Pneumococcal carriage and transmission in Karonga district, Malawi, before and after introduction of 13-valent pneumococcal conjugate vaccination

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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My role

I, Ellen Heinsbroek, 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.

I conducted the background literature review (Chapter 1) and the systematic literature review on transmission of pneumococcal carriage in children under-five years of age (Chapter 4) with input from my PhD supervisors Neil French and Jonathan Read.

Chapters 5 and 6 on pneumococcal carriage in the pre-vaccination period consist of analyses on pre-existing datasets. I performed the analyses with input from Neil French and Jonathan Read. Statistics support was provided by Peter Diggle.

The work on vaccine uptake and timeliness (Chapter 7) has been conducted together with Hazzie Mvula and Naor Bar-Zeev. I performed the analyses on vaccine timeliness and supervised Hazzie Mvula on the analyses on vaccine uptake and the literature review. Writing the manuscript of this work was done together with Hazzie Mvula and Naor Bar-Zeev. The manuscript has been adapted for this thesis.

I adapted existing protocols and data collection forms for the collection of samples in the post-vaccination period (Chapter 8). I wrote the study protocol and applied for ethical approval. I trained and supervised a team of four interviewers and two data clerks. Laboratory support for the collection and storage of samples in Karonga was provided by Amos Phiri and his team. Laboratory analysis of samples was performed at MLW by Maaike Alaerts and Todd Swarthout and their team. I performed the data management and data cleaning. I performed the analyses with input from Neil French and Jonathan Read.

The mathematical modelling work (Chapter 9 and 10) was done together with Jonathan Read, with input from Neil French. Statistics support was provided by Chris Jewell from Lancaster University. Abel Heinsbroek helped to prepare my model for parallel processing using a high performance computing cluster. I used the ‘chadwick’ cluster for parallel processing which is managed by Cliff Addison and Dave Love.

Abstract

Streptococcus pneumoniae (pneumococcus) is a leading cause of childhood morbidity and mortality worldwide. Thirteen-valent pneumococcal conjugate vaccine (PCV-13) was introduced in the Malawian infant immunisation programme in November 2011. PCV-13 is currently given at a "3+0" schedule: doses are given at 6, 10 and 14 weeks and no booster dose is currently implemented. The aim of this thesis was to study pneumococcal carriage and transmission in Karonga District, Malawi, before and after introduction of PCV-13, in order to review the effect of the current pneumococcal vaccination programme on carriage, and to give recommendations on the implementation of different vaccination strategies in Malawi.

Pneumococcal carriage studies were conducted in Karonga between 2008 and 2014, with a focus on infants born to an HIV-positive mother, and HIV-positive adults, both of whom are at high risk of invasive pneumococcal disease. We found no difference in pneumococcal acquisition in infants by maternal HIV-status. A greater proportion of infant pneumococcal acquisition was attributable to carriage in other children <5 years in the household than to maternal carriage. Pneumococcal carriage in HIV-positive adults in Malawi remained high despite up to two years of antiretroviral treatment, indicating a failure of reconstitution of respiratory mucosal immune response.

An analysis on the uptake and timeliness of PCV-13 vaccination showed that despite high vaccination coverage in this setting, delays in vaccination were common. Infants born to lower educated or farming mothers and those living more remotely were at greater risk of being not fully vaccinated and being vaccinated late.

Carriage studies conducted in 2014, two years post PCV-13 introduction, showed that carriage of vaccine type (VT) pneumococci had decreased in vaccinated children and unvaccinated older age groups, but that a reservoir of VT carriage was still present. VT carriage had not decreased in unvaccinated children <5 years. Carriage of non-vaccine type pneumococci (NVT) had increased in vaccinated children. Our results suggest that a herd immunity effect is taking place albeit slowly in comparison to other countries. Waning immunity seemed to occur in vaccinated children 1-4 years.

