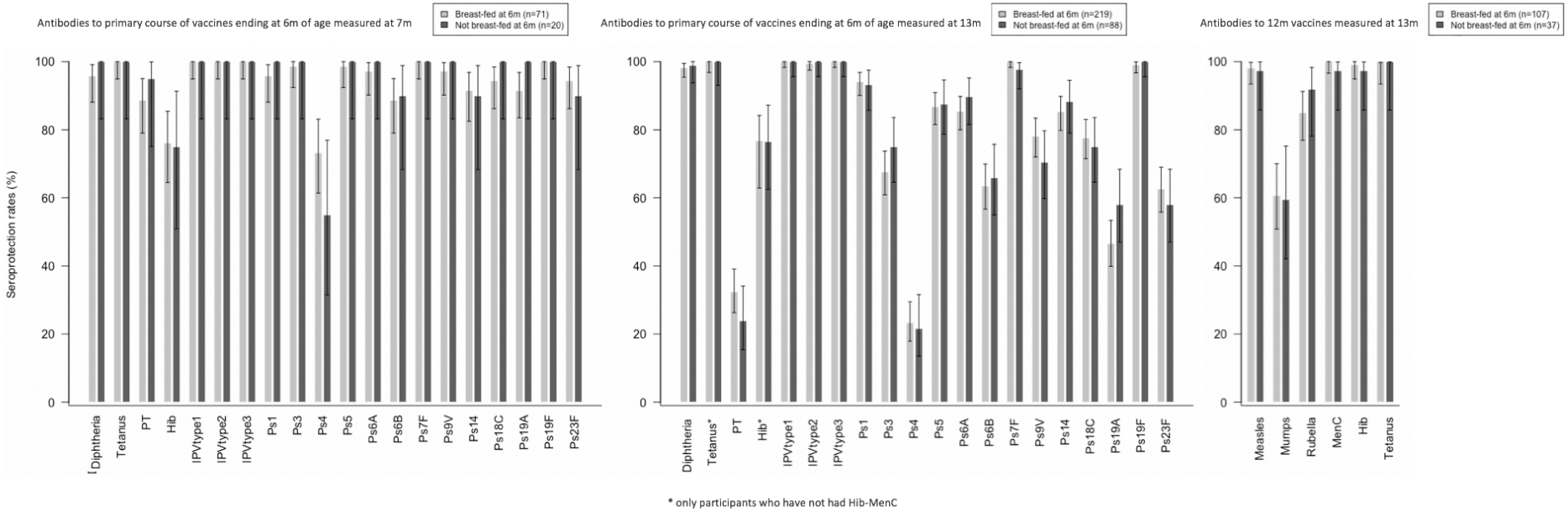


* only participants who have not had Hib-MenC

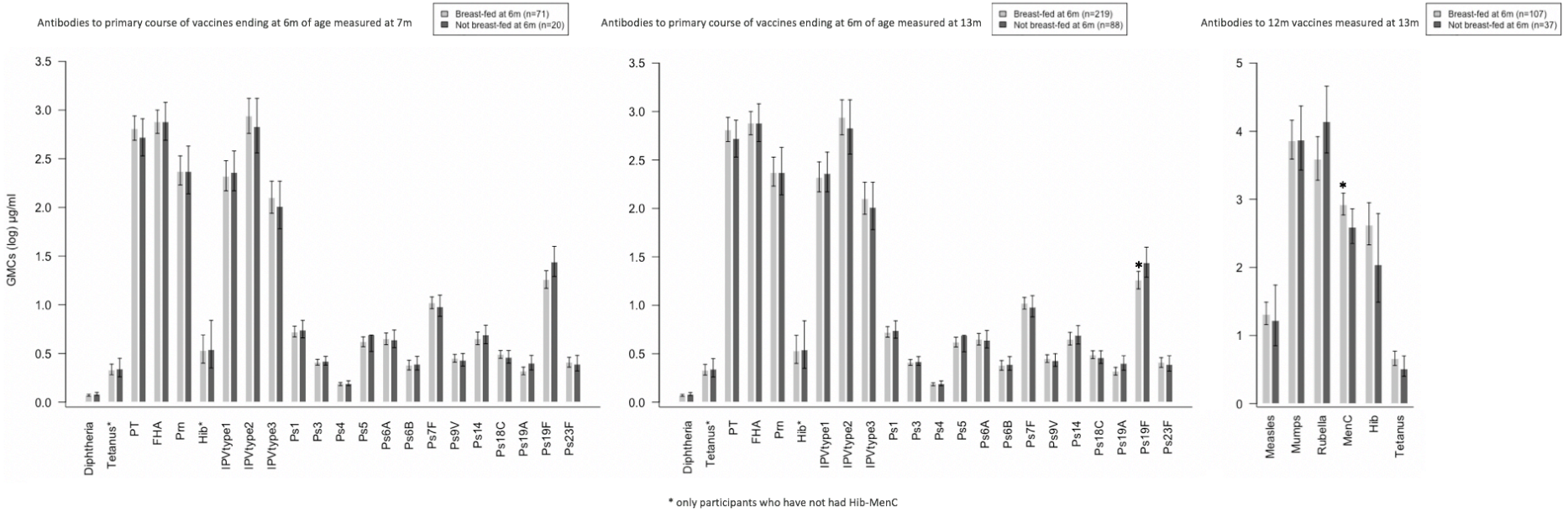


* only participants who have not had Hib-MenC

Figure 3.4 Seroprotection rates and GMCs in infants who were or were not still breastfed at 6 months of age



* only participants who have not had Hib-MenC



* only participants who have not had Hib-MenC

Figure 3.5 Seroprotection rates and GMCs in infants whose mothers did or did not receive antibiotics during delivery

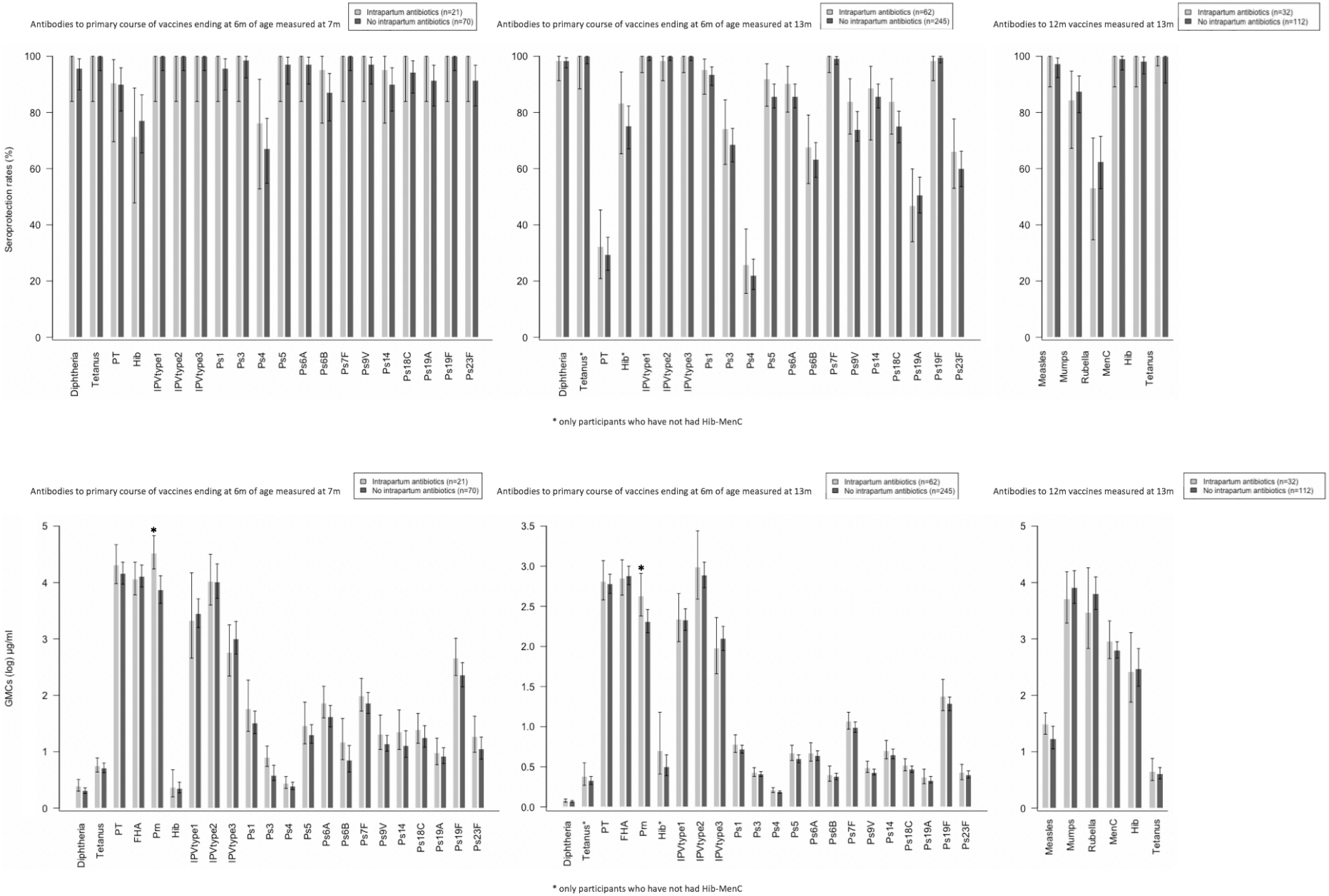
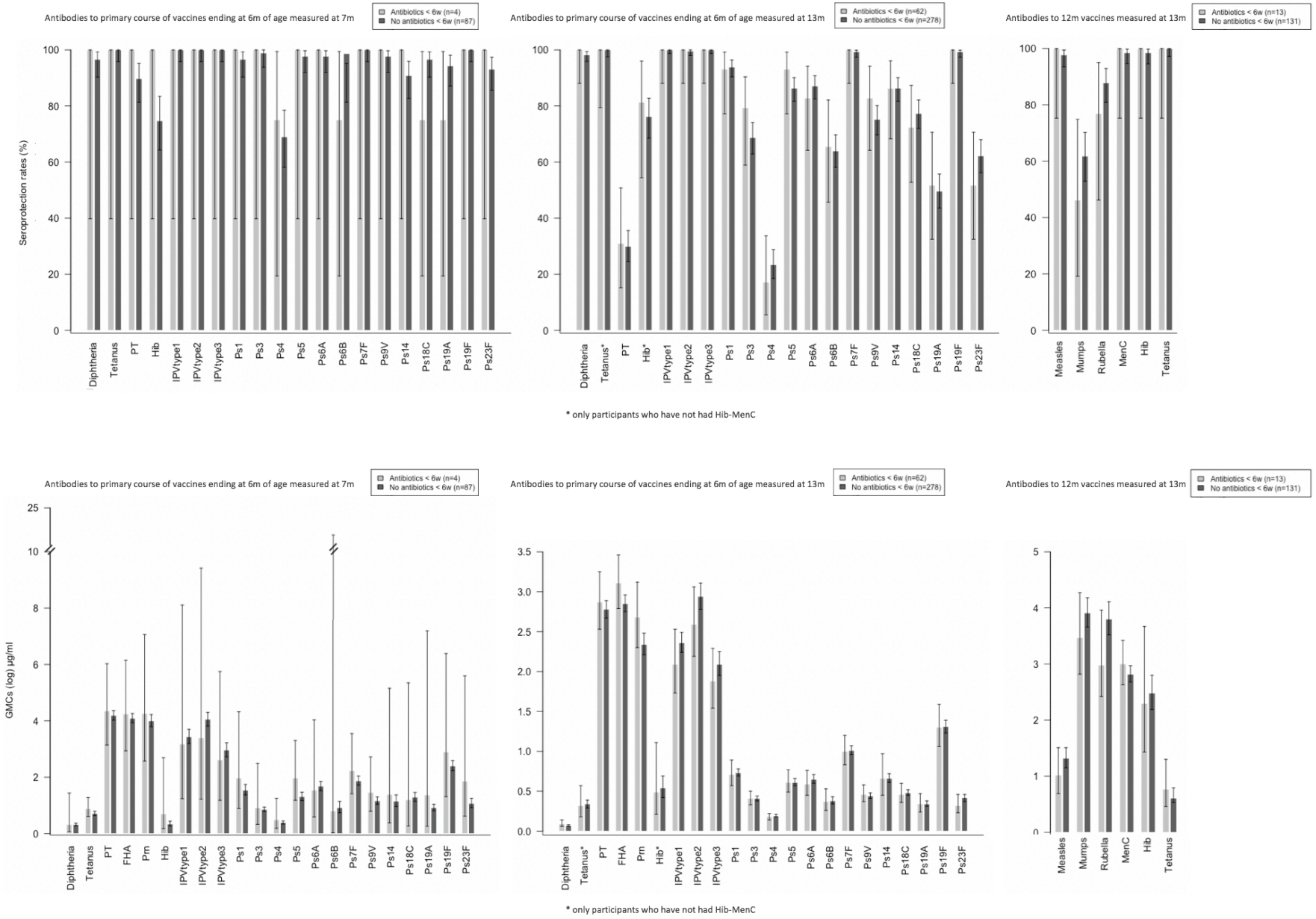


Figure 3.6 Seroprotection rates and GMCs in infants who did or did not receive antibiotics before their 6-weeks immunisation



CHAPTER 4. THE INFLUENCE OF NEONATAL BACILLUS CALMETTE-GUÉRIN (BCG) IMMUNSATION ON HETEROLGOUS VACCINE RESPONSES IN INFANTS

4.1 SYSTEMATIC REVIEW

This literature review was done and published while our study on the influence of BCG at birth on heterologous vaccine responses in infants was being designed. It summarises studies that have investigated the influence of previous or co-administered BCG on heterologous vaccine antibody responses in children and adults.

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The influence of neonatal Bacille Calmette-Guérin (BCG) immunisation on heterologous vaccine responses in infants

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ABSTRACT

Introduction: Bacillus Calmette-Guérin vaccine (BCG), one of the most widely used vaccines, does not only provide protection against tuberculosis and other mycobacterial infections, but also has non-specific (heterologous) immunomodulatory effects. In participants in a randomised trial, we investigated the effect of neonatal BCG immunisation on antibody responses to routine infant vaccines given in the first year of life.

Methods: Antibodies against antigens in the diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b (Hib), and the 13-valent pneumococcal conjugate vaccines were measured in 91 (45 BCG-vaccinated, 46 BCG-naïve) infants one month after, and in 310 (169 BCG-vaccinated, 141 BCG-naïve) infants seven months after immunisation at 6 weeks, 4 and 6 months of age. In addition, antibodies against meningococcus C, Hib, measles, mumps, and rubella were measured in 147 (78 BCG-vaccinated, 69 BCG-naïve) infants one month after immunisation at 12 months of age. The seroprotection rates for each vaccine and the geometric mean concentrations (GMC) of antibodies were compared in BCG-vaccinated and BCG-naïve infants.

Results: At 7 months of age, seroprotection rates were high in both BCG-vaccinated and BCG-naïve infants. At 13 months of age, seroprotection rates were lower than at 7 months of age, particularly for pertussis and a number of pneumococcal antigens, with generally higher rates for the latter in BCG-vaccinated infants. Although not statistically significant, antibody responses in BCG-vaccinated infants were consistently higher against diphtheria, tetanus, and pneumococcal antigens at both 7 and 13 months of age, and against measles and mumps at 13 months of age, but were lower against Hib one month after immunisation at both 7 and 13 months of age.

Conclusion: The immunomodulatory effect of BCG on antibody responses to heterologous vaccines adds to the evidence that BCG immunisation at birth has broad heterologous effects on the infant immune system.

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1. Introduction

Bacillus Calmette-Guérin (BCG) vaccine, is given to over 160 million infants each year, making it one of the most widely used vaccines worldwide. The vaccine is primarily considered to protect

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against tuberculosis (TB), particularly infant TB [1,2] and other mycobacterial infections [3]. However, BCG has powerful non-specific (heterologous) immunomodulatory effects, which are of particular importance for the early development of the immune system [4,5]. Randomised trials have shown that BCG reduces childhood mortality from causes other than TB by approximately one quarter in developed countries [6] and by approximately half in high-mortality settings [7–9]. The reduced mortality is attributed to decreased deaths from pneumonia, sepsis and diarrhoea [7,10,11]. Additionally, BCG immunisation is reported to decrease hospitalisation rates due to non-TB respiratory infections and sepsis [12,13]. Moreover, the immunomodulatory effects of BCG might decrease the risk for atopic dermatitis [14] and allergies [15]; are proposed to beneficial in the treatment of multiple sclerosis [16] and diabetes mellitus [17]; and are used in the prevention and treatment of bladder cancer [18], melanoma [19], and leukaemia [20].

The immunomodulatory effects of BCG also influence responses to heterologous immunisations [21]. Previous or concurrent administration of BCG with other vaccines has been associated with significantly higher concentrations of antibodies against hepatitis B (HepB), polio, pneumococcus and influenza [22–26], but not with altered antibody responses to diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b (Hib) and typhoid [23,24,27,28]. One study reported lower antibodies concentrations against HepB in infants who were vaccinated with BCG compared to BCG-naïve infants [23].

Using samples from participants in a randomised trial, we investigated the effect of BCG immunisation at birth on humoral responses to routine infant immunisations given in the first year of life.

2. Methods

2.1. Study design and population

Participants were a subset of infants from The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR). This randomised trial comprises 1272 healthy infants recruited antenatally from 2013 to 2016 to investigate whether BCG immunisation at birth protects against childhood infection, allergy and asthma. Inclusion criteria were: greater than 32 weeks gestation, birth weight greater than 1500 g, mother not HIV positive, absence of symptoms or signs of illness, and no known contact with TB. Twins of participants were excluded, as they were not considered independent. Three-monthly parent questionnaires were used to prospectively collect demographic and other data.

2.2. Intervention

Infants in MIS BAIR were randomised within 10 days of birth to either receive BCG-Denmark (Statens Serum Institut, Copenhagen) 0.05 ml intradermally in the deltoid region of the left arm (BCG-vaccinated) or no BCG (BCG-naïve). The infants included in this study were not randomised specifically for the measurement of vaccine responses, but were a subset of the randomised participants.

2.3. Routine immunisation

All infants received routine immunisations according to the Australian National Immunisation Program: at birth: intramuscular HepB vaccine (H-B-Vax II Paediatric® (*bioCSL*)); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa)-HepB-inactivated polio (IPV)-Hib vaccine (Infanrix® hexa (*GlaxoSmithKline*)), intramuscular 13-valent conjugate pneumococcal vaccine (PCV13) (Prevenar13® (*Wyeth*)), and oral rotavirus vaccine (RotaTeq® (*Merck*)); at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (Priorix® (*GlaxoSmithKline*)) and intramuscular combined meningococcal C (MenC) and Hib vaccine (Menitorix® (*GlaxoSmithKline*)). Vaccine records were obtained from individual immunisation records and/or the Australian Immunisation Register.

2.4. Blood collection

From the subset of participants whose parent/guardian provided consent, blood samples were collected at study visits one month after the scheduled 6-month routine vaccines ('7 months') and the scheduled 12-month routine vaccines ('13 months') in tubes containing sodium-heparin (S-monovette® (*Sarstedt*)). Only participants who had a blood sample taken 28 ± 14 days after their 6-month and/or 12-month routine vaccinations were included in the analysis of immediate post-vaccination responses. Plasma was stored at −80 °C until analysis.

2.5. Antibody measurement

Plasma samples were analysed at the National Institute for Health and Environment, in Bilthoven, the Netherlands. Immunoglobulin (Ig) G antibodies were measured using fluorescent bead-based multiplex immune-assays (Luminex xMAP technology) [29–34] against 26 vaccine antigens: (diphtheria, tetanus, pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), meningococcus type C (MenC), measles, mumps and rubella). In all assays, international or in-house reference controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

2.6. Statistical analysis

The proportion of infants with an antibody concentration above the standard protective correlate value for each vaccine was calculated (seroprotection rate) for the BCG-vaccinated and BCG-naïve groups [32,35,36]. The Clopper-Pearson method was used to estimate the 95% confidence intervals (CIs) of the seroprotection rates. For FHA and PRN, no accepted protective correlate value exists. Proportions were compared using Fisher's exact test and 95% confidence interval (CI) for differences in proportions estimated. In each group, the geometric mean concentration (GMC) for each vaccine antibody (IgG) was calculated. Geometric mean ratios (GMR) with 95% CI were derived from the anti-logged coefficient using a linear regression with log-concentrations as outcome and BCG immunisation status as covariate. Effect modification was assessed using the following pre-specified factors: maternal BCG immunisation status, maternal dTpa (*Boostrix*® (GlaxoSmithKline)) immunisation in pregnancy, gestational age, delivery mode, sex, birth weight, age at randomisation (pre-specified as < 48 h versus ≥ 48 h), age at first DTPa-HepB-IPV-Hib/PCV13 vaccination, MMR immunisation status (for responses at 13 months of age), age at blood sampling and time between 6-month or 12-month immunisation and blood sampling. The GMR for each vaccine antigen was adjusted for those factors that both differed between groups and had an effect on that vaccine antigen response. As the Hib-MenC vaccine includes a tetanus toxoid (as carrier protein) and a Hib component, infants who had received this vaccine before blood sampling at 13 months of age were excluded from the

analysis of persistence of antibodies against these two vaccine antigens. A 5%-significance level was used. All statistical analyses were done using R version 3.4.3.

2.7. Ethics

Informed consent was obtained from participants' parents or legal guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation (33025). MIS BAIR is registered with the Australia & New Zealand Clinical Trials Registry (1051228) and the U.S. National Institutes of Health (NCT01906853).

3. Results

Of the 471 potentially eligible participants, seven were excluded because they were a twin of another participant, four because they were not vaccinated according to the standard schedule, one because he received BCG outside the study and one because vaccine records were not available (Fig. 1). Of the remaining participants, 90 were excluded because their blood samples were not taken 28 ± 14 days after their 6-month and 12-month routine vaccines. For the final analysis, at 7 months of age, 91 (45 BCG-vaccinated and 46 BCG-naïve) participants were included for measurement of antibodies following their primary course of vaccines ending at 6 months of age. At 13 months of age, 147 (78 BCG-vaccinated and 69 BCG-naïve) were included for measurement of antibodies following their 12-month vaccines. In addition, at 13 months of age, a total of 310 (169 BCG-vaccinated and 141 BCG-naïve) participants had their samples analysed for persistence of antibodies following their primary course of vaccines. For these participants, blood was taken 7 months ± 23 days after their 6-month vaccinations. Baseline characteristics of participants are summarised in Table 1.

3.1. The effect of BCG immunisation on seroprotection rates

One month after immunisation, the large majority of infants had antibody concentrations above the standard protective correlate, except for Hib and pneumococcus serotype 4 at 7 months of age, and mumps at 13 months of age. In contrast, at 13 months of age, for immunisations given in the first 6 months, a high proportion of infants did not have antibody concentrations above the protective correlate, particularly for PT and pneumococcus serotype 4 (Table 2). There was no consistent difference in seroprotection rates between BCG-vaccinated and BCG-naïve infants at either 7 or 13 months of age. Although there was a suggestion of higher seroprotection rates in BCG-vaccinated infants for some

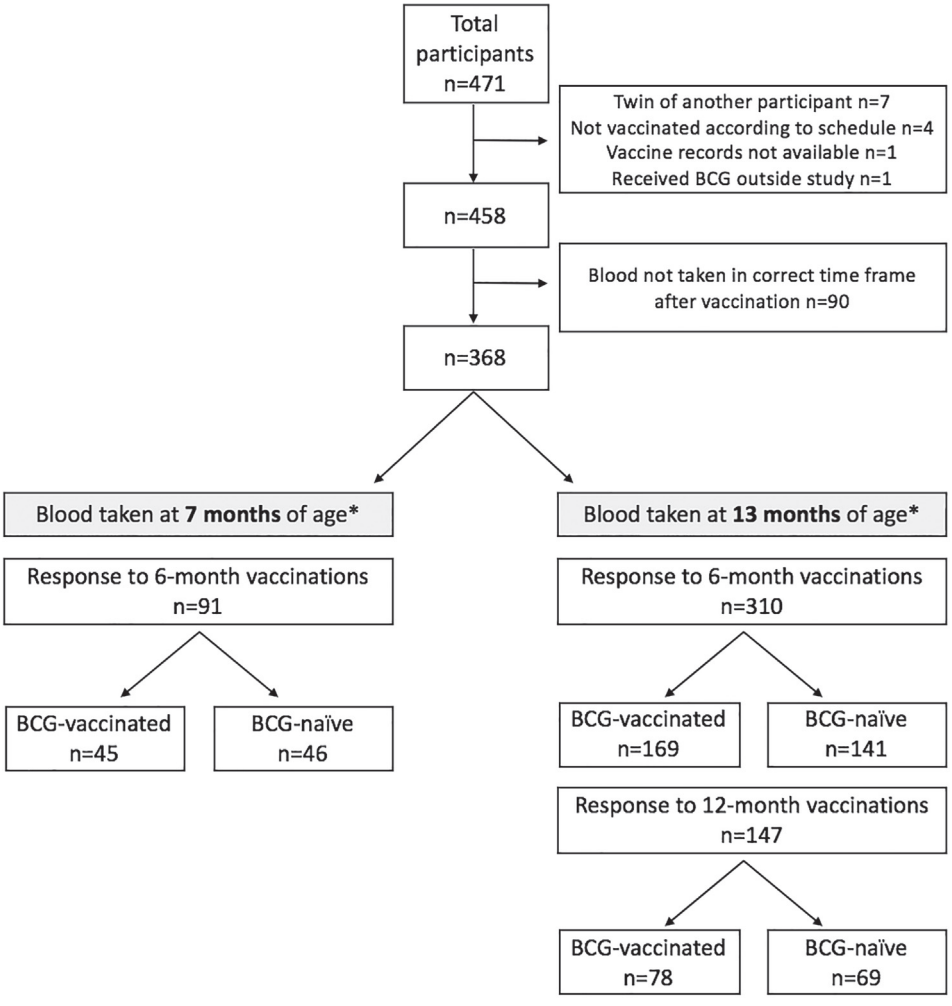


Fig. 1. Selection of participants.

Table 1
Characteristics of participants.

	Samples at 7 months of age for antibodies to primary course of vaccines ending at 6 months of age			Samples at 13 months of age for persistence of antibodies to primary course of vaccines ending at 6 months of age			Samples at 13 months of age for antibodies to 12-month vaccines		
	BCG-vaccinated (n = 45) n (%) or median (IQR)	BCG-naïve (n = 46) n (%) or median (IQR)	p-value	BCG-vaccinated (n = 169) n (%) or median (IQR)	BCG-naïve (n = 141) n (%) or median (IQR)	p-value	BCG-vaccinated (n = 78) n (%) or median (IQR)	BCG-naïve (n = 69) n (%) or median (IQR)	p-value
Mother BCG-vaccinated	13 (30)[1]	9 (21)[2]	0.48	35 (22)[3]	30 (22)[4]	0.92	22 (29)[5]	12 (18)[2]	0.24
Maternal dTpa in pregnancy	18 (40)	28 (61)	0.25	85 (50)	61 (43)	0.46	39 (50)	30 (43)	0.63
Gestational age (weeks)	39.1 (38.5–40.4)	40.0 (38.7–40.5)	0.46	39.4 (38.5–40.3)	39.2 (38.3–40.3)	0.25	39.4 (38.5–40.5)	39.2 (38.3–40.3)	0.23
Caesarean section	21 (47)	14 (30)	0.29	62 (37)	55 (39)	0.78	27 (35)	27 (39)	0.70
Sex (male)	20 (44)	23 (50)	0.75	79 (47)	72 (51)	0.66	36 (46)	36 (52)	0.67
Birth weight (kg)	3.44 (3.06–3.62)	3.49 (3.22–3.81)	0.44	3.46 (3.14–3.79)	3.42 (3.12–3.75)	0.27	3.45 (3.13–3.68)	3.36 (3.14–3.64)	0.48
Age at randomisation (hours)	33.5 (20.4–53.7)	31.1 (19.6–58.1)	0.87	26.2 (17.3–51.1)	25.6 (19.3–56.3)	0.31	24.9 (16.9–47.8)	26.5 (19.6–50.8)	0.32
MMR/Hib-MenC-vaccinated	–	–		110 (65)	110 (71)	0.30	78 (1 0 0)	69 (1 0 0)	1
Age at routine immunisation (days)									
– 6-week vaccines	44 (42–48)	45 (43–47)[6]	0.94	46 (43–50)	45 (43–49)	0.94	45 (43–48)	45 (43–48)	0.77
– 4-month vaccines	123 (118–128)	123 (115–126)	0.16	125 (120–133)	124 (121–131)	0.12	124 (119–132)	124 (122–130)	0.71
– 6-month vaccines	188 (183–194)	186 (178–193)	0.12	191 (185–208)	191 (184–200)	0.07	190 (184–207)	190 (185–197)	0.14
– 12-month vaccines	–	–		377 (371–385)	374 (368–381)	0.37	377 (370–385)	374 (368–381)	0.09
Interval between (days)									
– 6-month vaccines to 7-month blood sample	30 (24–36)	26 (20–38)	0.40	–	–		–	–	
– 6-month vaccines to 13-month blood sample	–	–		209 (189–223)	210 (191–227)	0.05	–	–	
– 12-month vaccines to 13-month blood sample	–	–		–	–		30 (24–35)	27 (21–37)	0.45
Age at blood sampling (days)	222 (210–227)	216 (206–226)	0.05	402 (386–416)	400 (389–418)	0.24	409 (399–415)	402 (394–412)	0.07

p-values were determined by *t*-test for continuous variables and Pearson's Chi-squared test for categorical variables.
BCG = Bacille Calmette-Guérin vaccine, dTpa = diphtheria-tetanus-acellular pertussis vaccine.
[1] 1 mother with unknown BCG status.
[2] 3 mothers with unknown BCG status.
[3] 7 mothers with unknown BCG status.
[4] 6 mothers with unknown BCG status.
[5] 2 mothers with unknown BCG status.
[6] Date not available for 1 participant.

Table 2
Seroprotection rates in participants at 7 and 13 months of age.

Vaccine antigen	Protective correlate	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
		BCG-vaccinated (n = 45) % (n); (95% CI)	BCG-naïve (n = 46) % (n); (95% CI)	Difference % (95% CI)	p-value	BCG-vaccinated (n = 169) % (n); (95% CI)	BCG-naïve (n = 141) % (n); (95% CI)	Difference % (95% CI)	p-value
Diphtheria	0.1 IU/mL[1]	100 (45); (92.1, 100)	93.5 (43); (82.1, 98.6)	6.5 (−1.7, 17.6)	0.24	98.8 (167); (95.6, 99.9)	97.9 (138); (93.9, 99.6)	0.9 (−2.3, 5.0)	0.66
Tetanus	0.1 IU/mL[1]	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (93); (96.1, 100)[2]	100 (73); (95.0, 100)[2]	0 (−4.0, 5.0)[2]	–
PT	25 IU/mL	88.9 (40); (75.9, 96.3)	91.3 (42); (79.2, 97.8)	−2.4 (−16.2, 11.0)	0.74	30.2 (51); (23.4, 37.7)	30.5 (43); (23.0, 38.8)	−0.3 (−10.7, 9.9)	1
Hib	0.15 μg/mL	71.1 (32); (55.7, 83.6)	80.4 (37); (66.1, 90.6)	−9.3 (−26.9, 8.5)	0.34	73.1 (68); (62.9, 81.8)[2]	80.8 (59); (70.0, 89.1)[2]	−7.7 (−20.3, 5.5)[2]	0.27
Polio type 1	0.23 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	100 (141); (97.4, 100)	0 (−2.2, 2.6)	–
Polio type 2	0.29 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	99.4 (168); (96.7, 100)	100 (141); (97.4, 100)	−0.6 (−3.3, 2.1)	1
Polio type 3	0.12 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	100 (141); (97.4, 100)	0 (−2.2, 2.6)	–
Pn 1	0.35 μg/mL	97.8 (44); (88.2, 99.9)	95.7 (44); (85.2, 99.5)	2.0 (−7.8, 12.7)	1	92.3 (156); (87.2, 95.8)	95.0 (134); (90.0, 98.0)	−2.7 (−8.5, 3.1)	0.36
Pn 3	0.35 μg/mL	97.8 (44); (88.2, 99.9)	100 (46); (92.2, 100)	−2.2 (−11.6, 5.7)	0.49	71.6 (121); (64.2, 78.3)	67.4 (95); (59.0, 75.0)	4.2 (−6.0, 14.6)	0.46
Pn 4	0.35 μg/mL	77.8 (35); (62.9, 88.8)	60.9 (28); (45.4, 74.9)	16.9 (−2.2, 35.0)	0.11	26.0 (44); (19.6, 33.3)	19.9 (28); (13.6, 27.4)	6.2 (−3.3, 15.4)	0.23
Pn 5	0.35 μg/mL	97.8 (44); (88.2, 99.9)	97.8 (45); (88.5, 99.9)	0 (−9.7, 9.4)	1	87.0 (147); (81.0, 91.7)	87.2 (123); (80.6, 92.3)	−0.3 (−7.8, 7.6)	1
Pn 6A	0.35 μg/mL	100 (45); (0.92, 100)	95.7 (44); (0.85, 99.5)	4.3 (−3.8, 14.6)	0.49	87.6 (148); (81.6, 92.1)	85.1 (85); (78.1, 90.5)	2.5 (−5.2, 10.5)	0.62
Pn 6B	0.35 μg/mL	91.1 (41); (78.8, 97.5)	87.0 (40); (73.7, 95.1)	4.2 (−9.7, 18.3)	0.74	67.5 (114); (59.8, 74.5)	60.3 (120); (51.7, 68.4)	7.2 (−3.5, 17.9)	0.19
Pn 7F	0.35 μg/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	98.6 (139); (95.0, 99.8)	1.4 (−0.8, 5.0)	0.21
Pn 9V	0.35 μg/mL	100 (45); (92.1, 100)	95.7 (44); (85.2, 99.5)	4.3 (−3.8, 14.6)	0.49	78.1 (132); (71.1, 84.1)	73.0 (103); (64.9, 80.2)	5.1 (−4.5, 14.8)	0.35
Pn 14	0.35 μg/mL	91.1 (41); (78.8, 97.5)	91.3 (42); (79.2, 97.6)	−0.2 (−13.4, 12.9)	1	86.4 (146); (80.3, 91.2)	86.5 (122); (79.8, 91.7)	−0.1 (−7.8, 7.8)	1
Pn 18C	0.35 μg/mL	97.8 (44); (88.2, 99.9)	93.5 (43); (82.1, 98.6)	4.3 (−5.9, 15.7)	0.62	75.7 (128); (68.6, 82.0)	78.0 (110); (70.3, 84.5)	−2.3 (−11.6, 7.3)	0.69
Pn 19A	0.35 μg/mL	95.6 (43); (84.9, 99.5)	91.3 (42); (79.2, 97.6)	4.3 (−7.4, 16.7)	0.68	53.3 (90); (45.4, 61.0)	46.1 (65); (37.7, 54.7)	7.2 (−4.0, 18.2)	0.25
Pn 19F	0.35 μg/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	98.8 (167); (95.8, 99.9)	100 (141); (97.4, 100)	−1.2 (−4.2, 1.5)	0.50
Pn 23F	0.35 μg/mL	95.6 (43); (84.9, 99.5)	91.3 (42); (79.2, 97.6)	4.3 (−7.4, 16.7)	0.68	64.5 (109); (56.8, 71.7)	57.4 (81); (48.8, 65.7)	7.1 (−3.8, 17.8)	0.24
						Antibodies to 12-month vaccines measured at 13 months of age			
						BCG-vaccinated (n = 78) % (n); (95% CI)	BCG-naïve (n = 69) % (n); (95% CI)	Difference % (95% CI)	p-value
Measles	0.12 IU/mL					98.7 (77); (93.1, 100)	97.1 (67); (89.9, 99.6)	1.6 (−4.4, 8.8)	0.60
Mumps	45 IU/mL					65.4 (51); (53.8, 75.8)	56.5 (39); (44.0, 68.4)	8.9 (−6.9, 24.4)	0.31
Rubella	10 IU/mL					88.5 (69); (79.2, 94.6)	85.5 (59); (75.0, 92.8)	3.0 (−8.2, 14.7)	0.66
MenC	2 μg/mL					98.7 (77); (93.1, 100)	98.6 (68); (92.2, 100)	0.2 (−5.6, 6.6)	1
Hib	0.15 μg/mL					98.7 (77); (92.1, 100)	98.6 (68); (92.2, 100)	0.2 (−5.6, 6.6)	1
Tetanus	0.01 IU/mL					93.6 (73); (85.7, 97.9)	95.7 (66); (87.8, 99.1)	−2.1 (−10.5, 6.5)	0.72

BCG = Bacille Calmette-Guérin vaccine, CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PT = pertussis toxin.
[1] 0.01 IU/mL at 13 months of age.
[2] Only participants who had not had Hib-MenC.

pneumococcal serotypes at 7 months of age and for mumps and rubella at 13 months of age, these differences were not statistically significant (Table 2, Fig. 2).

3.2. The effect of BCG immunisation on concentration of antibodies to the primary course of vaccines

GMCs for antibodies to immunisations given in the first 6 months at 7 months of age and their persistence at 13 months of age are shown in Tables 3 and Supplementary Data, Figs. 1–2. After adjusting for pre-specified factors, GMRs were consistently above one, indicating higher average antibody responses in BCG-vaccinated infants, for diphtheria, tetanus and pneumococcal antigens at both 7 months and 13 months, though these findings were not statistically significant. In contrast, for pertussis and polio, there was no consistent direction of change at both time points: GMRs for pertussis antigens were mostly above one at both time points; GMRs for polio antigens were below one for all three antigens at 13 months of age (Table 3, Fig. 3).

3.3. The effect of BCG immunisation on concentration of antibodies to the 12-month vaccines

GMCs at 13 months of age for antibodies to immunisations given at 12 months of age in BCG-vaccinated and BCG-naïve infants are shown in Table 3 and Supplementary Data Fig. 3. After adjustment for pre-specified factors, GMRs were above one in BCG-vaccinated infants for measles and mumps (Table 3, Fig. 3). In contrast, the GMR for Hib at 13 months of age indicated a lower response to this booster dose in BCG-vaccinated infants, consistent with the finding at 7 months of age of a diminished response to the primary course of Hib immunisations. These findings did not, however, reach statistically significance.

3.4. Effect modifiers

Age at bleeding, time between immunisation and bleeding, and maternal dTpa immunisation during pregnancy were the strongest effect modifiers of infant vaccine responses. We also observed a moderate effect of biological sex and gestational age on vaccine responses, while maternal BCG status, age at randomisation, birth weight and delivery mode had little effect.

3.5. Effect modification by 'age at randomisation'

Seroprotection rates and GMCs/GMRs of infants randomised on day 0–1 of life and those randomised on day 2–10 of life are shown in Supplementary Data Tables 1–4 and in Supplementary Data Fig. 4. In infants randomised on day 2–10, at 7 months of age, BCG-vaccinated infants had significantly higher antibody responses to pneumococcal serotype 9V, 19F and 23F compared with BCG-naïve infants (Supplementary Data Table 4). In infants randomised on day 0–1, at 13 months of age, BCG-vaccinated infants had significantly higher antibody responses (persistence of antibodies) against tetanus compared with BCG-naïve infants (Supplementary Data Table 2).

Within the group of infants who were BCG-vaccinated, at 7 months of age, antibody concentrations against all 22 vaccine antigens were higher in those randomised to BCG on day 2–10 of life compared to those randomised on day 0–1 of life. This was statistically significant for pneumococcal serotype 9V (GMR 0.67, p-value = 0.04) and 19A (GMR 0.50, p-value = 0.03) (Supplementary Data Table 5). At 13 months of age, there was no apparent difference in GMRs between participants randomised to BCG on day 0–1 and those randomised on day 2–10 (Supplementary Data Table 5).

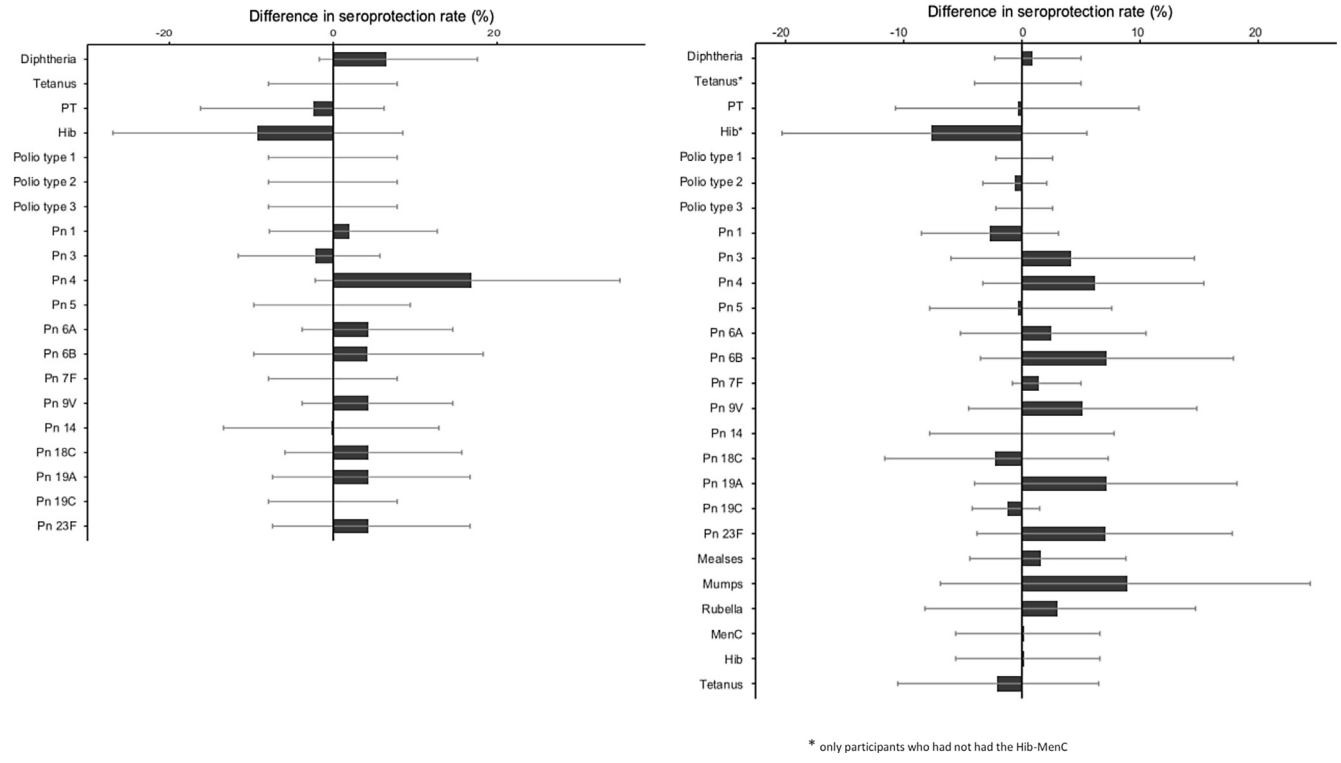


Fig. 2. Differences with 95% CI in seroprotection rates for 26 vaccine antigens in participants at 7 (left panel) and 13 months (right panel) of age. A positive difference indicates a higher seroprotection rate in BCG-vaccinated infants. In right panel, first 20 results relate to responses to primary course of vaccines ending at 6 months of age; last 6 results relate to responses to 12-month vaccines.

