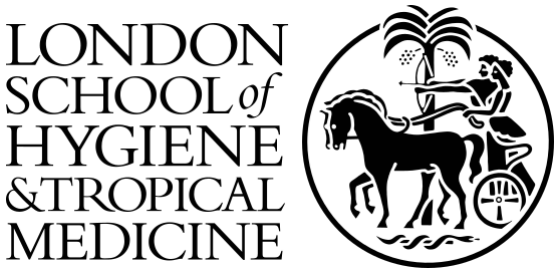

Pneumococcal vaccination for developing countries: PCV10 or PCV13?

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Declaration

I, Beth Temple, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed: 

Date: 25 January 2023

Abstract

Streptococcus pneumoniae (pneumococcus) is the most common cause of pneumonia and a major cause of meningitis globally. Pneumococcal conjugate vaccines (PCVs) offer protection against disease caused by selected pneumococcal serotypes and the World Health Organization (WHO) recommends the introduction of PCV as a priority. PCV10 and PCV13 have been available for over a decade, yet there is a paucity of head-to-head data to assist countries with decision-making regarding vaccine choice. We designed and conducted a randomised controlled trial in Ho Chi Minh City, Vietnam, to directly compare the immunogenicity, reactogenicity, and effect on pneumococcal carriage of a 2+1 schedule of PCV10 and PCV13.

We showed that both vaccines are safe, highly immunogenic, and reduce carriage of vaccine serotypes. Some differences between vaccines were observed. PCV10 tended to be more immunogenic after the first dose and PCV13 tended to be more immunogenic post-primary series and post-booster. The clinical implications of these differences are unknown. There were trends towards a greater impact on carriage of PCV10-serotypes with PCV10 and a greater impact on carriage of PCV13-only-serotypes with PCV13. This resulted in a similar overall impact on carriage of PCV13-serotypes with both vaccines that is likely to generate substantial herd protection effects. Shared-serotype 6B and PCV13-only-serotype 6A were the two most commonly carried serotypes among unvaccinated children. The relative contribution of these two serotypes to serogroup 6 disease could be an important factor in vaccine choice in this setting. Overall, based on data from our trial, we would expect the introduction of either PCV10 or PCV13 in a 2+1 schedule to have significant public health benefits, offering both direct protection to vaccinees and indirect herd protection to the broader population. With the introduction of new PCVs to the market, more head-to-head data will be needed to assist with growing vaccine choice.

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This work would not have been possible without the contributions of the research team at the Pasteur Institute of Ho Chi Minh City, Vietnam (Pasteur), and the staff at the study clinics in District 4 and District 7 of Ho Chi Minh City. Particular thanks go to Tran Ngoc Huu, Nguyen Trong Toan, and Doan Y Uyen. A special thanks also to Nguyen Thi Kieu Chinh and Lam Trung Duc for brightening up the Vietnam Pneumococcal Project Office and to Kathryn Bright for her never-ending (and sometimes daily) support. I acknowledge the vital role of the staff from the pneumococcal laboratory at Pasteur, led by Vo Thi Trang Dai, in receiving and processing the 4,860 blood samples and 7,052 nasopharyngeal swabs collected during the Vietnam Pneumococcal Trial. Thanks also to staff from the Child Health laboratory at Menzies School of Health Research, Darwin, Australia, and the New Vaccines Group and Translational Microbiology Group laboratories at the Murdoch Children's Research Institute, Melbourne, Australia for their role in establishing and overseeing the pneumococcal laboratory at Pasteur and in conducting the opsonophagocytic assays and molecular serotyping by microarray. I especially appreciate Heidi Smith-Vaughan's patience in putting up with my many questions about pneumococcal culture data.

I acknowledge the contributions of all the co-authors of the research papers included in this thesis. I am also very grateful to Monica Nation for taking the time to read through a draft of this thesis and for her ability to notice missing words and typos. Special gratitude goes to the Vietnam Pneumococcal Trial participants and their families, without whom there would be no research to present. Finally, I would like to thank my family for their support throughout my studies and their patience with me, particularly during the final stages of preparing this thesis.

Preface

This thesis is a combination of a book style thesis and a research paper style thesis, written in accordance with the guidelines and regulations specified by the London School of Hygiene and Tropical Medicine. The first three chapters are written in book style format and provide a general introduction and background to the topic, an outline of the PhD objectives, and a description of how the research project was developed and established. The next three chapters comprise three research papers published in peer-reviewed journals: one protocol paper published in BMJ Open and two results papers published in The Lancet Infectious Diseases and Vaccine. There is, by necessity, some repetition of material in the published manuscripts. The final chapter integrates the research findings to draw conclusions regarding the choice of pneumococcal vaccine for Vietnam and other developing countries. Literature reviews were conducted using PubMed. The first and final chapters include literature published up to and including 1 December 2022. The timeframes for the literature reviews contained with each research paper are noted at the start of the respective chapters.

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List of Abbreviations

Abbreviation	Full description
AMC	Advanced Market Commitment
ANSORP	Asian Network for Surveillance of Resistant Pathogens
ARI	acute respiratory infection
Bld	venous blood sample
CHC	Commune Health Centre
CI	confidence interval
CRM_{197}	Cross-Reactive Material-197
CSF	cerebrospinal fluid
DTaP	diphtheria-tetanus-acellular pertussis vaccine
DTP	diphtheria-tetanus-pertussis vaccine
DTwP	diphtheria-tetanus-whole-cell pertussis vaccine
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Program on Immunization
FDA	United States Food & Drug Administration
GMC	geometric mean concentration
GMOI	geometric mean opsonisation index
HbOC	Hib vaccine: PRP-mutant diphtheria toxin
HBV	hepatitis B vaccine
Hexa	DTaP-HBV-IPV-Hib vaccine (INFANRIX-HEXA)
Hib	*Haemophilus influenzae* type b vaccine
HIC	high-income country
IgG	immunoglobulin G
IPD	invasive pneumococcal disease
IPV	inactivated polio vaccine
Lao PDR	Lao People's Democratic Republic
LMIC	low- and middle-income country
LRTI	lower respiratory tract infection
m	months of age
MCRI	Murdoch Children's Research Institute, Melbourne, Australia
MenC	meningitis C vaccine
MR	measles-rubella vaccine
NIHE	National Institute of Hygiene and Epidemiology
NP	nasopharyngeal

Abbreviation	Full description
NTHi	non-typeable *Haemophilus influenzae*
OI	opsonisation index
OM	otitis media
OPA	opsonophagocytic assay
OPV	oral polio vaccine
Pasteur	Pasteur Institute of Ho Chi Minh City, Ho Chi Minh City, Vietnam
PCV	pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine (PREVENAR-7)
PCV10	10-valent pneumococcal conjugate vaccine (SYNFLORIX)
PCV13	13-valent pneumococcal conjugate vaccine (PREVENAR-13)
PCV15	15-valent pneumococcal conjugate vaccine (VAXNEUVANCE)
PCV20	20-valent pneumococcal conjugate vaccine (PREVENAR-20)
PPV23	23-valent pneumococcal polysaccharide vaccine (PNEUMOVAX)
PRP	polyribosylribitol phosphate (Hib polysaccharide)
PRP-OMP	Hib vaccine: PRP-*Neisseria meningitidis* outer membrane protein
PRP-T	Hib vaccine: PRP-tetanus toxoid
Quin	DTwP-HBV-Hib vaccine (QUINVAXEM)
RCT	randomised controlled trial
SIIPL-PCV	10-valent pneumococcal conjugate vaccine (PNEUMOSIL)
Spn	*Streptococcus pneumoniae*
UK	United Kingdom
URTI	upper respiratory tract infection
US	United States
VPT-II	Vietnam Pneumococcal Trial II
VT	vaccine-type
w	weeks of age
WHO	World Health Organization

Chapter 1: Background

1.1 Introduction to Streptococcus pneumoniae

Streptococcus pneumoniae (pneumococcus) is a bacterium that causes a range of diseases, including meningitis, bacteraemia, pneumonia, acute otitis media and sinusitis.[1] Invasive pneumococcal disease (IPD) is defined by the isolation of *S. pneumoniae* from a normally sterile body site. *S. pneumoniae* is a major cause of morbidity and mortality worldwide and pneumococcal diseases are most common in young children and among the elderly.[1] Global estimates of the burden of pneumococcal disease modelled 318,000 pneumococcal deaths among children less than five years of age in 2015, with 81% of these attributable to pneumococcal pneumonia.[2] The Global Burden of Disease Study 2017 produced higher mortality estimates among the same age group, with 380,931 deaths attributed to pneumococcal pneumonia alone.[3]

S. pneumoniae is a Gram-positive encapsulated diplococcus (Figure 1.1). The capsule consists of polysaccharides, which determine the virulence of the bacterium.[4,5] Differences in capsular structure give rise to different serotypes of pneumococcus. Capsular polysaccharide elicits an immune response by stimulating the production of serotype-specific antibody. By 1995, 90 different serotypes had been identified by the Quellung reaction.[6,7] Since then, technological developments have enabled further analysis of the capsular structure, and there are now 100 known pneumococcal serotypes.[8] Whilst most serotypes have the ability to cause disease, a relatively small number are responsible for the majority of cases of IPD.

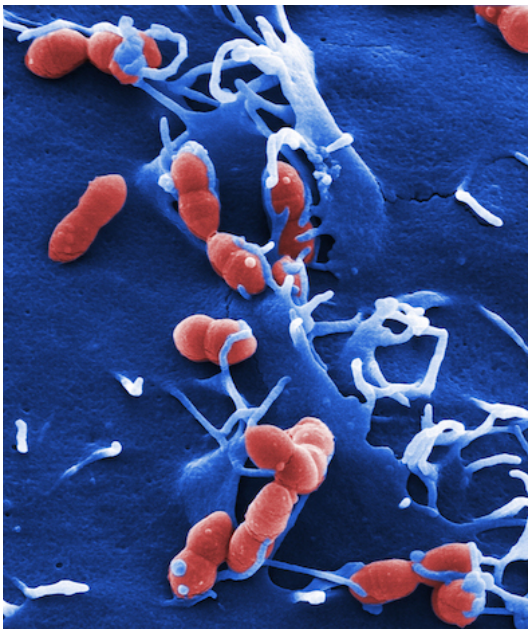


Figure 1.1: *S. pneumoniae* on a pharyngeal epithelial cell

(Source: Manfred Rohde, Helmholtz Centre for Infection Research, Germany, 2014)

1.2 Polysaccharide and conjugate pneumococcal vaccines

In the 1970s, several vaccines containing capsular polysaccharide from multiple pneumococcal serotypes were developed and tested on gold miners in South Africa.[9] This led to the licensure of the first pneumococcal vaccine in 1977, a 14-valent pneumococcal polysaccharide vaccine (Merck & Company), which was superseded in 1983 by a 23-valent PPV that is still in use today (PPV23; PNEUMOVAX, Merck & Company). PPV23 was initially licensed for the prevention of pneumococcal disease in adults but was subsequently also approved for use in children greater than 2 years of age. PPV23 is not approved for use in children less than 2 years of age because pneumococcal polysaccharides are T-cell independent antigens and do not elicit protective immune responses in this age group due to immaturity of the immune system.[10] By conjugating polysaccharides to proteins, the immune response is altered from T-cell independent to T-cell dependent, eliciting both a strong primary response and a booster response in infants.[11] The first licensed pneumococcal conjugate vaccine (PCV) contained seven serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) conjugated to Cross-Reactive Material-197 protein (CRM_{197}), a non-toxic variant of diphtheria toxin (PCV7; PREVENAR-7, Wyeth Vaccines, now Pfizer). Infant vaccination against pneumococcus has been available since the licensure of PCV7 in the United States (US) in the year 2000, and has been recommended by the World Health Organization (WHO) for introduction into national immunisation programmes since 2006.[12]

The introduction of PCV7 was associated with dramatic reductions in pneumococcal disease.[13-16] The benefits of vaccination were not only seen amongst vaccinated individuals (direct effects), but also in the wider population (indirect or herd protection effects). These indirect effects were significant; for example twice as many cases of pneumococcal disease in the US were prevented as a result of herd protection as were prevented directly through vaccination.[17-19] The serotypes included in PCV7 were selected on the basis of IPD serotype coverage in the US: these seven serotypes were responsible for 80% of IPD infections among children less than 6 years of age pre-licensure.[11] However, geographical variation in serotype distribution[20-23] and an increase in IPD caused by non-PCV7 serotypes following vaccine introduction[24] necessitated the development of higher valency PCVs.

1.3 The current choice of PCV

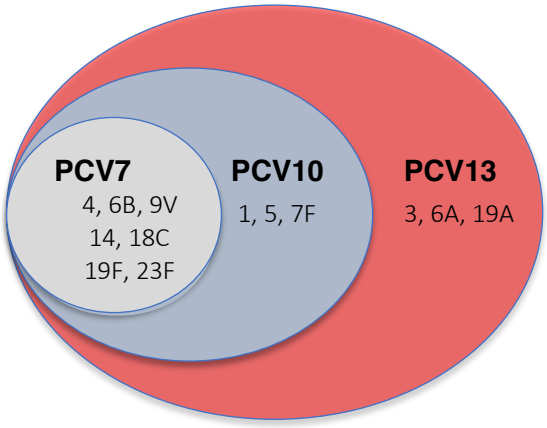


Figure 1.2: Serotypes included in the first and second-generation PCVs

Following the success of PCV7 introduction, two PCVs with increased serotype coverage were developed: a 10-valent PCV (PCV10; SYNFLORIX, PHiD-CV, GlaxoSmithKline Biologicals) and a 13-valent PCV (PCV13; PREVENAR-13, Pfizer). Both PCV10 and PCV13 contain the seven serotypes in PCV7 with the addition of serotypes 1, 5 and 7F, and PCV13 also includes serotypes 3, 6A and 19A (Figure 1.2). PCV10 and PCV13 received World Health Organization (WHO) prequalification in October 2009 and August 2010, respectively. Prequalification is the process through which WHO evaluates candidate vaccines to determine their acceptability in terms of both safety and efficacy. As well as the number of serotypes included, PCV10 and PCV13 differ in relation to their carrier proteins and methods of conjugation. PCV13 was built on PCV7 and, like its predecessor, all of the serotypes are conjugated to CRM_{197}. One 0.5mL dose of PCV13 contains 2.2µg of saccharides from each serotype except 6B (4.4µg). In PCV10, eight of the serotypes are conjugated to Protein D, a highly conserved nonlipidated cell-surface lipoprotein from non-typeable *Haemophilus influenzae* (NTHi). The remaining two serotypes are conjugated to tetanus toxoid (serotype 18C) and diphtheria toxoid (serotype 19F). The inclusion of Protein D may confer protection against NTHi infections. One 0.5mL dose of PCV10 contains 1µg of saccharides from serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3µg of saccharides from serotypes 4, 18C and 19F.

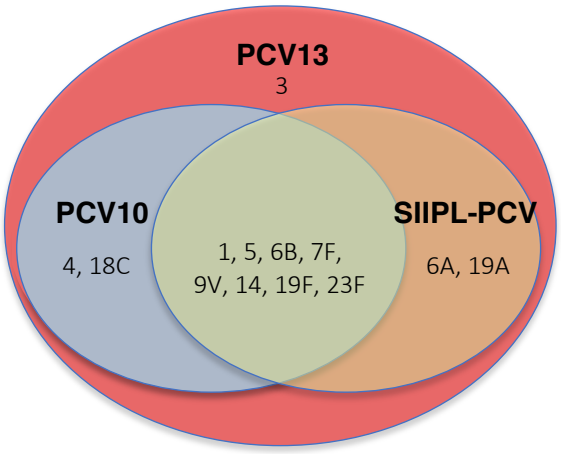


Figure 1.3: Serotypes included in the three PCVs with WHO prequalification

In December 2019, a second 10-valent PCV (SIIPL-PCV; PNEUMOSIL, Serum Institute of India) received WHO prequalification. SIIPL-PCV contains 10 of the serotypes in PCV13 (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F and 23F), eight of which are also included in PCV10 (Figure 1.3). The two 10-valent vaccines differ in their inclusion of serotypes 4 and 18C (in PCV10) and serotypes 6A and 19A (in SIIPL-PCV). SIIPL-PCV is currently only in use in India, where introduction began in 2021, and is not the focus of this thesis.

1.4 Global PCV introduction

Initially, the high cost of PCV restricted its use to high-income countries (HICs). To increase the availability of PCV, the Gavi Alliance (formerly known as the Global Alliance for Vaccines and Immunizations) made funding available to support PCV introduction in countries with the lowest income. Funding is provided through the Advanced Market Commitment (AMC), a mechanism that ensures an affordable and sustainable supply of vaccine. In 2009, Rwanda became the first Gavi-eligible country to introduce PCV to its routine immunisation programme. As of October 2022, PCV had been introduced nationally in 154 countries and sub-nationally or to at-risk populations in a further 11 countries. 17 countries were either planning PCV introduction or planning to apply for Gavi funding for PCV introduction, leaving 12 countries with no current plans regarding PCV introduction (Figure 1.4).

Despite the introduction of PCV into the national immunisation programmes of a large number of countries, global access to PCV remains poor. Based on 2022 pneumococcal vaccine introduction data, 60% of surviving infants globally have access to PCV by living in countries or regions where PCV has been introduced.[25] Of course, not all children in those countries or regions receive routine immunisations, including many of those at greatest risk of

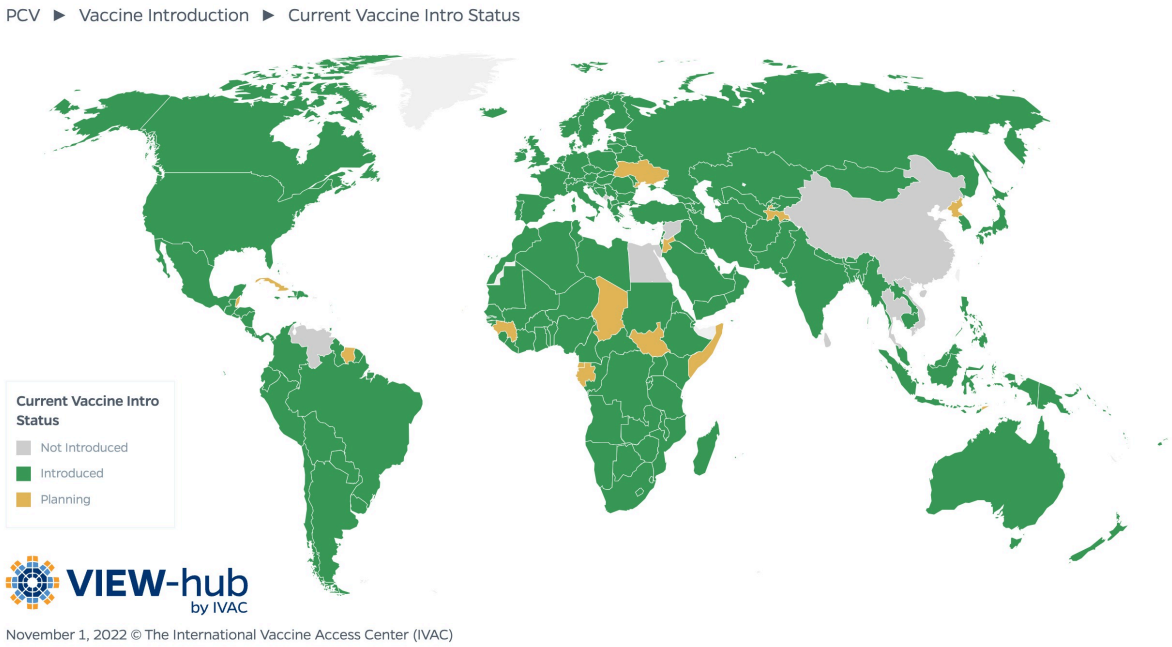


Figure 1.4: Map of PCV introduction status by country
Source: https://view-hub.org

pneumococcal disease. Based on 2022 WHO/UNICEF estimates of national immunisation coverage, 67% of children with theoretical access to PCV actually receive the vaccine, leaving 60% of the world's children (81.4 million) unvaccinated. Two approaches are needed to improve global access to PCV: better access for children living in countries where PCV has been introduced, and introduction of PCV into the remaining countries. For countries yet to introduce PCV, the choice of vaccine remains a key question.

1.5 PCV immunogenicity

WHO prequalification and regulatory approval of PCVs are based on immunogenicity, and require demonstration of immunological non-inferiority to a licensed PCV.[26-28] Assessment of non-inferiority is based primarily on the proportion of children with serotype-specific antibody concentration immunoglobulin G (IgG) ≥0.35μg/mL four weeks post-primary series. The cut-off of 0.35μg/mL was determined as the correlate of protection for IPD in a pooled analysis of data from three efficacy trials involving PCV7 and an investigational 9-valent PCV.[28] The 9-valent PCV, manufactured by Wyeth Vaccines, had the same composition as PCV7 with the addition of serotypes 1 and 5; these serotypes were dropped from the formulation pre-licensure. Some demonstration of the functional capacity of the antibodies produced, such as through opsonophagocytic assay (OPA), and the ability to elicit a booster response are also recommended. Data from the literature showing the comparative immunogenicity of PCV7, PCV10, and PCV13 are summarised below.

1.5.1 Comparative immunogenicity of PCV13 and PCV7

PCV13 was developed by the manufacturers of PCV7 and was built as a successor to that vaccine. Eleven studies have reported a direct comparison of the post-primary series immunogenicity of PCV13 and PCV7 in terms of the percentage of responders (with IgG ≥0.35μg/mL) post-primary series (Table 1.1).[29-39] These comprise ten randomised controlled trials and one observational study. Nine of the trials report the difference in percentage of responders between groups, including the two pivotal studies used for the approval of PCV13 in the US and Europe.[29,30] For both pivotal studies, non-inferiority for each of the shared serotypes was defined by a lower limit of the 95% confidence interval (CI) for the difference in the percentage of responders (PCV13-PCV7) post-primary series of greater than -10.0%.[40] Non-inferiority was shown for all shared serotypes except for 6B (in both studies) and 9V (in the US study; Appendix A, Table S1a). Applying the same criteria of non-inferiority to the other seven trials that report the difference in the percentage of responders, PCV13 was non-inferior to PCV7 for all shared serotypes in all studies with only one exception (serotype 23F in the Taiwan study; Appendix A, Table S1a). Six trials, including the two pivotal studies, have been included in a meta-analysis of the percentage of responders post-primary series.[41] The aggregate percentage of responders in the PCV13 groups across all studies was greater than 93% for each of the shared serotypes except 6B (89%), with no differences between the PCV13 and PCV7 groups.

Table 1.1: Characteristics of studies comparing the percentage of responders following primary series vaccination with PCV13 or PCV7

Study	Design	Country	Primary series		N for post-primary series immunogenicity analysis	
			PCV schedule	Co-administered vaccines	PCV13 group	PCV7 group
Keininger et al. 2010[29]	RCT‡	Germany	2, 3, 4m	DTaP-HBV-IPV-Hib	285	279
Yeh et al. 2010[30]	RCT‡	United States	2, 4, 6m	DTaP-HBV-IPV + Hib	252	252
Bryant et al. 2010[31]	RCT‡	United States	2, 4, 6m	DTaP-HBV-IPV + Hib	94	106-108
Snape et al. 2010[32]*	RCT	United Kingdom	2, 4m	DTaP-IPV-Hib at 2, 3, 4m MenC at 2, 4m	104-111	101-102
Weckx et al. 2012[33]	RCT‡	Brazil	2, 4, 6m	DTwP-Hib + OPV at 2, 4m DTwP-Hib + OPV + HBV at 6m	156	152-158
Huang et al. 2012[34]	RCT‡	Taiwan	2, 4, 6m	DTaP-IPV-Hib at 2, 4m DTaP-HBV-IPV-Hib at 6m	80	83
Kim et al. 2013[35]	RCT	Korea	2, 4, 6m	DTaP	83	85
Amdekar et al. 2013[36]	RCT‡	India	6, 10, 14w	DTwP-Hib-HBV + OPV	185-206	185-195
Grant et al. 2013[37]*†	Observational	United States	2, 4, 6m	not reported	85	32
Togashi et al. 2015[38]	RCT	Japan	3 doses 4-8w apart from 3-6m of age	DTaP	174-177	124-176
Zhu et al. 2016[39]	RCT	China	3, 4, 5m	none	444-446	441-446

* Study does not report the difference in the percentage of responders between groups. † Observational study conducted among American Indian infants. ‡ Trial included in meta-analysis.[41] PCV = pneumococcal conjugate vaccine. PCV13 = 13-valent PCV. PCV7 = 7-valent PCV. RCT = randomised controlled trial. m = months of age. w = weeks of age. DTaP = diphtheria-tetanus-acellular pertussis vaccine. HBV = hepatitis B vaccine. IPV = inactivated polio vaccine. Hib = *Haemophilus influenzae* type b vaccine. DTwP = diphtheria-tetanus-whole-cell pertussis vaccine. OPV = oral polio vaccine. MenC = meningitis C vaccine.

For the six additional PCV13 serotypes not included in PCV7, non-inferiority comparisons in the two pivotal studies were made with the PCV7 serotype that produced the lowest percentage of responders. Non-inferiority was shown for all additional serotypes in both studies except for serotype 3 in the US study. Across the other comparative studies, more than 85% of participants had IgG ≥0.35µg/mL to all additional serotypes following a three-dose primary series of PCV13 (Appendix A, Table S1b). In the meta-analysis, the aggregate percentage of responders across studies was greater than 93% for each of the additional serotypes except serotype 3 (88%).

Several other studies have evaluated the post-primary series immunogenicity of PCV13 across a range of settings (Appendix A, Table S2).[42-52] These include studies from Europe, North America, Asia, and Africa, studies evaluating two-dose and three-dose primary series, and a study of pre-term infants. All these studies show PCV13 to be immunogenic, with a high percentage of responders post-primary series both for the serotypes shared with PCV7 (>90% across all studies after three doses for all serotypes except 6B and 23F) and for the additional six PCV13 serotypes (>80% across all studies for all serotypes except 3 and 5).

The trials comparing the immunogenicity of PCV13 and PCV7 also show good functional antibody activity post-primary series, measured by OPA, and strong booster responses. The percentage of participants with an opsonisation index (OI) ≥8 post-primary series was assessed in both pivotal studies. The cut-off of 8 was determined as a result of correlating with protection in a mouse model and in PCV7-vaccinated infants, and corresponding to IgG levels of 0.2-0.35µg/mL.[53] In both studies, more than 90% of PCV13-recipients achieved an OI ≥8 for all 13 serotypes, and geometric mean OIs (GMOIs) were comparable between PCV13- and PCV7-recipients for all shared serotypes (on the basis of overlapping CIs).[29,30] Four of the other comparative trials also report post-primary series GMOIs with similar results, the only differences between PCV13- and PCV7-recipients (on the basis of non-overlapping CIs) being for serotypes 4 (lower among PCV13- than PCV7-recipients in one study) and 19F (higher among PCV13- than PCV7-recipients in two studies).[31,35,38,39] The booster response was formally assessed in the pivotal US study in terms of the ratio of the geometric mean concentrations (GMCs) of antibody, with non-inferiority shown for all shared serotypes.[30]

On the basis of favourable comparative immunogenicity data with PCV7, PCV13 received European Commission authorisation in December 2009 and US Food and Drug Administration (FDA) approval in February 2010.

1.5.2 Comparative immunogenicity of PCV10 and PCV7

Six studies have reported a direct comparison of the post-primary series immunogenicity of PCV10 and PCV7 in terms of the percentage of responders post-primary series (Table 1.2).[54-59] All six used an enzyme-linked immunosorbent assay (ELISA) with 22F-inhibition and defined responders as serotype-specific IgG ≥0.20µg/mL; this level has been shown to be equivalent to a level of 0.35µg/mL using the WHO reference laboratory ELISA without 22F-inhibition.[60] For the pivotal study used for the approval of PCV10,[54] non-inferiority for each of the shared serotypes was defined by a lower limit of the 96.5% CI for the difference in the percentage of responders (PCV10-PCV7) post-primary series of greater than -10.0%. For the three additional PCV10 serotypes not included in PCV7, non-inferiority comparisons were made with the aggregate response in the PCV7 group (the percentage of responders across all seven serotypes). Overall non-inferiority was demonstrated if found for at least seven of the ten individual serotypes. As such, the one-sided alpha of 0.025 was adjusted by 7/10; hence 96.5% CIs were used. Non-inferiority was met for eight serotypes (all except serotypes 6B and 23F, Appendix A, Table S3a); thus overall non-inferiority was demonstrated. Only one other study reports non-inferiority,[58] using the same definition as used in the pivotal study, and non-inferiority was met for all serotypes. The other four comparative studies do not report the difference in the percentage of responders, but results are consistent with the two non-inferiority trials. More than 93% of participants had IgG ≥0.20µg/mL to the shared serotypes 4, 9V, 14, 18C, and 19F, and to the additional three serotypes following three doses of PCV10 across all studies. For serotypes 6B and 23F the percentage of responders ranged from 62 to 94% and from 75 to 97%, respectively, and tended to be lower in the PCV10 group than the PCV7 group (Appendix A, Table S3a).

Five of the six comparative studies also report the response to serotypes 6A and 19A. These two serotypes are known to cross-react with vaccine serotypes 6B and 19F, respectively;[61,62] some protective immunity to these serotypes may therefore be conferred by vaccination. Across the five studies, low-to-moderate responses were seen to cross-reactive serotypes 6A and 19A, with the percentage of responders ranging from 22 to 67% for 6A and from 23 to 68% for 19A (Appendix A, Table S3b).

Many other studies have evaluated the post-primary series immunogenicity of PCV10 across a range of settings (Appendix A, Table S4).[63-83] These encompass studies from Europe, North and South America, Asia, and Africa. They include studies evaluating two-dose and three-dose primary series, studies of specific populations (based on gestational age, HIV status, and sickle cell disease), and studies with a range of co-administered medications and vaccines. Despite

Table 1.2: Characteristics of studies comparing the percentage of responders following primary series vaccination with PCV10 or PCV7

Study	Design	Country	Primary series		N for post-primary series immunogenicity analysis	
			PCV schedule	Co-administered vaccines	PCV10 group	PCV7 group
Vesikari et al. 2009[54]*	RCT	Finland, France, Poland	2, 3, 4m	DTaP-HBV-IPV-Hib	1107	375
Wysocki et al. 2009[55]	RCT	Germany, Poland, Spain	2, 3, 4m	MenC-CRM + DTaP-HBV-IPV-Hib *or* MenC-TT + DTaP-HBV-IPV-Hib *or* Hib-MenC + DTaP-HBV-IPV	169 175 173	 170
Bermal et al. 2009[56]	RCT	The Philippines	6, 10, 14w	DTwP-HBV-Hib + OPV	285	94-95
		Poland	2, 4, 6m	DTwP-HBV-Hib + IPV	284-285	96
van den Bergh et al. 2011[57]	RCT	The Netherlands	2, 3, 4m	DTaP-HBV-IPV-Hib *or* DTaP-IPV-Hib	194 189	 192
Kim et al. 2011[58]*	RCT	Korea	2, 4, 6m	Hib	344	123
Knuf et al. 2012[59]	RCT	Germany	2, 3, 4m	DTaP-HBV-IPV-Hib	53	48

* Only these two trials report the difference in percentage of responders between groups. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV7 = 7-valent PCV. RCT = randomised controlled trial. m = months of age. w = weeks of age. DTaP = diphtheria-tetanus-acellular pertussis vaccine. HBV = hepatitis B vaccine. IPV = inactivated polio vaccine. Hib = *Haemophilus influenzae* type b vaccine. MenC = meningitis C vaccine. DTwP = diphtheria-tetanus-whole-cell pertussis vaccine. OPV = oral polio vaccine.

the disparities between these studies, the results are largely consistent. With the exception of a small study from the Netherlands,[83] over 90% of participants across the studies had IgG levels above the threshold for all shared serotypes except 6B and 23F. Similar results were seen for the additional three PCV10 serotypes, with over 90% of participants over the threshold in all bar two studies.[81,82]

The trials comparing the immunogenicity of PCV10 and PCV7 also show good OPA responses post-primary series, and strong booster responses. All six comparative trials report post-primary series OPA data.[54-59] More than 80% of participants in all studies achieved an OI ≥8 for nine of the ten serotypes; the exception being serotype 1, for which the percentage ranged from 43 to 82% across the studies. Comparing PCV10- and PCV7-recipients, GMOIs tended to be similar between groups or to be lower among PCV10-recipients (on the basis of overlapping or non-overlapping CIs, respectively) for most serotypes. GMOIs were lower in PCV10- than PCV7-recipients for serotypes 4 (two studies), 6B (four studies), 9V (one study), 14 (three studies), and 23F (six studies), and similar in the remaining studies. For serotype 18C, GMOIs were lower in PCV10- than PCV7-recipients in two studies, higher in one study, and similar in three studies. For serotype 19F, GMOIs were higher in PCV10- than PCV7-recipients in five of the six studies. Strong booster responses were also demonstrated, although no studies formally assessed non-inferiority of the booster response.

On the basis of favourable comparative immunogenicity data with PCV7, PCV10 received European Commission authorisation in March 2009.

1.5.3 Comparative immunogenicity of PCV10 and PCV13

Despite the availability of both PCV10 and PCV13 for over a decade, there is a paucity of data directly comparing their immunogenicity. At the time of commencing this PhD, no such data had been published. Since then, there have been three head-to-head studies that evaluate the comparative immunogenicity of PCV10 and PCV13 (Table 1.3).[84-86] Two report post-primary series data: a trial of a novel schedule at 1, 2, and 3 months of age in Papua New Guinea,[84] and a trial of a 2-, 4-, and 6-month schedule among First Nations Australians.[86] The third, a non-randomised trial of a 3+1 schedule at 2, 3, 4, and 11 months of age in the Netherlands, reports only pre- and post-booster data.[85] These studies found some differences in antibody levels between the two vaccines, but with no consistent pattern between vaccines or across serotypes. Four weeks post-primary series more than 90% of PCV10- and PCV13-recipients had IgG ≥0.35μg/mL to almost all shared serotypes, the only exceptions being 23F in the

Table 1.3: Characteristics of studies with immunogenicity data following vaccination with PCV10 or PCV13

Study	Design*	Country	Primary series		N post-primary series	
			PCV schedule	Co-administered vaccines	PCV10 group	PCV13 group
Pomat et al. 2019[84]	Comparative	Papua New Guinea	1, 2, 3m	DTwP-HBV-Hib + OPV	109	102
Leach et al. 2021[86]	Comparative	Australia	2, 4, 6m	DTaP-HBV-IPV-Hib	115	117
Wijmenga-Monsuur et al. 2015[85]	Comparative	The Netherlands	2, 3, 4m	DTaP-HBV-IPV-Hib	57†	31†
Prymula et al. 2017[87]	Non-comparative	Czech Republic, Germany, Poland, Sweden	6-14w, 3m, 4m	DTaP-HBV-IPV-Hib	132-137	132
Carmona Martinez et al. 2019[88]	Non-comparative	Czech Replublic, Germany, Poland, Spain	2, 3, 4m	DTaP-HBV-IPV-Hib	208-210	218-219
Odutola et al. 2019[89]	Non-comparative	The Gambia	2, 3, 4m	DTwP-HBV-Hib + OPV	182-196	190-195
de Los Santos et al. 2020[90]	Non-comparative	Mexico	2, 4m	not reported	79-86	80-85
Mahdi et al. 2020[91]	Non-comparative	South Africa	6, 14w	DTaP-HBV-IPV-Hib	93	95

* Design is described as “Comparative” if the study was designed to compare PCV10 and PCV13, and as “Non-comparative” if the study was not designed to compare PCV10 and PCV13 but included groups of both products. † First blood sample collected pre-booster, seven months post-primary series. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV. m = months of age. w = weeks of age. DTwP = diphtheria-tetanus-whole-cell pertussis vaccine. HBV = hepatitis B vaccine. Hib = *Haemophilus influenzae* type b vaccine. OPV = oral polio vaccine. DTaP = diphtheria-tetanus-acellular pertussis vaccine. IPV = inactivated polio vaccine.

PCV10 group in the Papua New Guinea trial and 6B in the PCV13 group in the Australia trial (Appendix A, Table S5). Similar IgG GMCs were observed between groups for 6/10 serotypes in the Papua New Guinea trial, with higher levels in the PCV13 group for 3/10 serotypes (7F, 19F, and 23F) and higher levels in the PCV10 group for 1/10 serotypes (14). In the Australia trial, similar IgG levels were observed for 2/10 serotypes (6B and 18C), with higher levels in the PCV13 group for 7/10 serotypes, and higher levels in the PCV10 group for 1/10 serotypes (19F). The Papua New Guinea and Netherlands trials report data six to seven months post-primary series. In the Papua New Guinea trial, similar IgG levels were observed for four serotypes six months post-primary series (at 9 months of age), with higher levels in the PCV13 group for three serotypes (1, 5, and 7F), and higher levels in the PCV10 group for three serotypes (6B, 18C, and 19F). In the Netherlands trial, similar IgG levels were observed for four serotypes seven months post-primary series (pre-booster data at 11 months of age), with higher levels in the PCV13 group for one serotype (19F) and higher levels in the PCV10 group for five serotypes (1, 6B, 9V, 18C, and 23F).[85]

There have also been five trials that include groups of both PCV10 and PCV13 but were not designed to make comparisons between products (Table 1.3). Three are trials of investigational vaccines that include control groups of both PCV10 and PCV13,[87-89] one evaluates mixed regimens of PCV10 and PCV13,[90] and one compares different schedules of PCV10 or PCV13.[91] Across these trials, both vaccines generated a high percentage of participants with protective levels of antibody post-primary series for most serotypes, with few observable differences between groups (Appendix A, Table S5). Broadly speaking, these data support the findings from the studies comparing the immunogenicity of these higher valency vaccines with PCV7 that both are highly immunogenic.

Differences in post-primary series antibody GMCs were more commonly observed. Responses tended to favour PCV13, albeit with variation across the studies. In at least three of the five studies, higher GMCs were observed (on the basis of non-overlapping CIs) with PCV13 than with PCV10 for serotypes 1, 7F, and 23F. In the two studies evaluating two-dose primary series, GMCs were similar between groups for seven of the shared serotypes, higher in the PCV13 group for one[91] or two[90] serotypes, and higher in the PCV10 group for one[90] or two[91] serotypes.

Strong post-primary series OPA responses were observed with both PCV10 and PCV13. More than 80% of participants achieved an OI ≥8 across the three trials with PCV10 and PCV13 control groups for all serotypes except serotype 1 (which was <80% in the PCV10 group in two trials and in the PCV13 group in one trial).[87-89] Similar to the antibody GMCs by ELISA, OPA

GMOIs to serotypes 1, 7F and 23F were higher with PCV13 than PCV10 in at least two of the three trials. GMOIs to serotype 19F were lower with PCV13 than PCV10 in all three trials. Following a two-dose primary series, GMOIs were higher with PCV13 for serotype 7F and lower for serotype 19F.[90]

The head-to-head trial from the Netherlands showed that PCV10 and PCV13 both generate a strong booster response,[85] which is supported by post-booster data from three of the non-comparative trials.[87,88,91] Post-booster antibody levels tended to favour PCV13, with higher GMCs observed in the PCV13 group for between five and seven serotypes across the four studies, and lower GMCs observed for two serotypes in one study.

Overall, the limited comparative data on the immunogenicity of PCV10 and PCV13 show that both vaccines induce strong immune responses. Post-primary series, a high percentage of participants achieve protective levels of antibody to most serotypes with either vaccine. Antibody GMCs are generally higher for more serotypes with PCV13 than PCV10, both post-primary series and post-booster, albeit with some variation across the serotypes affected and the different studies.

1.6 Non-immunological comparisons of PCV10 and PCV13

1.6.1 Comparative effect of PCV10 and PCV13 on pneumococcal carriage

Carriage data provide an important tool for the evaluation of PCVs, especially in low- and middle-income countries (LMICs) where disease surveillance is often not feasible.[92] Carriage is a prerequisite for disease, so the effect of PCV on pneumococcal carriage offers a proxy measure for the expected effects on pneumococcal disease among vaccinees.[93] A reduction in carriage leads to reduced transmission of vaccine-type (VT) pneumococci, thereby generating herd protection.[14,19,94] The effect of PCV on carriage therefore also offers a proxy measure for the expected herd protection effects of vaccination. Evaluating the post-introduction effects on carriage also provides a means of monitoring serotype replacement, the phenomenon of an increase in carriage and disease of non-vaccine serotypes that commonly accompanies the vaccine-induced decrease in VT carriage and disease.

The direct effect of PCV on carriage can only be estimated prior to (or shortly following) vaccine introduction, before indirect herd protection effects arise in the population. A review of pre-licensure efficacy trials shows that vaccination leads to a direct effect of around a 50% reduction in VT carriage.[93] After vaccine introduction, the effect on carriage among vaccinees represents the total (direct and indirect) effect, and the effect on carriage among the unvaccinated population represents the indirect herd protection effect. Two recent systematic reviews of carriage in LMICs show that substantial reductions in VT carriage are consistently observed among the target age group for vaccination following introduction of either PCV10 or PCV13.[92,95] Declines in VT carriage among unvaccinated age groups (children too young to be vaccinated, older children, and adults) are also commonly observed.[92] Together these data show that PCV10 and PCV13 have substantial direct and indirect effects on pneumococcal carriage.

At the time of commencing this PhD, there were no published data that directly compared the effect of PCV10 and PCV13 on pneumococcal carriage. Since then, the head-to-head trials from Papua New Guinea and Australia have reported short-term carriage outcomes.[84,96] At one and six months post-primary series in the Papua New Guinea trial, and at one-month post-primary series in the Australia trial, there were no differences in VT carriage between PCV10- and PCV13-recipients. There have also been two observational studies from countries where the use of PCV7 was replaced with the simultaneous use of PCV10 and PCV13. Healthy Cypriot children and Korean children hospitalised with respiratory infections had similar

carriage rates if they were vaccinated with PCV10 or PCV13.[97,98] A review comparing the effect of PCV10 and PCV13 introduction on carriage (based on observational studies) also found similar magnitude declines in VT carriage with either vaccine.[99]

1.6.2 Comparative effectiveness of PCV10 and PCV13 against disease

The ultimate goal of PCV programmes is to reduce pneumococcal disease and the recommended measure to evaluate the impact of PCV introduction is IPD surveillance.[100] There have been no head-to-head studies on the effects of PCV10 and PCV13 on pneumococcal disease, but the efficacy and effectiveness of both vaccines have been extensively reviewed for a variety of clinical endpoints in a range of settings. PCV7 introduction led to a rapid and substantial decline in IPD due to vaccine serotypes (VT-IPD). Feikin et al. (2013) conducted a meta-analysis of IPD surveillance data from HICs that showed a 66% reduction in VT-IPD one-year after PCV7 introduction and a greater-than 90% reduction from three-years post-introduction onwards.[101] Izurieta et al. (2018) later showed that a switch from PCV7 to either PCV10 or PCV13 led to an additional reduction in VT-IPD in HICs.[102] Cohen et al. (2017) reported both PCV10 and PCV13 to be effective in preventing IPD and pneumonia, with comparable effectiveness observed in HICs and LMICs (reductions in VT-IPD of 70 to 95% in HICs and of 60 to 90% in LMICs).[103] de Oliveira et al. (2016) reviewed the effectiveness of PCV10 and PCV13 on hospitalisation and death due to IPD and pneumonia in Latin American and Caribbean countries and found that both vaccines led to substantial reductions in all endpoints examined, with no evidence that one vaccine was superior to the other.[104] This finding is consistent with IPD laboratory surveillance data from the same region (Sistema Regional de Vacunas, SIREVA), which showed a substantial reduction in the number of VT-IPD isolates in both PCV10 and PCV13 countries.[105] Neither publication from this region provides a direct comparison of the two vaccines; heterogeneity between studies precluded a meta-analysis in the review, and the surveillance data lacked vaccine status information (so vaccine effectiveness could not be calculated) and came from countries with varying vaccination polices (so could not be averaged across sites).[106] Berman-Rosa et al. (2020) provided a review of studies (randomised controlled trials, case-control, and cohort studies) that estimated the direct effectiveness or efficacy of PCV10 and PCV13 in children less than 5 years of age.[107] Similar to previous reviews, they found that both vaccines demonstrated high effectiveness against VT-IPD across a range of schedules and settings (73 to 100% with PCV10 and 67 to 96% with PCV13 against their respective VT-IPD) but were unable to perform a meta-analysis due to heterogeneity between studies.

IPD surveillance data from a network of European countries (SpIDnet) was recently used to estimate the serotype-specific effectiveness of PCV10 and PCV13.[108] Few sites used PCV10 (with PCV13 sites contributing more than five times as many IPD cases as PCV10 sites), which limited the outcomes that could be evaluated. High effectiveness against PCV13-only serotype 19A and vaccine-related serotype 6C IPD was observed in PCV13 sites, with point estimates greater than 80%. Conversely, a non-significant 33% effectiveness against 19A and no effectiveness against 6C was observed in PCV10 sites. The overall effectiveness of the two vaccines against their respective VT-IPD was very similar (85% for at least one dose of PCV10 and 84% for at least one dose of PCV13).

Countries that have either switched between PCV10 and PCV13 or used both simultaneously provide another source of data on the comparative effectiveness of PCV10 and PCV13. In Quebec, Canada, PCV7 was introduced in 2004 and was replaced with PCV10 in 2009 before a switch to PCV13 in 2011. The vaccine effectiveness against VT-IPD was high with both higher-valency vaccines (97% with PCV10 and 86% with PCV13).[109] PCV10 was also effective against serotype 19A-IPD and had similar effectiveness to PCV13 against PCV13-type IPD. In Belgium, PCV7 was introduced in 2008 and was replaced with PCV13 in 2011 before a switch to PCV10 in 2015 or 2016 (region-dependent). Both vaccines were highly effective against VT-IPD, but serotype 19A-IPD increased ten-fold following the switch from PCV13 to PCV10 to become the most common serotype among children less than 2 years of age.[110] Consequently, Belgium switched back to PCV13 in 2019. In New Zealand, PCV7 was introduced in 2008 and was replaced with PCV10 in 2011 before a switch to PCV13 in 2014 and a switch back to PCV10 in 2017. Although comparative vaccine effectiveness data have not been reported, it has been shown that, similar to the findings in Belgium, the rate of serotype 19A-IPD increased following the switch back from PCV13 to PCV10.[111] Effectiveness data from the simultaneous use of PCV10 and PCV13 have been reported from one country. In Sweden, PCV product is chosen at the county level, and PCV7 (introduced between 2007 and 2009) was replaced (in October 2009) with PCV10 in some counties and PCV13 in others. Consistent with the SpIDnet data, effectiveness against serotype 19A- and 6C-IPD was demonstrated in PCV13- but not PCV10-counties, but the effectiveness against total IPD was similar with both vaccines.[112]

Otitis media (OM) is a common disease of childhood. OM can be caused by a range of bacterial pathogens, the most common of which are *S. pneumoniae* and NTHi. Both PCV10 and PCV13 have the potential to protect against OM caused by VT pneumococci, and the Protein D carrier protein in PCV10 could also confer protection against OM caused by NTHi. A recent review of the effects (efficacy, effectiveness, and impact) of PCV10 and PCV13 found that both vaccines

offer some protection against OM.[113] Four studies were identified that directly compare PCV10 and PCV13 (two in Sweden, one in Korea, and one among First Nations Australians), but all of these had design limitations that impede their ability to draw conclusions on the relative effects of the two products. The specific impact of Protein D-containing PCVs (the licensed PCV10 and its predecessor 11-valent formulation) on NTHi OM and carriage has also been reviewed.[114] The review concludes that Protein D-containing PCVs may decrease NTHi OM, but any impact appears modest and more evidence is needed. The effect on NTHi carriage is variable and, where observed, tends to be small and transient. The potential effect on NTHi carriage density is yet to be evaluated.

Overall, despite a multitude of data demonstrating that both PCV10 and PCV13 are highly effective at preventing IPD and other pneumococcal diseases caused by serotypes included in the vaccine, there remains little information on the comparative effects of these two vaccines to aid decision-makers regarding vaccine choice. The current WHO position paper on PCVs concludes that there is insufficient evidence to determine if the two vaccines differ in their overall impact on pneumococcal disease, but that PCV13 may offer advantages over PCV10 against serotypes 19A and 6C.[12] It suggests that vaccine choice should be based on logistical factors, such as vaccine price and supply, and the local and regional epidemiology of pneumococcal serotypes.

1.7 Serotype distribution in Vietnam

The relative contribution of different serotypes both to pneumococcal carriage and disease varies geographically, so local and regional data are useful to aid decision-making regarding PCV product choice. Vietnam is yet to introduce PCV into the national immunisation programme. Both PCV10 and PCV13 are available on the private market, but neither are widely used.

1.7.1 Invasive pneumococcal disease serotypes

There are few data on the serotypes that cause pneumococcal disease in Vietnam (Table 1.4). The only published data on IPD serotypes come from 60 isolates identified and serotyped from blood and/or cerebrospinal fluid (CSF) from patients of all ages admitted to the Hospital for Tropical Diseases in Ho Chi Minh City between 1993 and 2002.[115] Serotype 23F was the most common (n=23, 38%) followed by serotype 14 (n=8, 14%), with three (5%) or fewer of the other serotypes detected. IPD surveillance data (unpublished) from Central and Southern Vietnam identified and serotyped 63 isolates from children less than 5 years of age between 2012 and 2016.[116] Serotype 19F and serogroup 6 (serotypes 6A and 6B) were the most common (n=18, 29% each), followed by serotypes 23F (n=7, 11%) and 14 (n=6, 10%).