Our mathematical modelling studies provided further evidence for waning immunity. An immunity half-life between 6 months and 1 year was found to fit best with the observed post-vaccination carriage prevalence. If the immunity half-life were to be increased to 2 years, this would have a large impact on VT carriage decline in vaccinated and

unvaccinated age groups. In the stochastic individual-based transmission models, which included explicit household transmission, an indirect vaccine effect was observed immediately after introduction of PCV-13. Adding a booster dose to the current three-dose schedule (3+1 schedule) would be beneficial for vaccinated and unvaccinated groups. Replacing the current 3+0 schedule with a 2+1 schedule (booster dose at 9 months) initially resulted in slower VT decline, but seemed to be associated with a longer-term gain in lower VT carriage in 1-4 year olds. Maternal vaccination did not result in additional VT carriage reduction in the infant.

Ongoing surveillance is required to assess VT carriage in this population and monitor waning immunity and serotype replacement in vaccination individuals. A review of the vaccination schedule may be required to optimise total population impact in this high carriage setting. There is need for cheaper, more effective, serotype-independent pneumococcal vaccines. Potentially cheaper PCVs produced in India are in the pipeline, as well as a couple of new generation vaccines that are currently in phase 2 clinical trials. The next decade is promising to be an exciting time for pneumococcal researchers and policy makers worldwide, as new vaccines will pose interesting possibilities to further decrease pneumococcal carriage and disease worldwide.

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List of Acronyms

ABC	Approximate Bayesian computation
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CD4	CD4+ lymphocyte count
CI	Confidence interval
CT	Cycle threshold
DCC	Day care centre
HIV	Human immunodeficiency virus
HR	Hazard ratio
IgG	Immunoglobulin type G
IPD	Invasive pneumococcal disease
IQR	Inter-quartile range
KHDSS	Karonga Health and Demographic Surveillance System
KPS	Karonga Prevention Study
MCMC	Markov Chain Monte Carlo
MLEE	Multi-locus enzyme electrophoresis
MLST	Multi-locus sequence typing
MLW	Malawi Liverpool Wellcome Trust Clinical Research Programme
NVT	Non-vaccine type
OR	Odds ratio
PR	Prevalence ratio
RFEL	Restriction fragment end labelling
RR	Rate ratio
PAF	Population attributable fraction
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PFGE	Pulsed-field gel electrophoresis
SD	Standard deviation
SIS	Susceptible – Infectious - Susceptible
SMC	Sequential Monte Carlo
QC	Quality Control
VE	Vaccine efficacy
VT	Vaccine type
UOL	University of Liverpool
WHO	World Health Organisation

List of Supporting Publications and Presentations

H. Mvula*, **E. Heinsbroek*,** M. Chichana, A.C. Crampin, S. Kabuluz, G. Chirwa, C. Mwansambo, A. Costello, N.A. Cunliffe, R.S. Heyderman, N. French, N. Bar-Zeev, *Predictors of Uptake and Timeliness of Newly Introduced Pneumococcal and Rotavirus Vaccines, and of Measles Vaccine in Rural Malawi: a Population Cohort Study.* PLoS One. 2016 May 6;11(5) *Co-first authors

E. Heinsbroek, T. Tafatatha, C. Chisambo, A. Phiri, O. Mwiba, B. Ngwira, A.C. Crampin, J.M. Read, N. French, *Pneumococcal acquisition in HIV-exposed and HIV-unexposed infants in rural Malawi: a longitudinal household study,* Am J Epidemiol. 2016 Jan 1;183(1):70-8

E. Heinsbroek, T. Tafatatha, A. Phiri, B. Ngwira, A.C. Crampin, J.M. Read, N. French, *Persisting high prevalence of pneumococcal carriage among HIV-positive adults receiving antiretroviral therapy in Malawi: a cohort study.* AIDS. 2015;29:1837-44.

Pneumococcal carriage and prolonged antiretroviral treatment in HIV-positive adults in Malawi, International Symposium on Pneumococci and Pneumococcal Diseases, Glasgow, UK, 2016 (oral e-poster presentation).

Pneumococcal carriage in households in Karonga District Malawi, before and after introduction of pneumococcal conjugate vaccination, European Society for Paediatric Infectious Diseases Meeting, Brighton, UK, 2016 (oral presentation).

Decrease in pneumococcal carriage before introduction of pneumococcal conjugate vaccine in Karonga district, Malawi, International Symposium on Pneumococci and Pneumococcal Diseases, Hyderabad, India, 2014 (poster presentation).