Table 3
Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) in participants at 7 and 13 months of age.

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	BCG-vaccinated (n = 45) GMC (95% CI)	BCG-naïve (n = 46) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value	BCG-vaccinated (n = 169) GMC (95% CI)	BCG-naïve (n = 141) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
Diphtheria[1]	**0.47 (0.38, 0.58)**	**0.34 (0.26, 0.43)**	**1.40 (1.02, 1.92)**	**0.04**	1.18 (0.87, 1.61)[b,c,g]	0.28	0.08 (0.07, 0.10)	0.07 (0.06, 0.08)	1.19 (0.95, 1.50)	0.14	1.16 (0.94, 1.43)[b,g,h,j]	0.17
Tetanus[1]	1.22 (1.01, 1.48)	1.01 (0.81, 1.26)	1.22 (0.91, 1.62)	0.18	1.23 (0.93, 1.64)[e]	0.14	0.48 (0.37, 0.63)[3]	0.39 (0.31, 0.50)[3]	1.24 (0.87, 1.77)[3]	0.24	1.33 (0.95, 1.86)[3,j]	0.10
PT[1]	77.68 (59.87, 100.80)	63.36 (51.12, 78.54)	1.23 (0.88, 1.71)	0.23	1.06 (0.78, 1.45)[b]	0.70	17.81 (15.24, 20.81)	17.13 (14.45, 20.30)	1.04 (0.83, 1.31)	0.74	1.03 (0.83, 1.27)[b,g,h,i,j]	0.80
FHA[1]	64.25 (50.50, 81.73)	63.23 (49.11, 81.42)	1.02 (0.72, 1.43)	0.93	0.91 (0.66, 1.26)[b,c,e]	0.58	19.77 (16.87, 23.17)	18.31 (15.82, 21.18)	1.08 (0.87, 1.34)	0.49	1.09 (0.88, 1.34)[b,e,g,h]	0.43
PRN[1]	66.20 (51.24, 85.53)	55.38 (40.44, 75.82)	1.20 (0.80, 1.78)	0.38	1.05 (0.71, 1.56)[b,c,f]	0.79	12.54 (10.41, 15.11)	11.53 (9.42, 14.11)	1.09 (0.83, 1.43)	0.55	1.08 (0.83, 1.41)[b,e,h]	0.54
Hib[2]	0.54 (0.32, 0.93)	0.48 (0.32, 0.72)	1.13 (0.58, 2.17)	0.72	0.92 (0.48, 1.75)[c,e,g]	0.80	1.05 (0.65, 1,69)[3]	1.22 (0.72, 2.05)[3]	0.86 (0.42, 1.75)[3]	0.68	1.06 (0.54, 2.08)[3,c,h]	0.86
Polio type 1[1]	36.40 (27.76, 47.75)	32.94 (23.23, 46.70)	1.11 (0.71, 1.71)	0.65	1.06 (0.69, 1.63)[c,f]	0.80	10.47 (8.82, 12.42)	11.98 (9.92, 14.47)	0.87 (0.68, 1.13)	0.30	0.86 (0.67, 1.10)[b,h,j]	0.22
Polio type 2[1]	65.26 (49.67, 85.75)	60.83 (42.60, 86.86)	1.07 (0.69, 1.67)	0.75	1.01 (0.65, 1.56)[b,h]	0.97	19.72 (16.21, 24.00)	23.95 (19.51, 29.40)	0.82 (0.62, 1.09)	0.18	0.82 (0.62, 1.09)[b,h]	0.17
Polio type 3[1]	20.20 (14.50, 28.14)	22.72 (16.22, 31.81)	0.89 (0.56, 1.42)	0.62	0.94 (0.60, 1.49)[h]	0.80	8.68 (7.09, 10.62)	9.18 (7.41, 11.36)	0.95 (0.71, 1.27)	0.71	0.92 (0.69, 1.23)[b,e,h,j]	0.57
Pn 1[2]	5.30 (4.16, 6.74)	3.62 (2.55, 5.12)	1.47 (0.96, 2.23)	0.07	1.18 (0.79, 1.75)[a,g,h]	0.42	1.23 (1.07, 1.42)	1.10 (0.96, 1.26)	1.12 (0.92, 1.36)	0.27	1.08 (0.89, 1.30)[b,d,h]	0.44
Pn 3[2]	1.47 (1.21, 1.79)	1.40 (1.14, 1.72)	1.05 (0.79, 1.40)	0.71	1.05 (0.79, 1.40)	0.71	0.57 (0.50, 0.65)	0.51 (0.45, 0.58)	1.12 (0.93, 1.34)	0.23	1.07 (0.90, 1.28)[h]	0.43
Pn 4[2]	0.55 (0.44, 0.69)	0.48 (0.37, 0.63)	1.14 (0.80, 1.62)	0.45	1.02 (0.73, 1.44)[g]	0.89	0.23 (0.21, 0.26)	0.20 (0.18, 0.22)	1.14 (0.98, 1.33)	0.08	1.11 (0.96, 1.29)[b,c,h,j]	0.16
Pn 5[2]	**3.90 (3.07, 4.95)**	**2.57 (1.92, 3.44)**	**1.52 (1.05, 2.20)**	**0.03**	1.28 (0.89, 1.83)[a,g,h]	0.18	0.98 (0.85, 1.13)	0.85 (0.74, 0.99)	1.15 (0.93, 1.41)	0.19	1.11 (0.91, 1.36)[b,g,h,j]	0.30
Pn 6A[2]	5.69 (4.65, 6.97)	4.09 (3.04, 5.48)	1.39 (0.98, 1.98)	0.07	1.21 (0.85, 1.72)[b,g]	0.29	1.07 (0.92, 1.25)	0.93 (0.78, 1.09)	1.16 (0.92, 1.45)	0.21	1.10 (0.90, 1.37)[b,g,h]	0.35
Pn 6B[2]	2.39 (1.55, 3.68)	1.54 (0.97, 2.46)	1.55 (0.83, 2.89)	0.17	1.23 (0.66, 2.31)[d,g]	0.51	0.56 (0.47, 0.67)	0.47 (0.39, 0.57)	1.19 (0.92, 1.55)	0.19	1.16 (0.90, 1.49)[b,h]	0.26
Pn 7F[2]	6.64 (5.35, 8.25)	5.67 (4.44, 7.24)	1.17 (0.85, 1.62)	0.33	1.04 (0.75, 1.44)[b,g]	0.81	1.97 (1.75, 2.22)	1.76 (1.55, 2.01)	1.12 (0.94, 1.33)	0.20	1.08 (0.92, 1.28)[b,g,h]	0.34
Pn 9V[2]	**3.00 (2.47, 3.64)**	**2.03 (1.52, 2.70)**	**1.48 (1.05, 2.09)**	**0.03**	1.25 (0.90, 1.75)[a,g,h]	0.18	0.62 (0.55, 0.71)	0.57 (0.49, 0.66)	1.10 (0.90, 1.33)	0.35	1.04 (0.86, 1.25)[g,h,j]	0.69
Pn 14[2]	2.87 (1.92, 4.29)	2.48 (1.76, 3.51)	1.16 (0.69, 1.95)	0.58	1.16 (0.69, 1.95)	0.58	1.09 (0.93, 1.28)	1.00 (0.85, 1.17)	1.09 (0.87, 1.37)	0.44	1.04 (0.83, 1.30)[g,h,j]	0.74
Pn 18C[2]	3.11 (2.39, 4.05)	2.78 (2.07, 3.75)	1.12 (0.75, 1.65)	0.58	1.01 (0.69, 1.50)[g]	0.94	0.67 (0.59, 0.77)	0.62 (0.55, 0.71)	1.08 (0.89, 1.30)	0.43	1.04 (0.87, 1.24)[b,g,i,j]	0.70
Pn 19A[2]	2.00 (1.47, 2.70)	1.49 (1.15, 1.92)	1.34 (0.91, 1.99)	0.14	1.25 (0.84, 1.85)[b,c]	0.27	0.49 (0.40, 0.61)	0.43 (0.34, 0.53)	1.16 (0.86, 1.56)	0.32	1.07 (0.81, 1.42)[h,j]	0.64
Pn 19F[2]	13.37 (10.29, 17.37)	9.97 (7.67, 12.97)	1.34 (0.93, 1.93)	0.12	1.15 (0.80, 1.66)[b,g]	0.44	3.24 (2.73, 3.83)	2.93 (2.47, 3.47)	1.10 (0.87, 1.40)	0.42	1.04 (0.82, 1.31)[g,h,j]	0.75
Pn 23F[2]	2.96 (2.19, 4.00)	1.86 (1.29, 2.66)	1.59 (1.00, 2.54)	0.05	1.43 (0.90, 2.28)[g]	0.13	0.62 (0.51, 0.74)	0.51 (0.42, 0.62)	1.21 (0.92, 1.59)	0.17	1.15 (0.87, 1.50)[b,e,f,h]	0.32

Vaccine antigen	Antibodies to 12-month vaccines measured at 13 months of age					
	BCG-vaccinated (n = 78) GMC (95% CI)	BCG-naïve (n = 69) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
Measles[1]	3.34 (2.75, 4.06)	2.73 (2.01, 3.72)	1.22 (0.86, 1.74)	0.26	1.20 (0.84, 1.70)[e]	0.31
Mumps[1]	67.50 (50.13, 90.88)	52.81 (38.48, 72.49)	1.28 (0.83, 1.97)	0.26	1.14 (0.80, 1.62)[g,h]	0.47
Rubella[1]	54.23 (40.61, 72.42)	52.56 (36.57, 75.53)	1.03 (0.65, 1.63)	0.89	0.90 (0.63, 1.27)[g,h]	0.54
MenC[2]	16.60 (13.43, 20.53)	17.81 (13.76, 23.05)	0.93 (0.67, 1.29)	0.67	0.94 (0.68, 1.30)[e,h]	0.70
Hib[2]	13.71 (9.47, 19.85)	18.39 (12.98, 26.06)	0.75 (0.45, 1.24)	0.26	0.72 (0.44, 1.19)[c,g,h]	0.20
Tetanus[1]	0.95 (0.72, 1.25)	1.07 (0.81, 1.41)	0.89 (0.60, 1.31)	0.55	0.89 (0.60, 1.31)	0.55

BCG = Bacille Calmette-Guérin vaccine, CI = confidence interval, FHA = filamentous haemagglutinin, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PT = pertussis toxin, PRN = pertactin.

[1] IU/ml.
[2] μg/ml.
[3] Only participants who have not had Hib-MenC.
[a] Maternal BCG status.
[b] Maternal dTpa during pregnancy.
[c] Gestational age.
[d] Delivery mode.
[e] Sex.
[f] Birth weight.
[g] Age at bleeding.
[h] Time between immunisation and bleeding.
[i] Age at randomisation.
[j] MMR/Hib-MenC immunisation status.

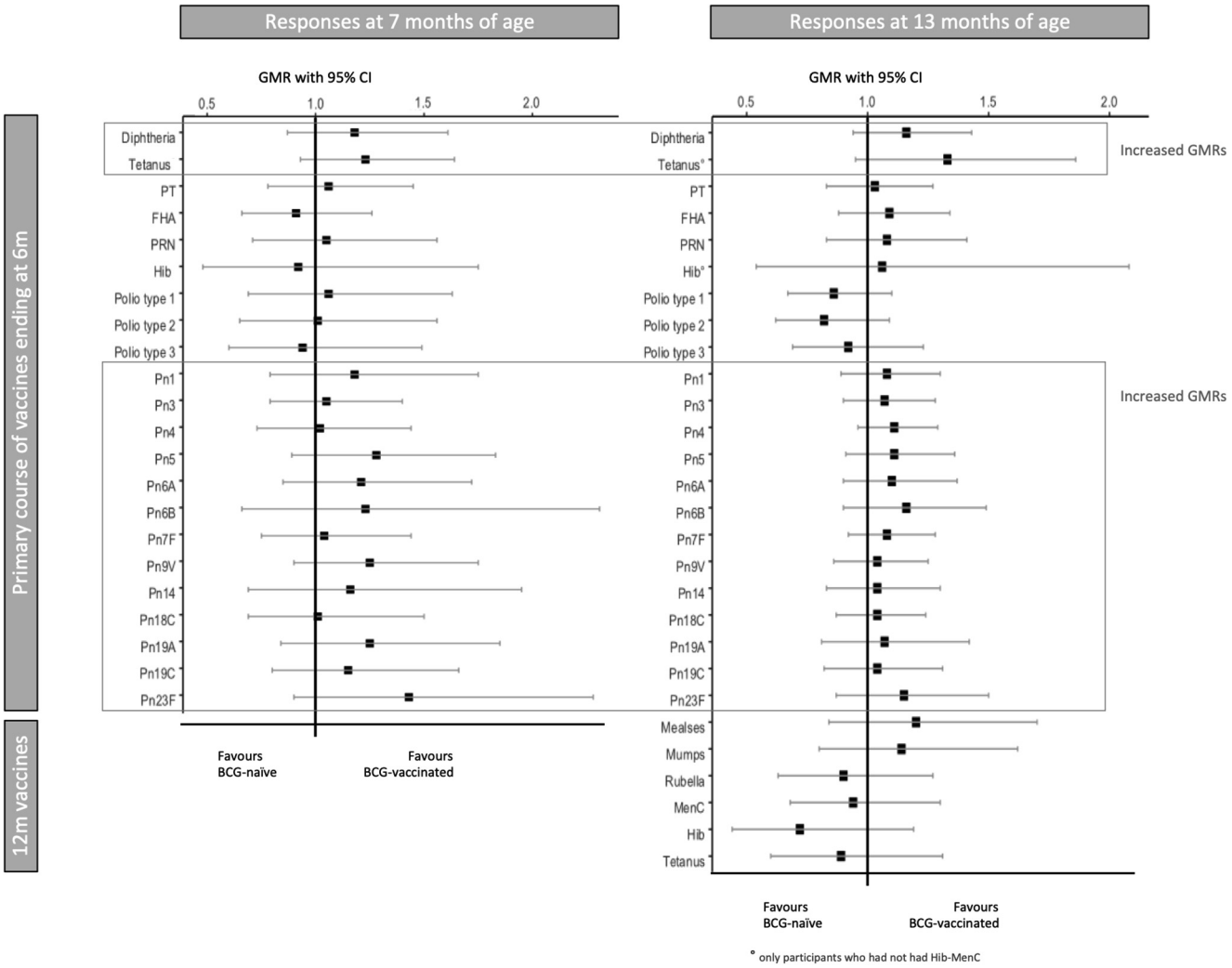


Fig. 3. GMRs with 95% CI for 26 vaccine antigens in participants at 7 and 13 months of age.

4. Discussion

BCG immunisation has heterologous effects on the infant immune system [37]. It is a low-cost and readily available intervention with a well-established safety profile. To date, only a few studies have investigated the effect of previous or concurrent administration of BCG on heterologous vaccines responses [21]. A number of these studies have reported that BCG immunisation is associated with increased vaccine responses to HepB, polio, pneumococcus and influenza [23–26]. However, a recent study in Denmark found higher GMCs against pertussis and pneumococcus (statistically significant for serotypes 9V, 18C and 19F) only when BCG was given after the first day of life [27].

Our study includes the broadest range of vaccine responses tested to date for BCG immunomodulatory effects and is the first to include responses to meningococcal and MMR immunisation, as well as to three different pertussis and polio antigens. Our results suggest that neonatal BCG immunisation might lead to higher GMCs and seroprotection rates for diphtheria, tetanus and pneumococcal antigens, and a reduced response and seroprotection rate for Hib. Although our findings only reached statistical significance for a few of the pneumococcal serotypes (9V, 19F and 23F) when BCG was given after the first day of life, the consistent direction of change in GMRs at both 7 and 13 months of age suggest a possible effect of BCG on antibody responses. Our results are consistent with those from a recent trial in Denmark [27] which reported a stronger effect on vaccine responses when BCG vaccine was administered after the first day of life.

Although the observed differences in GMCs measured approximately one month after immunisation were relatively small, these differences in antibody response might influence the duration of protection. This is highlighted by the differences in the proportion of participants who had antibody concentrations above the protective threshold at 13 months of age, although the clinical significance of these differences is uncertain.

The precise mechanisms underlying the immunomodulatory effects of BCG immunisation are the subject of ongoing investigation. Studies show that BCG immunisation affects both innate [24,38,39] and T cell immunity [40]. Epigenetic reprogramming by BCG alters innate immune cells that my function as antigen-presenting cells [41,42]. Changes in innate immune function early in life, together with increased expression of co-stimulatory molecules or cytokine responses, might mechanisms by which BCG influences adaptive immune responses to other vaccines [38,43].

Although the effects of BCG on T-cell immunity are well established [5,24,40], few studies have investigated the influence of BCG on cellular responses to heterologous vaccines. A case-control study showed that BCG given at birth leads to an increase in interferon (IFN)-gamma-producing spot-forming cells (SFC) to tetanus and polio, but not to HepB antigens [44], and a randomised trial

of BCG-Tice given at birth led to an increase in IFN-gamma (as well as interleukin (IL)-5- and IL-13-) producing T cells to HepB antigens [24]. Augmented cellular responses induced by BCG is therefore another possible mechanism by which BCG influences vaccine responses, especially in infants, who have reduced T-helper 1 cell responses to many pathogens and toxins [45]. The possibility that BCG only has indirect effects on B-cells through innate immunity and T-cells [24,44] might be one explanation for the small magnitude of the differences in heterologous vaccine responses between BCG-vaccinated and BCG-naïve infants.

Different BCG vaccine strains are associated with differences in vaccine efficacy. BCG-Japan has been suggested to have superior efficacy compared to BCG-Russia in protecting against TB [46]. TB-specific responses (mycobacterial-specific polyfunctional and cytotoxic T cells) are higher after immunisation with BCG-Denmark and BCG-Japan compared with BCG-Russia [47]. BCG-Denmark also leads to higher *in vitro* cytokine responses to both specific and non-specific stimuli compared with BCG-Russia and BCG-Bulgaria [47,48]. It is therefore possible that different BCG vaccine strains also have dissimilar heterologous effects. To date, most studies investigating the effect of BCG on heterologous vaccine responses, including the recent study from Denmark [27] and ours, have used BCG-Denmark, which is a strain that has been associated with superior efficacy for both specific and non-specific effects [49].

The strengths of our study are the use of samples from a randomised trial, the detailed participant demographic and clinical data allowing for adjustment for possible confounding factors, the homogeneity in the vaccines given and the range of measured antibody responses. The limitations of the study, aside from the small sample size and consequent risk of type-2 error, include the fact that participants were not specifically randomised for the outcome of this study, that not all participants had antibodies measured exactly 4 weeks after immunisation, and that antibody concentrations were not measured pre vaccination.

The results of this study add to the evidence that BCG immunisation at birth modulates the early-life immune system, including the humoral response to heterologous vaccines. The immunomodulatory effects of BCG might be used to improve vaccine efficacy and duration of protection on a population level. They also need to be taken into account when considering replacing BCG with new vaccines, as well as when deciding on recommendations for discontinuation of universal BCG immunisation programmes [3]. Future studies investigating the immunomodulatory effects of BCG should focus on the differences between BCG vaccine strains and on the optimal timing of administration.

5. Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.016.

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4.2 ORIGINAL STUDY OF PARTICIPANTS WITHIN MIS BAIR

This chapter contains the published version of a study that investigated the effect of BCG immunisation at birth on heterologous vaccine responses to routine infant vaccines given in the first year of life.

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REVIEW

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The influence of BCG on vaccine responses – a systematic review

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ABSTRACT

Introduction: Bacillus Calmette-Guérin (BCG) vaccine is one of the most widely used vaccines worldwide. In addition to providing protection against tuberculosis, it has non-specific (heterologous) immunomodulatory effects.

Areas covered: In our systematic review, we found eight studies, involving 2439 participants, investigating the influence of BCG vaccination on the humoral response to 16 different vaccines. A beneficial effect of BCG on vaccine responses was reported in five of the eight studies. Previous or concurrent administration of BCG was associated with significantly higher levels of antibodies against hepatitis B, polio type 1, pneumococcus, and influenza in some studies. No study reported a statistically significant increase in the response to other vaccines, though in many cases higher antibody levels were observed in the BCG-vaccinated group. One study reported lower levels of antibodies against hepatitis B in BCG-vaccinated participants.

Expert commentary: The studies in this review suggest that BCG modulates humoral responses to other vaccines. Future studies should focus on the influence of BCG vaccine strain and the optimal timing of administration to exploit the immunomodulatory effects of BCG to improve vaccine efficacy and duration of protection.

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1. Introduction

The immune response to vaccinations given in infancy varies substantially between individuals. The serological response to hepatitis B vaccine, for example, varies more than 100-fold at 7 months of age and some infants do not have any measurable antibodies [1,2]. Similarly, the response to pneumococcal and Hib vaccination at 6 months of age varies up to 40-fold [1]. This has implications for both protective efficacy and duration of protection. Many factors are known to contribute to variation in vaccine response, including age [3–5], gender [6], genetics [7–9], geographic location [10], and route of administration [11,12]. Another important factor that might contribute to differences in the response of vaccines is co-administration of other vaccines [13,14].

Bacillus Calmette-Guérin vaccine (BCG) is one of the most widely used vaccines worldwide. In addition to providing protection from tuberculosis, it has non-specific (heterologous) immunomodulatory effects [15]. BCG vaccination reduces all-cause infant mortality in certain settings [16], decreases hospitalization rates due to respiratory infections and sepsis [17,18], is used in the treatment of bladder cancer [19], might decrease the incidence of eczema [20] and other allergies [21], and is proposed to be beneficial in the treatment of autoimmune diseases [22,23].

This systematic review summarizes studies in humans that have investigated the influence of BCG vaccination on humoral responses to other vaccines.

2. Literature review methods

In September 2017, MEDLINE (1946 to present) and Embase (1947 to present) were searched using the Ovid interface with the following search terms: (Bacillus Calmette-Guérin OR BCG) AND (vaccin* OR immun* OR antibod* OR humoral OR B-lymphocytes). This identified 959 and 3804 studies, respectively. Among these, eight fulfilled our inclusion criteria of articles published in English reporting results of studies investigating the effect of previous or concurrent administration of BCG on humoral vaccines responses in plasma or serum in humans. References were hand-searched for additional publications and no further relevant studies were found. One study, published only in abstract form, was identified through personal communication [24].

3. Literature review results

A total of eight studies were included, involving 2439 participants and 1249 controls, investigating the influence of BCG on the humoral response to 19 vaccine antigens following administration of 16 different vaccines (oral polio vaccine (OPV) (1), hepatitis B vaccine (HBV) (2), diphtheria-tetanus-whole cell pertussis (DTwP) (1), DT-acellular pertussis (DTaP) (1), DTaP-inactivated polio vaccine-*Haemophilus influenzae* type b (DTaP-IPV-Hib) (1), DTaP-HBV-IPV-Hib (1), inactivated influenza vaccine (IIV) (3), live attenuated influenza vaccine (LAIV) (2),

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chromatographic influenza vaccine (CWV) (1), heptavalent pneumococcal conjugate vaccine (PCV7) (1), PCV13 (1), typhoid fever vaccine (TFV) (1)) [5,24–29]. Three studies used BCG-Denmark [24,27,28], two studies used BCG-Pasteur [5,29], one used BCG-Denmark, BCG-Japan, and BCG-Russia [1], and two studies did not specify the strain that was used [25,26]. All of the studies were done prospectively and were specifically designed to investigate the influence of BCG on vaccine responses. All but one study [5] used control participants for comparison. In four of the eight studies, participants were randomized to either receive BCG or no BCG [24,27–29]. A placebo (NaCl 0.9%) was used in the control group in only one study (Table 1) [28].

3.1. Effect of BCG on antibody responses to diphtheria, tetanus, pertussis, polio, and Hemophilus influenzae type b vaccination

Four studies investigated the influence of BCG on the response to DTP vaccination (with *H. influenzae* type b and polio components in two studies and hepatitis B in one study) in a total of 949 neonates [1,5,27,29]. In two studies, BCG was administered within the first week of life [1,27], in one study BCG was administered at birth or at the age of 2 or 4.5 months [29], and in one study during the second month of life [5]. All of the studies measured antibody levels to individual components after three doses of the vaccine. Three studies measured antibody levels against tetanus toxin [1,27,29] and two studies each against diphtheria toxin [27,29], *H. influenzae* type b [1,27], and pertussis toxin [5,27]. Although higher antibody levels against tetanus toxin and *H. influenzae* type b were reported in one study, these differences were not statistically significant [1]. BCG did not influence any of the other antibody levels (Tables 1 and 2).

3.2. Effect on the response to hepatitis B vaccination

Two studies investigated the influence of BCG on the response to hepatitis B vaccination in a total of 259 neonates [1,29]. In both studies, BCG was administered at birth together with a monovalent hepatitis B vaccine, followed by two doses of a monovalent hepatitis B vaccine in one study [29], and three doses of a combined DTaP-HBV-IPV-Hib vaccine in the other study [1]. The hepatitis B vaccine was given at intervals of 2 months [1,29]. The first study included a second group of participants in whom BCG was given at the age of 2 months [29]. The first study found significantly higher levels of antibodies against hepatitis B surface antigen at 2 and 4.5 months after the last dose of hepatitis B vaccine in neonates receiving BCG at birth, but not in infants receiving BCG at the age of 2 months [29]. In contrast, the second study found lower antibody levels at 1 month after the last dose of the hepatitis B vaccine [1], (Tables 1 and 2).

3.3. Effect on the response to oral polio vaccination

One study investigated levels of antibodies against polio type 1 after oral polio vaccination in 151 neonates [29]. BCG was either administered at birth or at the age of 2 months. The polio vaccine was administered at birth, followed by subsequent doses at the age of 1, 2, and 3 months. When BCG was given at birth, it had no influence on polio type 1 antibody levels. However, participants who were given BCG at the age of 2 months had higher antibody levels against polio type 1 at the age of 4.5 months (Tables 1 and 2).

3.4. Effect on the response to pneumococcal vaccination

Two studies investigated the influence of BCG on the response to pneumococcal vaccination in a total of 408 neonates [1,27]. In both studies, BCG was given at birth. In the first, the pneumococcal vaccine was given at the age of 3, 5, and 12 months [27], while in the second study it was given at the age of 2, 4, and 6 months [1]. The first study found higher antibodies against pneumococcal polysaccharide antigens for all tested serotypes (statistically significant only for serotypes 9V and 18C) 1 month after the third dose of the pneumococcal vaccine [1], while the second study did not find differences in pneumococcal antibody levels [27] (Tables 1 and 2).

3.5. Effect on the response to influenza vaccination

Three studies investigated the influence of BCG the response to influenza vaccination in a total of 1470 adults [25,26,28]. One study used a live, an inactivated, and a chromatographic (not currently used) influenza vaccine [26], one study used a live and an inactivated influenza vaccine [25], and one study used only an inactivated influenza vaccine [28]. In the first two studies, BCG and influenza vaccine were administered simultaneously [25,26], while in the third study BCG was administered 2 weeks before the influenza vaccine [28]. Antibody levels were measured 2 weeks to 1 month [28] or 8 months [25,26] after the administration of the influenza vaccine. In participants receiving the inactivated influenza vaccine after receiving BCG, all three studies found significantly higher hemagglutination-inhibiting antibodies levels, as well as more rapid and higher seroconversion rates [25,26,28]. However, in participants receiving the live attenuated influenza vaccine, BCG had no effect on antibody levels or seroconversion rates [25,26] (Tables 1 and 2).

3.6. Effect on the response to typhoid vaccination

One study investigated the influence of BCG vaccination the response to inactivated typhoid fever vaccine in 20 adults [24]. BCG was administered 2 weeks before the typhoid vaccine. The study found higher seroconversion rates in BCG-vaccinated participants 2 weeks after the typhoid vaccine but these findings were not statistically significant. There was no difference in antibody levels 2 weeks and 3 months after vaccination (Tables 1 and 2).

3.7. Effect of different BCG strains

In the two of the five studies that showed a beneficial effect of BCG on humoral vaccine responses, the BCG strain was not specified [25,26]. BCG-Denmark and BCG-Pasteur were each used in one study only [28,29]. One study used BCG-Denmark,

Table 1. Effect of BCG vaccination on humoral vaccine responses (statistically significant findings indicated in bold).

Participants (controls)	Study design	Timing of BCG vaccine BCG strain	Timing of other vaccinations after BCG vaccine	Timing of blood sample after last vaccination	Vaccine responses	Reference Publication year Country
300 neonates (122)	Randomized controlled	Birth BCG-Denmark	DTaP-IPV-Hib 3m, 5m, 12m PVC13 3m, 5m, 12m	1m	**BCG at birth**: - no influence on anti-TT, anti-DT, anti-PT, and anti-Hib IgG levels at 1m (p=0.43, 0.72, 0.31, 0.34) - no influence on anti-Pn Ps IgG levels for serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F IgG levels at 1m (p=0.77, 0.59, 0.83, 0.47, 0.20, 0.99, 0.71)	Nissen 2007 [27] Denmark
20 adults (10)	Randomized controlled	0w BCG-Denmark	TFV 2w	0w, 2w, 3m	**BCG before TFV**: - no influence on anti-Vi IgG levels at 2w (p-value NS) and 3m (p=0.78) - higher seroconversion rates* at 2w (p=0.07)	Blok 2016 [24] The Netherlands
40 adults (20)	Randomized placebo-controlled	0w BCG-Denmark	IIV 2w	–2w, 0w, 1w, 2w, 1m	**BCG before IIV**: - **higher anti-Hi IgG levels against A(H1N1)pdm09 at 2w to 1m (p=0.04)** - no influence on anti-Hi IgG levels against A(H3N2)2012 and B/2012 at 1w to 1m (p=0.58, 0.56) - **more rapid seroconversion* against A(H1N1)pdm09 (p=0.08**), but not against A(H3N2)2012 and B/2012 (p=0.99, 0.61)	Leentjens 2015 [28] The Netherlands
108 neonates (52)	Non-randomized controlled	Birth BCG-Denmark BCG-Japan BCG-Russia	HBV birth PVC7 2m, 4m, 6m DTaP-HBV-IPV-Hib 2m, 4m, 6m	1m	**BCG at birth**: - higher anti-TT IgG levels at 7m (p=0.19) - higher anti-Hib IgG levels at 7m (p=0.37) - **higher anti-Pn Ps IgG levels for serotypes 9V and 18C at 7m (p<0.01, 0.04)** - higher anti-Pn Ps IgG levels for serotypes 4, 6B, 11A, 14, 19F, and 23F at 7m (p=0.71, 0.06, 0.50, 0.43, 0.18, 0.50) - **lower anti-HBs IgG levels at 7 m (p=0.03)**	Ritz 2013 [1] Australia
151 neonates (47)	Randomized controlled	Birth, 2m or 4.5m BCG-Pasteur	HBV 0m, 2m, 4m OPV 0m, 1m, 2m, 3m DTwP 2m, 3m, 4m	0m, 2m, 4.5m	**BCG at birth**: - no influence on anti-TT IgG levels at 2m (p=0.25) and at 4.5m (p=0.61) - no influence on anti-DT IgG levels at 2m (p=0.97) and at 4.5m (p=0.85) - no influence on anti-PV1 IgG levels at 2m (p=0.37) and at 4.5m (p=0.38) - **higher HBs IgG levels at 2 m (p=0.03) and 4.5m (p=0.004)** **BCG at 2m**: - no influence on anti-TT IgG levels 4.5m (p=0.55) - no influence on anti-DT IgG levels 4.5m (p=0.50) - **higher anti-PV1 IgG levels at 4.5m (p=0.002)** - no influence on HBs IgG levels at 4.5m (p-=0.80)	Ota 2001 [29] The Gambia
390 neonates	Randomized non-controlled	1–2m BCG-Pasteur	DTaP 2m, 4m, 6m or 2m, 3m, 4m	2–3m, 5–6m, 7–8m	**BCG before DTPa**: - no influence on anti-PT IgG levels at 7m whether BCG was given simultaneously with first dose of DTPa or 3w before first dose of DTPa (p-values NS)	Simondon1999 [5] Senegal

(*Continued*)

Table 1. (Continued).

Participants (controls)	Study design	Timing of BCG vaccine BCG strain	Timing of other vaccinations after BCG vaccine	Timing of blood sample after last vaccination	Vaccine responses	Reference Publication year Country
1325 adults (933)	Non-randomized controlled	0w Not specified	IIV 0w LAIV 0w	8m	**BCG together with IIV**: - **higher anti-Hi IgG levels at 8m (p<0.05)** - **higher seroconversion* rates at 8m (p<0.05)** **BCG together with LAIV**: - no effect on anti-Hi IgG levels or seroconversion rates (p-values NS) **BCG together with CWV**: - no effect on anti-Hi IgG levels or seroconversion rates (p-values NS)	Zykov 1985 [26] Russia
105 adults (65)	Non-randomized controlled	0w Not specified	IIV 0w LAIV 0w	8m	**BCG together with IIV**: - **higher anti-Hi IgG levels at 8m (p<0.01)** - **higher seroconversion* rates at 8m (p<0.01)** **BCG together with LAIV**: - no effect on anti-Hi IgG levels or seroconversion rates (p-values NS)	Zykov 1981 [25] Russia

*4-fold increase in antibody titer compared to baseline
anti-DT IgG = immunoglobulin G antibodies to diphtheria toxin
anti-HBs IgG = immunoglobulin G antibodies to hepatitis B surface antigen
anti-Hi IgG = haemagglutination-inhibiting immunoglobulin G antibodies
anti-Hib IgG = immunoglobulin G antibodies to *Haemophilus influenzae* type b antigen
anti-Pn Ps IgG = immunoglobulin G antibodies to *Pneumococcus* polysaccharide antigen
anti-PT IgG = immunoglobulin G antibodies to pertussis toxin
anti-PV1 IgG = immunoglobulin G antibodies to polio type 1
anti-TT IgG = immunoglobulin G antibodies to tetanus toxid
anti-Vi IgG = immunoglobulin G antibodies to *Salmonella* Vi antigen
CWV = chromatographic influenza vaccine
DTaP = diphtheria-acellular pertussis-tetanus vaccine
DTwP = diphtheria-whole-cell pertussis-tetanus vaccine
DTaP-HBV-IPV-Hib = diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio *H. influenzae* type b
DTaP-IPV-Hib = diphtheria-tetanus-acellular pertussis-inactivated polio-*H. influenzae* type b
HBV = hepatitis B vaccine
IgG = immunoglobulin G
IIV = inactivated influenza vaccine
LAIV = live attenuated influenza vaccine
m = months
NS = not stated
OPV = oral polio vaccine
PVC7 = 7-valent pneumococcal conjugate vaccine
PVC13 = 13-valent pneumococcal conjugate vaccine
TFV = typhoid fever vaccine
w = weeks

Table 2. Summary of the findings of studies investigating the influence of BCG vaccination on the humoral response to other vaccines (number of BCG-vaccinated participants. non BCG-vaccinated controls in each study with p-value for the comparison between the antibody response in participants and controls; statistically significant findings indicated in bold).

Responses to vaccination	Tetanus	Diphtheria	Pertussis	*H. influenzae* type b	Polio type 1	Pneumococcus	Hepatitis B	Influenza, inactivated	Influenza, live attenuated	Influenza, chromatographic	Typhoid fever
Increased	56, 52; p=0.19 [1]			56, 52; p=0.37 [1]	**29, 28; p=0.002** [29]	**56, 52; serotype 9V, p<0.01; 18C, p=0.04** 4, p=0.71; 6B, p=0.06; 11A p=0.50; 14, p=0.43; 19F, p=0.18; 23F, p=0.50 [1]	**34, 34; p=0.03** [29] **27, 28; p=0.004** [29]	**20, 20; A(H1N1)pdm09, p=0.04** [28] **132, 117; p<0.05** [26] **12, 20; p<0.01** [25]			
Not influenced	178, 122; p=0.43 [27] 35, 35; p=0.25 [29] 28, 28; p=0.61 [29] 29, 28; p=0.55 [29]	178, 122; p=0.72 [27] 35, 35; p=0.97 [29] 28, 28; p=0.85 [29] 29, 28; p=0.50 [29]	178, 122; p=0.31 [27] 390, 0; p-value NS [5]	178, 122; p=0.34 [27]	34, 34; p=0.37 [29] 27, 28; p=0.38 [29]	178, 122; serotype 4, p=0.77; 6B, p=0.59, 9V, p=0.83; 14, p=0.47; 18C, p=0.20; 19F, p=0.99; 23F, p=0.71 [27]	29, 28; p=0.80 [29]	20, 20; A(H3N2)2012, p=0.56 [28] 20, 20; B/2012, p=0.58 [28]	20, 13; p-value NS [26] 13, 20; p-value NS [25]	240, 260; p-value NS [26]	10, 10; p=0.78 [24]
Decreased							**56, 52; p=0.03** [28]				

NS = not specified

BCG-Japan, and BCG-Russia [1]. In the three studies which showed no influence of BCG on humoral vaccine responses, BCG-Denmark was used in two [24,27] and BCG-Pasteur in one [5].

4. Discussion

In our systematic review of the studies that have investigated the influence of BCG vaccination on humoral responses to other vaccines, a beneficial effect was reported in five of eight studies [1,25,26,28,29]. Previous or concurrent administration of BCG was associated with significantly higher levels of antibodies against hepatitis B, polio, pneumococcus, and influenza. Although no study reported a statistically significant increase in the antibody response to diphtheria, tetanus, pertussis, and *H. influenzae* type b vaccines following BCG vaccination, in many cases higher antibody levels were observed in the BCG-vaccinated group. Although this may reflect an absence of any influence of BCG, it is possible that these studies were underpowered to detect a statistically significant difference.

The mechanisms underlying the immunomodulatory effects of BCG vaccination are uncertain [30], but are postulated to include altered cytokine responses [29,31] (for example, an increase in interferon (IFN)-gamma production) [32], as well as other changes in innate and T-cell immunity [33]. One distinct feature of the neonatal innate immune response to Toll-like receptor stimulation is reduced production of IFN-alpha, IFN-gamma, and interleukin(IL)-12, but increased production of cytokines inducing T helper 17 cells, including IL-6 and IL-23 [34]. As these cytokines are involved in the differentiation of adaptive immune responses and memory development [35], it is possible that BCG-induced changes in innate immune function in early life is one mechanism by which this vaccine influences adaptive immune responses to vaccines. Another potential mechanism is through epigenetic reprogramming of innate immune cells [36,37] that provides the innate immune system with memory, a phenomenon termed 'trained immunity' [38,39].

In addition to influencing humoral vaccine responses, BCG also affects innate and T-cell immunity [29,30,33]. Few studies have investigated the influence of BCG on cellular responses or cytokine production after vaccination. However, one study showed that BCG given at birth leads to an increase in interferon-gamma producing T-helper 1 cells to tetanus toxoid and polio, but not to hepatitis B surface antigen [40]. Neonates have reduced T-helper 1 cell responses to many pathogens and toxins [41], so an increase in these responses induced by BCG is a possible mechanism by which BCG improves vaccine efficacy.

The number of studies investigating the influence of BCG on vaccine responses is limited and there is variation in study design, particularly in the timing of vaccinations as well as the time between BCG and administration of the other vaccines. Further the use of different BCG vaccine strains potentially introduces more variation as there are differences in the immune response induced by different strains [42]. For example, BCG-Denmark and BCG-Japan induce higher frequencies of mycobacterial-specific polyfunctional and cytotoxic T cells and higher concentrations of Th1 cytokines [43]. Additionally, BCG-Denmark leads to higher cytokine responses to both specific and non-specific stimuli *in vitro* [44]. The small number of studies in our review that included information about the BCG strain used precludes drawing any conclusions about the influence of strain in this setting.

Recent efforts to develop more effective TB vaccines have investigated recombinant vaccines [45] or recombinant BCG strains [46,47]. The immunomodulatory effects of BCG need to be taken into consideration when assessing new vaccines designed to replace BCG, especially recombinant vaccines. New TB vaccines should not only be evaluated for protection against TB, but also for their immunomodulatory effects. Replacing BCG with a vaccine that has superior protection against TB but which does not have its beneficial non-specific effects could have a paradoxical deleterious impact on all-cause infant mortality.

The results of the studies in this review suggest that BCG modulates the humoral response to other vaccines. Future studies should focus on identifying the influence of BCG vaccine strain and the optimal timing of administration to exploit the immunomodulatory effects of BCG to improve vaccine efficacy and duration of protection. The immunomodulatory effects of BCG need to be taken into consideration when deciding on recommendations for discontinuations of BCG vaccination programs and when assessing new vaccines designed to replace BCG.