Table 1.4: Serotyping data from pneumococcal isolates from IPD and lower respiratory tract specimens in Vietnam

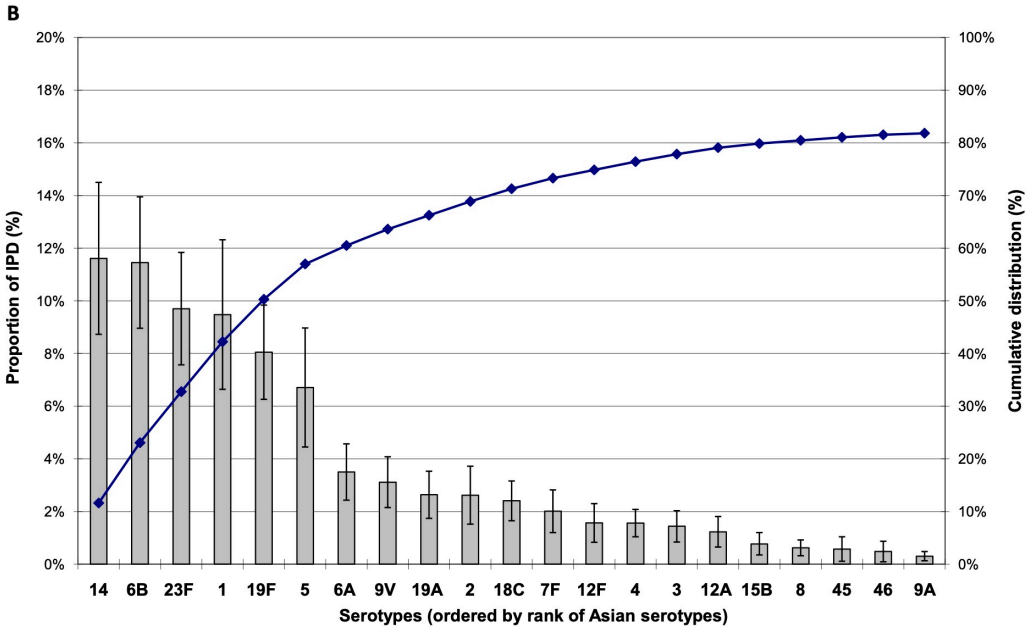
Study	Description	Year of study	Age	N	Most common serotypes
Parry et al. 2002[115]	Blood/CSF samples from 1 hospital in Ho Chi Minh City	1993-2002	All ages	60	23F (38%), 14 (13%)
Dai et al. 2017[116]	CSF samples from 2 hospitals in Ho Chi Minh City	2012-2016	<5yrs	63	19F (29%), 6A/B (29%), 23F (11%), 14 (10%)
Song et al. 2004[117] (ANSORP)	Sterile sites & lower respiratory tract specimens from 1 centre in Ho Chi Minh City	2000-2001	All ages	63	19F (41%), 23F (27%), 14 (17%)
Kim et al. 2012[118] (ANSORP)	Sterile sites & lower respiratory tract specimens from 7 hospitals in 3 cities	2008-2009	All ages	233	19F (34%), 23F (20%), 14 (10%)

IPD = invasive pneumococcal disease. CSF = cerebrospinal fluid. ANSORP = Asian Network for Surveillance of Resistant Pathogens.

Two studies of IPD in children less than 16 years of age from a single hospital in the neighbouring country of Cambodia between 2007 and 2012 and between 2013 and 2014 identified serotypes 1, 6B, 23F, 14, and 19A as the most common from 93 isolates serotyped across the two studies.[119,120] Similarly, analysis of stored isolates from IPD cases among children and adults in Cambodia between 2005 and 2014 identified serotypes 1, 14, 19A, 19F, and 23F as the most common from 26 isolates.[121] One study from the neighbouring country of the Lao People's Democratic Republic (Lao PDR) reports serotyping data from IPD isolates from patients of all ages from a single hospital between 2003 and 2009. Of only 28 isolates with serotyping data, serotypes 1 and 5 were the most common (n=6, 21% and n=4, 14%, respectively).[122]

A limitation of these studies from Vietnam and neighbouring countries on the serotypes causing IPD is the small number of isolates included. Several publications report regional estimates for IPD serotype distribution and/or expand the permissible samples to include lower respiratory tract specimens such as sputum and bronchoalveolar lavage fluid. The Asian Network for Surveillance of Resistant Pathogens (ANSORP) reports serotyping data from 11 countries (Korea, Japan, China, Thailand, Taiwan, India, Sri Lanka, Singapore, Malaysia, Indonesia, and Vietnam), from isolates recovered from normally sterile body sites and lower respiratory tract specimens in children and adults. These include 63 isolates collected between 2000 and 2001 and 233 isolates collected between 2008 and 2009 from Vietnam, with serotypes 19F, 23F, and 14 the most common in both periods (Table 1.4).[117,118] These three serotypes were also the most common across all ANSORP sites in 2000 to 2001, and in the top four serotypes (along with 19A) in 2008 to 2009, which was after the introduction of PCV in some sites.

Serotypes 19F, 23F, and 14 were also the most common in a review of IPD serotyping data from Association of South East Asian Nations countries (ASEAN: Brunei, Cambodia, Indonesia, Malaysia, Myanmar, Lao PDR, the Philippines, Singapore, Thailand, and Vietnam) published to March 2012, followed by serotypes 6B, 1, and 19A.[123] The Pneumococcal Global Serotype Project estimated the global and regional distributions of serotypes causing IPD in children less than 5 years of age from studies conducted between 1980 and 2007 in 70 different countries.[124] Of all regions, Asia had greatest diversity of serotypes, with 18 serotypes responsible for 80% of IPD compared with 15 in Africa and only 10 and 8 in Europe and North America, respectively. Serotypes 14, 6B, 23F, 1, 19F, and 5 were each responsible for more than 5% of IPD (Figure 1.5).



Source: GSP Version 2 Dec 5, 2008 AMC/TPP analyses

Figure 1.5: Proportion of IPD in young children due to the 21 most common or important serotypes in Asia

Error bars indicate the 95% confidence intervals, line indicates the cumulative proportion of IPD. *(Source: https://doi.org/10.1371/journal.pmed.1000348.s002)*

1.7.2 Pneumococcal carriage serotypes

Six distinct studies report nasopharyngeal (NP) carriage data from children less than five years of age with respiratory infections in Vietnam between 1993 and 2009 (Table 1.5). Among inpatients and outpatients with upper and lower respiratory tract infections, serotypes 19F, 23F, 6B (or 6A/B), and 14 were consistently the most commonly carried serotypes.[125-130] These serotypes are also commonly carried among children in the community in Vietnam, as shown in six studies reporting community pneumococcal carriage between 1997 and 2016 (Table 1.5). Serogroups/serotypes 19, 6, 23, and 14 represent the top four serogroups/serotypes (in varying order) in three studies from Southern Vietnam,[131-133] and serotypes 6A/B and 19F were the most commonly carried in three studies from Nha Trang along with serotypes 14, 15B/C, and 23F.[129,134,135] These data are consistent with pre-PCV introduction carriage data from outpatients aged 1 month to 15 years attending a single outpatient facility for minor illnesses in Cambodia, among whom serotypes 6B, 19F, 6A, 19A, and 23F were most commonly identified.[120] Similarly, a recent systematic review among children less than five years of age in Southeast Asia identified serotypes 6A/B, 23F and 19F as the most commonly carried pre-PCV introduction.[136] A broader systematic review of carriage in LMICs, including healthy and sick adults and children, noted that a wide spectrum of serotypes was identified, but serotypes 6A, 6B, 19A, 19F, and 23F were the most commonly isolated across 22 studies.[137]

Table 1.5: Pneumococcal carriage and serotyping data from nasopharyngeal swabs in Vietnam

Study	Description	Year of study	Age	Carriage prevalence	Most common serotypes
Respiratory infections					
Tran et al. 1998[125]	URTI outpatients from 1 hospital in Ho Chi Minh City	1993 & 1996	6m-<5yrs	32% (84/259)	6A, 6B, 14, 19F, 23F (% not reported)
Bogaert et al. 2002[126]	URTI outpatients from 3 centres in Hanoi	1997-1999	<5yrs	20% (84/410)	23F (32%), 19F (21%), 6B (13%), 14 (10%)
Watanabe et al. 2008[127]	LRTI inpatients from 2 hospitals in Hanoi	2001-2002	<5yrs	24% (53/220)	19F (47%), 23F (32%), 6B (6%)
Nguyen et al. 2008[128]	Inpatients from 1 hospital in Hai Phong (includes 4 invasive specimens)	2006-2007	<5yrs	Not reported (84 Spn positive)	19F (30%), 23F (18%), 14 (13%), 6B (13%)
Vu et al. 2011[129]	LRTI inpatients from 1 hospital in Nha Trang	2007-2008	<5yrs	41% (225/550)	19F, 6A/B, 23F, 14 (only selected % reported)
Dhoubhadel et al. 2014[130]	ARI inpatients from 1 hospital in Nha Trang	2008-2009	<5yrs	32.6% (194/595)	19F, 6A/B, 23F, 14 (% only reported graphically)
Community carriage					
Parry et al. 2000[131]	6 sites (2 kindergartens, 3 primary schools, 1 village) in Southern Vietnam	1997	1-16yrs	44% (404/911)	[n=125] serogroups 19 (21%), 23 (19%), 14 (15%), 6 (10%)
Lee et al. 2001[132]	1 site (daycare & outpatient attendees) in Ho Chi Minh City	1998-1999	<5yrs	35% (104/295)	[n=92] serogroups 6 (27%), 19 (18%), 23 (16%), 14 (10%)
Schultsz et al. 2007[133]	14 sites (8 kindergartens, 5 health centres, 1 primary school) in Southern Vietnam	2003-2004	<7yrs	38% (536/1422)	[n=178] serogroups 19 (34%), 23 (21%), 6 (10%), 14 (6%)
Vu et al. 2011[129]	Healthy community controls from LRTI study in Nha Trang	2008 (Jan)	<5yrs	50% (176/350)	6A/B, 19F, 15B/C, 14, 23F (only selected % reported)
Nguyen et al. 2019[134]	Healthy community controls from ARI study in Nha Trang	2008 (Jul)	<5yrs	29% (95/331)	[n=92] serogroup 6, 19F, 14, 15B/C (% only reported graphically)
Mohamed et al. 2021[135]	Community study in Nha Trang	2016 (Oct)	4-23m	30% (212/698)	[n=202] 6A (34%), 19F (20%), 6B (14%), 23F (9%), 14 (5%)

Carriage prevalence data are the proportion of swabs (n/N) positive for *Streptococcus pneumoniae*, expressed as a percentage. URTI = upper respiratory tract infection. LRTI = lower respiratory tract infection. ARI = acute respiratory infection. Spn = *Streptococcus pneumoniae*.

1.8 Summary

Two PCVs are in widespread use globally, PCV10 and PCV13. Both were licensed based on immunological non-inferiority to PCV7. There have been only three head-to-head trials of PCV10 and PCV13, two reporting post-primary series immunogenicity and carriage data, the other reporting post-booster immunogenicity data. These trials show no consistent pattern of differences between the two vaccines and no evidence for a differential effect on carriage. Post-introduction data from a range of settings show that both are highly effective at reducing VT-IPD and other pneumococcal diseases. On the basis of current evidence, WHO concludes that PCV10 and PCV13 have a similar overall impact on pneumococcal disease. In Vietnam, the most commonly carried serotypes among healthy children and those with respiratory infections are 19F, 6A/B, 23F, and 14, with serotypes 19F, 23F, and 14 also the most commonly identified among IPD cases. The use of either PCV10 or PCV13 in Vietnam can therefore be expected to be highly effective. We aimed to provide head-to-head data on these two vaccines in Vietnam in order to provide local data to support decision-making regarding product choice.

Chapter 2: PhD Objectives and Structure

2.1 Overview

Pneumococcus is the leading vaccine-preventable cause of death in young children, and WHO recommends the introduction of PCV as a priority.[12] PCVs have been available for over 25 years, yet 60% of the world's children are missing out on receiving this life-saving vaccine as they live in countries that are yet to introduce it or have limited vaccine coverage.[25] Introduction in Asia has been particularly slow, in part due to a lack of local data to facilitate decision-making. Notwithstanding the recent prequalification of SIIPL-PCV, countries considering PCV introduction are faced with a choice between the two currently available vaccines, PCV10 and PCV13, with two schedules recommended by WHO (a 3+0 schedule, with three doses in infancy and no booster dose, or a 2+1 schedule, with two doses in infancy and a booster dose given at 9 months or later). The overall purpose of this research is to generate data to aid decision-making regarding which of PCV10 and PCV13 would be more appropriate for Vietnam and other countries in the region.

2.1.1 Aim

The aim of this PhD is to design and conduct a trial to provide head-to-head data comparing PCV10 and PCV13.

2.1.2 Specific Objectives

The specific objectives to address the aim of this PhD are:

1. to design a randomised controlled trial to assess differences in the immunogenicity, reactogenicity, and effect on carriage of PCV10 and PCV13;
2. to directly compare the immunogenicity of a 2+1 schedule of PCV10 or PCV13 up to 18 months of age;
3. to directly compare the reactogenicity of a 2+1 schedule of PCV10 or PCV13;
4. to evaluate the effect of a 2+1 schedule of PCV10 or PCV13 on pneumococcal carriage in the first two years of life;
5. to describe which pneumococcal serotypes are most commonly carried by unvaccinated children in the first two years of life.

2.2 Data sources

2.2.1 Objective 1

Objective 1 is addressed in Chapters 3 and 4. This objective, to design a trial to directly compare PCV10 and PCV13, does not involve any data per se. The different designs that we considered and the process for site selection are described in Chapter 3. The published protocol for the trial "Evaluation of different infant vaccination schedules incorporating pneumococcal vaccination" (the Vietnam Pneumococcal Trial) forms Chapter 4.

The Vietnam Pneumococcal Trial was designed to answer two questions: firstly, what is the optimal infant PCV10 schedule; and secondly, how do the responses to vaccination with PCV10 and PCV13 compare? Of these two distinct and independent aims of the trial, the comparison of PCV10 and PCV13 contributes towards this thesis.

Briefly, the Vietnam Pneumococcal Trial involved 1,201 infants, recruited at 2 months of age, and 199 children, recruited at 18 months of age. Infants were randomised (in a 3:3:5:4:5:4 ratio) to one of six PCV schedules: PCV10 in a 3+1, 3+0, 2+1 or two-dose schedule; PCV13 in a 2+1 schedule; and controls that receive two doses of PCV10 administered at 18 and 24 months of age (Table 2.1). When the trial started, it was planned to follow participants up to 18 months of age. Follow-up was later extended to 24 months of age, necessitating recruitment of an additional group to serve as unvaccinated controls from 18 months onwards. This group received a single dose of PCV10 on completion of the study at 24 months of age.

The data that contribute to each of the specific objectives 2 to 5 are sourced from the Vietnam Pneumococcal Trial and are outlined below.

Table 2.1: Overall schedule of study vaccines and samples in the Vietnam Pneumococcal Trial

ARM	2m	3m	4m	5m	6m	7m	9m	9.5m	10m	12m	18m	19m	24m
A 3+1 (n=150)	BldX* NP1 PCV10 Hexa	PCV10 Hexa	PCV10 Hexa	Bld1	NP2		Bld2 NP3 PCV10 measles		Bld3	NP4	Bld4* NP5 MR	Hexa	NP6
B 3+0 (n=150)	NP1 PCV10 Hexa	PCV10 Hexa	PCV10 Hexa	Bld1	BldX NP2		Bld2* NP3 measles		Bld3	NP4	Bld4* NP5 MR	Hexa	NP6
C 2+1 (n=250)	NP1 PCV10 Hexa		PCV10 Hexa	Bld1	BldX* NP2		Bld2 NP3 measles	PCV10 Hexa	Bld3	NP4	Bld4* NP5 MR	Hexa	NP6
D Two-dose (n=200)	NP1 PCV10 Hexa	Bld1	Hexa		Bld2 NP2 PCV10 Hexa3	Bld3	BldX* NP3 measles			NP4	Bld4* NP5 MR	Hexa	NP6
E 2+1 PCV13 (n=250)	NP1 PCV13 Hexa	BldX*	PCV13 Hexa	Bld1	NP2		Bld2 NP3 measles	PCV13 Hexa	Bld3	NP4	Bld4* NP5 MR	Hexa	NP6
F Control 1 (n=200)	NP1 Hexa	Hexa	Hexa		NP2		NP3 measles			NP4	Bld4 NP5 PCV10 Hexa	BldX MR	BldY NP6 PCV10
G Control 2 (n=200)	Hexa or Quin†	Hexa or Quin†	Hexa or Quin†								Bld4 NP5 Hexa	BldX MR	BldY NP6 PCV10

* Samples taken for a subset of participants only – each participant provides a total of 4 bloods. † 3 doses of infant DTP-containing vaccines are required for enrolment into the study at 18m, which can be INFANRIX-HEXA or QUINVAXEM. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV. Hexa = INFANRIX-HEXA (hexavalent diphtheria, tetanus, pertussis, hepatitis B, polio, and *Haemophilus influenzae* type b vaccine). Quin = QUINVAXEM (pentavalent diphtheria, tetanus, pertussis, hepatitis B, and *Haemophilus influenzae* type b vaccine) + oral polio vaccine. MR = measles-rubella vaccine. NP = nasopharyngeal swab. Bld = venous blood sample.

2.2.2 Objective 2

Objective 2 is addressed in Chapter 5, the research paper "Immunogenicity and reactogenicity of ten-valent versus 13-valent pneumococcal conjugate vaccines among infants in Ho Chi Minh City, Vietnam: a randomised controlled trial".

The data for Objective 2, to compare the immunogenicity of PCV10 and PCV13, are sourced from venous blood samples collected between 2 and 18 months of age. When the trial was designed, the PCV10-manufacturers requested that the primary comparisons in the trial be made with their recommended schedule at that time, a 3+1 schedule. As such, the primary objective in the protocol was based on the 2+1 PCV13 schedule versus the 3+1 PCV10 schedule, with the 2+1 PCV13 schedule versus 2+1 PCV10 schedule listed as a key secondary objective despite being the main comparison of interest. By the time the trial was completed, the 2+1 schedule was well-established and the head-to-head comparison could be reported as the main output, although both comparisons are reported side-by-side in the publication.

The Vietnam Ministry of Health Ethics Committee stipulated that a maximum of four blood samples could be collected per participant. In order to maximise the questions that could be addressed by the trial, the timepoints for the collection of blood samples varied both between and within study groups. Consequently, the PCV10 immunogenicity data is sourced from different study groups at different timepoints (Table 2.2).

Table 2.2: Vietnam Pneumococcal Trial groups and samples that contribute to Objective 2 – the comparative immunogenicity of PCV10 and PCV13

Timepoint	Sample description	PCV10 Group	PCV13 Group
2 months	Pre-PCV	A*†	A*†
3 months	Post-dose one PCV	D	E*
5 months	Post-primary series	A+B (three-dose primary series)	E
		C (two-dose primary series)	
9 months	Pre-booster	C	E
10 months	Post-booster	C	E
18 months	18 months of age	C*	E*

* Samples collected from a subset of participants only (at 18 months, n = 50 participants per group; at other timepoints, n = [total minus 50] participants per group). † Pre-PCV samples were only collected from Group A but should be representative of the entire study population due to randomisation. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV.

Immunogenicity was assessed using both binding assays (ELISA) and functional assays (OPA). From all blood samples, the concentrations of serotype-specific IgG antibodies to all PCV13 serotypes were determined at the pneumococcal laboratory at the Pasteur Institute of Ho Chi Minh City (Pasteur) using a modified third-generation standardised ELISA.[138] From a subset of post-primary series and post-booster blood samples (half of the participants in each group), functional antibody responses to all PCV13 serotypes were assessed at the New Vaccines Group laboratories at the Murdoch Children's Research Institute, Melbourne, Australia (MCRI) using a multiplexed OPA method.[139] More detailed laboratory methods are included in Appendix C, the Supplementary Appendix to the protocol paper that forms Chapter 4.

2.2.3 Objective 3

Objective 3 is addressed in Chapter 5, the research paper "Immunogenicity and reactogenicity of ten-valent versus 13-valent pneumococcal conjugate vaccines among infants in Ho Chi Minh City, Vietnam: a randomised controlled trial".

The data for Objective 3, to compare the reactogenicity of a 2+1 schedule of PCV10 and PCV13, are sourced from diary cards that were provided to the parents at each vaccination visit to record temperature, erythema, and other reactions on days 0-3 post-vaccination. Reactogenicity information following primary series immunisations at 2 and 4 months of age were compared between groups that received PCV10 and INFANRIX-HEXA (hexavalent diphtheria, tetanus, pertussis, hepatitis B, polio, and *Haemophilus influenzae* type b vaccine, DTaP-HBV-IPV-Hib; Group C), PCV13 and DTaP-HBV-IPV-Hib (Group E), or DTaP-HBV-IPV-Hib alone (Group F; Table 2.3). Reactogenicity information following booster immunisations at 9.5 months of age were compared between groups that received PCV10 and DTaP-HBV-IPV-Hib (Group C) or PCV13 and DTaP-HBV-IPV-Hib (Group E). There was no group that received DTaP-HBV-IPV-Hib alone at the booster timepoint for comparison.

Table 2.3: Vietnam Pneumococcal Trial groups that contribute to Objective 3 – the comparative reactogenicity of PCV10 and PCV13

Timepoint	Vaccines administered		
	PCV10 and DTaP-HBV-IPV-Hib	PCV13 and DTaP-HBV-IPV-Hib	DTaP-HBV-IPV-Hib alone
Post-2-month dose	C	E	F
Post-4-month dose	C	E	F
Post-booster dose	C	E	n/a

PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV. DTaP-HBV-IPV-Hib = hexavalent diphtheria, tetanus, pertussis, hepatitis B, polio, and *Haemophilus influenzae* type b vaccine. n/a = not applicable.

2.2.4 Objective 4

Objective 4 is addressed in Chapter 6, the research paper "Effect of a 2+1 schedule of ten-valent versus 13-valent pneumococcal conjugate vaccine on pneumococcal carriage: Results from a randomised controlled trial in Vietnam".

The data for Objective 4, to evaluate the impact of 2+1 schedules of PCV10 and PCV13 on pneumococcal carriage, are sourced from NP swabs collected at 2, 6, 9, 12, 18, and 24 months of age. Pneumococcal carriage rates were compared between PCV10-recipients (Group C), PCV13-recipients (Group E), and unvaccinated controls (Table 2.4). As described in Section 2.2, participant follow-up was initially planned to finish at 18 months of age, at which time Group F (control) participants received their first dose of PCV. When follow-up was extended to 24 months of age, an additional control group (Group G) was recruited to serve as unvaccinated controls from 18 to 24 months of age. Thus, data for unvaccinated controls are sourced from Group F from 2 to 12 months of age, Group F and G combined at 18 months of age, and Group G at 24 months of age.

NP swabs from the 2- to 12-month timepoints were cultured at the pneumococcal laboratory at Pasteur, or by Pasteur scientists at the Child Health laboratory at Menzies School of Health Research, Darwin, Australia, and serotyped using latex agglutination and Quellung reaction.[140,141] NP swabs from the 18- and 24-month timepoints underwent molecular testing at the Translational Microbiology Group laboratories at MCRI.[142,143] These samples were screened using *lytA* real-time quantitative PCR before culture amplification and serotyping by DNA microarray from the resultant growth. More detailed laboratory methods are included in Appendix C, the Supplementary Appendix to the protocol paper that forms Chapter 4.

Table 2.4: Vietnam Pneumococcal Trial groups that contribute to Objective 4 – the comparative effect of PCV10 and PCV13 on pneumococcal carriage

Timepoint	2+1 PCV10	2+1 PCV13	Unvaccinated controls
2 months	C	E	F
6 months	C	E	F
9 months	C	E	F
12 months	C	E	F
18 months	C	E	F and G
24 months	C	E	G

PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV.

2.2.5 Objective 5

Objective 5 is addressed in Chapter 6, the research paper "Effect of a 2+1 schedule of ten-valent versus 13-valent pneumococcal conjugate vaccine on pneumococcal carriage: Results from a randomised controlled trial in Vietnam".

The data for objective 5, to describe the most commonly carried pneumococcal serotypes in the absence of vaccination, come from the same collection of NP swabs used for the Objective 4 analyses. All swabs collected from participants prior to receipt of their first dose of PCV were included. As such, the data are sourced from Groups C, E, and F combined at 2 months of age (as all participants were unvaccinated at the time of their 2-month swab), Group F at 6, 9, and 12 months of age, Groups F and G combined at 18 months of age, and Group G at 24 months of age.

2.3 My role in the Vietnam Pneumococcal Trial

My role in the Vietnam Pneumococcal Trial was Trial Manager. I was intrinsically involved in all stages of the trial, from the concept and design phase through to the data analysis and publication phase. I wrote the funding application, the ethics applications, and the trial protocol, with input from the investigators. I wrote the Plain Language Statement and Informed Consent Form, the study forms (including the data collection forms), and the standard operating procedures, in conjunction with the Study Coordinator, Kathryn Bright. As Trial Manager I worked closely with the Study Coordinator in the day-to-day running of the trial. I was responsible for all reporting requirements for the ethics committees and funding bodies.

I oversaw the data management for the trial. I designed an EpiData database (version 3.1, Odense, Denmark) for entry of the clinical and demographic data collected at each study visit, and worked with the Clinical Epidemiology & Biostatistics Unit at MCRI in the design of a Microsoft Access database for data management and retention. I designed and generated queries in the Access database to check and validate the data in preparation for analysis. I also ran checks on the immunology and microbiology data. With the exception of the density analysis (included in Appendix E, the Supplementary Appendix to the research paper in Chapter 6), I performed all statistical analyses and generated all figures presented in this thesis, using Stata statistical software (release 14 or 15, StataCorp LLC, TX). I wrote or co-wrote the research papers that form Chapters 4-6 and responded to the peer review comments for each paper. My role in each of the research papers is summarised in the Research Paper Cover Sheets included at the start of each of these chapters.

Approval for the Vietnam Pneumococcal Trial was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia, the Institutional Review Board at the Pasteur Institute of Ho Chi Minh City, Vietnam, and the Ethical Review Committee for Bio-medical Research, Ministry of Health, Vietnam. Approval to base my thesis on the Vietnam Pneumococcal Trial was also obtained from the London School of Hygiene & Tropical Medicine (Appendix B).

Chapter 3: The Vietnam Pneumococcal Trial

3.1 Evolution of the trial design

The Vietnam Pneumococcal Trial was initially conceived to follow on from the Fiji Pneumococcal Project, an earlier randomised controlled trial of different pneumococcal vaccine schedules. That trial evaluated reduced-dose primary series vaccination with PCV7, with or without a booster dose of PPV23 at 12 months of age, and with a 20% micro-dose of PPV23 administered at 17 months of age.[144] Participants received no doses, one dose (at 14 weeks of age), two doses (at 6 and 14 weeks of age), or three doses (at 6, 10, and 14 weeks of age) of PCV7. Participants also received three doses of diphtheria, tetanus, whole-cell pertussis, *Haemophilus influenzae* type B, and Hepatitis B vaccine (DTwP-Hib-HBV) at 6, 10, and 14 weeks of age.

Data from the Fiji Pneumococcal Project showed similar immunogenicity following two doses or three doses of PCV7.[144] Evidence from Hib conjugate vaccines[145,146] and from trials of an investigational 11-valent PCV[147] suggests that the immunogenicity of PCVs could be enhanced by co-administration with whole cell pertussis compared with acellular pertussis. With a trend towards using acellular pertussis-containing combination vaccines for infants in LMICs, and the development of PCV13 to supersede PCV7, we designed a trial involving a two-dose or three-dose primary series of PCV13, co-administered with a three-dose primary series of a combination vaccine containing whole cell or acellular pertussis. To build on the Fiji Pneumococcal Project, we included four different booster options in this design: PCV13 at 12 months of age, a full-dose or a micro-dose (20%) of PPV23 at 12 months of age, or a micro-dose of PPV23 at 9 months of age (Table 3.1).

Following the development of this design, it emerged that WHO was considering recommending 2+1 childhood immunisation schedules for LMICs, with two doses in early infancy and a booster dose late in the first year of life. At the same time, preliminary data from the Fiji Pneumococcal Project showed excellent responses to the micro-dose of PPV23 administered at 17 months of age in previously un-boosted children, supporting the use of this lower dose as a booster. We therefore modified the design to include PCV13 in a two-dose primary series at 6 and 14 weeks of age, with a booster dose of either PCV13 or PPV23 at 9 months of age (Table 3.2).

Groups would receive a combination vaccine containing whole cell pertussis at 6, 10, and 14 weeks of age (the EPI schedule at the time), whole cell pertussis in a 2+1 schedule (at 6 weeks, 14 weeks, and 9 months of age), or acellular pertussis in a 2+1 schedule (at 6 weeks, 14 weeks, and 9 months of age).

Table 3.1: Proposed PCV and DTP vaccination schedules in the initial trial design

Primary Series				**Booster**		
Group*	**6 weeks**	**10 weeks**	**14 weeks**	**Group***	**9 months**	**12 months**
A	PCV13 DTwP	PCV13 DTwP	PCV13 DTwP	1		PCV13
B	PCV13 DTaP	PCV13 DTaP	PCV13 DTaP	2		PPV23
C	PCV13 DTwP	DTwP	PCV13 DTwP	3		mPPV23
D	PCV13 DTaP	DTaP	PCV13 DTaP	4	mPPV23	

* All combinations of primary series groups (A, B, C, and D) and booster groups (1, 2, 3, and 4) to be evaluated. PCV = pneumococcal conjugate vaccine. DTP = diphtheria-tetanus-pertussis combination vaccine. PCV13 = 13-valent PCV. DTwP = DTP containing whole cell pertussis. DTaP = DTP containing acellular pertussis. mPPV23 = micro-dose of 23-valent pneumococcal polysaccharide vaccine.

Table 3.2: Proposed PCV and DTP vaccination schedules in the second trial design

Group	**Primary Series**			**Booster**
	6 weeks	**10 weeks**	**14 weeks**	**9 months**
A	PCV13 DTwP		PCV13 DTwP	PCV13 or mPPV23 DTwP
B	PCV13 DTaP		PCV13 DTaP	PCV13 or mPPV23 DTaP
C	PCV13 DTwP	DTwP	PCV13 DTwP	PCV13 or mPPV23

PCV = pneumococcal conjugate vaccine. DTP = diphtheria-tetanus-pertussis combination vaccine. PCV13 = 13-valent PCV. DTwP = DTP containing whole cell pertussis. DTaP = DTP containing acellular pertussis. mPPV23 = micro-dose of 23-valent pneumococcal polysaccharide vaccine.

Full analysis of data from the Fiji Pneumococcal Project, comparing children who had and had not received a booster dose of PPV23 at 12 months of age, showed evidence of hyporesponsiveness to the micro-dose of PPV23 at 17 months of age.[148] As a consequence, we removed PPV23 from the proposed schedules in the new trial. Another key finding from the Fiji Pneumococcal Project data came from the group that received a single-dose primary series of PCV7 at 14 weeks of age. Antibody responses to this dose reached protective levels for five of the seven serotypes and were significantly higher than those of unvaccinated controls for all serotypes.[144] Furthermore, the booster response to PPV23 was higher following a single-dose primary series for four of the seven serotypes than following a two-dose or three-dose primary series, and was comparable for the other three serotypes.[149] These data suggested that schedules using two doses (with a significant interval between doses) may provide better long-term protection, and warranted further investigation.

In light of these data, we redesigned the trial to incorporate a two-dose schedule of PCV13 at 6 weeks and 6 months of age, to be compared with a 2+1 schedule of PCV13 at 6 weeks, 14 weeks, and 6 months of age. We retained one group with a three-dose primary series of DTP and included 2+1 schedules of PCV13 co-administered with either DTwP or DTaP (Table 3.3).

Table 3.3: Proposed PCV and DTP vaccination schedules in the third trial design

Group	Primary Series			Booster	
	6 weeks	10 weeks	14 weeks	6 months	9 months
A	PCV13 DTwP		PCV13 DTwP		PCV13 DTwP
B	PCV13 DTwP		PCV13 DTwP	PCV13 DTwP	
C	PCV13 DTwP		 DTwP	PCV13 DTwP	
D	PCV13 DTwP	 DTwP	PCV13 DTwP		PCV13
E	PCV13 DTaP		PCV13 DTaP		PCV13 DTaP

PCV = pneumococcal conjugate vaccine. DTP = diphtheria-tetanus-pertussis combination vaccine. PCV13 = 13-valent PCV. DTwP = DTP containing whole cell pertussis. DTaP = DTP containing acellular pertussis. mPPV23 = micro-dose of 23-valent pneumococcal polysaccharide vaccine.

After this redesign, discussions with Pfizer regarding the provision of PCV13 for the trial came to a standstill. By contrast, GlaxoSmithKline Biologicals developed an interest in the study and agreed to donate PCV10. We therefore modified the design to include multiple PCV10 schedules, whilst maintaining a single PCV13 schedule to provide the first planned head-to-head comparison of the two vaccines.

We also felt, following discussions with and feedback from both researchers within the pneumococcal field and potential funding bodies, that the applicability of the data generated in this trial and its utility in guiding government decision-making regarding pneumococcal vaccine introduction in the region would be enhanced by moving the trial location from Fiji, a small Pacific Island nation, to Asia. We selected Vietnam as the new location for the trial as a country with a strong health system, a track record of conducting relevant clinical trials and a government with a strong interest both in the trial and in introducing PCV in the near future. Consultation with WHO also determined that trial results from Vietnam would be considered to be applicable to other countries in the region.

One consequence of relocating the trial site from Fiji to Vietnam was that inclusion of DTwP and DTaP comparisons were no longer viable, due to a lack of two licensed DTP-combination vaccines that differ only in their pertussis component. The presence of other differences in the vaccines would make interpretation of the results difficult; we therefore decided to give all participants four doses of INFANRIX-HEXA (DTaP-HBV-IPV-Hib). After several further iterations of the design, taking into account the timing of the routine Expanded Program on Immunzation (EPI) schedule in Vietnam and the growing interest in the impact of a booster dose of PCV, we arrived at the design of the Vietnam Pneumococcal Trial, involving four different PCV10 schedules (a 3+1, 3+0, 2+1 and a two-dose schedule), a 2+1 PCV13 schedule, and a control group (Table 3.4; see Chapter 4 for details).

Table 3.4: PCV and DTP vaccination schedules in the Vietnam Pneumococcal Trial

Group	2m	3m	4m	6m	9/9.5m	18m	19m	24m
A	PCV10 DTP	PCV10 DTP	PCV10 DTP		PCV10		DTP	
B	PCV10 DTP	PCV10 DTP	PCV10 DTP				DTP	
C	PCV10 DTP		PCV10 DTP		PCV10 DTP		DTP	
D	PCV10 DTP		DTP	PCV10 DTP			DTP	
E	PCV13 DTP		PCV13 DTP		PCV13 DTP		DTP	
F	DTP	DTP	DTP			PCV10 DTP		PCV10
G						DTP		PCV10

PCV = pneumococcal conjugate vaccine. DTP = diphtheria-tetanus-pertussis combination vaccine. m = months of age. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV.

3.2 Selection of a clinical trial site

Vietnam is a Southeast Asian country on the Indochina Peninsula. It comprises 63 administrative units: 58 provinces and five municipalities, which are divided into districts and further divided into communes. Each commune has a Commune Health Centre (CHC) that provides preventive health services including EPI immunisations. CHCs have excellent knowledge about their population, including records of the number of births.

Initially, our partner institution was the National Institute of Hygiene and Epidemiology (NIHE), based in the northern municipality of Hanoi (Figure 3.1). The first trial site we explored was in Khánh Hòa Province on the south-central coast, around the province capital city of Nha Trang (Figure 3.1). NIHE had a history of conducting research in this province; however the long distance to transport samples to NIHE for processing was a major disadvantage of this site. Furthermore, we were aware of a large pneumonia surveillance study being conducted in Nha Trang to provide baseline data for potential future pneumococcal vaccine introduction studies, and did not want to influence the results of that study in any way by vaccinating a portion of the community.

The second site we explored was the rural northern province of Phú Thọ (Figure 3.1). This site had the advantage of being closer to NIHE, although the transport of samples would still have taken several hours, especially during the wet season. Another interesting feature of this site was that one of the minority ethnic groups in Vietnam, the Mường, was over-represented, accounting for around one third of the local population despite making up only 1.5% of the total population of Vietnam. However, the rural nature of this site meant that communes were small but disperse, and we would have had to conduct the trial in 12 different CHCs to achieve an adequate recruitment rate. This would have posed serious logistical difficulties, especially in relation to vaccine and sample transport.

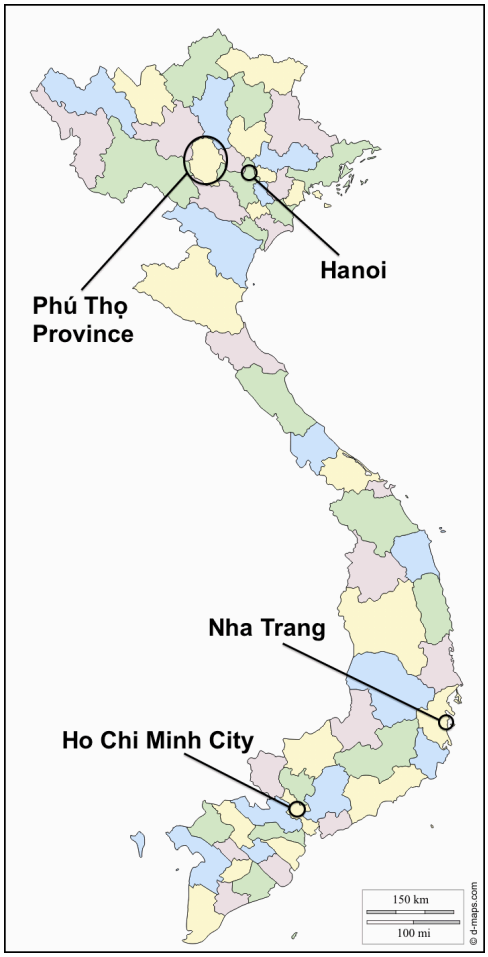


Figure 3.1: Map of Vietnam showing potential trial sites

The problems encountered in Phú Thọ province led us to consider a site in a municipality. NIHE were not willing to consider Hanoi as a potential site, as they believed that participant recruitment and retention rates would be too low to make the trial viable. We therefore explored sites within the southern municipality of Ho Chi Minh City (Figure 3.1), and changed our partner institution to Pasteur, located in District 3 of Ho Chi Minh City (Figure 3.2). Along with other members of the investigator team, I visited health centres within different districts to examine their records and assess the suitability of the site for conducting a vaccine trial. Factors considered included: the number of births, the proportion of the population who receive vaccinations through the private sector, the proportion of the population classed as transient, the timeliness of infant vaccinations, and the proximity to Pasteur. As a result of this process, Districts 4 and 7 were selected as sites for the trial (Figure 3.2). We established a study clinic at one CHC in each study district, and participants were drawn from all communes within that district. We also established a project office at Pasteur, from where the trial was managed, and constructed three laboratories at Pasteur for the processing, storage and analysis of trial samples (bloods and NP swabs).

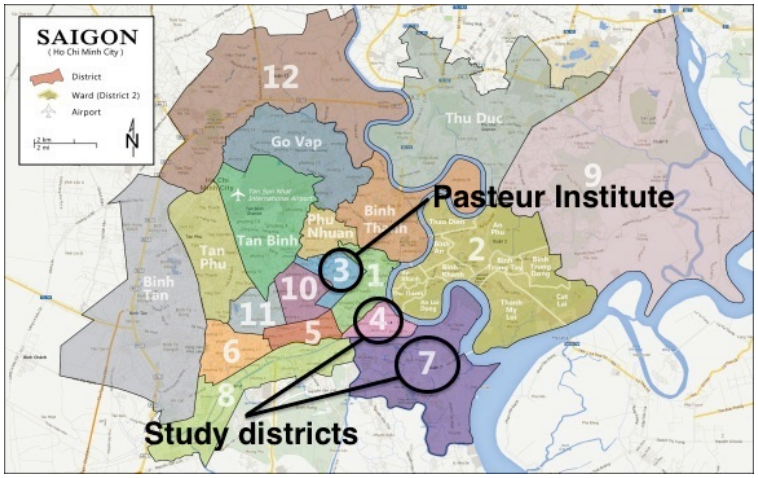


Figure 3.2: Map of Ho Chi Minh City showing the location of the study districts and the Pasteur Institute

Chapter 4: Research paper - The Vietnam Pneumococcal Trial protocol

The protocol for the Vietnam Pneumococcal Trial was published in BMJ Open in 2018 and is linked to Objective 1 of this thesis: to design a randomised controlled trial to assess differences in the immunogenicity, reactogenicity, and effect on carriage of PCV10 and PCV13. The timeframe for the literature review contributing to this manuscript is to 31 August 2017. The supplementary material from the publication is included in Appendix C. My contribution to this paper is detailed in the Research Paper Cover Sheet.

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Student ID Number	082622	**Title**	Miss
First Name(s)	Ellinor Beth		
Surname/Family Name	Temple		
Thesis Title	Pneumococcal vaccination for developing countries: PCV10 or PCV13?		
Primary Supervisor	Kim Mulholland		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	BMJ Open		
When was the work published?	2018		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was intrinsically involved in the development of the trial design and wrote the full trial protocol. I drafted the protocol manuscript and responded to peer review comments.

SECTION E

Student Signature	[redacted]
Date	6/1/23

Supervisor Signature	K. Mulholland
Date	25/1/23

BMJ Open

Evaluation of different infant vaccination schedules incorporating pneumococcal vaccination (The Vietnam Pneumococcal Project): protocol of a randomised controlled trial

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ABSTRACT

Introduction WHO recommends the use of pneumococcal conjugate vaccine (PCV) as a priority. However, there are many countries yet to introduce PCV, especially in Asia. This trial aims to evaluate different PCV schedules and to provide a head-to-head comparison of PCV10 and PCV13 in order to generate evidence to assist with decisions regarding PCV introduction. Schedules will be compared in relation to their immunogenicity and impact on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Methods and analysis This randomised, single-blind controlled trial involves 1200 infants recruited at 2 months of age to one of six infant PCV schedules: PCV10 in a 3+1, 3+0, 2+1 or two-dose schedule; PCV13 in a 2+1 schedule; and controls that receive two doses of PCV10 and 18 and 24 months. An additional control group of 200 children is recruited at 18 months that receive one dose of PCV10 at 24 months. All participants are followed up until 24 months of age. The primary outcome is the post-primary series immunogenicity, expressed as the proportions of participants with serotype-specific antibody levels ≥0.35 µg/mL for each serotype in PCV10.

Ethics and dissemination Ethical approval has been obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (EC00153) and the Vietnam Ministry of Health Ethics Committee. The results, interpretation and conclusions will be presented to parents and guardians, at national and international conferences, and published in peer-reviewed open access journals.

Trial registration number NCT01953510; Pre-results.

Strengths and limitations of this study

- This study is specifically designed to address two independent questions within a single study: which schedule to use for the provision of pneumococcal conjugate vaccine (PCV), and which PCV to use.
- This study includes a head-to-head comparison of the two licensed PCVs, allowing a direct assessment of their relative immunogenicity and impact on nasopharyngeal carriage.
- The primary outcome is the criteria used for the licensing and varying of PCV schedules.
- This study has relatively low power for the secondary nasopharyngeal carriage outcomes, so the ability to draw conclusions relating to these outcomes is vulnerable in the event of lower-than-anticipated carriage rates.

INTRODUCTION

Background and rationale

Streptococcus pneumoniae (pneumococcus) remains a leading vaccine preventable cause of serious infection in young children, despite the availability of effective vaccines. The first infant pneumococcal vaccine, the 7-valent pneumococcal conjugate vaccine (PCV7), was licensed in the USA in the year 2000. Introduction of PCV7 has been associated with dramatic reductions in pneumococcal disease.[1–3] However, geographical variation in serotype distribution[4–7] and an increase in invasive pneumococcal disease (IPD) caused by non-PCV7 serotypes following vaccine introduction[8] necessitated the development of higher valency PCVs.

There are currently two licensed PCVs: PCV10, a 10-valent pneumococcal vaccine that uses non-typeable *Haemophilus influenzae* (NTHi) protein D as a carrier protein for 8 of the 10 serotypes (*Synflorix*, PHiD-CV; GSK); and PCV13, a 13-valent pneumococcal CRM_{197} conjugate vaccine (*Prevnar-13/Prevenar-13*; Pfizer). Both have been shown to be non-inferior to PCV7 in terms of post-primary series immunogenicity for the shared serotypes.[9–11]

Despite the availability of both PCV10 and PCV13 for several years, there have been no published studies to date directly comparing their post-primary series immunogenicity or impact on nasopharyngeal (NP) carriage.

The cost of PCVs is a major barrier to vaccine introduction in low-income to middle-income countries; therefore, investigation of alternative schedules with a reduced number of doses is of great importance. The uptake of PCV introduction in Asia has been particularly slow. Three schedules are currently in routine use around the world for PCV introduction: a 3+1 schedule (a three-dose primary series followed by a booster dose in the second year of life), a 3+0 schedule (a three-dose primary series without a booster dose) and a 2+1 schedule (a two-dose primary series followed by a booster dose in the second year of life). Data from periods of PCV7 shortage in the USA show high vaccine effectiveness of a two-dose primary series against IPD,[12 13] and trial data of CRM_{197}-conjugated PCVs show comparable immunogenicity following a two-dose or three-dose primary series, although antibody levels to serotypes 6B and 23F tend to be lower after two doses.[14 15] Trials of PCV10 and PCV13 also support the use of a two-dose primary series. A trial of PCV10 in Europe directly comparing the immunogenicity of a two-dose and three-dose primary series showed a similar proportion of participants achieving protective antibody levels (≥0.2 µg/mL) for all 10 serotypes.[16] In a trial of PCV13 in Mexico, over 93% of participants achieved protective antibody levels (≥0.35 µg/mL) for most of the 13 serotypes following two doses, with the exception of serotypes 6B and 23F.[17] Four trials in Europe directly comparing PCV13 and PCV7 responses showed comparable immune responses between the vaccines following two doses.[18]

In developing countries, a 2+1 schedule with a booster dose in the first year of life may be advantageous. This modified schedule would likely increase compliance, would provide full immunisation closer to the peak incidence of pneumococcal disease and could enable the booster dose to coincide with measles vaccination. Alternatively, a further reduced PCV schedule with only two doses may be optimal for pneumococcal vaccination. Our previous trial in Fiji showed that protective antibody levels were reached for five of the seven serotypes following a single dose of PCV7 at 14 weeks of age.[15] Furthermore, a booster dose of the 23-valent pneumococcal polysaccharide vaccine at 12 months of age was more immunogenic following a single dose primary series of PCV7 compared with a two-dose or three-dose primary series for four serotypes, and comparable for the other three serotypes.[19] A trial of PCV9 from South Africa also showed that one dose at 6 weeks of age elicited a significant response for seven serotypes,[20] and modelling data from the USA suggest that a single dose of PCV could prevent up to 62% of IPD.[21] More recently, in the UK, where routine infant PCV vaccination has been in place for over 10 years, a 1+1 schedule of PCV13 was shown to elicit equivalent or superior post-booster responses compared with a 2+1 schedule for nine serotypes.[22]

Carriage of pneumococci in the nasopharynx is commonly a prerequisite for IPD and is the usual means of transmission of the bacteria. The herd effect of pneumococcal vaccination is mediated by the impact on NP carriage.[23] Vaccination with PCVs generally results in a decrease in vaccine type (VT) pneumococcal carriage, which is most commonly observed after a booster dose and often accompanied by a compensatory increase in non-VT carriage.[23–27] There have been few trials that evaluate the effect of different PCV schedules on carriage. A trial from the Netherlands showed that a two-dose primary series with or without a booster reduced VT carriage at 12 months of age compared with controls.[28] VT carriage was further reduced at 18 months in the group that received the booster dose, compared with the group that did not receive the booster, although this difference did not persist at 24 months of age. Similarly, our trial in Fiji showed that a two-dose or three-dose primary series with or without a booster reduced VT carriage at 12 months of age compared with controls, but no difference was seen at 17 months of age (F Russell, personal communication).

It has been hypothesised that the protein D carrier in PCV10 may result in an impact on *H. influenzae* carriage. A recent review of the impact of protein D-containing PCVs on NTHi carriage concludes that any such impact is likely to be small and transient, although changes in the density of carriage are yet to be evaluated. Two large phase III trials (POET trial of an 11-valent PCV and COMPAS trial of PCV10) showed trends towards a reduction in NTHi carriage following a booster dose of PCV, along with a trial of PCV10 in toddlers in Kenya, but other trials conducted in Finland, the Netherlands and the Czech Republic showed no impact of PCV10 on NTHi carriage.[29]

This trial includes six infant vaccination schedules: four different PCV10 schedules (arm A, a 3+1 schedule at 2, 3, 4 and 9 months of age; arm B, a 3+0 schedule at 2, 3 and 4 months; arm C, a 2+1 schedule at 2, 4 and 9.5 months; and arm D, a two-dose schedule at 2 and 6 months); a 2+1 PCV13 schedule at 2, 4 and 9.5 months (arm E); and a control group that receives two doses of PCV10 at 18 and 24 months (arm F). In response to more recent interest in schedules with only one or two doses of PCV, which may be sufficient to maintain herd immunity at the population level, an additional control group is recruited at 18 months of age for comparison with the initial control group (arm G).