5. Expert commentary

BCG, one of the most widely used vaccines worldwide, has important non-specific immunomodulatory effects. The mechanisms underlying these effects remain uncertain, but include altered cytokine responses and epigenetic reprogramming (trained immunity), as well as other changes in innate and T-cell immunity. There is only a limited number of studies investigating the influence of BCG on vaccine responses and there is variation in their study design, particularly in the timing of vaccinations, as well as in the time between BCG and administration of the other vaccines. Despite these limitations, our review indicates that previous or concurrent administration of BCG vaccine can influence the immune response to other vaccines. This property of BCG could be used to improve vaccine efficacy and duration of protection. The influence of different BCG vaccine strains and the optimal timing of administration of BCG to exploit its immunomodulatory effects are priorities for future studies.

6. Five-year view

The protective efficacy of BCG vaccination against *M. tuberculosis* is suboptimal and new vaccines are in development designed to replace BCG. The immunomodulatory effects of BCG need to be taken into account when considering replacing BCG with new TB vaccines, as well as when deciding on recommendations for discontinuation of universal BCG vaccination programs.

Key issues

- Bacillus Calmette-Guérin (BCG) vaccine is one of the most widely used vaccines worldwide. In addition to protecting against tuberculosis, the vaccine has other important immunomodulatory effects.
- BCG vaccination reduces all-cause infant mortality in certain settings, decreases hospitalization rates due to respiratory infections and sepsis, is used in the treatment of bladder cancer, might decrease the incidence of eczema and other allergies, and is proposed to be beneficial in the treatment of autoimmune diseases.
- This review shows that BCG also influences the immune response to heterologous antigens: previous or concurrent administration of BCG vaccine is associated with higher levels of antibodies against hepatitis B, polio, pneumococcus, and influenza.
- Future studies should focus on investigating the influence of BCG vaccine strain and on the optimal timing of administration to exploit the immunomodulatory effects of BCG to improve vaccine efficacy and duration of protection.

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Declaration of interest

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Reviewer disclosures

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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4.3 ADDITIONAL INFORMATION

Table 4.1 Seroprotection rates at 7 and 13 months of age in participants who were randomised to BCG or no BCG on day 0-1 of life (post-hoc analysis)

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**BCG-vaccinated** (n=16) **% (n); (95% CI)**	**BCG-naïve** (n=18) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**	**BCG-vaccinated** (n=70) **% (n); (95% CI)**	**BCG-naïve** (n=63) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**
Diphtheria	0.1 IU/mL[1]	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	95.2 (60); (86.7, 99.0)	4.8 (0.6, 13.1)	0.10
Tetanus	0.1 IU/mL[1]	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (35); (90.0, 100)[2]	100 (34); (89.7, 100)[2]	0 (-10.0, 10.3)[2]	-
PT	25 IU/mL	93.8 (15); (69.8, 99.8)	94.4 (17); (72.7, 99.9)	-0.7 (-24.2, 21.0)	1	35.7 (25); (24.6, 48.0)	34.9 (22); (23.3, 48.0)	0.8 (-15.5, 16.9)	1
Hib	0.15 µg/mL	62.5 (10); (35.4, 84.8)	72.2 (13); (46.5, 90.3)	-9.7 (-40.1, 21.7)	0.72	77.1 (27); (59.9, 89.6)[2]	85.3 (29); (68.9, 95.0)[2]	-8.2 (-27.0, 11.0)[2]	0.54
Polio type 1	0.23 IU/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	100 (63); (94.3, 100)	0 (-5.2, 5.8)	-
Polio type 2	0.29 IU/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	100 (63); (94.3, 100)	0 (-5.2, 5.8)	-
Polio type 3	0.12 IU/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	100 (63); (94.3, 100)	0 (-5.2, 5.8)	-
Pn 1	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	90.0 (63); (80.5, 95.9)	95.2 (60); (86.7, 99.0)	-5.2 (-15.2, 4.4)	0.33
Pn 3	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	68.6 (48); (56.4, 79.1)	63.5 (40); (50.4, 75.3)	5.1 (-11.0, 2.1)	0.58
Pn 4	0.35 µg/mL	68.7 (11); (41.3, 89.0)	66.7 (12); (41.0, 86.7)	2.1 (-29.4, 32.5)	1	28.6 (20); (18.4, 40.6)	23.8 (15); (14.0, 36.2)	4.8 (-10.4, 19.6)	0.56
Pn 5	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	88.6 (62); (78.7, 94.9)	87.3 (55); (76.5, 94.4)	1.3 (-10.2, 13.3)	1
Pn 6A	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	90 (63); (80.5, 95.9)	85.7 (54); (74.6, 93.3)	4.3 (-7.1, 16.4)	0.59
Pn 6B	0.35 µg/mL	81.3 (13); (54.4, 96.0)	88.9 (16); (65.3, 98.6)	-7.6 (-34.6, 18.3)	0.65	68.6 (48); (56.4, 79.1)	60.3 (38); (47.2, 72.4)	8.3 (-8.0, 24.3)	0.37
Pn 7F	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	99.4 (62); (91.5, 100)	1.6 (-3.7, 8.5)	0.47
Pn 9V	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	82.9 (58); (72.0, 90.8)	76.2 (48); (63.8, 86.0)	6.7 (-7.1, 20.8)	0.39
Pn 14	0.35 µg/mL	87.5 (14); (61.7, 98.4)	83.3 (15); (58.6, 96.4)	4.2 (-22.9, 29.9)	1	82.9 (58); (72.0, 90.8)	85.7 (54); (74.6, 93.3)	-2.9 (-15.5, 10.1)	0.81
Pn 18C	0.35 µg/mL	93.8 (15); (69.8, 99.8)	100 (18); (81.5, 100)	-6.3 (-28.8, 23.4)	0.47	80.0 (56); (68.7, 88.6)	79.4 (50); (67.3, 88.5)	0.6 (-13.1, 14.7)	1
Pn 19A	0.35 µg/mL	87.5 (14); (61.7, 98.4)	100 (18); (81.5, 100)	-12.5 (-36.4, 6.8)	0.21	52.9 (37); (40.6, 64.9)	44.4 (28); (31.9, 57.5)	8.4 (-8.6, 24.9)	0.39
Pn 19F	0.35 µg/mL	100 (16); (79.4, 100)	100 (18); (81.5, 100)	0 (-19.8, 18.0)	-	100 (70); (94.9, 100)	100 (63); (94.3, 100)	0 (-5.2, 5.8)	-
Pn 23F	0.35 µg/mL	87.5 (14); (61.7, 98.4)	94.4 (17); (72.7, 99.9)	-6.9 (-32.0, 15.9)	0.59	57.1 (40); (44.7, 68.9)	63.5 (40); (50.4, 75.3)	16.8 (-22.6, 10.3)	0.48
						Antibodies to 12-month vaccines measured at 13 months of age			
						BCG-vaccinated (n=35) **% (n); (95% CI)**	**BCG-naïve** (n=29) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**
Measles	0.12 IU/mL					100 (35); (90.0, 100)	96.6 (28); (82.2, 99.9)	3.4 (-6.8, 17.3)	0.45
Mumps	45 IU/mL					68.6 (24); (50.7, 83.1)	58.6 (17); (38.9, 76.5)	10.0 (-13.6, 32.9)	0.44
Rubella	10 IU/mL					91.4 (32); (76.9, 98.2)	82.8 (24); (64.2, 94.2)	8.7 (-8.3, 2.8)	0.45
MenC	2 µg/mL					97.1 (34); (85.1, 99.9)	100 (29); (88.1, 100)	-2.9 (-14.7, 9.2)	1
Hib	0.15 µg/mL					100 (35); (90.0, 100)	100 (29); (88.1, 100)	0 (-10.0, 11.9)	-
Tetanus	0.01 IU/mL					94.3 (33); (80.8, 99.3)	93.1 (27); (77.2, 99.2)	1.2 (-13.0, 17.2)	1

[1] 0.01 IU/mL at 13 months of age [2] only participants who have not had Hib-MenC

BCG = Bacillus Calmette-Guérin vaccine, CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 4.2 Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) at 7 and 13 months of age in participants who were randomised to BCG or no BCG on day 0-1 of life (post-hoc analysis)

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**BCG-vaccinated** (n=16) **GMC (95% CI)**	**BCG-naïve** (n=18) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**	**BCG-vaccinated** (n=70) **GMC (95% CI)**	**BCG-naïve** (n=63) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Diphtheria[1]	0.42 (0.29, 0.61)	0.34 (0.25, 0.47)	1.22 (0.77, 1.96)	0.39	1.09 (0.70, 1.70)[b,g]	0.69	0.09 (0.07, 0.12)	0.06 (0.05, 0.08)	1.40 (1.00, 1.96)	0.05	1.27 (0.93, 1.75)[b,h]	0.13
Tetanus[1]	1.17 (0.97, 1.43)	0.88 (0.60, 1.28)	1.34 (0.88, 2.04)	0.17	1.34 (0.88, 2.04)	0.17	0.64 (0.43, 0.95)[3]	0.42 (0.29, 0.60)[3]	1.52 (0.90, 2.57)[3]	0.11	**1.72 (1.06, 2.82)[3,f,j]**	**0.03**
PT[1]	72.45 (49.56, 105.91)	66.62 (46.86, 94.71)	1.09 (0.66, 1.79)	0.73	0.99 (0.62, 1.59)[g]	0.97	22.13 (17.43, 28.10)	16.98 (13.00, 22.18)	1.30 (0.91, 1.86)	0.14	1.33 (0.95, 1.87)[b]	0.10
FHA[1]	54.19 (38.50, 76.29)	61.33 (42.34, 88.83)	0.88 (0.54, 1.44)	0.61	0.87 (0.55, 1.36)[e,g]	0.52	20.39 (16.33, 25.47)	16.56 (13.35, 20.55)	1.23 (0.90, 1.68)	0.18	1.15 (0.85, 1.55)[b,h]	0.36
PRN[1]	66.36 (44.78, 98.32)	49.92 (30.06, 82.92)	1.32 (0.71, 2.49)	0.36	0.83 (0.49, 1.39)[b,f]	0.47	14.73 (11.26, 19.27)	10.74 (7.86, 14.69)	1.37 (0.91, 2.06)	0.13	1.39 (0.94, 2.08)[b]	0.10
Hib[2]	0.38 (0.15, 0.98)	0.36 (0.20, 0.66)	1.06 (0.37, 3.05)	0.91	1.06 (0.37, 3.05)	0.91	1.34 (0.67, 2.68)[3]	1.49 (0.72, 3.12)[3]	0.89 (0.33, 2.41)[3]	0.82	1.32 (0.50, 3.49)[3,g,h]	0.57
Polio type 1[1]	32.55 (18.27, 58.00)	22.97 (11.83, 44.61)	1.42 (0.60, 3.34)	0.41	1.42 (0.60, 3.34)	0.41	11.04 (8.46, 14.40)	11.87 (8.80, 16.01)	0.93 (0.63, 1.38)	0.72	0.91 (0.62, 1.33)[j]	0.61
Polio type 2[1]	57.85 (36.12, 92.64)	48.94 (26.25, 91.24)	1.18 (0.55, 2.54)	0.66	1.18 (0.55, 2.54)	0.66	20.91 (15.25, 28.69)	25.56 (18.97, 34.44)	0.82 (0.53, 1.26)	0.36	0.82 (0.53, 1.26)	0.36
Polio type 3[1]	16.20 (10.45, 25.12)	15.75 (7.49, 33.13)	0.95 (0.39, 2.31)	0.90	0.95 (0.39, 2.31)	0.90	8.81 (6.44, 12.06)	10.44 (7.72, 14.10)	0.84 (0.55, 1.30)	0.84	0.84 (0.55, 1.30)	0.84
Pn 1[2]	4.50 (2.99, 6.78)	4.25 (2.32, 7.77)	1.06 (0.52, 2.18)	0.87	0.88 (0.46, 1.70)[g,h]	0.69	1.33 (1.04, 1.70)	1.09 (0.89, 1.32)	1.23 (0.89, 1.68)	0.20	1.23 (0.89, 1.68)	0.20
Pn 3[2]	1.34 (0.99, 1.80)	1.50 (1.00, 2.26)	0.89 (0.54, 1.46)	0.63	0.89 (0.54, 1.46)	0.63	0.56 (0.46, 0.67)	0.52 (0.43, 0.63)	1.07 (0.82, 1.39)	0.62	1.07 (0.82, 1.39)	0.62
Pn 4[2]	0.54 (0.35, 0.84)	0.59 (0.37, 0.95)	0.92 (0.49, 1.72)	0.78	0.92 (0.49, 1.72)	0.78	0.25 (0.21, 0.29)	0.21 (0.18, 0.25)	1.16 (0.91, 1.48)	0.22	1.17 (0.92, 1.49)[b]	0.19
Pn 5[2]	3.38 (2.32, 4.93)	2.75 (1.70, 4.46)	1.23 (0.68, 2.24)	0.49	1.05 (0.61, 1.81)[g,h]	0.85	1.06 (0.85, 1.34)	0.86 (0.69, 1.07)	1.24 (0.91, 1.70)	0.18	1.24 (0.91, 1.70)	0.18
Pn 6A[2]	5.68 (3.93, 8.21)	4.89 (3.14, 7.61)	1.16 (0.66, 2.04)	0.59	1.07 (0.62, 1.85)[h]	0.59	**1.18 (0.92, 1.52)**	**0.81 (0.64, 1.03)**	**1.45 (1.03, 2.05)**	**0.03**	1.31 (0.94, 1.84)[b,h]	0.11
Pn 6B[2]	1.77 (0.68, 4.61)	1.83 (0.93, 3.59)	0.97 (0.32, 2.93)	0.95	0.97 (0.32, 2.93)	0.95	0.59 (0.44, 0.80)	0.45 (0.34, 0.59)	1.33 (0.88, 2.01)	0.18	1.34 (0.89, 2.02)[b]	0.15
Pn 7F[2]	6.31 (4.40, 9.07)	6.56 (4.48, 9.60)	0.96 (0.58, 1.60)	0.88	0.86 (0.54, 1.37)[g]	0.51	2.07 (1.73, 2.47)	1.70 (1.42, 2.04)	1.21 (0.94, 1.56)	0.13	1.13 (0.88, 1.46)[b,h]	0.32
Pn 9V[2]	2.44 (1.68, 3.54)	2.63 (1.64, 4.24)	0.92 (0.51, 1.68)	0.80	0.79 (0.46, 1.36)[g,h]	0.38	0.66 (0.54, 0.80)	0.56 (0.45, 0.69)	1.18 (0.89, 1.57)	0.24	1.09 (0.82, 1.45)[h,j]	0.54
Pn 14[2]	1.82 (0.90, 3.69)	2.59 (1.38, 4.85)	0.70 (0.28, 1.74)	0.43	0.60 (0.26, 1.37)[h]	0.21	1.00 (0.75, 1.33)	0.91 (0.71, 1.18)	1.10 (0.75, 1.69)	0.63	1.10 (0.75, 1.69)	0.63
Pn 18C[2]	2.36 (1.33, 4.20)	3.23 (2.13, 4.91)	0.73 (0.37, 1.43)	0.35	0.64 (0.35, 1.19)[h]	0.15	0.74 (0.59, 0.91)	0.63 (0.51, 0.78)	1.17 (0.86, 1.57)	0.31	1.15 (0.86, 1.54)[j]	0.35
Pn 19A[2]	1.22 (0.65, 2.28)	1.90 (1.27, 2.84)	0.64 (0.32, 1.29)	0.21	0.64 (0.32, 1.29)	0.21	0.53 (0.39, 0.71)	0.37 (0.29, 0.46)	1.44 (0.98, 2.10)	0.06	1.44 (0.98, 2.10)	0.06
Pn 19F[2]	9.65 (5,78, 16.11)	12.50 (8.53, 18.34)	0.77 (0.42, 1.41)	0.39	0.77 (0.42, 1.41)	0.39	3.33 (2.57, 4.31)	2.90 (2.28, 3.70)	1.15 (0.81, 1.63)	0.44	1.06 (0.74, 1.30)[h,j]	0.74
Pn 23F[2]	2.41 (1.28, 4.54)	2.62 (1.47, 4.67)	0.92 (0.40, 2.09)	0.84	0.92 (0.40, 2.09)	0.84	0.56 (0.42, 0.74)	0.54 (0.40, 0.74)	1.02 (0.67, 1.55)	0.91	1.02 (0.68, 1.56)[f]	0.88
							Antibodies to 12-month vaccines measured at 13 months of age					
							BCG-vaccinated (n=35) **GMC (95% CI)**	**BCG-naïve** (n=29) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Measles[1]							3.44 (2.59, 4.57)	2.82 (1.58, 5.03)	1.22 (0.67, 2.22)	0.51	1.22 (0.67, 2.22)	0.51
Mumps[1]							91.03 (63.10, 131.32)	51.33 (30.59, 86.11)	1.77 (0.97, 3.25)	0.06	1.43 (0.87, 2.35)[d,f,h]	0.16
Rubella[1]							57.90 (39.54, 84.79)	42.13 (22.69, 78.22)	1.37 (0.69, 2.73)	0.36	1.19 (0.67, 2.10)[g,h]	0.55
MenC[2]							14.19 (9.72, 20.74)	18.64 (14.25, 24.38)	0.76 (0.47, 1.22)	0.26	0.95 (0.61, 1.49)[c,e,f,g]	0.82
Hib[2]							11.58 (6.45, 20.81)	19.35 (12.18, 30.73)	0.59 (0.28, 1.27)	0.18	0.64 (0.31, 1.31)[e]	0.21
Tetanus[1]							0.85 (0.58, 1.25)	0.71 (0.50, 1.02)	1.19 (0.71, 2.02)	0.50	1.19 (0.71, 2.02)	0.50

[1] IU/ml [2] µg/ml [3] only participants who have not had Hib-MenC

[a] maternal BCG status
[b] maternal dTpa during pregnancy
[c] gestational age
[d] delivery mode
[e] sex
[f] birth weight
[g] age at bleeding
[h] time between immunisation and bleeding
[i] age at randomisation
[j] MMR/MenC-Hib immunisation status

BCG = Bacillus Calmette-Guérin vaccine, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin, PRN = pertactin

Table 4.3 Seroprotection rates at 7 and 13 months of age in participants who were randomised to BCG or no BCG on day 2 to 10 of life (post-hoc analysis)

		Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
Vaccine antigen	**Protective correlate**	**BCG-vaccinated** (n=29) **% (n); (95% CI)**	**BCG-naïve** (n=28) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**	**BCG-vaccinated** (n=98) **% (n); (95% CI)**	**BCG-naïve** (n=78) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**
Diphtheria	0.1 IU/mL[1]	100 (29); (88.1, 100)	89.3 (25); (71.8, 97.7)	10.7 (-1.8, 27.4)	0.11	98.0 (96); (92.8, 99.8)	100 (78); (93.9, 99.6)	-2.0 (-7.2, 2.7)	0.50
Tetanus	0.1 IU/mL[1]	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	100 (57); (93.7, 100)[2]	100 (39); (91.0, 100)[2]	0 (-6.4, 9.1)[2]	-
PT	25 IU/mL	86.2 (25); (68.8, 96.1)	89.3 (25); (71.8, 97.7)	-3.1 (-21.9, 15.9)	1	26.5 (26); (18.1, 36.4)	26.9 (21); (17.5, 38.2)	-0.4 (-12.2, 16.7)	0.53
Hib	0.15 µg/mL	75.9 (22); (56.5, 89.7)	85.7 (24); (67.3, 96.0)	-9.9 (-30.7, 11.5)	0.50	70.2 (40); (56.6, 81.6)[2]	76.9 (30); (60.7, 88.9)[2]	-6.7 (-23.9, 11.9)[2]	0.49
Polio type 1	0.23 IU/mL	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	100 (98); (96.3, 100)	100 (78); (95.4, 100)	0 (-3.8, 4.7)	-
Polio type 2	0.29 IU/mL	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	99.0 (97); (94.4, 100)	100 (78); (95.4, 100)	-1.0 (-5.6, 3.7)	1
Polio type 3	0.12 IU/mL	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	100 (98); (96.3, 100)	100 (78); (95.4, 100)	0 (-3.8, 4.7)	-
Pn 1	0.35 µg/mL	96.6 (28); (82.2, 99.9)	92.9 (26); (85.2, 99.5)	3.7 (-11.2, 19.9)	0.61	93.9 (92); (87.1, 97.7)	94.9 (74); (90.0, 98.0)	-1.0 (-8.4, 7.1)	1
Pn 3	0.35 µg/mL	96.6 (28); (82.2, 99.9)	100 (28); (87.7, 100)	-3.4 (-17.4, 9.0)	1	73.5 (72); (63.6, 81.9)	70.5 (55); (59.1, 80.3)	3 (-10.3, 16.5)	0.74
Pn 4	0.35 µg/mL	82.8 (24); (64.2, 94.2)	57.1 (28); (37.2, 75.5)	25.6 (-1.7, 4.7)	0.05	24.5 (24); (16.4, 34.2)	16.7 (13); (9.2, 26.8)	7.8 (-4.5, 19.6)	0.26
Pn 5	0.35 µg/mL	96.6 (28); (82.2, 99.9)	96.4 (27); (81.7, 99.9)	0.1 (-14.2, 14.9)	1	85.7 (84); (77.2, 92.0)	87.2 (68); (77.7, 93.7)	-1.5 (-11.7, 9.4)	0.83
Pn 6A	0.35 µg/mL	100 (29); (88.1, 100)	92.9 (26); (76.5, 99.1)	7.1 (-5.2, 22.8)	0.24	85.7 (84); (77.2, 92.0)	84.6 (66); (74.7, 91.8)	1.1 (-9.5, 12.5)	0.84
Pn 6B	0.35 µg/mL	96.6 (28); (82.2, 99.9)	85.7 (24); (67.3, 96.0)	10.8 (-5.1, 28.8)	0.19	67.3 (66); (57.81 76.5)	60.3 (47); (48.5, 71.2)	7.2 (-3.5, 17.9)	0.35
Pn 7F	0.35 µg/mL	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	100 (98); (96.3, 100)	98.7 (77); (93.1, 100)	1.3 (-2.5, 6.9)	0.44
Pn 9V	0.35 µg/mL	100 (29); (88.1, 100)	92.9 (26); (76.5, 99.1)	7.1 (-52, 22.8)	0.24	74.5 (73); (64.7, 82.8)	70.5 (55); (59.1, 80.3)	4 (-9.2, 17.5)	0.61
Pn 14	0.35 µg/mL	93.1 (27); (78.8, 97.5)	96.4 (27); (81.7, 99.9)	-3.3 (-19.1, 11.9)	1	88.8 (87); (80.8, 94.3)	87.2 (68); (77.7, 93.7)	1.6 (-8.1, 12.1)	0.82
Pn 18C	0.35 µg/mL	100 (29); (88.1, 100)	89.3 (25); (71.8, 97.7)	10.7 (-1.8, 27.4)	0.11	72.4 (71); (68.6, 82.0)	76.9 (60); (66.0, 85.7)	-4.5 (-17.1, 8.7)	0.60
Pn 19A	0.35 µg/mL	100 (29); (88.1, 100)	85.7 (24); (67.3, 96.0)	14.3 (-1.5, 31.7)	0.05	53.1 (52); (42.7, 63.2)	47.4 (37); (36.0, 59.1)	5.6 (-9.2, 20.2)	0.54
Pn 19F	0.35 µg/mL	100 (29); (88.1, 100)	100 (28); (87.7, 100)	0 (-11.9, 12.3)	-	98.0 (96); (92.8, 99.8)	100 (78); (93.9, 99.6)	-2.0 (-7.2, 2.7)	0.50
Pn 23F	0.35 µg/mL	100 (29); (88.1, 100)	89.3 (25); (71.8, 97.7)	10.7 (-1.8, 27.4)	0.11	**69.4 (68); (59.3, 78.3)**	**52.6 (41); (40.9, 64.0)**	**16.8 (-3.8, 17.8)**	**03.0**
						Antibodies to 12-month vaccines measured at 13 months of age			
						BCG-vaccinated (n=43) **% (n); (95% CI)**	**BCG-naïve** (n=40) **% (n); (95% CI)**	**Difference % (95% CI)**	**p-value**
Measles	0.12 IU/mL					97.7 (42); (87.7, 99.9)	97.5 (39); (86.8, 99.9)	0.2 (-15.5, 25.2)	1
Mumps	45 IU/mL					62.8 (27); (46.7, 77.0)	55.0 (55); (38.5, 70.7)	7.8 (-15.5, 25.2)	0.51
Rubella	10 IU/mL					86.0 (37); (72.0, 94.7)	87.5 (35); (72.2, 95.8)	-1.5 (-15.5, 25.2)	1
MenC	2 µg/mL					100 (43); (91.8, 100)	97.5 (39); (86.8, 99.9)	2.5 (-5.9, 13.0)	0.48
Hib	0.15 µg/mL					97.7 (42); (87.7, 99.9)	97.5 (39); (86.8, 99.9)	0.2 (-9.9, 10.9)	1
Tetanus	0.01 IU/mL					93.0 (40); (80.9, 98.5)	97.5 (39); (86.8, 99.9)	-4.5 (-16.6, 6.8)	0.62

[1] 0.01 IU/mL at 13 months of age [2] only participants who have not had Hib-MenC

BCG = Bacillus Calmette-Guérin vaccine, CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin

Table 4.4 GMCs and geometric mean antibody ratios GMRs at 7 and 13 months of age in participants who were randomised to BCG or no BCG on day 2 to 10 of life (post-hoc analysis)

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**BCG-vaccinated** (n=29) **GMC (95% CI)**	**BCG-naïve** (n=28) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**	**BCG-vaccinated** (n=98) **GMC (95% CI)**	**BCG-naïve** (n=78) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Diphtheria[1]	0.50 (0.39, 0.65)	0.33 (0.23, 0.48)	1.51 (0.98, 2.33)	0.06	1.21 (0.78, 1.87)[b,g]	0.39	0.08 (0.07, 0.10)	0.08 (0.06, 0.10)	1.07 (0.78, 1.47)	0.65	1.08 (0.81, 1.43)[b,h]	0.61
Tetanus[1]	1.25 (0.95, 1.66)	1.10 (0.82, 1.47)	1.14 (0.77, 1.69)	0.51	1.14 (0.77, 1.69)	0.51	0.41 (0.29, 0.58)[3]	0.37 (0.27, 0.51)[3]	1.10 (0.67, 1.80)[3]	0.71	1.16 (0.73, 1.84)[3,j]	0.52
PT[1]	80.73 (56.22, 115.93)	61.36 (45.98, 81.87)	1.32 (0.84, 2.07)	0.23	1.04 (0.69, 1.57)[b]	0.85	15.44 (12.59, 18.94)	17.24 (13.78, 21.57)	0.90 (0.66, 1.21)	0.47	0.92 (0.70, 1.21)[b,g,h]	0.55
FHA[1]	70.57 (50.62, 98.38)	64.49 (45.04, 92.33)	1.09 (0.68, 1.76)	0.71	0.82 (0.52, 1.27)[b,c]	0.36	19.69 (15.75, 24.61)	19.85 (16,25, 24.24)	0.99 (0.73, 1.35)	0.96	1.03 (0.77, 1.38)[b,c,h]	0.84
PRN[1]	66.11 (46.55, 93.91)	59.19 (38.72, 90.50)	1.12 (0.65, 1.91)	0.68	0.83 (0.49, 1.39)[b,f]	0.47	11.21 (8.65, 14.52)	12.21 (9.34, 15.96)	0.92 (0.63, 1.33)	0.55	0.97 (0.68, 1.37)[b,e,h]	0.85
Hib[2]	0.66 (0.34, 1.29)	0.58 (0.34, 1.02)	1.13 (0.48, 2.66)	0.77	0.80 (0.36, 1.78)[g]	0.58	0.87 (0.45, 1.69)[3]	1.02 (0.47, 2.17)[3]	0.85 (0.31, 2.35)[3]	0.76	0.97 (0.36, 2.60)[3,g,h]	0.95
Polio type 1[1]	38.72 (28.56, 52.50)	41.53 (27.97, 61.65)	0.93 (0.57, 1.52)	0.77	0.93 (0.57, 1.52)	0.77	10.06 (7.99, 12.67)	12.07 (9.43, 15.44)	0.83 (0.60, 1.17)	0.29	0.85 (0.61, 1.18)[b,g,h]	0.32
Polio type 2[1]	65.26 (49.67, 85.75)	69.96 (44.53, 109.92)	1.00 (0.57, 1.74)	0.99	0.85 (0.49, 0.97)[b]	0.57	18.88 (14.61, 24.41)	22.72 (17.05, 30.28)	0.83 (0.57, 1.22)	0.34	0.85 (0.59, 1.24)[b,h]	0.41
Polio type 3[1]	23.18 (16.45, 32.66)	27.77 (18.19, 42.40)	0.83 (0.49, 1.42)	0.50	0.83 (0.49, 1.42)	0.50	8.64 (6.60, 11.33)	8.27 (6.19, 11.21)	1.04 (0.70, 1.57)	0.83	0.92 (0.69, 1.23)[b,g,h]	0.57
Pn 1[2]	**5.80 (4.24, 7.92)**	**3.26 (2.08, 5.11)**	**1.78 (1.05, 3.03)**	**0.03**	1.47 (0.89, 1.80)[g]	0.13	1.16 (0.98, 1.38)	1.11 (0.93, 1.34)	1.04 (0.81, 1.34)	0.75	1.03 (0.81, 1.31)[b,h]	0.80
Pn 3[2]	1.56 (1.19, 2.03)	1.34 (1.05, 1.70)	1.16 (0.82, 1.66)	0.39	1.16 (0.82, 1.66)	0.39	0.58 (0.48, 0.70)	0.50 (0.42, 0.59)	1.16 (0.89, 1.50)	0.26	1.13 (0.88, 1.44)[h]	0.33
Pn 4[2]	0.56 (0.42, 0.73)	0.42 (0.30, 0.60)	1.31 (0.85, 2.02)	0.21	1.18 (0.77, 1.81)[g]	0.44	0.22 (0.19, 0.25)	0.19 (0.17, 0.22)	1.14 (0.93, 1.38)	0.21	1.10 (0.91, 1.33)[c,h]	0.31
Pn 5[2]	**4.21 (3.06, 5.81)**	**2.46 (1.67, 3.63)**	**1.71 (1.05, 2.80)**	**0.03**	1.49 (0.89, 1.83)[g]	0.11	0.93 (0.77, 1.12)	0.85 (0.69, 1.04)	1.09 (0.82, 1.44)	0.54	1.08 (0.83, 1.40)[b,g,h]	0.58
Pn 6A[2]	5.70 (4.40, 7.38)	3.64 (2.42, 5.47)	1.57 (0.98, 2.50)	0.06	1.26 (0.78, 2.02)[b,g]	0.34	1.00 (0.82, 1.21)	1.03 (0.81, 1.30)	0.97 (0.72, 1.31)	0.87	0.96 (0.73, 1.26)[b,g,h]	0.76
Pn 6B[2]	2.81 (1.78, 4.44)	1.38 (0.72, 2.68)	2.03 (0.93, 4.43)	0.07	1.69 (0.76, 3.70)[g]	0.18	0.55 (0.44, 0.69)	0.49 (0.38, 0.64)	1.11 (0.79, 1.56)	0.55	1.10 (0.79, 1.52)[b,h]	0.58
Pn 7F[2]	6.83 (5.13, 9.10)	5.16 (3.70, 7.21)	1.32 (0.86, 2.03)	0.20	1.32 (0.86, 2.03)	0.20	1.92 (1.63, 2.25)	1.82 (1.51, 2.18)	1.06 (0.83, 1.34)	0.66	1.03 (0.82, 1.30)[g,h]	0.81
Pn 9V[2]	**3.36 (2.68, 4.21)**	**1.71 (1.18, 2.48)**	**1.96 (1.29, 2.99)**	**<0.01**	**1.77 (1.16, 2.68)[g]**	**<0.01**	0.60 (0.51, 0.71)	0.58 (0.47, 0.72)	1.04 (0.79, 1.36)	0.78	1.00 (0.78, 1.29)[h]	0.98
Pn 14[2]	3.70 (2.26, 6.06)	2.42 (1.57, 3.73)	1.53 (0.80, 2.91)	0.19	1.53 (0.80, 2.91)	0.19	1.17 (0.97, 1.42)	1.08 (0.87, 1.33)	1.09 (0.82, 1.44)	0.56	1.06 (0.81, 1.38)[g,h]	0.69
Pn 18C[2]	3.62 (2.76, 4.75)	2.53 (1.65, 3.87)	1.43 (0.88, 2.34)	0.15	1.27 (0.78, 2.07)[g]	0.33	0.64 (0.54, 0.75)	0.62 (0.52, 0.74)	1.03 (0.80, 1.31)	0.84	0.99 (0.79, 1.24)[g,h]	0.93
Pn 19A[2]	**2.62 (1.93, 3.56)**	**1.27 (0.90, 1.79)**	**2.06 (1.32, 3.23)**	**<0.01**	1.55 (0.98, 2.45)[b,c,g]	0.06	0.46 (0.35, 0.61)	0.48 (0.34, 0.68)	0.96 (0.62, 1.48)	0.85	0.90 (0.60, 1.36)[g,h,j]	0.62
Pn 19F[2]	**16.01 (11.92, 21.51)**	**8.63 (6.00, 12.39)**	**1.86 (1.18, 2.93)**	**<0.01**	**1.68 (1.06, 2.66)[g]**	**0.03**	3.21 (2.55, 4.04)	2.96 (2.32, 3.76)	1.09 (0.78, 1.51)	0.62	1.05 (0.76, 1.44)[h]	0.77
Pn 23F[2]	**3.31 (2.37, 4.64)**	**1.49 (1.93, 2.83)**	**2.23 (1.27, 3.91)**	**<0.01**	**2.23 (1.27, 3.91)**	**<0.01**	0.66 (0.52, 0.85)	0.48 (0.37, 0.64)	1.38 (0.95, 2.00)	0.09	1.33 (0.93, 1.90)[h]	0.12
							Antibodies to 12-month vaccines measured at 13 months of age					
							BCG-vaccinated (n=43) **GMC (95% CI)**	**BCG-naïve** (n=43) **GMC (95% CI)**	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Measles[1]							3.26 (2.46, 4.31)	2.67 (1.88, 3.79)	1.22 (0.78, 1.90)	0.37	1.11 (0.72, 1.89)[e,h]	0.63
Mumps[1]							52.91 (33.75, 82.96)	53.92 (35.54, 81.82)	0.98 (0.54, 1.80)	0.95	0.90 (0.56, 1.46)[g,h]	0.67
Rubella[1]							51.42 (33.22, 79.57)	61.70 (39.20, 97.09)	0.83 (0.44, 1.55)	0.56	0.68 (0.44, 1.05)[e,g,h]	0.08
MenC[2]							18.87 (14.85, 23.96)	17.23 (11.43, 25.98)	1.09 (0.69, 1.37)	0.70	1.09 (0.69, 1.37)	0.70
Hib[2]							15.73 (9.64, 25.68)	17.73 (10.58, 29.71)	0.89 (0.44, 1.79)	0.74	0.97 (0.36, 2.60)[c,f]	0.95
Tetanus[1]							1.04 (0.70, 1.55)	1.44 (0.98, 2.12)	0.72 (0.42, 1.25)	0.24	0.80 (0.47, 1.38)[e]	0.42

[1] IU/ml [2] µg/ml [3] only participants who have not had Hib-MenC

[a] maternal BCG status
[b] maternal dTpa during pregnancy
[c] gestational age
[d] delivery mode
[e] sex
[f] birth weight
[g] age at bleeding
[h] time between immunisation and bleeding
[i] age at randomisation
[j] MMR/MenC-Hib immunisation status

BCG = Bacillus Calmette-Guérin vaccine, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin, PRN = pertactin

Table 4.5 GMCs and GMRs at 7 and 13 months of age in participants who were randomised to BCG on day 0-1 or on day 2-10 of life (post-hoc analysis)

	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
Vaccine antigen	**GMC (95% CI) Randomised day 0-1** (n=16)	**GMC (95% CI) Randomised day 2-10** (n=29)	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**	**GMC (95% CI) Randomised day 0-1** (n=70)	**GMC 95% CI) Randomised day 2-10** (n=98)	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Diphtheria[1]	0.42 (0.29, 0.61)	0.50 (0.39, 0.65)	0.83 (0.55, 1.28)	0.39	0.83 (0.55, 1.24)[b,c,g]	0.34	1.27 (0.93, 1.75)[b,h]	1.08 (0.81, 1.43)[b,h]	1.10 (0.80, 1.51)	0.57	1.04 (0.78, 1.39)[b,g,h]	0.78
Tetanus[1]	1.17 (0.97, 1.43)	1.25 (0.95, 1.66)	0.94 (0.63, 1.49)	0.74	0.94 (0.63, 1.49)	0.74	1.72 (1.06, 2.82)[3,f,j]	1.16 (0.73, 1.84)[3,j]	1.57 (0.92, 2.68)	0.09	1.59 (0.99, 2.55)[3,j]	0.06
PT[1]	72.45 (49.56, 105.91)	80.73 (56.22, 115.93)	0.90 (0.52, 1.56)	0.69	1.01 (0.59, 1.73)[e]	0.97	**1.33 (0.95, 1.87)[b]**	**0.92 (0.70, 1.21)[b,g,h]**	**1.43 (1.05, 1.96)**	**0.02**	1.32 (0.99, 1.77)[b,h]	0.06
FHA[1]	54.19 (38.50, 76.29)	70.57 (50.62, 98.38)	0.77 (0.46, 1.27)	0.30	0.88 (0.55, 1.41)[e]	0.58	1.15 (0.85, 1.55)[b,h]	1.03 (0.77, 1.38)[b,c,h]	1.04 (0.75, 1.43)	0.83	0.97 (0.71, 1.32)[b,h]	0.84
PRN[1]	66.36 (44.78, 98.32)	66.11 (46.55, 93.91)	1.00 (0.58, 1.73)	0.99	1.19 (0.71, 1.99)[b,c,f]	0.49	1.39 (0.94, 2.08)[b]	0.97 (0.68, 1.37)[b,e,h]	1.31 (0.90, 1.92)	0.16	1.24 (0.87, 1.79)[b,h]	0.24
Hib[2]	0.38 (0.15, 0.98)	0.66 (0.34, 1.29)	0.58 (0.19, 1.75)	0.32	0.49 (0.18, 1.34)[g,h]	0.16	1.32 (0.50, 3.49)[3,g,h]	0.97 (0.36, 2.60)[3,g,h]	1.54 (0.57, 4.18)	0.39	1.61 (0.78, 3.30)[3,b,i]	0.19
Polio type 1[1]	32.55 (18.27, 58.00)	38.72 (28.56, 52.50)	0.84 (0.47, 1.49)	0.54	1.07 (0.61, 1.88)[c,f]	0.80	0.91 (0.62, 1.33)[j]	0.85 (0.61, 1.18)[b,g,h]	1.10 (0.77, 1.56)	0.60	1.10 (0.78, 1.56)[h]	0.58
Polio type 2[1]	57.85 (36.12, 92.64)	65.26 (49.67, 85.75)	0.83 (0.47, 1.47)	0.51	0.90 (0.52, 1.58)[b,c]	0.71	0.82 (0.53, 1.26)	0.85 (0.59, 1.24)[b,h]	1.11 (0.74, 1.66)	0.62	1.04 (0.70, 1.55)[b,h]	0.83
Polio type 3[1]	16.20 (10.45, 25.12)	23.18 (16.45, 32.66)	0.68 (0.34, 1.36)	0.27	0.79 (0.40, 1.58)[f]	0.50	0.84 (0.55, 1.30)	0.92 (0.69, 1.23)[b,g,h]	1.02 (0.67, 1.54)	0.93	0.98 (0.65, 1.46)[b,e,h]	0.91
Pn 1[2]	4.50 (2.99, 6.78)	5.80 (4.24, 7.92)	0.78 (0.47, 1.28)	0.32	0.70 (0.44, 1.11)[g]	0.13	1.23 (0.89, 1.68)	1.03 (0.81, 1.31)[b,h]	1.15 (0.86, 1.53)	0.34	1.12 (0.86, 1.48)[b,h]	0.39
Pn 3[2]	1.34 (0.99, 1.80)	1.56 (1.19, 2.03)	0.86 (0.57, 1.29)	0.46	0.88 (0.56, 1.36)	0.46	1.07 (0.82, 1.39)	1.13 (0.88, 1.44)[h]	0.96 (0.73, 1.26)	0.77	0.98 (0.76, 1.26)[e,h]	0.86
Pn 4[2]	0.54 (0.35, 0.84)	0.56 (0.42, 0.73)	0.98 (0.60, 1.58)	0.93	1.02 (0.73, 1.44)[g]	0.55	1.17 (0.92, 1.49)[b]	1.10 (0.91, 1.33)[c,h]	1.12 (0.90, 1.40)	0.31	1.11 (0.90, 1.37)[b,h]	0.34
Pn 5[2]	3.38 (2.32, 4.93)	4.21 (3.06, 5.81)	0.80 (0.49, 1.32)	0.38	0.73 (0.45, 1.16)[g]	0.18	1.24 (0.91, 1.70)	1.08 (0.83, 1.40)[b,g,h]	1.15 (0.85, 1.54)	0.36	1.12 (0.85, 1.47)[b,h]	0.44
Pn 6A[2]	5.68 (3.93, 8.21)	5.70 (4.40, 7.38)	1.00 (0.65, 1.53)	0.99	1.00 (0.65, 1.53)	0.99	1.31 (0.94, 1.84)[b,h]	0.96 (0.73, 1.26)[b,g,h]	1.18 (0.87, 1.62)	0.29	1.15 (0.87, 1.52)[b,e,h]	0.33
Pn 6B[2]	1.77 (0.68, 4.61)	2.81 (1.78, 4.44)	0.63 (0.26, 1.55)	0.31	0.63 (0.26, 1.55)	0.31	1.34 (0.89, 2.02)[b]	1.10 (0.79, 1.52)[b,h]	1.08 (0.75, 1.57)	0.68	1.05 (0.74, 1.49)[b,e,h]	0.77
Pn 7F[2]	6.31 (4.40, 9.07)	6.83 (5.13, 9.10)	0.92 (0.58, 1.46)	0.73	0.92 (0.58, 1.46)	0.73	1.13 (0.88, 1.46)[b,h]	1.03 (0.82, 1.30)[g,h]	1.08 (0.85, 1.37)	0.54	1.06 (0.85, 1.32)[b,e,g,h]	0.62
Pn 9V[2]	2.44 (1.68, 3.54)	3.36 (2.68, 4.21)	0.73 (0.49, 1.08)	0.11	**0.67 (0.46, 0.97)[g]**	**0.04**	1.09 (0.82, 1.45)[h,j]	1.00 (0.78, 1.29)[h]	1.09 (0.84, 1.42)	0.50	1.12 (0.88, 1.41)[e,h,i]	0.36
Pn 14[2]	1.82 (0.90, 3.69)	3.70 (2.26, 6.06)	0.49 (0.22, 1.12)	0.09	0.49 (0.22, 1.12)	0.09	1.10 (0.75, 1.69)	1.06 (0.81, 1.38)[g,h]	0.86 (0.62, 1.19)	0.35	0.87 (0.64, 1.20)[e,h]	0.40
Pn 18C[2]	2.36 (1.33, 4.20)	3.62 (2.76, 4.75)	0.65 (0.38, 1.12)	0.12	0.65 (0.38, 1.12)	0.12	1.15 (0.86, 1.54)[j]	0.99 (0.79, 1.24)[g,h]	1.16 (0.88, 1.51)	0.29	1.12 (0.88, 1.43)[b,f,g,h,i]	0.37
Pn 19A[2]	**1.22 (0.65, 2.28)**	**2.62 (1.93, 3.56)**	**0.47 (0.26, 0.85)**	**0.01**	**0.50 (0.27, 0.94)[c,g]**	**0.03**	1.44 (0.98, 2.10)	0.90 (0.60, 1.36)[g,h,j]	1.14 (0.76, 1.72)	0.53	1.17 (0.80, 1.72)[h,j]	0.42
Pn 19F[2]	9.65 (5,78, 16.11)	16.01 (11.92, 21.51)	0.60 (0.35, 1.03)	0.06	0.60 (0.35, 1.03)	0.06	1.06 (0.74, 1.30)[h,j]	1.05 (0.76, 1.44)[h]	1.04 (0.73, 1.46)	0.84	0.99 (0.71, 1.37)[d,g,h,i]	0.94
Pn 23F[2]	2.41 (1.28, 4.54)	3.31 (2.37, 4.64)	0.73 (0.39, 1.36)	0.31	0.73 (0.39, 1.36)	0.31	1.02 (0.68, 1.56)[f]	11.33 (0.93, 1.90)[h]	0.84 (0.57, 1.23)	0.36	0.82 (0.57, 1.17)[b,e,h]	0.27
							Antibodies to 12-month vaccines measured at 13 months of age					
							GMC (95% CI) Randomised day 0-1 (n=35)	**GMC(95% CI) Randomised day 2-10** (n=43)	**Unadjusted GMR (95% CI)**	**p-value**	**Adjusted GMR (95% CI)**	**p-value**
Measles[1]							1.22 (0.67, 2.22)	1.11 (0.72, 1.89)[e,h]	1.06 (0.71, 1.57)	0.78	1.09 (0.75, 1.60)[e,h]	0.64
Mumps[1]							1.43 (0.87, 2.35)[d,f,h]	0.90 (0.56, 1.46)[g,h]	1.72 (0.95, 3.10)	0.07	1.57 (0.95, 2.59)[h]	0.08
Rubella[1]							1.19 (0.67, 2.10)[g,h]	0.68 (0.44, 1.05)[e,g,h]	1.13 (0.63, 2.02)	0.69	1.00 (0.63, 1.59)[g,h]	0.99
MenC[2]							0.95 (0.61, 1.49)[c,e,f,g]	1.09 (0.69, 1.37)	0.75 (0.49, 1.15)	0.19	0.80 (0.52, 1.22)[e]	0.30
Hib[2]							0.64 (0.31, 1.31)[e]	0.97 (0.36, 2.60)[c,f]	0.74 (0.35, 1.55)	0.42	0.84 (0.40, 1.74)[e]	0.63
Tetanus[1]							1.19 (0.71, 2.02)	0.80 (0.47, 1.38)[e]	0.82 (0.47, 1.42)	0.47	0.82 (0.47, 1.42)	0.47

[1] IU/ml [2] µg/ml [3] only participants who have not had Hib-MenC

[a] maternal BCG status
[b] maternal dTpa during pregnancy
[c] gestational age
[d] delivery mode
[e] sex
[f] birth weight
[g] age at bleeding
[h] time between immunisation and bleeding
[i] MMR/MenC-Hib immunisation status

BCG = Bacillus Calmette-Guérin vaccine, CI = confidence interval, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, FHA = filamentous haemagglutinin, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcal serotype, PT = pertussis toxin, PRN = pertactin

Figure 4.1 GMCs of antibodies against DTPa-IPV-Hib in participants at 7 months of age. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

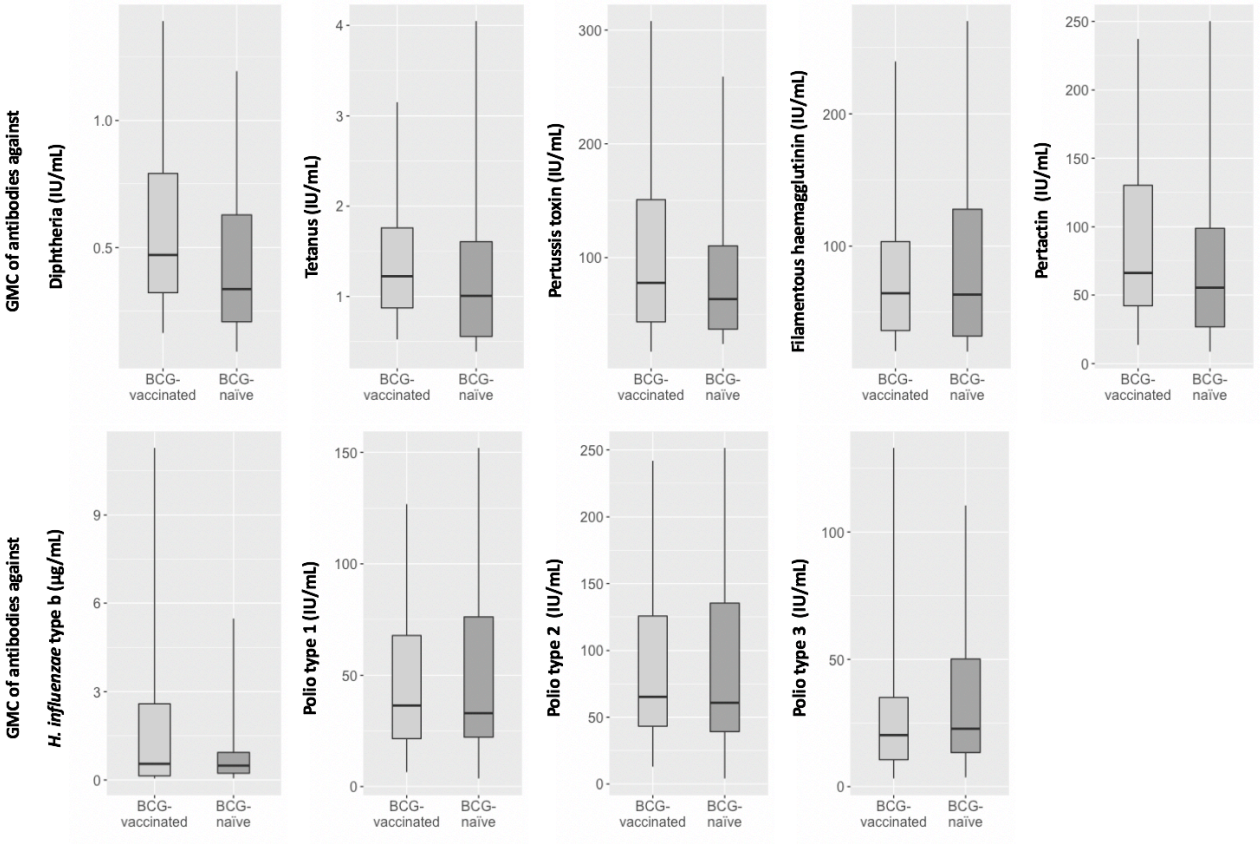


Figure 4.2 GMCs of antibodies against pneumococcus in participants at 7 months of age. Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

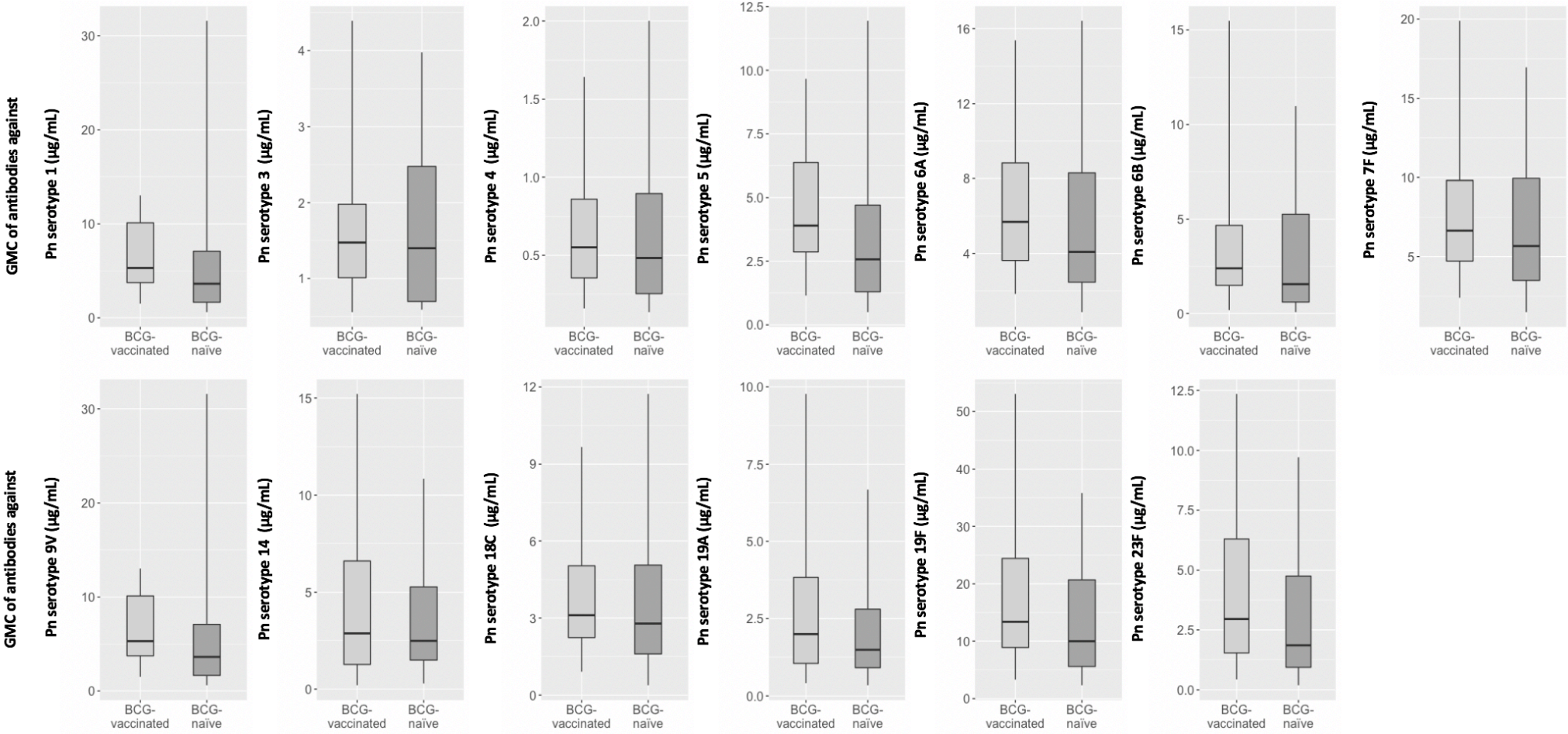


Figure 4.3 GMCs of antibodies against DTPa-IPV in participants at 13 months of age (persistence of antibodies). Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

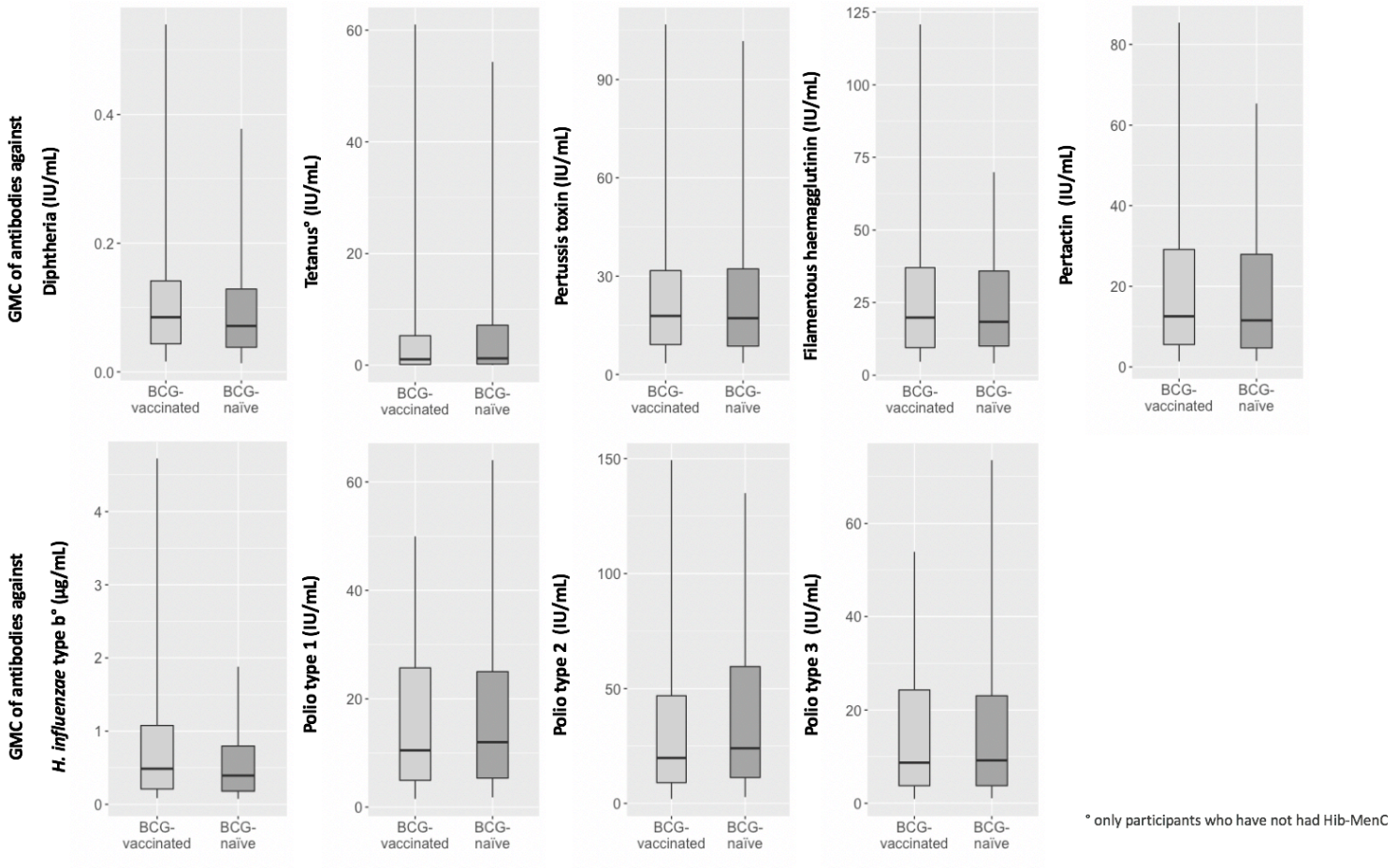


Figure 4.4 GMCs of antibodies against pneumococcus in participants (persistence of antibodies). Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

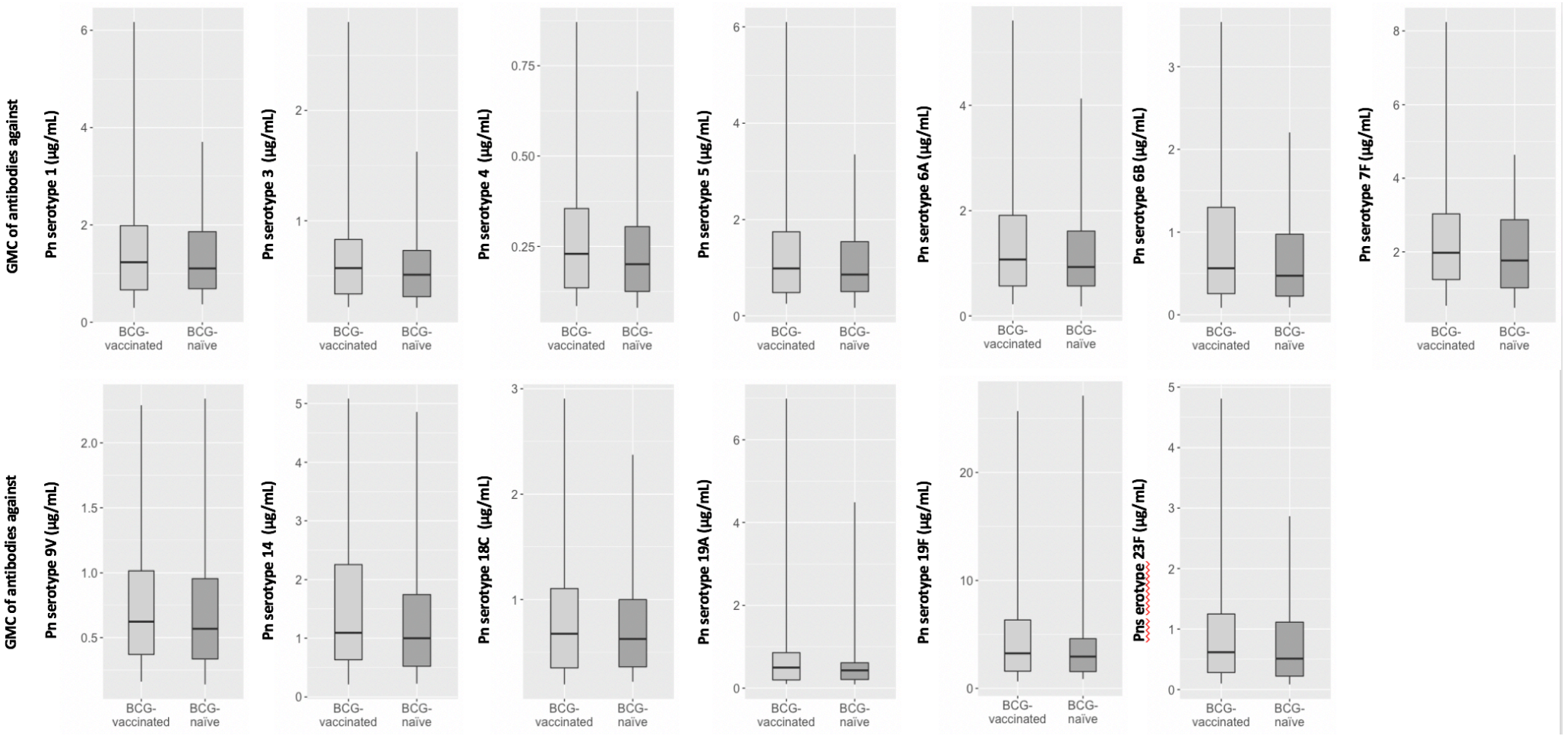


Figure 4.5 GMCs of antibodies against MMR, MenC and Hib in participants at 13 months of age (antibodies against 12-months immunisations). Boxes depict interquartile range (IQR) and whiskers depict 1.5-times the IQR.

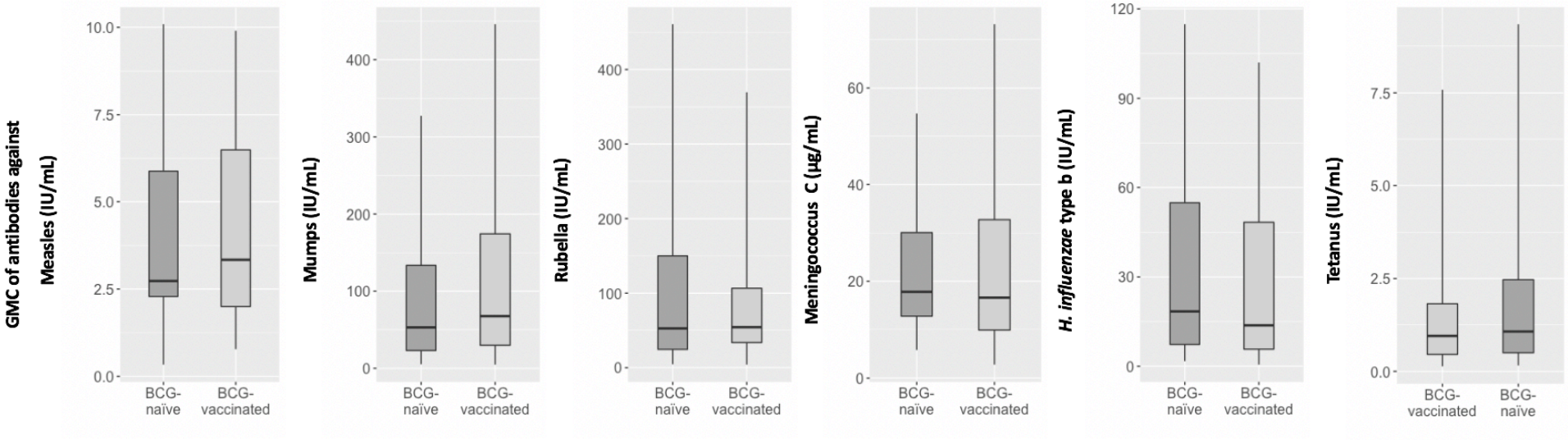
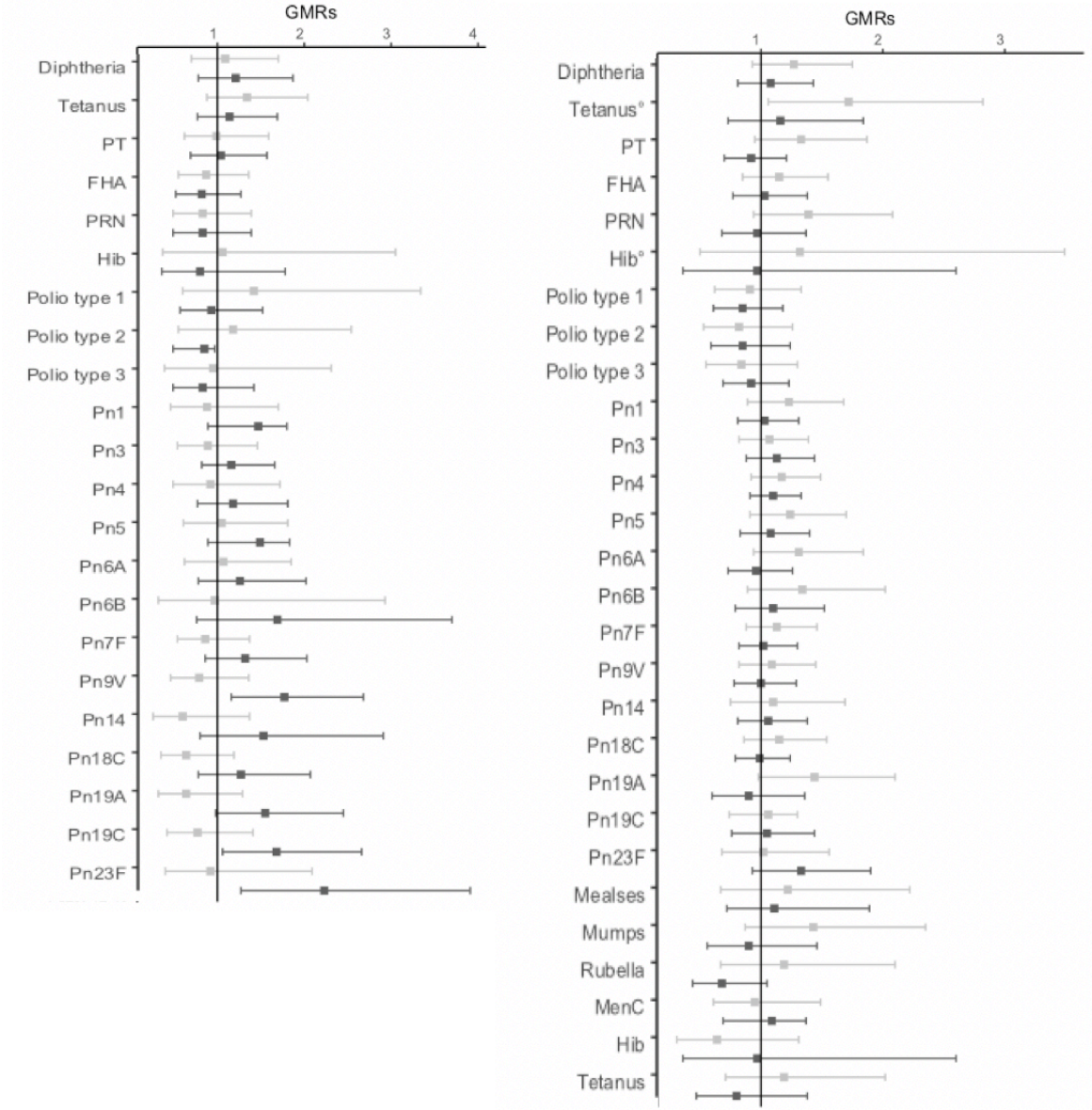


Figure 4.6 GMRs against 22 vaccine antigens in participants at 7 months (left panel) and against 26 vaccine antigens 13 months (right panel) of age in those randomised to BCG immunisation on day 0–1 (light grey) and day 2–10 (dark grey) of life. Error bars depict 95% CI.



CHAPTER 5. CORRELATION OF VACCINE RESPONSES

5.1 INTRODUCTION

This chapter contains the published version of a study that investigated the correlation of antibody responses to the routine vaccines given within the Australian standard immunisation schedule in healthy infants.

Correlation of vaccine responses

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Introduction

There are many different methods to quantify the immune response to vaccines including innate, humoral, cellular and cytokine responses. However, the most frequently used is the measurement of antibody concentrations. There is a wide variation between individuals in antibody responses induced by the same vaccine, and numerous intrinsic (including age, sex and genetics), external (including geographic location, family size and toxins) and behavioural (including nutrition, exercise, smoking and alcohol consumption) factors influence vaccine responses.[1] The magnitude of response is also influenced by the type of vaccine. For example, live attenuated vaccines induce strong antibody responses, similar to an infection with a wild-type pathogen, leading to induction of memory cells and often life-long protection. In contrast, inactivated, subunit or toxoid vaccines induce lower responses, requiring booster doses. The response to different subunit vaccines also varies: polysaccharide-protein conjugated vaccines have superior immunogenicity (including the induction of long-term protection) compared with polysaccharide vaccines which only induce short-lived T cell-independent antibody responses.[2]

Currently, it is unknown whether there is a correlation between antibody responses to different vaccines. It is also unknown whether there is any consistency within individuals in the magnitude of antibody responses to all vaccines or to vaccines of a certain type (e.g. subunit, live or attenuated vaccines). In clinical practice, e.g. when investigating for primary immunodeficiency, measuring antibody responses to diphtheria, tetanus and pneumococcal vaccine antigens is common practice.[3] When the vaccination status of an individual is unknown, antibody responses to tetanus is often used as a surrogate marker.[4] However, there is no data available to show that antibody responses to these vaccines correlate with responses to other vaccines.

In this study, we investigated the correlation of antibody responses between routine vaccine antigens in infants.

Methods

Participants and antibody measurement

Participants were a subset of infants from a randomised controlled trial (The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR)), which investigated whether Bacillus Calmette-Guérin vaccine (BCG) immunisation at birth protects against childhood infection, allergy and asthma. All infants were vaccinated according to the Australian National Immunisation Program: at birth: intramuscular hepatitis B (HepB) vaccine (H-B-Vax II Paediatric® (*bioCSL*)); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa), HepB, polio (IPV), and *Haemophilus influenzae* type b (Hib) vaccine (Infanrix® hexa (*GlaxoSmithKline*)), intramuscular 13-valent conjugate pneumococcal vaccine (conjugated to CRM197, a diphtheria toxoid) (PCV13) (Prevenar13® (*Wyeth*)), and oral rotavirus vaccine (RotaTeq® (*Merck*)); at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (Priorix® (*GlaxoSmithKline*, Abbotsford, Victoria)) or M-M-R® II (*Seqirus*, Parkville, Victoria)) and intramuscular combined meningococcal C (MenC) and Hib vaccine (conjugated to tetanus toxoid) (Menitorix® (*GlaxoSmithKline*)). Vaccine records were obtained from individual immunisation records and/or the Australian Immunisation Register.

From the subset of participants whose parent/guardian provided consent, blood samples were obtained in sodium-heparin tubes (S-monovette® (*Sarstedt*)) during study visits at 7 and 13 months of age (one month after the administration of routine scheduled immunisations). Plasma was stored at -80°C until analysis. Only participants who had blood taken 28 ± 14 days after their 6-month and 12-month routine vaccines, respectively, were included in the final analysis. Additionally, persistence of antibodies was measured 7 months after the 6-month vaccines. For this, only participants who had their blood taken between 210 ± 14 days after their 6-month vaccinations were included, and, for the responses to tetanus and Hib only, participants who

had not yet received their 12-month vaccinations, as Menitorix (Hib-MenC) given at 12 months of age includes a tetanus toxoid as carrier protein.

Blood samples were analysed at the National Institute for Health and Environment, in Bilthoven, the Netherlands. Immunoglobulin (Ig) G antibodies against 26 vaccine antigens (diphtheria, tetanus, pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (types 1, 2, 3), Hib, pneumococcal ((Pn) serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), MenC, measles, mumps and rubella) were measured using fluorescent bead-based multiplex immune-assays (Luminex xMAP technology).[5-10] In all assays, an international or in-house reference, controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

The geometric mean concentration (GMC) for each vaccine antibody (immunoglobulin G) was calculated at one and seven months after vaccination. To investigate the correlation between antibody responses to different vaccines within the study cohort, pairwise Spearman's rank correlation coefficients (ρ) with 95% confidence intervals (CI) were calculated between responses to each vaccine antigen. Correlations were categorised as follows: 'very weak' ρ = 0.00 - 0.19, 'weak' ρ = 0.20 - 0.39, 'moderate' ρ = 0.40 - 0.59, 'strong' ρ = 0.60 - 0.79 and 'very strong' ρ = 0.80 - 1.00.

Heatmaps were created to visualise the relationship between antibody responses to different vaccine antigens amongst all infants. Each individual's response to a vaccine antigen was scaled by subtracting the mean of all responses to that vaccine antigen and dividing by the standard deviation [(individual vaccine response – mean vaccine response) / standard

deviation]. A 5% significance level was used. All statistical analyses were done using R version 3.4.3.

Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation (38124A).

Results

In total, 91 participants had antibody responses to their 6-month vaccinations measured one month after vaccination and 141 participants seven months after vaccination (for Hib and tetanus, only the 48 participants who had not yet received the MenC-Hib vaccination were included at the second time point). Additionally, 148 participants had antibody responses to their 12-month vaccinations measured one month after vaccination. The background characteristics of the participants are summarised in Table 1.

Antibody responses in individuals one and seven months after 6-month vaccinations are shown in Figure 1 and 2. Antibody responses in individuals one month after the 12-month vaccinations are shown in Figure 3. Low responders are depicted at the top of the heatmap and high responders at the bottom.

Correlation of antibody responses one month after the 6-month vaccinations

Results of pairwise comparisons of antibody responses to the 22 vaccine antigens included in the 6-month vaccinations measured one month after vaccination are shown in Figure 4 and Supplementary Table 1. One month after the 6-month vaccinations, ρ varied between 0.03 (between FHA and Pn14) and 0.86 (between Pn1 and Pn5). The strongest correlations were observed between antibody responses to different polio serotypes (strong to very strong) and

different pneumococcal serotypes (moderate to very strong), with the exception of serotypes 3 and 14 (weak to strong). For the correlation between responses to different pneumococcal serotypes, the strongest correlations were observed for serotypes 1 (ρ = 0.52 - 0.86), 18C (ρ = 0.60 - 0.80) and 23F (ρ = 0.49 - 0.79). For antibody responses to pertussis, there was a moderate correlation between FHA and PRN (ρ = 0.51), and between FHA and PT (ρ = 0.52), but only a weak correlation between responses to PT and PRN (ρ = 0.25).

Antibody responses to diphtheria had moderate to strong correlations with pneumococcal serotypes, except pneumococcal serotypes 3 and 14 (weak). Additionally, antibody responses to diphtheria had moderate correlations with responses to FHA and PRN (ρ = 0.41 - 0.45), while the correlations between responses to other vaccine antigens were weak. Antibody responses to tetanus, Hib and polio serotypes 1-3 had very weak to weak correlations with responses to all the other vaccine antigens.

Correlation of antibody responses seven months after the 6-month vaccinations

Results of pairwise comparisons between antibody responses to the 22 vaccine antigens in the 6-month vaccinations measured seven months after vaccination are shown in Figure 4 and Supplementary Table 1. Seven months after the 6-month vaccinations, correlations between antibody responses were weaker than one month after the vaccinations, except for correlations between tetanus and Hib, and between tetanus and diphtheria for which correlations were stronger at seven months compared with one month after vaccination (0.74 vs 0.24, 0.39 vs 0.22) (Figure 1 and Supplementary Table 1). Overall, 7 months after vaccination, ρ varied between -0.19 (between polio serotype 1 and Hib) and 0.77 (between Pn6A and Pn6B). The strongest correlations were observed between antibody responses to different pneumococcal serotypes (mostly moderate to strong with the exception of responses to serotypes 14, 19A and 19F and other serotypes, for which correlations were weak to moderate (except for the strong correlation between responses to 19A and 19F)). Moderate to strong correlations were also

observed between responses to different polio serotypes and between different pertussis antigens.

Correlations between antibody responses to pneumococcal serotypes and diphtheria were weak to moderate. Antibody responses to tetanus had a strong correlation with responses to Hib, but weak to moderate correlations for responses to all the other antigens. The correlation between responses to tetanus and polio serotypes 1-3 were weakly negative, as was the correlation between responses to polio serotypes 1-3 and Hib. All other correlations between responses to polio and other vaccine antigens were very weak to weak.

Correlation of antibody responses one month after the 12-month vaccinations

Results of pairwise comparisons of antibody responses to the 6 vaccine antigens measured one month after the 12-month vaccination are shown in Figure 5 and Supplementary Table 2. Overall, correlations between antibody responses to the 12-month vaccinations were weaker than those between responses to the 6-month vaccinations (Figure 2 and Supplementary Data Table 2). There was a high correlation between antibody responses to mumps and rubella (ρ = 0.61) and moderate correlations between responses to tetanus and MenC (ρ = 0.52), between tetanus and Hib (ρ = 0.5), and between MenC and Hib (ρ = 0.48). Almost all of the correlations between the live-vaccines measles, mumps, rubella and non-live vaccines (MenC, tetanus, Hib) were weakly negative.

Discussion

In this study, we observed a wide variation in antibody responses to vaccines and that overall, correlations between responses to different vaccines are weak. Strong correlations were found only between similar antigens (e.g. pneumococcal or polio serotypes) or between antigens that were conjugated to a protein carrier of the same or similar antigen. For example, in the

conjugate pneumococcal vaccine, pneumococcal antigens are conjugated to CRM197, which is a diphtheria toxoid. This likely explains the moderate correlations between antibody responses to pneumococcal serotypes and diphtheria toxoid after the 6-month vaccinations. Similarly, for the combined Hib and MenC vaccine, both antigens are conjugated to a tetanus toxoid, which likely explains the moderate correlation between antibody responses to tetanus and Hib, and between tetanus and MenC observed after the 12-month vaccinations. However, after the 6-month vaccinations, there was only a weak correlation between the response to tetanus and Hib despite the latter being conjugated to tetanus toxoid. In contrast, this correlation was strong seven months after vaccination. We cannot explain this difference between immediate immune response and persistence of antibodies.

Despite being administered in the same vaccine, there were only weak correlations between antibody responses to tetanus, pertussis, polio and Hib antigens. Interestingly, we found negative correlations between responses to the live-vaccines measles, mumps and rubella, and non-live vaccines (MenC, tetanus, Hib). The finding is not easily explained as several studies have shown that simultaneous or delayed administration of MMR does not influence antibody concentrations to non-live vaccines.[11-14]

Although infants were vaccinated simultaneously with two vaccines at each time point (which excludes confounding factors such as such as age, nutritional status, microbiota, time of day of administration etc.), correlations might be explained by the influence of factors such as adjuvants in the vaccines, vaccination site, needle size etc. Nevertheless, the vaccines in the study were given exactly as in clinical practice. Therefore, our findings are relevant to patient care. As the correlation between responses to different pneumococcal serotypes is strong, it may not be necessary to measure multiple responses to all serotypes. Serotypes which could potentially be used as a surrogate marker for response to pneumococcal vaccination are 18C, 1

and 23F. Responses to serotypes 14 and 3 should not be used as a surrogate, as responses to these antigens have the weakest correlations with responses to other serotypes. These findings are consistent with a recent study that reported correlations between 0.43 and 0.67 for responses to PCV13.[15] This study also observed the lowest correlations for antibody responses against pneumococcal serotype 14 and other serotypes.[15]

Antibody responses to pneumococcal antigens may also be used to predict responses to diphtheria vaccination, especially when measured one month after vaccination, but not for responses to other vaccine antigens as other correlations were weak. Similarly, our finding of a strong correlation between antibody responses to different polio serotypes means measuring responses to one serotype can be used to predict responses to other polio serotypes.

In contrast, antibody responses to pertussis, tetanus, polio and Hib were only weakly correlated with other vaccines responses, and therefore should not be used as surrogates for vaccine immunity. Notably, our results indicate that responses to non-live vaccines do not predict responses to live-vaccines.

As routine vaccinations include the administration of many different vaccine types, including toxoid, conjugated polysaccharide, inactivated and live vaccines, it is difficult to evaluate any consistency in the magnitude of antibody responses to vaccines of a certain type. Correlation between antibody responses to toxoid vaccines (diphtheria and tetanus) were weak at both one and seven months after vaccinations. Correlation between antibody responses to polysaccharide vaccines (Hib and pneumococcus) were mostly very weak to weak (with a few exceptions). Correlation between antibody responses to inactivated vaccine (polio) and purified proteins (pertussis) could not be compared with other vaccines of the same type. Furthermore, except

the strong correlation that was found between responses to mumps and rubella, correlations between live vaccines were weak.

The strengths of this study include the broad range of measured antibody responses and the novel approach to comparing responses between different vaccine antigens. The limitations include the fact that only antibody concentration and not function was measured, that not all responses were measured exactly four weeks after vaccination, and that responses to HepB vaccination was not measured (as this antigen is not included in the multiplex assay and we had insufficient sample volume for standard assays).

In summary, the correlation between responses to similar antigens in the same vaccine (such as different serotypes of a bacteria or virus), as well as responses to antigens conjugated to similar carrier proteins is strong. In contrast, the correlation between responses to other vaccines is weak. Measuring antibody responses to one or a few vaccine antigens therefore does not offer a reliable surrogate marker of responses to unrelated vaccines.

Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

PZ, NR, KP and NC were responsible for designing the study. NM was responsible for selecting the samples. FK was responsible for analysing the samples. PZ performed the statistical analysis, which was supervised by NR, KP and NC. PZ drafted the initial manuscript. All authors critically revised the manuscript and approved the final manuscript as submitted.