Explanation for choice of comparators

There was no PCV licensed in Vietnam at the time the protocol was finalised in 2013. The inclusion of control groups that receive no infant doses of PCV is therefore justified. Control group participants recruited in infancy receive two doses of PCV10, at 18 and 24 months of age. Control group participants recruited at 18 months of age receive a single dose of PCV10 at 24 months of age. Intervention group participants receive at least two doses of PCV in the first year of life. All participants receive pneumococcal immunisation that is likely to be effective and is not otherwise available in Vietnam. The specific regimens to be evaluated are based on likely future global

Table 1 Schedule of enrolment, interventions and assessments

Age (months)	2	3	4	5	6	7	9	9.5	10	12	18	19	24
Enrolment													
Informed consent	X										X*		
Eligibility assessment	X										X*		
Allocation	X												
Interventions													
PCV10—group A	X	X	X				X						
PCV10—group B	X	X	X										
PCV10—group C	X		X					X					
PCV10—group D	X				X								
PCV13—group E	X		X					X					
PCV10—group F											X		X
PCV10—group G													X
Assessments													
Demographics	X										X*		
Household characteristics	X										X*		
Nasopharyngeal swab	X				X		X			X	X		X
Blood sample—group A	X†			X			X		X		X†		
Blood sample—group B				X	X		X†		X		X†		
Blood sample—group C				X	X†		X		X		X†		
Blood sample—group D		X			X	X	X†				X†		
Blood sample—group E		X†		X			X		X		X†		
Blood sample—group F											X	X	X
Blood sample—group G											X	X	X
General health	X	X	X	X	X	X	X	X	X	X	X	X	X

*Group G only. Any events occurring before 18 months do not apply to group G.
†Each participant provides only one of these blood samples (the last 50 participants per group enrolled into groups A–E provide this blood sample at 18 months; the remainder provide it at the other time point).

recommendations and to directly compare the two licensed PCVs.

Both PCV10 and PCV13 have been shown to be non-inferior to PCV7 for the serotypes common to both vaccines, and to have the potential to provide protection against the additional serotypes included.[9–11] For both vaccines, the most common adverse reactions are redness at the injection site and irritability, which are common following administration of other vaccines. Other adverse reactions may include drowsiness; temporary loss of appetite; pain, redness or swelling at the injection site; and fever. Such reactions are usually temporary.

OBJECTIVES

This trial has been designed to answer two independent questions concurrently, relating to the evaluation of different schedules incorporating PCV10 and the comparison of PCV10 and PCV13:

1. What is the optimal schedule for provision of EPI vaccines with the incorporation of PCV10; and
2. How do the responses to vaccination with PCV10 or PCV13 compare?

The primary endpoint for both study questions is the post-primary series immunogenicity. For this endpoint, data from arms A and B are combined, as they receive an identical three-dose primary series (see table 1 for a detailed description of the trial arms). The primary analysis for each study question is to assess non-inferiority of the post-primary series immunogenicity (in terms of the proportion of participants achieving protective levels of serotype-specific IgG of ≥0.35 µg/mL), using arms A+B as the comparator (see below for details). Non-inferiority is assessed for each of the 10 serotypes in PCV10, and an overall conclusion of non-inferiority drawn if found for at least 7 of the 10 serotypes.

What is the optimal schedule for provision of Expanded Program of Immunisation (EPI) vaccines with the incorporation of PCV10?

Primary objective

The primary objective is to compare a 2+1 schedule at 2, 4 and 9.5 months of age with a 3+1 schedule at 2, 3, 4 and 9 months of age. The primary hypothesis is that the proportion of participants with protective levels of antibody is non-inferior following a two-dose primary series

(arm C) compared with a three-dose primary series (arms A+B). The schedules will also be compared in relation to the geometric mean concentrations (GMCs) of IgG and opsonophagocytosis post-primary series; the proportion of participants with protective levels of antibody, the GMCs of IgG and opsonophagocytosis post-booster; the memory B-cell responses; and the impact on nasopharyngeal (NP) carriage rates and density of bacteria of interest.

Key secondary objectives

- To investigate an experimental two-dose schedule at 2 and 6 months of age (arm D), compared with a 3+1 schedule (arm A±B) and a 2+1 schedule (arm C); and
- To assess the impact of a booster dose on NP carriage of pneumococcus and NTHi, comparing a 3+1 schedule (arm A) with a 3+0 schedule (arm B) and with unvaccinated controls (arm F).

How do the responses to vaccination with PCV10 or PCV13 compare?

Primary objective

The primary objective is to compare a PCV13 schedule at 2, 4 and 9.5 months of age with a PCV10 schedule at 2, 3, 4 and 9 months of age. The primary hypothesis is that the proportion of participants with protective levels of antibody is non-inferior following a two-dose primary series of PCV13 (arm E) compared with a three-dose primary series of PCV10 (arms A+B). The schedules will also be compared in relation to the GMCs of IgG and opsonophagocytosis post-primary series; the proportion of participants with protective levels of antibody, the GMCs of IgG and opsonophagocytosis post-booster; the memory B-cell responses; and the impact on nasopharyngeal (NP) carriage rates and density of bacteria of interest.

Key secondary objectives

- To compare PCV10 (arm C) and PCV13 (arm E) in a 2+1 schedule at 2, 4 and 9.5 months of age; and
- To compare the responses to a single dose of PCV10 (arm D) and PCV13 (arm E).

Additional objectives

Additional objectives relating to the second control group (arm G) are:

- To evaluate a single dose of PCV10 at 18 months of age, comparing serotype-specific antibody levels in arms F and G at 18, 19 and 24 months of age; and
- To compare the immunogenicity and reactogenicity of *Infanrix-hexa* at 18 months of age in children who have received three doses of *Infanrix-hexa* or *Quinvaxem* in infancy (arm G).

Trial design

The Vietnam Pneumococcal Project is a single-blind, open-label, randomised controlled phase II/III non-inferiority trial to investigate simplified childhood vaccination schedules that are more appropriate for developing country use. This is a seven-arm trial that includes six different infant vaccination schedules (arms A–F) and an additional control group (arm G) recruited at 18 months of age (table 1). Arm A receives PCV10 at 2, 3, 4 and 9 months of age (3+1); arm B receives PCV10 at 2, 3 and 4 months (3+0); arm C receives PCV10 at 2, 4 and 9.5 months (2+1); arm D receives PCV10 at 2 and 6 months (two-dose); arm E receives PCV13 at 2, 4 and 9.5 months (2+1); arm F receives two doses of PCV10 at 18 and 24 months; and arm G receives one dose of PCV10 at 24 months. Participants also receive *Infanrix-hexa* (DTP-Hib-HBV-IPV) instead of the routine EPI vaccine *Quinvaxem* (DTP-Hib-HBV): four doses for participants in arms A–F and one dose for arm G participants.

METHODS AND ANALYSIS

Study setting

PCV introduction in Asia has been slow, in part due to a lack of local or regional data on the effect of PCV. We selected the Southeast Asian country of Vietnam as the location for the trial as a country with a strong health system, a track record of conducting relevant clinical trials, and a Government with strong interest both in the trial and in introducing PCV in the near future. Furthermore, trial results from Vietnam are likely to be considered as applicable to other countries in the region. This is the first trial involving infants to take place within Ho Chi Minh City, the largest city in Vietnam. The trial is conducted in two districts, District 4 and District 7. Districts are divided into communes, each of which has a health centre that provides preventive health services including EPI immunisations, along with some primary healthcare services. The study is conducted in one commune health centre in each district, with participants drawn from the surrounding communes within that district.

Eligibility criteria

Inclusion criteria

Subjects must meet all of the following inclusion criteria in order to be eligible to participate: aged between 2 months and 2 months plus 2 weeks (arms A–F) or aged between 18 months and 18 months plus 4 weeks (arm G); no significant maternal or perinatal history; born at or after 36 weeks' gestation; written informed consent from the parent/legal guardian; lives within approximately 30 min of the commune health centre; anticipates living in the study area for the next 22 months (arms A–F) or 6 months (arm G); and received three doses of either *Quinvaxem* or *Infanrix-hexa* in infancy (arm G only).

Exclusion criteria

Subjects meeting any of the following exclusion criteria at baseline will be excluded from study participation: known allergy to any component of the vaccine; allergic or anaphylactic reaction to any previous vaccine; known immunodeficiency disorder; known HIV-infected mother; known thrombocytopenia or coagulation disorder; on immunosuppressive medication; administration or planned administration of any immunoglobulin or blood

product since birth; severe birth defect requiring ongoing medical care; chronic or progressive disease; seizure disorder; history of invasive pneumococcal, meningococcal or *H. influenzae* type b diseases, or tetanus, measles, pertussis or diphtheria infections; receipt of any 2-month vaccines through the EPI programme (arms A–F), or receipt of PCV (arm G); or family plans on giving the infant *Quinvaxem* (arms A–F).

Interventions

PCV schedules

Eligible participants recruited in infancy are randomised to one of six different vaccination schedules (table 1). Participants randomised to arms A–D receive PCV10 in a 3+1 schedule at 2, 3, 4 and 9 months of age; a 3+0 schedule at 2, 3 and 4 months of age; a 2+1 schedule at 2, 4 and 9.5 months of age; or a two-dose schedule at 2 and 6 months of age, respectively. Participants randomised to arm E receive PCV13 in a 2+1 schedule at 2, 4 and 9.5 months of age. Control group participants receive PCV10 at 18 and 24 months of age if randomised to arm F, or PCV10 at 24 months of age if recruited to arm G at 18 months of age. PCV is administered by intramuscular injection into the anterolateral thigh in children less than 18 months old and in the deltoid muscle of the arm in children aged 18 months and over. All vaccinations are performed by nurses specifically trained in infant vaccine administration.

PCV10

PCV10 (*Synflorix*) is a 10-valent pneumococcal polysaccharide conjugate vaccine using protein D (a highly conserved surface protein from NTHi) as the main carrier protein. PCV10 is presented as a turbid white suspension in a two-dose phial. One dose consists of 0.5 mL of the liquid vaccine, containing 1 µg of pneumococcal polysaccharide from serotypes 1, 5, 6B, 7F, 9V, 14 and 23F and 3 µg of pneumococcal polysaccharide from serotypes 4, 18C and 19F. Serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F are conjugated to protein D; serotype 18C is conjugated to tetanus toxoid carrier protein; and serotype 19F is conjugated to diphtheria toxoid carrier protein.

PCV13

PCV13 (*Prevnar-13*) is a 13-valent pneumococcal polysaccharide conjugate vaccine using non-toxic diphtheria CRM_{197} carrier protein. PCV13 is presented as a 0.5 mL suspension in a single-dose pre-filled syringe. One dose contains approximately 2.2 µg of pneumococcal polysaccharide from serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F and 4.4 µg of pneumococcal polysaccharide from serotype 6B.

Criteria for discontinuing or modifying allocated interventions

There is no modification of doses for participants in this study. If a participant has an allergic or anaphylactic response to vaccination, they will be withdrawn from the study. Participants may also be withdrawn voluntarily by the parent/legal guardian at any time, or by the study staff if they refuse any further study procedures or develop any of the exclusion criteria during the course of the study.

Strategies to improve and monitor adherence

Scheduled visit dates are noted on a health record card kept by the parent. If a participant does not attend a scheduled visit, a reminder phone call is made from the study clinic. If the participant cannot be contacted directly, their local commune health centre is contacted for further follow-up by phone or by home visit.

Relevant concomitant care

Participants receive *Infanrix-hexa*, which is only available on the private market, instead of the routine EPI vaccine *Quinvaxem*. Participants in arms A–F receive four doses in one of the following schedules: 2, 3, 4 and 19 months (arms A and B); 2, 4, 9.5 and 19 months (arms C and E); 2, 4, 6 and 19 months (arm D); or 2, 3, 4 and 18 months (arm F); and participants in arm G receive one dose at 18 months of age. The routine EPI measles and measles–rubella immunisations are also provided during the course of the study: measles at 9 months of age and measles–rubella at 18 (arms A–E) or 19 (arms F–G) months of age. Participants allocated to one of the 2+1 vaccination schedules (arms C and E) receive measles at 9 months of age and receive PCV and *Infanrix-hexa* 2 weeks later. For visits with two vaccinations, the vaccines are administered in different limbs. Other vaccinations are permitted in this study with a 2-week interval from study vaccines, with the exception of *Quinvaxem* in arms A–F. Other medications are also permitted, with the exception of immunosuppressive medication and medications listed as contraindicated to the study vaccines.

Outcomes

Primary outcome measure

The primary outcome measure is the concentration of serotype-specific IgG for the 10 serotypes common to both PCV10 and PCV13, assessed 4 weeks post-primary series and measured using a modified third-generation standardised ELISA.[30] Primary comparisons between arms are made in terms of the proportion of children with antibody concentration ≥0.35 µg/mL for individual serotypes. The cut-off of 0.35 µg/mL was determined as a result of a pooled analysis of data from efficacy trials[31] and is used as the basis for non-inferiority assessments for the approval of new PCVs.[32–34]

Secondary immunogenicity outcome measures

- Serotype-specific IgG antibody concentrations for all PCV13 serotypes are measured by ELISA from all blood samples (table 1) and are summarised in terms of both the proportion of children with antibody concentration ≥0.35 µg/mL and the GMC.
- Opsonisation indices (OIs) for all PCV13 serotypes are measured by opsonophagocytic assay (OPA)[35] for 100 participants per intervention group (arms A–E) 4 weeks post-primary series and 4 weeks post-booster,

and are summarised in terms of the proportion of participants with OI ≥8 and the geometric mean titre.
- Polysaccharide-specific memory B cells for serotypes 1, 5, 6B, 14, 18C, 19A and 23F are enumerated by ELISPOT[35] for 50 participants per intervention group (arms A–E) post-booster and at 18 months of age, and for 100 participants per control group (arms F and G) at 18 and 24 months of age. The results are summarised as the median number of antibody-secreting cells.

Nasopharyngeal carriage outcome measures

- NP carriage of pneumococcal serotypes is measured by traditional culture (colonial morphology, α-haemolysis, the optochin test and *lytA* PCR where indicated)[36] and latex agglutination using type-specific antisera at 2, 6, 9 and 12 months of age in all groups and at 18 and 24 months of age in the control groups (arms F and G). NP carriage and density of pneumococcal serotypes are measured by quantitative real-time PCR (qPCR) targeting *lytA* and microarray at 18 and 24 months of age.[37 38] Overall, capsular, vaccine-type and serotype-specific carriage rates are described. The antimicrobial resistance of pneumococcal isolates is determined at 12 months of age by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method, for oxacillin, erythromycin, trimethoprim/sulfamethoxazole, ofloxacin, clindamycin, vancomycin, tetracycline and chloramphenicol. E-tests are conducted for penicillin, ceftriaxone and vancomycin where indicated, and CLSI breakpoints applied.
- NP carriage of *H. influenzae* is measured by traditional culture (colonial morphology, X and V dependence, *SiaT* PCR for discrimination from *H. haemolyticus* and the Phadebact Haemophilus coagglutination test) at 12 months of age in all groups, at 6 and 9 months of age in arms A and C, and from all swabs in the control groups (arms F and G). Overall density of *H. influenzae* carriage is measured by qPCR targeting *hpd* and *SiaT* diagnostic targets at 18 and 24 months of age.[39 40]

Immunogenicity of *Infanrix-hexa*

Immunogenicity of *Infanrix-hexa* is measured in terms of IgG levels to diphtheria, tetanus, Hib PRP antigen, hepatitis B surface antigen and *Bordetella pertussis*. IgG levels will be determined by ELISA, using commercial test kits.

An overview of the procedures for collection, transportation and laboratory analyses of the blood and NP samples can be found in online supplementary appendix 1.

Sample size

The target sample size for infant recruitment (groups A–F) is 1200 with an allocation ratio of 3:3:5:4:5:4, resulting in target group sizes of A=150, B=150, C=250, D=200, E=250 and F=200. An additional target of 200 children aged 18 months are recruited into group G. Sample size calculations are based on the primary outcome of post-primary series immunogenicity (proportion of participants with serotype-specific antibody concentrations ≥0.35 µg/mL) for each of the two study questions. A non-inferiority margin of a 10% difference in absolute risk is deemed clinically significant, as used by regulatory authorities. Non-inferiority is assessed for each of the 10 serotypes in PCV10 (comparing groups A+B with group C or group E), and an overall conclusion of non-inferiority is drawn if the alternative hypotheses are accepted for at least 7 of the 10 serotypes. This sample size provides >99% power for the overall conclusion of non-inferiority with a 5% one-sided type I error rate, estimated by simulation using a tailor-made program written for implementation in Stata with 10 000 replications.[41] Powers for serotype-specific hypotheses range from 83% to >99%, calculated in PASS Software 2002 using the Farrington-Manning (1990) method.[42] Based on findings from our earlier work in Fiji and from data available in the literature,[43–45] the assumed probabilities of antibody concentration ≥0.35 µg/mL are 95% for serotypes 1, 4, 5, 7F, 9V, 14 and 19F; 90% for serotype 18C; 80% for serotype 23F; and 75% for serotype 6B. The within-subject correlation between the multiple binary endpoints is captured by a subject-level variation term with SD 1.7 in a random-effect logistic regression model, and the loss to follow-up rate is assumed to be 5% post-primary series and 10% at 12 months of age. The sample size also provides 98% power to detect a difference in post-primary series immunogenicity following two doses of PCV10 or PCV13, defined by a 10% difference in absolute risk based on Fisher's exact test (5% two-sided).

Carriage outcomes

The sample size provides 76% and 71% power to detect a difference in NTHi carriage rates at 12 months of age between groups A and F and groups A and B, respectively, and 64% and 59% power to detect a difference in vaccine-type pneumococcal carriage rates between groups A and F and groups A and B, respectively. Difference in carriage is defined by a relative risk of 0.6. The calculations were based on Fisher's exact tests (5% one-sided), assuming carriage rates in group F (controls) of 30% for NTHi and 24% for vaccine-type pneumococci, based on data from Vietnam (L Yoshida, personal communication).

Recruitment

Participants in groups A–F are recruited from infants born in the study communes during the enrolment period. Commune health centre staff identify potential participants from the commune health centre birth records. Based on the expected number of births, around a quarter of infants born in the study communes need to be enrolled to complete recruitment within the target enrolment period of 12 months. Recruitment rates will be monitored on a monthly basis and meetings held with study staff and commune health centre staff to discuss any significant declines in recruitment rates. Commune health centre staff visit the home of potential participants

when the infant is approximately 6 weeks old and provide verbal and written information about the trial, in Vietnamese. Those interested in participating are referred to the study clinic when the infant is approximately 2 months old. At this time, written informed consent is obtained (online supplementary appendix 2), after which a study nurse/doctor examines the infant to ensure that all the eligibility criteria are met. Participants in group G are recruited from children turning 18 months old in the study communes in parallel to the children in groups A–F turning 18 months.

Allocation

The allocation sequence for groups A–F is produced using a computer-generated list of random numbers using a block randomisation scheme, stratified by district. The group allocation is contained within a sealed envelope at the study clinic, with sequential ID numbers written on the outside of the envelope. The allocation sequence is generated at Menzies School of Health Research. A study doctor will enrol participants and assign them to a study group by selecting the next available envelope. The envelope is not opened until after completion of the informed consent and eligibility assessment processes.

Blinding

All laboratory staff are blinded to the study group allocation as the key outcome measures that address the study objectives are all laboratory based. Laboratory samples are labelled with the ID number, which does not identify the study group. Given the different timing of the vaccination schedules in the different groups, the study nurses, vaccine administrators and participants will not be blinded to the study group allocation.

Data collection methods

Standardised carbon copy data collection forms are used and are completed by dedicated, trained study staff. The original is transported to the trial office for data entry, with the carbon copy filed at the clinic. Blood samples and NP swabs are collected by staff specifically trained in the collection of samples from infants, and the volume of blood collected and the swab quality are recorded.

Retention: Appointments are documented on a parent-held health record card and a reminder phone call made the week before the scheduled visit. If a participant fails to attend an appointment, a follow-up phone call is made to rebook the visit. Participants are given a small payment towards the transport costs of coming to the clinic for each study visit. Participants who miss a study visit will continue to be followed up for both sample collection and vaccine administration where possible, with attempts made to contact them until such time as they would have completed the study.

Data management

Data collection forms are double-entered by dedicated data entry staff into pre-coded EpiData V.3.1 files with built-in range and consistency checks. Entered data are validated monthly and then uploaded to a central Microsoft Access database, stored on a secure server. Immunology results are double-entered in a Microsoft Excel spreadsheet. NP culture results are entered in a Microsoft Access database and qPCR and microarray results exported from SentiNET into a Microsoft Excel database. The data collection forms and laboratory results are linked at the time of analysis.

Statistical methods

Analysis of primary and secondary outcomes

For each of the two study questions, the primary objective is to compare a 2+1 schedule of (1) PCV10 and (2) PCV13, with a 3+1 schedule of PCV10. The primary outcome is the proportion of participants with serotype-specific antibody concentrations ≥0.35 µg/mL, 4 weeks post-primary series (at 5 months of age). Data from arms A and B are combined to form the three-dose post-primary series group. The primary analyses assess the non-inferiority of (1) two doses of PCV10 at 2 and 4 months of age (arm C) compared with three doses at 2, 3 and 4 months of age (arms A+B); and (2) two doses of PCV13 at 2 and 4 months of age (arm E) compared with three doses of PCV10 at 2, 3 and 4 months of age (arms A+B). The proportion of children achieving protective levels of serotype-specific IgG (≥0.35 µg/mL) 4 weeks post-primary series is determined for each of the 10 PCV10 serotypes. The non-inferiority margin is defined by a 10% difference in absolute risk. The serotype-specific risk differences (arms A+B/arm C) with 90% CIs are calculated using the Newcombe Score method and the null hypothesis rejected if the upper bound of the CI is <10%. Overall non-inferiority is declared if at least 7 of the 10 individual null hypotheses are rejected at a one-sided 5% level of significance. Secondary data analyses to address the primary objective include the ratio of GMCs post-primary series (arm C/arms A+B and arm E/arms A+B) with 95% CIs, and the booster response analysed by analysis of covariance, adjusting for pre-booster levels.

Analysis of key secondary objectives for study question 1

- A single dose of PCV10 at 2 months of age (arm D) will be assessed for non-inferiority to three doses at 2, 3 and 4 months of age (arms A+B), as described for the primary objective.
- The impact of a booster dose on pneumococcal and NTHi carriage will be assessed at 12 months of age. Overall pneumococcal, capsular pneumococcal, PCV10 type and NTHi carriage rates will be determined. Proportions will first be compared between the 3+1 group (arm A) and the control group (arm F), using Fisher's exact test. Where significant differences are found, rates will then be compared between the 3+0 group (arm B) and controls and between the 3+1 and 3+0 groups.

Analysis of key secondary objectives for study question 2

- The immunogenicity of two doses of PCV10 or PCV13 will be compared in relation to the proportion of

participants with serotype-specific antibody concentrations ≥0.35 µg/mL (to the 10 shared serotypes), 4 weeks post-primary series (at 5 months of age). A significant difference will be indicated by a 10% difference in absolute risk, comparing PCV10 (arm C) with PCV13 (arm E), and an overall difference will be declared if at least 7 of the 10 individual null hypotheses are rejected and the seven differences are in the same direction.

- The immunogenicity of a single dose of PCV10 or PCV13 will be compared, as described for the immunogenicity of two doses.

Additional analyses

Descriptive analyses at the group level will be conducted on the OPA, ELISPOT and microarray data.

Populations of analysis

Analyses will be on a per-protocol population. The primary non-inferiority analyses will be repeated on an intention-to-treat population (ITT), with all participants analysed in the group they were randomised to. Any differences between the per-protocol and ITT analyses will be reported. For each outcome, all available data will contribute to the analyses. To investigate whether data are missing completely at random, we will explore whether attrition varies across the study arms based on baseline covariates. If differential attrition is dependent on baseline variables, we will use a modelling approach to adjust for any such baseline factors and we will present the adjusted results along with the primary analysis.

Additional populations of analysis

- OPAs will be conducted on a subset of 100 participants per group. The first 100 participants per group with both post-primary series and post-booster blood samples available will contribute to the OPA analysis.
- B-cell assays will be conducted on a subset of 50 participants per group for arms A–E and 100 participants per group for arms F and G. The last 50/100 participants enrolled per group will have blood samples collected for the B-cell analysis.

Data monitoring

Data monitoring committee: Safety oversight is under the direction of an independent Data Safety and Monitoring Board (DSMB), in accordance with a DSMB Charter kept in the trial office. The DSMB will meet approximately three times a year to review aggregate and individual participant data related to safety, data integrity and overall conduct of the trial, including a detailed review of all serious adverse events (SAEs).

Interim analyses and stopping guidelines: No interim analyses are planned. Stopping guidelines are based on safety. An extraordinary meeting of the DSMB will be called in the event that serious safety issues emerge, to provide recommendations regarding termination of the trial. A final decision to terminate rests with the Principal Investigators and the Sponsor.

Harms

Data on SAEs will be collected throughout the study, with parents asked about hospitalisations and significant signs and symptoms at each study visit and through a regular review of hospital records. Details of any SAEs will be recorded on the standard reporting form from the Vietnam Ministry of Health and reported to the Principal Investigators and the Ethics Committees. Participants will be kept under observation for 30 min following vaccine administration to monitor for any adverse reactions, and information on reactogenicity in the 72 hours following vaccine administration will be recorded on parent held diary cards.

Auditing

External site monitoring will be provided by FHI360, to independently assess protocol and good clinical practice (GCP) compliance. Monitoring visits will occur at study initiation, close-out and approximately twice a year in each study clinic. 100% of Informed Consent Forms and SAEs and a random selection of approximately 20% of participant folders will be monitored, along with the Trial Regulatory File and laboratory records.

Patient and public involvement

Patients were not involved in the development, design, recruitment or conduct of the study. Community consultation took place at the district level during the design phase, as well as discussion and approval of the design from the district and city level Ministry of Health and the People's Committee of Ho Chi Minh City. Participants will be informed of the overall study results by post, with a postal address collected at the final study visit.

ETHICS AND DISSEMINATION

Research ethics approval

The protocol, the Plain Language Statement (PLS) and the Informed Consent Form (ICF) have approval from the Institutional Review Board at the Pasteur Institute of Ho Chi Minh City, the Vietnam Ministry of Health Ethical Review Committee and the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies School of Health Research. Both Ethics Committees receive annual reports on the trial progress, for continuing approval of the trial.

Protocol amendments

Any modifications to the protocol that may impact on the conduct of the study will be documented in a formal protocol amendment and approved by both Ethics Committees prior to implementation of the changes. The modified protocol will be given a new version number and date. The Ethics Committees will also be notified of any minor corrections/clarifications or administrative changes to the protocol, which will be documented in a protocol amendment letter. Significant protocol changes will also be updated in the ClinicalTrials.gov record.

Consent

Obtaining consent

The consent process is undertaken by specifically trained study staff. The study staff will go through the PLS and ICF, translated into Vietnamese, in detail with the potential participant's parent/legal guardian. The study staff will then discuss the trial further and answer any questions that may arise. Written informed consent is required prior to enrolment of the infant into the study. Consent is obtained from the parent/legal guardian as the participants are too young to provide consent themselves. A copy of the PLS and ICF will be given to the parent/legal guardian for their records.

Ancillary studies

Specific consent for the indefinite storage of blood and NP samples for future research related to the trial will be obtained from the parent/legal guardian and recorded on the ICF. Any future research will undergo ethical review. Any samples for which indefinite storage is not consented to will be destroyed at the close of the trial.

Confidentiality

All study-related information will be stored securely and held in strict confidence. All documents kept at the study clinics, including the ICFs and participant folders, are stored in locked cabinets. All documents kept centrally are stored in the trial office, which is kept locked. Electronic data is stored in the trial office and on a secure password protected server. The electronic data and laboratory samples are coded by a unique participant number and do not contain the participant name. Access to participants' information will be granted to FHI360 for monitoring purposes, and to the Ethics Committees or DSMB if required.

Access to data

The final trial dataset will be under the custody of the trial sponsor, Murdoch Children's Research Institute (MCRI). The Principal Investigator, trial manager and trial statistician will have access to the full anonymised final dataset.

Ancillary and post-trial care

Participants are advised to come to the study clinic for ancillary care, or to Children's Hospital Number 2 in Ho Chi Minh City, where they will not be charged for treatment and services. All participants are covered by clinical trials insurance for trial related harms.

Dissemination policy

Plans

Participants will be informed of the overall study results by post, with a postal address collected at the final study visit. Following completion of the trial, the results will be submitted for publication in peer-reviewed journals, and presented at relevant international conferences. Agreements between MCRI and each of the Pasteur Institute of Ho Chi Minh City and GSK Biologicals SA provide that a party must obtain the prior approval of the other parties in advance of submitting a manuscript for publication, and that such approval will not be unreasonably withheld.

Authorship

A publication subcommittee will consider all proposed publications, with the final decision on content and authorship resting with the Principal Investigator. The role of each author will be published. Group authors may be used where appropriate. There are no plans for the use of professional writers.

Reproducible research

There are no plans to grant public access to the full protocol, participant-level dataset or statistical code.

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Contributors BT was involved with the study design, led the funding and ethics applications, has been involved in the day-to-day management of the trial and data analysis, and drafted the protocol and this manuscript. NTT advised on the study design and location, was involved in the approval processes in Vietnam and has been involved in the day-to-day management and implementation of the trial. DYU advised on the study design and location and has been involved in the day-to-day implementation of the trial. AB advised on the study design, assisted with the funding applications, and advised on and provided oversight of the immunology laboratory procedures. KB advised on the study design and location and has been responsible for the day-to-day management and implementation of the trial. YBC advised on the study design and funding applications, especially the statistical aspects of the trial. PL advised on the study design, assisted with the funding applications, and advised on and provided oversight of the immunology laboratory procedures. CDN advised on the study design and statistical analysis plan. NTMP advised on the study design and location, was involved in the approval processes in Vietnam and has been involved in the day-to-day management of the trial. CS advised on the study design, assisted with the funding applications, and advised on and provided oversight of the microbiology laboratory procedures. HS-V advised on the study design, assisted with the funding applications, and advised on and provided oversight of the microbiology laboratory procedures. TQHV advised on the study design and advised on and provided oversight of the laboratory procedures at Pasteur. TNH advised on the study design and location, undertook consultations, was involved in the approval processes in Vietnam and has had overall responsibility for the conduct of the trial in Vietnam as Site Principal Investigator. EKM conceived the study, undertook consultations, provided oversight for the funding and ethics applications, provided oversight for the conduct of the trial and data analysis, and has had overall responsibility for all aspects of the trial as the Principal Investigator. All authors contributed to refinement of the study protocol and reviewed and approved this manuscript.

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Patient consent Guardian consent obtained.

Ethics approval Vietnam Ministry of Health Ethics Committee and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research.

Provenance and peer review Not commissioned; externally peer reviewed.

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Chapter 5: Research paper - The comparative immunogenicity and reactogenicity of PCV10 and PCV13

The comparative immunogenicity and reactogenicity data were published in The Lancet Infectious Diseases in 2019 and are linked to Objectives 2 and 3 of this thesis: to directly compare the immunogenicity of a 2+1 schedule of PCV10 or PCV13 up to 18 months of age, and to directly compare the reactogenicity of a 2+1 schedule of PCV10 or PCV13. The timeframe for the literature review contributing to this manuscript is to 28 February 2019. The supplementary material from the publication is included in Appendix D. My contribution to this paper is detailed in the Research Paper Cover Sheet.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	082622	**Title**	Miss
First Name(s)	Ellinor Beth		
Surname/Family Name	Temple		
Thesis Title	Pneumococcal vaccination for developing countries: PCV10 or PCV13?		
Primary Supervisor	Kim Mulholland		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	The Lancet Infectious Diseases		
When was the work published?	2019		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

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SECTION C – Prepared for publication, but not yet published

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Please list the paper's authors in the intended authorship order:	
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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was involved in the design and day-to-day management of the research, was responsible for the data management, and analysed the data. I generated the tables and figures, wrote the first draft of the manuscript, refined the manuscript in response to input from co-authors, and responded to peer-review comments.

SECTION E

Student Signature	[redacted]
Date	6/1/23

Supervisor Signature	K. Mulholland
Date	25/1/23

Immunogenicity and reactogenicity of ten-valent versus 13-valent pneumococcal conjugate vaccines among infants in Ho Chi Minh City, Vietnam: a randomised controlled trial

Beth Temple, Nguyen Trong Toan, Vo Thi Trang Dai, Kathryn Bright, Paul Vincent Licciardi, Rachel Ann Marimla, Cattram Duong Nguyen, Doan Y Uyen, Anne Balloch, Tran Ngoc Huu, Edward Kim Mulholland**

Summary

Background Few data are available to support the choice between the two currently available pneumococcal conjugate vaccines (PCVs), ten-valent PCV (PCV10) and 13-valent PCV (PCV13). Here we report a head-to-head comparison of the immunogenicity and reactogenicity of PCV10 and PCV13.

Methods In this parallel, open-label, randomised controlled trial, healthy infants from two districts in Ho Chi Minh City, Vietnam, were randomly allocated (in a 3:3:5:4:5:4 ratio), with use of a computer-generated list, to one of six infant PCV schedules: PCV10 in a 3+1 (group A), 3+0 (group B), 2+1 (group C), or two-dose schedule (group D); PCV13 in a 2+1 schedule (group E); or no infant PCV (control; group F). Blood samples were collected from infants between 2 months and 18 months of age at various timepoints before and after PCV doses and analysed (in a blinded manner) by ELISA and opsonophagocytic assay. The trial had two independent aims: to compare vaccination responses between PCV10 and PCV13, and to evaluate different schedules of PCV10. In this Article, we present results pertaining to the first aim. The primary outcome was the proportion of infants with an IgG concentration of at least 0·35 µg/mL for the ten serotypes common to the two vaccines at age 5 months, 4 weeks after the two-dose primary vaccination series (group C *vs* group E, per protocol population). An overall difference among the schedules was defined as at least seven of ten serotypes differing in the same direction at the 10% level. We also assessed whether the two-dose primary series of PCV13 (group E) was non-inferior at the 10% level to a three-dose primary series of PCV10 (groups A and B). This trial is registered with ClinicalTrials.gov, number NCT01953510.

Findings Of 1424 infants screened between Sept 30, 2013, and Jan 9, 2015, 1201 were allocated to the six groups: 152 (13%) to group A, 149 (12%) to group B, 250 (21%) to group C, 202 (17%) to group D, 251 (21%) to group E, and 197 (16%) to group F. 237 (95%) participants in group C (PCV10) and 232 (92%) in group E (PCV13) completed the primary vaccination series and had blood draws within the specified window at age 5 months, at which time the proportion of infants with IgG concentrations of at least 0·35 µg/mL did not differ between groups at the 10% level for any serotype (PCV10–PCV13 risk difference –2·1% [95% CI –4·8 to –0·1] for serotype 1; –1·3% [–3·7 to 0·6] for serotype 4; –3·4% [–6·8 to –0·4] for serotype 5; 15·6 [7·2 to 23·7] for serotype 6B; –1·3% [–3·7 to 0·6] for serotype 7F; –1·6% [–5·1 to 1·7] for serotype 9V; 0·0% [–2·7 to 2·9] for serotype 14; –2·1% [–5·3 to 0·9] for serotype 18C; 0·0% [–2·2 to 2·3] for serotype 19F; and –11·6% [–18·2 to –4·9] for serotype 23F). At the same timepoint, two doses of PCV13 were non-inferior to three doses of PCV10 for nine of the ten shared serotypes (excluding 6B). Reactogenicity and serious adverse events were monitored according to good clinical practice guidelines, and the profiles were similar in the two groups.

Interpretation PCV10 and PCV13 are similarly highly immunogenic when used in 2+1 schedule. The choice of vaccine might be influenced by factors such as the comparative magnitude of the antibody responses, price, and the relative importance of different serotypes in different settings.

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Introduction

Streptococcus pneumoniae (pneumococcus) is a leading vaccine-preventable cause of serious infection in young children, and was estimated to cause 294 000 deaths among children younger than 5 years of age in 2015.[1] The greatest burden of pneumococcal disease and related mortality is in low-income and middle-income countries (LMICs).

Two pneumococcal conjugate vaccines (PCVs) are currently licensed for infant vaccination against pneumococcus. 13-valent PCV (PCV13) contains pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. Ten-valent PCV (PCV10) contains ten of these serotypes (except serotypes 3, 6A, and 19A), although there is evidence for some cross-protection against serotype 6A and 19A disease.[2–4] PCV10 and PCV13 have been shown to be immunologically non-inferior to the first-licensed, seven-valent PCV (PCV7),[5–7] but there are few data directly comparing

Research in context

Evidence before this study

The licensure of the two currently available pneumococcal conjugate vaccines (PCVs), the ten-valent PCV (PCV10) and the 13-valent PCV (PCV13), was based on demonstration of their immunological non-inferiority to seven-valent PCV. However, in itself, this non-inferiority does not preclude differences between these two second-generation PCVs. We searched PubMed from inception to Feb 28, 2019, using search terms including, but not limited to, "10-valent pneumococcal conjugate vaccine" OR "13-valent pneumococcal conjugate vaccine" AND "immunogenicity". Two studies have been published on the comparative immunogenicity of PCV10 and PCV13: one from the Netherlands comparing the booster response in a 3+1 schedule, and a trial of a novel schedule at 1 months, 2 month, and 3 months in Papua New Guinea. A further two European trials of investigational vaccines contained control groups that received PCV10 or PCV13 in a 3+1 schedule. These studies indicated that both vaccines are highly immunogenic. The vaccines differed little in terms of the proportions of children achieving protective levels of antibody, but differences in the geometric mean concentration of antibody were commonly observed and tended to favour PCV13, albeit with variations across the studies. Given the paucity of comparative data on PCV10 and PCV13, countries considering PCV introduction have little on which to base their decision, other than the relative cost of the vaccines.

Added value of this study

This is the first published study to compare the two currently licensed PCVs in a 2+1 schedule—a schedule increasingly used by low-income and middle-income countries (LMICs), and one of the WHO-recommended schedules. The results of this study will therefore have importance in LMIC settings, which often have a high burden of pneumococcal disease.

Implications of all the available evidence

The data from this randomised controlled trial in a LMIC support previous non-comparative data that both PCV10 and PCV13 are highly immunogenic in a 2+1 schedule, with similar reactogenicity. There are few differences between the two vaccines in relation to the 0·35 µg/mL correlate of protection, but the geometric mean concentrations of antibody, both post-primary series and post-booster, tend to be higher after vaccination with PCV13. It is hard to assess whether these differences would translate to differing degrees of protection afforded by the two vaccines, particularly for mucosal disease, in which a higher concentration of antibody might be required for protection. Vietnam and other LMICs considering vaccine introduction might wish to consider the immunological differences shown in this study in the context of their own pneumococcal epidemiology.

PCV10 with PCV13, despite these vaccines having been available for several years. A trial from Papua New Guinea compared three doses of PCV10 and PCV13 administered at 1 month, 2 months, and 3 months of age, with immunogenicity data obtained prevaccination, after dose three, and at 9 months of age.[8] Two European trials of investigational next-generation pneumococcal vaccines have included control groups of both PCV10 and PCV13, administered in a 3+1 schedule at 2 months, 3 months, 4 months, and 12–15 months of age, with immunogenicity data obtained post-primary series, pre-booster, and post-booster.[9,10] Two other trials with post-primary series immunogenicity data available are registered on ClinicalTrials.gov: a trial from The Gambia of investigational, protein-based pneumococcal vaccines administered in a 3+0 schedule that includes both PCV10 and PCV13 control groups (NCT01262872); and a trial from Mexico to evaluate mixed regimens that includes groups that received a two-dose primary series of either PCV10 or PCV13 (NCT01641133). In addition, a small, non-randomised study from the Netherlands compared booster responses to PCV10 and PCV13 given in a 3+1 schedule.[11] Broadly, these studies have shown that both PCV10 and PCV13 are highly immunogenic post-primary series and post-booster. Serotype-specific geometric mean concentrations (GMCs) of IgG antibody after vaccination with PCV13 tend to be higher post-primary series, lower pre-booster, and higher post-booster than GMCs after PCV10 vaccination, although these trends do not hold for all serotypes. Notably, of these studies, only the Papua New Guinean study[8] and the Dutch study[11] of the booster response were designed specifically to evaluate differences in the immunogenicity of the two vaccines.

Given the few comparative data, particularly data from LMICs, available to influence the choice of PCV, we did a randomised controlled trial in Vietnam (the Vietnam Pneumococcal Project) of different infant pneumococcal vaccination schedules, including a head-to-head comparison of PCV10 and PCV13 delivered in a 2+1 schedule, one of the schedules recommended by WHO.[12] The trial had two independent aims: to compare vaccination responses between PCV10 and PCV13, and to evaluate different schedules of PCV10. In this Article we present results pertaining to the first aim.

Methods

Study design and participants

We designed a parallel, open-label, randomised controlled trial to investigate simplified childhood vaccination schedules that are appropriate for use in LMICs. The trial was conducted in two districts within Ho Chi Minh City, Vietnam. Infants with no significant maternal or perinatal history and who were born at or after 36 weeks' gestation were enrolled at 2 months of age and followed up to 24 months of age. Infants were

excluded if they had any known allergy to any component of the vaccine or had had an allergic or anaphylactic reaction to any previous vaccine, had a known immunodeficiency disorder, or were born to a mother infected with HIV. Full details of the participant eligibility criteria and recruitment processes have been described previously.[13]

A parent or legal guardian of each participant provided written informed consent. The protocol was approved by the Institutional Review Board at the Pasteur Institute of Ho Chi Minh City, Vietnam, and ethical approval was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia, and the Ministry of Health Ethics Committee, Vietnam. The trial was overseen by an independent data safety and monitoring board. The protocol for this trial has been published elsewhere.[13]

Randomisation and masking

A computer-generated list of randomisation numbers was used in a block randomisation scheme, stratified by district, to allocate participants (in a 3:3:5:4:5:4 ratio) to one of six groups. This was a single-blind trial with all laboratory-based outcome assessors masked to the group allocation. Additional details of the randomisation and masking have been described previously.[13]

Procedures

Participants were assigned to receive one of six infant vaccination schedules: PCV10 in a 3+1 (group A), 3+0 (group B), 2+1 (group C), or two-dose (group D) schedule; PCV13 in a 2+1 schedule (group E); or a control group (group F) that received no infant doses of PCV (figure 1). The control group was included to contribute data primarily for the secondary nasopharyngeal carriage outcomes, which will be presented elsewhere. Participants also received four doses of the hexavalent diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b, and hepatitis B (DTaP-IPV-Hib-HepB) vaccine. Participants in groups A–E provided four blood samples over the course of the trial. The timepoints for the collection of blood samples varied both between and within study groups to enable more questions to be addressed within the confines of a maximum of four blood samples per participant (see appendix for the full schedule of vaccines and samples). As such, the number of blood samples varied by timepoint, and samples from different PCV10 study groups contributed to analyses of the comparative immunogenicity of PCV10 and PCV13 at different timepoints: pre-PCV from group A; 4 weeks after one dose of PCV from group D; post-primary series (4 weeks after two doses of PCV), pre-booster (at 9 months of age), and post-booster (4 weeks after a booster dose of PCV at 9·5 months of age) from group C; and 18 months of age from a subset of group C (figure 1). We assessed the concentrations of serotype-specific IgG antibodies to all 13 serotypes in PCV13 using a modified third-generation standardised ELISA.[14] Functional antibody response to all 13 serotypes were also assessed by opsonophagocytic assay.[15]

Outcomes

To compare vaccination responses between PCV10 and PCV13, we planned to fully evaluate the immunogenicity of a 2+1 schedule (PCV10 or PCV13 given at 2 months, 4 months, and 9·5 months of age) in a head-to-head manner. The primary outcome was the proportion of children with protective levels of antibody (defined as ≥0·35 µg/mL, assessed by ELISA). GMCs of antibodies were also recorded. The primary outcome timepoint was 4 weeks post-primary series (age 5 months). At this timepoint, we also compared the two-dose primary series of PCV13 (group E) with a three-dose primary series of PCV10 given at 2 months, 3 months, and 4 months of age (groups A and B). This comparison was listed in the protocol as the primary outcome because, at the time the trial was designed, the two-dose primary series was not an approved schedule for PCV10. Both comparisons are presented here.

Secondary outcomes also included functional antibody responses to all 13 serotypes, assessed by opsonophagocytic assay. The proportion of children with an opsonisation index of at least 8 and geometric mean opsonisation indices were recorded in a subset of participants at 4 weeks post-primary series and 4 weeks post-booster.

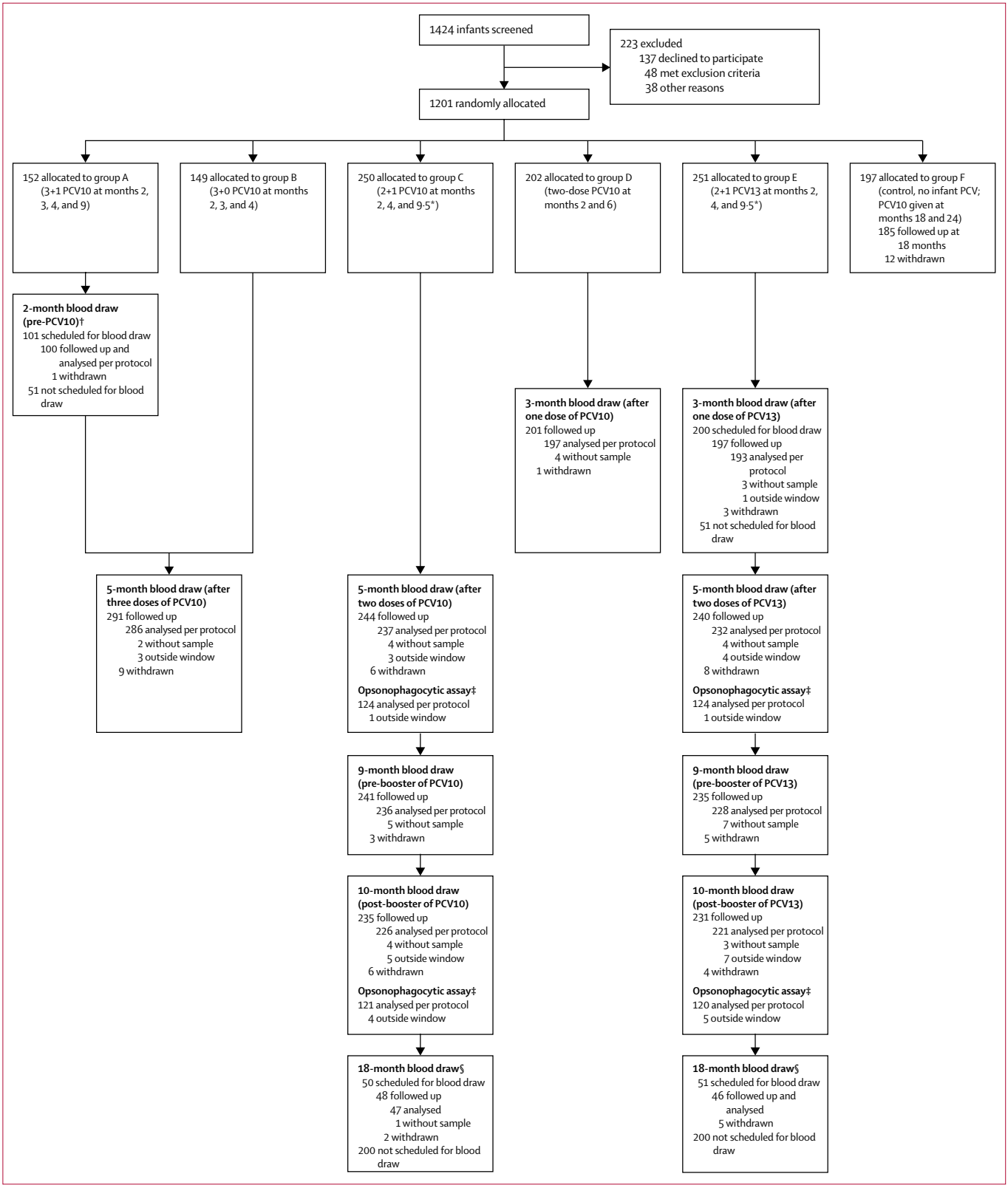
The comparative reactogenicity of PCV10 and PCV13 was also evaluated. Reactogenicity assessments included erythema at the PCV and DTaP-IPV-Hib-HepB vaccination sites and axillary temperature on days 0–4 post-vaccination, as measured by the parent or caregiver and recorded on a parent-held diary card.

A post-hoc analysis comparing the proportion of children with antibody concentrations of at least 1·00 µg/mL was done post-primary series and post-booster to explore whether the use of a higher threshold of protection would identify more differences between the vaccines.

Statistical analysis

The groups were primarily compared in terms of the proportions of children with a serotype-specific IgG concentration of at least 0·35 µg/mL at 4 weeks post-primary series (the threshold used for comparing PCV formulations). For the head-to-head comparison of two-dose primary series of PCV10 and PCV13, a 10% risk difference was considered clinically significant. Risk differences (PCV10 – PCV13) with 95% CIs were calculated with the Newcombe-Score method. The null hypothesis for each of the ten shared serotypes was that the risk difference was between –10% and 10%, with the null hypothesis rejected if the 95% CI of the risk difference was entirely outside of this range. An overall difference was considered demonstrated if at least seven of the ten individual null hypotheses were rejected in the same direction.