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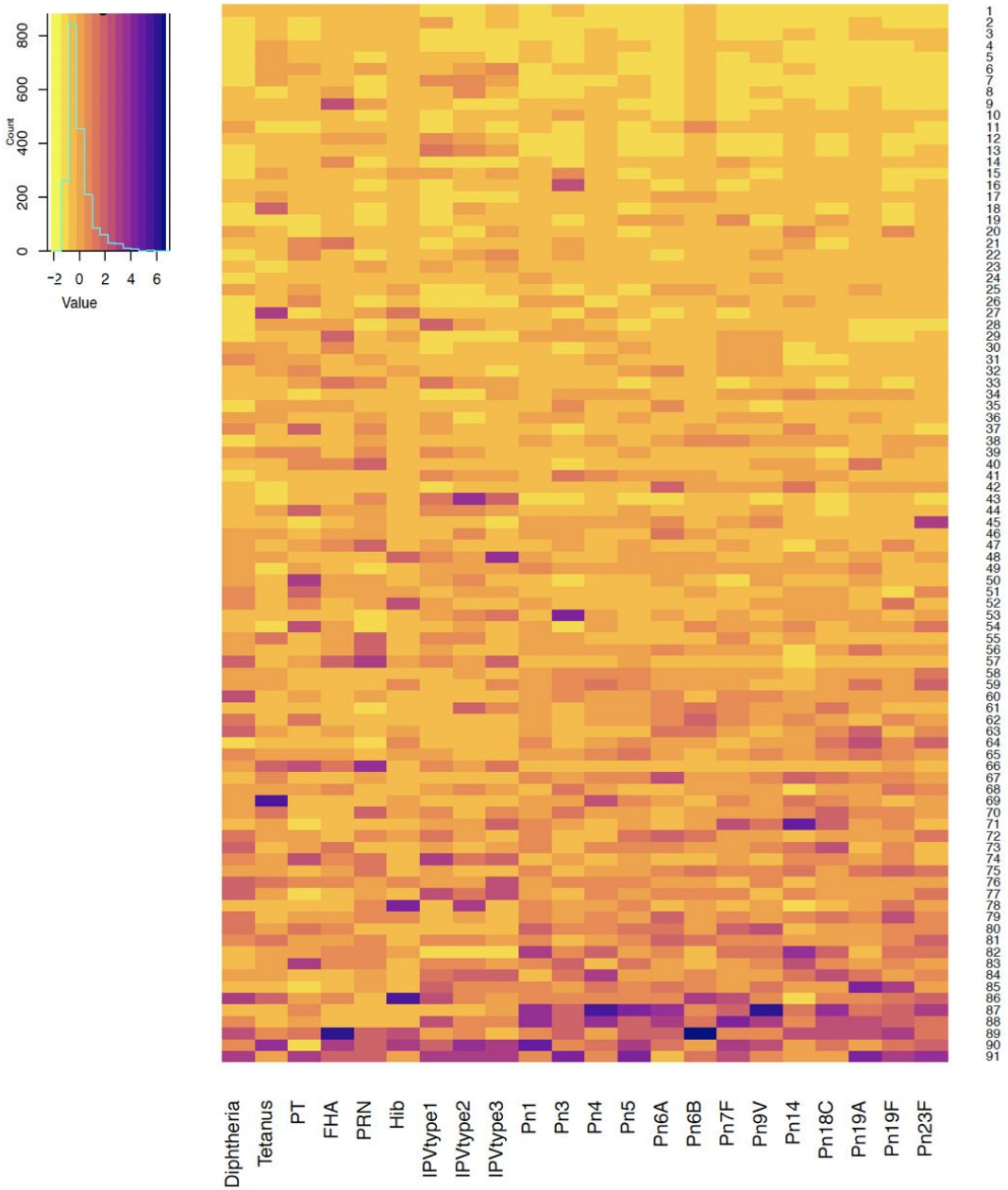
Table 1 Characteristics of study participants

	Samples at 7 months of age for antibodies	Samples at 13 months of age for persistence of antibodies	Samples at 13 months of age for antibodies
	to primary course of vaccines ending at 6 months of age		to 12-month vaccines
	(n=91) n (%) or median (IQR)	(n=141) n (%) or median (IQR)	(n=148) n (%) or median (IQR)
Sex (male)	43 (47)	73 (52)	73 (49)
Gestational age (weeks)	39.5 (38.6-40.5)	39.2 (38.3-40.4)	39.2 (38.4-40.4)
Birth weight (kg)	3.48 (3.10-3.78)	3.37 (3.08-3.67)	3.41 (3.14-3.66)
Caesarean section	35 (38)	50 (35)	54 (36)
BCG-vaccinated	45 (49)	82 (58)	78 (53)
MMR/MenC-Hib-vaccinated	-	93 (66)	148 (100)
Maternal dTpa vaccination in pregnancy	46 (51)	81 (57)	69 (47)
Maternal influenza vaccination in pregnancy	55 (60)	79 (52)	88 (59)
Age at routine vaccination (days)			
- 6-week vaccines	45 (43-48)[1]	46 (43-50)	45 (43-48)
- 4-month vaccines	123 (117-127)	124 (103-130)	124 (120-131)
- 6-month vaccines	187 (181-193)	189 (184-195)	190 (185-202)
- 12-month vaccines	-	-	376 (369-383)
Interval between (days)			
- 6-month vaccines and 7-month blood sample	28 (21-37)	-	-
- 6-month vaccines and 13-month blood sample	-	211 (204-218)	-
- 12-month vaccines and 13-month blood sample	-	-	29 (22-36)
Age at blood sampling (days)	218 (208-227)	400 (394-411)	406 (397-414)

[1] age for one participant not available

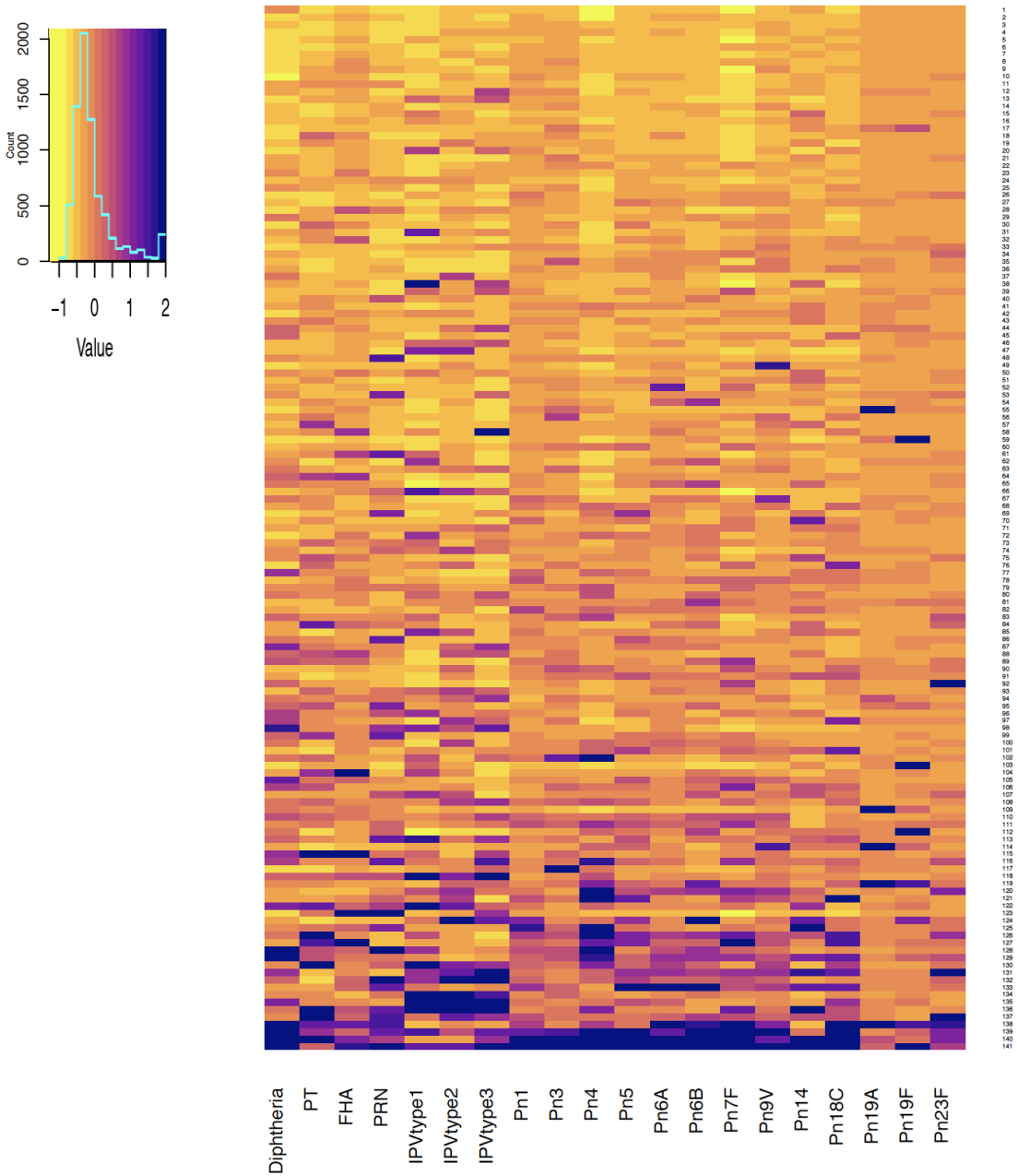
BCG = Bacille Calmette-Guérin, dTpa = diphtheria-tetanus-acellular pertussis vaccine, MMR = measles-mumps-rubella vaccine, MenC-Hib = meningococcal C-*Haemophilus influenzae* type b

Figure 1 Antibody responses to vaccine antigens measured one month after the 6-month vaccinations (at 7 months of age) in 91 participants. Each row depicts one participant with the colour in each column representing the 'scaled' vaccine antibody response in that individual.



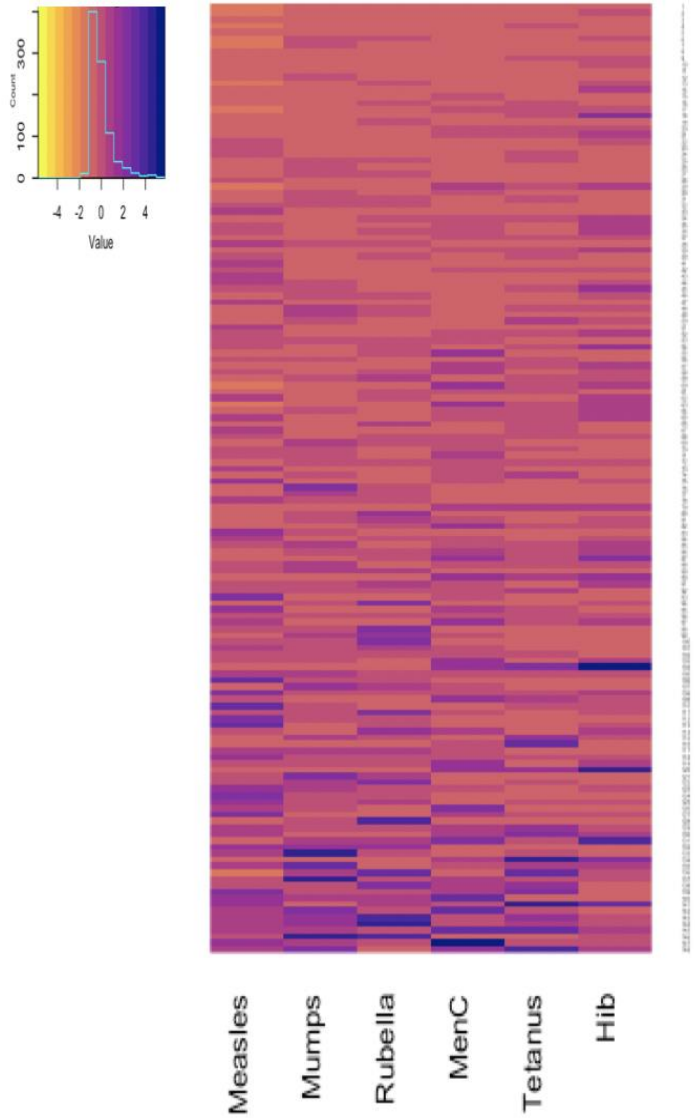
PT = pertussis toxin, FHA = filamentous haemagglutinin, PRN = pertactin, Hib = *Haemophilus influenzae* type b, IPV = polio, Pn = pneumococcal serotypes

Figure 2 Antibody responses to vaccine antigens measured 7 months after 6-month vaccinations (at 13 months of age, for persistence of responses) in 141 participants. Each row depicts one participant with the colour in each column representing the 'scaled' vaccine antibody response in that individual.



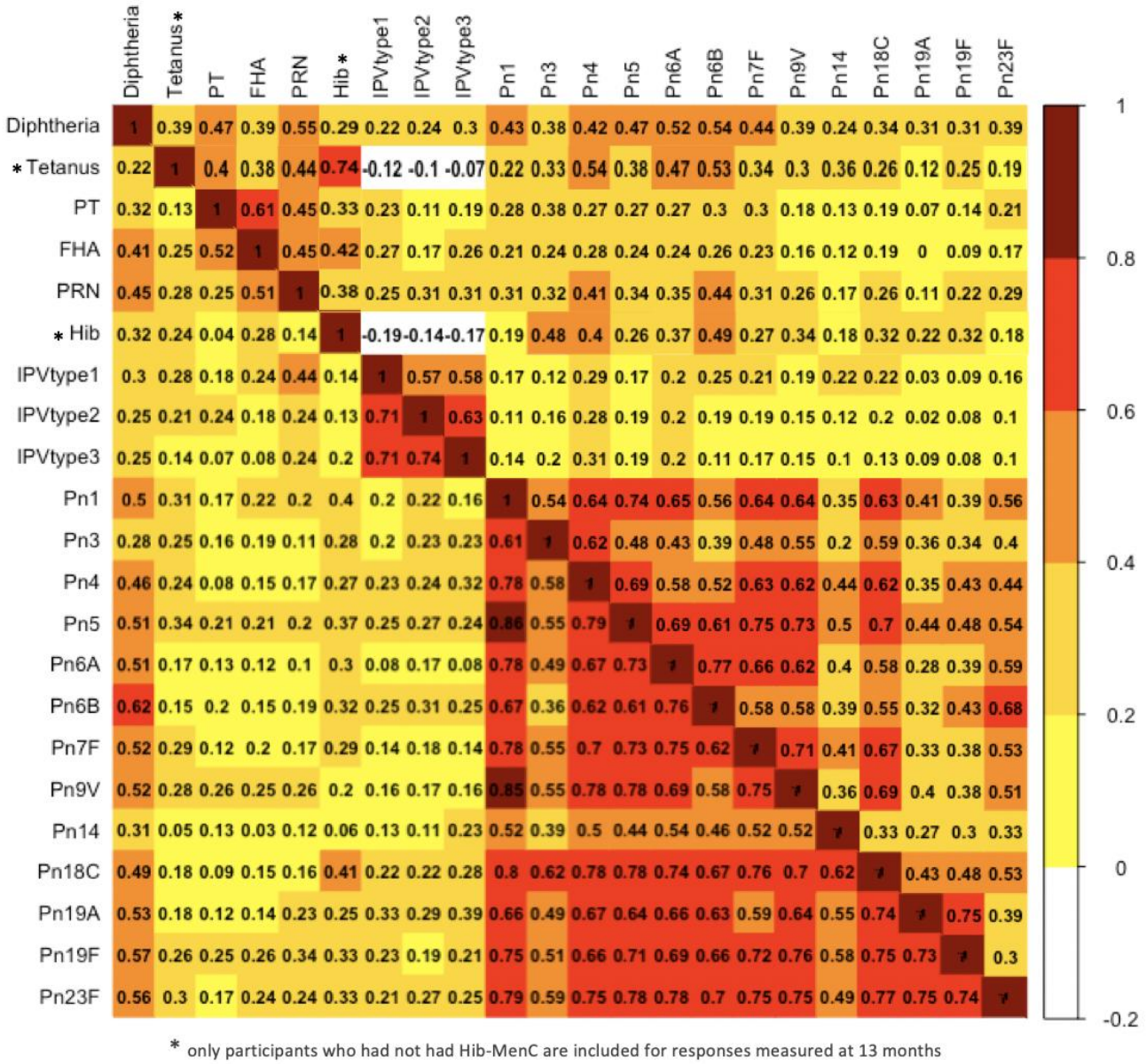
PT = pertussis toxin, FHA = filamentous haemagglutinin, PRN = pertactin, IPV = polio, Pn = pneumococcal serotypes

Figure 3 Antibody responses to 12-month vaccinations measured one month after vaccination in 148 participants. Each row depicts one participant with the colour in each column representing the 'scaled' vaccine antibody response in that individual.



MenC = Meningococcus C, Hib = *Haemophilus influenzae* type b

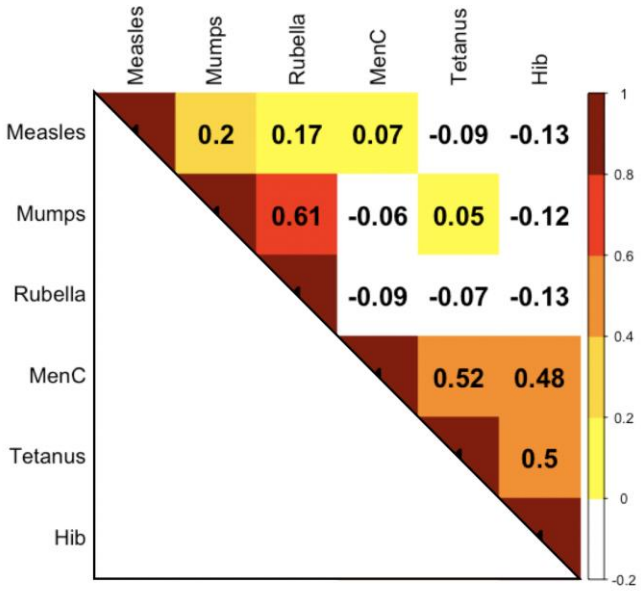
Figure 4 Correlation (expressed as Spearman correlation coefficients) for pairwise comparisons of antibody responses to the 6-month vaccinations measured at one (lower left) and seven (upper right) months after vaccination



* only participants who had not had Hib-MenC are included for responses measured at 13 months

PT = pertussis toxin, FHA = filamentous haemagglutinin, PRN = pertactin, Hib = *Haemophilus influenzae* type b, IPV = polio, Pn = pneumococcal serotypes

Figure 5 Correlation (expressed as Spearman correlation coefficients) for pairwise comparisons of antibody responses to the 12-month vaccinations at 13 months of age



MenC = Meningococcus C, Hib = *Haemophilus influenzae* type b

Table 5.1 Correlation (Spearman correlation coefficients with 95% CI and p-values) for pairwise comparisons of antibody responses to the 6-month immunisations measured 1 (lower left) and 7 (upper right) months after immunisation. *only participants who had not had MMR/Hib-MenC

	Diphtheria	Tetanus*	PT	FHA	PRN	Hib*	IPV type 1	IPV type 2	IPV type 3	Pn 1	Pn 3	Pn4	Pn 5	Pn 6A	Pn 6B	Pn 7F	Pn 9V	Pn 14	Pn 18C	Pn 19A	Pn 19F	Pn 23F
Diphtheria		**0.39 (0.12-0.61) <0.01**	**0.47 (0.32-0.59) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.55 (0.43-0.66) <0.01**	0.29 (0.01-0.53) 0.05	**0.22 (0.06-0.38) <0.01**	**0.24 (0.08-0.39) <0.01**	**0.30 (0.15-0.45) <0.01**	**0.43 (0.28-0.55) <0.01**	**0.38 (0.22-0.51) <0.01**	**0.42 (0.28-0.55) <0.01**	**0.47 (0.22-0.59) <0.01**	**0.52 (0.38-0.63) <0.01**	**0.54 (0.41-0.65) <0.01**	**0.44 (0.29-0.56) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.24 (0.08-0.39) <0.01**	**0.34 (0.19-0.48) <0.01**	**0.31 (0.15-0.45) <0.01**	**0.31 (0.15-0.45) <0.01**	**0.39 (0.24-0.52) <0.01**
Tetanus	**0.22 (0.02-0.41) 0.03**		**0.40 (0.13-0.61) 0.01**	**0.38 (0.11-0.60) 0.01**	**0.44 (0.18-0.64) <0.01**	**0.74 (0.57-0.84) <0.01**	-0.12 (-0.39-0.17) 0.41	-0.10 (-0.38-0.19) 0.48	-0.07 (-0.35-0.22) 0.64	0.22 (-0.07-0.47) 0.14	**0.33 (0.05-0.56) 0.02**	**0.54 (0.30-0.71) <0.01**	**0.38 (0.11-0.60) 0.01**	**0.47 (0.21-0.66) <0.01**	**0.53 (0.29-0.71) <0.01**	**0.34 (0.06-0.57) 0.02**	**0.30 (0.02-0.54) 0.04**	**0.36 (0.09-0.59) 0.01**	0.26 (-0.02-0.51) 0.07	0.12 (-0.17-0.39) 0.41	0.25 (-0.03-0.50) 0.08	0.19 (-0.10-0.45) 0.19
PT	**0.32 (0.12-0.49) <0.01**	0.13 (-0.08-0.33) 0.22		**0.61 (0.49-0.70) <0.01**	**0.45 (0.31-0.57) <0.01**	**0.33 (0.05-0.56) 0.02**	**0.23 (0.07-0.38) <0.01**	0.11 (-0.06-0.27) 0.21	**0.19 (0.03-0.35) 0.02**	**0.28 (0.12-0.43) <0.01**	**0.38 (0.22-0.51) <0.01**	**0.27 (0.11-0.41) <0.01**	**0.27 (0.11-0.42) <0.01**	**0.27 (0.11-0.42) <0.01**	**0.30 (0.14-0.44) <0.01**	**0.30 (0.14-0.44) <0.01**	**0.18 (0.01-0.33) 0.03**	0.13 (-0.04-0.29) 0.13	**0.19 (0.03-0.35) 0.02**	0.07 (-0.09-0.24) 0.38	0.14 (-0.02-0.30) 0.09	0.21 (0.04-0.36) 0.01
FHA	**0.41 (0.22-0.57) <0.01**	**0.25 (0.04-0.43) 0.02**	**0.52 (0.36-0.66) <0.01**		**0.45 (0.31-0.58) <0.01**	**0.42 (0.16-0.63) <0.01**	**0.27 (0.11-0.42) <0.01**	0.17 (0.00-0.32) 0.05	**0.26 (0.10-0.41) <0.01**	**0.21 (0.04-0.36) 0.01**	**0.24 (0.08-0.39) <0.01**	**0.28 (0.12-0.42) <0.01**	**0.24 (0.07-0.39) <0.01**	**0.24 (0.08-0.39) <0.01**	**0.26 (0.10-0.41) <0.01**	**0.23 (0.07-0.38) <0.01**	0.16 (-0.01-0.32) 0.06	0.12 (-0.04-0.28) 0.14	**0.19 (-0.03-0.35) 0.02**	0.00 (-16.5-16.6) 0.99	0.09 (-0.08-0.25) 0.29	0.17 (0.00-0.33) 0.05
PRN	**0.45 (0.27-0.60) <0.01**	**0.28 (0.07-0.46) <0.01**	**0.25 (0.05-0.44) 0.02**	**0.51 (0.34-0.65) <0.01**		**0.38 (0.10-0.60) <0.01**	**0.25 (0.09-0.40) <0.01**	**0.31 (0.15-0.45) <0.01**	**0.31 (0.15-0.45) <0.01**	**0.31 (0.15-0.45) <0.01**	**0.32 (0.17-0.47) <0.01**	**0.41 (0.26-0.53) <0.01**	**0.34 (0.19-0.48) <0.01**	**0.35 (0.20-0.49) <0.01**	**0.44 (0.30-0.57) <0.01**	**0.31 (0.16-0.46) <0.01**	**0.26 (0.10-0.41) <0.01**	**0.17 (0.01-0.33) 0.04**	**0.26 (0.10-0.41) <0.01**	**0.11 (-0.05-0.27) 0.18**	**0.22 (0.06-0.38) <0.01**	**0.29 (0.13-0.43) <0.01**
Hib	**0.32 (0.13-0.50) <0.01**	**0.24 (0.04-0.43) <0.01**	0.04 (-0.17-0.25) 0.70	**0.28 (0.07-0.46) <0.01**	0.14 (-0.07-0.34) 0.18		-0.19 (-0.45-0.10) 0.20	-0.14 (-0.41-0.15) 0.35	-0.17 (-0.43-0.12) 0.25	0.19 (-0.10-0.45) 0.19	**0.48 (0.22-0.67) <0.01**	**0.40 (0.13-0.61) <0.01**	0.26 (-0.02-0.51) 0.07	**0.37 (0.09-0.59) 0.01**	**0.49 (0.24-0.68) <0.01**	0.27 (-0.02-0.51) 0.06	**0.34 (0.07-0.57) 0.02**	0.18 (-0.11-0.44) 0.21	**0.32 (0.04-0.56) 0.02**	0.22 (-0.06-0.48) 0.13	**0.32 (0.04-0.56) 0.02**	0.18 (-0.11-0.44) 0.23
IPV type 1	**0.30 (0.10-0.47) <0.01**	**0.28 (0.08-0.46) <0.01**	0.18 (-0.02-0.37) 0.08	**0.24 (0.04-0.43) 0.02**	**0.24 (0.26-0.60) <0.01**	0.14 (-0.06-0.34) 0.18		**0.57 (0.44-0.67) <0.01**	**0.58 (0.46-0.68) <0.01**	0.17 (0.00-0.32) 0.05	0.12 (-0.04-0.28) 0.15	**0.29 (0.13-0.43) <0.01**	0.17 (0.00-0.32) 0.05	**0.20 (0.04-0.35) 0.02**	**0.25 (0.09-0.40) <0.01**	**0.21 (0.04-0.36) 0.01**	0.19 (0.03-0.35) 0.02	**0.22 (0.06-0.37) <0.01**	**0.22 (0.05-0.37) <0.01**	0.03 (-0.13-0.20) 0.68	**0.09 (-0.08-0.25) 0.30**	**0.16 (-0.01-0.31) 0.06**
IPV type 2	**0.25 (0.04-0.43) 0.02**	**0.21 (0.00-0.40) 0.05**	**0.24 (0.04-0.43) 0.02**	0.18 (-0.02-0.37) 0.08	**0.24 (0.03-0.43) 0.02**	0.13 (-0.07-0.33) 0.21	**0.71 (0.56-0.80) <0.01**		**0.63 (0.51-0.72) <0.01**	0.11 (-0.05-0.28) 0.17	0.16 (0.00-0.32) 0.05	**0.28 (0.12-0.42) <0.01**	**0.19 (0.02-0.34) 0.03**	**0.20 (0.04-0.36) 0.02**	**0.19 (0.02-0.34) 0.03**	**0.19 (0.03-0.35) 0.02**	0.15 (-0.02-0.30) 0.08	0.12 (-0.05-0.28) 0.16	**0.20 (0.03-0.35) 0.02**	0.02 (-0.14-0.19) 0.78	0.08 (-0.09-0.24) 0.34	**0.10 (-0.07-0.26) 0.25**
IPV type 3	**0.25 (0.10-0.47) <0.01**	0.14 (-0.06-0.34) 0.18	0.07 (-0.13-0.28) 0.49	0.08 (-0.13-0.28) 0.46	**0.24 (0.03-0.42) 0.02**	0.20 (0.00-0.39) 0.06	**0.71 (0.59-0.80) <0.01**	**0.74 (0.64-0.82) <0.01**		0.14 (-0.02-0.30) 0.09	**0.20 (0.03-0.35) 0.02**	**0.31 (0.16-0.45) <0.01**	**0.19 (0.02-0.34) 0.03**	**0.20 (0.04-0.36) 0.02**	0.11 (-0.06-0.27) 0.20	**0.17 (0.00-0.33) 0.04**	0.15 (-0.01-0.31) 0.07	0.10 (-0.07-0.26) 0.26	0.13 (-0.03-0.29) 0.12	0.09 (-0.08-0.25) 0.30	**0.08 (-0.09-0.24) 0.04**	**0.10 (-0.07-0.26) 0.24**
Pn 1	**0.50 (0.33-0.64) <0.01**	**0.31 (0.11-0.59) <0.01**	0.17 (-0.04-0.36) 0.11	**0.22 (0.01-0.41) 0.04**	0.20 (0.00-0.39) 0.06	**0.40 (0.21-0.56) <0.01**	0.20 (0.00-0.39) 0.06	**0.22 (0.01-0.41) 0.04**	0.16 (-0.04-0.36) 0.12		**0.54 (0.41-0.65) <0.01**	**0.64 (0.53-0.73) <0.01**	**0.74 (0.66-0.81) <0.01**	**0.65 (0.55-0.74) <0.01**	**0.56 (0.44-0.67) <0.01**	**0.64 (0.53-0.73) <0.01**	**0.64 (0.53-0.73) <0.01**	**0.35 (0.20-0.49) <0.01**	**0.63 (0.52-0.72) <0.01**	**0.41 (0.26-0.54) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.56 (0.44-0.67) <0.01**
Pn 3	**0.28 (0.07-0.46) <0.01**	**0.25 (0.05-0.43) 0.02**	0.16 (-0.04-0.35) 0.12	0.19 (-0.02-0.38) 0.07	0.11 (-0.10-0.31) 0.31	**0.28 (0.08-0.46) <0.01**	0.20 (0.00-0.39) 0.06	**0.23 (0.02-0.41) 0.03**	**0.23 (0.03-0.42) 0.03**	**0.61 (0.46-0.72) <0.01**		**0.62 (0.50-0.71) <0.01**	**0.48 (0.34-0.60) <0.01**	**0.43 (0.29-0.56) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.48 (0.34-0.60) <0.01**	**0.55 (0.42-0.66) <0.01**	**0.20 (0.37.0.35) <0.01**	**0.59 (0.47-0.69) <0.01**	**0.36 (0.21-0.49) <0.01**	**0.34 (0.19-0.48) <0.01**	**0.40 (0.25-0.53) <0.01**
Pn4	**0.46 (0.28-0.61) <0.01**	**0.24 (0.03-0.42) 0.02**	0.08 (-0.12-0.28) 0.43	0.15 (-0.06-0.34) 0.17	0.17 (-0.04-0.36) 0.11	**0.27 (0.07-0.45) 0.01**	**0.23 (0.02-0.41) 0.03**	**0.24 (0.04-0.42) 0.02**	**0.32 (0.12-0.49) <0.01**	**0.78 (0.78-0.85) <0.01**	**0.58 (0.43-0.70) <0.01**		**0.69 (0.59-0.77) <0.01**	**0.58 (0.46-0.68) <0.01**	**0.52 (0.39-0.63) <0.01**	**0.63 (0.52-0.72) <0.01**	**0.62 (0.51-0.72) <0.01**	**0.44 (0.30-0.57) <0.01**	**0.62 (0.51-0.71) <0.01**	**0.35 (0.20-0.49) <0.01**	**0.43 (0.28-0.55) <0.01**	**0.44 (0.30-0.57) <0.01**
Pn 5	**0.51 (0.33-0.64) <0.01**	**0.34 (0.14-0.51) <0.01**	**0.21 (0.00-0.40) 0.04**	**0.21 (0.01-0.40) 0.04**	**0.20 (0.00-0.39) 0.05**	**0.37 (0.18-0.54) <0.01**	**0.25 (0.05-0.43) 0.02**	**0.27 (0.07-0.45) <0.01**	**0.24 (0.04-0.43) 0.02**	**0.86 (0.79-0.91) <0.01**	**0.55 (0.39.0.68) <0.01**	**0.79 (0.69-0.85) <0.01**		**0.69 (0.59-0.77) <0.01**	**0.61 (0.49-0.70) <0.01**	**0.75 (0.66-0.81) <0.01**	**0.73 (0.64-0.80) <0.01**	**0.50 (0.37-0.62) <0.01**	**0.70 (0.60-0.77) <0.01**	**0.44 (0.30-0.56) <0.01**	**0.48 (0.34-0.60) <0.01**	**0.54 (0.41-0.64) <0.01**
Pn 6A	**0.51 (0.34-0.65) <0.01**	0.17 (-0.03-0.36) 0.11	0.13 (-0.08-0.33) 0.21	0.12 (-0.09-0.31) 0.27	0.10 (-0.10-0.30) 0.33	**0.30 (0.10-0.48) <0.01**	0.08 (-0.12-0.28) 0.43	0.17 (-0.03-0.37) 0.10	0.08 (-0.12-0.28) 0.46	**0.78 (0.69-0.85) <0.01**	**0.49 (0.31-0.63) <0.01**	**0.67 (0.54-0.77) <0.01**	**0.73 (0.61-0.81) <0.01**		**0.77 (0.69-0.83) <0.01**	**0.66 (0.55-0.74) <0.01**	**0.62 (0.51-0.72) <0.01**	**0.40 (0.25-0.53) <0.01**	**0.58 (0.46-0.68) <0.01**	**0.28 (0.12-0.42) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.59 (0.47-0.69) <0.01**
Pn 6B	**0.62 (0.47-0.73) <0.01**	0.15 (-0.05-0.35) 0.14	0.20 (-0.01-0.39) 0.06	0.15 (-0.05-0.35) 0.15	0.19 (-0.02-0.38) 0.07	**0.32 (0.12-0.49) <0.01**	**0.25 (0.05-0.43) 0.02**	**0.31 (0.11-0.48) <0.01**	**0.25 (0.04-0.43) 0.02**	**0.67 (0.54-0.77) <0.01**	**0.36 (0.17-0.53) <0.01**	**0.62 (0.47-0.73) <0.01**	**0.61 (0.46-0.72) <0.01**	**0.76 (0.66-0.84) <0.01**		**0.58 (0.46-0.68) <0.01**	**0.58 (0.46-0.68) <0.01**	**0.39 (0.24-0.52) <0.01**	**0.55 (0.42-0.65) <0.01**	**0.32 (0.17-0.47) <0.01**	**0.43 (0.29-0.56) <0.01**	**0.68 (0.58-0.76) <0.01**
Pn 7F	**0.52 (0.36-0.66) <0.01**	**0.29 (0.09-0-47) <0.01**	0.12 (-0.09-0.32) 0.27	0.20 (-0.01-0.29) 0.06	0.17 (-0.03-0.37) (0.10)	**0.29 (0.09-0.47) <0.01**	0.14 (.0.07-0.33) 0.20	0.18 (-0.03-0.37) 0.09	0.14 (-0.07-0.33) 0.20	**0.78 (0.69.0.85) <0.01**	**0.55 (0.38-0.68) <0.01**	**0.70 (0.57-0.79) <0.01**	**0.73 (0.62-0.81) <0.01**	**0.75 (0.65-0.83) <0.01**	**0.62 (0.48-0.73) <0.01**		**0.71 (0.61-0.78) <0.01**	**0.41 (0.26-0.54) <0.01**	**0.67 (0.57-0.75) <0.01**	**0.33 (0.17-0.47) <0.01**	**0.38 (0.23-0.51) <0.01**	**0.53 (0.40-0.64) <0.01**
Pn 9V	**0.52 (0.36-0.66) <0.01**	**0.28 (0.07-0.46) <0.01**	**0.26 (0.06-0.44) 0.01**	**0.25 (0.05-0.44) 0.01**	**0.26 (0.05-0.44) 0.01**	0.20 (-0.01-0.39) 0.06	0.16 (-0.05-0.35) 0.14	0.17 (-0.03-0.37) 0.10	0.16 (-0.05-0.35) 0.13	**0.85 (0.78-0.90) <0.01**	**0.55 (0.38-0.68) <0.01**	**0.78 (0.69-0.85) <0.01**	**0.69 (0.68-0.85) <0.01**	**0.58 (0.57-0.79) <0.01**	**0.36 (0.43-0.70) <0.01**	**0.75 (0.64-0.83) <0.01**		**0.36 (0.21-0.50) <0.01**	**0.69 (0.59-0.77) <0.01**	**0.40 (0.24-0.53) <0.01**	**0.38 (0.23-0.51) <0.01**	**0.51 (0.37-0.62) <0.01**
Pn 14	**0.31 (0.11-0.49) <0.01**	0.05 (-0.16-0.25) 0.66	0.13 (-0.08-0.33) 0.21	0.03 (-0.18-0.23) 0.78	0.12 (-0.08-0.32) 0.24	0.06 (-0.14-0.26) 0.57	0.13 (-0.08-0.33) 0.22	0.11 (-0.10-0.31) 0.31	**0.23 (0.03-0.42) 0.03**	**0.52 (0.36-0.66) <0.01**	**0.39 (0.20-0.55) <0.01**	**0.50 (0.33-0.64) <0.01**	**0.44 (0.25-0.59) <0.01**	**0.54 (0.38-0.67) <0.01**	**0.46 (0.28-0.61) <0.01**	**0.52 (0.36-0.66) <0.01**	**0.52 (0.35-0.65) <0.01**		**0.33 (0.18-0.47) <0.01**	**0.27 (0.11-0.42) <0.01**	**0.30 (0.14-0.44) <0.01**	**0.33 (0.18-0.47) <0.01**
Pn 18C	**0.49 (0.32-0.63) <0.01**	0.18 (-0.03-0.37) 0.0.9	0.09 (-0.12-0.29) 0.41	0.15 (-0.06-0.34) 0.16	0.16 (-0.05-0.36) 0.13	**0.41 (0.22-0.57) <0.01**	**0.22 (0.01-0.41) 0.04**	**0.22 (0.01-0.41) 0.04**	**0.28 (0.08-0.46) <0.01**	**0.80 (0.71-0.86) <0.01**	**0.62 (0.48.0.74) <0.01**	**0.78 (0.69-0.85) <0.01**	**0.78 (0.69.0.85) <0.01**	**0.74 (0.63.0.82) <0.01**	**0.67 (0.54-0.77) <0.01**	**0.76 (0.65-0.83) <0.01**	**0.70 (0.58-0.80) <0.01**	**0.62 (0.47-0.73) <0.01**		**0.43 (0.28-0.56) <0.01**	**0.48 (0.34-0.60) <0.01**	**0.53 (0.40-0.64) <0.01**
Pn 19A	**0.53 (0.37-0.67) <0.01**	0.18 (-0.02-0.37) 0.08	0.12 (-0.08-0.32) 0.24	0.14 (-0.07-0.34) 0.18	**0.23 (0.03-0.42) 0.03**	**0.25 (0.05-0.44) 0.01**	**0.33 (0.13-0.50) <0.01**	**0.29 (0.09-0.47) <0.01**	**0.39 (0.20-0.55) <0.01**	**0.66 (0.52-0.76) <0.01**	**0.49 (0.32-0.64) <0.01**	**0.67 (0.54-0.77) <0.01**	**0.64 (0.49-0.74) <0.01**	**0.66 (0.52-0.76) <0.01**	**0.63 (0.48-0.74) <0.01**	**0.59 (0.44-0.71) <0.01**	**0.64 (0.50-0.75) <0.01**	**0.55 (0.38-0.68) <0.01**	**0.74 (0.63-0.82) <0.01**		**0.75 (0.71-0.82) <0.01**	**0.39 (0.24-0.52) <0.01**
Pn 19F	**0.57 (0.41-0.69) <0.01**	**0.26 (0.05-0.44) 0.01**	**0.25 (0.05-0.43) 0.02**	**0.26 (0.05-0.44) 0.01**	**0.34 (0.14-0.51) <0.01**	**0.33 (0.13-0.50) <0.01**	**0.23 (0.03-0.42) 0.03**	0.19 (-0.01-0.38) 0.07	**0.21 (0.00.0.40) 0.04**	**0.75 (0.64-0.83) <0.01**	**0.51 (0.34-0.65) <0.01**	**0.66 (0.53-0.77) <0.01**	**0.71 (0.59-0.80) <0.01**	**0.69 (0.56-0.78) <0.01**	**0.66 (0.52-0.76) <0.01**	**0.72 (0.61-0.81) <0.01**	**0.76 (0.66-0.84) <0.01**	**0.58 (0.43-0.70) <0.01**	**0.75 (0.65-0.83) <0.01**	**0.73 (0.61-0.81) <0.01**		**0.30 (0.14-0.45) <0.01**
Pn 23F	**0.56 (0.40-0.68) <0.01**	**0.30 (0.10-0.48) <0.01**	0.17 (-0.04-0.36) 0.11	**0.24 (0.04-0.43) 0.02**	**0.24 (0.04-0.43) 0.02**	**0.33 (0.14-0.50) <0.01**	**0.21 (0.00-0.40) 0.05**	**0.27 (0.07-0.45) <0.01**	**0.25 (0.04-0.43) 0.02**	**0.79 (0.70-0.85) <0.01**	**0.59 (0.44-0.71) <0.01**	**0.75 (0.64-0.83) <0.01**	**0.78 (0.68-0.85) <0.01**	**0.78 (0.69-0.85) <0.01**	**0.70 (0.57-0.79) <0.01**	**0.75 (0.65-0.83) <0.01**	**0.75 (0.64-0.83) <0.01**	**0.49 (0.31-0.63) <0.01**	**0.77 (0.67-0.84) <0.01**	**0.75 (0.64-0.83) <0.01**	**0.75 (0.64-0.83) <0.01**	

Table 5.2 Correlation (expressed as Spearman correlation coefficients with 95% confidence interval and p-values) for pairwise comparisons of antibody responses to the12-month immunisations measured 1 month after immunisation.

	Measles	Mumps	Rubella	MenC	Tetanus	Hib
Measles		**0.20** **(0.04-0.35)** **0.01**	**0.17** **(0.01-0.33)** **0.04**	0.07 (-0.09-0.23) 0.39	-0.09 (-0.24-0.08) 0.30	-0.13 (-0.28-0.04) 0.13
Mumps			**0.61** **(0.50-0.70)** **<0.01**	-0.06 (-0.22-0.10) 0.45	0.05 (-0.11-0.21) 0.56	-0.12 (-0.28-0.04) 0.14
Rubella				-0.09 (-0.25-0.07) 0.25	-0.07 (-0.23-0.09) 0.38	-0.13 (-0.28-0.03) 0.12
MenC					**0.52** **(0.39-0.63)** **<0.01**	**0.48** **(0.35-0.60)** **<0.01**
Tetanus						**0.50** **(0.36-0.61)** **<0.01**

CHAPTER 6. GENERAL DISCUSSION AND FURTHER DIRECTIONS

The studies included in this thesis contribute to the understanding of factors that influence vaccine responses during the first year of life. I found that there is substantial variation within and between infants in antibody responses to routine vaccines. Measuring antibody responses to one antigen does not offer a surrogate marker of responses to other vaccines, as there is a weak correlation between antibody responses to different vaccines. Maternal dTpa immunisation during pregnancy, sex and the immunomodulatory effects of BCG influence humoral vaccine responses, while maternal TIV immunisation during pregnancy, delivery mode, breastfeeding and antibiotic exposure do not have an effect on antibody responses to routine immunisations in infants. Understanding the role of different factors in modulating vaccine responses has important implications for the future development and optimisation of vaccines and immunisation schedules and for individualising immunisation.

Maternal immunisation during pregnancy aims to protect infants during the period before they complete their primary immunisations and has been shown to be highly effective.[18-20] Nevertheless, my study shows that that antenatal dTpa immunisation influences antibody responses to subsequent infant vaccines. At both 7 and 13 months of age, infants of dTpa-immunised mothers had reduced specific (diphtheria and pertussis) and heterologous (polio and pneumococcal) vaccine antibody responses (but higher Hib responses at 7 months of age). In contrast, maternal TIV immunisation during pregnancy had no consistent effect on infant vaccine responses. One proposed mechanism for the inhibition of infant antibody production after antenatal immunisation is that maternal antibodies bind to vaccine epitopes and mask them from infant B lymphocytes, leading to attenuation of responses to these antigens. Hence, the ratio between maternal antibody concentrations and dose of vaccine antigen might influence the degree of inhibition.[133,134] As most vaccines induce high antibody responses, small differences in antibody concentrations may not be of clinical significance in terms of protective efficacy or may

only impact the duration of protection. I have recently shown that at 13 months of age, many infants vaccinated with a 3+0 schedule (with the last dose of PCV13 given at 6 months of age) have pneumococcal antibody concentrations below the standard threshold correlate of protection leaving them at risk for pneumococcal disease (Appendix 1).[135] There is a potential risk that the comprehensive implementation of maternal dTpa immunisation during pregnancy might further decrease antibody concentrations to pneumococcal vaccination. To retain the benefits of maternal immunisation whilst optimising protection against pneumococcal disease throughout early childhood and improving antibody persistence and indirect protective effects, immunisation schedules with additional booster doses of pneumococcal (and other) vaccines in the second year of life might need to be considered.

BCG has heterologous effects on the infant immune system.[136] The results from my study suggest that neonatal BCG immunisation might lead to higher GMCs and seroprotection rates for diphtheria, tetanus and pneumococcal antigens, and a reduced response and seroprotection rate for Hib. Although the findings only reached statistical significance for a few of the pneumococcal serotypes (9V, 19F and 23F), the consistent direction of change in GMRs at both 7 and 13 months of age suggests an effect of BCG on antibody responses. The results are also consistent with those from a recent trial in Denmark, which also found increased responses in BCG-vaccinated infants, albeit only statistically significant when BCG was administered after the first day of life.[137] To date, most studies investigating the effect of BCG on heterologous vaccine responses, including the recent study from Denmark[137] and ours, have used BCG-Denmark, which is a strain that has been associated with superior efficacy for both specific and non-specific effects.[138,139] The mechanisms underlying the immunomodulatory effects of BCG immunisation are the subject of ongoing investigation.[140] Studies show that BCG immunisation affects both innate[141-143] and T cell

immunity.[144] Notably, it has been shown that epigenetic reprogramming by BCG alters innate immune cells that might function as antigen-presenting cells.[145,146]

Apart from maternal immunisation and BCG, a further factor that I found to influence antibody responses to immunisation in infants, although to lesser extent, was biological sex. In my study, antibody concentrations were significantly lower at 7 months of age in females for responses to tetanus and filamentous haemagglutinin, and at 13 months of age for responses to pertactin. In contrast, at 13 months of age, antibody concentrations were significantly higher in females for polio type 3, pneumococcal serotype 6A and measles. My finding that females have lower antibody responses against tetanus, and higher responses against polio (type 3), is consistent with previous studies in adults. [91-93] It is difficult to compare my findings with the meta-analysis that has investigated the effect of biological sex on vaccine responses in infants and children up to the age of three years,[101] as many of the vaccine antigens for which I found significant differences between boys and girls (filamentous haemagglutinin, measles, polio) were not investigated in the meta-analysis. The meta-analysis did report "consistently higher or equivalent" vaccine antibody and memory B cells responses in girls compared to boys. However, similar to our study, two of the three trials included in the meta-analysis that compared antibody responses to tetanus vaccination also found lower responses in boys. In contrast, the studies included in the meta-analysis reported consistently higher antibody responses to pneumococcal vaccination in girls,[101] while I did not find sex differences in the response to pneumococcal vaccination, except for serotype 6A. This might be because a large part of our pneumococcal responses were measured 7 months after vaccination, while in the meta-analysis they were measured 1 month after vaccination. In adults, males have generally been reported to have higher antibody responses to pneumococcal vaccines.[147]

One proposed mechanism through which sex might affect vaccine responses is through hormones.[25,148] However, as shown in my literature review, sex differences in vaccine responses also occur before puberty and after menopause,[149] therefore further factors are likely involved. Many genes for proteins involved in the immune responses are encoded on the X chromosome, and mutations or polymorphisms of X-linked genes therefore have a greater impact in males.[150] A further mechanism through which sex differences in vaccine responses might occur, is through epigenetic programming, which is also influenced by sex hormones[148] or by sex differences in the intestinal microbiota.[151,152]

Maternal antibodies inhibit infant antibody responses to vaccines.[21] Although the majority of maternal antibody transfer is transplacental, breast milk also contains antibodies that might interfere with immune responses. Small studies have reported that after routine vaccination, breast-fed infants have higher serum IgG levels to diphtheria,[106] Hib[107,108] and oral polio virus (OPV)[106,153] vaccination, and higher salivary IgA levels to tetanus, diphtheria and OPV vaccination.[106] For ORV vaccine, reduced seroconversion rates were observed in breast-fed infants compared with formula-fed infants,[104,105] however, this was not confirmed in subsequent studies.[154] One difficulty with the investigation of the effect of breastfeeding on vaccine responses is that infants with mixed feeding are sometimes classified as breast-fed and sometimes as non-breast-fed.[154] Furthermore, IgA levels, as well as the neutralising activity of IgA in breast milk, may depend on geographic location.[155] These factors could explain some of the inconsistency in results from different studies. It is possible that our study was too small to detect any effect of breastfeeding on vaccine responses.

In recent years, considerable research has revealed the importance of the intestinal microbiota in the development of the immune system and regulation of immune responses.[112,156] Advances in sequencing technologies and bioinformatic analysis have facilitated the understanding of the

composition of the intestinal microbiota. Despite the evidence for the intimate relationship between the intestinal microbiota and the immune system, only a limited number of studies have investigated the effect of the composition of the intestinal microbiota on vaccine responses. These studies have been summarised in my recent review (Appendix 3).[157] These studies show that, in infants, a higher relative abundance of the phylum Actinobacteria (especially the species *Bifidobacterium longum*) is associated with both higher humoral and cellular vaccine responses to oral and parenteral vaccines,[158,159] while a higher relative abundance of the phylum Proteobacteria is associated with lower humoral and cellular responses to these vaccines.[158] In addition, in infants and adults, a higher relative abundance of the phylum Firmicutes is associated with higher humoral and cellular responses to oral vaccines,[160,161] while a higher relative abundance of the phylum Bacteroidetes is associated with lower humoral responses to oral vaccines in infants.[160] Currently, there no studies that have investigated the influence of the viral, fungal or parasitic intestinal microbiota on vaccine responses. However, children treated with anthelminthic drugs have been shown to have higher antibody responses after oral cholera immunisation,[162] while adults who are infected with *Ascaris lumbricoides* have been shown to have a suppressed interleukin-2 response after oral cholera immunisation.[163] It has also been shown that antenatal maternal helminth infections are associated with modestly increased IgA responses to oral vaccines in infants.[164] However, administration of anthelminthic drugs to pregnant women does influence oral vaccine responses in infants.[165]

There is evidence that the interaction between the microbiota and the immune system are of particular importance during early infancy, when they both develop concurrently.[166-168] Compared with adults, the microbiota in infants is less stable and is influenced by a number of factors, including gestational age, delivery mode, feeding, and maternal and infant antibiotic and probiotic exposure.[109,169-175] It is therefore likely that the intestinal microbiota has a greater influence on the immune response to immunisations given in infancy than later in life. Breastfed infants have a

higher relative abundance of *B. longum* subspecies *infantis* in their intestinal microbiota,[176-178] which in my recent review was associated with both increased humoral and cellular vaccine responses to both oral and parenteral vaccines (Appendix 4). In accordance with this, previous studies have reported that breastfed infants have higher vaccine-specific IgA and IgG concentrations in serum and saliva after parenteral vaccines.[106-108] In the study included in my thesis, I could not find a difference in antibody responses to routine immunisation between infants who were or were not still breastfed at 7 days, 1 month or 6 months of age. Antibiotics, which are frequently administered in early-life, have a substantial impact on the intestinal microbiota.[179,180] It is therefore plausible that antibiotics also influence the immune response to vaccines. However, in the study included in my thesis, I did not find a difference in antibody responses to routine immunisation in infants who were or were not exposed to intrapartum or early-life antibiotics.

To date, none of the studies that have investigated the association between the intestinal microbiota and vaccine responses has used whole genome sequencing, a technique that identifies a larger number of bacteria and, importantly, also viruses, fungi and parasites which potentially also influence vaccine responses. Moreover, in the few studies that did investigate the influence of the bacterial intestinal microbiota on vaccine responses, results were reported at various different taxonomic levels (phyla, order, family, genus, species) and it is likely that the relative abundance of certain genera or species, or specific microbial intestinal signatures are more important than phylum or class level changes in altering the response to vaccines. Further, well-designed, adequately powered studies using whole-genome sequencing are therefore needed to investigate in more detail the influence of the intestinal, respiratory and urinary microbiota on vaccine responses, including the effect of bacteria, archaea, eukaryotes and viruses. Whilst the microbiota remains relatively consistent over time in adults, in contrast, during the first 6 months of life, the numbers and type of microbes fluctuate markedly.[181,182] Therefore, future studies in infants should include frequent sampling, ideally both prior to and concurrent with measuring vaccine responses.

As shown in chapter 5, there was no correlation between vaccine responses to unrelated antigens. Therefore, surrogate antigens cannot be chosen and responses to a variety of vaccines need to be measured. Despite the lack of evidence that breastfeeding and antibiotic administration influence vaccine responses, these factors need to be considered when designing studies that investigate the effect of the microbiota on vaccine responses, as they are known to influence the microbiota.[183] Further factors that are known to influence the microbiota and need to be accounted for when designing studies that investigate the influence of the microbiota on vaccine responses, are feeding, administration of probiotics and other medications, siblings, day care attendance and pets. As live vaccines might influence the composition of the microbiota, it might be preferable to do a study which includes only infants that where not vaccinated with BCG or ORV. Identifying microbial signatures within the early-infant intestinal microbiota that are associated with differences in vaccine responses will make an important contribution to the growing field of research investigating the importance of the interplay between the microbiota and regulation of the human immune system. This will help identify strategies, such nutritional interventions, to improve vaccine effectiveness and duration of protection, particularly in infancy when the intestinal microbiota is more amenable to external influences.

While there have been only few studies on the effect of the intestinal microbiota on vaccine responses,[158-161,184] many studies have investigated the effect of concurrent administration of probiotics around the time of immunisation. Probiotics are live microbes which, when administered orally in adequate amounts are beneficial to the host.[185] I recently published a systematic review of studies that have investigated the effect of probiotics on the humoral response to immunisations (Appendix 4).[186] A beneficial effect of probiotics was reported in about half the studies, mostly in studies using oral vaccines or parenteral influenza immunisation.[186] Probiotics offer a relatively cheap intervention to improve vaccine effectiveness and duration of protection and have few adverse effects.[187] There is sufficient evidence from the studies in my

review to suggest this strategy is worth pursuing. However, future studies should focus on establishing optimal strains, doses and timing of probiotic administration in relation to immunisation.

Non-specific effects of vaccines are defined as effects induced by vaccines beyond protection against their targeted disease.[122] The best evidence for non-specific effects of vaccines exists for live-vaccines. For BCG, for example, it has been shown that it reduces all-cause infant mortality in certain settings[122] and that it decreases hospitalisation rates.[123,124] Measles-containing vaccines (MCV) also reduce childhood mortality in high-mortality settings from diseases other than measles by 26 to 49%.[122] To date, the exact mechanisms underlying the immunomodulatory effects of vaccines are uncertain,[188] but they include epigenetic reprogramming,[145,146] alteration in cytokine responses[141,143] and other changes in innate and T-cell immunity.[144,189] The immunomodulatory effect of neonatal BCG on antibody responses to heterologous vaccines found in my study adds to the evidence that BCG immunisation at birth has broad heterologous effects on the infant immune system, which should be further explored. In contrast to these findings, I have recently shown that MCV vaccine given at 12 months of age does not significantly influence the persistence of antibody concentrations to heterologous vaccines received in the first year of life (Appendix 2).[190]

The clinical significance of changes in humoral immunity induced by different factors needs to be interpreted in light of the fact that most vaccines lead to an antibody concentration significantly above the protective threshold in the majority of infants. Nonetheless, in the study described in Chapter 2, antenatal dTpa led to changes in antibody concentrations that were associated with diminished seroprotection rates, which might be clinically relevant. Furthermore, differences in antibody concentrations might be relevant for the duration of protection. Nonetheless, recognising factors which might influence vaccine antibody responses, is important for the design of vaccine studies and decisions on vaccine schedules. It also offers ways to optimise vaccine

immunogenicity and effectiveness, as well as the possibility to individualise immunisation. The complex interplay between different components of the immune system in vaccine responses is not yet fully understood, and future studies should focus on additional factors, including the innate and cellular immune system and the function of antibodies.

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Persistence of pneumococcal antibodies after primary immunisation with a polysaccharide–protein conjugate vaccine

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ABSTRACT

Introduction Despite immunisation, antibiotics and intensive care management, infection with *Streptococcus pneumoniae* remains a major cause of morbidity and mortality in children. The WHO currently recommends vaccinating infants with either a 3+0 schedule (6 weeks, 3–4 and 4–6 months of age) or 2+1 schedule (2 doses before 6 months of age, plus a booster dose at 9–15 months of age). This study investigated pneumococcal antibody responses, including persistence of antibodies, after immunisation of healthy infants with a 3+0 schedule.

Methods We measured pneumococcal antibody concentrations to all 13 antigens included in the 13-valent pneumococcal conjugate vaccine (PCV13) after immunisation with a 3+0 schedule in 91 infants at 7 months and in 311 infants at 13 months of age. The geometric mean concentrations (GMCs) and the proportion of infants with an antibody concentration above the standard threshold correlate of protection (seroprotection rate) were calculated at both time points.

Results At 7 months of age, GMCs varied between 0.52 µg/mLand 11.52 µg/mL, and seroprotection rates varied between 69% and 100%. At 13 months of age, GMCs had decreased to between 0.22 µg/mLand 3.09 µg/mL, with the lowest responses against serotype 4, followed by 19A, 3, 6B and 23F. Seroprotection rates at 13 months of age were below 90% for most serotypes, with the lowest rates for serotype 4 (23%) followed by 19A (50%), 23F (61%) and 6B (64%).

Conclusion Our study shows that at 13 months of age, many infants vaccinated with a 3+0 schedule have pneumococcal antibody concentrations below the standard threshold correlate of protection. To optimise protection against pneumococcal disease through early childhood and to improve antibody persistence and indirect protective effects, immunisation schedules with booster doses might be necessary.

What is already known on this topic?

- Despite immunisation, *Streptococcus pneumoniae* remains a major cause of global morbidity and mortality in children under 5 years of age.
- The WHO currently recommends vaccinating infants in the first year of life with either a 3+0 schedule or 2+1 schedule of 13-valent pneumococcal conjugate vaccine (PCV13).
- Most of the previous studies investigating responses to PCV13 have measured antibodies only at 4 weeks after immunisation; few have measured persistence of antibodies.

What this study adds?

- At 13 months of age, many infants vaccinated with a 3+0 PCV13 schedule ending at 6 months have pneumococcal antibody concentrations below the standard threshold correlate of protection, leaving them at risk for pneumococcal disease.
- In our cohort, 7 months after immunisation, the lowest concentrations for pneumococcal antibodies were observed for serotypes 4, 19A, 3 , 6B and 23F. . Previous studies have shown that higher threshold correlates of protection might be necessary for some of these serotypes. This might explain why breakthrough infections with these serotypes are observed between the second and fifth year of life.
- To optimise protection against pneumococcal disease throughout early childhood, and to improve antibody persistence and indirect protective effects, immunisation schedules with booster doses of pneumococcal vaccine should be considered.

INTRODUCTION

Streptococcus pneumoniae remains a major cause of respiratory tract infections (acute otitis media, sinusitis, bronchitis and pneumonia) and invasive disease (bacteraemia, meningitis and osteomyelitis) globally and is associated with considerable healthcare costs. The pathogen contributes significantly to the mortality of children under 5 years of age with an estimated 0.5 million deaths per year.[1] Infants are particularly vulnerable to invasive pneumococcal disease (IPD) and, in addition to elderly people, carry the highest incidence and case fatality rates (approximately 24% in children under 5 years of age).[2]

Based on its polysaccharide capsule, *S. pneumoniae* is grouped into over 90 serotypes. The serotypes that are most frequently associated with IPD depend on geographical location, vaccine use, age group and individual risk factors.[3] In children with IPD, for example, in Sweden, before the introduction of immunisation, serotypes 6B, 19F, 18C, 6A, 23F, 4 and 14 were most frequent.[4] In contrast, in the UK, after the introduction of the 13-valent pneumococcal

conjugate vaccine (PCV13), serotypes 19A, 7F and 3,[5] and in Australia, currently, serotypes 3 and 19A are the most common.[6]

The introduction of 7-valent pneumococcal polysaccharide–protein conjugated vaccine (PCV7), 10-valent pneumococcal polysaccharide–protein conjugated vaccine (PCV10) and PCV13 has significantly reduced the burden of IPD (protection rate >90%),[7 8] pneumonia (27%)[8–11] and vaccine serotype pneumococcal acute otitis media (57%–67%).[12–15] Furthermore, by decreasing nasopharyngeal carriage and antimicrobial resistance rates,[16] immunisation of infants with PCV also has indirect protective effects for vaccinated and unvaccinated older children and adults (herd immunity).[17]

Antibody concentrations necessary for protection against pneumococcal disease vary for protection against nasopharyngeal carriage, disease type and for cross-protection between serotypes. An antibody concentration of 0.35 µg/mL is conventionally used as the threshold correlate of protection value for IPD and during licencing of new PCVs.[18] An antibody concentration of 0.5 µg/mL is the estimated threshold correlate of protection value for protection against acute otitis media and pneumonia. Even higher concentrations (above 2 µg/mL) are estimated to be necessary for cross-protection between serotypes.[19] A recent study showed that the threshold correlate of protection value varies by serotype.[5]

Since 2007, the guidelines from the Expanded Programme on Immunization (EPI) from the WHO recommend vaccination with either three doses of PCV13 at 6 weeks, 3–4 and 4–6 months of age (3+0 schedule) or with two doses before 6 months of age, plus a booster dose at 9–15 months of age (2+1 schedule), depending on the local epidemiology of pneumococcal disease. Both schedules are estimated to protect against IPD for at least 2–3 years.[20] Currently, breakthrough cases of IPD in children who have been fully vaccinated with three doses of PCV13 with a 3+0 schedule are observed mostly between the second and fifth year of life,[6] which has, in Australia, led to moving the third dose into the second year of life.[6 21] In contrast, a study in the UK observed no difference in postbooster antibody concentrations between children who were vaccinated with a 1+1 or a 2+1 schedule for 9 of 13 serotypes.[22] It therefore remains unclear how long the protection lasts from a schedule with only primary doses in infancy and what the optimal timing is for a booster dose (or doses).

To date, most studies have measured antibody concentrations only 4 weeks after schedule completion,[23] and few have measured persistence of antibodies, which is important for protection against breakthrough infections in early childhood.[24–29] In this study, we measured pneumococcal antibody concentrations at the age of 7 months and 13 months of age (1 and 7 months after immunisation) following a 3+0 schedule with PCV13 to investigate whether antibody concentrations are still above the threshold correlate of protection 7 months after completing the schedule.

METHODS

Study design and population

Participants in this study were a subset of infants in the Melbourne Infant Study: BCG for Allergy and Infection Reduction from whom blood samples were collected at 7 months and 13 months of age. This randomised clinical trial comprises 1272 healthy infants recruited antenatally from 2013 to 2016 to investigate whether BCG immunisation at birth protects against childhood infection, allergy and asthma. Inclusion criteria were: greater than 32 weeks' gestation, birth weight greater than 1500 g, mother not HIV positive, absence of symptoms or signs of illness and no known contact with TB. Twins of participants were excluded from the study, as they were not considered as being independent

Immunisation

All infants received routine immunisations according to the Australian National Immunisation Program: at birth: intramuscular hepatitis B vaccine (H-B-Vax II Paediatric [*bioCSL*]); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa), hepatitis B, inactivated poliomyelitis and *Haemophilus influenzae* type b (Hib) vaccine (Infanrix Hexa [*GlaxoSmithKline*]), intramuscular conjugate pneumococcal vaccine (Prevenar13 [*Wyeth*]) and oral rotavirus vaccine (RotaTeq[Merck]); at 12 months of age: subcutaneous measles-mumps-rubella vaccine (Priorix [*GlaxoSmithKline*]) and intramuscular combined Hib and meningococcal C vaccine (Menitorix [*GlaxoSmithKline*]).

Blood collection

Plasma samples were available from 91 healthy participants at 7 months of age (1 month after completing the primary immunisation schedule) and from 311 participants at 13 months of age (7 months after completing the primary immunisation schedule). Blood was collected in sodium-heparin (S-monovette[*Sarstedt*]) tubes and stored at −80°C until analysis.

Antibody measurement

Pneumococcal-specific antibody concentration to all 13 antigens included in PCV13 were measured using a fluorescent bead-based multiplex immune-assay (Luminex xMAP technology). The geometric mean concentrations (GMCs) for each vaccine antibody was calculated, and the 95% confidence interval (CI) was estimated at both time points. The proportion of infants with an antibody concentration above the threshold correlate of protection value of 0.35 µg/mL[30] and 0.5 µg/mL[19] (seroprotection rate) was also calculated at both time points together with 95% CIs using the Clopper-Pearson method.

Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation 38124A).

RESULTS

The characteristics of participants included at 7 months and 13 months of age are summarised in table 1. There were no differences in sex, gestational age, birth weight or delivery mode between the infants included at 7 months and 13 months of age. Infants at 7 months of age had slightly higher rates of maternal influenza and DTPa immunisation during pregnancy. The age at immunisation was similar in both groups.

Antibodies at 7 months of age

At 7 months of age, GMCs varied between 0.52 µg/mL and 11.53 µg/mL. The highest responses were observed against serotypes 19F (11.53 µg/mL; 95% CI 9.50 to 13.86 µg/mL) and 7F (6.13; 95% CI 5.22 to 7.21), followed by 6A (4.81; 95% CI 4.02 to 5.77) and 1 (4.37; 95% CI 3.53 to 5.49). The lowest responses were observed against serotype 4 (0.52; 95% CI 0.43 to 0.61), followed by 3 (1.44; 95% CI 1.25 to 1.65), 19A (1.72; 95% CI 1.41 to 2.09) and 6B (1.91; 95% CI 1.40 to 2.62).

Table 1 Characteristics of participants with blood samples analysed at 7 months and 13 months

	Samples at 7 months (n=91) n (%) or median (IQR)	Samples at 13 months (n=311) n (%) or median (IQR)
Sex (male)	43 (47)	152 (49)
Gestational age (weeks)	39.5 (38.6–40.5)	39.3 (38.4–40.3)
Birth weight (kg)	3.48 (3.10–3.78)	3.44 (3.14–3.76)
Caesarean section	35 (38)	117 (38)
Maternal DTPa immunisation in pregnancy	46 (51)	146 (47)
Maternal influenza immunisation in pregnancy	55 (60)	175 (56)
Age at routine immunisation in days (median)		
6-week vaccines	45 (43–48)*	46 (43–49)
4-month vaccines	123 (117–127)	125 (121–132)
6-month vaccines	187 (181–193)	191 (185–204)
Interval between (days)		
6-month immunisation and 7-month blood sample	28 (21–37)	–
6-month immunisation and 13-month blood sample	–	209 (190–224)
Age at blood sampling (days)	218 (208–227)	400 (389–418)

*Age for one participant not available.
DTPa, diphtheria-tetanus-acellular pertussis vaccine.

GMCs against serotypes 5, 9V, 14, 18C and 23F were similar at 2–3 µg/mL (figure 1 and online supplementary table 1).

Seroprotection rates using a threshold correlate of protection value of 0.35 µg/mL varied between 69% and 100%. The lowest seroprotection rate was observed for serotype 4 (69%; 95% CI 59% to 78%), followed by serotype 6B (89%; 95% CI 81% to 95%). The rates for the remaining 11 serotypes were all above 90% (100% for serotypes 7F and 19F). Using a threshold correlate of protection value of 0.50 µg/mL, seroprotection rates were between 53% and 100%, with most between 85% and 89%. The lowest rates were again observed for serotype 4 (53%; 95% CI 19% to 29%) and 6B (85%; 95% CI 76% to 91%) (figure 2 and online supplementary table 2).

Antibodies at 13 months of age

At 13 months of age, GMCs were lower than at 7 months, varying between 0.22 µg/mL and 3.09 µg/mL. The highest response was again observed for serotype 19F, but the GMC at this age was considerably lower than that measured at 7 months of age (3.09 µg/mL vs 11.53 µg/mL) (figure 1 and online supplementary table 1). GMCs for all other serotypes were below 2.00 µg/mL, with many below 1.00 µg/mL (serotypes 3, 4, 5, 6B, 9V, 18C, 19A and 23F).

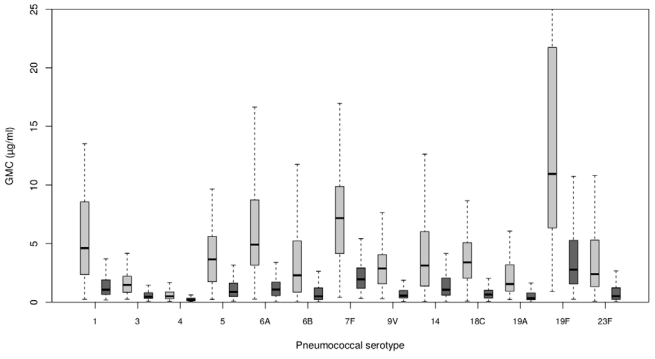


Figure 1 Mean geometric mean concentrations (GMCs) in µg/mL of antibodies against pneumococcal antigens included in PCV13 at 7 months (light grey bars) and 13 months (dark grey bars) of age. PCV13, 13-valent pneumococcal conjugate vaccine.

At 13 months of age, seroprotection rates using a threshold correlate of protection value of 0.35 µg/mL were below 90% for the majority of antigens, except serotypes 1, 7F and 19F (figure 2). The lowest seroprotection rate was observed for serotype 4 (23%; 95% CI 42% to 63%), followed by 19A (50%; 95% CI 44% to 56%), 23F (61%; 95% CI 56% to 67%) and 6B (64%; 95% CI 59% to 70%). Using a threshold correlate of protection value of 0.50 µg/mL, seroprotection rates varied between 13% and 98%. Seroprotection rates were below 80% for eight serotypes and below 60% for six serotypes. The lowest rate of were observed for serotype 4 (13%; 95% CI 9% to 17%), followed by 19A (37%; 95% CI 31% to 42%), 3 (47%; 95% CI 41% to 53%), 23F (50%; 95% CI 45% to 56%) and 6B (52%; 95% CI 46% to 58%) (figure 2 and online supplementary table 1). For 7F and 19F, for which the highest GMCs were observed at 7 months of age, there were very little differences in seroprotection rates between 7 months and 13 months of age (for a threshold correlate value of 0.35 µg/mL: 7F: 100% vs 98%, 19F: 100% vs 100%), while the largest differences were observed for serotypes 4 (69% vs 23%), 19A (93% vs 50%), 23F (93% vs 61%) and 3 (99% vs 70%).

DISCUSSION

Of WHO member states, 44% include PCV in their routine immunisation schedule.[31] However, the burden of mortality from IPD remains high, despite immunisation, antibiotics and intensive care management.[32] The EPI currently recommends the 3+0 vaccine schedule for countries in which the incidence of IPD peaks in infants within the first year of life (usually developing countries) and the 2+1 schedule for countries in which the peak incidence occurs beyond the first year of life, as it offers a longer duration of protection.[23 33]

Our finding that at 13 months of age (7 months after completing a 3+0 schedule) many infants have antibody concentrations below the standard threshold correlate of protection value of 0.35 µg/mL for a number of pneumococcal serotypes (with the lowest seroprotection rates observed for serotypes 4, 19A, 23F and 6B and the lowest antibody concentrations for serotypes 4, 19A, 23F and 3) is worrying.

Our finding of low GMCs and low seroprotection rates at 13 months of age with a 3+0 schedule is consistent with clinical surveillance data that show higher protection rates against vaccine-type IPD in children with a 3+1 or 2+1 schedule (up to 100%) than with a 3+0 schedule (up to 90%) after vaccination with PCV7 or PCV9.[7 34] Additionally, recent data show that children who were vaccinated with a 3+0 schedule more than 24 months earlier have a fivefold increased risk of contracting a vaccine-type IPD compared with children who were vaccinated within the previous 12 months.[35] In Australia, for pneumococcal disease, vaccine failures in children above the age of 1 year have increased since the introduction of a 3+0 schedule of PCV13.[35–37] Notably, a similar phenomenon was observed in relation to Hib disease in older children in the UK. An increased number of cases was observed after a schedule without a booster was used for nearly a decade. Subsequently, the schedule was changed to include a Hib booster dose.[38]

GMCs and seroprotection rates following a 3+0 schedule might be sufficiently high for some countries with high immunisation coverage, which benefit from strong herd immunity through decreased nasopharyngeal carriage. However, systematic

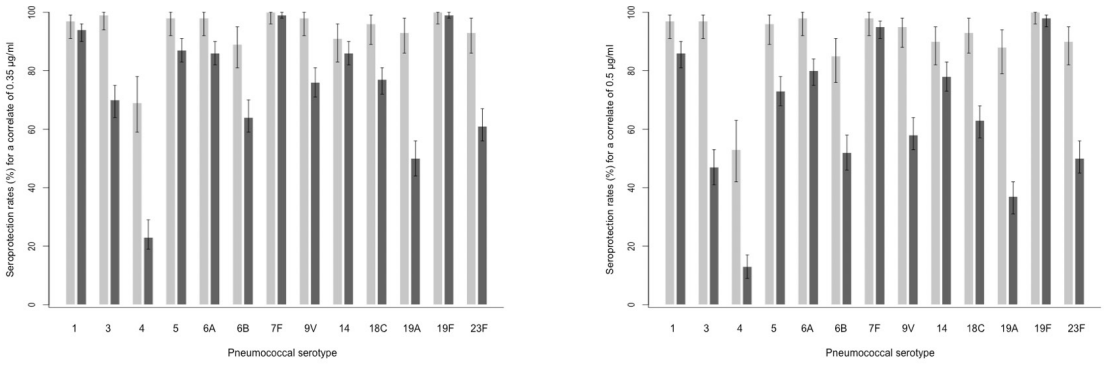


Figure 2 Seroprotection rates against pneumococcal antigens included in PCV13 at 7 months (light grey bars) and 13 months (dark grey bars) of age using a threshold correlate of protection value of 0.35 µg/mL (left) and 0.5 µg/mL (right). PCV13, 13-valent pneumococcal conjugate vaccine.

reviews show that while 3+1, 2+1 and 3+0 schedules reduce the incidence of pneumonia[9] and nasopharyngeal carriage with vaccine-type pneumococcal serotypes in children,[39] only a 3+1 schedule also has indirect benefits by reducing pneumonia and nasopharyngeal carriage in adults.[40] Since immunisation has a large influence on the circulating pneumococcal serotypes, the epidemiology is constantly changing, and it is difficult to compare studies from settings in which a variety of different vaccine schedules have been used.

After the introduction of PCV13, the largest reduction in IPD due to the additional serotypes included in PCV13 (compared with PCV7) was observed for serotype 19A, followed by 1 and 6A. The incidence of IPD due to serotype 3 did not change appreciably.[41 42] Currently, in PCV13-vaccinated populations, approximately 14% of IPD in children younger than 5 years of age is caused by vaccine serotypes in populations vaccinated with the 2+1 schedule[41] and 30% in populations vaccinated with the 3+0 schedule.[6] The most frequently isolated serotypes are 1, 19A and 23F in children vaccinated with 2+1 schedule[3] and 19A, 3, 19F and 23F in children vaccinated with the 3+0 schedule.[6] The most frequently isolated pneumococcal vaccine serotype isolated from children with pleural effusion who are appropriately vaccinated with PCV13 with either a 2+1 or 3+1 schedule is serotype 3.[43 44] This serotype is currently also the most frequent vaccine serotype isolated from patients of all ages with acute otitis media.[45] Consistent with this clinical observation, serotypes 19A, 3 and 23F, were, apart from serotype 4, the serotypes for which we observed the lowest seroprotection rates at 13 months of age. Even though, the same serotypes are still causing disease after the 2+1 and 3+1 schedule numbers are lower than after the 3+0 schedule.[6]

The antibody threshold correlate of protection value provides a benchmark for predicting protection against IPD and other pneumococcal diseases, but other factors, for example, antibody function (measured by opsonophagocytic assays) are also important when considering protective immunity. Nevertheless, for pneumococcal protection, there is a good correlation between total antibody concentrations and opsonophagocytic activity.[33 46] The antibody concentration needed to protect against pneumococcal disease depends on geographic location, age, disease type and serotype. In Africa, antibody concentrations required to prevent IPD are reported to be higher than in the USA.[30 47] Even though a threshold correlate of protection value of 0.35 µg/mL for IPD and 0.5 µg/mL for mucosal disease is used, these values may not be valid for all serotypes.[5] A recent study estimated necessary protection correlates for IPD to be below 0.35 µg/mL only for the vaccine serotypes 18C, 6B, 23F and 4. In contrast, the necessary protection correlates were estimated to be much higher for serotype 3 (2.83 µg/ml), 19F (1.17 µg/mL), 19A (1 µg/mL) and 7F (0.87 µg/mL).[5]

Recent studies show that antenatal immunisation with DTPa vaccine leads to significantly lower infant pneumococcal antibodies at 13 months of age.[48–50] Since antenatal immunisation is now routinely recommended, this might further decrease pneumococcal seroprotection rates in young children, leaving them exposed to pneumococcal disease after 12 months of age. This is of particular importance for children who have received a 3+0 PCV13 schedule without a booster.

In our study, 7 months after immunisation, the lowest concentrations for pneumococcal antibodies were observed for serotypes 4, 19A, 3, 6B and 23F. Together, with the fact that higher protection correlates might be necessary for serotype 3 and 19A and 7F,[5] this might explain why the highest breakthrough infections are observed for some of these serotypes.[3 6] Our findings suggest that following a 3+0 schedule of PVC13, the addition of a booster dose (or doses) might improve the duration of vaccine-induced protection against IPD, protection from mucosal disease, cross-protection from other serotypes and herd immunity.

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ORIGINAL ARTICLE

The immunomodulatory effects of measles-mumps-rubella vaccination on persistence of heterologous vaccine responses

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Abstract

It is proposed that measles-containing vaccines have immunomodulatory effects which include a reduction in all-cause childhood mortality. The antibody response to heterologous vaccines provides a means to explore these immunomodulatory effects. This is the first study to investigate the influence of measles-mumps-rubella (MMR) vaccine on the persistence of antibodies to a broad range of heterologous infant vaccinations given in the first year of life. In total, 319 children were included in the study. All infants received routine vaccinations at 6 weeks, 4 and 6 months of age. At 12 months of age, 212 children were vaccinated with MMR and *Haemophilus influenzae* type b-meningococcus C (Hib-MenC) vaccines while the remaining 99 children had not yet received these vaccines. In the MMR/Hib-MenC-vaccinated group, blood was taken 28 ± 14 days after receiving these vaccines. Antibodies against diphtheria, tetanus, pertussis [pertussis toxin (PT), filamentous hemagglutinin, pertactin], poliomyelitis (type 1, 2, 3) and 13 pneumococcal serotypes were measured. Seroprotection rates and geometric mean antibody concentrations were compared between MMR/MenC-Hib-vaccinated and MMR/MenC-Hib-naïve participants. In the final analysis, 311 children were included. Seroprotection rates were lower in MMR/Hib-MenC-vaccinated children against PT and pneumococcal serotype 19A. After adjustment for prespecified factors, MMR/Hib-MenC-vaccinated infants had significantly higher antibody concentrations against tetanus (likely explained by a boosting effect of the carrier protein, a tetanus toxoid), while for the other vaccine antigens there was no difference in antibody concentrations between the two groups. MMR vaccination given at 12 months of age in a developed country does not significantly influence antibody concentrations to heterologous vaccines received in the first year of life.

INTRODUCTION

It is proposed that vaccines, particularly live-vaccines such as Bacillus Calmette-Guérin (BCG) and measles-containing vaccines (MCV), have heterologous ("nonspecific") immunomodulatory effects.[1-4] These are effects induced by vaccines beyond protection against their targeted disease. The reduced mortality has been attributed to decreased deaths from respiratory illnesses, diarrhea and malnutrition.[5] For MCV, a recent meta-analysis reported reduced childhood mortality from diseases other than measles by 26–49% in high-mortality settings.[1] However, a

number of the included studies were deemed to be at risk of bias. More recent studies have also shown a beneficial effect of MCV on childhood survival, the effect being greater when MCV are given before the age of 12 months.[6,7] The immunomodulatory effects of MCV have also been used in the treatment of atopic dermatitis, for which the vaccine (given subcutaneously) has been reported to decrease serum immunological markers.[8] In addition, in the treatment of cutaneous warts, it been hypothesized that the injection of MCV into lesions accelerated the destruction of the papilloma virus and infected host cells.[9,10]

MCV induce both humoral and cell-mediated immune responses.[11,12] The mechanisms underlying the immunomodulatory effects of MCV remain unclear. The antibody response to heterologous vaccines provides one way to explore these effects. Only three studies have investigated the influence of MCV on heterologous vaccine responses and these reported inconsistent results.[13-15] In these studies, a MCV was given at the same time as the other vaccines. We hypothesized that through effects on innate immunity, stimulation of B- and T-lymphocytes and expansion of pre-existing memory cells, a MCV might alter heterologous responses to previously administered vaccines. The aim of our study was to investigate the effect of a MCV vaccine given at 12 months of age on persistence of antibodies to a broad range of heterologous routine vaccines given in the first year of life.

RESULTS

Of 319 eligible participants, five were excluded because they were a twin of another participant, two because they were not vaccinated according to the schedule and one because vaccine records were not available (Figure 1). Of the remaining 311 participants, 212 had received the measles-mumps-rubella (MMR)/Hib-MenC vaccine 28 $\pm$ 14 days before the study. The remainder were MMR/Hib-MenC vaccine-naïve (as they had not yet been taken to a clinic for vaccination). The background characteristics of participants are shown in Table 1. There were no differences in sex, gestational age, birth weight, Cesarean section rate, maternal combined diphtheria-tetanus-acellular pertussis vaccination in pregnancy or infant BCG-vaccination status between the two groups. However, the MMR/Hib-MenC-naïve infants received their vaccines on average later than the MMR/Hib-MenC-vaccinated infants. The time interval between the 6-month vaccines and blood sampling at 13 months of age was longer in the MMR/Hib-MenC-vaccinated infants compared with the MMR/Hib-MenC-naïve infants.

Infants who were vaccinated with MMR/Hib-MenC had a lower seroprotection rate for 15/19 (79%) antigens compared with those who were MMR/Hib-MenC vaccine-naïve. This difference was statistically significant for pertussis toxin (13%) and pneumococcal serotype 19A (17%) (Table 2 and Figure 2).

Unadjusted geometric mean antibody concentrations (GMCs) were lower in MMR/Hib-MenC-vaccinated children for 20/21 (95%) antigens. This reached statistical significance for 10/21 (48%) antigens. In contrast, MMR/Hib-MenC-vaccinated children had significantly higher GMCs against tetanus (Table 3, Figures 3 and 4). After adjusting for the prespecified factors, a geometric mean antibody ratio above 1, indicating higher average antibody concentrations against tetanus in MMR/Hib-MenC-vaccinated children was the only statistically significant finding (Table 3 and Figure 5).

DISCUSSION

Children who are infected with measles have a significantly higher mortality rate than children who are vaccinated against the disease.[16] One explanation for the decreased mortality after measles vaccination is that by preventing measles infection, the vaccine also prevents the transient immunosuppressive state (also called measles virus immune amnesia) that is observed after measles infection.[17] Measles virus commonly infects memory lymphocytes[18,19] and consequently, after measles infection, individuals lose their immune repertoire and are predisposed to a broad range of infections for a period of up to several weeks or even years.[17]

A further explanation for the decreased mortality after MCV is modulation of the immune system through the immunomodulatory effects of the vaccine.[9,10,20] The precise mechanisms underlying the immunomodulatory effects of live-vaccines, particularly BCG and MMR, are the subject of ongoing investigations. BCG vaccine, for example, influences the innate[21-23] and T-cell immunity[24]

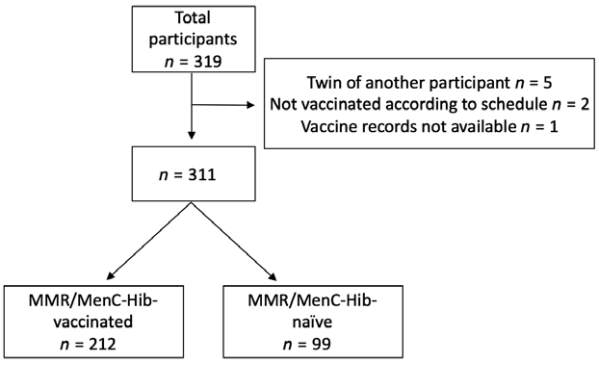


Figure 1. Selection of participants. MMR, measles-mumps-rubella; Hib-MenC, *Haemophilus influenzae* type b-meningococcus C.

Table 1. Characteristics of participants.

	MMR/Hib-MenC-vaccinated (n = 212) n (%) or median (IQR)	MMR/Hib-MenC-naïve (n = 99) n (%) or median (IQR)	P-value
Sex (male)	102 (48)	50 (50)	0.70
Gestational age (weeks)	39.2 (38.4–40.4)	39.3 (38.4–40.3)	0.77
Birth weight (kg)	3.42 (3.12–3.73)	3.55 (3.19–3.88)	0.29
Cesarean section	77 (36)	40 (40)	0.49
Maternal DTPa in pregnancy	101 (48)	45 (45)	0.72
BCG-vaccinated	111 (52)	58 (59)	0.31
Age at routine vaccinations (days)			
6-week vaccines	45 (43–48)	47 (44–52)	0.01
4-month vaccines	124 (120–130)	127 (123–137)	<0.01
6-month vaccines	189 (184–200)	193 (186–220)	<0.01
12-month vaccines	375 (369–383)	—	
Interval between (days)			
6-month vaccines to blood sampling at 13 months of age	218 (204–230)	190 (166–203)	<0.01
12-month vaccines to blood sampling at 13 months of age	32 (21–43)	—	
Age at blood sampling (days)	410 (397–421)	385 (378–398)	<0.01

P-values are from a t-test for continuous variables and a χ^2 test for categorical variables.
DTPa, diphtheria-tetanus-acellular pertussis vaccine; IQR, interquartile range; BCG, Bacillus Calmette-Guérin; MMR, measles-mumps-rubella; Hib-MenC, *Haemophilus influenzae* type b-meningococcus C.