See Online for appendix


1424 infants screened
223 excluded
137 declined to participate
48 met exclusion criteria
38 other reasons
1201 randomly allocated
152 allocated to group A (3+1 PCV10 at months 2, 3, 4, and 9)
149 allocated to group B (3+0 PCV10 at months 2, 3, and 4)
250 allocated to group C (2+1 PCV10 at months 2, 4, and 9·5*)
202 allocated to group D (two-dose PCV10 at months 2 and 6)
251 allocated to group E (2+1 PCV13 at months 2, 4, and 9·5*)
197 allocated to group F (control, no infant PCV; PCV10 given at months 18 and 24)
185 followed up at 18 months
12 withdrawn
2-month blood draw (pre-PCV10)†
101 scheduled for blood draw
100 followed up and analysed per protocol
1 withdrawn
51 not scheduled for blood draw
3-month blood draw (after one dose of PCV10)
201 followed up
197 analysed per protocol
4 without sample
1 withdrawn
3-month blood draw (after one dose of PCV13)
200 scheduled for blood draw
197 followed up
193 analysed per protocol
3 without sample
1 outside window
3 withdrawn
51 not scheduled for blood draw
5-month blood draw (after three doses of PCV10)
291 followed up
286 analysed per protocol
2 without sample
3 outside window
9 withdrawn
5-month blood draw (after two doses of PCV10)
244 followed up
237 analysed per protocol
4 without sample
3 outside window
6 withdrawn
Opsonophagocytic assay‡
124 analysed per protocol
1 outside window
5-month blood draw (after two doses of PCV13)
240 followed up
232 analysed per protocol
4 without sample
4 outside window
8 withdrawn
Opsonophagocytic assay‡
124 analysed per protocol
1 outside window
9-month blood draw (pre-booster of PCV10)
241 followed up
236 analysed per protocol
5 without sample
3 withdrawn
9-month blood draw (pre-booster of PCV13)
235 followed up
228 analysed per protocol
7 without sample
5 withdrawn
10-month blood draw (post-booster of PCV10)
235 followed up
226 analysed per protocol
4 without sample
5 outside window
6 withdrawn
Opsonophagocytic assay‡
121 analysed per protocol
4 outside window
10-month blood draw (post-booster of PCV13)
231 followed up
221 analysed per protocol
3 without sample
7 outside window
4 withdrawn
Opsonophagocytic assay‡
120 analysed per protocol
5 outside window
18-month blood draw§
50 scheduled for blood draw
48 followed up
47 analysed
1 without sample
2 withdrawn
200 not scheduled for blood draw
18-month blood draw§
51 scheduled for blood draw
46 followed up and analysed
5 withdrawn
200 not scheduled for blood draw

The two-dose primary series of PCV13 and the three-dose primary series of PCV10 were compared in terms of non-inferiority, based on a non-inferiority margin of a 10% risk difference, as used by regulatory authorities. The null hypothesis for each of the shared serotypes was that the risk difference was greater than 10%, with the null hypothesis rejected if the upper bound of the 90% CI was less than 10% (equivalent to using a 5% one-sided test). An overall conclusion of non-inferiority was drawn if the null hypotheses were rejected for at least seven of the ten shared serotypes.

The sample size provided 98% power for an overall conclusion on the difference between two doses of PCV10 and PCV13, and more than 99% power for an overall conclusion on the non-inferiority of two doses of PCV13 compared with three doses of PCV10. Details of the sample size calculations have been described previously.[13]

IgG concentrations between groups were also compared in terms of GMC ratios (PCV10 / PCV13) with 95% CIs, and were described as higher in one group if the 95% CI excluded a ratio of 1·00. Similarly, geometric mean opsonisation indexes were described as higher in one group if the 95% CI of the ratio of geometric mean opsonisation indexes (PCV10 / PCV13) excluded a ratio of 1·00. Risk differences were calculated for the proportion of children with an opsonisation index of at least 8, with a 10% difference considered significant, in line with the IgG comparisons. Beyond the primary outcome, our aim was to provide an overall description of the pattern of differences in immunogenicity between PCV10 and PCV13. As such, no formal adjustments for multiple comparisons were made, but we have deliberately avoided reporting p values. Comparisons of reactogenicity (proportions of participants with erythema or fever) between groups were done with Fisher's exact tests.

Statistical analyses were done in accordance with the protocol and the statistical analysis plan. All immunological analyses were done on the per-protocol population, and primary analyses were repeated on the intention-to-treat population. Reactogenicity analyses were done on the intention-to-treat population. Analyses were done using Stata statistical software (release 14).

The trial is registered at ClinicalTrials.gov, number NCT01953510.

	Group A (n=152)	Group B (n=149)	Group C (n=250)	Group D (n=202)	Group E (n=251)	Group F (n=197)
Sex						
Male	66 (43%)	73 (49%)	135 (54%)	91 (45%)	127 (51%)	100 (51%)
Female	86 (57%)	76 (51%)	115 (46%)	111 (55%)	124 (49%)	97 (49%)
District						
4	68 (45%)	67 (45%)	112 (45%)	90 (45%)	111 (44%)	87 (44%)
7	84 (55%)	82 (55%)	138 (55%)	112 (55%)	140 (56%)	110 (56%)
Birthweight, g*	3234 (424)	3212 (349)	3228 (370)	3234 (410)	3199 (357)	3208 (395)
Place of delivery						
Hospital	149 (98%)	149 (100%)	245 (98%)†	194 (96%)	247 (99%)†	192 (97%)
Other	3 (2%)	0	4 (2%)	8 (4%)	3 (1%)	5 (3%)
Type of delivery						
Normal	89 (59%)	85 (57%)	160 (64%)	130 (64%)	151 (60%)	121 (61%)
Elective caesarean	30 (20%)	30 (20%)	43 (17%)	36 (18%)	57 (23%)	34 (17%)
Emergency caesarean	27 (18%)	30 (20%)	40 (16%)	34 (17%)	42 (17%)	41 (21%)
Other or unknown	6 (4%)	4 (3%)	7 (3%)	2 (1%)	1 (0·4%)	1 (1%)
Cigarette smoker at residence						
No	57 (38%)	52 (35%)	81 (33%)†	74 (37%)	86 (34%)	72 (37%)
Yes	95 (63%)	97 (65%)	168 (67%)	128 (63%)	165 (66%)	125 (63%)
Breastfeeding at enrolment						
No	41 (27%)†	42 (28%)	55 (22%)	37 (18%)	56 (22%)†	56 (29%)†
Yes	110 (73%)	107 (72%)	195 (78%)	165 (82%)	194 (78%)	140 (71%)

Data are n (%) or mean (SD). *Birthweight data missing for ten participants (one from group B, three from group C, three from group D, two from group E, and one from group F). †Data missing for one participant.

Table 1: **Baseline characteristics by study group**

Figure 1: **Trial profile**
Samples collected outside the visit window (27–43 days post-vaccination) were included only in the intention-to-treat analyses. The most common reason for participants to be without a blood sample was that the nurse was unable to successfully find a vein (18 [49%] of 37 missing blood draws). PCV=pneumococcal conjugate vaccine. PCV10=ten-valent PCV. PCV13=13-valent PCV. *PCV (and the hexavalent diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b, and hepatitis B [DTaP-IPV-Hib-HepB] vaccine) were administered at 9·5 months in participants from groups C and E because the Vietnamese Ministry of Health does not permit co-administration of measles and DTaP-IPV-Hib-HepB (see appendix for full schedules of PCV and co-administered vaccines). †The 2-month blood sample from group A provided pre-PCV data; samples at this timepoint were only collected from one study group, with the assumption that all groups would be interchangeable at baseline as a result of randomisation. ‡125 participants from groups C and E contributed to the opsonophagocytic assay analyses, selected as the first 125 with both post-primary series and post-booster blood samples collected. §Participants allocated to groups A–E from the last 300 recruited provided a blood sample at 18 months of age, with the remainder providing a sample at an alternative timepoint (appendix).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

1424 infants were screened between Sept 30, 2013, and Jan 9, 2015, with 1201 (84%) enrolled (figure 1): 152 (13%) to group A, 149 (12%) to group B, 250 (21%) to group C, 202 (17%) to group D, 251 (21%) to group E, and 197 (16%) to group F. The groups were balanced with respect to baseline characteristics (table 1). Overall, 1179 (98%) participants completed their primary series vaccinations, 1146 (95%) received their booster dose of PCV or were followed up to 9 months of age, and

	Participants with IgG concentration ≥0·35 µg/mL, % (95% CI)			Risk difference, %		GMC, µg/mL (95% CI)			GMC ratio (95% CI)	
	Two-dose PCV10 (n=237)	Three-dose PCV10 (n=286)	Two-dose PCV13 (n=232)	Two-dose PCV10 minus PCV13 (95% CI)	Three-dose PCV10 minus PCV13 (90% CI)	Two-dose PCV10 (n=237)	Three-dose PCV10 (n=286)	Two-dose PCV13 (n=232)	Two-dose PCV10 / PCV13	Three-dose PCV10 / PCV13
Shared PCV serotypes										
1	97·9 (95·1 to 99·3)	98·3 (96·0 to 99·4)	100·0 (98·4 to 100·0)	−2·1 (−4·8 to −0·1)	−1·7 (−3·5 to −0·3)	2·21 (1·97 to 2·48)	2·79 (2·51 to 3·10)	4·88 (4·40 to 5·42)	0·45 (0·39 to 0·53)	0·57 (0·49 to 0·66)
4	98·7 (96·3 to 99·7)	99·0 (97·0 to 99·8)	100·0 (98·4 to 100·0)	−1·3 (−3·7 to 0·6)	−1·0 (−2·6 to 0·3)	3·21 (2·87 to 3·58)	3·85 (3·44 to 4·31)	4·82 (4·41 to 5·26)	0·67 (0·58 to 0·77)	0·80 (0·69 to 0·93)
5	95·8 (92·4 to 98·0)	98·6 (96·5 to 99·6)	99·1 (96·9 to 99·9)	−3·4 (−6·8 to −0·4)	−0·5 (−2·3 to 1·3)	1·17 (1·07 to 1·27)	1·81 (1·67 to 1·97)	2·20 (2·00 to 2·41)	0·53 (0·47 to 0·60)	0·83 (0·73 to 0·94)
6B	76·8 (70·9 to 82·0)	84·6 (79·9 to 88·6)	61·2 (54·6 to 67·5)	15·6 (7·2 to 23·7)	23·4 (17·0 to 29·6)	0·80 (0·69 to 0·92)	1·08 (0·95 to 1·23)	0·48 (0·42 to 0·55)	1·65 (1·36 to 1·99)	2·24 (1·86 to 2·69)
7F	98·7 (96·3 to 99·7)	99·3 (97·5 to 99·9)	100·0 (98·4 to 100·0)	−1·3 (−3·7 to 0·6)	−0·7 (−2·1 to 0·5)	2·07 (1·89 to 2·27)	3·04 (2·79 to 3·32)	3·33 (3·05 to 3·63)	0·62 (0·55 to 0·71)	0·91 (0·81 to 1·03)
9V	96·2 (92·9 to 98·2)	99·3 (97·5 to 99·9)	97·8 (95·0 to 99·3)	−1·6 (−5·1 to 1·7)	1·5 (−0·3 to 3·7)	1·63 (1·47 to 1·81)	2·47 (2·26 to 2·71)	3·27 (2·93 to 3·65)	0·50 (0·43 to 0·58)	0·76 (0·66 to 0·87)
14	98·3 (95·7 to 99·5)	100·0 (98·7 to 100·0)	98·3 (95·6 to 99·5)	0·0 (−2·7 to 2·9)	1·7 (0·4 to 3·8)	5·86 (5·11 to 6·73)	9·76 (8·79 to 10·83)	7·99 (6·82 to 9·37)	0·73 (0·60 to 0·90)	1·22 (1·02 to 1·47)
18C	96·6 (93·5 to 98·5)	98·6 (96·5 to 99·6)	98·7 (96·3 to 99·7)	−2·1 (−5·3 to 0·9)	−0·1 (−2·0 to 1·9)	1·86 (1·64 to 2·11)	3·87 (3·47 to 4·30)	3·14 (2·84 to 3·48)	0·59 (0·50 to 0·70)	1·23 (1·06 to 1·43)
19F	99·2 (97·0 to 99·9)	99·7 (98·1 to 100·0)	99·1 (96·9 to 99·9)	0·0 (−2·2 to 2·3)	0·5 (−0·8 to 2·2)	9·54 (8·37 to 10·87)	8·34 (7·52 to 9·24)	7·67 (6·78 to 8·68)	1·24 (1·04 to 1·49)	1·09 (0·93 to 1·27)
23F	77·6 (71·8 to 82·8)	90·6 (86·6 to 93·7)	89·2 (84·5 to 92·9)	−11·6 (−18·2 to −4·9)	1·3 (−3·0 to 5·9)	0·89 (0·78 to 1·02)	1·32 (1·18 to 1·48)	1·14 (1·01 to 1·29)	0·78 (0·65 to 0·94)	1·16 (0·98 to 1·37)
Additional PCV13 serotypes										
3	5·9 (3·3 to 9·7)	7·0 (4·3 to 10·6)	97·8 (95·0 to 99·3)	−91·9 (−94·6 to −87·3)	−90·9 (−93·2 to −87·2)	0·10 (0·09 to 0·11)	0·11 (0·10 to 0·12)	1·53 (1·40 to 1·68)	0·07 (0·06 to 0·08)	0·07 (0·06 to 0·08)
6A	40·5 (34·2 to 47·1)	50·3 (44·4 to 56·3)	94·8 (91·1 to 97·3)	−54·3 (−60·8 to −47·0)	−44·5 (−49·7 to −38·8)	0·31 (0·28 to 0·35)	0·37 (0·34 to 0·41)	1·94 (1·69 to 2·21)	0·16 (0·14 to 0·19)	0·19 (0·16 to 0·22)
19A	70·5 (64·2 to 76·2)	68·2 (62·4 to 73·5)	98·3 (95·6 to 99·5)	−27·8 (−34·0 to −21·8)	−30·1 (−34·9 to−25·3)	0·55 (0·49 to 0·62)	0·56 (0·51 to 0·62)	3·82 (3·34 to 4·36)	0·14 (0·12 to 0·17)	0·15 (0·12 to 0·17)

Immunogenicity data at 4 weeks after two doses of PCV10 (at 2 months and 4 months of age, group C), two doses of PCV13 (at 2 months and 4 months of age, group E), or three doses of PCV10 (at 2 months, 3 months, and 4 months of age, groups A and B). GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

Table 2: **Post-primary series immunogenicity in the per-protocol population**

1093 (91%) were followed up to 18 months of age. Of the 108 participants withdrawn before 18 months, the reasons for withdrawal were: moved away and lost to follow-up (55 [51%]); refused a study procedure (23 [21%]); voluntary withdrawal (22 [20%]); and other (eight [8%]).

At 5 months of age, among the 237 (95%) participants in group C (PCV10) and 232 (92%) in group E (PCV13) who completed the primary vaccination series and had blood draws within the specified time window (figure 1), the head-to-head comparison of two doses PCV13 and two doses of PCV10 showed no evidence of a difference in the proportion of infants with a serotype-specific IgG concentration of at least 0·35 µg/mL, with the CIs for the between-group differences overlapping with the –10% to 10% range for all ten shared serotypes (table 2, figure 2). In both groups, more than 95% of participants had protective IgG concentrations for all serotypes except 6B and 23F. Comparing the magnitude of the response on the basis of GMC ratio, GMCs were higher in the PCV10 group than in the PCV13 group for serotypes 6B and 19F, and higher in the PCV13 group than in the PCV10 group for the other eight shared serotypes (table 2).

We also showed that two doses of PCV13 were non-inferior to three doses of PCV10 in terms of the proportion of infants with protective serotype-specific IgG concentrations, with the upper bound of the CI for the between-group difference less than 10% for nine of the ten shared serotypes (table 2, figure 2). The point estimates for the risk differences for these nine serotypes were all within –2% and 2%. The exception was serotype 6B, for which the proportion of participants achieving a protective IgG concentration was 84·6% (95% CI 79·9–88·6) in the PCV10 group compared with 61·2% (54·6–67·5) in the PCV13 group (risk difference 23·4% [90% CI 17·0–29·6]). IgG GMCs were higher in the PCV10 group than in the PCV13 group for serotypes 6B, 14, and 18C, and higher in the PCV13 group than in the PCV10 group for serotypes 1, 4, 5, and 9V. The conclusions based on the results of the per-protocol analysis and the intention-to-treat analysis did not differ (appendix).

In addition to the post-primary series timepoint, we directly compared responses to PCV10 and PCV13 at 4 weeks after a single dose, pre-booster, 4 weeks post-booster, and 18 months of age (figure 3, appendix). At 2 months of age, pre-PCV, the highest GMCs of antibody were seen for serotypes 14 (0·64 µg/mL [95% CI 0·49 to 0·84]), 19F (0·45 µg/mL [0·39 to 0·53]), 19A (0·41 µg/mL [0·36 to 0·47]), and 6A (0·32 µg/mL [0·28 to 0·37]), and the proportion of participants with IgG concentrations of at least 0·35 µg/mL for these four serotypes ranged from 44·0% (95% CI 34·1 to 54·3), for serotype 6A, to 68·0% (57·9 to 77·0), for serotype 14 (appendix). Comparing pre-PCV and post-PCV GMCs, a single dose of either PCV10 or PCV13 elicited no response to the shared serotypes 6B, 14, and 23F, or to the non-PCV10 types 6A and 19A. After a single dose of either PCV10 or PCV13, more than half of participants had IgG concentrations of at least 0·35 µg/mL to serotypes 1, 4, 5, 7F, 14, and 19F in both groups, and to serotype 18C in the PCV13 group. Considering a 10% difference in the proportion of participants with IgG concentrations of at least 0·35 µg/mL as clinically significant, more participants had protective concentrations of IgG specific to serotype 19F in the PCV10 group than in the PCV13 group (risk difference 18·3% [11·4 to 25·2]), and more to serotype 18C in the PCV13 group than in the PCV10 group (risk difference –33·0% [–41·7 to –23·6]; appendix). Comparing the magnitude of the response (based on the ratio of GMCs for the ten shared serotypes), GMCs were higher in the PCV10 group for serotypes 1, 4, 5, 9V, and 19F, and higher in the PCV13 group for serotypes 7F and 18C (appendix).

At 9 months of age, pre-booster and 5 months post-primary series, most participants still had protective concentrations of antibody to most of the ten shared serotypes, ranging from 75·4% (69·4 to 80·8) to 100·0% [98·4 to 100·0] in the PCV10 group, and 68·9% (62·4 to 74·8) to 99·1% (96·9 to 99·9) in the PCV13 group. The proportion of participants with protective concentrations of serotype-specific antibody was higher in the PCV10 group than in the PCV13 group for serotype 6B (risk difference 18·6% [12·4 to 24·9]), and higher in the PCV13 group than in the PCV10 group for serotype 5 (risk difference –18·4% [–24·8 to –12·0]). GMCs were higher in the PCV10 group for serotypes 6B, 18C, 19F, and 23F, and higher in the PCV13 group for serotypes 1, 5 and 7F, 9V, and 14 (appendix).

Post-booster, the proportion of participants with IgG concentrations of at least 0·35 µg/mL was more than 97% for all ten shared serotypes in both groups (appendix). In terms of GMCs, the same pattern was seen post-booster dose as post-primary series for most serotypes, with GMCs higher in the PCV10 group than in the PCV13 group for serotype 19F, and higher in the PCV13 group than in the PCV10 group for serotypes 1, 5, 7F, 9V, 14, and 23F. By contrast with the post-primary series results, post-booster GMCs were higher in the PCV10 group than in the PCV13 group for serotype 18C, and higher in the PCV13 group than in the PCV10 group for serotype 6B, with no difference between groups for serotype 4 (appendix).

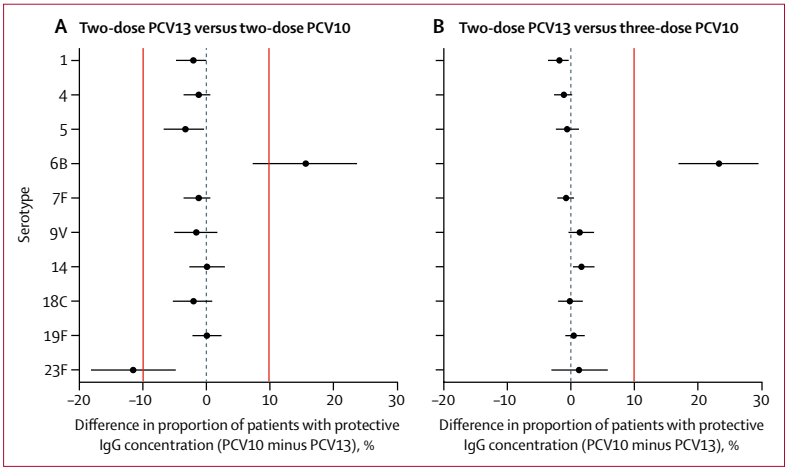


Figure 2: Comparative immunogenicity of PCV13 versus PCV10 at 4 weeks post-primary series
Data are differences (PCV10 minus PCV13) in the proportions of patients with protective serotype-specific IgG concentrations (≥0·35 µg/mL) in patients who received PCV13 versus those who received PCV10. (A) Two-dose primary series of PCV13 (at 2 months and 4 months; group E) versus two-dose primary series of PCV10 (at 2 months and 4 months; group C). (B) Two-dose primary series of PCV13 (group E) versus three-dose primary series of PCV10 (at 2 months, 3 months, and 4 months; groups A and B). Bars represent 95% CIs for two-sided tests of difference (A) or 90% CIs for one-sided tests of non-inferiority (B), with solid vertical lines indicating the predefined thresholds for determining differences or non-inferiority between groups. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

At 18 months of age, the proportion of participants with protective IgG concentrations was still greater than 95% for serotypes 14 and 19F (both groups) and serotype 6B (PCV10 group), and greater than 59% for all other shared serotypes, with no between-group differences at the 10% level (appendix). Differences in GMCs were only seen for serotypes 18C and 19F, which showed higher concentrations in the PCV10 group than in the PCV13 group (appendix).

For the non-PCV10 serotypes (3, 6A, and 19A), IgG concentrations of at least 0·35 µg/mL were seen in more than 94% of PCV13 recipients post-primary series (table 2), and more than 99% of PCV13 recipients post-booster (appendix). The GMC to serotype 3 was similar post-primary series (table 2) and post-booster (appendix) whereas GMCs for serotypes 6A and 19A increased substantially. PCV10 also elicited responses to serotypes 6A and 19A post-booster, with more than 90% of participants achieving IgG concentrations of at least 0·35 µg/mL (appendix). GMCs to all three non-PCV10 serotypes were higher in the PCV13 group than in the PCV10 group at all timepoints, with the exception of serotype 6A at 3 months of age (4 weeks post-one PCV dose) and serotype 19A at 18 months of age, for which there were no differences between the vaccine groups (appendix). The proportion of infants with serotype-specific IgG concentrations of at least 1·00 µg/mL were also compared post-primary series and post-booster

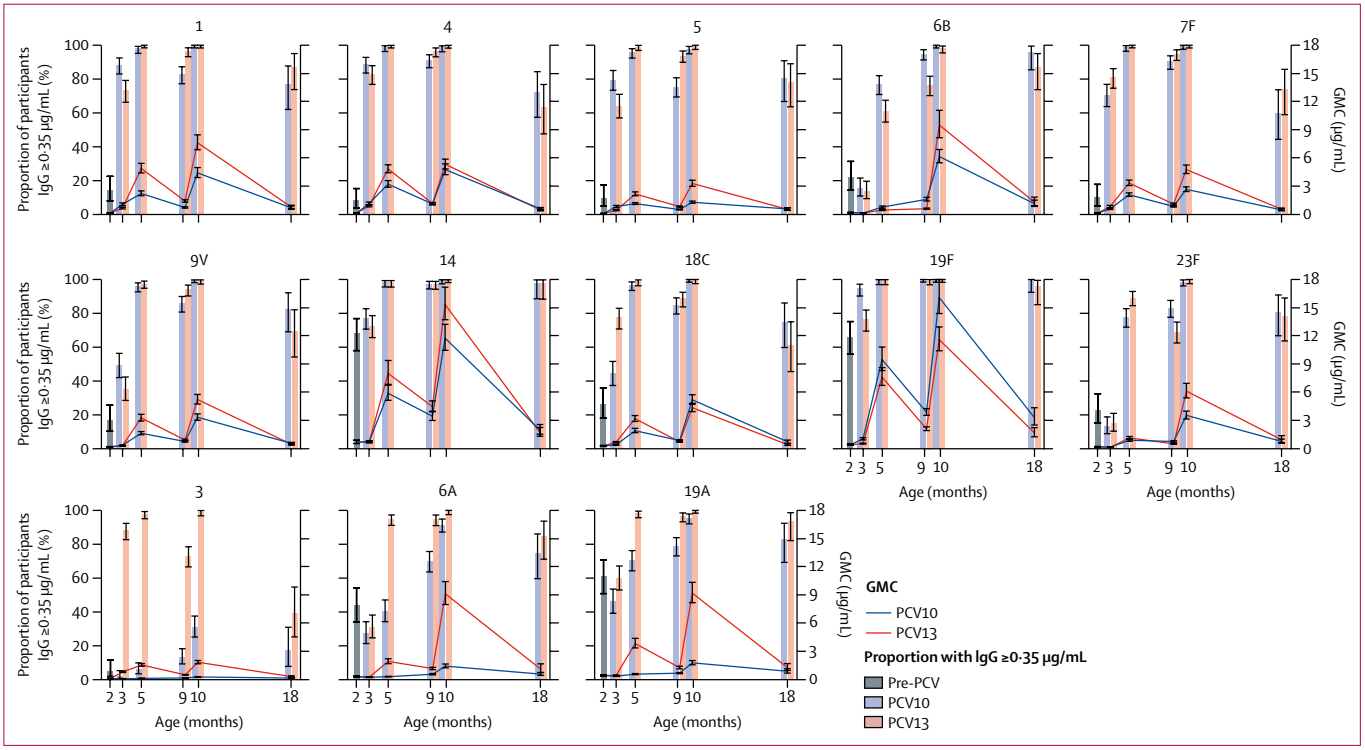


Figure 3: **Serotype-specific IgG concentrations before and after PCV10 or PCV13 vaccinations**
GMCs of serotype-specific IgG (lines) and proportion of participants with protective concentrations (≥0·35 µg/mL) of serotype-specific IgG (bars) over time, for the ten shared serotypes and the three additional serotypes in PCV13, with 95% CIs. Sources of data were as follows: group A at 2 months of age (pre-PCV); group D (PCV10) and group E (PCV13) at 3 months of age (after one dose); and group C (PCV10) and group E (PCV13) at 5 months (after two-dose primary series), 9 months (pre-booster), 10 months (post-booster), and 18 months of age (in a subset of participants). GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

(appendix). Post-primary series, more participants in the PCV13 group than in the PCV10 group had protective antibody concentrations for serotypes 1 and 5, and more participants in the PCV10 group than in the PCV13 group had protective concentrations for serotype 6B at the 10% level. Post-booster, more participants in the PCV13 group than in the PCV10 group had protective concentrations for serotype 5.

Differences in opsonophagocytic responses after the primary series of PCV10 or PCV13 vaccinations (table 3) broadly reflected those seen in the IgG concentrations. Geometric mean opsonisation indices were higher in the PCV10 group than in the PCV13 group for serotypes 6B and 19F, and higher in the PCV13 group than in the PCV10 group for all other serotypes except 14 (based on the ratio of geometric mean opsonisation indexes for the ten shared serotypes). The proportions of participants with an opsonisation index of 8 or more (table 3) also reflected the proportions of those with protective IgG concentrations for most serotypes, albeit with some exceptions: for serotype 1, more than 97% of infants in both groups had protective IgG concentrations (table 2), whereas the proportions achieving an opsonisation index of 8 or more were 66·1% (57·1–74·4) in the PCV10 group and 87·9% (80·8–93·1) in the PCV13 group (table 3). A similar pattern was seen for serotype 9V in the PCV10 group, with only 80·6% (72·6–87·2) achieving an opsonisation index of at least 8, compared with 96·2% (92·9–98·2) having a protective IgG concentration. Only serotypes 1 and 9V had differences between the PCV10 and PCV13 groups at the 10% level, with higher proportions of patients in the PCV13 group having opsonisation indices of 8 or more (table 3).

There were fewer between-group differences post-booster than post-primary series (table 3). Geometric mean opsonisation indices were higher in the PCV10 group than in the PCV13 group for serotype 19F, and higher in the PCV13 group than in the PCV10 group for serotypes 4, 6B, 7F, 9V, and 23F based on the ratio of geometric mean opsonisation indices. More than 90% of participants in both groups achieved an opsonisation index of at least 8 for the ten shared serotypes, including serotype 1, with no differences between groups at the 10% level.

PCV13 was immunogenic to each of the non-PCV10 serotypes after the primary series, with more than 92% of participants achieving an opsonisation index of at least 8, and increased responses were seen following the booster dose for serotypes 6A and 19A (table 3). As with the IgG responses, PCV10 generated little to no functional immunity for serotypes 3, 6A, and 19A post-primary series, but substantial opsonophagocytic

	Post-primary series						Post-booster					
	Participants with opsonisation index ≥8, %			Geometric mean opsonisation index			Participants with opsonisation index ≥8, %			Geometric mean opsonisation index		
	PCV10 (n=124)	PCV13 (n=124*)	Risk difference†	PCV10 (n=124)	PCV13 (n=124*)	Ratio‡	PCV10 (n=121)	PCV13 (n=120)	Risk difference†	PCV10 (n=121)	PCV13 (n=120)	Ratio‡
Shared PCV serotypes												
1	66·1 (57·1 to 74·4)	87·9 (80·8 to 93·1)	−21·8 (−31·6 to −11·4)	22 (17 to 28)	52 (40 to 67)	0·42 (0·30 to 0·61)	90·9 (84·3 to 95·4)	95·0 (89·4 to 98·1)	−4·1 (−11·1 to 2·7)	145 (106 to 198)	164 (127 to 211)	0·88 (0·59 to 1·32)
4	100·0 (97·1 to 100·0)	100·0 (97·1 to 100·0)	0·0 (−3·0 to 3·0)	922 (820 to 1036)	1320 (1188 to 1465)	0·70 (0·60 to 0·82)	99·2 (95·5 to 100·0)	100·0 (97·0 to 100·0)	−0·8 (−4·5 to 2·3)	1280 (1072 to 1529)	1771 (1560 to 2011)	0·72 (0·58 to 0·90)
5	97·6 (93·1 to 99·5)	98·4 (94·3 to 99·8)	−0·8 (−5·4 to 3·6)	351 (286 to 430)	476 (394 to 575)	0·74 (0·56 to 0·97)	98·3 (94·2 to 99·8)	100·0 (97·0 to 100·0)	−1·7 (−5·8 to 1·7)	768 (627 to 941)	929 (802 to 1076)	0·83 (0·64 to 1·06)
6B	71·8 (63·0 to 79·5)	60·5 (51·3 to 69·1)	11·3 (−0·5 to 22·6)	59 (40 to 86)	28 (20 to 40)	2·10 (1·26 to 3·50)	96·7 (91·8 to 99·1)	95·8 (90·5 to 98·6)	0·9 (−4·6 to 6·5)	299 (224 to 399)	826 (592 to 1153)	0·36 (0·23 to 0·56)
7F	96·8 (91·9 to 99·1)	98·4 (94·3 to 99·8)	−1·6 (−6·5 to 2·9)	250 (182 to 343)	570 (418 to 778)	0·44 (0·28 to 0·68)	100·0 (97·0 to 100·0)	100·0 (97·0 to 100·0)	0·0 (−3·1 to 3·1)	484 (369 to 636)	1231 (938 to 1615)	0·39 (0·27 to 0·58)
9V	80·6 (72·6 to 87·2)	99·2 (95·6 to 100·0)	−18·5 (−26·4 to −11·5)	73 (52 to 102)	267 (200 to 357)	0·27 (0·18 to 0·43)	94·2 (88·4 to 97·6)	99·2 (95·4 to 100·0)	−5·0 (−10·7 to −0·2)	308 (217 to 436)	742 (566 to 974)	0·41 (0·27 to 0·64)
14	89·5 (82·7 to 94·3)	93·5 (87·7 to 97·2)	−4·0 (−11·4 to 3·1)	132 (92 to 191)	220 (153 to 316)	0·60 (0·36 to 1·00)	96·7 (91·8 to 99·1)	96·7 (91·7 to 99·1)	0·0 (−5·3 to 5·3)	394 (293 to 531)	454 (328 to 628)	0·87 (0·56 to 1·34)
18C	88·7 (81·8 to 93·7)	96·8 (91·9 to 99·1)	−8·1 (−15·1 to −1·5)	124 (88 to 175)	242 (189 to 309)	0·51 (0·34 to 0·78)	99·2 (95·5 to 100·0)	100·0 (97·0 to 100·0)	−0·8 (−4·5 to 2·3)	732 (564 to 950)	561 (446 to 706)	1·31 (0·92 to 1·84)
19F	100·0 (97·1 to 100·0)	99·2 (95·6 to 100·0)	0·8 (−2·3 to 4·4)	1217 (1078 to 1375)	856 (728 to 1008)	1·42 (1·16 to 1·74)	100·0 (97·0 to 100·0)	98·3 (94·1 to 99·8)	1·7 (−1·6 to 5·9)	1579 (1380 to 1807)	1095 (877 to 1367)	1·44 (1·11 to 1·87)
23F	58·9 (49·7 to 67·6)	76·6 (68·2 to 83·7)	−17·7 (−28·7 to −6·1)	29 (21 to 41)	53 (38 to 75)	0·54 (0·33 to 0·88)	91·7 (85·3 to 96·0)	100·0 (97·0 to 100·0)	−8·3 (−14·5 to −3·4)	149 (109 to 202)	689 (534 to 890)	0·22 (0·14 to 0·32)
Additional PCV13 serotypes												
3	0·0 (0·0 to 2·9)	92·7 (86·6 to 96·6)	−92·7 (−96·1 to −86·0)	4 (4 to 4)	41 (34 to 50)	0·10 (0·08 to 0·12)	3·3 (0·9 to 8·2)	88·3 (81·2 to 93·5)	−85·0 (−90·0 to −76·5)	4 (4 to 5)	54 (43 to 68)	0·08 (0·06 to 0·10)
6A	31·5 (23·4–40·4)	97·6 (93·1 to 99·5)	−66·1 (−73·8 to −56·4)	18 (12 to 26)	1392 (1106 to 1752)	0·01 (0·01 to 0·02)	66·9 (57·8 to 75·2)	100·0 (97·0 to 100·0)	−33·1 (−41·8 to −24·7)	118 (74 to 189)	3847 (3311 to 4468)	0·03 (0·02 to 0·05)
19A	35·5 (27·1 to 44·6)	95·2 (89·8 to 98·2)	−59·7 (−68·0 to −49·4)	9 (7 to 11)	139 (106 to 181)	0·06 (0·04 to 0·09)	61·2 (51·9 to 69·9)	99·2 (95·4 to 100·0)	−38·0 (−46·9 to −29·0)	25 (18 to 34)	587 (461 to 748)	0·04 (0·03 to 0·06)

Data are point estimate (95% CI) for the proportion of participants with a serotype-specific opsonisation index of 8 or more, and geometric mean opsonisation indices at 4 weeks post-primary series and 4 weeks post-booster dose in participants given a 2+1 schedule of PCV10 or PCV13. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine. *n=123 for serotype 3 (one sample not tested because of insufficient sera). †Risk difference is PCV10–PCV13. ‡Ratio is PCV10 / PCV13.

Table 3: **Functional antibody responses post-primary series and post-booster**

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	2 months			4 months			9·5 months		
	N	Any	Severe*	N	Any	Severe*	N	Any	Severe*
Erythema									
At PCV10 site	244	23 (9%)	2 (1%)	235	26 (11%)	1 (<1%)	218	19 (9%)	1 (<1%)
At PCV13 site	237	17 (7%)	0	222	23 (10%)	1 (<1%)	211	12 (6%)	0
At DTaP-IPV-Hib-HepB site									
PCV10 group	244	13 (5%)	2 (1%)	236	21 (9%)	1 (<1%)	222	13 (6%)	1 (<1%)
PCV13 group	240	18 (8%)	1 (<1%)	225	29 (13%)	0	211	11 (5%)	0
Control group	192	11 (6%)	0	188	15 (8%)	2 (1%)	NA	NA	NA
Fever									
PCV10 and DTaP-IPV-Hib-HepB	237	104 (44%)	10 (4%)	235	102 (43%)	11 (5%)	225	87 (39%)	16 (7%)
PCV13 and DTaP-IPV-Hib-HepB	236	98 (42%)	9 (4%)	227	100 (44%)	20 (9%)	219	89 (41%)	21 (10%)
DTaP-IPV-Hib-HepB alone	186	35 (19%)	3 (2%)	187	18 (10%)	4 (2%)	NA	NA	NA

Data are n (%) and show participants reporting erythema at the vaccination site(s) and participants reporting axillary fever after vaccination at various timepoints among participants for whom data were available (N). 1809 diary cards were collected, of which 20 were excluded because they contained no data on erythema or fever. Otherwise, all available data contributed to the analysis. The maximum reported values for erythema and fever across days 0–4 were used. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine. DTaP-IPV-Hib-HepB=hexavalent diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b, and hepatitis B vaccine. NA=Not applicable. *Severe erythema was defined by a diameter of more than 30 mm, and severe fever was defined as a temperature of 38·5°C or higher.

Table 4: **Reactogenicity**

responses to serotypes 6A and 19A were seen after the booster dose of PCV10 (table 3).

Reactogenicity information was analysed at 2 months, 4 months, and 9·5 months of age in the 2+1 PCV10 group (group C) and the 2+1 PCV13 group (group E), and at 2 months and 4 months of age in the control group (group F; table 4). Diary cards were collected from more than 96% of participants vaccinated at each timepoint. The incidences of erythema at the PCV and the DTaP-IPV-Hib-HepB vaccination sites were both low. The incidence of erythema at the PCV site did not differ between the PCV10 and PCV13 groups at any timepoint (p=0·395 at 2 months, p=0·939 at 4 months, and p=0·346 at 9·5 months), and was similar to that at the DTaP-IPV-Hib-HepB site. Co-administration of DTaP-IPV-Hib-HepB with either PCV10 or PCV13 had no effect on the incidence of erythema at the DTaP-IPV-Hib-HepB site (p=0·590 at 2 months, p=0·100 at 4 months, and p>0·999 at 9·5 months; table 4).

The incidence of axillary fever (≥37·5°C) following PCV vaccination ranged from 39% to 44% (4–10% for severe fever [≥38·5°C]; table 4). Fever and severe fever did not differ in incidence between PCV10 recipients and PCV13 recipients at any timepoint (p=0·880 at 2 months, p=0·190 at 4 months, and p=0·643 at 9·5 months). In the PCV13 group, the proportion of fevers categorised as severe at 4 months and at 9·5 months was higher than that at 2 months (p=0·019). The incidence of fever after co-administration of PCV and DTaP-IPV-Hib-HepB was significantly higher than the incidence after DTaP-IPV-Hib-HepB vaccination alone (p<0·0001 at 2 months and at 4 months).

135 participants from groups A–F were hospitalised during the trial, in a total of 163 admissions (appendix). The most common reasons for hospitalisation were acute respiratory infection (70 [43%] of 163) and acute gastroenteritis (29 [18%]). 156 (94%) hospitalisations were unrelated to vaccination, and all resolved without sequelae. The reasons for hospitalisation (p=0·750) and the causality (in relation to vaccination; p=0·098) were similar across groups (appendix). No participants were withdrawn as a result of harms, and none died during the trial.

Discussion

PCVs are now in use in national immunisation programmes in 142 countries. Increasingly, countries are adopting a 2+1 schedule, with a two-dose primary series followed by a booster dose at or after 9 months of age. In this paper we present the results of the first head-to-head study comparing the two currently available PCVs in a 2+1 schedule, measuring both serotype-specific IgG and functional antibody levels to all 13 serotypes in PCV13. The immunological advantage of one vaccine over the other varied by serotype and by timepoint. The overall pattern that emerges is that PCV10 generally fares better for the shared serotypes after a single dose. After the two-dose primary series, responses to PCV13 are stronger, but wane similarly to PCV10 by 9 months of age. PCV13 produces stronger booster responses, but this effect is lost by 18 months of age.

Responses after a single dose allow us to judge protection in the interval between doses. This knowledge is important because many children will not present on time for the second dose, and because 1+1 schedules are currently under consideration.[16] After a single dose of either PCV10 or PCV13, there was no response to some serotypes (6B, 14, and 23F, and non-PCV10 types 6A and 19A). However, for most other serotypes, the majority of children responded beyond the protective concentration

of 0·35 µg/mL, consistent with the observation that there is some incomplete protection afforded to infants by a single dose. The magnitude of the response was greater with PCV10 for half of the shared serotypes.

Both vaccines produced strong responses post-primary series, with more than 95% of children responding to most serotypes (the exceptions being 6B and 23F, consistent with previous findings[17,18]), although the magnitude of the response was greater with PCV13 for eight of the shared serotypes. After the booster, almost all children had protective levels of antibody, but again the magnitude of the response was greater with PCV13 for seven of the shared serotypes. The concentration of 0·35 µg/mL was determined from a pooled analysis of data from efficacy trials, and was established as the basis for comparing new with existing PCVs post-primary series.[19] The true protective concentration of antibody varies geographically, by serotype, and by disease type.[20–22] Applying a more conservative concentration threshold of 1·00 µg/mL to our data, more than 80% of children responded to most serotypes post-primary series (the exceptions being serotypes 6B and 23F in both groups, and 5 in the PCV10 group), and more than 90% post-booster (the only exception being serotype 5 in the PCV10 group). At this threshold, PCV13 fared better for serotypes 1 (post-primary) and 5 (both post-primary and post-booster), and PCV10 for serotype 6B (post-primary).

In general, the opsonophagocytic assay titres reflected the ELISA titres, with similar proportions of infants protected by an IgG concentration of at least 0·35 µg/mL and infants with an opsonisation index of at least 8, but some important differences did emerge. With both vaccines, particularly PCV10, poor opsonophagocytic assay responses to serotype 1 were seen post-primary series, despite strong ELISA responses. This finding was reflected in the two European trials of investigational PCVs, in which 41% and 62% of participants in the PCV10 groups and 61% and 84% in the PCV13 groups had an opsonisation index of at least 8.[9,10] This disconnect between responses measured by opsonophagocytic assay and by ELISA is corrected after the booster dose, providing immunological evidence for the importance of a booster dose in protecting against disease. This is an important finding for Africa, where serotype 1 is a frequent cause of pneumococcal disease,[23] and where most countries use a 3+0 schedule without a booster dose. Analysis of serotype 1 immunogenicity in the context of reduced-dose PCV10 schedules with or without a booster will be reported elsewhere as part of the evaluation of different PCV schedules (the other aim of this trial).

Both vaccines were strongly immunogenic against serotype 19F; however, responses were stronger after PCV10 vaccination at all timepoints and according to both ELISA and opsonophagocytic assay. By contrast, findings from the Dutch study[11] showed that PCV13 produced stronger 19F booster responses by ELISA than did PCV10, although opsonophagocytic assay responses were similar. Serotype 19F has persisted in both carriage[24] and disease[25] in the USA, despite more than 15 years of vaccination, and has been the most common cause of vaccine failure in children.[26] In the original PCV7 efficacy trial,[27] effectiveness against invasive pneumococcal disease and ear disease for serotype 19F was lower than for other serotypes (along with serotype 6B), despite good circulating antibody levels. The sharp rise in serotype 19A disease after PCV7 introduction shows that the 19F component of PCV7 (and PCV13) provides no protection against 19A disease. By contrast, the 19F component of PCV10 appears to provide protection against 19A disease, although probably not carriage.[28,29]

PCV13 elicited strong responses to the non-PCV10 serotypes, with more than 94% of children responding post-primary series and more than 99% post-booster. Interestingly, PCV13 produced only modest increases in IgG and opsonophagocytic assay responses for serotype 3 post-booster compared with post-primary series, and these responses were considerably lower than those for other serotypes, a finding consistent with previous immunogenicity data.[18] The effectiveness of PCV13 against serotype 3 disease is in doubt.[30,31] Among PCV10 recipients, we found modest immunogenicity to serotypes 6A and 19A after the booster dose at 9 months, with more than 90% of children achieving an IgG concentration of at least 0·35 µg/mL, although the GMCs were significantly lower than those generated by PCV13. Opsonophagocytic assay responses were also lower but considerable. These results support findings from three experimental PCVs in the 1990s showing poor correlation between ELISA and opsonophagocytic assay results for cross-reactive serotypes,[32] but are consistent with some degree of protection afforded by PCV10 against both 6A and 19A disease. As part of this trial, we are evaluating the effects of vaccination on pneumococcal carriage, which will elucidate the capacity for PCV10 to protect against carriage of serotypes 6A and 19A.

One of the limitations of this study was the use of immunological endpoints rather than disease outcomes. However, given that both PCV10 and PCV13 have been in routine use in many countries for several years with demonstrated effectiveness, a direct comparison of the two vaccines on this basis is appropriate, and is enhanced by the inclusion of functional opsonophagocytic assays in addition to the standard IgG antibody measurement by ELISA. Another limitation is the inclusion of assessment of responses to multiple serotypes at several timepoints, leading to the likelihood that some of the observed differences arose by chance. This is a problem faced by all studies of PCVs. To compensate for this, we defined a single conclusion for the primary outcome, requiring a difference (or non-inferiority) in the proportion of participants with an IgG concentration of at least 0·35 µg/mL to be observed for seven of the ten

shared serotypes. Beyond the primary outcome, no formal adjustments for multiple comparisons were made. The inclusion of multiple outcomes in this study is also a strength. We have assessed the immunogenicity, with both ELISAs and opsonophagocytic assays, and reactogenicity of PCV10 and PCV13 in a 2+1 schedule, providing a comprehensive head-to-head comparison of these vaccines. For the reactogenicity assessments, a limitation of this study is the use of parent-held diary cards. However, the same potential issues of bias in self-reported symptoms apply to all study groups, and therefore would not affect the between-group comparisons. Furthermore, we reported a single measure for the occurrence of erythema and fever on days 0–4 post-vaccination to limit any effect of missing data; only 1% of diary cards were excluded from this analysis because of a lack of data.

In conclusion, PCV10 and PCV13 are highly immunogenic, consistent with their effectiveness, and show similar reactogenicity. The differences in immunogenicity described vary by serotype and timepoint. PCV13 tends to produce stronger responses post-primary series and post-booster, while PCV10 appears to produce stronger responses after a single dose. PCV10 produces reasonable responses to non-PCV10 types 6A and 19A, whereas PCV13 produces only modest responses to serotype 3. It has been argued that a higher antibody concentration is required to protect against mucosal disease than against invasive pneumococcal disease, but it is hard to assess whether or not the observed differences in immunogenicity would translate to differing degrees of protection afforded by the two vaccines. Further analysis of data from this trial will compare B-cell memory induced by PCV10 and PCV13 and will evaluate the effects of the two vaccines on the carriage of vaccine serotypes, vaccine-related serotypes, and other serotypes of pneumococcus, which might further elaborate the differences between the two vaccines.

Contributors

BT was involved in the design and day-to-day management of the trial, did the data analysis, and wrote the first draft of this manuscript with input from CDN and EKM. NTT, KB, and DYU were involved in the design, establishment, day-to-day management, and implementation of the trial. VTTD was responsible for the ELISA experiments. RAM was responsible for the opsonophagocytic assays. PVL and AB were involved in the design, and advised on and provided oversight of the immunology laboratory procedures. CDN advised on the statistical analyses and assisted with the figures. TNH was involved in the design and establishment, and had overall responsibility for the conduct of the trial in Vietnam as site principal investigator. EKM conceived the study, provided oversight for the conduct of the trial and data analysis, and had overall responsibility for all aspects of the trial as the principal investigator. All authors contributed to refinement of and approved the submitted manuscript.

Declaration of interests

All authors receive salary support from grants from the National Health and Medical Research Council of Australia or the Bill & Melinda Gates Foundation. Non-financial support (in the form of PCV10 vaccine doses) and funding for opsonophagocytic assays was provided by GlaxoSmithKline Biologicals SA. We declare no other competing interests.

Data sharing

The study protocol and informed consent form have been published previously and are freely available. Data will be made publicly available in accordance with the rules set out by the Bill & Melinda Gates Foundation.

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Chapter 6: Research paper - The comparative effect of PCV10 and PCV13 on pneumococcal carriage

Data on the comparative effect of PCV10 and PCV13 on pneumococcal carriage were published in Vaccine in 2021 and are linked to Objectives 4 and 5 of this thesis: to evaluate the effect of a 2+1 schedule of PCV10 or PCV13 on pneumococcal carriage in the first two years of life, and to describe which pneumococcal serotypes are most commonly carried by unvaccinated children in the first two years of life. The timeframe for the literature review contributing to this manuscript is to 30 April 2020. The supplementary material from the publication is included in Appendix E. My contribution to this paper is detailed in the Research Paper Cover Sheet.