Table 2. Proportions of participants at 13 months of age with antibody concentrations above protective correlate.

Vaccine antigen	Protective correlate	MMR/Hib-MenC-vaccinated (n = 212) % (n); 95% CI	MMR/Hib-MenC-naïve (n = 99) % (n); 95% CI	Difference % (95% CI)	P-value
Diphtheria	0.01 IU mL^{-1}	97.6 (207); (94.6, 99.2)	100 (99); (97.3, 100)	−2.4 (−4.5, 1.4)	0.18
Tetanus	0.01 IU mL^{-1}	100 (212); (98.3, 100)	100 (99); (96.3, 100)	0 (−1.8, 3.7)	—
Pertussis toxin	25 IU mL^{-1}	**26.4 (56); (20.6, 32.9)**	**39.4 (29); (29.7, 49.7)**	**−13.0 (−24.4, −1.9)**	**0.02**
Polio					
Type 1	0.23 IU mL^{-1}	100 (212); (98.3, 100)	100 (99); (96.3, 100)	0 (−1.8, 3.7)	—
Type 2	0.29 IU mL^{-1}	99.5 (211); (97.4, 100)	100 (99); (96.3, 100)	−0.5 (−2.6, 3.3)	1
Type 3	0.12 IU mL^{-1}	100 (212); (98.3, 100)	100 (99); (96.3, 100)	0 (−1.8, 3.7)	—
Pneumococcus (Pn)					
Serotype 1	0.35 µg mL^{-1}	92.0 (195); (87.5, 95.3)	97.0 (96); (91.4, 99.3)	−5.0 (−10.0, 1.1)	0.14
Serotype 3	0.35 µg mL^{-1}	69.3 (147); (62.7, 75.5)	70.8 (70); (60.7, 79.4)	−1.4 (−11.2, 9.9)	0.89
Serotype 4	0.35 µg mL^{-1}	20.3 (43); (15.0, 26.3)	30.3 (30); (21.5, 40.4)	−10.0 (−20.9, 0.1)	0.06
Serotype 5	0.35 µg mL^{-1}	85.8 (182); (80.4, 90.2)	89.9 (89); (82.2, 95.0)	−4.0 (−11.1, 4.5)	0.37
Serotype 6A	0.35 µg mL^{-1}	86.3 (183); (80.9, 90.6)	86.9 (86); (78.6, 92.8)	−0.6 (−8.1, 8.5)	1
Serotype 6B	0.35 µg mL^{-1}	63.7 (135); (56.8, 70.2)	65.7 (65); (55.4, 74.9)	−2.0 (−13.0, 9.7)	0.80
Serotype 7F	0.35 µg mL^{-1}	99.5 (211); (97.4, 100)	99.0 (98); (94.5, 100)	0.5 (−1.7, 5.1)	0.54
Serotype 9V	0.35 µg mL^{-1}	75.5 (160); (69.1, 81.1)	76.8 (76); (67.2, 84.7)	−1.3 (−10.9, 9.4)	0.89
Serotype 14	0.35 µg mL^{-1}	84.4 (179); (78.8, 89.0)	90.9 (90); (83.4, 95.8)	−6.5 (−13.6, 1.9)	0.15
Serotype 18C	0.35 µg mL^{-1}	73.6 (156); (67.1, 79.4)	83.8 (83); (75.1, 90.5)	−10.3 (−19.1, −0.1)	0.06
Serotype 19A	0.35 µg mL^{-1}	**44.3 (94); (37.5, 51.3)**	**61.6 (61); (51.3, 71.2)**	**−17.3 (−18.5, 5.3)**	**<0.01**
Serotype 19F	0.35 µg mL^{-1}	99.1 (210); (96.6, 99.9)	100 (99); (96.3, 100)	−0.9 (−3.4, 2.8)	1
Serotype 23F	0.35 µg mL^{-1}	60.8 (129); (53.9, 67.5)	62.6 (62); (52.3, 72.1)	−1.8 (−13.0, 10.0)	0.80

Bold denotes statistically significant results. P-values are from a t-test for continuous variables and a χ^2 test for categorical variables.
CI, confidence interval; MMR, measles-mumps-rubella; Hib-MenC, *Haemophilus influenzae* type b-meningococcus C.

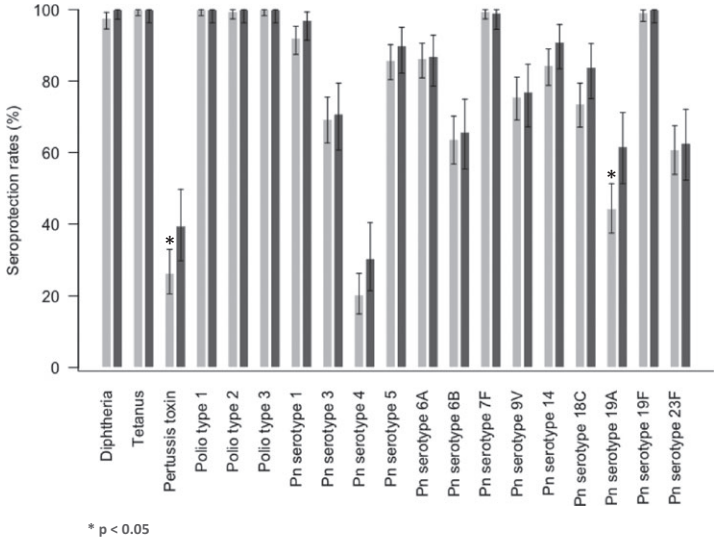


Figure 2. Seroprotection rates in measles-mumps-rubella (MMR)/*Haemophilus influenzae* type b-meningococcus C (Hib-MenC)-vaccinated (n = 212, dark gray bars) and MMR/Hib-MenC-naïve (n = 99, light gray bars) participants at 13 months of age.

by epigenetic reprogramming of immune cells[25,26] and by altering cytokine responses.[22,27] Fewer studies have investigated the mechanisms underlying the immunomodulatory effects of MCV. Measles virus induces a predominately T helper type 1 response, which leads to an increase in interferon-gamma and interleukin-12 production.[28,29] MCV induce both humoral and cell-mediated immune responses.[11,12] The mechanisms associated with the nonspecific immunomodulatory effects of MCV include a decrease in leukocytes[30] and lymphocytes, with a decrease in CD4 cells[30] and an increase in CD8 cells,[31] and induction of cross-reactive B- and T-lymphocyte responses.[32] Additionally, functional assays show enhanced lymphoproliferation after stimulation.[30] Furthermore, following MCV, increases in soluble CD8 molecule and β-microglobulin have been observed.[33] It is also proposed that MCV induces "trained" innate immune memory by epigenetic and transcriptional reprogramming of innate cells.[32]

One way to explore the immunomodulatory effects of vaccines is to study their influence on the antibody responses to heterologous vaccines. For BCG, it has been shown that the vaccine, when administered prior, increases antibody responses to a number of heterologous vaccines.[34,35] For MCV, much less data are available. Three studies have investigated the effect of co-administration of MCV with other vaccines. One study showed that female infants, who are vaccinated with high-dose Edmonstron-Zagreb measles vaccine at the same time as receiving a rabies vaccine, have higher antibodies against rabies 3 months after vaccination compared to female infants who do not receive the vaccine.[13] Another study showed that when MMR is given simultaneously with a yellow fever vaccine, antibody concentrations and seroprotection rates to yellow fever are lower than when the vaccines are given more than a month apart.[14] In contrast, concurrent administration of MMR with a live-attenuated influenza vaccine does not influence antibody responses to influenza.[15] In addition to the studies investigating the effect of co-administered MCV, one study investigated the effect of prior MCV on the response to a smallpox vaccine. Another study reported that when children received a varicella vaccination <30 days following MCV, they had a 2.5-fold increase in the incidence of breakthrough disease.[36]

In our study, we did not find any significant differences in GMCs to heterologous vaccines between infants who did or did not receive a MCV. In contrast to previous studies, which investigated the effect of co-administration of vaccines, we investigated the influence of a MCV vaccine on the persistence of antibodies to vaccines given earlier in life. In our study, MMR was given at 12 months of age, while in many developing countries a MCV is given at 9 months of age or earlier. While age at first MCV does not influence cellular vaccine responses,[28,37-39] younger age is associated with lower antibody responses, faster waning and lower avidity of antibodies.[40,41] In contrast, MCV were observed to have a stronger effect on the reduction in all-cause mortality when given at a younger age.[6,7] Further studies are needed to explore the effect of age of MCV vaccination on heterologous vaccine responses.

The lower unadjusted GMCs and seroprotection rates in MMR/MenC-Hib-vaccinated participants are most

Table 3. Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) in participants at 13 months of age.

Vaccine antigen	MMR/Hib-MenC-vaccinated (n = 212) GMC 95% CI	MMR/Hib-MenC-naïve (n = 99) GMC 95% CI	Unadjusted GMR (95% CI)	*P*-value	Adjusted GMR (95% CI)	Adjusted *P*-value
Diphtheria (IU mL^{-1})	**0.07 (0.06, 0.08)**	**0.09 (0.07, 0.12)**	**0.77 (0.60, 0.98)**	**0.03**	1.04 (0.81, 1.33)[a,g,h,i]	0.76
Tetanus (IU mL^{-1})	**0.90 (0.76, 1.06)**	**0.32 (0.26, 0.39)**	**2.80 (2.13, 3.69)**	**<0.01**	**2.82 (2.15, 3.71)[d]**	**<0.01**
Pertussis (IU mL^{-1})						
Pertussis toxin	**15.94 (13.96, 18.21)**	**21.48 (17.33, 26.63)**	**0.74 (0.58, 0.95)**	**0.02**	0.97 (0.75, 1.25)[a,g,h,i]	0.82
Filamentous hemagglutinin	18.03 (15.82, 20.54)	21.84 (17.98, 26.53)	0.83 (0.65, 1.04)	0.10	1.00 (0.79, 1.27)[a,d,g,i]	0.98
Pertactin	11.39 (9.72, 13.34)	13.84 (10.66, 17.96)	0.82 (0.61, 1.10)	0.19	1.04 (0.77, 1.40)[a,d,g,i]	0.80
Polio (IU mL^{-1})						
Type 1	**9.82 (8.41, 11.48)**	**14.84 (12.04, 18.30)**	**0.66 (0.51, 0.87)**	**<0.01**	0.76 (0.57, 1.01)[a,g,i]	0.06
Type 2	20.44 (17.09, 24.44)	24.54 (19.53, 30.84)	0.83 (0.61, 1.13)	0.24	1.00 (0.72, 1.38)[a,g,i]	0.98
Type 3	**8.08 (6.68, 9.76)**	**11.23 (9.06, 13.92)**	**0.72 (0.53, 0.98)**	**0.04**	0.88 (0.63, 1.22)[a,d,g,i]	0.45
Pneumococcus (Pn) (μg mL^{-1})						
Serotype 1	1.11 (0.98, 1.25)	1.31 (1.10, 1.55)	0.85 (0.69, 1.04)	0.12	1.02 (0.82, 1.26)[a,c,i]	0.87
Serotype 3	0.52 (0.47, 0.58)	0.58 (0.48, 0.70)	0.90 (0.74, 1.09)	0.26	1.06 (0.86, 1.30)[i]	0.57
Serotype 4	0.21 (0.19, 0.23)	0.24 (0.21, 0.27)	0.87 (0.74, 1.03)	0.11	1.02 (0.85, 1.22)[a,b,f,g,i]	0.85
Serotype 5	**0.85 (0.76, 0.96)**	**1.08 (0.89, 1.32)**	**0.79 (0.63, 0.98)**	**0.03**	0.95 (0.75, 1.20)[a,h,i]	0.67
Serotype 6A	0.95 (0.83, 1.08)	1.13 (0.92, 1.40)	0.83 (0.66, 1.06)	0.14	1.16 (0.90, 1.50)[a,g,h,i]	0.24
Serotype 6B	0.51 (0.44, 0.60)	0.53 (0.41, 0.69)	0.96 (0.73, 1.28)	0.80	1.30 (0.97, 1.74)[a,g,i]	0.08
Serotype 7F	1.80 (1.63, 2.00)	2.05 (1.74, 2.40)	0.88 (0.73, 1.06)	0.18	1.08 (0.88, 1.32)[a,h,i]	0.46
Serotype 9V	0.56 (0.50, 0.63)	0.68 (0.57, 0.81)	0.83 (0.68, 1.03)	0.09	1.04 (0.83, 1.29)[h,i]	0.75
Serotype 14	**0.96 (0.84, 1.10)**	**1.26 (1.02, 1.56)**	**0.77 (0.60, 0.98)**	**0.03**	0.94 (0.72, 1.23)[h,i]	0.67
Serotype 18C	**0.60 (0.54, 0.68)**	**0.76 (0.65, 0.90)**	**0.79 (0.65, 0.97)**	**0.02**	1.00 (0.81, 1.24)[a,g,h,i]	0.89
Serotype 19A	**0.39 (0.33, 0.47)**	**0.64 (0.49, 0.85)**	**0.61 (0.45, 0.84)**	**<0.01**	0.81 (0.58, 1.12)[i]	0.20
Serotype 19F	**2.79 (2.42, 3.20)**	**3.85 (3.07, 4.82)**	**0.72 (0.56, 0.93)**	**0.01**	0.90 (0.68, 1.19)[h,i]	0.46
Serotype 23F	0.55 (0.47, 0.65)	0.59 (0.46, 0.75)	0.94 (0.70, 1.26)	0.67	1.18 (0.87, 1.61)[a,d,e,i]	0.87

Bold denotes statistically significant results.

CI, confidence interval; MMR, measles-mumps-rubella; Hib-MenC, *Haemophilus influenzae* type b-meningococcus C.

[a]Maternal diphtheria-tetanus-acellular pertussis immunization during pregnancy.

[b]Gestational age.

[c]Delivery mode.

[d]Sex.

[e]Birth weight.

[f]Bacillus Calmette-Guérin immunization.

[g]Age at 6 weeks immunization.

[h]Age at bleeding.

[i]Time between immunization and bleeding.

likely explained by waning of antibody levels as the interval between the 6-month immunization and blood sampling at 13 months of age was an average of 28 days longer in this group. This was most marked in those vaccine antigens which wane most rapidly and therefore had the lowest seroprotection rates (pertussis toxin, pneumococcal serotypes 4 and 19A).

The higher seroprotection rate and GMC for tetanus in MMR/MenC-Hib-vaccinated infants are likely explained by a boosting effect of the carrier protein in the MenC-Hib vaccine, which is a tetanus toxoid.

The strengths of our study are the detailed participant demographic and clinical data allowing for adjustment for possible confounding factors, the homogeneity in the vaccines given and the wide range of measured antibody responses. The limitations of the study are the small sample size and consequent risk of type-2 error and the fact that MMR was co-administered with MenC-Hib.

METHODS

Study design and population

Participants were a subset of infants from a trial to determine whether neonatal BCG vaccination protects against childhood infection, allergy and asthma (the Melbourne Infant Study: BCG for Allergy and Infection Reduction). Inclusion criteria

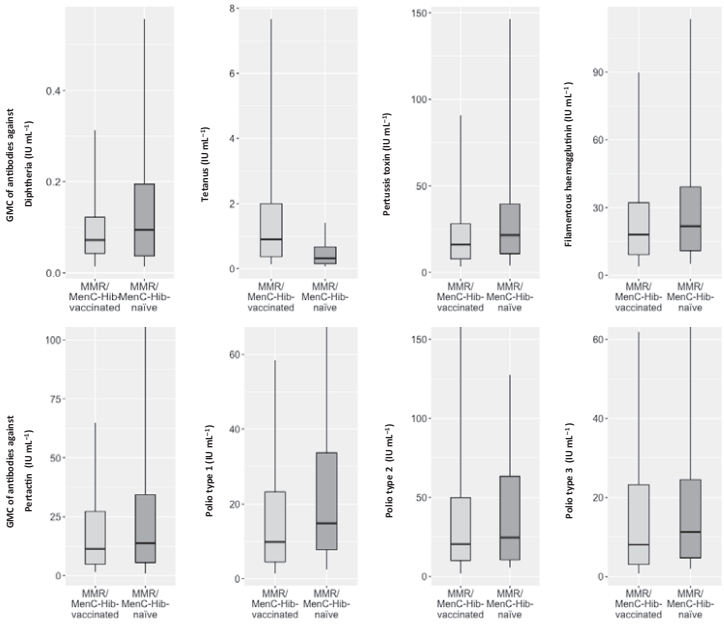


Figure 3. Geometric mean antibody concentrations (GMCs) of antibodies against diphtheria-tetanus-acellular pertussis-poliomyelitis in measles-mumps-rubella (MMR)/*Haemophilus influenzae* type b-meningococcus C (Hib-MenC)-vaccinated (n = 212) and MMR/Hib-MenC-naïve participants (n = 99) at 13 months of age (box-and-whisker plots showing interquartile range (IQR) with whiskers depicting 1.5-times the IQR).

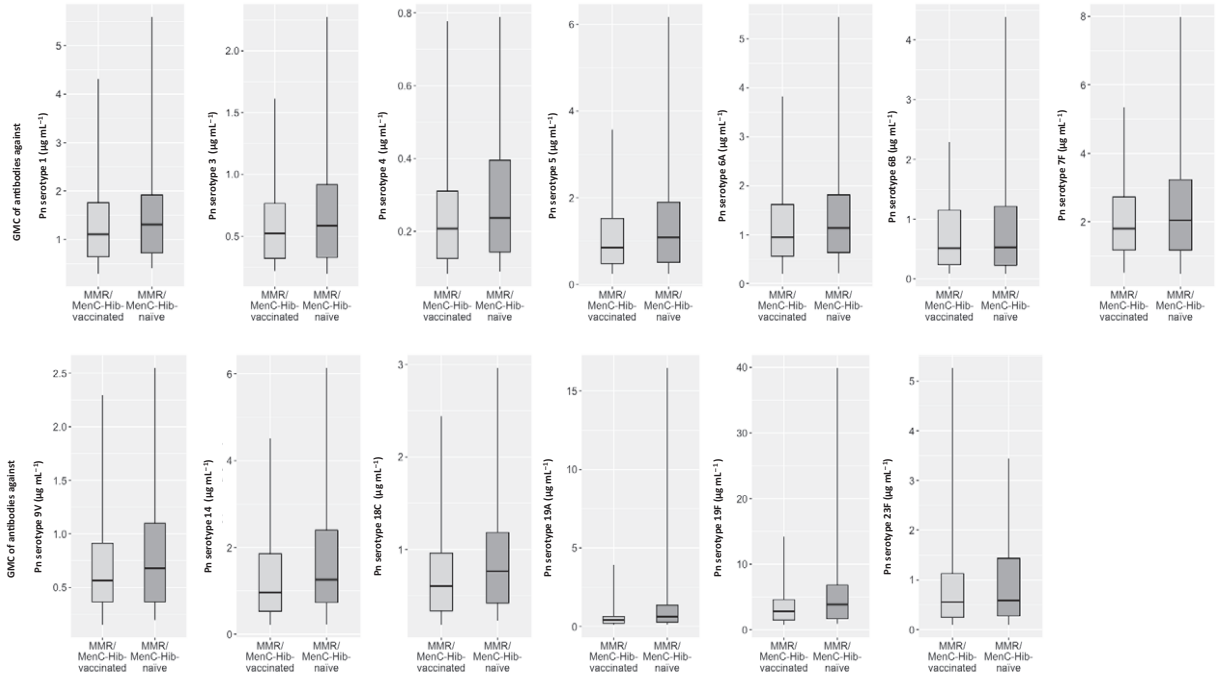


Figure 4. Geometric mean antibody concentrations (GMCs) of antibodies against pneumococcus in measles-mumps-rubella (MMR)/*Haemophilus influenzae* type b-meningococcus C (Hib-MenC)-vaccinated (n = 212) and MMR/Hib-MenC-naïve participants (n = 99) at 13 months of age (box-and-whisker plots showing interquartile range (IQR) with whiskers depicting 1.5 times the IQR).

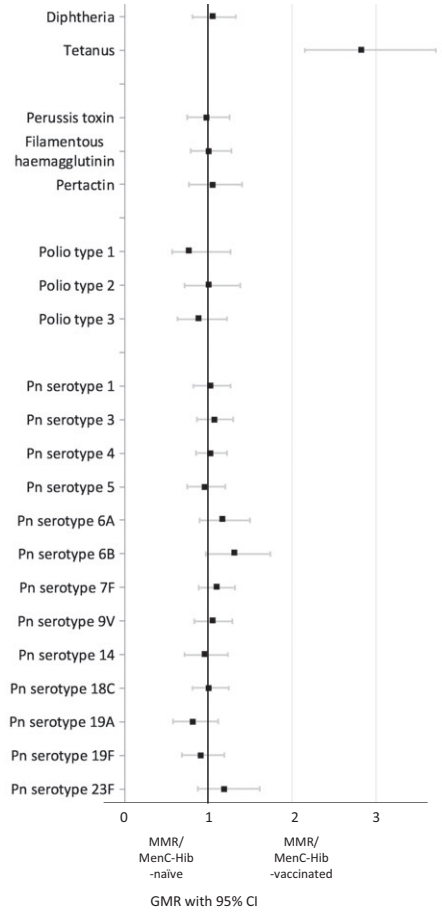


Figure 5. Geometric mean antibody concentrations (GMCs) with 95% CI of antibodies to vaccine antigens in participants at 13 months of age [measles-mumps-rubella (MMR)/*Haemophilus influenzae* type b-meningococcus C (Hib-MenC)-vaccinated (n = 212) and MMR/Hib-MenC-naïve participants (n = 99)].

included >32 weeks gestation and birth weight >1500 g. Parent questionnaires were used to prospectively collect data. All infants received routine vaccinations according to the Australian National Immunisation Program: at birth: intramuscular hepatitis B vaccine [H-B-Vax II Paediatric® (bioCSL, Parkville, VIC, Australia)]; at 6 weeks, 4 and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis, hepatitis B, poliomyelitis and *Haemophilus influenzae* type b (Hib) vaccine [Infanrix® Hexa (GlaxoSmithKline, Abbotsford, VIC, Australia)], intramuscular 13-valent conjugate pneumococcal vaccine (conjugated to CRM197, a diphtheria toxin) [Prevenar13® (Wyeth, West Ryde, NSW, Australia)] and oral rotavirus vaccine [RotaTeq® (Merck, Parkville, VIC, Australia)]. Approximately half of the infants also received intradermal BCG-Denmark shortly after birth as part of the Melbourne Infant Study: BCG for Allergy and Infection Reduction. At the time of the study, approximately two-thirds of the participants had received subcutaneous MMR vaccine [Priorix® (GlaxoSmithKline)] or M-M-R® II [Seqirus, Parkville, VIC, Australia)] and intramuscular combined Hib and meningococcal C (MenC) vaccine (conjugated to tetanus toxin) [Menitorix® (GlaxoSmithKline)] scheduled at 12 months of age, while the other third had not yet received these vaccines. Vaccine records were obtained from the National Australian Immunisation Register and/or individual vaccination records.

Blood samples were collected into sodium heparin (S-monovette, Sarstedt®, Mawson Lakes, SA, Australia) tubes during study visits at approximately 13 months of age and plasma stored at −80°C until analysis.

For the participants who had had their MMR/Hib-MenC before the study, only those who had blood taken 28 ± 14 days after the MMR/Hib-MenC vaccine were included in the final analysis. Participants of twins were excluded from the final analysis, because they were not considered independent.

Antibody assays

Blood samples were analyzed at the National Institute for Public Health and Environment, in Bilthoven, the Netherlands. Immunoglobulin G antibodies against 26 vaccine antigens (diphtheria, tetanus, pertussis pertussis toxin, filamentous hemagglutinin, pertactin), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), MenC, measles, mumps and rubella) were measured in duplicate using fluorescent bead-based multiplex immune-assays (Luminex xMAP technology). In all assays, an international or in-house reference, controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA, USA).[42-47] Laboratory staff were blinded to MMR/Hib-MenC vaccine status.

Statistical analysis

The antibody response to each of the scheduled routine vaccines was compared between the participants who had received their 12-months' MMR/MenC-Hib vaccine at the time of the study ("MMR/MenC-Hib-vaccinated") and those who had not ("MMR/MenC-Hib-naïve").

The proportion of children with an antibody concentration above the standard protective correlate value (seroprotection rate)[45,48,49] for each vaccine was calculated in each group (with the exception of filamentous hemagglutinin and pertactin for which no accepted protective correlate value exists). The Clopper–Pearson method was used to estimate the 95% confidence intervals (CIs) of the seroprotection rates. Proportions were compared using a Fisher's exact test and 95% CI for differences in proportions estimated. In each group, the GMCs for each vaccine antibody were calculated. The geometric mean antibody ratio with 95% CI was obtained as the anti-logged coefficient from a linear regression with log concentrations as outcome and MMR/Hib-MenC vaccination status as covariate.

Effect modification was assessed using the following prespecified factors: maternal vaccination (diphtheria-tetanus-acellular pertussis and influenza) during pregnancy, birth weight, sex, gestational age, age at first routine vaccination,

infant BCG-vaccination status, age at sampling and time from 12-month vaccination to blood sampling. The geometric mean antibody ratio for each vaccine antigen was adjusted for those factors that differed between groups and had an effect on that vaccine antigen response. A 5% significance level was used. All statistical analyses were done using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

Ethics

Informed consent was obtained from participants' parents or guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, Approval No. 38124A). Melbourne Infant Study: BCG for Allergy and Infection Reduction is registered with the Australia & New Zealand Clinical Trials Registry (1051228) and the US National Institutes of Health (NCT01906853).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Review

The influence of the intestinal microbiome on vaccine responses ☆

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ABSTRACT

There is substantial variation between individuals in the immune response to vaccinations. The intestinal microbiome plays a crucial rule in the development and regulation of the immune system and therefore its composition might affect how individuals respond to vaccinations. In this review, we summarise studies that investigated the influence of the intestinal microbiome on humoral and cellular vaccine responses.

To date, only four studies (three in infants and one in adults) have investigated the influence of the intestinal microbiome on vaccine responses. All found an association between the intestinal microbiome and vaccine responses. Despite the heterogeneity in study designs (including different vaccines, schedules, timing of collection of stool and blood samples, analysis methods and reporting of results on different taxonomic levels), findings across studies were consistent: a higher relative abundance of the phylum Actinobacteria (oral and parenteral vaccines) and Firmicutes (oral vaccines) was associated with both higher humoral and higher cellular vaccine responses, while a higher relative abundance of the phylum Proteobacteria (oral and parenteral vaccines) and Bacteroidetes (oral vaccines) was associated with lower responses.

Further, well-designed, adequately powered studies using whole-genome sequencing (to include the influence of viruses, fungi and parasites) are needed to investigate in more detail the influence of the intestinal microbiome on vaccine responses. This will help identify strategies to improve vaccine efficacy and duration of protection, particularly in infancy when the intestinal microbiome is more amenable to external influences and plays an important role in the development of the immune system.

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☆ **Once sentence summary:** The relative abundance of certain bacterial taxa in the intestinal microbiome influences both humoral and cellular immune responses to oral and parenteral vaccines.

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1. Introduction

There is substantial variation between individuals in the immune response to vaccinations. The serological response to hepatitis B vaccine, for example, varies more than 100-fold at seven months of age and some infants do not have any measurable antibodies [1,2]. Similarly, the response to pneumococcal and Hib vaccination at six months of age varies up to 40-fold [1]. This has implications for both protective efficacy and duration of protection. Factors contributing to the variation in vaccine response include age [3–5], gender [6], genetics [7–9], geographic location [10], time of day vaccine administered [11] and co-administered vaccines [12,13]. A further important factor which likely influences vaccine responses is the intestinal microbiome.

In recent years, considerable research has revealed the importance of the intestinal microbiome in the development of the immune system and regulation of immune responses [14,15]. Abundance of certain bacteria in the intestinal microbiome have been linked to susceptibility to neonatal sepsis [16], chronic inflammatory bowel disease [17], chronic obstructive pulmonary disease [18], diabetes mellitus [19], and eczema, allergies and asthma [20]. Advances in DNA sequencing technology and bioinformatic analysis have facilitated the ability to determine differences in the composition of the intestinal microbiome. Despite the evidence for the intimate relationship between the intestinal microbiome and the immune system, only a limited numbers of studies have investigated the effect of the composition of the intestinal microbiome on vaccine responses. These studies are summarised in this review.

2. Systematic review methods

In April 2017, MEDLINE (1946 to present) and Embase (1947 to present) were searched using the Ovid interface with the following search terms: (microbiome OR microbiota OR biodiversity OR Actinobacteria OR bacteroides OR *Bifidobacterium* OR *Enterobacteriaceae* OR *lactobacillus* OR Proteobacteria) AND (feces OR faeces OR meconium OR intestin* OR) AND (RNA, ribosomal OR sequence analysis, DNA OR culture or quantif*) AND (vaccin* OR immuniz* OR immunis* OR antibodies OR immunoglobulin OR immunity, humoral OR immunity, cellular OR mucosal immunity) without any language limitations. This identified 757 and 1062 studies, respectively. Of these, four fulfilled our inclusion criteria of studies in humans investigating the influence of the composition of the intestinal microbiome on humoral or cellular vaccines responses, in which no concomitant probiotics were given. References were hand-searched for additional publications and no further relevant studies were found.

3. Systematic review results

Three studies reporting results from 146 infants (206 stool samples) and one study reporting results from 17 adults (170 stool samples) met the inclusion criteria (Table 1). Two of the studies were done in developing countries [21,22]. Responses to oral vaccinations were investigated in two studies [22,23], to parenteral vaccination in one [24], and to both oral and parenteral in one [21]. Two studies measured humoral and cellular responses in serum [21,23], while one study each measured humoral responses in serum [22] or in stool [24]. Multiple methods were used to determine the intestinal microbiome, including bacterial culture [24], PCR for Bifidobacteria [21,24], Human Intestinal Tract Chip microarray [22], and 16S rRNA gene sequencing (Roche 454 [23] Illumina MiSeq [21]). An overview of the associations between the intestinal microbiome and vaccine responses is provided in Table 2.

3.1. Oral vaccination

Three studies investigated the influence of the intestinal microbiome on the immune response to oral vaccines; two of these were done in infants [21,22] and one in adults [23].

The first study compared the intestinal microbiome of infants in Ghana, using 39 participants who were responders to oral rotavirus vaccination (defined as serum rotavirus-specific immunoglobulin (Ig) A levels $\geq$ 20 IU/ml measured four weeks after the third vaccine dose) with 39 who were non-responders. The study found that responders had an increased relative abundance of *Streptococcus gallolyticus*, a decreased relative abundance of the phylum Bacteroidetes, and a higher Enterobacteria-*Bacteroides* ratio in their stools taken at 6 weeks of age (two weeks before the third vaccine dose). The study did not find a difference in the diversity of the intestinal microbiome between responders and non-responders [22].

The second study, investigated the influence of the intestinal microbiome on the immune response to oral poliovirus vaccination in 48 infants in Bangladesh. A higher relative abundance of the phylum Actinobacteria (families *Coriobacteriaceae* and *Bifidobacteriaceae* (species *Bifidobacterium longum* subspecies *infantis* and *B. longum* subspecies *longum*)) at the age of 15 weeks was associated with higher polio-specific T cell responses and IgG levels in serum at the same time points (one week after the fourth vaccine dose). In contrast, a higher relative abundance of the order Pseudomonadales was negatively associated with polio-specific T cell responses and IgG levels in serum [21].

The third study, investigated the influence of the intestinal microbiome on the immune response to oral *Salmonella* Typhi vaccination in 17 adults in the US one week before receiving the vaccine until two months after. The study found that participants with an overall more diverse, complex bacterial communities (mostly consisting of the order Clostridiales, predominantly the families *Lachnospiraceae* and *Ruminococcaceae*) had multiphasic cell-mediated immune responses, which is associated with a better response to the oral *Salmonella* Typhi vaccination. The study did not find differences in overall community diversity between humoral responders and non-responders (measured by serum typhoid-specific IgA and IgG levels). All the measurements were made within two months of administering the vaccine [23].

3.2. Parenteral vaccination

Two studies in infants investigated the influence of the intestinal microbiome on the immune response to intramuscular vaccination [21,24]. The first, investigating 20 infants in France, measured faecal polio-specific IgA levels after intramuscular vaccination with a pentavalent diphtheria-tetanus-acellular pertussis-

Table 1
Studies reporting the influence of the intestinal microbiome on vaccine responses.

Participants	Study design	Vaccine schedule	Time stool collected	Time vaccine response measured	Stool analysis technique	Main findings	Study Country Publication year
Studies in infants							
Infants (n = 78)	Prospective case-control study	Oral rotavirus vaccine Age 0, 4, 8 w	Age 6 w	Age 12 w	Human Intestinal Tract Chip (Microarray)	Responders (serum IgA $\geq$ 20 IU/ml) associated with • **increased relative abundance of *Streptococcus gallolyticus*** (FDR = 0.008) • **decreased relative abundance of phylum Bacteroidetes** (FDR = 0.003) • **higher Enterobacteria/Bacteroides ratio** (p = 0.04) • no difference in diversity (Shannon index) from non-responders (p = 0.87)	Harris [22] Ghana 2017
Infants (n = 48)	Prospective cohort study	Oral poliovirus vaccine Age 0, 6, 10, 14 w Intramuscular tetanus-hepatitis B vaccine Age 6, 10, 14 w Intradermal BCG vaccine Age 0 w	Age 6, 11, 15 w	Age 15 w	16S rRNA gene sequencing, V4 Illumina MiSeq *Bifidobacterium*-specific PCR and T-RFLP	**Higher relative abundance of Actinobacteria** (species ***Bifidobacterium longum***) at 15 w associated with • higher BCG-, polio-, and tetanus-specific T cell responses (p < 0.05) • higher hepatitis B-, and polio-specific IgG levels in serum (p < 0.05) **Higher relative abundance of Enterobacteriales** at 15 w associated with • lower BCG- and tetanus-specific T cell responses (p < 0.05) **Higher relative abundance of Pseudomonadales** at 15 w was associated with • lower BCG-, hepatitis B-, polio-, and tetanus-specific T cell responses (p < 0.05) • lower polio-specific IgG levels in serum (p < 0.05)	Huda [21] Bangladesh 2014
Infants (n = 20)	Randomised placebo-controlled trial	Intramuscular diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis-*Haemophilus influenzae* type b vaccine Age 8, 12, 16 w 9 infants were fed bifidogenic formula (fermented *B. breve* C50 and *S. thermophilus*)	Age 4, 8, 12, 16 w	Age 16 w	Culture Multiplex PCR for *Bifidobacteria*	Infants with **detectable levels *Bifidobacterium longum* subspecies *Bifdobacterium infantis*** at 16 w • higher faecal polio-specific IgA levels (p < 0.002)	Mullie [24] France 2004
Studies in adults							
Adults (n = 17)	Prospective case-control study	Oral *Salmonella* Typhi vaccine Schedule not specified 6 participants received 4 doses 7 participants received 1 dose 4 participants not vaccinated	−7, 0, 2, 4, 7, 10, 14, 28, 42, 56 d 0 = day of vaccination	−7, 0, 2, 4, 7, 10, 14, 28, 42, 56 d 0 = day of vaccination	16S rRNA gene sequencing, V1/V2 Roche 454	**More diverse, complex bacterial communities** (mostly within order Clostridiales, predominantly within the families ***Lachnospiraceae* and *Ruminococcaceae***) associated with • multiphasic cell-mediated immune response (p-value NS) • no differences in overall community diversity between humoral responders and non-responders (measured by IgA and IgG levels in serum)	Eloe-Fadrosh [23] US 2013

Abbreviations: BCG – Bacille Calmette-Guérin; d – days; FDR – false discovery rate; PCR – polymerase chain reaction; RNA – ribonucleic acid; T-RFLP – terminal restriction fragment length polymorphism; w – weeks.

Table 2
Associations between the intestinal microbiome and vaccine responses.

	Relative abundance associated with **higher** vaccine responses		Relative abundance associated with **lower** vaccine responses	
Phylum	**ACTINOBACTERIA**	**FIRMICUTES**	**PROTEOBACTERIA**	**BACTEROIDETES**
Order	**Actinomycetales** Higher relative abundance associated with • higher polio-specific IgG levels in serum [21]		**Enterobacteriales** Higher relative abundance associated with • lower tetanus-specific T cell response [21] • lower BCG-T cell response [21] **Pseudomonadales** Higher relative abundance associated with • lower polio-specific T cell response and polio-specific IgG levels in serum [21] • lower tetanus-specific T cell response [21] • lower hepatitis B-specific T cell response [21] • lower BCG-specific T cell response [21]	
Family	***Coriobacteriaceae*** Higher relative abundance associated with • higher polio-specific T cell response and polio-specific IgG levels in serum [21]	***Lachnospiraceae*** Higher relative abundance associated with • superior *Salmonella* Typhi-specific T cell response [23] ***Ruminococcaceae*** Higher relative abundance associated with • superior *Salmonella* Typhi-specific T cell response [23]		
Genus	***Corynebacterium*** Higher relative abundance associated with • higher tetanus-specific T cell response [21] ***Rothia*** Higher relative abundance associated with • higher hepatitis B-specific IgG levels in serum [21] • higher BCG-T cell response [21]			***Bacteroides*** Higher relative abundance associated with • lower rotavirus-specific IgA levels in serum [22] ***Prevotella*** Higher relative abundance associated with • lower rotavirus-specific IgA levels in serum [22]
Species	***Bifidobacterium longum* subspecies *infantis*** Higher relative abundance associated with • higher polio-specific T cell response and polio-specific IgG levels in serum [21] • higher tetanus-specific T cell response [21] • higher T cell response to PPD antigen [21] Detectable levels associated with • higher polio-specific IgA levels in stool [24] ***Bifidobacterium longum* subspecies *longum*** Higher relative abundance associated with • higher polio-specific T cell response and polio-specific IgG levels in serum [21]	***Streptococcus gallolyticus*** Higher relative abundance associated with • higher rotavirus-specific IgA levels in serum [22]		

inactivated poliomyelitis-*Haemophilus influenzae* type b vaccine. The study found that infants with detectable levels of *B. longum* subspecies *B. infantis* in their stools at the age of four months when receiving their third vaccine dose had higher faecal polio-specific IgA levels at the same time point [24].

In the second study, which was the study from Bangladesh that also measured responses to oral poliovirus vaccination, the immune response to intramuscular tetanus-hepatitis B vaccination was investigated. The study found that a higher relative abundance of the phylum Actinobacteria (including the genera *Bifidobacterium* (*B. longum* subspecies *infantis*) and *Corynebacterium*) was associated with higher tetanus-specific T cell responses at 15 weeks of age. A higher relative abundance of the phylum Actinobacteria was also associated with higher tetanus-specific IgG levels, but within the phylum only the order Actinomycetales, and not the genus *Bifidobacterium* was positively associated with higher humoral responses. When comparing the immune response to hepatitis B vaccination, no correlation was found between the composition of the intestinal microbiome and hepatitis B-specific T cell responses. However, a higher relative abundance of Actinobacteria (genus *Rothia*) was associated with higher hepatitis B-specific IgG levels. In contrast, a higher relative abundance of the order Pseudomonadales was associated with both lower tetanus-specific and hepatitis B-specific T cell responses. Further, a high bacterial diversity and a higher relative abundance of the order *Enterobacteriales* was associated with lower tetanus-specific T cell responses [21].

3.3. Bacille Calmette-Guérin vaccination

The above mentioned study from Bangladesh also investigated the influence of the intestinal microbiome on immune response to Bacille Calmette-Guérin (BCG) vaccination given at birth in 48 infants. The study found a positive association between the prevalence of the phylum Actinobacteria at 15 weeks of age and BCG vaccine responses, including higher T cell responses to purified protein derivative (PPD) antigen (*Bifidobacterium*, species *B. longum* subspecies *infantis*) and superior PPD skin test responses (Actinomycetales, genus *Rothia*). Consistent with the response to other vaccine investigated in the study, a higher relative abundance of the order Pseudomonadales was associated with lower PPD-specific T cell responses and the order Enterobacteriales was associated with a lower PPD skin test responses [21].

4. Discussion

Despite the increasing recognition of the influence of the microbiome on immunity, to date, only three studies that included a total of 146 infants and one study of 17 adults have investigated the influence of the composition of the intestinal microbiome on vaccine responses. These studies show that, in infants, a higher relative abundance of the phylum Actinobacteria (order Actinomycetales, family *Coriobacteriaceae*, genera *Corynebacterium* and *Rothia*, and especially the species *B. longum*) is associated with both higher humoral and cellular vaccine responses to oral, and parenteral vaccines [21,24], while a higher relative abundance of the phylum Proteobacteria (order Enterobacteriales and order Pseudomonadales) is associated with lower humoral and cellular responses to these vaccines [21]. In addition, in infants and adults, a higher relative abundance of the phylum Firmicutes (families *Lachnospiraceae* and *Ruminococcaceae*, and the species *S. gallolyticus*) is associated with higher humoral and cellular responses to oral vaccines [22,23], while a higher relative abundance of the phylum Bacteroidetes is associated with lower humoral responses to oral vaccines in infants [22].

The findings of the studies included in this review showing that the intestinal microbiome influences vaccine responses are consistent with the findings from many animal studies [25]. For example, a study in macaques immunised with live attenuated *Shigella dysenteriae* vaccines shows that a higher diversity in the intestinal microbiome correlates with improved protection from *S. dysenteriae* infection [26]. A study in mice shows that Toll-like receptor (TLR) 5-mediated sensing of flagellin produced by the intestinal microbiome is necessary for antibody formation following influenza vaccination. Mice which are deficient in TLR5, germ-free mice and mice treated with antibiotics have impaired vaccine responses to influenza vaccination. When the intestinal microbiome of germ-free or antibiotic-treated mice is restored with flagellated *Escherichia coli*, but not flagellin-mutated bacteria, antibody responses are restored [27]. However, antibiotic treatment does not affect antibody responses to either tetanus-diphtheria-pertussis or yellow fever vaccine [27]. Further, another study in mice shows that stimulation of a different pattern recognition receptor (Nod2) through metabolites produced by the intestinal microbiome (muramyl dipeptide) influences vaccine response to intranasal vaccination with cholera toxin [28].

Interestingly, a study in human volunteers shows that expression of TLR5 in blood three days post influenza vaccination correlates with the antibody response 28 days later [29]. Since influenza vaccine does not stimulate TLR5 directly, but many intestinal microbes do, it is possible that stimulation of TRL5 is one of the pathways through which the intestinal microbiome enhances immunogenicity of vaccines. However, the interplay between the microbiome, TLR stimulation and immune responses is complex. For example, children growing up in rural areas with high exposure to microbial products, have higher expression of TLR in their blood [30]. However, another study shows that infants in South Africa produce lower levels of cytokines in response to TLR4 stimulation compared with age-matched children in North America or Europe, which might influence vaccine effectiveness [31].

There is evidence that the interaction between the intestinal microbiome and the immune system are of particular importance during early infancy, when they both develop concurrently [32–34]. Compared with adults, the microbiome in infants is much less stable and is influenced by a number of factors, including gestational age, delivery mode, feeding, and maternal and infant antibiotic exposure [35–42]. It is therefore likely that the intestinal microbiome has a greater influence on the immune response to vaccinations given in infancy than later in life. Breast-fed infants have a higher relative abundance of *B. longum* subspecies *infantis* [43–45], which the studies in this review suggest is associated with both increased humoral and cellular vaccine responses to both oral and parenteral vaccines. In accordance with this, breast-fed infants have significantly higher vaccine-specific IgA and IgG levels in serum, saliva and faeces after oral and parenteral vaccines [46,47]. However, as the microbiome and the immune system develop in parallel, it is possible that reported associations between the two are associative rather than causally linked.

Many studies show that oral vaccines (cholera, poliovirus, rotavirus) have lower immunogenicity and efficacy in developing countries [48–50]. Factors postulated to explain this include malnutrition and micronutrient deficiencies, a higher prevalence of tuberculosis and HIV infections, interference of higher pre-vaccination immunoglobulin levels (acquired through either transplacental transfer or transfer with breast-milk) [51,52], and increased exposure to microorganisms early in life leading to a tolerance to vaccine antigens [53]. Both of the two studies in our review that were done in developing countries found that the composition of the intestinal microbiome influences the efficacy of oral vaccination suggesting this may be an additional factor explaining

the inferior efficacy of oral vaccines in developing countries. A recent review found that probiotics have a beneficial effect on the immune response to oral vaccinations and may offer a relatively cheap intervention to improve vaccine efficacy and duration of protection in this setting [54].

Antibiotics, which are frequently administered in early-life, have a substantial impact on the intestinal microbiome [55,56]. It is therefore plausible that antibiotics also influence the immune response to vaccines. However, to date, this has only been investigated in animal studies. Interestingly, while antibiotic administration decreases humoral responses to intramuscular tetanus, pneumococcal and hepatitis B vaccinations in mice [57], it increases humoral response to oral vaccination in mice and chicken [57–59].

There are also no studies investigating the influence of the viral or fungal intestinal microbiome on vaccine responses. However, children treated with antihelminthics show higher seroconversion rates after oral cholera vaccination [60], while adults who are infected with *Ascaris lumbricoides* have a suppressed interleukin-2 response after oral cholera vaccination [61]. While one study showed that antenatal maternal helminth infections were associated with modestly increased IgA responses to oral vaccines in infants [62], another study did not find an influence of antihelminthics during pregnancy on oral vaccine responses in infants [63].

An important aspect to note is that there was considerable heterogeneity in study design between the four studies. In addition to using different vaccines and vaccine schedules, the timing of the collection of stool samples was sometimes prior and sometimes consecutive with the time at which vaccine responses were measured. Limitations of the studies include small sample sizes and the lack of accounting for potential confounding factors which might influence vaccine responses, including sex, delivery mode, maternal antibodies, feeding method and exposure to medications (e.g. antibiotics, probiotics or corticosteroids). The study investigating the influence of the intestinal microbiome on responses to BCG vaccination (measured by specific T cell proliferation and skin test reaction) did not test for possible infection with *Mycobacterium tuberculosis,* which would be an important confounding factor [21].

The methods used to analyse the stool microbiome also varied between the studies. No studies used whole genome sequencing, a technique that identifies a larger number of bacteria and, importantly, also viruses, fungi and parasites which potentially also influence vaccine responses. Moreover, results were reported at various different taxonomic levels (phyla, family, genus, species) and it is likely that the relative abundance of certain genera or species, or specific microbial intestinal signatures is more important than phylum or class level changes in altering the response to vaccines. Further, well-designed, adequately powered studies using whole-genome sequencing (to include the influence of viruses, fungi and parasites) are therefore needed to investigate in more detail the influence of the intestinal microbiome on vaccine responses. Whilst the intestinal microbiome remains relatively consistent over time in adults, in contrast, during the first six months of life, the numbers and species of bacteria fluctuate markedly [64,65]. Therefore, future studies in infants should include more frequent sampling, ideally both prior to and concurrent with measuring vaccine responses.

5. Conclusion

The studies included in this review support the notion that the intestinal microbiome influences vaccine responses, especially early in life. Further well-designed studies determining the influence of the intestinal microbiome, including the influence of viruses, fungi and parasites, on vaccine responses are needed. This will help identify strategies, such nutritional interventions, to improve vaccine efficacy and duration of protection, particularly in infancy when the intestinal microbiome is more amenable to external influences and plays an important role in the development of the immune system.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

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Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PZ drafted the initial manuscript, and approved the final manuscript as submitted. NC critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Review

The influence of probiotics on vaccine responses – A systematic review

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ABSTRACT

The immunomodulatory effects of probiotics may influence the response to vaccines. We systematically reviewed prospective randomised placebo-controlled studies in humans that have investigated the effect of probiotics on humoral vaccine responses.

We found 26 studies, involving 3812 participants, investigating the effect of 40 different probiotic strains on the response to 17 different vaccines. A beneficial effect of probiotics was reported in about half of the studies. The evidence for a beneficial effect of probiotics on vaccine response was strongest for oral vaccinations and for parenteral influenza vaccination. However, there was substantial variation between studies in the choice of probiotic, strain, dose, viability, purity, and duration and timing of administration. The one study that investigated the effect of probiotic administration to mothers during pregnancy found lower vaccine responses in their infants.

The studies in our review suggest that probiotics offer a relatively cheap intervention to improve vaccine efficacy and duration of protection. Future studies should focus on establishing optimal strains, doses and timing of administration in relation to vaccination.

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1. Introduction

The immune response to vaccinations varies substantially between individuals. This has implications for both protective efficacy and duration of protection. Factors contributing to the variation in vaccine responses include age [1–3], gender [4], genetics [5–7], geographic location [8], time of day vaccine administered [9], and co-administered vaccines [10,11].

In recent years, considerable research has revealed the importance of the intestinal microbiota in the development of the immune system and regulation of immune responses [12,13]. While there have been only few studies on the effect of the intesti-

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nal microbiota on vaccine responses [14–18], many studies have investigated the effect of probiotics around the time of vaccination.

Probiotics are defined as live microorgansims which, when administered orally in adequate amounts (thought to be $\geq 1 \times 10^9$ colony forming units (cfu) daily), are beneficial to the host [19]. The most frequently used microorgansims are *Lactobacillus spp*, *Bifidobacterium spp*, and *Saccharomyces boulardii*. The mechanism of action of probiotics include normalisation of perturbed microbiota, regulation of intestinal transit, increased turnover of enterocytes, gut barrier reinforcement, colonisation resistance, acid and short-chain fatty acid production, vitamin synthesis, and bile salt metabolism [19]. Probiotics enhance both innate and adaptive immunity [20,21], and have been found to be beneficial in treatment of acute gastroenteritis [22,23], in prevention of antibiotic-associated diarrhoea [24], in reduction of infection in children attending day care centres [25–27], and in prevention of eczema and allergies [28,29]. However, most studies investigating the influence of probiotics on the immune response in humans have been small in size or limited. Despite this, there has been an explosion in the use of probiotics, promoted by the pharmaceutical industry; the global probiotics market size exceeded US$35 billion in 2015. An evidence base to guide interventions is critically needed. Here, we systematically review studies investigating the influence of probiotics on vaccine responses.

2. Systematic search method

In April 2017, MEDLINE (1946 to present) and Embase (1947 to present) were searched using the Ovid interface with the following search terms: (probiotics OR *Lactobacillus* OR *Bifidobacterium*) AND (vaccin* OR immun* OR antibod* OR humoral) without any language limitations. This identified 2366 studies. Of these, 25 fulfilled our inclusion criteria of prospective randomised placebo-controlled studies in humans measuring humoral vaccines responses in plasma or stool after administration of probiotics. References were hand-searched for additional publications and 1 further relevant study was found (Fig. 1). A p-value ≤ 0.05 was used to define statistically significant findings.

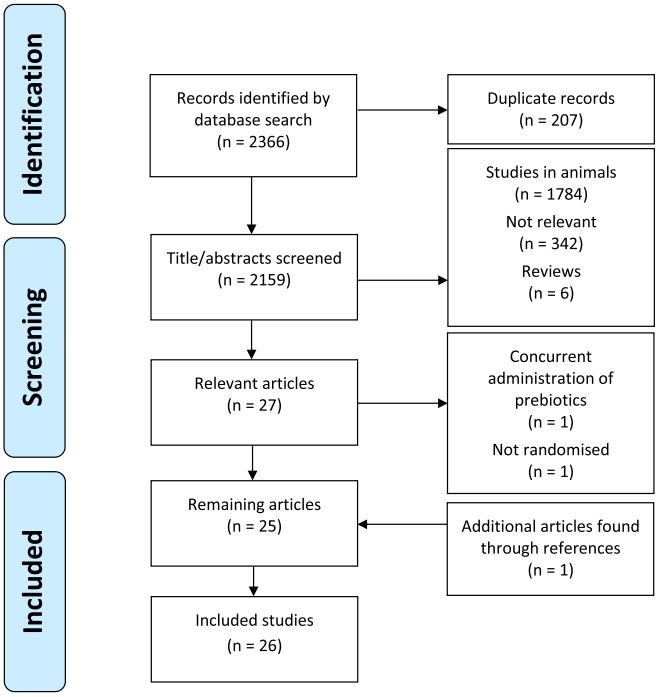


Fig. 1. Flow diagram of selection of articles included in the review.

3. Results

A total of 26 studies were reviewed, involving 3812 participants, investigating the use of 40 different probiotic strains on the efficacy of 17 different vaccines (DTP (2), DTwP, DTaP-Hib (2), DTaP-IPV-Hib (2), HAV, HBV (2), Hib, LAIV, MMRV, OCV (2), OPV, ORV, PCV7, PPV23 (2), Polio, TIV (11), Ty21a). The dose of probiotic used in each study varied between 10^8 and 10^{13} colony forming units cfu per day.

3.1. Effect of probiotics on vaccine responses in neonates

The effect of probiotic administration to neonates on humoral vaccine responses has been investigated in 4 studies in a total of 573 infants. Probiotics were administered for a duration of between 4 and 12 months [18,30–33]. One study showed a higher seroconversion rate for *Haemophilus influenzae* type b-specific immunoglobulin (Ig) G (50% vs. 21%, p = 0.020) after administering a combination of 4 probiotic strains, *B. breve*, *L. rhamnosus* GG, *L. rhamnosus* and *P. freudenreichii* ($2–5 \times 10^9$ cfu each), for the first 6 months of life [33]. Another study found higher poliomyelitis-specific IgA levels in stool at the age of 4 months (p < 0.020) after administering a combination of *B. breve* and *Streptococcus thermophiles* (dose not specified) for the first 4 months [18]. The two other studies did not find any effect of probiotics on specific IgG levels to routine vaccinations (one for diphtheria, tetanus, pertussis, poliomyelitis and HBV-specific IgG and one for HBV-specific IgG) [30,31].

3.2. Effect of probiotics on vaccine responses in children

Five studies, that included 541 children, reported on vaccine responses after administration of probiotics started beyond the neonatal period [34–38]. The age at which probiotics were started differed substantially between studies (from 2-months to 10-years of age) and the duration for which they were administered also varied considerably (between 6 days and 9 months). Three studies investigated the effect of probiotics on responses to parenteral vaccines. One reported higher diphtheria-specific IgG levels at 6.5 months after vaccination (p = 0.044) with administration of *L. paracasei spp paracasei* ($10^8–10^{10}$ cfu) for 9 months [37]. The remaining two reported no effect of probiotics on responses to measles-mumps-rubella-varicella vaccination and to tetanus and pneumococcocal vaccination, respectively [34,36]. Of the two studies that investigated the effect of probiotics on responses to orally administered vaccines, one found a beneficial effect. This study looking at oral rotavirus vaccination following intake of *L. casei* (10^{11} cfu) for 6 days, found a higher number of rotavirus-specific IgM secreting cells (p = 0.020) and higher IgA seroconversion rates (93% vs. 74%, p = 0.050) 8 days after vaccination [38]. In contrast, the other study found lower cholera toxin B subunit IgA levels 42 days after oral cholera vaccination (p = 0.016) with administration of *B. breve* for 3 weeks [35].

3.3. Effect of probiotics administered during pregnancy on vaccine responses in infants

Only one study has investigated the effect of administration of probiotics to mothers during pregnancy on vaccine responses in infants [39]. The mothers were given *L. rhamnosus* GG (2×10^{10} cfu). Unexpectedly, the study found lower pneumococcal-specific IgG levels (serotype 4 p = 0.027, 6 B p = 0.040, 18 C p = 0.032,

Table 1
Prospective randomised placebo-controlled trials reporting the influence of probiotic administration on vaccine responses (statistically significant findings indicated in bold).

Participants (analysed/ randomised) Age range	Probiotic strain	Daily probiotic dose Duration of administration (starting time)	Vaccine schedule	Effects of probiotic on vaccine response (measured in blood unless indicated other)	Author Country Publication Year
Probiotics administered to neonates					
264/300 Neonates	*B. longum* BB536	$1x10^7$/g formula 12m	DTP, schedule NS Polio, schedule NS HBV, schedule NS	No difference in diphtheria, tetanus, pertussis, polio, HBV IgG levels at 7m (p=0.466, 0.880, 0.209, 0.423, 0.665) No difference in diphtheria, tetanus, pertussis, HBV IgG levels at 11m (p=0.570, 0.934, 0.279, 0.307)	Wu [30] China 2016
202/253 Neonates	*B. longum* BL999 *L. rhamnosus* LPR	$2.8x10^8$ cfu total 6m	A: HBV 0, 1, 6m, DTaP 6m B: HBV 0, 1, 6m	Trend towards increased HBV IgG levels at 12m in Group A (p=0.069) No difference in HBV IgG levels at 12m in Group B (p=0.996) No difference in seroconversion rates for HBV IgG at 12m in Group A or Group B (p=1, 0.259)	Soh [31] Singapore 2010
87/145 Neonates	*B. breve* Bbi99 *L. rhamnosus* GG *L. rhamnosus* LG705 *P. freudenreichii*	2-$5x10^9$ cfu each 6m[1]	DTwP 3, 4, 5m Hib 4m	**Higher seroconversion rates for Hib IgG at 6m (50% vs. 21%, p=0.020)** Trend for higher Hib IgG levels at 6m (p=0.064) No difference in diphtheria and tetanus IgG levels at 6m (p=0.449, 0.310)	Kukkonen [33] Finland 2006
20/30 Neonates	*B. breve* C50 *S. thermophilus*	NS 4m	DTaP-IPV-Hib 2, 3, 4m	**Higher polio IgA levels in stool at 4m (p<0.020)**	Mullié [18] France 2004
Probiotics administered to children beyond the neonatal period					
47/56 Infants 8-10m	*B. bifum* *B. infantis* *B. longum* *L. acidophilus*	$3x10^9$ cfu each 5m (2m pre vaccination)	MMRV 12m	Higher total IgG levels at 3m (92% vs. 83%, p=0.052) No difference in seroconversion rates for rubella, mumps, measles, varicella IgG at 3m (p=0.226, 0.392, 0.187, 0.117)	Youngster [34] Israel 2010
126/128 Children 2-5y	*B. breve* BBG-01	$4x10^9$ cfu 1m (3w pre vaccination)	OCV d21 and 35	**Lower cholera toxin B subunit IgA levels at 6w after vaccination (p=0.016)** No difference in *Vibrio cholera* O1 Ogawa lipopolysaccharides IgG, IgA and cholera toxin B subunit IgG levels 6w after vaccination (p-values NS)	Matsuda [35] Bangladesh 2010
140/162 Children 9m-10y	*L. casei* CRL431 *L. acidophilus* CRL730 *S. thermophilus*	9.5x107-109 cfu each >4m (time NS)	DTaP-Hib 18m PPV2318m	No difference in tetanus and pneumococcus IgG levels 4w after vaccination (p=0.913, p=0.671) No difference in total IgA, IgE, IgG, IgM levels 4w after vaccination (p=0.085, 0.964, 0.599, 0.082)	Perez [36] Argentina 2009
171/180 Infants 4m	*L. paracasei spp paracasei* F19	$1x10^8$-10^{10} cfu 9m (from 4m of age)	DTaP-IPV-Hib 3, 5.5, 12m	**Higher diphtheria IgG levels at 6.5 and 12m after vaccination (p=0.044, 0.072)** No difference in tetanus or Hib polysaccharide IgG levels at 6.5 and 12m after vaccination (p-values NS)	West [37] Sweden 2007
57/60 Infants 2-5m	*L. casei* GG	$1x10^{11}$ cfu 6d (on day of vaccination)	ORV d1	**Higher number of rotavirus IgM secreting cells 8d after vaccination (p=0.020)** **Higher seroconversion rates for rotavirus IgA (93% vs. 74%) 8d after vaccination (p=0.050)** Higher seroconversion rates for rotavirus IgM (96% vs. 85%) 8d after vaccination (p=0.150)	Isolauri [38] Finland 1995
Probiotics administered in pregnancy					
61/61 Women	*L. rhamnosus* GG	$2x10^{10}$ cfu >36w of pregnancy until delivery	DTaP-Hib, schedule NS PCV7, schedule NS	**Lower pneumococcal IgG levels for serotype 4, 6B, 18C, 19F, 23F at 12m (p=0.027, 0.040, 0.032, 0.041, 0.019)** **Lower seroconversion rates for pneumococcal IgG against serotypes 4, 9V, 18C, 23F at 12m (p<0.050)** **Lower tetanus IgG levels at 12m (p=0.042)** No difference in Hib IgG levels at 12m (p-values NS) **Higher tolerogenic T regulatory (Treg) responses at 12m (51% vs. 36%, p=0.016)**	Licciardi [39] Australia 2013
Probiotics administered to adults on vaccine responses					
123/138 Adults 20-45y	*L. coryniformis* CECT5711	$2.8x10^9$ cfu 2w (pre vaccination)	HAV	**Increase in HAV IgG and IgM levels 4w after vaccination (p=0.017)** No difference in seroconversion rates for HAV IgG 4we after vaccination (p-values NS)	Redondo [11] Spain 2017
42/45 Adults ≥65y	*L. paracasei* MCC1849 (heat-killed)	$1x10^{13}$ cfu 6w (3w pre vaccination)	TIV A/California/7/2009 (H1N1), A/Texas/50/2012 (H3N2), B/Massachusetts/2/2012	No difference in A/H1N1, A/H3N2 and B strain IgG levels 6w after vaccination (p= 0.643, 0.767, 0.828) No difference in total IgA, IgG, and IgM levels 6w after vaccination (p=0.632, 0.821, 0.329) No difference in NK-cell, neutrophil bactericidal and phagocytic activity 6w after vaccination (p=0.814, 0.217, 0.560)	Maruyama [40] Japan 2016
1066/1104 Adults 18-60y	*L. paracasei* 431	$\geq 1x10^9$ cfu 6w (3w pre vaccination)	TIV (2011/2012) A/California/7/2009-like, A/Perth/16/2009-like, B/Brisbane/60/2008-like	No difference in A/H1N1, A/H3N2 and B strain IgG levels 3w after vaccination (p-values NS) No difference in A/H1N1, A/H3N2 and B strain IgA levels in saliva 3w after vaccination (p-values NS) No difference in seroconversion rates for IgG against all strains 3w after vaccination (p-values NS)	Jespersen [41] Germany Denmark 2015
15/15 Adults 75.6±6.7y	*L. paracasaei* MoLac-1 (heat-killed)	$1x10^{12}$ cfu 3m (3w pre vaccination)	TIV (A/H1N1, A H3N2, B)	No difference in NK-cell, neutrophil bactericidal and phagocytic activity 3 and 9w after vaccination (p-values NS) No difference in IgA, IgG and IgM levels 3 and 9w after vaccination (p-values NS) Trend towards higher H3N2 IgG levels 3w after vaccination (p=0.090) No difference in seroconversion rates for IgG against all strains 6w after vaccination (50.0% vs. 28.6%, 87.5% vs 42.9%, and 37.5% vs 14.3%, p-values NS)	Akatsu [42] Japan 2013
40/60 Adults 65-85y	*L. plantarum* CECT7315/7316	$5x10^8$-10^9 cfu 3m (3-4m after vaccination)	TIV (2006/2007)	**Increase in influenza IgA levels 6m after vaccination (p=0.008 for $5x10^9$, 0.039 for $5x10^8$)** **Increase in influenza IgG levels 6m after vaccination (p=0.023 for $5x10^9$)** Trend towards increase in influenza IgM levels (p=0.054 for $5x10^9$)	Bosch [43] Spain 2012
554/737 Adults ≥65y	*L. casei* Shirota	$\geq 1.3x10^{10}$ cfu 5.7m (3w pre vaccination)	TIV	No difference in A/H1N1, A/H3N2 and B strain IgG levels 4w and 5.7m after vaccination (p-values NS) No difference in seroconversion rates for IgG against all strains 4w and 5.7m after vaccination (p-values NS)	Van Puyenbroeck [44] Belgium 2012
211/221 Adults 19-60y	*B. lactis* BB-12 *L. paracasei* 431	$1x10^9$ cfu total 6w (2w pre vaccination)	TIV (2008/2009)	**Increase in influenza IgG, IgG1, IgG3 levels 4w after vaccination (p≤0.010)** **Higher seroconversion rates for IgG, IgG1, IgG3 (p<0.001)** **Higher influenza IgA levels in saliva 4w after vaccination (*L. paracasei* p=0.017, *B. lactis* p=0.035)** No difference in NK-cells activity, number of CD4+ T-lymphocytes and phagocytosis (p-values NS) No difference in INF-ɤ, IL-2 and IL-10 levels 4w after vaccination (p-values NS)	Rizzardini [45] Italy 2012
39/42 Adults 18-49y	*L. rhamnosus* GG	$1x10^{10}$ cfu 4w (on day of vaccination)	LAIV (2007/2008)	**Increase in haemagglutinin titres to H3N2 strain 4w after vaccination** (p=0.048) No difference in seroconversion rates for IgG against all strains 4w after vaccination (p=0.320, 0.360, 1) and at d56 after vaccination (p-values NS)	Davidson [46] USA 2011
27/27 Adults ≥65y	*B. longum* BB536	$1x10^{11}$ cfu 20w (3w pre vaccination)[2]	TIV (2004/2005) A/New Caledonia/20/99 (H1N1), A/Wyoming/3/2003 (H3N2), B/Shanghai/361/2002	No difference in seroconversion rates for IgG against all strains 17w after vaccination (23% vs. 29%, 85% vs. 86%, 39% vs. 29%, p-values NS) No difference in NK-cell, neutrophil bactericidal and phagocytic activity 17w after vaccination (p-values NS)	Namba [47] Japan 2010
86/136 Adults ≥70y	*L. casei* DN-114 001 *L. bulgaricus* *S. thermophilus*	$2x10^{10}$ cfu total 7w (4w pre vaccination)	TIV (2004/2005)	Higher A/H1N1, A/H3N2 and B strain IgG levels 21d after vaccination (p-values NS)	Boge [48] France 2009
222/241 Adults ≥70y	*L. paracasei* DN-114 001 *L. bulgaricus* *S. thermophilus*	$2x10^{10}$ cfu total 13w (4w pre vaccination)	TIV (2006/2007)	**Higher B strain IgG levels 3, 6 and 9w after vaccination (p=0.029, 0.027, 0.025)** **Higher seroconversion rates for IgG against B strain 6 and 9w after vaccination (p=0.006, 0.017)**	Boge [48] France 2009
83/83 Adults 18-62y	*B. lactis* Bi-07 *B. lactis* Bl-04 *L. acidophilus* La-14 *L. acidophilus* NCFM *L. plantarum* Lp-115 *L. paracasei* Lpc-37 *L. salivarius* Ls-33	$2x10^{10}$ cfu each 3w (1w pre vaccination)	OCV d7 and 14	**Higher cholera IgG levels 1w after vaccination with *B. lactis* Bl-04 (p=0.010) and *L. acidophilus* La-14 (p=0.010)** **Higher cholera IgA and IgM levels 2w after vaccination with *L. acidophilus* NCFM (p=0.030, 0.020)** No difference in overall vaccination titres, cholera IgA or IgM levels 1-2w after vaccination (p-values NS) Lower cholera IgM levels 14d after vaccination with *B. lactis* Bl-04 and *L. plantarum* Lp-115 (p=0.090, 0.010)	Paineau [52] France 2007
50/50 Adults 22-56y	*L. fermentum* CECT5716	$1x10^{10}$ cfu 4w (2w pre vaccination)	TIV d14 (2004/2005)	**Increase in influenza IgA levels 4w after vaccination (p<0.050)** **Higher total IgG and IgM levels 4w after vaccination (p<0.050)** No difference in total IgA levels and influenza IgG and IgM levels 2 and 4w after vaccination (p-values NS) **Higher TNF-α levels 2 and 4w after vaccination (p<0.050)** No difference in B-/T-lymphocytes, NK-cell or IL-10/IL-12/INF-ɤ levels 4w after vaccination (p-values NS)	Olivares [49] Spain 2007

(*continued on next page*)

Table 1 (*continued*)

50/60 Adults ≥70y	*L. paracasei* NCC 2461	1x10^{9} cfu 6m (4m pre vaccination)	TIV PPV23	No difference in influenza A, influenza B or pneumococcal IgG levels for serotype 1, 2, 4, 5, 6B, 9V, 14, 18C, 19F, 23F 2m after vaccination (p=0.770, 0.480, 0.060-0.360)	Bunout [50] Switzerland 2004
64/66 Adults 20-30y	*L. acidophilus* CRL431 *L. rhamnosus* GG	1x10^{10} cfu total 5w (1w pre vaccination)	OPV	**Increase in poliovirus neutralizing Ig levels 2-4w after vaccination (p=0.014-0.048)** **Increase in poliovirus IgA and IgM levels 2-4w after vaccination (p=0.036-**0.860, **0.040 -**0.720) No change in poliovirus IgG levels 2-4w after vaccination (p= 0.083-0.960)	de Vrese [53] Germany 2004
29/30 Adults 20-50y	*Lactobacillus* GG *L. lactis*	4x10^{10} cfu 3.4x10^{10} cfu 1w (on day of vaccination)	Ty21a d1, 3 and 5	No difference in typhoid IgA, IgG or IgM secreting cells 1w after vaccination (p=0.810, 0.660, 0.670) **Higher CR3 receptor expression on neutrophils 1w after vaccination with *L. lactis* (p=0.030)**	Fang [54] Finland 2000

[1] also given to pregnant women in last month of pregnancy
[2] placebo group received probiotics during first 6 w of study

cfu – colony forming units
d – days
DTaP – diphtheria-tetanus- acellular pertussis vaccine
DTwP – diphtheria-tetanus-whole cell pertussis vaccine
DTPa-HBV-IVP-Hib – combined diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliomyelitis-*Haemophilus influenza* type b vaccine
DTaP-Hib – combined diphtheria-tetanus-acellular pertussis-*Haemophilus influenza* type b vaccine
HAV – hepatitis A vaccine
HBV – hepatitis B vaccine
Hib – *Haemophilus influenza* type b vaccine
LAIV – nasal live attenuated trivalent influenza vaccine
m – months
MMRV – measles-mumps-rubella-varicella vaccine NK – natural killer
NS – not specified
OCV – oral cholera vaccine
OPV – oral polio vaccine
ORV – oral rotavirus vaccine
PCV7 – 7-valent pneumococcal conjugate vaccine
PPV23 – 23-valent pneumococcal polysaccharides vaccine
TIV – inactivated trivalent influenza vaccine
Ty21a – oral *Salmonella* Typhi
w – weeks
y – years

19 F p = 0.041, 23 F p = 0.019), lower seroconversion rates for pneumococcal serotypes 4, 9 V, 18 C, 23 F (p < 0.050) and lower tetanus toxoid-specific IgG levels (p = 0.042) at the age of 12 months. However, of note, infants had higher tolerogenic T regulatory (Treg) responses (51% vs. 36%, p = 0.016) at the age of 12 months.

3.4. Effect of probiotics on vaccine responses in adults

Sixteen studies investigated vaccine responses after administration of probiotics in a total of 2637 adults. Twelve of these studies investigated the response to influenza vaccination [40–50], one study the response to hepatitis A vaccination [51] and three the response to oral vaccinations (cholera [52], polio [53], and *Salmonella* typhi [54]). In 5 of the 12 studies, probiotics enhanced the response to influenza vaccine. After taking *B. lactis* and *L. paracasei* (10^{9} cfu) for 6 weeks, an increase in influenza-specific IgG, IgG1, IgG3 levels ($p \leq 0.01$), higher seroconversion rates for influenza-specific IgG, IgG1, IgG3 (p < 0.010) and higher influenza-specific IgA levels in saliva were noted 4 weeks following trivalent inactivated influenza vaccination (TIV) (*B. casei* p = 0.017, *B. lactis* p = 0.035) [45]. Further, higher B strain-specific IgG levels 3, 6 and 9 weeks after vaccination (p = 0.029, 0.027, 0.025) and higher seroconversion rates 6 and 9 weeks after TIV (p = 0.006, 0.017) were noted after taking *L. paracasei*, *L. bulgaricus* and *S. thermophilus* (2×10^{10}) for 13 weeks [48]. After the taking *L. plantarum* (5×10^{8}–5×10^{9}) for 12 weeks, higher influenza-specific IgA and IgG levels were reported 6 months after TIV vaccination

Table 2
Strains and doses of probiotics tested in randomised placebo-controlled trials reporting the influence of probiotic administration on vaccine responses.

	Increase in vaccine response	No change in vaccine response	Decrease in vaccine response
***B. breve* BBG-01**			4x10^{9} cfu [35]
***B. breve* Bbi99**	2-5x10^{9} cfu, with *L. rhamnosus* GG, *L. rhamnosus* LG705, and *P. freundenreichii* [33]		
***B. breve* C50**	Dose NS, with *S. thermophiles* [18]		
B. bifum		3x10^{9} cfu, with *B. infantis, B. longum,* and *L. acidophilus* [55]	
B. infantis		3x10^{9} cfu, with *B. bifum, B. longum,* and *L. acidophilus* [55]	
***B. lactis* BB-12**	1x10^{9} cfu, with *L. paracasei* 431 [45]		
***B. lactis* Bi-07**		2x10^{10} cfu [52]	
***B. lactis* B1-04**	2x10^{10} cfu [52]		
B. longum		3x10^{9} cfu, with *B. bifum. B. infantis,* and *L. acidophilus* [55]	
***B. longum* BB536**		1x10^{7}/g formula [30] 1x10^{11} cfu [47]	
***B. longum* BL999**		2.8x10^{8} cfu, with *L. rhamnosus* LPR [31]	
***Lactobacillus* GG**		4x10^{10} cfu, with *L. lactis* [54]	
L. acidophilus		3x10^{9} cfu, with *B. bifum. B. infantis,* and *B. longum* [55]	
***L. acidophilus* CRL431**	1x10^{10} cfu, with *L. rhamnosus* GG [53]		
***L. acidophilus* CRL730**		9.5x10^{7}-10^{9} cfu, with *L. casei* CRL431, and *S. thermophiles* [36]	
***L. acidophilus* La-14**	2x10^{10} cfu [52]		
***L. acidophilus* NCFM**	2x10^{10} cfu [52]		
L. bulgaricus	2x10^{10} cfu, with *L. paracasei* DN-114 001, and *S. thermophiles* [48]	2x10^{10} cfu, with *L. casei* DN-114 001, and *S. thermophiles* [48]	
***L. casei* CRL431**		9.5x10^{7}-10^{9} cfu. with *L. acidophilus* CRL730, and *S. thermophiles* [36]	
***L. casei* DN-114 001**		2x10^{10} cfu, with *L. bulgaricus,* and *S. thermophiles* [48]	
***L. casei* GG**	1x10^{11} cfu [38]		
***L. casei* Shirota**		≥1.3x10^{10} cfu [44]	
***L. coryniformis* CECT5711**	2.8x10^{9} cfu [51]		
***L. fermentum* CECT5716**	1x10^{10} cfu [49]		
***L. paracasei* DN-114 001**	2x10^{10} cfu, with *L. bulgaricus*, and *S. thermophiles* [48]		
***L. paracasei* Lpc-37**		2x10^{10} cfu [52]	
***L. paracasei* MCC1849** (heat-killed)		1x10^{13} cfu [48]	
***L. paracasei* NCC 2461**		1x10^{9} cfu [50]	
***L. paracasaei* MoLac-1** (heat-killed)		1x10^{12} cfu [42]	
***L. paracasei* 431**	1x10^{9} cfu with *B. lactis* BB-12 [45]	≥1x10^{9} cfu [41]	
***L. paracasei spp paracasei* F19**	1x10^{8}-10^{10} cfu [37]		
***L. plantarum* CECT7315/7316**	5x10^{8} -10^{9} cfu [43]		
***L. plantarum* Lp-115**		2x10^{10} cfu [52]	
***L. rhamnosus* GG**	2-5x10^{9} cfu, with *B. breve* Bbi99, *L. rhamnosus* LG705, and *P. freundenreichii* [33] 1x10^{10} cfu, with *L. acidophilus* CRL431 [53] 1x10^{10} cfu [46]		2x10^{10} cfu [39]
***L. rhamnosus* LG705**	2-5x10^{9} cfu, with *B. breve* Bbi99, *L. rhamnosus* GG, and *P. freundenreichii* [33]		
***L. rhamnosus* LPR**		2.8x10^{8} cfu, with *B. longum* BL999 [31]	
***L. salivarius* Ls-33**		2x10^{10} cfu [52]	
L. lactis	3.4x10^{10} cfu [54]		
P. freundenreichii	2-5x10^{9} cfu, with *B. breve* Bbi99, *L. rhamnosus* GG, and *L. rhamnosus* LG705 [33]		
S. thermophiles	Dose NS, with *B. breve* C50 [18] 2x10^{10} cfu, with *L. paracasei* DN-114 001, and *L. bulgaricus* [48]	9.5x10^{7}-10^{9} cfu, with *L. casei* CRL431, and *L. acidophilus* CRL730 [36] 2x10^{10} cfu, with *L. casei* DN-114 001, and *L. bulgaricus* [48]	

(p = 0.008–0.039, p = 0.023, respectively) [43]. Similarly, higher influenza-specific IgA levels were reported 4 weeks following TIV vaccination (p < 0.050) after taking *L. fermentum* (10^{10} cfu) for 4 weeks [49]. Finally, higher hemagglutinin titers to the H3N2 strain were reported 4 weeks following vaccination (p = 0.048) with nasal live attenuated trivalent influenza vaccine after taking *L. rhamnosus* GG (10^{10} cfu) for 4 weeks [46].

The study investigating the effect of probiotic intake on responses to hepatitis A vaccination reported higher total specific hepatitis A Ig levels 4 we following vaccination (p = 0.017) after the taking *L. coryniformis* (2.8×10^9 cfu) for for 2 weeks [51].

The studies looking at the effect of probiotics on responses to oral vaccines mostly reported favourable outcomes. Taking *L. acidophilus* and *L. rhamnosus* GG (10^{10} cfu) for 5 weeks was associated with an increase in poliovirus neutralising antibody levels and an increase in poliovirus-specific IgA and IgM levels 14–28 days following vaccination (p = 0.014–0.048, 0.036–0.860, 0.040–0.720, respectively) [53]. In a study comparing 7 different probiotic strains taken for 3 weeks (*B. lactis, L. acidophilus, L. plantarum* and *L. paracasei*, 2×10^{10} cfu each), however, improved responses to oral cholera vaccination were seen with only 3 of the 7 strains: higher cholera-specific IgG levels 7 days after vaccination with *B. lactis* (p = 0.010) and *L. acidophilus* La-14 (p = 0.010) and higher cholera-specific IgA and IgM levels (p = 0.030, 0.020) 14 days after vaccination with *L. acidophilus* NCFM [52]. The intake of *Lactobacillus* (4×10^{10} cfu) and *Lactococcus lactis* (3.4×10^{10} cfu) around the time of *Salmonella* typhi vaccination was not associated with an increase in typhoid-specific Ig levels, but was associated with higher CR3 receptor expression on neutrophils 7 days following vaccination (p = 0.030), in case of *L. lactis* [54]. Table 1

3.5. Effect of different doses and strains

Table 2 summaries the strains and doses of probiotics tested in the different trials. Because of the great heterogeneity between studies, it is not possible to make any conclusion. However, there is a trend for a favourable outcome with *B. breve, B. lactis, L. acidophilus,* and *L. rhamnosus*. On the contrary, studies with *B. longum, L. casei,* and *L. paracasei* at similar doses did not find any significant changes in vaccine responses.

4. Discussion

In our systematic review of the studies that have reported the effect of probiotics on the humoral response to either parenteral or oral vaccinations, a beneficial effect was reported in about half (in 3 of 7 studies investigating parental vaccinations in neonates and children [18,33,37], in 5 of 12 investigating responses to influenza vaccination [43,45,46,48,49], in the one study investigation response to hepatitis A vaccination [55], and in 3 of 5 studies assessing oral vaccinations in children and adults [38,52,53]). The one study that investigated the effect of probiotic administration to mothers during pregnancy found lower vaccine response in infants [39].

Some of the variation in the reported effect of probiotics in the studies in our review is likely to result from the substantial variation between studies in the choice of probiotics, strain, dose, viability, purity, and duration and timing of administration. Notably, a total of 40 different probiotic microorganisms were used in the 26 studies. The choice of strain and/or combination has a major influence, as suggested by studies investigating the effects of probiotics on other outcomes. For example, of the 12 randomised controlled trials investigating the effect of probiotics on infant crying, 6 report a positive effect. The majority of those that a beneficial effect used *L. reuteri*, while trials using *L. rhamnosus* or *Bifidobacterium* spp. showed no effect [56]. Similarly, a meta-analysis investigation the effect of early life probiotic administration on atopy found that overall, probiotics have a significantly reduce the risk of atopic sensitisation, while, in contrast, the administration of *L. acidophilus* is associated with an increased risk [57].

Interestingly, even though, probiotics are defined as live microorganisms, two of the studies included in this review used heat-killed microorganisms [40,42]. These did not show any significant effect on vaccine responses. However, in animal studies it has been shown that heat- killed and live probiotics have identical anti-inflammatory effects [58,59], suggesting that non-viable bacteria might act as antigens influencing the immune system.

Studies which looked at the stool microbiome after the intake of probiotics showed an increase in the quantity of the administered strains (*B. breve, B. longum, B. longum/B. infantis, L. casei*) and sometimes a decrease in Enterobactericeae [18,30,35,36,60]. This supports the concept that probiotics affect the vaccine response through alterations in the composition of the intestinal microbiota. Whether the intestinal microbiome influences vaccine responses, has, to date, only been investigated in 4 small studies [14–18]. One of these studies reported that a higher abundance of *B. longum-infantis* and *B. breve* is associated with an increase in faecal polio-specific IgA after parental polio vaccine in infants [18]. Another study reported that abundance of *Actinobacteria* (including *Bifidobacterium longum)* is associated with improved humoral and T-cell responses to Bacillus Calmette–Guérin, polio, and tetanus toxoid vaccines, while bacterial diversity and the abundance of *Enterobacteriales, Pseudomonadales* and *Clostridiales* is associated with lower vaccine responses [15]. In contrast, a small study in adults showed that individuals with more diverse intestinal microbiota had superior cell mediated immune responses to oral typhoid vaccination [16]. Finally, a study investigating the serological response to rotavirus vaccine showed an increased abundance of *Streptococcus bovis* and a decrease in *Bacteroidetes phylum* was associated with vaccine responders [14]. It has been suggested that the different abundance of these bacteria in low-income countries might partly explain the significantly lower efficacy of oral vaccines, such as rotavirus, polio and cholera [14]. The administration of probiotics might be of particular benefits in these settings.

The suggestion from our review that probiotics increase responses to influenza vaccination [43,45,46,48,49] raises the possibility that probiotics might be helpful in elderly people, in whom it is known that seroconversion rates to influenza vaccination are lower in comparison to younger people.

Probiotics offer a relatively cheap intervention to improve vaccine efficacy and duration of protection and have few adverse effects [35]. There is sufficient evidence from the studies in our review to suggest this strategy is worth pursuing. However, future studies should focus on establishing optimal strains, doses and timing of administration in relation to vaccination.

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Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

PZ drafted the initial manuscript, and approved the final manuscript as submitted. NC critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

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