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Student ID Number	082622	**Title**	Miss
First Name(s)	Ellinor Beth		
Surname/Family Name	Temple		
Thesis Title	Pneumococcal vaccination for developing countries: PCV10 or PCV13?		
Primary Supervisor	Kim Mulholland		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Vaccine		
When was the work published?	2021		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I was involved in the design and day-to-day management of and data management for the research. I co-wrote the first draft of the manuscript with Monica Nation. Ms Nation drafted the Introduction and Methods sections and I drafted the Abstract, Results and Discussion sections. I conducted the carriage prevalence analyses and generated the carriage figures. I refined the manuscript in response to comments from co-authors and responded to the peer-review comments.

SECTION E

Student Signature	[redacted]
Date	6/1/23

Supervisor Signature	K. Mulholland
Date	25/1/23

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Effect of a 2+1 schedule of ten-valent versus 13-valent pneumococcal conjugate vaccine on pneumococcal carriage: Results from a randomised controlled trial in Vietnam

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ABSTRACT

Background: Pneumococcal conjugate vaccines (PCVs) generate herd protection by reducing nasopharyngeal (NP) carriage. Two PCVs, PCV10 and PCV13, have been in use for over a decade, yet there are few data comparing their impact on carriage. Here we report their effect on carriage in a 2+1 schedule, compared with each other and with unvaccinated controls.

Methods: Data from four groups within a parallel, open-label randomised controlled trial in Ho Chi Minh City contribute to this article. Three groups were randomised to receive a 2+1 schedule of PCV10 (n = 250), a 2+1 schedule of PCV13 (n = 251), or two doses of PCV10 at 18 and 24 months (controls, n = 197). An additional group (n = 199) was recruited at 18 months to serve as controls from 18 to 24 months. NP swabs collected at 2, 6, 9, 12, 18, and 24 months were analysed (blinded) for pneumococcal carriage. This study aimed to determine if PCV10 and PCV13 have a differential effect on pneumococcal carriage, a secondary outcome of the trial. We also describe the serotype distribution among unvaccinated participants. Trial registration: ClinicalTrials.gov NCT01953510.

Findings: Compared with unvaccinated controls, a 2+1 schedule of PCV10 reduced PCV10-type carriage by 45–62% from pre-booster through to 24 months of age, and a 2+1 schedule of PCV13 reduced PCV13-type carriage by 36–49% at 12 and 18 months of age. Compared directly with each other, there were few differences between the vaccines in their impact on carriage. Vaccine serotypes accounted for the majority of carriage in unvaccinated participants.

Interpretation: Both PCV10 and PCV13 reduce the carriage of pneumococcal vaccine serotypes. The introduction of either vaccine would have the potential to generate significant herd protection in this population.

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1. Introduction

Streptococcus pneumoniae (the pneumococcus) causes significant morbidity and mortality in children under five years of age, with pneumococcal pneumonia estimated to be responsible for over 380,000 deaths among that age group in 2017 [1]. There are

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100 pneumococcal serotypes, and pneumococcal conjugate vaccines (PCVs) protect against a subset that most commonly cause invasive pneumococcal disease. In addition to providing direct protection to the vaccinee, PCVs result in powerful herd protection by reducing nasopharyngeal carriage and transmission of vaccine-type pneumococci to unvaccinated individuals [2].

Two PCV formulations are licenced for paediatric use. Ten-valent PCV (PCV10, Synflorix®, GSK) includes serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F. 13-valent PCV (PCV13, Prevenar®, Pfizer) includes the same serotypes, as well as 3, 6A, and 19A. A third PCV, Pneumosil® (10-valent: serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, and 23F) received World Health Organization (WHO) pre-qualification in December 2019. Assessment of carriage is essential to fully evaluate the benefits of vaccination, as carriage is considered a prerequisite for disease and underpins herd protection [3,4]. However, despite the availability of both PCV10 and PCV13 for over a decade, only one clinical trial has directly compared the effect of these vaccines on pneumococcal carriage [5]. In Papua New Guinea, a setting with high pneumococcal carriage, all participants received either PCV10 or PCV13 at 1, 2, and 3 months of age (with no unvaccinated control group), with carriage assessed at 4 and 9 months of age. Overall pneumococcal carriage transiently decreased in the PCV13 group compared with the PCV10 group, but no differences in vaccine-type carriage were observed. In Cyprus and Korea, PCV7-use was replaced with the simultaneous use of both PCV10 and PCV13. Observational studies in these two settings (among healthy children in Cyprus and among children hospitalised with respiratory infections in Korea) showed similar carriage rates with either vaccine, although a non-significant 63% reduction in the carriage of additional PCV13 serotypes was noted among PCV13-recipients compared with PCV10-recipients in Cyprus [6,7]. A review of several other observational studies reports that introduction of either PCV10 or PCV13 leads to a reduction in vaccine-type carriage of a similar magnitude among vaccinees for the serotypes included in the vaccine [8].

As there are limited data to guide vaccine formulation choice, we undertook a randomised controlled trial of alternative PCV schedules that included a comparison of PCV10 and PCV13 in a 2+1 schedule (administered at 2, 4, and 9.5 months of age) in Ho Chi Minh City, Vietnam. Previously, we found both vaccines were safe and highly immunogenic [9]. Here, we aimed to determine if vaccine formulation had a differential effect on nasopharyngeal pneumococcal carriage and density in children during the first two years of life, comparing PCV10-recipients, PCV13-recipients, and unvaccinated controls. We also evaluate the most common serotypes carried by unvaccinated participants over time, to describe the serotypes circulating in the absence of vaccination.

2. Methods

2.1. Study design and participants

Vietnam is a lower-middle income country in South-East Asia with a population of over 95 million [10]. The burden of childhood pneumonia mortality is high, and PCV is not currently included in the national immunisation program [11]. We conducted an open label randomised controlled trial ('The Vietnam Pneumococcal Project'), in districts 4 and 7 in Ho Chi Minh City, Vietnam. A detailed protocol describing the trial aims, study design, study population, and sample size has been published [12]. Infants were enrolled at two months of age and randomised to one of six vaccination schedules (Appendix Table S1), including a 2+1 PCV10 schedule at 2, 4, and 9.5 months of age (group C), a 2+1 PCV13 schedule at 2, 4, and 9.5 months of age (group E), and a control group that received two doses of PCV10 at 18 and 24 months of age (group F). Participants originally consented to be followed up to 18 months of age. Follow-up was later extended to 24 months of age, with an additional group (group G) enrolled at 18 months of age to serve as unvaccinated controls between 18 and 24 months. Group G participants received a single dose of PCV10 at 24 months of age. Here we describe the microbiological outcomes for participants who received a 2+1 schedule of PCV10 (group C), a 2+1 schedule of PCV13 (group E), and unvaccinated controls (groups F and G). Ethical approval was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia, and the Ministry of Health Ethics Committee, Vietnam.

2.2. Randomisation and masking

As described previously, the allocation sequence for groups A–F was generated using computerised block randomisation, stratified by district [12]. Allocation concealment was maintained using sealed envelopes with sequential study numbers on the outside of the envelope. Group G participants were recruited at 18 months of age from the study districts, concurrent with group A-F participants turning 18 months. The participants and study nurses were not blinded to group allocation, as the trial arms had different vaccination schedules. All laboratory staff were blinded to group allocation.

2.3. Study procedures and laboratory analyses

Study staff collected demographic information using data collection forms. Demographic data were double-entered into an EpiData v3.1 database, with validation checks completed before upload into a Microsoft Access database. Laboratory data were entered into a Microsoft Access (2–12 month time points) or Excel (18 and 24 month time points) database.

Nasopharyngeal swabs were collected at 2, 6, 9, 12, 18, and 24 months of age, and stored and tested consistent with WHO guidelines [13]. Samples collected at 2, 6, 9, and 12 months were cultured on Columbia Colistin Naladixic Acid Horse Blood agar, and *S. pneumoniae* identified based on colony morphology including α-haemolysis and susceptibility to optochin [14]. Serotyping was conducted on isolates using latex agglutination and Quellung reaction with a complete set of antisera [15]. At 18 and 24 months we performed a detailed assessment of the long-term effect of PCV on pneumococcal carriage and density using molecular methods. Samples were screened for pneumococci by quantitative real-time PCR (qPCR) targeting the autolysin (*lytA*) gene [16]. Samples with presumptive pneumococci were cultured on selective agar before molecular serotyping by microarray (Senti-SP version 1.5, BUGS Bioscience) [17]. Pneumococci were designated as non-typeable if no serotype was identified using phenotypic testing, or if microarray identified a non-encapsulated lineage (NT1, NT2, NT3a, NT3b, NT4a, NT4b, NT2/NT3b). Samples were excluded from all analyses if serotyping could not be conducted or a serotyping result could not be determined.

2.4. Carriage outcomes

Vaccine-type carriage was defined as carriage of a serotype contained in the vaccine formulation; PCV10-type carriage (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F), or PCV13-type carriage (serotypes in PCV10, and 3, 6A, and 19A). Non-vaccine-type carriage was defined as carriage of a serotype not in the corresponding vaccine (excluding non-typeable pneumococci). Samples that contained both vaccine-type and non-vaccine-type serotypes were considered positive for both vaccine-type and non-vaccine-type carriage. Ser-

otypes 15B and 15C were reported as 15B/C as these serotypes are known to interconvert [18], and '11F-like' was reported as 11A [19]. Serotype-specific density at 18 and 24 months was derived by multiplying pneumococcal density (determined by *lytA* qPCR) with the relative abundance of the serotype (determined by microarray).

2.5. Statistical analyses

We determined the prevalence of overall pneumococcal, PCV10-type, PCV13-type, serotype 3/6A/19A (additional PCV13-type), non-PCV10-type, and non-PCV13-type carriage at 2, 6, 9, 12, 18, and 24 months for group C (2+1, PCV10), group E (2+1, PCV13), and controls. The control group varied by timepoint and was based on vaccination status: Group F (2–12 months), Groups F and G combined (18 months), or Group G (24 months). We also determined the overall probability of carriage, with participants defined as carriers if they had a positive swab at any time point. Carriage among PCV10-recipients (Group C), PCV13-recipients (Group E), and controls who were recruited at 2 months of age (Group F) was ascertained between 6 months of age (post-primary series) and 18 months of age (the time of first PCV dose in controls). Individual time point carriage prevalences and the overall probability of carriage in each of the vaccine groups were compared with controls, and a head-to-head comparison of PCV10 and PCV13 was also conducted. Prevalence ratios (PR) and 95% confidence intervals (CI) were calculated, and groups were compared using Fisher's exact tests (5% level); one-sided when vaccine groups were compared with controls, and two-sided when vaccine groups were compared. Density data for pneumococcal carriers were $\log_{10}$-transformed and reported as $\log_{10}$ genome equivalents per ml ($\log_{10}$ GE per ml). As the transformed density data were not normally distributed, groups were compared using the non-parametric Mann-Whitney *U* test. Statistical analyses were conducted using Stata version 15.1 (StataCorp LLC). The trial is registered at ClinicalTrials.gov, number NCT01953510.

3. Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

4. Results

Between Sept 30, 2013, and Jan 9, 2015, 1201 two-month-old infants were enrolled and randomised to groups A to F (Fig. 1). Between Apr 14, 2015, and May 12, 2016, 199 18-month-old children were recruited to the additional control group (group G). Participants from groups C (2+1 PCV10, n = 250), E (2+1 PCV13, n = 251), F (controls $\leq$ 18 months of age, n = 197), and G (controls $\geq$ 18 months of age, n = 199) contribute data to this article. The groups were balanced with respect to participant demographics at baseline and to most characteristics at 18 months of age (Appendix Table S2). The exceptions were age at the 18 month visit (18.3 months in group G, compared with 18.1 months in each of groups C, E, and F, $p < 0.001$) and antibiotic use in the fortnight prior to the 18 month visit (20.4% in group G, compared with 12.4% in group C, 11.6% in group E, and 10.9% in group F, $p = 0.020$).

Of the 897 participants in this study, 106 were withdrawn and 12 did not consent to the extended follow-up beyond 18 months

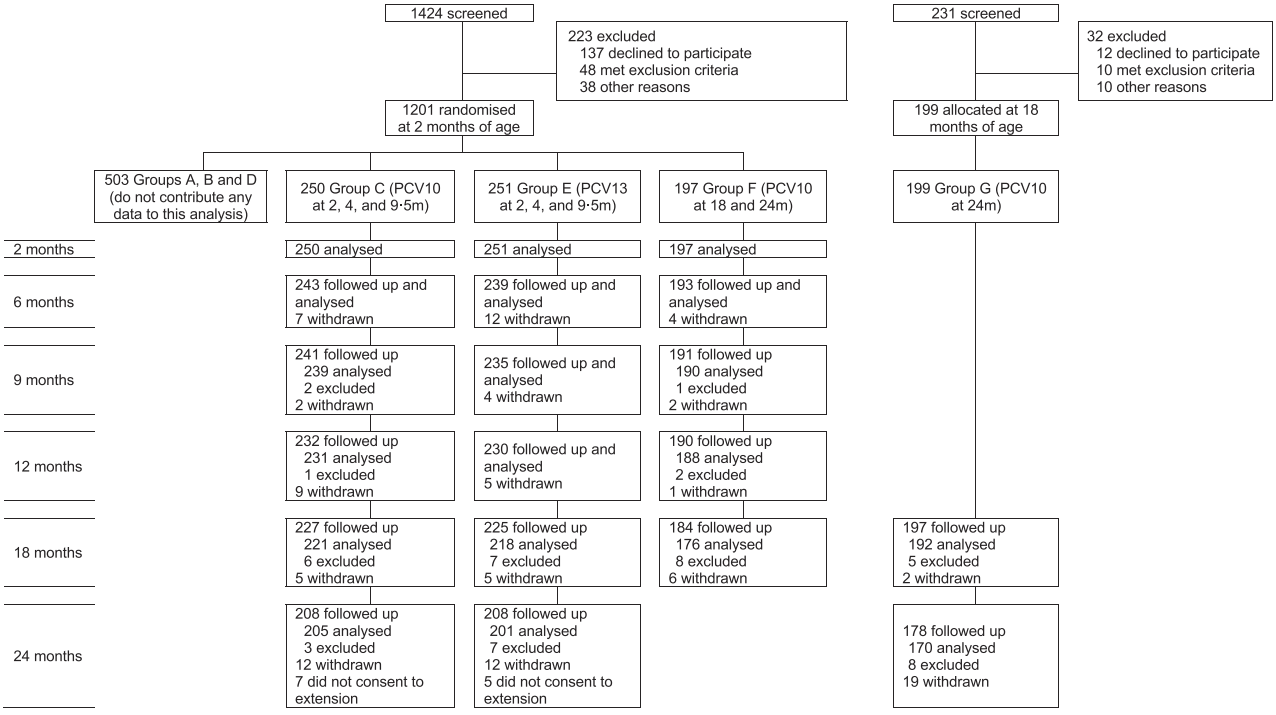

Fig. 1. CONSORT diagram. Reasons withdrawn (n = 106): moved away and lost to follow-up (n = 67, 63%); refused a study procedure (n = 19, 18%); 16 (15%) voluntary withdrawal (n = 16, 15%); and other (n = 4, 4%). Reasons excluded: no sample (either participants missed the study visit or attended the visit but had no sample collected, n = 16); insufficient DNA for microarray (n = 24); pneumococcal carriage status could not be determined (n = 6); cultured isolate was irretrievable from freezer storage (n = 1); and excluded as a result of a protocol violation (PCV was administered outside the trial or the sample was collected after administration of PCV, n = 3). Participants who "did not consent to extension" completed the study at 18 months of age, as per the original study design. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV.

(Fig. 1). In all, 96.7% of participants (675/698) were followed up at 6 months, 95.6% (667/698) at 9 months, 93.4% (652/698) at 12 months, 92.9% (833/897) at 18 months, and 86.3% (594/688, excluding group F and those that did not consent to the extension) at 24 months. A total of 4103 swabs were collected, of which 4069 (99.2%) were included in the analyses. Of the 34 swabs not included, 24 had insufficient DNA for microarray, pneumococcal carriage status could not be determined in six, three were excluded due to protocol violations, and one isolate was irretrievable from freezer storage.

Overall, 616/4069 (15.1%) swabs contained capsular pneumococci. The majority of swabs (591/616, 95.9%) contained a single serotype. Of the 25 instances of multiple serotype carriage, two serotypes were identified in 23, and three serotypes were identified in each of the remaining two samples. In all, 30 different serotypes were identified (Appendix Table S3). A total of 175 nontypeable pneumococci were also identified, with two different genetic lineages detected (NT2 and NT4b, determined at 18 and 24 months only).

We examined the prevalence of pneumococcal carriage over time among PCV10-vaccinated participants, PCV13-vaccinated participants, and controls. Participant characteristics at the time of each swab were similar across groups (Table 1). The exceptions were current antibiotic use at the 9 month visit (p = 0.047), age at the 18 month visit (p = 0.016), and current symptoms of upper respiratory tract infection (URTI) at 24 months of age (p = 0.024).

Overall pneumococcal carriage was low at 2 months of age among all three groups, ranging from 1.5-6.0% (Fig. 2, Table 2). Carriage increased steadily to 12 months of age in all groups, peaking at 24.5% in controls and at 18.2% and 19.6% in the PCV10 and PCV13 groups, respectively. At 18 months of age, overall pneumococcal carriage was significantly lower in both vaccinated groups than controls, but this was not sustained out to 24 months of age. PCV10-type carriage was reduced by 60% among PCV10-recipients compared with controls at 9 months of age (prevalence ratio (PR) 0.40 [95% CI 0.16–0.97] p = 0.029), and this continued through to 24 months of age with reductions of 45–62% (Table 2). PCV13-type carriage was reduced by 36% and 49% among PCV13-recipients compared with controls at 12 and 18 months of age, respectively (Table 2). For both vaccines, the most profound differences were seen at 18 months of age. There was a consistent trend towards reduced carriage of the additional PCV13 serotypes (3, 6A, and 19A) among PCV13-recipients compared with controls from 12 months of age onwards, with no such trend observed among PCV10-recipients. In relation to non-vaccine-type carriage, non-PCV10-type carriage appears higher among PCV10-recipients compared with controls at 24 months of age, and non-PCV13-type carriage appears consistently higher among PCV13-recipients compared with controls from 12 months of age onwards, although these differences do not reach statistical significance.

The head-to-head comparison of PCV10 and PCV13 showed few differences between vaccines (Fig. 2, Table 2). PCV10-type carriage was consistently lower among PCV10-recipients than PCV13-recipients from 9 months of age onwards (statistically significant at 9 months of age). Among PCV13-recipients, serotype 3/6A/19A carriage remained relatively constant over time, ranging from 3.0 to 3.8% between 6 and 24 months of age, and was generally lower than among PCV10-recipients from 9 months of age onwards (statistically significant at 24 months of age). Serotype 6A carriage ranged from 1.7 to 2.9%, serotype 19A carriage from 0.8 to 1.0%, and there was only one occurrence of serotype 3 carriage (at 18 months of age; Appendix Table S3). Serotype 3/6A/19A carriage fluctuated more among PCV10-recipients, ranging from 2.7 to 9.8% between 6 and 24 months of age. Serotype 6A carriage ranged from 1.6 to

Table 1
Characteristics of participants analysed, by time point.

	Time point	PCV10 group	PCV13 group	Control group*	p-value
Age, months	2 m	2.1 (1.9–2.4)	2.1 (1.9–2.4)	2.1 (2.0–2.5)	>0.999
	6 m	6.1 (5.7–6.9)	6.1 (5.7–7.0)	6.1 (5.0–6.8)	>0.999
	9 m	9.1 (9.0–10.1)	9.1 (8.8–10.1)	9.1 (9.0–11.2)	0.117
	12 m	12.1 (12.0–14.0)	12.1 (11.8–13.1)	12.1 (12.0–13.2)	>0.999
	18 m	18.1 (17.9–20.9)	18.1 (17.7–20.0)	18.2 (17.4–20.3)	0.018
	24 m	24.1 (23.9–25.9)	24.1 (23.6–28.3)	24.1 (23.4–26.9)	0.321
Any current breastfeeding	2 m	195/250 (78.0%)	194/250† (77.6%)	140/196† (71.4%)	0.208
	6 m	129/243 (53.1%)	117/239 (49.0%)	91/193 (47.2%)	0.437
	9 m	91/239 (38.1%)	88/235 (37.4%)	70/190 (36.8%)	0.966
	12 m	71/231 (30.7%)	62/230 (27.0%)	52/188 (27.7%)	0.637
	18 m	30/220† (13.6%)	28/218 (12.8%)	52/368 (14.1%)	0.908
	24 m	9/205 (4.4%)	13/200† (6.5%)	10/170 (5.9%)	0.636
Presence of URTI symptoms	2 m	18/250 (7.2%)	14/251 (5.6%)	10/197 (5.1%)	0.603
	6 m	43/243 (17.7%)	37/239 (15.5%)	27/193 (14.0%)	0.564
	9 m	38/239 (15.9%)	51/235 (21.7%)	28/190 (14.7%)	0.118
	12 m	50/231 (21.6%)	44/230 (19.1%)	34/188 (18.1%)	0.635
	18 m	23/220† (10.5%)	35/218 (16.1%)	59/368 (16.0%)	0.134
	24 m	31/205 (15.1%)	44/200† (22.0%)	20/170 (11.8%)	0.024
Antibiotic use in past fortnight	2 m	6/250 (2.4%)	12/251 (4.8%)	4/197 (2.0%)	0.178
	6 m	21/243 (8.6%)	21/239 (8.8%)	17/193 (8.8%)	0.994
	9 m	36/239 (15.1%)	41/235 (17.4%)	26/190 (13.7%)	0.551
	12 m	25/231 (10.8%)	20/230 (8.7%)	22/188 (11.7%)	0.575
	18 m	28/220† (12.7%)	25/218 (11.5%)	59/368 (16.0%)	0.255
	24 m	18/205 (8.8%)	18/200† (9.0%)	22/170 (12.9%)	0.337
Current antibiotic use	2 m	3/250 (1.2%)	6/251 (2.4%)	4/197 (2.0%)	0.602
	6 m	5/243 (2.1%)	9/239 (3.8%)	7/193 (3.6%)	0.684
	9 m	10/239 (4.2%)	18/235 (7.7%)	5/190 (2.6%)	0.047
	12 m	17/231 (7.4%)	14/230 (6.1%)	14/188 (7.4%)	0.820
	18 m	13/220† (5.9%)	13/218 (6.0%)	17/368 (4.6%)	0.709
	24 m	12/205 (5.9%)	9/200† (4.5%)	10/170 (5.9%)	0.788

Data are median (range) or n/N (%). p-values based on quantile regression with bootstrapped standard errors (for comparisons of medians) or chi-squared test (for comparisons of proportions). PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. URTI = upper respiratory tract infection (presence of runny nose and/or cough at the time of swab collection). *Data for controls comes from Group F (2–12 months), Groups F and G combined (18 months), or Group G (24 months). †Data missing for one participant.

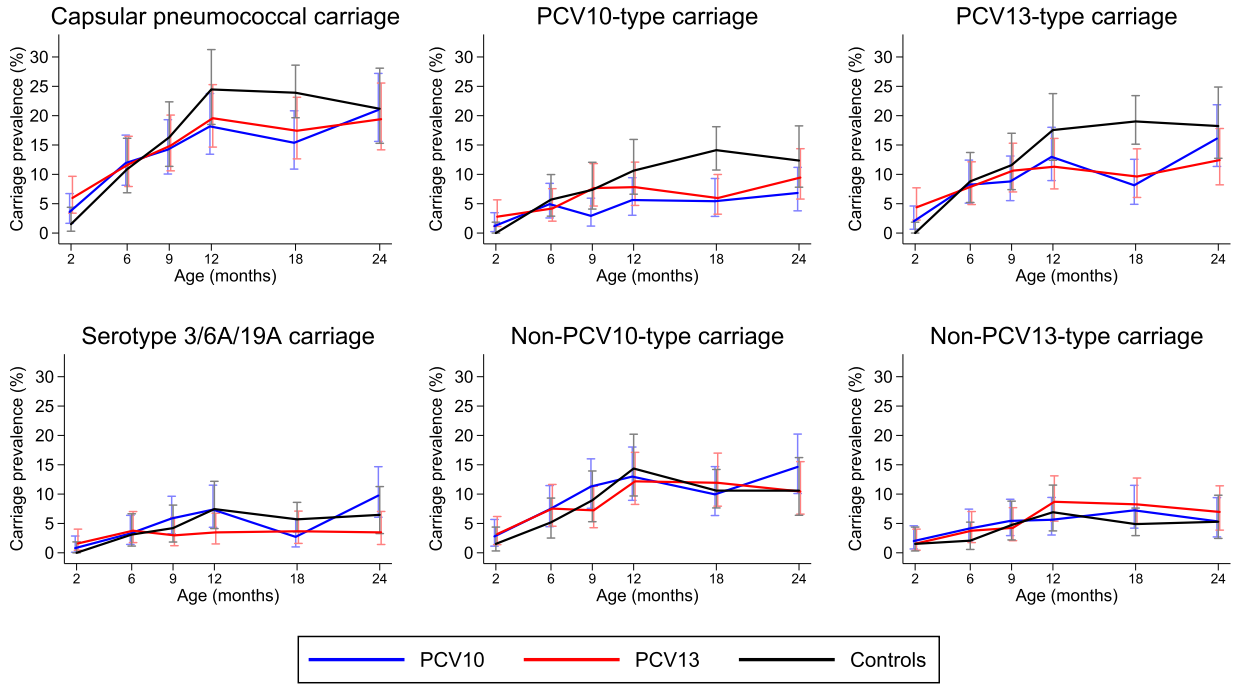

Fig. 2. Pneumococcal carriage prevalence over time. Prevalence (95% CI) of capsular, PCV10-type, PCV13-type, serotype 3/6A/19A, non-PCV10-type, and non-PCV13-type carriage at 2, 6, 9, 12, 18, and 24 months of age, among participants who received a 2+1 schedule (at 2, 4, and 9.5 months of age) of PCV10, a 2+1 schedule of PCV13, or unvaccinated controls. CI = confidence interval. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. Control group data come from: Group F (2–12 months); Groups F and G combined (18 months); or Group G (24 months).

6.8%, serotype 19A carriage from 0.9 to 3.0%, and there were three occurrences of serotype 3 carriage (all at 12 months of age; Appendix Table S3).

In response to lower-than-anticipated pneumococcal carriage rates we performed an additional analysis of the overall probability of carriage at any time between 6 and 18 months of age (Appendix Table S4). The overall probabilities of carriage generally reflect the trends observed over time. PCV10- and PCV13-recipients were 34% (95% CI 0–56%) and 29% (−6 to 3%) less likely to be positive for PCV10-type carriage at any time between 6 and 18 months than controls, respectively, and were 24% (−2 to 44%) and 27% (1–46%) less likely to be positive for PCV13-type carriage (Appendix Table S4). There were no differences in the overall probabilities of carriage comparing PCV10 and PCV13-recipients.

Pneumococcal density was evaluated at 18 and 24 months of age in pneumococcal carriers. Overall pneumococcal density was similar at 18 and 24 months of age, with no differences between PCV10-recipients, PCV13-recipients, and controls (Appendix Figure S1). Similarly, no differences were observed in PCV10-type, PCV13-type, serotype 3/6A/19A, non-PCV10-type, or non-PCV13-type carriage density in PCV10-recipients compared with PCV13-recipients, or between either PCV group compared with the control group.

We also examined the most common serotypes carried by unvaccinated participants over time. Between 2 and 24 months of age, a total of 22 capsular serotypes were identified among unvaccinated participants, with the greatest diversity (15 different serotypes) seen at 12 months of age. Over time, the most commonly carried serotypes were 6A, 6B, 19F, 23F, 19A, 23A, 15A, and 14 (Fig. 3). These serotypes were responsible for 231 of the 266 (86.8%) pneumococci identified among unvaccinated participants. Across all time points, PCV10 serotypes accounted for 50.8% of pneumococci, and PCV13 serotypes for 75.6%.

5. Discussion

PCV is included universally in the national immunisation schedules of 136 countries [20]. PCV13 is used in three times as many countries as PCV10, although the total number of recipients is similar. In this paper we report the first head-to-head comparison of the effect of PCV10 and PCV13 on pneumococcal carriage in a 2+1 schedule. This schedule is becoming increasingly adopted by countries, as the booster dose may increase the duration of protection and lead to greater herd effects [21]. We show that, compared with unvaccinated controls, both vaccines reduced carriage of pneumococcal serotypes included in the corresponding vaccine. In the head-to-head comparison, PCV10 and PCV13 generally had a similar impact on carriage.

At 9 months of age (prior to the booster dose), vaccination with PCV10 resulted in a 60% reduction in PCV10-type carriage compared with unvaccinated controls. This was sustained out to 24 months of age, with reductions of 45–62%. Vaccination with PCV13 resulted in 32–49% reductions in PCV13-type carriage after the booster dose (from 12 to 24 months of age). Interestingly, vaccination with PCV10 led to consistently lower levels of PCV10-type carriage than vaccination with PCV13 from 9 months onwards, although the differences were only statistically significant at 9 months of age.

Considering the serotypes unique to PCV13, vaccination with PCV13 resulted in a consistent (albeit not statistically significant) 36–55% reduction in 3/6A/19A carriage compared with controls. Such a trend was not observed with PCV10 vaccination, despite our findings of modest immunogenicity to serotypes 6A and 19A [9], two serotypes that are known to cross-react with serotypes 6B and 19F. In the head-to-head comparison of PCV10 and PCV13, carriage of 3/6A/19A was 64% lower in the PCV13 group than the PCV10 group at 24 months of age, driven primarily by ser-

Table 2
Pneumococcal carriage prevalence % (95% CI), prevalence ratio (95% CI), and Fisher's exact p-value, by time point.

	Carriage prevalence, % (95% CI)			PCV10 vs controls		PCV13 vs controls		PCV13 vs PCV10	
	2+1 PCV10	2+1 PCV13	Controls*	Prevalence ratio (95% CI)	p-value†	Prevalence ratio (95% CI)	p-value†	Prevalence ratio (95% CI)	p-value†
Any pneumococcal serotype carriage									
2 m	3.6 (1.7–6.7)	6.0 (3.4–9.7)	1.5 (0.3–4.4)	2.36 (0.65–8.62)	0.146	3.92 (1.15–13.37)	0.013	1.66 (0.74–3.72)	0.296
6 m	11.9 (8.1–16.7)	11.7 (7.9–16.5)	10.9 (6.9–16.2)	1.10 (0.65–1.86)	0.426	1.08 (0.63–1.83)	0.454	0.98 (0.60–1.60)	>0.999
9 m	14.2 (10.1–19.3)	14.9 (10.6–20.1)	16.3 (11.4–22.4)	0.87 (0.56–1.36)	0.320	0.91 (0.59–1.42)	0.393	1.05 (0.68–1.62)	0.897
12 m	18.2 (13.4–23.8)	19.6 (14.6–25.3)	24.5 (18.5–31.3)	0.74 (0.51–1.08)	0.074	0.80 (0.56–1.15)	0.138	1.08 (0.74–1.57)	0.722
18 m	15.4 (10.9–20.8)	17.4 (12.6–23.1)	23.9 (19.6–28.6)	0.64 (0.45–0.92)	0.008	0.73 (0.52–1.03)	0.040	1.13 (0.74–1.73)	0.607
24 m	21.0 (15.6–27.2)	19.4 (14.2–25.6)	21.2 (15.3–28.1)	0.99 (0.67–1.47)	0.531	0.92 (0.61–1.37)	0.384	0.93 (0.63–1.36)	0.712
PCV10-type carriage									
2 m	1.2 (0.2–3.5)	2.8 (1.1–5.7)	0.0 (0.0–1.9)	(..)	0.174	(..)	0.017	2.32 (0.61–8.89)	0.339
6 m	4.9 (2.6–8.5)	4.2 (2.0–7.6)	5.7 (2.9–10.0)	0.87 (0.39–1.92)	0.443	0.73 (0.32–1.69)	0.306	0.85 (0.37–1.92)	0.828
9 m	2.9 (1.2–5.9)	7.7 (4.6–11.8)	7.4 (4.1–12.1)	0.40 (0.16–0.97)	0.029	1.04 (0.53–2.04)	0.531	2.62 (1.11–6.14)	0.024
12 m	5.6 (3.0–9.4)	7.8 (4.7–12.1)	10.6 (6.6–16.0)	0.53 (0.27–1.04)	0.044	0.74 (0.40–1.35)	0.205	1.39 (0.70–2.77)	0.360
18 m	5.4 (2.8–9.3)	6.0 (3.2–10.0)	14.1 (10.7–18.1)	0.38 (0.21–0.70)	0.001	0.42 (0.24–0.76)	0.001	1.10 (0.51–2.35)	0.840
24 m	6.8 (3.8–11.2)	9.5 (5.8–14.4)	12.4 (7.8–18.3)	0.55 (0.29–1.05)	0.049	0.77 (0.43–1.37)	0.233	1.38 (0.71–2.68)	0.368
PCV13-type carriage									
2 m	2.0 (0.7–4.6)	4.4 (2.2–7.7)	0.0 (0.0–1.9)	(..)	0.054	(..)	0.002	2.19 (0.77–6.22)	0.203
6 m	8.2 (5.1–12.4)	7.9 (4.9–12.1)	8.8 (5.2–13.7)	0.93 (0.50–1.73)	0.481	0.90 (0.48–1.69)	0.440	0.97 (0.53–1.76)	>0.999
9 m	8.8 (5.5–13.1)	10.6 (7.0–15.3)	11.6 (7.4–17.0)	0.76 (0.43–1.34)	0.213	0.92 (0.54–1.58)	0.438	1.21 (0.70–2.10)	0.537
12 m	13.0 (8.9–18.0)	11.3 (7.5–16.1)	17.6 (12.4–23.8)	0.74 (0.47–1.17)	0.123	0.64 (0.40–1.04)	0.046	0.87 (0.53–1.42)	0.669
18 m	8.1 (4.9–12.6)	9.6 (6.1–14.3)	19.0 (15.1–23.4)	0.43 (0.26–0.70)	<0.001	0.51 (0.32–0.80)	0.001	1.18 (0.65–2.16)	0.618
24 m	16.1 (11.3–21.9)	12.4 (8.2–17.8)	18.2 (12.7–24.9)	0.88 (0.57–1.38)	0.340	0.68 (0.42–1.11)	0.080	0.77 (0.48–1.25)	0.322
3/6A/19A carriage									
2 m	0.8 (0.1–2.9)	1.6 (0.4–4.0)	0.0 (0.0–1.9)	(..)	0.312	(..)	0.097	1.99 (0.37–10.78)	0.686
6 m	3.3 (1.4–6.4)	3.8 (1.7–7.0)	3.1 (1.1–6.6)	1.06 (0.37–3.00)	0.569	1.21 (0.44–3.34)	0.461	1.14 (0.45–2.91)	0.810
9 m	5.9 (3.2–9.6)	3.0 (1.2–6.0)	4.2 (1.8–8.1)	1.39 (0.60–3.25)	0.294	0.71 (0.26–1.92)	0.335	0.51 (0.21–1.24)	0.180
12 m	7.4 (4.3–11.5)	3.5 (1.5–6.7)	7.4 (4.1–12.2)	0.99 (0.50–1.95)	0.559	0.47 (0.20–1.09)	0.056	0.47 (0.21–1.07)	0.098
18 m	2.7 (1.0–5.8)	3.7 (1.6–7.1)	5.7 (3.6–8.6)	0.48 (0.20–1.16)	0.066	0.64 (0.29–1.43)	0.184	1.35 (0.48–3.83)	0.599
24 m	9.8 (6.1–14.7)	3.5 (1.4–7.0)	6.5 (3.3–11.3)	1.51 (0.74–3.06)	0.168	0.54 (0.21–1.36)	0.137	0.36 (0.15–0.83)	0.016
Non-PCV10-type carriage									
2 m	2.8 (1.1–5.7)	3.2 (1.4–6.2)	1.5 (0.3–4.4)	1.84 (0.48–7.02)	0.284	2.09 (0.56–7.79)	0.207	1.14 (0.42–3.09)	>0.999
6 m	7.4 (4.4–11.5)	7.5 (4.5–11.6)	5.2 (2.5–9.3)	1.43 (0.68–3.03)	0.229	1.45 (0.69–3.08)	0.216	1.02 (0.54–1.91)	>0.999
9 m	11.3 (7.6–16.0)	7.2 (4.3–11.3)	8.9 (5.3–13.9)	1.26 (0.71–2.25)	0.263	0.81 (0.42–1.54)	0.319	0.64 (0.36–1.14)	0.154
12 m	13.0 (8.9–18.0)	12.2 (8.2–17.1)	14.4 (9.7–20.2)	0.90 (0.56–1.47)	0.394	0.85 (0.52–1.39)	0.303	0.94 (0.58–1.52)	0.888
18 m	10.0 (6.3–14.7)	11.9 (7.9–17.0)	10.6 (7.6–14.2)	0.94 (0.57–1.54)	0.461	1.13 (0.71–1.80)	0.357	1.20 (0.70–2.05)	0.543
24 m	14.6 (10.1–20.2)	10.4 (6.6–15.5)	10.6 (6.4–16.2)	1.38 (0.80–2.39)	0.156	0.99 (0.54–1.79)	0.549	0.71 (0.42–1.20)	0.232
Non-PCV13-type carriage									
2 m	2.0 (0.7–4.6)	1.6 (0.4–4.0)	1.5 (0.3–4.4)	1.31 (0.32–5.43)	0.498	1.05 (0.24–4.62)	0.631	0.80 (0.22–2.93)	0.751
6 m	4.1 (2.0–7.4)	3.8 (1.7–7.0)	2.1 (0.6–5.2)	1.99 (0.63–6.23)	0.177	1.82 (0.57–5.81)	0.232	0.92 (0.38–2.21)	>0.999
9 m	5.4 (2.9–9.1)	4.3 (2.1–7.7)	4.7 (2.2–8.8)	1.15 (0.50–2.63)	0.460	0.90 (0.37–2.17)	0.496	0.78 (0.35–1.75)	0.670
12 m	5.6 (3.0–9.4)	8.7 (5.4–13.1)	6.9 (3.7–11.5)	0.81 (0.39–1.71)	0.365	1.26 (0.64–2.46)	0.314	1.55 (0.79–3.03)	0.212
18 m	7.2 (4.2–11.5)	8.3 (5.0–12.7)	4.9 (2.9–7.6)	1.48 (0.77–2.84)	0.158	1.69 (0.90–3.17)	0.073	1.14 (0.60–2.18)	0.724
24 m	5.4 (2.7–9.4)	7.0 (3.9–11.4)	5.3 (2.4–9.8)	1.01 (0.43–2.39)	0.581	1.32 (0.58–2.96)	0.329	1.30 (0.60–2.79)	0.541

Carriage determined by culture and latex agglutination/Quellung testing (2–12 months) and by DNA microarray (18–24 months). Samples that could not be serotyped are excluded. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. * Control data sourced from Group F (2–12 month time points), Group F and G combined (18 months), or Group G (24 months). †Two-sided p-values were calculated for PCV10 vs PCV13 comparisons; one-sided p-values were calculated for comparisons with controls.

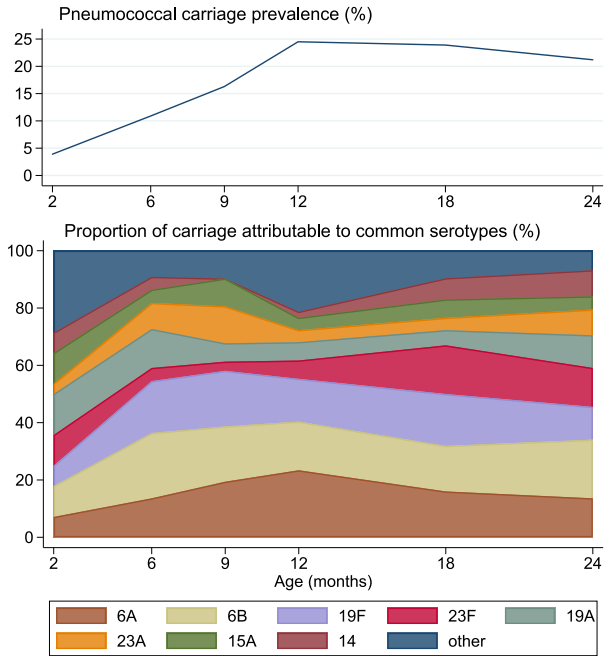

Fig. 3. Capsular pneumococcal carriage in unvaccinated participants. The top panel shows the capsular pneumococcal carriage prevalence over time among unvaccinated participants. The bottom panel shows the proportion of carriage at each time point attributable to each of the eight most commonly carried serotypes. Data come from: all groups (2 months); Group F (2–12 months); Groups F and G combined (18 months); or Group G (24 months).

otype 6A. This is similar to the non-significant 63% reduction in 3/6A/19A observed among PCV13-recipients compared with PCV10-recipients in Cyprus [6]. The long-term impact of a PCV10 schedule in Vietnam is unknown. Post-vaccine introduction data from elsewhere on the impact of PCV10 on carriage of serotypes 6A and 19A vary. In Brazil, carriage of both 6A and 19A had not changed three-years post-introduction of PCV10 [26], but by seven-years 6A carriage had reduced and 19A carriage had increased [27]. In Kenya, no effects were found on carriage of 6A or 19A two-years post-introduction of PCV10 [22]. Six-years post-introduction in Kenya there was still no effect on 6A carriage but 19A carriage had increased [23], similar to findings 1½-2 years post-introduction in Mozambique and three-years post-introduction in Fiji [17,24] By contrast in Palestine, at three-years post-introduction of PCV10 there was a decrease in 3/6A/19A carriage driven by 6A, with no change in 19A [25]. It may be that the impact of PCV10 on serotypes 6A and 19A depends on the local pneumococcal epidemiology. For serotype 3, for which PCV13 is generally not considered to be effective, we observed only five instances of carriage during the trial; it is therefore not possible to determine whether PCV13 impacted serotype 3 carriage.

Serotype 19F is the most common cause of vaccine failure in children [28], and has persisted in carriage in the wake of widespread and long-term vaccination [29,30]. Our earlier immunogenicity analyses showed that PCV10 produced better antibody and functional antibody responses to serotype 19F than PCV13 at all time points [9]. Here we found no evidence of a differential impact on 19F carriage between the two vaccines, and both PCV10 and PCV13 appear to impact the carriage of 19F to a similar degree as other vaccine types. However, the low serotype-specific carriage rates (0.9% and 2.2% at 12 months of age, 1.8% and 1.8% at 18 months of age, and 1.5% and 2.0% at 24 months of age for 19F in the PCV10 and PCV13 groups, respectively) and relatively short follow-up time make it difficult to predict the long-term consequences of our findings.

In many settings, PCV introduction has resulted in serotype replacement, whereby the reduction in vaccine-type carriage has been offset by an increase in non-vaccine-type carriage. In Vietnam, where there is still no routine PCV use, we show no definitive evidence of serotype replacement up to 24 months of age, although the increase in non-PCV10-type carriage at 24 months of age among PCV10 recipients and the trend towards higher non-PCV13-type carriage among PCV13-recipients than controls from 12 months of age onwards suggest that post-introduction surveillance for serotype replacement will be important. We did not observe any differences in pneumococcal density between groups at 18 or 24 months of age.

Data from unvaccinated participants provide information on the serotypes circulating in the absence of pneumococcal vaccination. The majority (87%) of carriage was attributable to only eight serotypes: vaccine-types 6B, 14, 19F, and 23F, additional PCV13-types 6A and 19A, and non-vaccine-types 15A and 23A. PCV10 and PCV13 serotypes represent half and three-quarters of pneumococci carried. Although it is not known what level of carriage impact is required to translate into herd protection effects, our observed reductions in vaccine-type carriage combined with the high representation of vaccine serotypes among unvaccinated participants suggest that immunisation with either vaccine is likely to impact the populations of pneumococci circulating in the community.

This trial provided a rare opportunity to evaluate the impact of vaccination with either PCV10 or PCV13 using an unvaccinated comparator group. One limitation of the study design is the use of different control groups at different time points, although few differences in characteristics were observed between groups, supporting the validity of this approach. Due to funding constraints we were not able to perform DNA microarray for all time points. However, the same method was used for all groups at any given time point. Lastly, we observed much lower pneumococcal carriage rates than anticipated; some of the non-significant differences seen between groups may therefore be due to a lack of power to detect these differences.

In conclusion, we have shown that, compared with unvaccinated controls, 2+1 schedules of PCV10 and PCV13 each reduce the carriage of pneumococcal vaccine serotypes, with the greatest impact seen at 18 months of age. There was a trend towards PCV10 having a greater impact on PCV10-type carriage than PCV13, and a trend towards PCV13 reducing serotype 3/6A/19A carriage that was not seen with PCV10. The majority of pneumococci identified from unvaccinated participants were vaccine-type, so the introduction of either PCV10 or PCV13 would have the potential to generate significant herd protection in the population.

6. Contributors

BT and MLN did the statistical analyses, interpreted the results with input from KM, CS, and HSV, and co-wrote the first draft of the manuscript. HSV and CS oversaw the microbiology with JB, EMD, JH and BO. VTTD managed and performed laboratory testing at the Pasteur Institute laboratory, with PTH, JL, TVP, and HNLT also contributing to laboratory testing. CDN advised on the statistical analyses and BT, MLN, JB and BO verified the underlying data. KB, NTT, and DYU were involved in the design, establishment, day-to-day management, and implementation of the trial. TNH was the site principal investigator, was involved in the design and establishment of the trial, and had overall responsibility its conduct in Vietnam. KM conceived the study, provided oversight for the conduct of the trial and the data analysis, and had overall responsibility for all aspects of the trial as the principal investigator. All authors contributed to refinement of and approved the submitted manuscript.

7. Data sharing

The study protocol and informed consent form have been published previously and are freely available. Data will be made publicly available in accordance with the rules set out by the Bill & Melinda Gates Foundation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors except JB and JH received salary support from National Health and Medical Research Council of Australia (NHMRC) and/or Bill & Melinda Gates Foundation grants. KM has received grant funding for a collaborative study on PCV impact on adult pneumonia from Pfizer. PCV10 vaccine doses were donated by GlaxoSmithKline Biologicals SA. We declare no other competing interests.

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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.2021.02.043.

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Chapter 7: Discussion

Despite the availability of PCV10 and PCV13 for over a decade, there remains a paucity of head-to-head data directly comparing these two products. The main basis for evaluation of new PCVs is their immunogenicity following the primary infant series. However, aside from our Vietnam Pneumococcal Trial, there have been only two randomised controlled trials directly comparing an infant series of PCV10 and PCV13. Of these, one evaluated the immunogenicity and effect on carriage up to 9 months of age of a 3+0 schedule administered at 1, 2, and 3 months in Papua New Guinea, a schedule that is not used anywhere else in the world.[84] The other compared the post-primary series immunogenicity and effect of carriage up to 7 months of age of a 3+0 schedule at 2, 4, and 6 months of age among First Nations Australians.[86,96] There are five other trials reporting post-primary series immunogenicity data that include both PCV10 and PCV13 groups, but none make comparisons between the two products.[87-91] Comparative data are also reported from three countries that have used PCV10 and PCV13 simultaneously. IPD effectiveness data are reported from Sweden, where PCV product is non-randomly chosen at the county level.[112] Data on the impact on carriage are reported in a study of children hospitalised with respiratory infections in Korea, during a time when PCV7 was part of the national immunisation programme and both PCV10 and PCV13 were available on the private market.[98] Carriage data are also reported in a study of healthy children in Cyprus that does not specify how individuals chose the vaccine product.[97] All in all, the data presented in this thesis represent a significant contribution to the evidence on the comparability of the two PCVs that are in widespread use globally.

7.1 Summary of research findings

Detailed discussion of the research findings is contained within the two results research papers (Chapters 5 and 6). This section provides a summary of the key findings in relation to the specific objectives 2-5 of this PhD. The Vietnam Pneumococcal Trial itself represents the outcome for objective 1 (to design a randomised controlled trial to assess differences in the immunogenicity, reactogenicity, and effect on carriage of PCV10 and PCV13).

Objective 2: to directly compare the immunogenicity of a 2+1 schedule of PCV10 or PCV13 up to 18 months of age (Chapter 5)

Immunogenicity assessments form the basis for the regulatory approval and licensure of PCVs. They enable direct comparison of different products irrespective of prior PCV use. We undertook a comprehensive evaluation of the immunogenicity of a 2+1 schedule of PCV10 and PCV13 administered at 2, 4, and 9.5 months of age. We collected blood samples pre-PCV, post-dose one, post-primary series (post-dose two), pre-booster, post-booster, and at 18 months of age. The post-dose one blood samples represent the first comparison of a single-dose of PCV10 and PCV13 in infancy. Both vaccines elicited strong immune responses. For our primary endpoint of the proportion of participants with protective levels of serotype-specific IgG (≥0.35μg/mL) post-primary series, there were no differences between products. Comparison of the magnitude of the responses (GMC of IgG and functional antibody) revealed some differences, but these varied by serotype and by timepoint. Overall, we found that after the first dose, serotype-specific IgG antibody levels (four weeks post-dose one) tended to be higher with PCV10 than with PCV13. Conversely, after the second dose (four weeks post-primary series), both IgG and functional antibody (OPA) levels tended to be higher with PCV13 than with PCV10. By five months post-primary series (pre-booster), IgG levels showed no clear pattern of differences between the two products. Post-booster, both IgG and OPA levels again tended to be higher with PCV13 than with PCV10, but by 18 months of age there were few differences in IgG levels between the two products. In conclusion, both vaccines are highly immunogenic. Serotype-specific differences between products do exist but it is unclear if these would translate to differing degrees of protection afforded by these two vaccines.

Objective 3: to directly compare the reactogenicity of a 2+1 schedule of PCV10 or PCV13 (Chapter 5)

Both PCV10 and PCV13 have been widely used in many countries over a number of years with no safety concerns. At the time the Vietnam Pneumococcal Trial commenced, PCV10 had been recently licensed for use on the private market in Vietnam and PCV13 was unlicensed. The trial provided the unique opportunity to generate local data on the comparative reactogenicity of the two products. We determined the frequency of erythema at the vaccine administration site and axillary fever on days 0-3 post-PCV using parent-held diary cards. The parent/caregiver was provided with a measuring stick and thermometer and trained on how to measure and record erythema and temperature. Across all timepoints, 9% of participants reported erythema at the PCV administration site, with similar frequencies observed following the 2-month, 4-month, and 9.5-month doses. There were no differences comparing vaccination with PCV10 or PCV13 at any timepoint. Across all timepoints, 42% of participants reported axillary fever (≥37.5°C) following vaccination with PCV (co-administered with DTaP-IPV-Hib-HBV), with similar frequencies observed following the 2-month, 4-month, and 9.5-month doses. Between 4 and 10% of participants reported severe fever (≥38.5°C). There were no differences in fever or severe fever comparing vaccination with PCV10 or PCV13 at any timepoint, but the coadministration of either PCV10 or PCV13 with DTaP-IPV-Hib-HBV resulted in a higher frequency of fever than administration of DTaP-IPV-Hib-HBV alone. Overall, we found no evidence for any difference in the safety profiles of PCV10 and PCV13.

Objective 4: to evaluate the effect of a 2+1 schedule of PCV10 or PCV13 on pneumococcal carriage in the first two years of life (Chapter 6)

Pneumococcal carriage is a prerequisite for disease so the effect of vaccination on carriage provides a useful marker for the expected impact on disease. Reduced carriage also leads to reduced transmission, which is the mechanism by which PCVs generate herd protection in the broader unvaccinated community. We collected NP swabs at 2, 6, 9, 12, 18, and 24 months of age from children who received a 2+1 schedule of PCV10 or PCV13 and from unvaccinated controls. The inclusion of unvaccinated controls was a unique aspect of this study and enabled us to determine the vaccine efficacy against carriage (or percent reduction in carriage, calculated as [1 – prevalence ratio] x 100). The vaccine efficacy of PCV10 against PCV10-type carriage was 45 to 62% from 9 months onwards. The vaccine efficacy of PCV13 against PCV13-type carriage was 32 to 49% from 12 months onwards (although it did not reach statistical significance at 24 months). Considering the post-booster period (12-24 months), the

maximum efficacy against carriage for both vaccines was observed at 18 months of age, at which timepoint VT carriage (both PCV10-type and PCV13-type) was at its lowest in both vaccinated groups but at its peak among unvaccinated controls. At this timepoint PCV10 significantly reduced PCV13-type carriage and PCV13 significantly reduced PCV10-type carriage. Directly comparing carriage among PCV10- and PCV13-recipients, few statistically significant differences were seen. However, there was a trend towards a greater effect on PCV10-type carriage with PCV10 than PCV13, and a trend towards a reduction in serotype 3/6A/19A carriage with PCV13 that was not seen with PCV10. Overall, our findings suggest that the introduction of either vaccine would generate substantial individual and herd protection effects.

Objective 5: to describe which pneumococcal serotypes are most commonly carried by unvaccinated children in the first two years of life (Chapter 6)

The inclusion of unvaccinated controls in the Vietnam Pneumococcal Trial enabled us to describe the natural history of pneumococcal carriage over the first two years of life and to determine the most commonly carried serotypes among young children in this population in the absence of widespread vaccination. Participants from the 2+1 PCV10, 2+1 PCV13, and control groups contributed swabs from any pre-PCV timepoints (2 months for the 2+1 PCV10 and 2+1 PCV13 groups, 2-18 months for the control group recruited in infancy, and 18-24 months for the additional control group recruited at 18 months of age). During the first two years of life the most commonly carried serotypes were 6B (representing 46 of the 266 pneumococci identified [17.3%]), 6A (16.2%), 19F (15.4%), 23F (11.3%), 19A (8.3%), 23A (6.4%), 15A (6.4%), and 14 (5.6%). Together, these eight serotypes represented 87% of pneumococci identified, with the remaining 13% (n=35) made up from 14 different serotypes (serotypes 3, 4, 6C, 7C, 8, 11A, 13, 15B/C, 17F, 18C, 19B, 19C, 34, and 35B). Across all timepoints, vaccine serotypes accounted for 51% (PCV10-types) and 76% (PCV13-types) of pneumococci identified. Similarly, during the post-booster period only (12-24 months), vaccine serotypes accounted for 54% (PCV10-types) and 78% (PCV13-types) of pneumococci identified. These data suggest that the introduction of either PCV10 or PCV13 would substantially impact the populations of pneumococci circulating in the community.

7.2 Limitations

The design limitations of the Vietnam Pneumococcal Trial are discussed within the research papers (Chapters 5 and 6). This section provides discussion around the broader limitations of the use of these outcome measures for decision-making regarding the choice of PCV.

7.2.1 Immunogenicity data

The accepted and recommended comparative measure of PCV immunogenicity is the proportion of participants with serotype-specific IgG ≥0.35µg/mL post-primary series. This cut-off was determined to be the threshold that correlates with protection against VT-IPD in a pooled analysis of data from three efficacy trials involving PCV7 and an investigational 9-valent PCV.[28] However, it is recognised that the level of antibody required to protect against IPD varies by serotype and that the threshold does not necessarily predict protection at the individual level. In the United Kingdom (UK), PCV13 serotype-specific correlates of protection were determined through a post-licensure indirect cohort study combined with immunogenicity and PCV7 effectiveness data.[150] Correlates of protection were found to be higher than 0.35µg/mL for serotypes 1, 3, 7F, 9V, 14, 19A, and 19F, exactly 0.35µg/mL for serotype 4, and lower than 0.35µg/mL for serotypes 6A, 6B, 18C, and 23F. A correlate of protection could not be calculated for serotype 5 as there were no cases of serotype 5 IPD during the study period. The aggregate correlate of protection across all PCV7 serotypes was calculated as 0.59µg/mL and across all PCV13 serotypes plus 6C was calculated as 0.98µg/mL. These data suggest that the 0.35µg/mL correlate of protection is too low. In addition to our primary analysis using the standard 0.35µg/mL threshold, we conducted an analysis using a 1.00µg/mL threshold, reported in the manuscript (Chapter 5). This 1.00µg/mL threshold was selected as being very close to the aggregate correlate for PCV13 serotypes from the UK study and to allow comparison with other PCV studies that have used this as an additional threshold. With this more conservative 1.00µg/mL threshold there were still few differences post-primary series between PCV10- and PCV13-recipients. A higher percentage of participants achieved IgG ≥1.00µg/mL with PCV10 for serotype 6B, and with PCV13 for serotypes 1 and 5. In the manuscript we did not analyse the data according to the serotype-specific correlates of protection derived in the UK. Immunological responses to PCV are known to vary significantly by population, so the utility of applying thresholds derived in one population to data from another population is questionable. However, for completeness, these data are presented below and show no differences between products at the 10% level for the shared serotypes (Table 7.1).

Table 7.1: Percentage of participants with IgG above serotype-specific correlates of protection at four weeks post-primary series, comparing PCV10- and PCV13-recipients in the Vietnam Pneumococcal Trial

	Correlate of protection, µg/mL	Participants with IgG ≥correlate of protection, %		Risk difference, % (95% CI)
		PCV10 (n=237)	PCV13 (n=232)	PCV10-PCV13
Shared serotypes				
1	0.78	87.3%	97.4%	-10.1 (-15.1, -5.4)
4	0.35	98.7%	100%	-1.3 (-3.7, 0.6)
5*	..	..	..	..
6B	0.16	92.8%	88.8%	4.0 (-1.3, 9.4)
7F	0.87	89.5%	95.7%	-6.2 (-11.2, -1.5)
9V	0.62	86.9%	94.8%	-7.9 (-13.3, -2.7)
14	0.46	98.3%	98.3%	0.0 (-2.7, 2.9)
18C	0.14	99.2%	99.1%	0.0 (-2.2, 2.3)
19F	1.17	94.9%	96.1%	-1.2 (-5.2, 2.8)
23F	0.20	90.3%	97.8%	-7.5 (-12.2, -3.3)
Additional PCV13 serotypes				
3	2.83	0.8%	15.9%	-15.1 (-20.4, -10.4)
6A	0.16	79.3%	98.7%	-19.4 (-25.1, -14.1)
19A	1.00	21.5%	89.2%	-67.7 (-73.5, -60.4)

The serotype-specific correlates of protection were derived from a post-licensure indirect cohort study in the UK.[150] * A serotype-specific correlate of protection could not be calculated for serotype 5 as there were no cases of serotype 5 IPD during the study period. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV.

Recognising the limitations of using a single threshold to describe protective antibody responses, our comparison of PCV10 and PCV13 focuses more on the levels of antibody (GMCs) over time and the patterns of differences between products. With this approach it is important to recognise that differences between groups can be detected even if both groups produce high levels of antibody that could be sufficient to generate protection. It is a challenge with comparative immunogenicity data to assess whether any observed differences would translate to differing degrees of protection afforded by the two products.

7.2.2 Reactogenicity data

The main limitation with the reactogenicity data in the Vietnam Pneumococcal Trial was the use of parent-held diary cards. Self-reporting could bias the results through an under-reporting of reactions, as only symptoms that are reported can be included in the analysis, or an over-reporting of reactions, as parents are actively looking for symptoms. To minimise potential bias we selected two relatively objective measures, erythema and axillary temperature, and provided training on the use of a measuring stick and thermometer to assess these symptoms.

Study staff also made a follow-up phone call on day 1 post-PCV to ask about any reactions and to check that the diary card was being completed. The limitations of using self-reported reactogenicity data apply equally to all study groups and should therefore not affect the between-group comparisons. Both PCV10 and PCV13 have a globally accepted safety profile. However, at the time of the trial, PCV10 had only been recently licensed and PCV13 was unlicensed in Vietnam. The main purpose of this outcome was therefore to provide local data on the comparative safety of the two vaccines in this population.

7.2.3 Carriage data

Carriage data provide a useful proxy measure for the expected direct and indirect effects of PCV vaccination, as carriage is a precursor to disease.[93] Detecting changes in pneumococcal carriage is also important for monitoring serotype replacement following vaccine introduction. However, pneumococcal serotypes vary in their frequency and duration of carriage and their invasiveness.[151] Some serotypes, such as 1, 5, and 7, are highly invasive but are rarely identified in carriage data. This could be the result of a short duration of carriage or carriage as a sub-dominant serotype. Conversely, some serotypes are commonly carried in the nasopharynx but rarely cause disease. This reveals the complexity of the relationship between carriage and disease and highlights a limitation of using carriage data to predict disease outcomes. Nonetheless, carriage data have successfully been used to model post-vaccination changes in IPD,[152] and are particularly useful for monitoring vaccine impact in LMICs where disease surveillance is often not feasible.[92] In the Vietnam Pneumococcal Trial, molecular microarray serotyping following *lytA* qPCR screening and culture amplification was used to identify pneumococci at the 18- and 24-month timepoints (with latex agglutination and Quellung serotyping following traditional culture at the earlier timepoints due to funding constraints). These sensitive molecular methods are more likely to detect sub-dominant serotypes, especially those carried at low abundance.[153] The use of these methods should therefore increase the concordance between carriage and IPD serotypes.[93] In this trial, the use of carriage data combined with immunogenicity data out to 24 months of age provides a comprehensive comparative evaluation of PCV10 and PCV13.

As noted in Chapter 6, pneumococcal carriage rates observed in the Vietnam Pneumococcal Trial were considerably lower than anticipated. Data from Nha Trang in south-central Vietnam from the time the trial was designed showed pneumococcal carriage rates (including non-typeable pneumococci) of 42% among healthy children in both the 0-12 and the 13-24 month age categories. Across all age groups up to 5 years of age, 68% of serotypes carried were

PCV13-serotypes. We believed that similar carriage rates and serotype distribution would be found in Ho Chi Minh City, so predicted a VT carriage rate of 24% (40% overall pneumococcal carriage, 60% of which would be vaccine serotypes). The observed proportion of pneumococci identified that were PCV13-serotypes among unvaccinated controls in the Vietnam Pneumococcal Trial was slightly lower than that observed in Nha Trang: 56% and 63% at 12 and 18 months of age, respectively. However, the overall pneumococcal carriage rates (including non-typeable pneumococci) were much lower: 29% and 26% at 12 and 18 months of age, respectively; leading to reduced power for the carriage outcomes. Despite this, we were still able to show a statistically significant reductions in VT carriage during the second year of life with either PCV10 or PCV13 compared with controls. We were not able to draw any definitive conclusions on the comparative effect of the two vaccines, but it is likely that even in the context of higher carriage rates a much larger study would be required to tease out such differences. A recent review of pneumococcal carriage in Southeast Asia pre-PCV introduction calculated a pooled pneumococcal carriage prevalence of 36% among healthy children less than 5 years of age, with estimates ranging from 8 to 68%.[136] Our observed carriage rates, whilst lower than predicted, were not dissimilar to those observed elsewhere within the region.

Our examination of carriage among unvaccinated children revealed that the majority (87%) of pneumococci identified were one of only eight serotypes. This analysis included multiple swabs collected from control group participants at three- to six-month intervals, and the same serotype was occasionally detected in consecutive swabs from the same individual. A longitudinal study of pneumococcal carriage during the first year of life from Indonesia found a median carriage duration of 132 days (interquartile range 77-217 days).[154] It is difficult to know whether the cases of consecutive carriage of the same serotype in our data represent single carriage episodes of long duration or multiple shorter carriage episodes with the same serotype. Part of our planned future work is to answer this question using genome sequencing. In the meantime, single carriage episodes of long duration could bias our results by making the affected serotypes appear more prevalent. To assess the impact this could have on our results, I conducted an additional analysis on the overall prevalence of carriage of the eight most commonly carried serotypes. Overall prevalence was defined by carriage of a given serotype at any timepoint between 6 and 18 months, in line with previously reported analyses (Chapter 6). A total of 85/193 (44%) control group participants carried any capsular pneumococcus some time between 6 and 18 months of age. Of these, 75 (88%) carried one of the eight most common serotypes, in line with the previously identified figure of 87%. According to this analysis, the hierarchy of the most common serotypes changed slightly (from 6B, 6A, 19F, 23F, 19A, 23A, 15A, 14, to 6A, 6B, 19F, 19A, 23F*, 23A*, 15A, 14 [*equal prevalence]), but the conclusions remain largely unchanged.

7.3 Reflections

This section offers some personal reflections on the work that contributes to this thesis and key learnings from the process of establishing and conducting the Vietnam Pneumococcal Trial.

7.3.1 The research site

Establishing the first randomised controlled trial to ever be conducted within Ho Chi Minh City was a considerable feat. The approval process in-country was complex and lengthy, involving many different levels of government. The protocol was first reviewed by the Institutional Review Board at the Pasteur Institute, before going to the Ethical Review Committee for Biomedical Research within the Vietnam Ministry of Health. The Ministry of Health ethics review process involved an in-person presentation and defence of the trial protocol followed by several rounds of revisions and re-review. The research plan and budget also had to be separately approved by the Ministry of Health finance department. Additionally, formal written approvals were required at the district level (from the Preventive Medicine Centre within the District 4 and District 7 governments) and at the city level (from the People's Committee of Ho Chi Minh City). Parallel to this, a separate approval process was required for the importation and regulatory testing of the trial vaccines, along with the development of a formal agreement with Children's Hospital Number 2 in Ho Chi Minh City for management of serious adverse events. However, once all the approvals were in place and the trial commenced, it ran very smoothly. We had a high participant retention rate and the majority of visits were conducted on time. Furthermore, there was excellent protocol adherence and sample management. On reflection, despite the time and effort required to get the project up and running, Ho Chi Minh City was an excellent place to conduct this research. If I was to undertake another trial in this setting, a key step would be to use a Contract Research Organisation to navigate and facilitate the ever-changing government approval processes.

7.3.2 Statistics and sample size

In the Vietnam Pneumococcal Trial we used a combination of one-sided and two-sided tests for the between-group comparisons. For the immunogenicity outcomes, one-sided tests were used for assessments of non-inferiority (two-dose versus three-dose primary series comparisons) and two-sided tests were used for assessments of difference (2+1 PCV10

versus 2+1 PCV13 comparisons). For the carriage outcomes, one-sided tests were used for comparisons with controls (vaccinated versus unvaccinated participants) and two-sided tests were used for comparisons of the two vaccines (PCV10-vaccinated versus PCV13-vaccinated participants). Whilst statistically valid, this approach added a level of complexity when presenting the trial results. On reflection, it may have been preferable to use two-sided tests for all comparisons, although this would have reduced the power for the non-inferiority immunogenicity comparisons and the control group carriage comparisons and may therefore have increased the sample size requirements above what was possible within the funding constraints.

7.3.3 Trial modifications

The Vietnam Pneumococcal Trial was modified after recruitment had started. Our initial funding was sufficient to follow participants up to 18 months of age and to conduct standard laboratory analyses (immunological assays by ELISA and OPA and pneumococcal carriage assessment by traditional culture methods). We secured additional funding that enabled us to extend the follow up to 24 months of age and to expand the laboratory work to include B cell assays and molecular microarray serotyping at the later timepoints. At that time, there was growing recognition of the importance of the herd protection effects of pneumococcal vaccination and an impact on carriage at 24 months was believed to be a good predictor of herd protection. To determine the effect of vaccination on carriage at 24 months, we had to recruit an additional unvaccinated control group (as the original control group received a dose of PCV at 18 months of age). By necessity, this non-randomised group was recruited at 18 months of age. Although this was not ideal, it also allowed us to incorporate another objective into the trial: the evaluation of a single dose of PCV10 at 18 months of age.[155] The concept of single-dose PCV schedules was another area of increasing interest, particularly in the context of humanitarian crises and remote settings. On reflection, it would have been ideal to recruit all participants in infancy and to conduct the same microbiological assays at all timepoints. However, this was not feasible with the funds available at the start of the trial, so we made pragmatic choices to maximise the public health value of this research when we were able to secure additional funding.

7.4 Areas for future research

Strategies to increase the accessibility and affordability of PCVs are urgently needed. Sixty percent of the world's children are unvaccinated and pneumococcal disease remains a major cause of mortality in children less than five years of age, despite the availability of effective vaccines. Reduced-dose vaccination schedules, new vaccine products, and mixed-regimen schedules (combining different products) are all areas of interest.

7.4.1 Reduced-dose schedules

PCV schedules consisting of fewer doses ('reduced-dose' schedules) are of increasing interest globally. The Vietnam Pneumococcal Trial included a group that received a reduced-dose schedule of PCV10 consisting of two doses at 2 and 6 months of age. Encouraging immunogenicity and carriage data with this schedule,[156,157] coupled with our earlier finding in Fiji that a single dose in infancy is better at priming for a booster dose than multiple doses for some serotypes,[149] led us to design a second trial specifically to evaluate reduced-dose PCV schedules. The Vietnam Pneumococcal Trial II (VPT-II) included 1+1 (one primary dose plus booster) and 0+1 (single-dose) schedules of PCV10 and PCV13.[158] The 1+1 schedule was administered at 2 and 12 months and the 0+1 schedule at 12 months of age. Recruitment in VPT-II began in March 2017, at which time there were no published data on 1+1 schedules and only one study on 0+1 schedules, a study that evaluated a single-dose of PCV10 among children aged 1 to 4 years in Kenya.[159] A manuscript reporting the main findings from VPT-II has recently been published in The Lancet Infectious Diseases.[160] Briefly, this trial demonstrates that 1+1 schedules of PCV10 or PCV13 dramatically reduce vaccine-type carriage in the second year of life, which is a key period for pneumococcal transmission. Substantial herd protection effects can therefore be expected, supporting a switch to 1+1 schedules for countries with mature PCV programmes. This trial also shows that 1+1 PCV schedules offer some degree of individual protection during the period between doses and generate strong immune responses to the booster dose. This, combined with the carriage findings, suggests that a 1+1 schedule could be considered for countries yet to introduce PCV, in conjunction with a comprehensive catch-up campaign. Like the immunogenicity findings reported in this thesis, VPT-II found that responses to the first dose tend to be stronger with PCV10 than PCV13 and responses to the booster dose tend to be stronger with PCV13 than PCV10. A 1+1 schedule could therefore potentially be most effective given as a mixed regimen of PCV10 followed by PCV13 (see Section 7.4.4). VPT-II also showed that 0+1 schedules of PCV10 or PCV13 provide some impact on carriage and are immunogenic. For the many

children living in hard-to-reach settings, such as humanitarian crises and remote areas, such a schedule could be valuable as a means of providing some protection to these vulnerable populations.

Two other trials, from the UK and South Africa, have evaluated the immunogenicity of 1+1 PCV schedules.[91,161] Post-booster responses were found to be similar with a 1+1 or a 2+1 schedule for most serotypes, with PCV13 in the UK and both PCV10 and PCV13 in South Africa. In 2020, the UK became the first country to implement a reduced-dose schedule of PCV; a 1+1 PCV13 schedule with doses at 3 and 12 months of age. How the pneumococcal epidemiology in the UK is affected by this schedule change over the next few years will be of great interest to many countries.

7.4.2 New vaccine products

Aside from PCV10 and PCV13, which form the subject of this thesis, there is one other PCV that has received WHO prequalification. SIIPL-PCV was developed by the Serum Institute of India, in partnership with PATH (formally Program for Appropriate Technology in Health), as a more affordable alternative to PCV10 and PCV13. SIIPL-PCV is a CRM_{197}-conjugated 10-valent PCV with a comparable safety and immunogenicity profile to PCV10 and PCV13.[162,163] SIIPL-PCV contains eight of the serotypes included in PCV10 (1, 5, 6B, 7F, 9V, 14, 19F, and 23F) and two of the additional serotypes in PCV13 (6A and 19A). The Gavi price for SIIPL-PCV is 2 USD per dose, which is over 30% lower than that for PCV10 or PCV13, making it the likely future vaccine of choice for LMICs.

Several higher-valency PCVs are either recently approved or currently undergoing clinical trials. A 15-valent PCV (PCV15; VAXNEUVANCE, Merck) recently received approval for paediatric use from the European Commission and the FDA. PCV15 is a CRM_{197}-conjugated PCV that contains all the PCV13 serotypes plus serotypes 22F and 33F; the additional serotypes were selected as early data from the US suggested these were important replacement serotypes in children.[164] A 20-valent PCV (PCV20; PREVENAR-20, Pfizer) has been developed as a successor to PCV13. PCV20 is a CRM_{197}-conjugated PCV that contains all the PCV13 serotypes, serotypes 22F and 33F that are also included in PCV15, and serotypes 8, 10A, 11A, 12F, and 15B. PCV20 has been approved for use in adults and is likely to be filed for approval for use in children with both the European Commission and the FDA by the end of 2022.[165,166] Other higher-valency PCVs currently undergoing clinical trials include a 21-valent PCV (Merck), two 24-valent PCVs (Affinivax and Vaxcyte), and a 25-valent PCV

(Inventprise). The cost of all these higher-valency PCVs is likely to prohibit their use in LMICs, so the choice will remain between the three WHO-prequalified vaccines for the foreseeable future.

7.4.3 Mixed-regimen schedules

There is increasing interest in the interchangeability of PCV10 and PCV13 in infant vaccination schedules. The current WHO position is that ideally the same product should be utilised for all doses, but where this is not possible the other product should be used to complete the schedule.[12] Several trials have evaluated mixed-regimen schedules that use a combination of PCV10 and PCV13 doses and found them to be safe and immunogenic (Table 2).[86,90,167-169] These comprise trials from the UK, the Czech Republic, Slovakia, Mexico, and Australia, and include both schedules with mixed-regimen primary series and schedules with a primary series of one product followed by a booster dose of a different product (Table 7.2). Two trials, from Mexico and Australia, included groups with mixed-regimen primary series. In Mexico, the mixed-regimen (PCV13 then PCV10, PS) tended to produce lower antibody levels than single-product-regimens of either PCV10 (SS) or PCV13 (PP).[90] In Australia, the mixed-regimen (SSSP) produced higher antibody levels than either single-product-regimen (SSS or PPP), albeit with an extra dose in the primary series.[86] All the trials included post-booster evaluations, either comparing the same booster product following different primary series products, or comparing different booster products following the same primary series product. Generally, booster responses with a given product were similar regardless of which product was used for the primary series.[90,168,169] Following a PCV10 primary series, booster responses tended to be stronger with PCV13 than PCV10.[169] Following a PCV13 primary series, booster responses were either stronger with PCV13 than PCV10,[167] or were similar between products.[169]

7.4.4 Reduced-dose schedules, new vaccine products, and mixed-regimen schedules

None of the mixed-regimen studies to date have evaluated a primary series combination with PCV10 as the first dose. In the Vietnam Pneumococcal Trial we found that immune responses to the first dose tend to be better with PCV10 than PCV13. This finding has since been replicated in VPT-II and in post-dose one head-to-head data from Australia and South Africa.[86,91,160] This is also supported by carriage data from VPT-II, where we found that PCV10 but not PCV13 reduced VT carriage at 6 months of age, following a single dose of vaccine at

Table 7.2: Trials evaluating mixed-regimen PCV10/PCV13 schedules

Study	Country	Schedule	Regimen	Evaluation timepoint
Trück et al. 2016[166]	UK	2+1 at 2, 4 & 12m	PP+P, PP+S	Post-booster
Urbancikova et al. 2017[167]	Czech Republic, Slovakia	3+1 at 2, 3, 4 & 12-15m (Czech Republic), 2+1 at 2, 4 & 11-12m (Slovakia)	SSS+P, PPP+P, SS+P, PP+P	Post-booster
de los Santos et al. 2020[90]	Mexico	2+1 at 2, 4 & 12-15m	PP+S, PS+S, SS+S	Post-primary series, post-booster
Leach et al. 2021[86], Leach et al. 2022[168]	Australia	3+1 at 2, 4, 6 & 12m, 4+1 at 1, 2, 4, 6 & 12m	PPP+P, PPP+S, SSS+P, SSS+S, SSSP+P, SSSP+S	Post-primary series, post-booster

PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV. m = months of age. P = PCV13. S = PCV10.

2 months of age. Data from VPT-II following a second dose of PCV show that, contrary to the post-dose one findings, immune responses to a second dose tend to be better with PCV13 than PCV10. This is consistent with data from South Africa (on the basis of non-overlapping 95% CIs as this trial did not report any head-to-head comparisons).[91] Together, these findings suggest that a mixed-regimen 1+1 schedule of PCV10 followed by PCV13 could be a way to increase the efficiency of a reduced-dose PCV schedule.

The concept of mixed-regimen schedules is not new. A trial from the 1990s of different Hib conjugate vaccines found that mixed-regimen schedules induced higher antibody levels than schedules with a single product.[170] Three vaccines were evaluated: Hib polysaccharide polyribosylribitol phosphate (PRP) conjugated to outer-membrane protein of *Neisseria meningitidis* (PRP-OMP), PRP conjugated to mutant diphtheria toxin (HbOC), and PRP conjugated to tetanus toxoid (PRP-T). Only PRP-OMP induced an immune response to the first dose, and schedules comprising a first dose of PRP-OMP and second and third doses of either HbOC or PRP-T were equally or more immunogenic than schedules comprising any single product for all three doses. A mixed-regimen schedule of PRP-OMP for the first dose (for the early protection afforded) and HbOC for subsequent doses (for the increased immunogenicity after the complete regimen) was used in Alaska for several years.[171] More recently, in the context of the SARS-CoV-2 pandemic, there is increasing evidence that mixed-regimen COVID-19 vaccine schedules offer superior immunogenicity to single-product schedules,[172] and such schedules are specifically mentioned in the latest WHO recommendations.[173]

Given that PCV13 is likely to become redundant in the medium-term with the development of PCV20 by the same company, a 1+1 PCV10 + PCV13 schedule would be unlikely to come into use. SIIPL-PCV uses the same construct as PCV13, so it can be hypothesised that substituting PCV13 with SIIPL-PCV in this schedule may produce similar results. This question could be answered with a head-to-head immunogenicity trial comparing 1+1 schedules of SIIPL-PCV alone (as the product most likely to be used in LMICs), PCV10 + SIIPL-PCV (as this could enhance the protection afforded by a 1+1 schedule), and PCV10 + PCV13 (for comparison), coupled with modelling studies to predict disease impact.

7.5 Conclusions

Both PCV10 and PCV13 have been in widespread use for a number of years. Post-introduction data from countries using either PCV10 or PCV13 show that both vaccines are highly effective at preventing VT-IPD, not only among vaccinees, through direct protection, but also among the broader population, through indirect herd protection. However, such data do not allow comparative evaluation of these two vaccines, as they come from different populations at different times. The paucity of head-to-head data, despite the availability of both vaccines for over a decade, leaves countries with little information on which to base vaccine choice.

This research has shown that, consistent with previous findings, both vaccines are highly immunogenic in a 2+1 schedule and reduce the carriage of vaccine serotypes. PCV13 contains an additional three serotypes not included in PCV10 (serotypes 3, 6A, and 19A). Serotypes 6A and 19A were commonly carried among unvaccinated children in the Vietnam Pneumococcal Trial, together accounting for around a quarter of pneumococci identified. Serogroup 6 was also a common cause of IPD among children less than 5 years of age in Central and Southern Vietnam, along with serotypes 19F, 23F, and 14, which are common to both vaccines.[116] The relative contribution of serotypes 6A and 6B (and also of 6C) to the serogroup 6 IPD is unknown but could have important implications for product choice in this setting.

Given the different serotype composition, the comparative effect of PCV10 and PCV13 needs to be considered both in relation to the ten shared serotypes and the additional PCV13 serotypes. Both vaccines elicit strong immune responses to the shared serotypes and, as noted previously, it is unclear whether any immunological differences observed between products would translate to clinically-relevant differences. PCV13 generates strong immune responses to the PCV13-only serotypes, although post-booster responses to serotype 3 (both IgG and OPA) are appreciably lower than to all other serotypes, in line with the observed limited and variable effectiveness of PCV13 against serotype 3 IPD.[174] PCV10 generates modest immune responses to 6A and 19A after the booster dose, as a result of cross-protection from vaccine serotypes 6B and 19F. In terms of the comparative effect on carriage, carriage of the shared serotypes was significantly reduced compared with unvaccinated controls at 9, 12, 18, and 24 months with PCV10, but only at 18 months with PCV13. Carriage of PCV13-only serotypes was consistently (albeit not significantly) lower than unvaccinated controls from 9 months onwards with PCV13 but fluctuated with PCV10. It therefore appears that PCV10 has a greater impact on carriage of the shared serotypes than PCV13 and PCV13 has a greater

impact on carriage of the additional PCV13 serotypes than PCV10. This results in a mostly similar overall effect on PCV13-type carriage with both vaccines in this setting.

PCV10 and PCV13 also differ in their carrier proteins. Eight of the serotypes in PCV10 are conjugated to the NTHi Protein D. This has the potential to confer protection against NTHi infection and disease, although previous data on the effect of PCV10 on NTHi OM and carriage are inconclusive. As part of the Vietnam Pneumococcal Trial, we determined the Protein D immunogenicity and the NTHi carriage prevalence and density. We are in the process of writing up these data for publication. Briefly, we found that a 2+1 PCV10 schedule elicited a strong immune response to Protein D both post-primary series and post-booster that was not seen with a 2+1 PCV13 schedule.[175] However, this did not translate to a difference in NTHi carriage prevalence at 12 months of age between PCV10- and PCV13-recipients (12.9% among PCV10-recipients compared with 11.3% among PCV13-recipients).[176] We also found no difference in the concentration of Protein D IgG between infants who carried NTHi and those who did not.[175] At 18 months of age, we observed no difference in the prevalence or density of *Haemophilus influenzae* carriage comparing PCV10-recipients with unvaccinated controls.[177] The relationship between Protein D IgG concentration and disease is unknown, so it is not possible to draw conclusions about any protection conferred against NTHi disease, but we show no evidence for additional protection against NTHi or *Haemophilus influenzae* carriage provided by PCV10.

Pneumococcal isolates identified from the 12-month NP swabs in the Vietnam Pneumococcal Trial underwent antibiotic susceptibility testing. Of the pneumococcal-positive swabs collected across all study groups, 204 out of 218 (94%) contained penicillin-non-susceptible pneumococci and 221 out of 234 (94%) contained MDR pneumococci (defined as non-susceptibility to three or more classes of antibiotic).[178] Regardless of which PCV is introduced into Vietnam in future, surveillance of pneumococcal serotypes and antimicrobial resistance in carriage and disease is recommended to identify and monitor changes in epidemiology and antibiotic resistance patterns following vaccine introduction.

Based on evidence generated in the Vietnam Pneumococcal Trial and elsewhere, the introduction of a 2+1 schedule of either PCV10 or PCV13 to the national immunisation programme would offer significant public health benefits. 2+1 schedules of SIIPL-PCV and 1+1 schedules of PCV10 + SIIPL-PCV have the potential to offer more cost-effective ways to introduce PCV and should be evaluated. However, this should not be at the cost of delaying the introduction of PCV, which should be considered as a priority.

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Appendix A: Chapter 1 supplementary data

Table of Contents

Table S1: Percentage of responders post-primary series from comparative studies of PCV13 and PCV7

a) Percentage of responders (defined as serotype-specific IgG ≥0.35µg/mL) following a primary series of PCV13 or PCV7 and the difference in percentage of responders (PCV13-PCV7) for the shared serotypes

Study		Serotype						
		4	**6B**	**9V**	**14**	**18C**	**19F**	**23F**
Keininger et al. 2010[1]	PCV13 group	98.2	77.5	98.6	98.9	97.2	95.8	88.7
	PCV7 group	98.2	87.1	96.4	97.5	98.6	96.0	89.5
	Difference (95% CI)	0.0 (-2.5,2.6)	**-9.6 (-16.0,-3.3)**	2.2 (-0.4,5.2)	1.5 (-0.9,4.1)	-1.4 (-4.2,1.2)	-0.3 (-3.8,3.3)	-0.8 (-6.0,4.5)
Yeh et al. 2010[2]	PCV13 group	94.4	87.3	90.5	97.6	96.8	98.0	90.5
	PCV7 group	98.0	92.8	98.4	97.2	98.4	97.6	94.0
	Difference (95% CI)	-3.6 (-7.3,-0.1)	**-5.5 (-10.9,-0.1)**	**-7.9 (-12.4,-4.0)**	0.4 (-2.7,3.5)	-1.6 (-4.7,1.2)	0.4 (-2.4,3.4)	-3.6 (-8.5,1.2)
Bryant et al. 2010[3]	PCV13 group	96.8	88.3	96.8	97.9	96.8	97.9	94.7
	PCV7 group	99.1	88.8	99.1	97.2	99.1	97.2	95.4
	Difference (95% CI)	-2.3 (-8.1,2.3)	-0.5 (-9.9,8.7)	-2.3 (-8.1,2.3)	0.7 (-5.0,6.2)	-2.3 (-8.2,2.4)	0.7 (-5.0,6.2)	-0.7 (-7.8,5.9)
Snape et al. 2010[4]*	PCV13 group	95.3	40.2	85.6	92.5	92.8	93.6	66.7
	PCV7 group	97.0	50.5	91.2	96.1	87.3	93.1	65.7
Weckx et al. 2012[5]	PCV13 group	100.0	96.8	98.7	98.1	97.4	94.2	96.8
	PCV7 group	100.0	95.6	100.0	97.5	98.1	98.7	93.0
	Difference (95% CI)	0.0 (-2.4,2.3)	1.2 (-3.4,6.1)	-1.3 (-4.6,1.1)	0.6 (-3.2,4.7)	-0.7 (-4.7,3.2)	-4.5 (-9.5,-0.4)	3.8 (-1.2,9.3)
Huang et al. 2012[6]	PCV13 group	98.8	100	98.8	100	100	98.8	95.0
	PCV7 group	100	100	100	100	100	100	100
	Difference (95% CI)	-1.2 (-6.8,3.3)	0 (-4.6,4.3)	-1.2 (-6.8,3.3)	0 (-4.6,4.3)	0 (-4.6,4.3)	-1.2 (-6.8,3.3)	**-5.0 (-12.3,-0.3)**
Kim et al. 2013[7]	PCV13 group	100.0	98.8	100.0	100.0	100.0	97.6	98.8
	PCV7 group	100.0	100.0	100.0	100.0	100.0	98.8	98.8
	Difference (95% CI)	0.0 (-4.4,4.4)	-1.2 (-6.5,3.2)	0.0 (-4.4,4.4)	0.0 (-4.4,4.5)	0.0 (-4.4,4.4)	-1.2 (-7.3,4.2)	0.0 (-5.5,5.3)
Amdekar et al. 2013[8]	PCV13 group	97.0	84.7	92.6	91.4	95.1	95.0	90.1
	PCV7 group	97.9	87.1	94.4	93.5	93.1	94.7	89.2
	Difference (95.2% CI) †	-0.9 (-4.5,2.7)	-2.4 (-9.5,4.7)	-1.7 (-6.9,3.4)	-2.2 (-8.0,3.4)	2.0 (-2.8,7.2)	0.3 (-4.4,5.2)	0.9 (-5.4,7.2)
Grant et al. 2013[9]*	PCV13 group	96.5	97.6	96.4	97.6	96.4	100.0	96.4
	PCV7 group	100.0	96.9	100.0	100.0	100.0	100.0	100.0
Togashi et al. 2015[10]	PCV13 group	100.0	97.7	100.0	100.0	100.0	98.9	97.7
	PCV7 group	100.0	99.4	100.0	100.0	100.0	96.6	98.3
	Difference (95% CI)	0.0 (-2.2,2.1)	-1.7 (-5.2,1.1)	0.0 (-2.1,2.1)	0.0 (-2.1,2.1)	0.0 (-2.1,2.2)	2.3 (-1.1,6.3)	-0.6 (-4.2,2.9)
Zhu et al. 2016[11]	PCV13 group	100.0	93.2	99.8	99.6	98.6	99.8	96.2
	PCV7 group	99.8	96.1	99.8	100.0	99.1	89.2	96.8
	Difference (97.5% CI)‡	0.2 (-0.9,1.5)	-2.9 (-6.5,0.5)	0.0 (-1.3,1.3)	-0.4 (-1.9,0.6)	-0.5 (-2.4,1.4)	10.6 (7.5,14.3)	-0.7 (-3.6,2.2)

Cells in bold indicate that non-inferiority was not met at the 10% level, with the lower bound of the CI less than -10%. * The difference between groups was not reported in these studies, so non-inferiority was not assessed. † The CI was adjusted for the fact that an interim analysis was performed. ‡ A 97.5% CI was reported in accordance with the Chan and Zhang non-inferiority procedure[12]. PCV = pneumococcal conjugate vaccine. PCV13 = 13-valent PCV. PCV7 = 7-valent PCV. Difference = difference (PCV13-PCV7) in the proportion of responders between groups. CI = confidence interval.

b) Percentage of responders (defined as serotype-specific IgG ≥0.35µg/mL) following a primary series of PCV13 for the additional PCV13 serotypes

Study	Serotype					
	1	3	5	6A	7F	19A
Keininger et al. 2010[1]	96.1	98.2	93.0	91.9	98.6	99.3
Yeh et al. 2010[2]	95.6	63.5	89.7	96.0	98.4	98.4
Bryant et al. 2010[3]	97.9	98.9	100.0	96.8	98.9	100.0
Snape et al. 2010[4]	97.2	86.0	89.3	79.2	94.4	92.7
Weckx et al. 2012[5]	99.4	87.1	98.7	97.4	100.0	99.4
Huang et al. 2012[6]	98.8	97.5	98.8	100.0	100.0	100.0
Kim et al. 2013[7]	100.0	100.0	100.0	97.6	100.0	100.0
Amdekar et al. 2013[8]	96.6	87.6	85.1	90.0	98.0	99.5
Grant et al. 2013[9]	98.8	96.4	97.6	97.6	100.0	98.8
Togashi et al. 2015[10]	100.0	99.4	99.4	98.3	100.0	100.0
Zhu et al. 2016[11]	99.5	99.3	99.6	98.2	99.8	99.6

PCV13 = 13-valent pneumococcal conjugate vaccine

Table S2: Percentage of responders post-primary series from additional studies of PCV13

Study	Country	Primary series	N (by sub-groups)	Shared PCV7/PCV13 serotypes							Additional PCV13 serotypes					
				4	6B	9V	14	18C	19F	23F	1	3	5	6A	7F	19A
[13]	Italy	3, 5m	258-264	96.6	58.4	94.7	94.2	92.4	95.1	68.6	96.6	92.8	91.6	86.5	98.5	98.5
[14]	Poland	2, 3, 4m	127-128 (lot 1)	97.7	77.3	98.4	92.9	96.1	98.4	82.8	93.0	93.7	90.6	85.2	100.0	99.2
			128-131 (lot 2)	96.9	74.0	96.2	94.5	93.1	97.7	81.7	90.8	95.4	88.5	86.3	100.0	99.2
[15]	France	2, 3, 4m	236-244	91.4	72.6	92.9	94.9	90.5	97.9	82.8	90.8	96.3	84.0	85.6	97.5	97.5
[16]	Canada	2, 4, 6m	272-277	97.1	93.1	95.3	98.2	96.4	98.5	90.2	95.7	79.6	87.0	96.4	98.6	97.8
[17]	Spain	2, 4, 6m	199 (post-dose 2)	92.5	27.9	89.9	91.0	88.9	100.0	55.8	96.0	73.8	86.4	80.8	94.5	92.9
			199 (post-dose 3)	98.5	94.9	97.0	97.0	99.0	99.0	93.0	98.5	86.2	96.0	99.0	100.0	99.5
[18]	Mexico	2, 4, 6m	124-162 (post-dose 2)	100.0	81.4	95.0	98.8	93.2	98.1	77.5	98.1	96.9	97.5	94.4	98.8	99.4
			149-162 (post-dose 3)	100.0	97.5	98.1	98.8	98.8	98.1	93.1	99.4	94.4	98.1	98.8	98.8	99.4
[19]	Japan	3 doses†	188	100.0	98.3	100.0	100.0	100.0	97.2	97.7	100.0	100.0	100.0	100.0	100.0	100.0
[20]	Spain	2, 4, 6m	260-273 (post-dose 2)	96.7	57.3	91.9	98.5	91.8	97.8	68.1	96.3	88.0	87.4	84.4	98.5	98.1
			260-273 (post-dose 3)	98.9	98.5	99.3	97.4	98.1	99.3	94.6	99.3	90.3	97.3	97.4	100.0	99.6
[21]	Poland, Spain	2, 3, 4m	98-99 (<37w gestation)	97.0	72.7	97.0	100.0	97.0	99.0	85.9	93.9	85.9	71.7	82.7	99.0	99.0
			97 (≥37w gestation)	99.0	87.6	96.9	97.9	94.8	99.0	92.8	95.9	90.7	90.7	94.8	99.0	99.0
[22]*	Burkina Faso	6, 14w	137	97	72	95	89	86	99	81	97	89	90	96	99	96
		6, 10, 14w	136	99	93	99	99	97	99	96	98	99	91	97	100	99
[23]	China	3, 5m	223-224	99.6	70.1	98.2	99.1	95.5	98.7	90.6	100.0	99.6	98.2	97.3	100.0	98.7
		3, 4, 5m‡	444-446	100.0	93.2	99.8	99.6	98.6	99.8	96.2	99.5	99.3	99.6	98.2	99.8	99.6
		2, 4, 6m	436-440	100.0	94.7	99.5	99.5	98.4	98.2	95.4	100.0	97.3	99.1	97.9	100.0	99.3

Responders are defined as serotype-specific IgG ≥0.35µg/mL. * Study reports the proportion of responders to 2 decimal places (dp), which converts to a percentage with no dp. † Doses were administered at least 4 weeks apart in children aged between 2 and 12 months. ‡ Data for this group also reported in Tables S1 and S2 (Zhu et al. 2016). PCV = pneumococcal conjugate vaccine. PCV13 = 13-valent PCV. PCV7 = 7-valent PCV. m = months. w = weeks.

Table S3: Percentage of responders post-primary series from comparative studies of PCV10 and PCV7

a) Percentage of responders following a primary series of PCV10 or PCV7 and the difference in percentage of responders (PCV10-PCV7) for the shared serotypes

Study		Serotype						
		4	**6B**	**9V**	**14**	**18C**	**19F**	**23F**
Vesikari et al. 2009[24]	PCV10 group	97.1 (94.8)	65.9 (54.8)	98.1 (94.0)	99.5 (99.0)	96.0 (90.7)	95.4 (89.1)	81.4 (66.6)
	PCV7 group	100.0 (99.2)	79.0 (70.7)	99.5 (98.4)	99.5 (97.9)	98.9 (97.6)	99.2 (98.1)	94.1 (87.2)
	Difference (96.5% CI)	-2.9 (-4.2,-1.7)	**-13.1 (-18.3,-7.5)**	-1.4 (-2.6,0.3)	0.1 (-0.7,1.7)	-2.9 (-4.6,0.9)	-3.8 (-5.5,-1.9)	**-12.7 (-16.1,-8.9)**
Wysocki et al. 2009[25]	PCV10 (MenC-CRM)	100.0 (100.0)	94.1 (87.0)	98.8 (98.8)	100.0 (98.2)	98.8 (97.0)	98.2 (98.2)	95.9 (94.1)
	PCV10 (MenC-TT)	99.4 (98.9)	88.6 (81.1)	97.7 (95.4)	100.0 (99.4)	98.9 (98.3)	99.4 (98.9)	96.0 (88.6)
	PCV10 (Hib-MenC)	98.3 (97.7)	87.3 (75.7)	98.3 (96.5)	100.0 (98.3)	99.4 (98.3)	98.8 (97.7)	92.5 (83.8)
	PCV7 (Hib-MenC)	100.0 (100.0)	92.9 (87.0)	98.8 (98.2)	99.4 (97.0)	98.8 (97.0)	100.0 (99.4)	94.1 (91.1)
Bermal et al. 2009[26]	PCV10 (Philippines)	99.3 (99.3)	91.2 (81.8)	99.6 (99.3)	100.0 (98.2)	99.6 (99.3)	100.0 (99.6)	97.2 (94.7)
	PCV7 (Philippines)	100.0 (99.3)	86.3 (81.1)	100.0 (100.0)	100.0 (98.9)	100.0 (98.9)	98.9 (97.9)	94.7 (91.6)
	PCV10 (Poland)	98.9 (96.8)	85.6 (78.2)	100.0 (97.5)	100.0 (98.2)	98.6 (97.2)	98.9 (97.9)	94.4 (88.8)
	PCV7 (Poland)	100.0 (99.0)	94.8 (91.7)	100.0 (99.0)	100.0 (99.0)	99.0 (00.0)	99.0 (96.9)	99.0 (96.9)
van den Bergh et al. 2011[27]	PCV10 (DTaP-HBV-IPV/Hib)	96.1	68.9	95.1	99.5	94.3	95.2	74.9
	PCV10 (DTaP-IPV-Hib)	97.3	64.9	97.3	100.0	94.2	95.1	76.0
	PCV7 (DTaP-IPV-Hib)	99.0	68.9	98.4	100.0	97.9	100.0	92.9
Kim et al. 2011[27]	PCV10 group	99.7	92.4	99.7	99.4	99.7	98.8	96.2
	PCV7 group	100.0	98.4	99.2	100.0	100.0	100.0	98.4
	Difference*	-0.3	-5.9	0.5	-0.6	-0.3	-1.2	-2.2
Knuf et al. 2012[28]	PCV10 group	96.2	62.3	94.3	96.2	100.0	98.1	75.5
	PCV7 group	100.0	81.3	97.9	100.0	100.0	100.0	93.8

Responders defined as ≥0.20µg/mL, with percent ≥0.35µg/mL shown in brackets where reported. * CI around the difference only reported graphically, but all serotypes met criteria for non-inferiority based on 96.5% CI. PCV = pneumococcal conjugate vaccine. PCV13 = 13-valent PCV. PCV7 = 7-valent PCV. Difference = difference (PCV10-PCV7) in the proportion of responders between groups, based on the ≥0.20µg/mL data. CI = confidence interval. MenC = meningococcal serogroup C vaccine. MenC-CRM = MenC with CRM_{197} carrier protein. MenC-TT = MenC with tetanus toxoid carrier protein. Hib-MenC = combined *Haemophilus influenzae* type b (Hib) and MenC vaccine. DTaP = diphtheria-tetanus-acellular pertussis vaccine. HBV = hepatitis B vaccine. IPV = inactivated polio vaccine.

b) Percentage of responders following a primary series of PCV10 for the additional PCV10 serotypes and cross-reactive serotypes

Study	Sub-group	Additional PCV10 serotypes			Cross-reactive serotypes	
		1	5	7F	6A	19A
Vesikari et al. 2009[24]	N/A	97.3 (90.2)	99.0 (95.5)	99.5 (97.4)	22.2 (9.7)	22.6 (8.2)
Wysocki et al. 2009[25]	MenC-CRM	96.4 (90.5)	100.0 (98.8)	100.0 (100.0)	52.7 (41.3)	59.3 (43.1)
	MenC-TT	97.7 (88.5)	100.0 (98.9)	99.4 (98.9)	44.3 (33.3)	59.5 (37.6)
	Hib-MenC	93.1 (84.4)	98.8 (97.1)	98.8 (97.1)	44.2 (28.5)	45.0 (32.2)
Bermal et al. 2009[26]	Philippines	100 (99.6)	100.0 (100.0)	99.6 (99.6)	63.2 (45.6)	68.4 (51.2)
	Poland	98.2 (91.9)	98.9 (96.1)	100.0 (99.3)	44.7 (30.9)	62.0 (43.7)
van den Bergh et al. 2011[27]	DTaP-HBV-IPV/Hib	96.1	100.0	99.0	31.4	31.1
	DTaP-IPV-Hib	98.9	98.9	99.5	29.0	28.2
Kim et al. 2011[27]	N/A	100.0	100.0	100.0	67.4	59.0
Knuf et al. 2012[28]	N/A	100.0	100.0	100.0	-	-

Responders defined as ≥0.20µg/mL, with percent ≥0.35µg/mL shown in brackets where reported. PCV10 = 10-valent pneumococcal conjugate vaccine. N/A = not applicable. MenC = meningococcal serogroup C vaccine. MenC-CRM = MenC with CRM_{197} carrier protein. MenC-TT = MenC with tetanus toxoid carrier protein. Hib-MenC = combined *Haemophilus influenzae* type b (Hib) and MenC vaccine. DTaP = diphtheria-tetanus-acellular pertussis vaccine. HBV = hepatitis B vaccine. IPV = inactivated polio vaccine. - = not reported.

Table S4: Percentage of responders post-primary series from additional studies of PCV10

Study	Country	Primary series	N (by sub-groups)	Shared PCV7/PCV13 serotypes											
				4	6B	9V	14	18C	19F	23F	1	5	7F	6A	19A
[29]	Denmark, Norway, Slovakia, Sweden	3, 5m	158	98.0	55.7	93.4	96.1	96.1	92.8	69.3	97.4	96.1	96.7	-	-
				(94.8)	(45.0)	(86.2)	(90.8)	(87.5)	(91.4)	(55.6)	(86.3)	(94.7)	(92.8)		
		3, 4, 5m	154	99.3	63.1	99.3	100.0	99.3	96.1	77.6	98.7	100.0	99.3	-	-
				(94.8)	(49.0)	(96.1)	(98.0)	(97.4)	(94.7)	(64.5)	(90.7)	(99.3)	(99.3)		
[30]	Czech Republic	3, 4, 5m	204-208 (paracetamol)	99.5	62.1	98.0	99.5	95.7	97.6	80.4	97.6	99.5	99.0	-	-
			225-227 (no paracetamol)	99.6	75.6	98.7	99.6	99.6	100.0	87.1	99.1	99.6	99.6	-	-
[31]	Chile	2, 4, 6m	117	99.1	93.2	99.1	100.0	99.1	100.0	94.0	100.0	100.0	100.0	50.0‡	86.8‡
[32]	Spain, Greece	2, 4, 6m	40-42 (27-30w gestation)	97.6	92.7	97.6	100.0	100.0	100.0	95.1	97.6	100.0	100.0	38.1	23.8
			79-82 (31-36w gestation)	98.8	95.1	100.0	100.0	100.0	100.0	96.3	100.0	100.0	100.0	49.4	49.4
			128-132 (≥37w gestation)	100.0	93.9	100.0	100.0	98.5	100.0	95.4	99.1	100.0	100.0	52.7	58.0
[33]	Mexico	2, 4, 6m	218-219	100.0	93.1	100.0	99.1	99.5	99.1	95.0	100.0	100.0	100.0	58.9	55.3
[34]	Mali, Nigeria	6, 10, 14w	217	100.0	82.0	97.2	99.1	99.5	98.6	87.6	100.0	100.0	99.5	25.8	43.8
[35]	India	6, 10, 14w	229	98.3	77.7	99.1	100.0	99.1	99.1	89.5	99.6	98.7	99.6	41.5	63.8
[36]	Taiwan	1.5, 3, 6m	218-219	99.5	95.4	100.0	99.5	100.0	100.0	97.2	100.0	100.0	100.0	78.5	64.7
[37]	Argentina, Panama	2, 4, 6m	334	99.4	93.1	98.8	98.2	98.8	97.3	96.1	99.7	99.7	99.7	64.4	61.1
[38]	Singapore, Malaysia	2, 3, 5m	218-219 (clinical lot)	100.0	96.3	100.0	99.5	100.0	99.5	98.2	100.0	99.5	100.0	69.4	61.6
			217-218 (commercial lot)	100.0	93.6	100.0	100.0	99.5	99.5	97.2	100.0	100.0	100.0	60.6	54.6
[39*]	Nepal	6, 14w	91-107				100.0				98.9	100.0		-	-
				(97.1)	(53.0)	(96.1)	(97.1)	(96.0)	(98.0)	(62.0)	(95.0)	(100.0)	(98.1)		
		6, 10, 14w	84-109				100.0				100.0	98.8		-	-
				(98.0)	(61.9)	(98.0)	(99.0)	(99.0)	(97.9)	(78.4)	(97.9)	(99.0)	(100.0)		
[40]	Japan	3, 4, 5m	229-231	100.0	92.6	99.6	100.0	100.0	99.6	94.8	100.0	100.0	100.0	70.0	76.6
[41]	South Africa	6, 10, 14w	70 (HIV-infected)	98.6	87.1	97.1	98.6	100.0	97.1	90.0	98.6	100.0	98.6	32.9	37.7
			91 (HIV-exposed-uninfected)	98.9	87.9	98.9	98.9	98.9	98.9	92.3	98.9	98.9	98.9	27.5	57.1
			93 (HIV-unexposed-uninfected)	100.0	79.6	100.0	100.0	100.0	100.0	89.2	100.0	100.0	100.0	28.0	54.8
[42]	Romania	3, 4, 5m	154 (ibuprofen)	99.3	84.0	99.3	100.0	99.3	100.0	91.9	100.0	100.0	99.4	44.2	53.1
			158 (delayed ibuprofen)	100.0	87.1	100.0	99.4	99.4	98.7	89.2	100.0	100.0	100.0	47.4	52.0
			164 (no ibuprofen)	99.4	84.7	98.7	99.4	98.7	99.4	92.0	99.4	99.4	100.0	43.4	40.1
			55 (paracetamol)	96.4	79.2	100.0	100.0	98.1	100.0	87.0	96.3	100.0	100.0	35.8	41.5
			55 (delayed paracetamol)	100.0	72.5	100.0	100.0	100.0	100.0	81.1	98.0	100.0	100.0	30.0	50.0
			56 (no paracetamol)	100.0	87.3	98.1	100.0	100.0	100.0	90.9	100.0	100.0	100.0	49.1	56.6
[43]	Burkina Faso	2, 3, 4m	48 (sickle cell disease)	100.0	85.4	100.0	97.8	100.0	100.0	89.6	100.0	100.0	100.0	-	-
			46 (no sickle cell disease)	100.0	91.3	100.0	97.8	100.0	97.8	89.1	100.0	100.0	100.0	-	-
[44]	South Africa	6, 14w	96-97	99.0	82.5	94.8	97.9	97.9	97.9	86.6	99.0	97.9	99.0	24.7	58.8
		6, 10, 14w	185-187	100.0	84.4	100.0	100.0	100.0	100.0	89.8	100.0	100.0	100.0	29.6	58.4
[45]	Bangladesh	6, 10, 18w	154 (4-dose vial)	100.0	84.4	98.7	99.4	100.0	98.7	89.0	99.4	100.0	100.0	61.7	81.8
			146 (1-dose vial)	100.0	84.9	97.9	100.0	100.0	99.3	94.5	100.0	100.0	100.0	58.2	80.1
[46]	Ghana, Burkina Faso	8, 12, 16w	140-141 (with RTS,S)	99.3	87.2	97.2	100.0	98.6	98.6	92.1	100.0	100.0	100.0	-	-
			134-135 (with HBV)	100.0	87.4	99.3	98.5	100.0	96.3	89.6	100.0	100.0	100.0	-	-

Study	Country	Primary series	N (by sub-groups)	Shared PCV7/PCV13 serotypes											
				4	6B	9V	14	18C	19F	23F	1	5	7F	6A	19A
[47†]	Estonia, Germany, Spain	2, 3, 4m	95-96 (3 x MenACWY-TT)	97.9	74.0	95.8	100.0	93.8	95.8	87.4	96.9	87.5	97.9	-	-
			103-104 (2 x MenACWY-TT)	95.2	78.8	95.2	100.0	97.1	90.4	79.8	91.3	82.7	95.2	-	-
			93-95 (2 x MenC-CRM)	97.9	80.6	94.6	100.0	94.7	94.7	81.9	95.7	86.0	100.0	-	-
			103 (2 x MenC-TT)	96.1	78.6	96.1	100.0	98.1	96.1	83.5	88.3	89.3	100.0	-	-
[48†]	Nepal	6, 10w	140-141	95.0	47.1	92.9	98.5	91.4	97.1	47.1	87.2	90.0	93.6	-	-
		6, 14w	144-145	96.5	65.2	93.7	96.5	100.0	99.3	63.5	97.2	89.6	97.9	-	-
[49†]	The Netherlands	3, 5m	52 (maternal pre-natal DTaP)	71.2	67.3	88.5	96.2	71.2	90.4	73.1	92.3	90.4	98.1	13.5	7.7
			50 (maternal post-natal DTaP)	74.0	54.0	94.0	94.0	80.0	96.0	66.0	94.0	90.0	98.0	14.0	18.0

Responders defined as ≥0.20µg/mL, with percent ≥0.35µg/mL shown in brackets where reported, unless indicated. * Proportion ≥0.20µg/mL shown graphically except for serotypes 14, 1, and 5; values >90% for all serotypes except 6B and 23F. † Values reported are percentage ≥0.35µg/mL as WHO reference laboratory ELISA used. ‡ n=54 and 53 for serotypes 6A and 19A, respectively. PCV = pneumococcal conjugate vaccine. PCV13 = 13-valent PCV. PCV7 = 7-valent PCV. m = months. w = weeks. HIV = human immunodeficiency virus. RTS,S = RTS,S/AS01 malaria vaccine. HBV = hepatitis B vaccine. MenACWY-TT = quadrivalent meningococcal vaccine with tetanus toxoid carrier protein. MenC = meningococcal serogroup C vaccine. MenC-CRM = MenC with CRM_{197} carrier protein. MenC-TT = MenC with tetanus toxoid carrier protein. DTaP = diphtheria-tetanus-acellular pertussis vaccine.

Table S5: Percentage of responders post-primary series from studies that contain groups vaccinated with PCV10 or PCV13

Study		Shared PCV10/PCV13 serotypes										Additional PCV13 serotypes		
		1	4	5	6B	7F	9V	14	18C	19F	23F	3	6A	19A
Pomat et al. 2019[50]*	PCV10	98.2 (95.6,99.5)	96.3 (92.7,99.5)	99.1 (97.2,99.5)	92.2 (86.9,97.4)	99.1 (97.2,99.5)	95.4 (91.4,99.5)	100 (100,100)	97.3 (94.1,99.5)	99.1 (97.2,99.5)	83.5 (76.3,90.7)	45.0 (35.3,54.6)	35.8 (26.5,45.1)	89.0 (82.9,95.1)
	PCV13	99.0 (97.1,99.5)	93.1 (88.2,98.0)	99.0 (97.1,99.5)	92.2 (86.9,97.4)	100 (100,100)	95.1 (90.9,99.3)	99.0 (97.1,99.5)	94.1 (89.6,98.7)	100 (100,100)	91.2 (85.7,96.7)	81.4 (73.8,88.9)	77.5 (69.3,85.6)	98.0 (95.4,99.5)
Leach et al. 2021[51]*†	PCV10	100	100	92	97	100	97	98	97	97	95	37	54	78
	PCV13	99	97	97	89	100	98	99	97	99	96	96	98	100
Prymula et al. 2017[52]	PCV10	98.5 (94.7,99.8)	97.7 (93.5,99.5)	99.3 (95.9,100)	72.9 (64.5,80.3)	99.3 (95.9,100)	97.8 (93.7,99.5)	100 (97.3,100)	97.8 (93.6,99.5)	100 (97.3,100)	83.0 (75.5,88.9)	13.6 (8.3,20.7)	33.8 (25.9,42.5)	46.3 (37.6,55.1)
	PCV13	97.7 (93.5,99.5)	97.0 (92.4,99.2)	96.2 (91.4,98.8)	75.8 (67.5,82.8)	97.7 (93.5,99.5)	97.0 (92.4,99.2)	97.0 (92.4,99.2)	97.0 (92.4,99.2)	97.0 (92.4,99.2)	91.7 (85.6,95.8)	97.7 (93.5,99.5)	96.2 (91.4,98.8)	97.7 (93.5,99.5)
Carmona Martinez et al. 2019[53]	PCV10	98.6 (95.9,99.7)	96.7 (93.3,98.6)	99.5 (97.4,100)	75.2 (68.8,80.9)	99.5 (97.4,100)	99.0 (96.6,99.9)	100 (98.3,100)	98.1 (95.2,99.5)	97.6 (94.5,99.2)	83.8 (78.1,88.5)	-	30.3 (24.1,37.0)	47.4 (40.4,54.4)
	PCV13	99.5 (97.5,100)	100 (98.3,100)	99.1 (96.7,99.9)	78.4 (72.4,83.7)	100 (98.3,100)	100 (98.3,100)	99.5 (97.5,100)	100 (98.3,100)	100 (98.3,100)	94.5 (90.6,97.1)	-	99.5 (97.5,100)	99.5 (97.5,100)
Odutola et al. 2019[54]	PCV10	100 (98.1,100)	99.5 (97.2,100)	99.5 (97.1,100)	82.3 (76.1,87.4)	99.0 (96.3,99.9)	98.0 (94.9,99.4)	99.5 (97.1,100)	99.5 (97.1,100)	97.4 (94.1,99.2)	86.8 (81.1,91.3)	10.2 (6.3,15.5)	28.9 (22.5,35.9)	53.8 (46.3,61.2)
	PCV13	100 (98.1,100)	100 (98.1,100)	100 (98.1,100)	92.7 (88.0,95.9)	100 (98.1,100)	98.5 (95.5,99.7)	100 (98.1,100)	98.4 (95.5,99.7)	100 (98.1,100)	97.4 (94.0,99.1)	100 (98.1,100)	99.5 (97.1,100)	98.4 (95.5,99.7)
de Los Santos et al. 2020[55]	PCV10	100 (95.8,100)	100 (95.8,100)	100 (95.8,100)	94.2 (87.0,98.1)	100 (95.8,100)	100 (95.8,100)	100 (95.8,100)	98.8 (93.7,100)	98.8 (93.7,100)	94.2 (87.0,98.1)	12.9 (6.6,22.0)	56.0 (44.7,66.8)	62.8 (51.7,73.0)
	PCV13	100 (95.9,100)	98.8 (93.6,100)	100 (95.8,100)	70.6 (59.7,80.0)	100 (95.8,100)	100 (95.8,100)	100 (95.8,100)	98.8 (93.6,100)	100 (95.8,100)	92.9 (85.3,97.4)	100 (95.8,100)	94.1 (86.8,98.1)	97.6 (91.8,99.7)
Mahdi et al. 2020[56]*	PCV10	100 (96.0,100)	96.8 (90.9,98.9)	94.6 (88.0,97.7)	76.3 (66.8,83.8)	96.8 (90.9,98.9)	94.6 (88.0,97.7)	95.7 (89.5,98.3)	81.7 (72.7,88.3)	97.8 (92.5,99.4)	76.3 (66.8,83.8)	4.3 (1.7,10.5)	14.0 (8.4,22.5)	23.7 (16.2,33.2)
	PCV13	95.8 (89.7,98.4)	91.6 (84.3,95.7)	91.6 (84.3,95.7)	61.1 (51.0,70.2)	96.8 (91.1,98.9)	85.3 (76.8,91.0)	84.2 (75.6,90.2)	83.2 (74.4,89.4)	96.8 (91.1,98.9)	75.8 (66.3,83.3)	84.2 (75.6,90.2)	80.0 (70.9,86.8)	92.6 (85.6,96.4)

Data are percentages (95% confidence intervals [CIs]). * These studies report the percentage of participants with serotype-specific IgG ≥0.35µg/mL; all other studies report the percentage ≥0.20µg/mL. † CIs not reported. PCV = pneumococcal conjugate vaccine. PCV10 = 10-valent PCV. PCV13 = 13-valent PCV.

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Appendix B: LSHTM ethics approval

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Observational / Interventions Research Ethics Committee

Miss Beth Temple
LSHTM

22 September 2016

Dear Beth

Study Title: Pneumococcal vaccination for developing countries: PCV10 or PCV13?

LSHTM Ethics Ref: 10378

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Advertisements	Brochure _v3.0_en	22/11/2013	3.0
Local Approval	MOH approval 18.08.2014_en	18/08/2014	na
Local Approval	HREC Approval letter Amendment request approved 10.09.14	10/09/2014	na
Information Sheet	02 ICF_v4.0_en	27/10/2014	4.0
Information Sheet	01 PLS_v5.0_en	05/03/2015	5.0
Protocol / Proposal	Protocol_v10.0	03/06/2015	10.0
Information Sheet	01b PLS_v3.0 control group G	17/06/2015	3.0
Local Approval	MOH approval 24.07.2015_en	24/07/2015	na
Local Approval	HREC Approval Letter Amendment 10.08.15	10/08/2015	na
Local Approval	IRB approval 28-08-15	28/08/2015	na
Local Approval	HREC Approval-Notification Letter 28.09.15	28/09/2015	na
Investigator CV	CVs_LSHTM ethics	06/10/2015	1.0
Protocol / Proposal	Explanatory letter	24/03/2016	n/a
Covering Letter	Covering letter_requested clarification	23/08/2016	na

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

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Improving health worldwide

Appendix C: Supplementary appendix to Chapter 4 research paper

Table of Contents

APPENDIX 1

Biological Specimens

Specimens include NP swabs, bacterial isolates cultured from NP swabs, serum from whole blood, plasma from whole blood and peripheral blood mononuclear cells (PBMCs). Long-term storage of specimens is at the Pneumococcal Laboratory at MCRI or at the Pasteur Institute of Ho Chi Minh City at -80°C. No genetic or HIV testing will be performed on stored samples and they will not be used to establish a tissue bank. Consent for the long-term storage of samples and their use in potential future studies is recorded on the ICF.

Sample Collection

Blood samples are collected using a butterfly needle into gel vacutainer tubes or sodium heparin vacutainer tubes. The volume of blood collected at different ages is as follows: 2.0ml at 2 months of age; 3.5ml from 3-10 months and 19 months of age; and 3.5ml or 7.5ml at 18 months and 24 months of age, depending on the assays to be conducted. Blood samples collected into gel vacutainer tubes are kept chilled in a cooler box and transported to the Pasteur Institute laboratory the same day. On arrival at the laboratory the samples are centrifuged and the sera divided into up to three aliquots, stored in micro-tubes and frozen at -80°C prior to analysis. For blood samples where plasma cell and memory B cell responses are assessed, samples are collected into sodium heparin vacutainer tubes and transported to the Pasteur Institute laboratory at room temperature the same day. On arrival at the laboratory plasma and PBMCs are separated from each heparinized blood sample by density gradient centrifugation. Plasma are divided into up to four aliquots and stored at -80°C prior to analysis.

NP samples are collected and transported according to standard guidelines.[1] In brief, NP samples are collected using sterile swabs and placed immediately into 1000µL Skim Milk Tryptone Glucose Glycerol (STGG) transport medium. The samples are kept chilled in a cooler box and transported to the Pasteur Institute laboratory the same day. On arrival at the laboratory two aliquots are removed and the aliquots and original sample are frozen at -80°C prior to analysis.

Serotype-specific IgG

Serotype-specific anti-pneumococcal IgG levels to each of the 13 serotypes in 13v-PCV are measured using a modified 3rd generation standardized ELISA at the Pasteur Institute laboratory.[2] Briefly, microtiter wells are coated with 2.5-10 mg/mL pneumococcal polysaccharide, depending on the serotype. This is diluted in phosphate buffered saline by incubating at 22° C overnight. To neutralize unspecified cell wall polysaccharide antibodies, 1/100 diluted serum samples are incubated overnight with 10 mg/mL of cell wall polysaccharide and 30mg/mL of serotype 22F, before further dilutions. A reference serum (89-SF, Food and Drug Administration, Bethesda MD) is used and incubated overnight with 10 mg/mL of cell wall polysaccharide. Horse radish peroxidase conjugated anti-human IgG and the TMB Peroxidase Substrate system is used for detection. Results are expressed as µg/mL of serotype-specific IgG. Three control sera will be used on each plate to assess inter-assay variation.

Opsonophagocytic Assay (OPA)

OPAs are conducted at the Pneumococcal Laboratory at MCRI.[3] Serial dilutions of a heat-inactivated sera, in Hanks balanced salt solution with Mg^{++}, Ca^{++} and gelatine, are made in a 96-well sterile microtitre plate. Frozen stock of pneumococci are thawed, washed and diluted to 5×10^4 CFU/serotype/mL. Standard bacterial dilutions are added to all wells and the plate incubated at RT for 30 min. At 30 min, baby rabbit complement, thawed just prior to use, followed by HL-60 cells (2×10^7 cells/ml) is added to all test wells. A bacterial control (heat inactivated foetal calf serum in place of human sera and no complement) and complement control (no sera) are included on all plates. Plates are placed on a horizontal shaker and incubated for 45 min at 37°C in 5% CO_2. The reaction is stopped at 45 min by placing the plate on ice. A 10µL aliquot of this mixture is then spotted onto Todd-Hewitt broth–yeast extract (0.5%) agar plates. After application of an overlay THYE agar containing selective antibiotic (Optochin, Spectinomycin, Streptomycin or Trimethoprim) and 2,3,5-Triphenyltetrazolium chloride (TTC), the plates are incubated overnight at 37°C in 5% CO_2. After overnight incubation, plates are counted and the results expressed as opsonisation indices (OI) where the OI is defined as the interpolated dilution of serum that kills 50% of bacteria.

Memory B cells

Analysis of the memory B cell response is undertaken at the Pasteur Institute laboratory, by ELISPOT assay.[3] PBMCs are re-suspended in RPMI Foetal Calf Serum (FCS) at a concentration of $2x10^6$ cells/mL and 100µL added to each well of the culture plate containing an antigen cocktail (Staphylococcus aureus Cowan strain – Pansorbin cells (SAC; 1:5000), 2.5µg/mL CpG and 83ng/mL pokeweed mitogen). Plates are incubated at 37°C with 5% CO_2 and 95% humidity for 5 days. At day 5, cells are harvested and washed and the cell pellet re-suspended in 1mL RPMI-FCS and counted by trypan blue. Cells are then made up to a final concentration of $2x10^6$ cells/mL for seeding onto antigen-coated ELISPOT plates. Multiscreen hydrophobic polyvinyldene difluoride (PVDF) membrane ELISPOT plates coated with anti-IgG (10µg/mL), tetanus toxoid (5µg/mL), diphtheria toxoid (10µg/mL) or pneumococcal polysaccharides conjugated to methylated human serum albumin at concentrations in the range 10-20µg/mL are sealed and incubated overnight at 4°C. ELISPOT plates are then washed and blocked with RPMI-FCS for 30 minutes at 37°C with 5% CO2 and 95% humidity. Cultured cells or *ex vivo* PBMCs are washed and seeded at 200 to $2x10^5$ cells/well of the antigen-coated ELISPOT plates in RPMI-FCS and incubated overnight at 37°C with 5% CO_2 and 95% humidity. Cells are then washed with PBS-T and bound IgG detected with an alkaline phosphatase-conjugated IgG for 4 hours at RT. ELISPOT plates are washed again before addition of an alkaline phosphatase substrate solution (nitroblue tetrazolium plus 5-bromo-4-chloro-3-indoylphosphate in dimethyl formamide). The reaction is stopped with two washes in distilled water. Cells are visualized and counted using an automated ELISPOT reader and software. The total frequency of IgG-secreting antibody-forming cells (AFCs) is used as the positive control and 1,000 IgG AFCs/10^6 cultured PBMCs is the lower cut-off for inclusion in the analysis. Up to $15x10^6$ cells/mL are used for the memory B cell assay at the Pasteur Institute and the remainder of the PBMCs are cryopreserved in liquid nitrogen in aliquots of $8\text{-}10x10^6$ cells/mL for planned T cell assays.

S. pneumoniae identification and serotyping

Identification of *S. pneumoniae* is conducted in line with WHO guidelines.[1] In brief, 50µl swab is plated onto Columbia colistin-nalidixic acid blood agar plates, and identification is primarily based on colonial morphology (flat, with a dimple, 1-3mm in size), α-haemolysis and optochin sensitivity. One colony, plus any additional colonies if morphologically distinct, is sub-cultured onto horse blood agar with an optochin disc. Any colonies that are optochin resistant or intermediately resistant but

otherwise appear to be *S. pneumoniae* are subject to *lytA* PCR,[1] following DNA preparation using the InstaGene matrix (BioRad). All presumptive pneumococci are serotyped, primarily by latex agglutination using reagents produced in-house using antisera from the Statens SerumInstitut, as previously described.[4 5] In summary, pneumococcal culture is made to a 4-5 McFarland density standard and then 10µL of the suspension mixed with 10µL of latex reagent on clear glass slides and rotated for 1 minute. A positive test is indicated by aggregation of latex particles and clearing of the suspension. Isolates that do not react with antisera are subject to *lytA* PCR.

H. influenzae identification

Identification of *H. influenzae* is made from 50µl swab plated onto bacitracin-vancomycin-clindamycin-chocolate-agar. One presumptive *H. influenzae* colony, plus any additional colonies if morphologically distinct, is selected. Colonies are identified as grayish, semi-opaque, smooth, flat or convex, 1-3mm in size. Confirmation is initially demonstrated by X and V growth factor dependence. Capsular and NTHi strains are discriminated using the Phadebact® Haemophilus coagglutination test. All NTHi isolates are tested for beta-lactamase production using nitrocefin.[6] Following identification of presumptive NTHi, DNA is extracted using the InstaGene matrix (BioRad)[7] and tested by *siaT* and *hypD* PCR for discrimination between NTHi and *H. haemolyticus*.[8]

Quantification of *H. influenzae* and pneumococcus

DNA is extracted from 100µl of STGG medium using high-throughput systems (MagNA Pure LC, Roche) using the DNA Isolation Kit II (Bacteria, Fungi) (Roche) incorporating enzymatic digestion. Quantification of *H. influenzae* and pneumococci is then performed using real-time quantitative PCR (qPCR).[9] qPCR targeting the *hpd3* and/or *siaT* gene (*H. influenzae*) or *lytA* gene (pneumococcus) is conducted in 25µl reactions containing 2µl of template DNA on a Stratagene Mx3005 machine using Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technologies) according to the manufacturer's instructions. The density of each bacterial species is assessed in comparison to a set of approximately five reference standards run with each assay to give the density of carriage.

Microarray serotyping

Samples that contain pneumococci are tested by DNA microarray as described previously with minor modifications.[4] Following a culture amplification step (on selective agar such as horse blood agar with 5 µg/ml gentamicin), DNA is extracted

using the Qiacube HT platform (Qiagen). When only a single α-haemolytic colony grows, it is sub-cultured before DNA extraction for microarray. DNA is labelled and then hybridised to the Senti-SP microarray (formally BUGS microarray), scanned on an Agilent scanner, and uploaded to Senti-Net (a cloud based software platform). Serotype-specific density is calculated by multiplying pneumococcal density (measured by *lytA* qPCR) by the relative abundance of each serotype (determined by microarray).

Immunogenicity of *Infanrix-hexa*

The specific IgG to *Haemophilus influenzae* type b (Hib) will be measured by ELISA. High binding ELISA plates are coated with Hib polysaccharide (HBO-HA, the PRP capsular linked to human albumin) antigen and incubated at 37°C for 2 hours and then overnight at 4°C. The plates are washed and blocked with 1% Gelatin in PBS, then loaded with dilutions of standards and patient samples. Following two hours incubation at 37°C, the plates are washed and peroxidase-labelled anti-human IgG is added to each well. Bound specific antibody is detected using the substrate TMB. After the substrate reaction, the intensity of the colour developed is proportional to the amount of IgG-specific antibodies detected in the sample. Results for the samples are determined directly using a standard curve and expressed as µg/mL. Three control sera will be used on each plate to assess inter-assay variation.

The specific IgG to tetanus and diphtheria will be measured using a commercial solid phase ELISA (Genzyme Virotech). The wells are coated with antigen. Specific antibodies of the sample bind to the antigen coated wells and are detected by a secondary enzyme conjugated antibody specific for human IgG. After the substrate reaction, the intensity of the colour developed is proportional to the amount of IgG-specific antibodies detected in the sample. Results for the samples are determined directly using a standard curve and expressed as IU/mL. Two control sera will be used on each plate to assess inter-assay variation.

The Hepatitis B surface antibodies will be measured using AxSym analyzer system. Patient serum is incubated with Micro-particles coated with recombinant HbsAg. Antibody present in the serum binds with antigen on the particles. When this reaction mixture is transferred to the matrix cell, the micro-particles bind irreversibly to the glass fibre matrix. Biotinylated rHBsAg is then added forming an antigen-antibody-antigen complex. Anti-Biotin: Alkaline phosphatase conjugate is dispensed onto the matrix cell and binds with any microparticle-bound antigen-antibody-antigen complex.

The matrix cell is washed to remove any unbound antibody and the substrate 4-Methylumbelliferyl Phosphate is added. The alkaline phosphatase-labelled conjugate catalyses the removal of a phosphate group from the substrate, yielding a fluorescent product, 4-Methylumbelliferone. This fluorescent product is measured and the concentration of anti-HBs in the sample is determined from a calibration curve and will be reported in IU/mL. A positive and negative control will be included in each assay.

The specific IgG to *B. pertussis* (PT) will be measured using a commercial solid phase ELISA (Genzyme Virotech). The wells are coated with antigen. Specific antibodies of the sample bind to the antigen coated wells and are detected by a secondary enzyme conjugated antibody specific for human IgG. After the substrate reaction, the intensity of the colour developed is proportional to the amount of IgG-specific antibodies detected in the sample. Results for the samples are derived using the optical density ratio of the cut-off control and the patient sample and expressed in VE or Virotech Units which have been calibrated with the reference standard IgG anti-Pertussis toxin (Lot 3, 200 U/ml) of the Centre for Biologic Evaluation and Research (CBER), FDA. Three control sera will be used on each plate to assess inter-assay variation.

Appendix 1 - References

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APPENDIX 2

Plain Language Statements and Informed Consent Form

These materials were translated into Vietnamese, and back-translated into English, by FHI360. This trial uses two Plain Language Statements, one for participants enrolled at 2 months of age and randomised into Arms A-F, and one for participants enrolled at 18 months of age into Arm G. The same Informed Consent Form is used for participants in all Arms.

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ABN: 70 413 542 847

INFORMATION SHEET: Vietnam Pneumococcal Vaccine Study

This is for you to keep.

Principal Investigators:
Assoc. Prof. Tran Ngoc Huu
Prof. Edward Kim Mulholland

Research Partners:
Pasteur Institute, Ho Chi Minh City
Menzies School of Health Research
Murdoch Childrens Research Institute

Introduction

Health research helps us to understand diseases and find ways to prevent them. Vaccines (like the routine baby injections) are an important way to prevent diseases. Pneumonia is a common problem in Vietnam and throughout the developing world. In the developing world it is the leading cause of death amongst under 5 year olds. A number of germs cause pneumonia but the most common germ is a bacteria called pneumococcus. Pneumococcus can also cause ear infections as well as other, more severe diseases like meningitis (infection around the brain). This germ normally lives in the nose of humans and is spread from person to person by touching or sneezing. There are more than 90 types of this germ but only some types cause serious infections in young children.

Why are we doing the study?

There are vaccines available to protect against infection with pneumococcus. These are called pneumococcal vaccines. Many countries around the world give all their babies a pneumococcal vaccine that protects against 7 types of the pneumococcal disease (7v-PCV). There are two new vaccines which have been developed. Both new vaccines give more protection against pneumococcal disease than the 7v-PCV. Both vaccines have completed all their tests and are licensed and being used by many countries in Europe and the United States. The clinical trials have shown that these vaccines are safe; therefore there is little danger to any child participating in this study. The vaccines are likely to provide some protection from ear infections and pneumonia. Unfortunately the costs of these vaccines are very high, so not all countries in the world can afford them. We are doing this study to find the best ways to protect babies from this germ and also to make it cheaper for countries, like Vietnam, to afford to buy the vaccine.

Benefits of the study

By joining the study your baby can be protected from the commonest pneumococcal germs. Both these vaccines are very expensive and are not presently available to other babies in Vietnam. They have been especially made for use in babies and young children and will protect the babies from the common diseases caused by the pneumococcus. We hope to find a schedule that works and which countries like Vietnam can afford. In addition children will receive 4 doses of *Infanrix-Hexa*: 3 doses during early infancy and a booster dose at either 18 or 19 months of age.

What does the study involve?

The study will include 1400 babies and we will be looking at 7 different vaccine schedules in this study. 1200 babies will be enrolled at 2 months old and will be randomly allocated to 1 of 6 groups. An additional 200 babies will be enrolled at 18 months old to act as controls.

Consent: A study doctor or nurse will discuss the study with each child's parent or legal guardian. They will explain what is involved and ask some questions about the baby's health. If you agree to join the study she will ask you to sign a consent form which says that you agree for your baby to join. If you consent to taking part in the study, she will perform a health check of your baby to make sure your baby is healthy to take part.

Vaccinations & health checks: If you agree to your baby to take part in the study you will need to come to the clinic between 9 and 11 times over a period of 22 months. The study nurse will remind you when you need to come. Like rolling a dice your baby will be allocated to 1 of 6 groups. Your baby will get between one and four doses of one of the two types of Pneumococcal vaccine, either the Prevnar-13 (13v-PCV which covers 13 types of the pneumococcal germ) or the 10v-Synflorix vaccine (which covers 10 types of the pneumococcal germ and may be better at protecting against pneumonia). Depending on which group your baby is randomly placed in will depend on when, how many doses and what type of Pneumococcal vaccine your baby will receive. Your baby will also get an infant vaccine (Infanrix-hexa 6-1) that covers all the diseases (diphtheria, tetanus, pertussis, hepatitis B, polio virus and *Haemophilus influenzae* type B) that are covered by the standard vaccines used in VIetnam. Vaccines will be given by staff from Pasteur Institute Ho Chi Minh City. Your baby will also have regular health checks during the study.

Questionnaire: At the start of the study you will be asked some general questions about your family and your baby's health. These are simply to help us understand how the vaccines work best. The results will be kept confidential (see below).

Blood tests: Up to four blood tests will be taken during the study, by staff from Children's Hospital Number 2. The blood tests are to check the response to the vaccines. If you would prefer, we can put local anesthetic cream on your baby's skin before taking the blood test so that it doesn't hurt as much. The amount of blood taken will vary depending on the age of the child: 2.0mls at 2 months of age; 3.5mls from 3 to 10 and 19 months of age; and 3.5mls or 7.5mls at 18 and 24 months of age.

Nose swabs: Six nose swabs will be taken during the study, at 2, 6, 9, 12, 18 and 24 months of age. The nose swabs are to see if the vaccine will help stop the spread of the pneumococcus from child to child. This will involve putting a cotton wool swab (like a cotton bud) into the baby's nose for a couple of seconds. This may make the baby sneeze and possibly cry briefly – it tickles quite a lot, but doesn't really hurt.

Summary of changes: Additional procedures and vaccines

Groups A-E	*18 months*	*Measles and Rubella given*
	19 months	*Infanrix Hexa given*
	24 months	*Nose swab taken*
Group F	*18 months*	*Infanrix Hexa given*
	19 months	*Measles and Rubella given* *Blood taken*
	24 months	*Nose swab taken* *Blood taken* *Synflorix given*

Hospital record review: If your baby becomes unwell during the study, the staff may need to look at your child's medical records.

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Are there any risks?

The vaccines we are using are licensed many countries. As with all vaccines there is likely to be some pain felt, and there is a small risk of soreness and redness where the vaccine was given. Babies in the study will get up to 4 extra injections than they would routinely get. We will check the babies to make sure they don't have any unexpected reactions. We also have a study doctor who will be keeping a record of any serious illnesses that are unlikely to occur during the study.

Confidentiality

All information collected in this study will remain confidential and will be used for research purposes only. All information will be kept secure. Your baby will be given an identification number at the start of the study. Any information collected will use this number and will not include your baby's name. The samples we collect will be sent to overseas laboratories to have further tests. These laboratories will not be given your child's name. We will ask your permission if it is alright for your baby's blood and nose swab samples to be stored indefinitely for other similar tests in the future. This would help us to perform any new pneumococcal test that may be developed in the future. The results of the study will be published in scientific journals and presented at conferences. There will never be details published that would identify your baby.

Voluntary Participation and Withdrawal from the Study

Your baby does not have to take part in the study. Your baby will get the best treatment available and the full attention of the health staff even if they do not participate. You are free to withdraw your baby from the study at any point. This will not affect any of your baby's further health care treatment and there will be no harmful consequences for your baby. If your baby has not had all their pneumococcal vaccines they may not be fully protected against the pneumococcal germs which most commonly affect infants. However, they will still gain some protection from the doses of vaccine received.

Compensation

We will pay 200,000VND towards the transport cost for coming to the clinic for each study visit. If your baby becomes ill or injured as a result of taking part in this clinical study, medical treatment will be provided.

Ethical Approval

This study has been approved by the People's Committee of Ho Chi Minh City. This study has also been approved by the Vietnam Ministry of Health Ethics Committee and the Menzies School of Health Research Ethics Committee, Australia. The ethics committees make sure that the study is being done in the best and safest way. If you have any concerns or complaints regarding the conduct of the research project you are invited to contact:

Vietnam Ministry of Health
Ethics Committee
Phone: 04 62732156

OR

Human Research Ethics Committee of the NT
Department of Health and Menzies School of Health Research
PO Box 41096, Casuarina, NT 0811, Australia
Phone: 61 8 8922 7922
Email: ethics@menzies.edu.au

How is the study funded?

The funding to perform the study is from the National Health and Medical Research Council, Australia and the Bill & Melinda Gates Foundation.

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Your Right to Ask Questions

Please feel free to contact us if you have any questions or concerns.

If you have any questions regarding the study activities, please phone:

If you have any questions regarding adverse events, please phone:

Commune Health Centre Number:

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INFORMATION SHEET: Vietnam Pneumococcal Vaccine Study (Control group)

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Assoc. Prof. Tran Ngoc Huu
Prof. Edward Kim Mulholland

Research Partners:
Pasteur Institute, Ho Chi Minh City
Menzies School of Health Research
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Introduction

Health research helps us to understand diseases and find ways to prevent them. Vaccines (like the routine baby injections) are an important way to prevent diseases. Pneumonia is a common problem in Vietnam and throughout the developing world. In the developing world it is the leading cause of death amongst under 5 year olds. A number of germs cause pneumonia but the most common germ is a bacteria called pneumococcus. Pneumococcus can also cause ear infections as well as other, more severe diseases like meningitis (infection around the brain). This germ normally lives in the nose of humans and is spread from person to person by touching or sneezing. There are more than 90 types of this germ but only some types cause serious infections in young children.

Why are we doing the study?

There are vaccines available to protect against infection with pneumococcus. These are called pneumococcal vaccines. Many countries around the world give all their babies a pneumococcal vaccine that protects against 7 types of the pneumococcal disease (7v-PCV). There are two new vaccines which have been developed. Both new vaccines give more protection against pneumococcal disease than the 7v-PCV.Both vaccines have completed all their tests and are licensed and being used by many countries in Europe and the United States. The clinical trials have shown that these vaccines are safe; therefore there is little danger to any child participating in this study. The vaccines are likely to provide some protection from ear infections and pneumonia. Unfortunately the costs of these vaccines are very high, so not all countries in the world can afford them. We are doing this study to find the best ways to protect babies from this germ and also to make it cheaper for countries, like Vietnam, to afford to buy the vaccine.

Benefits of the study

By joining the study your baby can be protected from the commonest pneumococcal germs. Both these vaccines are very expensive and are not presently available to other babies in Vietnam. They have been especially made for use in babies and young children and will protect the babies from the common diseases caused by the pneumococcus. We hope to find a schedule that works and which countries like Vietnam can afford. In addition your baby will receive a dose of Infanrix-hexa at 18 months of age.

What does the study involve?

The study will include 200 babies to act as comparisons to participants in an existing study of six different vaccine schedules.

Consent: A study doctor or nurse will discuss the study with each child's parent or legal guardian. They will explain what is involved and ask some questions about the baby's health. If you agree to join the study she will ask you to sign a consent form which says that you agree for your baby to join. If you consent to taking part in the study, she will perform a health check of your baby to make sure your baby is healthy to take part.

Vaccinations & health checks: If you agree to your baby to take part in the study you will need to come to the clinic 3 times over a period of 6 months. The study nurse will remind you when you need to come. Your baby will get a single dose of (Infanrix-hexa 6-1) that covers six diseases (diphtheria, tetanus, pertussis, hepatitis B, polio virus and *Haemophilus influenzae* type B) at 18 months of age, a single dose of Measles and Rubella (MR) at 19 months of age and a single dose of Pneumococcal vaccine (10v-Synflorix vaccine, which covers 10 types of the pneumococcal germ) at 24 months of age. Vaccines will be given by staff from Pasteur Institute Ho Chi Minh City. Your baby will also have a doctor's health check at each study visit.

Questionnaire: At the start of the study you will be asked some general questions about your family and your baby's health. These are simply to help us understand how the vaccines work best. The results will be kept confidential (see below).

Blood tests: Three blood tests will be taken over the six months, by staff from Children's Hospital Number 2. The blood tests are to check the response to the vaccines. If you would prefer, we can put local anesthetic cream on your baby's skin before taking the blood test so that it doesn't hurt as much. The amount of blood taken will be 3.5 or 7.5mls at 18 and 24 months of age; and 3.5mls at 19 months of age.

Nose swabs: Two nose swabs will be taken during the study, at 18 and 24 months of age. The nose swabs are to see if the vaccine will help stop the spread of the pneumococcus from child to child. This will involve putting a cotton wool swab (like a cotton bud) into the baby's nose for a couple of seconds. This may make the baby sneeze and possibly cry briefly – it tickles quite a lot, but doesn't really hurt.

Hospital record review: If your baby becomes unwell during the study, the staff may need to look at your child's medical records.

Are there any risks?

The vaccines we are using are licensed many countries. As with all vaccines there is likely to be some pain felt, and there is a small risk of soreness and redness where the vaccine was given. We will check the babies to make sure they don't have any unexpected reactions. We also have a study doctor who will be keeping a record of any serious illnesses that are unlikely to occur during the study.

Confidentiality

All information collected in this study will remain confidential and will be used for research purposes only. All information will be kept secure. Your baby will be given an identification number at the start of the study. Any information collected will use this number and will not include your baby's name. The samples we collect will be sent to overseas laboratories to have further tests. These laboratories will not be given your child's name. We will ask your permission if it is alright for your baby's blood and nose swab samples to be stored indefinitely for other similar tests in the future. This would help us to perform any new pneumococcal test that may be developed in the

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future. The results of the study will be published in scientific journals and presented at conferences. There will never be details published that would identify your baby.

Voluntary Participation and Withdrawal from the Study

Your baby does not have to take part in the study. Your baby will get the best treatment available and the full attention of the health staff even if they do not participate. You are free to withdraw your baby from the study at any point. This will not affect any of your baby's further health care treatment and there will be no harmful consequences for your baby. If your baby has not had all their pneumococcal vaccines they may not be fully protected against the pneumococcal germs which most commonly affect infants. However, they will still gain some protection from the doses of vaccine received.

Compensation

We will pay 200,000VND towards the transport cost for coming to the clinic for each study visit. If your baby becomes ill or injured as a result of taking part in this clinical study, medical treatment will be provided.

Ethical Approval

This study has been approved by the People's Committee of Ho Chi Minh City. This study has also been approved by the Vietnam Ministry of Health Ethics Committee and the Menzies School of Health Research Ethics Committee, Australia. The ethics committees make sure that the study is being done in the best and safest way. If you have any concerns or complaints regarding the conduct of the research project you are invited to contact:

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CONSENT FORM

This means you can say NO

Screening Number:	|___|___|___|___|
Participant ID:	|___|___|___|___|
Date:	__ __ /__ __ /__ __ dd / mm / yy

Principal Investigators:

Assoc. Prof. Tran Ngoc Huu
Prof. Edward Kim Mulholland

Research Partners:

Pasteur Institute, Ho Chi Minh City
Menzies School of Health Research

This form is to record if you agree for your infant to take part in the **"Evaluation of Different Infant Vaccination Schedules Incorporating Pneumococcal Vaccination"**. You should only sign this form if you are happy that the information about the study has been clearly explained to you, you have received enough information about the study and you have had all your questions answered satisfactorily.

Please record the name of the person you have spoken to about the study:

_____

By agreeing for your infant to take part in the study, you understand that:

- You are free to withdraw your child from the study at any time without having to give a reason;
- Your child will be vaccinated against all the diseases that are covered by the standard vaccines used in Vietnam, although these vaccines may be given at different times;
- If your child becomes sick, their hospital records will be reviewed by the study doctor or other designated study staff; and
- The samples taken in this study will be sent to overseas laboratories to test vaccine responses and carriage of bacteria

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Screening Number: |___|___|___|___|

Participant ID: |___|___|___|___|

Date: __ __ /__ __ /__ __

Consent:

❑ YES, I agree for my infant to take part in this study.

❑ NO, I do not agree for my infant to take part in this study.

Use of samples:

❑ YES, you may indefinitely store my unused identified samples for future work in the same general area of research that has obtained ethics committee approval.

❑ NO, you may **NOT USE** my samples for future research. Destroy my unused samples at the close of the study.

Signed (parent/legal guardian): _____ Date: __ __ /__ __ /__ __
dd / mm / yy

Name of parent/legal guardian: _____ Time: __ __ :__ __
hh : mm

Relationship to infant: _____

Name of infant or baby of: _____

Infant Sex: male / female Infant DOB: __ __ /__ __ /__ __
dd / mm / yy

Signed (study nurse): _____ Date: __ __ /__ __ /__ __
dd / mm / yy

If illiterate: *A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team).*

I have witnessed the accurate reading of the consent form to the parent of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Signed (witness): _____ Date: __ __ /__ __ /__ __
dd / mm / yy

Name of witness: _____

Appendix D: Supplementary appendix to Chapter 5 research paper

THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Temple B, Toan NT, Dai VTT, et al. Immunogenicity and reactogenicity of ten-valent versus 13-valent pneumococcal conjugate vaccines among infants in Ho Chi Minh City, Vietnam: a randomised controlled trial. *Lancet Infect Dis* 2019; published online April 8. http://dx.doi.org/10.1016/S1473-3099(18)30734-5.

LIST OF APPENDIX TABLES

Table S1: Schedule of vaccines and samples for infants enrolled into the Vietnam Pneumococcal Project

Group	2m	3m	4m	5m	6m	7m	9m	9·5m†	10m	12m	18m	19m	24m
A (3+1 PCV10)	Bld* NP PCV10 *Hexa*	PCV10 *Hexa*	PCV10 *Hexa*	Bld	NP		Bld NP PCV10 MV		Bld	NP	Bld* NP MR	*Hexa*	NP
B (3+0 PCV10)	NP PCV10 *Hexa*	PCV10 *Hexa*	PCV10 *Hexa*	Bld	Bld NP		Bld* NP MV		Bld	NP	Bld* NP MR	*Hexa*	NP
C (2+1 PCV10)	NP PCV10 *Hexa*		PCV10 *Hexa*	Bld	Bld* NP		Bld NP MV	PCV10 *Hexa*	Bld	NP	Bld* NP MR	*Hexa*	NP
D (2-dose PCV10)	NP PCV10 *Hexa*	Bld	*Hexa*		Bld NP PCV10 *Hexa*	Bld	Bld* NP MV			NP	Bld* NP MR	*Hexa*	NP
E (2+1 PCV13)	NP PCV13 *Hexa*	Bld*	PCV13 *Hexa*	Bld	NP		Bld NP MV	PCV13 *Hexa*	Bld	NP	Bld* NP MR	*Hexa*	NP
F (controls)	NP *Hexa*	*Hexa*	*Hexa*		NP		NP MV			NP	Bld NP PCV10 *Hexa*	Bld MR	NP PCV10 Bld

Bld = blood sample. NP = nasopharyngeal swab sample. PCV10 = ten-valent pneumococcal conjugate vaccine. PCV13 = 13-valent pneumococcal conjugate vaccine. *Hexa* = hexavalent diphtheria, tetanus, pertussis, polio, Haemophilus influenzae type b, and hepatitis B vaccine (DTaP-IPV-Hib-HepB). MV = measles vaccine. MR = measles-rubella vaccine.

* Each participant provides only one of these blood samples (participants allocated to groups A-E from the last 300 recruited provide this sample at 18 months of age; the remainder provide it at the other time point).

† The Vietnam Ministry of Health does not permit co-administration of measles and DTaP-IPV-Hib-HepB; therefore PCV and DTaP-IPV-Hib-HepB were administered at 9·5 months in participants from groups C and E.

Table S2: Post-primary series immunogenicity on the intention-to-treat population

Immunogenicity data at 4 weeks after two doses of PCV10 (at 2 months and 4 months of age, group C), two doses of PCV13 (at 2 months and 4 months of age, group E), or three doses of PCV10 (at 2 months, 3 months, and 4 months of age, group A and B). GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

	Participants with IgG concentration ≥0·35µg/mL, % (95% CI)			**Risk difference, %**		**GMC, µg/mL (95% CI)**			**GMC ratio (95% CI)**	
	Two-dose PCV10 (n=240)	Three-dose PCV10 (n=289)	Two-dose PCV13 (n=236)	Two-dose PCV10 to PCV13 (95% CI)	Three-dose PCV10 to PCV13 (90% CI)	Two-dose PCV10 (n=240)	Three-dose PCV10 (n=289)	Two-dose PCV13 (n=236)	Two-dose PCV10/PCV13	Three-dose PCV10/PCV13
Shared PCV serotypes										
1	97.9	98.3	100	-2.1	-1.7	2.22	2.78	4.86	0.46*	0.57*
	(95.2, 99.3)	(96.0, 99.4)	(98.4, 100)	(-4.8, -0.1)	(-3.5, -0.3)	(1.98, 2.48)	(2.51, 3.08)	(4.38, 5.39)	(0.39, 0.53)	(0.49, 0.66)
4	98.8	99.0	100	-1.3	-1	3.21	3.85	4.79	0.67*	0.8*
	(96.4, 99.7)	(97.0, 99.8)	(98.4, 100)	(-3.6, 0.6)	(-2.6, 0.3)	(2.88, 3.58)	(3.45, 4.30)	(4.39, 5.23)	(0.58, 0.77)	(0.69, 0.93)
5	95.8	98.6	99.2	-3.3	-0.5	1.17	1.82	2.2	0.53*	0.83*
	(92.5, 98.0)	(96.5, 99.6)	(97.0, 99.9)	(-6.7, -0.4)	(-2.3, 1.3)	(1.07, 1.27)	(1.67, 1.98)	(2.01, 2.41)	(0.47, 0.60)	(0.73, 0.94)
6B	77.1	84.4	61.0	16.1	23.4‡	0.8	1.08	0.49	1.65†	2.22†
	(71.2, 82.2)	(79.7, 88.4)	(54.5, 67.3)	(7.8, 24.1)	(17.1, 29.6)	(0.70, 0.92)	(0.95, 1.23)	(0.43, 0.55)	(1.37, 2.00)	(1.84, 2.66)
7F	98.8	99.3	100	-1.3	-0.7	2.07	3.03	3.31	0.63*	0.92
	(96.4, 99.7)	(97.5, 99.9)	(98.4, 100)	(-3.6, 0.6)	(-2.1, 0.5)	(1.89, 2.26)	(2.78, 3.30)	(3.03, 3.60)	(0.55, 0.71)	(0.81, 1.04)
9V	96.3	99.3	97.9	-1.6	1.4	1.63	2.47	3.24	0.50*	0.76*
	(93.0, 98.3)	(97.5, 99.9)	(95.1, 99.3)	(-5.1, 1.6)	(-0.3, 3.6)	(1.47, 1.81)	(2.26, 2.70)	(2.91, 3.62)	(0.43, 0.58)	(0.66, 0.88)
14	98.3	100	98.3	0	1.7	5.92	9.72	7.83	0.76*	1.24†
	(95.8, 99.5)	(98.7, 100)	(95.7, 99.5)	(-2.7, 2.8)	(0.4, 3.7)	(5.17, 6.78)	(8.76, 10.79)	(6.68, 9.18)	(0.61, 0.93)	(1.03, 1.49)
18C	96.7	98.6	98.7	-2.1	-0.1	1.87	3.86	3.12	0.60*	1.24†
	(93.5, 98.6)	(96.5, 99.6)	(96.3, 99.7)	(-5.3, 0.8)	(-1.9, 1.9)	(1.65, 2.12)	(3.47, 4.29)	(2.82, 3.45)	(0.51, 0.70)	(1.07, 1.44)
19F	99.2	99.7	99.2	0	0.5	9.56	8.22	7.6	1.26†	1.08
	(97.0, 99.9)	(98.1, 100)	(97.0, 99.9)	(-2.2, 2.3)	(-0.8, 2.2)	(8.40, 10.88)	(7.42, 9.12)	(6.72, 8.59)	(1.05, 1.50)	(0.92, 1.27)
23F	77.9	90.3	89.4	-11.5	0.9	0.89	1.32	1.14	0.78*	1.16
	(72.1, 83.0)	(86.3, 93.5)	(84.8, 93.0)	(-18.1, -4.9)	(-3.4, 5.4)	(0.78, 1.02)	(1.18, 1.48)	(1.01, 1.29)	(0.65, 0.94)	(0.98, 1.37)
Additional PCV13 serotypes										
3	5.8	6.9	97.9	-92	-91	0.1	0.11	1.54	0.07	0.07
	(3.2, 9.6)	(4.3, 10.5)	(95.1, 99.3)	(-94.7, -87.4)	(-93.3, -87.4)	(0.09, 0.11)	(0.10, 0.12)	(1.41, 1.68)	(0.06, 0.08)	(0.06, 0.08)
6A	40.8	50.5	94.9	-54.1	-44.4	0.31	0.37	1.94	0.16	0.19
	(34.6, 47.3)	(44.6, 56.4)	(91.3, 97.3)	(-60.5, -46.8)	(-49.6, -38.8)	(0.28, 0.35)	(0.34, 0.41)	(1.70, 2.22)	(0.14, 0.19)	(0.16, 0.22)
19A	70	67.8	98.3	-28.3	-30.5	0.55	0.56	3.8	0.14	0.15
	(63.8, 75.7)	(62.1, 73.2)	(95.7, 99.5)	(-34.5, -22.3)	(-35.2, -25.7)	(0.49, 0.61)	(0.50, 0.62)	(3.33, 4.33)	(0.12, 0.17)	(0.13, 0.17)

* indicates a GMC ratio with a 95% CI excluding 1.00, PCV13 higher
† indicates a GMC ratio with a 95% CI excluding 1.00, PCV10 higher
‡ indicates a risk difference with upper bound of the 90% CI ≥10%

Table S3: Comparison of responses to a single dose of PCV10 or PCV13

Immunogenicity data before and at 4 weeks after a single dose of PCV at 2 months of age. GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

	Participants with IgG concnetration ≥0·35µg/mL, % (95% CI)			Risk difference (95% CI)	GMC, µg/mL (95% CI)			GMC ratio (95% CI)
	Pre-PCV (n=100)	Post-PCV10 (n=197)	Post-PCV13 (n=193)	Post-PCV (PCV10-PCV13)	Pre-PCV (n=100)	Post-PCV10 (n=197)	Post-PCV13 (n=193)	Post-PCV (PCV10/PCV13)
Shared serotypes								
1	14·0 (7·9, 22·4)	88·3 (83·0, 92·5)	73·1 (66·2, 79·2)	15·3 (7·5, 22·9)	0·12 (0·10, 0·15)	1·05 (0·91, 1·20)	0·64 (0·56, 0·73)	1·64† (1·35, 1·99)
4	8·0 (3·5, 15·2)	88·8 (83·6, 92·9)	82·9 (76·8, 87·9)	5·9 (-1·0, 12·9)	0·09 (0·07, 0·10)	1·12 (0·98, 1·29)	0·88 (0·77, 1·00)	1·28† (1·06, 1·55)
5	10·0 (4·9, 17·6)	79·7 (73·4, 85·1)	64·2 (57·0, 71·0)	15·4 (6·5, 24·0)	0·11 (0·10, 0·13)	0·85 (0·74, 0·97)	0·46 (0·40, 0·53)	1·83† (1·51, 2·23)
6B	22·0 (14·3, 31·4)	15·7 (10·9, 21·6)	14·0 (9·4, 19·7)	1·7 (-5·4, 8·9)	0·21 (0·18, 0·24)	0·18 (0·16, 0·20)	0·17 (0·15, 0·19)	1·02 (0·87, 1·20)
7F	10·0 (4·9, 17·6)	70·6 (63·7, 76·8)	80·8 (74·6, 86·1)	-10·3 (-18·6, -1·7)	0·11 (0·09, 0·13)	0·57 (0·50, 0·66)	0·81 (0·70, 0·94)	0·71* (0·58, 0·86)
9V	17·0 (10·2, 25·8)	49·2 (42·1, 56·4)	35·2 (28·5, 42·4)	14·0 (4·2, 23·4)	0·18 (0·15, 0·20)	0·35 (0·31, 0·39)	0·28 (0·25, 0·31)	1·25† (1·07, 1·46)
14	68·0 (57·9, 77·0)	77·2 (70·7, 82·8)	72·5 (65·7, 78·7)	4·6 (-4·0, 13·2)	0·64 (0·49, 0·84)	0·69 (0·60, 0·78)	0·65 (0·55, 0·76)	1·06 (0·86, 1·30)
18C	26·0 (17·7, 35·7)	44·2 (37·1, 51·4)	77·2 (70·6, 82·9)	-33·0§ (-41·7, -23·6)	0·24 (0·21, 0·28)	0·34 (0·30, 0·38)	0·62 (0·55, 0·70)	0·54* (0·46, 0·65)
19F	66·0 (55·8, 75·2)	94·4 (90·2, 97·2)	76·2 (69·5, 82·0)	18·3‡ (11·4, 25·2)	0·45 (0·39, 0·53)	1·09 (0·97, 1·21)	0·58 (0·53, 0·64)	1·87† (1·62, 2·16)
23F	23·0 (15·2, 32·5)	13·2 (8·8, 18·7)	15·0 (10·3, 20·9)	-1·8 (-8·8, 5·1)	0·19 (0·17, 0·23)	0·16 (0·14, 0·18)	0·15 (0·13, 0·17)	1·04 (0·88, 1·23)
Additional PCV13-types								
3	5·0 (1·6, 11·3)	2·0 (0·6, 5·1)	88·1 (82·7, 92·3)	-86·1 (-90·1, -79·9)	0·07 (0·06, 0·09)	0·06 (0·05, 0·07)	0·80 (0·72, 0·89)	0·07 (0·06, 0·09)
6A	44·0 (34·1, 54·3)	27·4 (21·3, 34·2)	31·1 (24·6, 38·1)	-3·7 (-12·6, 5·3)	0·32 (0·28, 0·37)	0·25 (0·23, 0·28)	0·25 (0·23, 0·28)	0·99 (0·87, 1·14)
19A	61·0 (50·7, 70·6)	46·2 (39·1, 53·4)	60·1 (52·8, 67·1)	-13·9 (-23·4, -4·0)	0·41 (0·36, 0·47)	0·33 (0·30, 0·37)	0·43 (0·38, 0·47)	0·79 (0·68, 0·91)

* indicates a GMC ratio with a 95% CI excluding 1.00, PCV13 higher
† indicates a GMC ratio with a 95% CI excluding 1.00, PCV10 higher
‡ indicates a risk difference with 95% CI entirely below -10% (PCV13 better)
§ indicates a risk difference with 95% CI entirely above 10% (PCV10 better)

Table S4: Pre- and post-booster responses to a 2+1 schedule of PCV10 or PCV13

Immunogenicity data before and at 4 weeks after a booster dose of PCV at 9.5 months of age. GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

a) Participants with serotype-specific IgG ≥0·35μg/mL before and at 4 weeks after a booster dose of PCV at 9·5 months of age, % (95% CI)

	PCV10		PCV13		Pre-booster risk difference (95% CI) (PCV10-PCV13)	Post-booster risk difference (95% CI) (PCV10-PCV13)
	Pre-booster (n=236)	Post-booster (n=226)	Pre-booster (n=228)	Post-booster (n=221)		
Shared serotypes						
1	82·6 (77·2, 87·2)	100 (98·4, 100)	96·5 (93·2, 98·5)	100 (98·3, 100)	-13·9 (-19·5, -8·5)	0 (-1·7, 1·7)
4	91·1 (86·7, 94·4)	98·7 (96·2, 99·7)	96·5 (93·2, 98·5)	100 (98·3, 100)	-5·4 (-10·0, -1·0)	-1·3 (-3·8, 0·6)
5	75·4 (69·4, 80·8)	97·8 (94·9, 99·3)	93·9 (89·9, 96·6)	99·5 (97·5, 100)	-18·4 (-24·8, -12·0)‡	-1·8 (-4·6, 0·7)
6B	94·9 (91·3, 97·3)	100 (98·4, 100)	76·3 (70·3, 81·7)	98·2 (95·4, 99·5)	18·6 (12·4, 24·9)§	1·8 (-0·2, 4·6)
7F	90·3 (85·7, 93·7)	99·6 (97·6, 100)	94·7 (91·0, 97·3)	100 (98·3, 100)	-4·5 (-9·5, 0·4)	-0·4 (-2·5, 1·3)
9V	86·0 (80·9, 90·2)	100 (98·4, 100)	94·3 (90·4, 96·9)	99·5 (97·5, 100)	-8·3 (-13·8, -2·9)	0·5 (-1·3, 2·5)
14	97·5 (94·5, 99·1)	99·6 (97·6, 100)	97·4 (94·4, 99·0)	100 (98·3, 100)	0·1 (-3·1, 3·4)	-0·4 (-2·5, 1·3)
18C	84·7 (79·5, 89·1)	100 (98·4, 100)	88·6 (83·7, 92·4)	99·5 (97·5, 100)	-3·9 (-10·1, 2·4)	0·5 (-1·3, 2·5)
19F	100 (98·4, 100)	100 (98·4, 100)	99·1 (96·9, 99·9)	100 (98·3, 100)	0·9 (-0·8, 3·1)	0 (-1·7, 1·7)
23F	83·1 (77·6, 87·6)	98·7 (96·2, 99·7)	68·9 (62·4, 74·8)	99·5 (97·5, 100)	14·2 (6·4, 21·8)	-0·9 (-3·4, 1·4)
Additional PCV13-types						
3	13·1 (9·1, 18·1)	31·0 (25·0, 37·4)	72·8 (66·5, 78·5)	99·1 (96·8, 99·9)	-59·7 (-66·2, -51·8)	-68·1 (-73·8, -61·4)
6A	69·9 (63·6, 75·7)	91·6 (87·2, 94·9)	94·7 (91·0, 97·3)	99·5 (97·5, 100)	-24·8 (-31·3, -18·2)	-8·0 (-12·3, -4·3)
19A	78·8 (73·0, 83·8)	95·6 (92·0, 97·9)	96·5 (93·2, 98·5)	100 (98·3, 100)	-17·7 (-23·6, -11·9)	-4·4 (-8·0, -1·8)

b) GMCs before and at 4 weeks after a booster dose of PCV at 9·5 months of age, μg/mL (95% CI)

	PCV10		PCV13		Pre-booster GMC ratio (95% CI) (PCV10/PCV13)	Post-booster GMC ratio (95% CI) (PCV10/PCV13)
	Pre-booster (n=236)	Post-booster (n=226)	Pre-booster (n=228)	Post-booster (n=221)		
Shared serotypes						
1	0·71 (0·64, 0·79)	4·40 (3·91, 4·97)	1·40 (1·28, 1·53)	7·62 (6·86, 8·45)	0·51 (0·44, 0·58)*	0·58 (0·49, 0·68)*
4	1·09 (0·98, 1·22)	4·75 (4·20, 5·37)	1·14 (1·04, 1·24)	5·32 (4·82, 5·87)	0·96 (0·83, 1·11)	0·89 (0·76, 1·04)
5	0·54 (0·49, 0·59)	1·31 (1·20, 1·43)	0·85 (0·78, 0·92)	3·31 (3·00, 3·66)	0·63 (0·56, 0·72)*	0·40 (0·35, 0·45)*
6B	1·63 (1·44, 1·83)	6·17 (5·50, 6·92)	0·63 (0·56, 0·70)	9·51 (8·16, 11·09)	2·60 (2·21, 3·05)†	0·65 (0·54, 0·78)*
7F	0·83 (0·76, 0·91)	2·65 (2·41, 2·91)	1·07 (0·98, 1·17)	4·76 (4·33, 5·24)	0·78 (0·68, 0·88)*	0·56 (0·49, 0·64)*
9V	0·75 (0·68, 0·84)	3·34 (3·02, 3·69)	0·91 (0·83, 1·00)	5·23 (4·75, 5·77)	0·82 (0·72, 0·95)*	0·64 (0·55, 0·73)*
14	3·41 (2·96, 3·94)	11·76 (10·45, 13·24)	4·43 (3·89, 5·05)	15·37 (13·73, 17·21)	0·77 (0·63, 0·94)*	0·77 (0·65, 0·90)*
18C	0·81 (0·72, 0·90)	5·16 (4·68, 5·70)	0·67 (0·62, 0·73)	4·31 (3·89, 4·79)	1·19 (1·04, 1·37)†	1·20 (1·04, 1·38)†
19F	3·94 (3·59, 4·31)	16·16 (14·45, 18·08)	2·16 (1·97, 2·37)	11·68 (10·48, 13·02)	1·82 (1·60, 2·08)†	1·38 (1·18, 1·62)†
23F	0·76 (0·68, 0·86)	3·55 (3·15, 3·99)	0·51 (0·46, 0·57)	6·12 (5·40, 6·94)	1·49 (1·27, 1·75)†	0·58 (0·49, 0·69)*
Additional PCV13-types						
3	0·15 (0·13, 0·16)	0·25 (0·23, 0·29)	0·48 (0·45, 0·51)	1·82 (1·65, 2·01)	0·31 (0·27, 0·35)	0·14 (0·12, 0·16)
6A	0·57 (0·51, 0·65)	1·44 (1·25, 1·66)	1·18 (1·06, 1·31)	9·13 (7·99, 10·43)	0·49 (0·42, 0·57)	0·16 (0·13, 0·19)
19A	0·66 (0·60, 0·73)	1·76 (1·55, 2·00)	1·24 (1·11, 1·39)	9·18 (8·16, 10·33)	0·53 (0·46, 0·61)	0·19 (0·16, 0·23)

* indicates a GMC ratio with a 95% CI excluding 1.00, PCV13 higher
† indicates a GMC ratio with a 95% CI excluding 1.00, PCV10 higher
‡ indicates a risk difference with 95% CI entirely below -10% (PCV13 better)
§ indicates a risk difference with 95% CI entirely above 10% (PCV10 better)

Table S5: Antibody levels at 18 months of age

Immunogenicity data in a subset of participants at 18 months of age, following a 2+1 schedule of PCV10 or PCV13 at 2, 4, and 9·5 months of age. GMC=geometric mean concentration. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

	Participants with IgG concentration ≥0·35µg/mL, % (95% CI)		**Risk difference (95% CI)** (PCV10-PCV13)	**GMC, µg/mL (95% CI)**		**GMC ratio (95% CI)** (PCV10/PCV13)
	PCV10 (n=47)	PCV13 (n=46)		PCV10 (n=47)	PCV13 (n=46)	
Shared serotypes						
1	76·6 (62·0, 87·7)	87·0 (73·7, 95·1)	-10·4 (-25·8, 5·6)	0·68 (0·53, 0·87)	0·77 (0·61, 0·96)	0·88 (0·63, 1·23)
4	72·3 (57·4, 84·4)	63·0 (47·5, 76·8)	9·3 (-9·5, 27·3)	0·56 (0·45, 0·71)	0·43 (0·34, 0·54)	1·31 (0·96, 1·79)
5	80·9 (66·7, 90·9)	78·3 (63·6, 89·1)	2·6 (-13·8, 19·0)	0·61 (0·49, 0·74)	0·56 (0·44, 0·70)	1·09 (0·80, 1·47)
6B	95·7 (85·5, 99·5)	87·0 (73·7, 95·1)	8·8 (-3·4, 21·8)	1·15 (0·87, 1·54)	1·32 (0·93, 1·86)	0·88 (0·56, 1·36)
7F	59·6 (44·3, 73·6)	73·9 (58·9, 85·7)	-14·3 (-32·0, 4·7)	0·46 (0·35, 0·59)	0·53 (0·43, 0·66)	0·86 (0·62, 1·20)
9V	83·0 (69·2, 92·4)	69·6 (54·2, 82·3)	13·4 (-3·9, 29·9)	0·55 (0·45, 0·67)	0·45 (0·36, 0·58)	1·21 (0·89, 1·64)
14	97·9 (88·7, 99·9)	97·8 (88·5, 99·9)	0·0 (-9·1, 9·4)	1·94 (1·49, 2·52)	1·67 (1·27, 2·20)	1·16 (0·80, 1·69)
18C	74·5 (59·7, 86·1)	60·9 (45·4, 74·9)	13·6 (-5·3, 31·3)	0·67 (0·53, 0·86)	0·36 (0·28, 0·46)	1·85 (1·32, 2·61)†
19F	100 (92·5, 100)	95·7 (85·2, 99·5)	4·3 (-3·8, 14·5)	3·36 (2·56, 4·40)	1·73 (1·31, 2·28)	1·94 (1·32, 2·86)†
23F	80·9 (66·7, 90·9)	78·3 (63·6, 89·1)	2·6 (-13·8, 19·0)	0·77 (0·59, 1·01)	0·95 (0·65, 1·38)	0·81 (0·51, 1·29)
Additional PCV13-types						
3	17·0 (7·6, 30·8)	39·1 (25·1, 54·6)	-22·1 (-38·7, -3·8)	0·14 (0·11, 0·18)	0·29 (0·22, 0·38)	0·47 (0·33, 0·67)
6A	74·5 (59·7, 86·1)	84·8 (71·1, 93·7)	-10·3 (-26·2, 6·3)	0·59 (0·46, 0·76)	1·12 (0·75, 1·68)	0·53 (0·33, 0·84)
19A	83·0 (69·2, 92·4)	93·5 (82·1, 98·6)	-10·5 (-24·3, 3·2)	0·86 (0·66, 1·13)	1·26 (0·94, 1·68)	0·69 (0·46, 1·01)

† indicates a GMC ratio with a 95% CI excluding 1.00, PCV10 higher

Table S6: Percentage of participants with serotype-specific IgG ≥1.0µg/mL

Post-hoc analysis of immunogenicity data at 4 weeks post-primary series and 4 weeks post-booster, in participants that received a 2+1 schedule of PCV10 or PCV13 at 2, 4, and 9·5 months of age. PCV10=ten-valent pneumococcal conjugate vaccine. PCV13=13-valent pneumococcal conjugate vaccine.

	Post-primary series			Post-booster		
	PCV10 (n=237)	PCV13 (n=232)	Risk difference (95% CI) (PCV10-PCV13)	PCV10 (n=226)	PCV13 (n=221)	Risk difference (95% CI) (PCV10-PCV13)
Shared serotypes						
1	81.4 (75.9, 86.2)	97 (93.9, 98.8)	-15.5 (-21.2, -10.1)§	92.5 (88.2, 95.6)	98.6 (96.1, 99.7)	-6.2 (-10.5, -2.4)
4	90.3 (85.8, 93.7)	98.7 (96.3, 99.7)	-8.4 (-12.9, -4.4)	95.1 (91.5, 97.5)	99.1 (96.8, 99.9)	-4 (-7.7, -0.8)
5	64.1 (57.7, 70.2)	84.9 (79.6, 89.3)	-20.8 (-28.2, -13.0)§	65.5 (58.9, 71.7)	95.9 (92.4, 98.1)	-30.4 (-37.1, -23.6)§
6B	42.2 (35.8, 48.8)	20.7 (15.7, 26.5)	21.5 (13.2, 29.4)‡	97.8 (94.9, 99.3)	94.6 (90.7, 97.2)	3.2 (-0.5, 7.2)
7F	86.9 (82.0, 90.9)	94.8 (91.1, 97.3)	-7.9 (-13.3, -2.7)	92.5 (88.2, 95.6)	99.1 (96.8, 99.9)	-6.6 (-10.9, -3.0)
9V	76.8 (70.9, 82.0)	91.8 (87.5, 95.0)	-15 (-21.5, -8.5)	96.5 (93.1, 98.5)	98.2 (95.4, 99.5)	-1.7 (-5.2, 1.5)
14	93.2 (89.3, 96.1)	93.5 (89.6, 96.3)	-0.3 (-5.0, 4.4)	99.1 (96.8, 99.9)	98.6 (96.1, 99.7)	0.5 (-2.0, 3.1)
18C	77.2 (71.3, 82.4)	93.1 (89.0, 96.0)	-15.9 (-22.2, -9.6)	99.6 (97.6, 100.0)	95.9 (92.4, 98.1)	3.6 (0.8, 7.1)
19F	95.4 (91.8, 97.7)	96.6 (93.3, 98.5)	-1.2 (-5.1, 2.6)	100 (98.4, 100.0)	99.1 (96.8, 99.9)	0.9 (-0.9, 3.2)
23F	50.2 (43.7, 56.7)	53.4 (46.8, 60.0)	-3.2 (-12.2, 5.8)	91.6 (87.2, 94.9)	96.4 (93.0, 98.4)	-4.8 (-9.5, -0.3)
Additional PCV13-types						
3	2.1 (0.7, 4.9)	77.6 (71.7, 82.8)	-75.5 (-80.5, -69.1)	9.7 (6.2, 14.4)	79.6 (73.7, 84.7)	-69.9 (-75.7, -62.5)
6A	10.5 (6.9, 15.2)	75 (68.9, 80.4)	-64.5 (-70.6, -57.0)	60.6 (53.9, 67.0)	97.7 (94.8, 99.3)	-37.1 (-43.7, -30.3)
19A	21.5 (16.5, 27.3)	89.2 (84.5, 92.9)	-67.7 (-73.5, -60.4)	73.5 (67.2, 79.1)	99.1 (96.8, 99.9)	-25.6 (-31.8, -19.8)

‡ indicates a risk difference with 95% CI entirely below -10% (PCV13 better)
§ indicates a risk difference with 95% CI entirely above 10% (PCV10 better)

Table S7: Hospitalisations

Number (%) of hospitalisations by study group, and breakdown of reason for hospitalisation and causality (in relation to vaccination), n (%) within each study group

	Group A	Group B	Group C	Group D	Group E	Group F	Total
All hospitalisations, n (%)	21 (13%)	22 (13%)	39 (24%)	29 (18%)	28 (17%)	24 (15%)	163
Reason for hospitalisation, n (%)							
Acute respiratory infection	12 (57%)	7 (32%)	16 (41%)	13 (45%)	13 (46.5%)	9 (38%)	70 (43%)
Acute gastroenteritis	3 (14%)	6 (27%)	7 (18%)	5 (17%)	6 (21.5%)	2 (8%)	29 (18%)
Other	6 (29%)	9 (41%)	16 (41%)	11 (38%)	9 (32%)	13 (54%)	64 (39%)
Causality, n (%)							
Unrelated to vaccination	20 (95%)	21 (95%)	38 (97%)	27 (93%)	26 (93%)	24 (100%)	156 (96%)
Unlikely related to vaccination	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (7%)	0 (0%)	2 (1%)
Possibly related to vaccination	1 (5%)	0 (0%)	0 (0%)	2 (7%)	0 (0%)	0 (0%)	3 (2%)
Probably related to vaccination	0 (0%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (<1%)
Related to vaccination	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	1 (<1%)

Appendix E: Supplementary appendix to Chapter 6 research paper

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Table S1: Vaccination schedules and nasopharyngeal swabs in the Vietnam Pneumococcal Project

Time point			**2m**	**3m**	**4m**	**6m**	**9m***	**12m**	**18m**	**24m**
NP swabs			X			X	X	X	X	X
PCV doses										
Group	**Schedule**	**Vaccine**								
A	3+1	PCV10	X	X	X		X			
B	3+0	PCV10	X	X	X					
C	2+1	PCV10	X		X		X			
D	Two-dose	PCV10	X			X				
E	2+1	PCV13	X		X		X			
F	Controls	PCV10							X	X
G †	Controls	PCV10								X

PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. NP = nasopharyngeal. * Booster dose of PCV administered at 9 months of age in group A and at 9·5 months of age in groups C and E. † Group G recruited at 18 months of age.

Table S2: Comparison of participant demographics and characteristics between groups

	Group C (PCV10 at 2, 4 & 9·5m)	**Group E** (PCV13 at 2, 4 & 9·5m)	**Group F** (PCV10 at 18 & 24m)	**Group G** (PCV10 at 24m)	p-value
Participant demographics, at enrolment					
N (at enrolment)	250	251	197	199	
Age (months) [median (range)]	2·1 (1·9-2·4)	2·1 (1·9-2·4)	2·1 (1·9-2·5)	18·3 (17·4-20·3)	NA
Sex					0·528
Male	135 (54·0%)	127 (50·6%)	100 (50·8%)	113 (56·8%)	
Female	115 (46·0%)	124 (49·4%)	97 (49·2%)	86 (43·2%)	
District					0·140
4	112 (44·8%)	111 (44·2%)	87 (44·2%)	107 (53·8%)	
7	138 (55·2%)	140 (55·8%)	110 (55·8%)	92 (46·2%)	
Birthweight (g)* [mean (sd)]	3228 (370)	3199 (357)	3208 (395)	3264 (423)	0·326
Place of delivery*					0·424
Hospital	196 (78·7%)	198 (79·2%)	144 (73·1%)	152 (76·8%)	
Other	53 (21·3%)	52 (20·8%)	53 (26·9%)	46 (23·2%)	
Type of delivery*					0·063
Vaginal	160 (65·0%)	151 (60·2%)	121 (61·7%)	104 (53·1%)	
Elective caesarean	43 (17·5%)	57 (22·7%)	34 (17·3%)	44 (22·4%)	
Emergency caesarean	40 (16·3%)	42 (16·7%)	41 (20·9%)	43 (21·9%)	
Other/unknown	3 (1·2%)	1 (0·4%)	0 (0·0%)	5 (2·6%)	
Cigarette smoker in house*					0·840
No	81 (32·5%)	86 (34·3%)	72 (36·5%)	70 (35·2%)	
Yes	168 (67·5%)	165 (65·7%)	125 (63·5%)	129 (64·8%)	
Participant characteristics, at 18 months					
N (followed up at 18m)	227†	225†	185†	197	
Age (months) [median (range)]	18·1 (17·9-22·8)	18·1 (17·7-20·8)	18·1 (17·9-19·9)	18·3 (17·4-20·3)	<0·001
Any current breastfeeding	31 (13·7%)	29 (12·9%)	21 (11·4%)	32 (16.2%)	0·571
Presence of URTI symptoms	24 (10·6%)	36 (16·1%)	28 (15·2%)	31 (15·7%)	0·318
Antibiotic use in past fortnight	28 (12·4%)	26 (11·6%)	20 (10·9%)	40 (20·3%)	0·023
Current antibiotic use	13 (5·8%)	14 (6·3%)	10 (5·4%)	8 (4·1%)	0·787

Data are n (%) unless specified. p-values based on chi-squared test (for comparisons of proportions), ANOVA (for comparisons of means), or quantile regression with bootstrapped standard errors (for comparisons of medians). PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. NA = not applicable, as participants intentionally recruited at different ages. URTI = upper respiratory tract infection (presence of runny nose and/or cough). * Birthweight data missing for 9 participants (3, 2, 1, and 3 from Groups C, E, F, and G, respectively); Place of delivery data missing for 3 participants (1 each from Groups C, E, and G); Type of delivery data missing for 8 participants (4, 1, and 3 from Groups C, F, and G, respectively); Cigarette smoker data missing for 1 participant from Group C. † No information other than age available at 18 months for 1 participant from Group C, and no information available at 18 months for 1 participant from each of Groups E and F.

Table S3: Serotype-specific carriage prevalence, by time point

Vaccine serotypes	Group	Carriage prevalence (n/N)					
		2 months	6 months	9 months	12 months	18 months	24 months
PCV10-types							
6B	PCV10	0·4 (1/250)	1·2 (3/243)	1·3 (3/239)	2·6 (6/231)	0·9 (2/221)	1·5 (3/205)
	PCV13	0·8 (2/251)	2·5 (6/239)	3·8 (9/235)	3·0 (7/230)	1·4 (3/218)	3·5 (7/201)
	Control*		2·6 (5/193)	3·2 (6/190)	4·3 (8/188)	4·1 (15/368)	5·3 (9/170)
14	PCV10	0·4 (1/250)					
	PCV13	0·4 (1/251)			0·4 (1/230)	0·5 (1/218)	0·5 (1/201)
	Control*		0·5 (1/193)		0·5 (1/188)	1·9 (7/368)	2·4 (4/170)
19F	PCV10		0·4 (1/243)	0·4 (1/239)	0·9 (2/231)	1·8 (4/221)	1·5 (3/205)
	PCV13	0·8 (2/251)	1·3 (3/239)	2·1 (5/235)	2·2 (5/230)	1·8 (4/218)	2·0 (4/201)
	Control*		2·1 (4/193)	3·2 (6/190)	3·7 (7/188)	4·6 (17/368)	2·9 (5/170)
23F	PCV10	0·4 (1/250)	3·3 (8/243)	1·3 (3/239)	2·2 (5/231)	2·7 (6/221)	3·4 (7/205)
	PCV13	0·8 (2/251)	0·4 (1/239)	1·7 (4/235)	2·2 (5/230)	2·3 (5/218)	3·0 (6/201)
	Control*		0·5 (1/193)	0·5 (1/190)	1·6 (3/188)	4·3 (16/368)	3·5 (6/170)
Additional PCV13-types							
3	PCV10				1·3 (3/231)		
	PCV13					0·5 (1/218)	
	Control*					0·3 (1/368)	
6A	PCV10		1·6 (4/243)	3·3 (8/239)	3·0 (7/231)	1·8 (4/221)	6·8 (14/205)
	PCV13	0·8 (2/251)	2·9 (7/239)	2·1 (5/235)	1·7 (4/230)	1·8 (4/218)	2·5 (5/201)
	Control*		1·6 (3/193)	3·2 (6/190)	5·9 (11/188)	4·1 (15/368)	3·5 (6/170)
19A	PCV10	0·8 (2/250)	1·6 (4/243)	2·9 (7/239)	3·0 (7/231)	0·9 (2/221)	2·9 (6/205)
	PCV13	0·8 (2/251)	0·8 (2/239)	0·9 (2/235)	1·7 (4/230)	1·4 (3/218)	1·0 (2/201)
	Control*		1·6 (3/193)	1·1 (2/190)	1·6 (3/188)	1·4 (5/368)	2·9 (5/170)
Other vaccine-types†	PCV10					0·5 (1/221)	0·5 (1/205)
	PCV13						0·5 (1/201)
	Control*		0·5 (1/193)	0·5 (1/190)	0·5 (1/188)		

Non-vaccine serotypes	Group	Carriage prevalence (n/N)					
		2 months	6 months	9 months	12 months	18 months	24 months
15A	PCV10	0·8 (2/250)	0·4 (1/243)	1·3 (3/239)	2·2 (5/231)	1·8 (4/221)	1·5 (3/205)
	PCV13		0·4 (1/239)	0·9 (2/235)	1·3 (3/230)	2·8 (6/218)	2·0 (4/201)
	Control*	0·5 (1/197)	0·5 (1/193)	1·6 (3/190)	1·1 (2/188)	1·6 (6/368)	1·2 (2/170)
15B/C	PCV10			0·4 (1/239)	0·4 (1/231)	2·3 (5/221)	2·4 (5/205)
	PCV13	0·4 (1/251)	0·4 (1/239)		2·2 (5/230)	2·8 (6/218)	1·0 (2/201)
	Control*				2·1 (4/188)	0·5 (2/368)	1·2 (2/170)
23A	PCV10	0·4 (1/250)	0·8 (2/243)			0·9 (2/221)	0·5 (1/205)
	PCV13		0·4 (1/239)	1·3 (3/235)	0·4 (1/230)	0·5 (1/218)	0·5 (1/201)
	Control*		1·0 (2/193)	2·1 (4/190)	1·1 (2/188)	1·1 (4/368)	2·4 (4/170)
34	PCV10			0·8 (2/239)	1·3 (3/231)	0·5 (1/221)	0·5 (1/205)
	PCV13	0·4 (1/251)	0·8 (2/239)	0·9 (2/235)	1·3 (3/230)	0·5 (1/218)	1·5 (3/201)
	Control*		0·5 (1/193)	1·1 (2/190)	0·5 (1/188)	1·1 (4/368)	
Other non-vaccine-types‡	PCV10	0·8 (2/250)	2·9 (7/243)	2·5 (6/239)	2·2 (5/231)	1·8 (4/221)	0·5 (1/205)
	PCV13	0·8 (2/251)	1·7 (4/239)	1·3 (3/235)	3·9 (9/230)	1·8 (4/218)	2·0 (4/201)
	Control*	1·0 (2/197)			2·1 (4/188)	0·5 (2/368)	0·6 (1/170)

Blank cells indicate no carriage. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. * Control data sourced from Group F (2–12 month time points), Group F and G combined (18 months), or Group G (24 months). † The **6 other vaccine-types** comprised: **4 x serotype 4** (1at 9m [Group F], 1 at 12m [Group F], 2 at 24m [1 in Group C, 1 in Group E]); **1 x serotype 18C** (at 6m [Group F]); and **1 x serotype 9V** (at 18m [Group C]). ‡ The **46 other non-vaccine-types** comprised: **25 x serotype 11A** (2 at 2m [Group C], 4 at 6m [3 in Group C, 1 in Group E], 5 at 9m [4 in Group C, 1 in Group E], 8 at 12m [4 in Group C, 3 in Group E, 1 in Group F], 4 at 18m (2 in Group C, 1 in Group E, 1 in Group F/G], and 2 at 24m [Group E]); **7 x serotype 35B** (2 at 6m [1 in Group C, 1 in Group E], 1 at 9m [Group E], 1 at 12m [Group E], 2 at 18m [1 in Group C, 1 in Group E], and 1 at 24m [Group G]); **4 x serotypes 7C, 13, and 19C** (7C: 1 at 2m [Group E], 1 at 6m [Group E], 1 at 12m [Group F], 1 at 24m [Group E]; 13: 1 at 9m [Group C], 2 at 18m [1 in Group C, 1 in Group F/G], 1 at 24m [Group E]; 19C: 1 at 2m [Group F], 3 at 12m [1 in Group C, 2 in Group E]); **3 x serotypes 6C and 17F** (6C: 1 at 2m, 9m, and 12m [all in Group E]; 17F: 2 at 12m [1 in Group E, 1 in Group F], 1 at 18m [Group E]); **2 x serotypes 16F and 35F** (16F: 1 at 9m [Group C], 1 at 12m [Group E]; 35F: 2 at 6m [1 in Group C, 1 in Group E]); and **1 x serotypes 8** (12m [Group F]), **19B** (2m [Group F]), **20** (9m [Group C]), **35A** (18m [Group E]), **37** (6m [Group C]), and **38** (24m [Group C]).

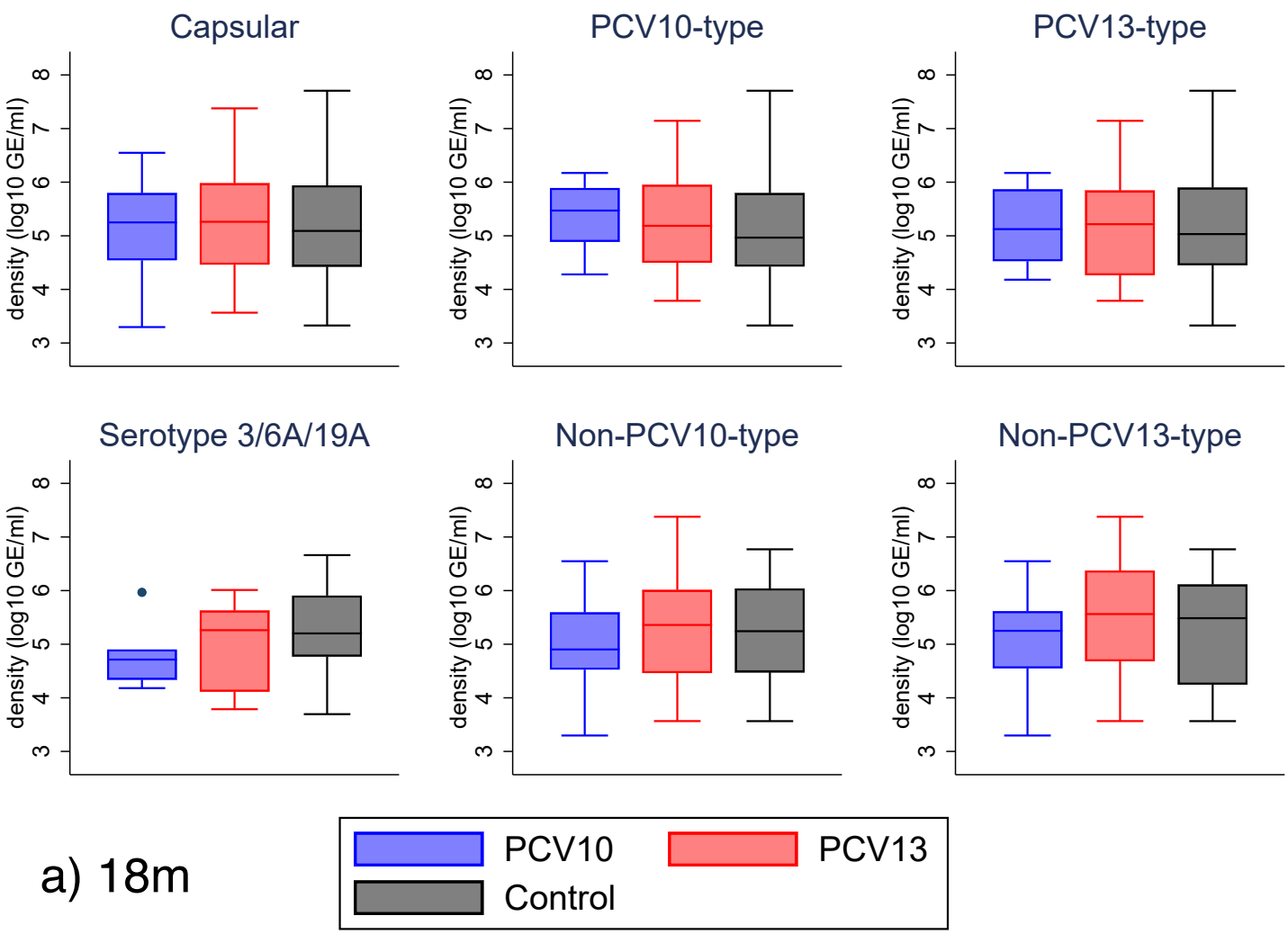
Table S4: Overall probability of carriage between 6 and 18 months of age

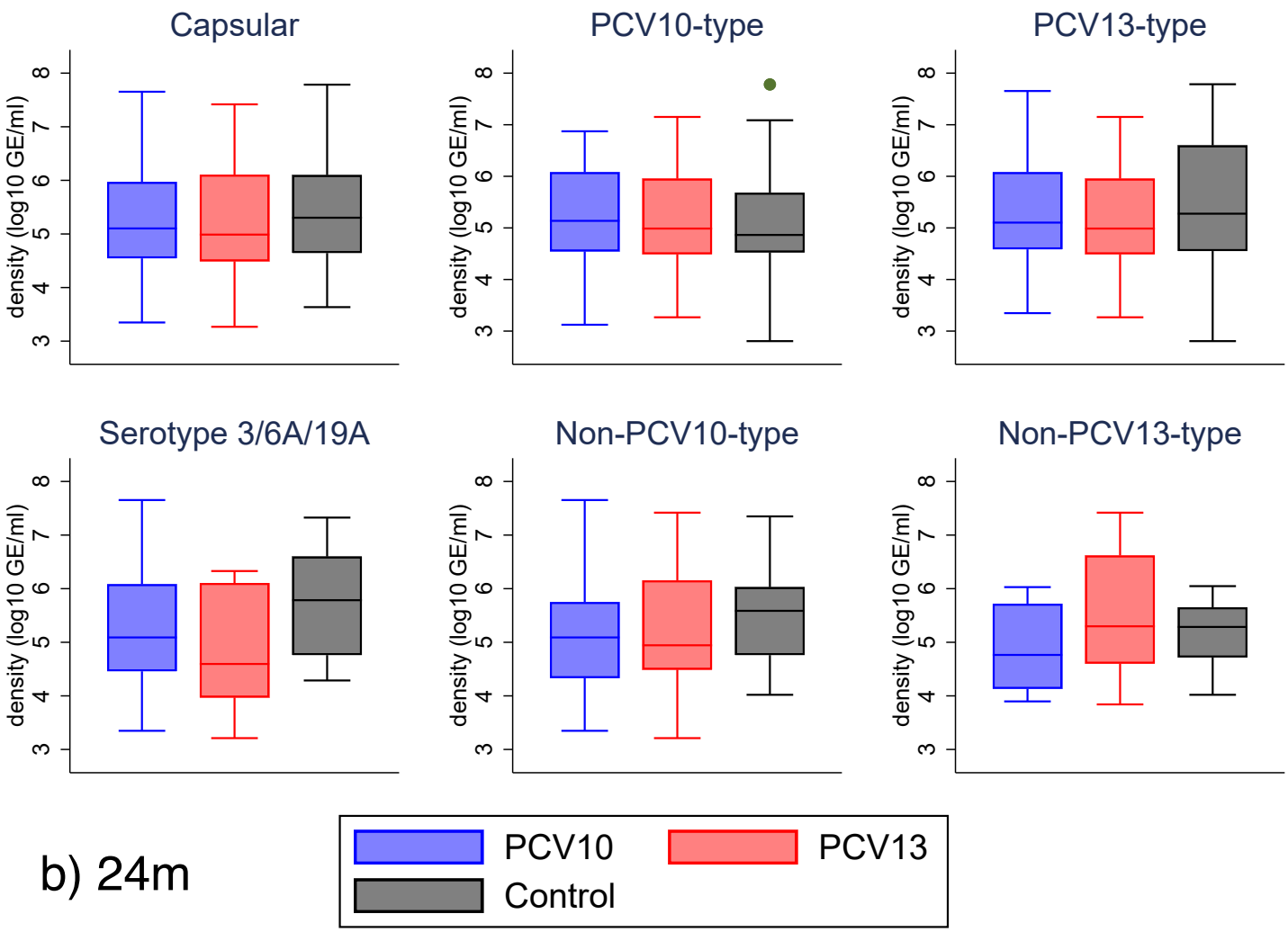
	Carriage prevalence, % (95% CI)			PCV10 vs Controls		PCV13 vs Controls		PCV13 vs PCV10	
	2+1 PCV10	2+1 PCV13	Controls*	Prevalence ratio (95% CI)	p-value†	Prevalence ratio (95% CI)	p-value†	Prevalence ratio (95% CI)	p-value†
Any pneumococcal serotype carriage	36·6 (30·6-43·0)	36·4 (30·3-42·8)	44·0 (36·9-51·3)	0·83 (0·66-1·05)	0·071	0·83 (0·66-1·04)	0·065	0·99 (0·79-1·26)	>0·999
PCV10-type carriage	14·0 (9·9-19·0)	15·1 (10·8-20·2)	21·2 (15·7-27·7)	0·66 (0·44-1·00)	0·031	0·71 (0·47-1·06)	0·062	1·08 (0·70-1·66)	0·796
PCV13-type carriage	24·7 (19·4-30·6)	23·8 (18·6-29·8)	32·6 (26·1-39·7)	0·76 (0·56-1·02)	0·042	0·73 (0·54-0·99)	0·028	0·97 (0·70-1·32)	0·833
3/6A/19A carriage	12·3 (8·5-17·2)	9·6 (6·2-14·1)	15·0 (10·3-20·9)	0·82 (0·51-1·32)	0·25	0·64 (0·38-1·07)	0·059	0·78 (0·47-1·30)	0·383
Non-PCV10-type carriage	24·7 (19·4-30·6)	24·7 (19·4-30·7)	27·5 (21·3-34·3)	0·90 (0·65-1·24)	0·292	0·90 (0·65-1·24)	0·293	1·00 (0·73-1·37)	>0·999
Non-PCV13-type carriage	15·2 (11·0-20·4)	16·3 (11·9-21·6)	13·0 (8·6-18·5)	1·18 (0·73-1·88)	0·297	1·26 (0·79-2·01)	0·2	1·07 (0·71-1·62)	0·803

Overall probability of carriage defined as the percentage of participants with any positive swab between 6 and 18 months of age. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. * Control data restricted to Group F. † Two–sided Fisher's exact test used for PCV10 vs PCV13 comparisons; one–sided Fisher's exact test used for comparisons with controls.

Appendix Figure S1: Pneumococcal carriage density among pneumococcal carriers a) at 18 months of age and b) at 24 months of age

Median (IQR) density (log_{10} genome equivalents per ml) of capsular, PCV10-type, PCV13-type, serotype 3/6A/19A, non-PCV10-type, and non-PCV13-type carriage, among pneumococcal carriers at a) 18 months of age and b) 24 months of age who received a 2+1 schedule of PCV10, a 2+1 schedule of PCV13, or unvaccinated controls. IQR = interquartile range. PCV = pneumococcal conjugate vaccine. PCV10 = ten-valent PCV. PCV13 = 13-valent PCV. IQR = inter-quartile range. Control group data come from: Group F (2-12 months); Groups F and G combined (18 months); or Group G (24 months). ● denotes a datapoint greater than the 75^{th} percentile plus 1·5 times the IQR.


Capsular
PCV10-type
PCV13-type
Serotype 3/6A/19A
Non-PCV10-type
Non-PCV13-type
density (log10 GE/ml)
3
4
5
6
7
8
a) 18m
PCV10
PCV13
Control


Capsular
PCV10-type
PCV13-type
Serotype 3/6A/19A
Non-PCV10-type
Non-PCV13-type
density (log10 GE/ml)
3
4
5
6
7
8
b) 24m
PCV10
PCV13
Control