Effect of antiretroviral therapy on pneumococcal carriage in HIV-positive adults in Karonga District, Malawi, International Symposium on Pneumococci and Pneumococcal Diseases, Hyderabad, India, 2014 (poster presentation).

Pneumococcal transmission to infants in HIV-exposed and HIV-unexposed households in Malawi, Epidemics-4, Amsterdam, The Netherlands, 2013 (poster presentation).

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I am very grateful to my supervisors Neil French and Jonathan Read. I am thankful to Neil for creating all the opportunities I've had during my PhD; both in access to existing datasets and the possibility of six months field work to collect new data. I have greatly enjoyed our lengthy supervisor meetings and have learnt a lot from discussing not only my own PhD, but also past, ongoing and future studies in the field. I am thankful to Jon for teaching me the wonderful art of mathematical modelling, a field completely new to me before embarking on this PhD. Not only have I learnt a new skill set from this analysis, I have learnt proper coding practises, and how to persevere when facing a steep learning curve. Many thanks for your patience and perseverance!

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1. Introduction

1.1 Streptococcus pneumoniae

Streptococcus pneumoniae (pneumococcus) is a leading cause of childhood morbidity and mortality worldwide. As the major cause of bacterial pneumonia, meningitis and sepsis, the pneumococcus was estimated in 2000 to cause 11% of all-cause mortality in children 1-59 months worldwide (1). In 2013, pneumococcal pneumonia accounted for the largest proportion of lower respiratory infection deaths and was estimated to cause 264.000 deaths in children younger than 5 years worldwide (2). Pneumococcal meningitis was estimated to cause an additional 79,100 deaths in under-fives worldwide (2). In addition, pneumococci are estimated to cause 30-50% of all episodes of otitis media, a vast, but poorly defined disease burden worldwide (3).

Currently, more than 90 different serotypes of pneumococci are known, classified by differences in the polysaccharide capsule. The polysaccharide capsule protects the bacteria from phagocytosis and is the most important virulence factor (4). The natural reservoir of the pneumococcus is the human nasopharynx. Although colonisation with pneumococci is mostly asymptomatic, nasopharyngeal carriage is thought to be a pre-requisite for disease (5). The pneumococcus interacts with the human immune system in a balance between carriage in the nasopharynx, evading the host's immune response, and causing non-invasive and invasive disease. Virulence and carriage characteristics differ amongst serotypes, with less than 10 serotypes estimated to be responsible for more than 75% of all episodes of invasive pneumococcal disease (IPD) (3). Some invasive serotypes are frequently carried but have a low carrier-case ratio (serotype 6B, 19F, 23F), whereas other serotypes have high IPD rates and can cause outbreaks in all age groups but are rarely found in carriage (serotype 1, 5) (3, 6).

Carriage in the nasopharynx also acts as the main reservoir for pneumococcal transmission, with person-to-person spread occurring in close contact (5). Pneumococcal carriage acquisition occurs very early in life in low-income countries, with an observed median time to acquisition of only 38.5 days, 45.5 days or 8 weeks reported from studies in Kenya, Thailand-Myanmar and Bangladesh respectively (7-9). This early and intense exposure in infancy is likely to play a role in the high disease incidence also experienced in these settings.

1.2 Pneumococcal vaccines

A 23-valent polysaccharide vaccine (PPV-23), containing polysaccharides from the capsular antigens of 23 different serotypes has been available since 1983. PPV-23 is only licensed for use in individuals aged >2 years, due to poor T-cell independent immunogenicity in the younger age group.

A 7-valent pneumococcal conjugate vaccine (PCV-7) containing serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, has been licensed since 2000 (7). Since then, two new conjugate vaccines have been introduced: a 10-valent vaccine (PCV-10) containing additional serotypes 1,5 and 7F, and a 13-valent vaccine (PCV-13) containing additional serotypes 3, 6A and 19A (Figure 1). PCVs contain polysaccharides of the capsular antigens linked a non-toxoid bacterial carrier protein (diphtheria CRM197 protein in PCV-7 and PCV-13; non-typeable *Haemophilus Influenza* protein D and tetanus and diphtheria toxoids in PCV-10) (10, 11). The presence of the carrier proteins elicits a T-cell dependent antibody response to the polysaccharide capsular antigens, thereby providing protection against vaccine-type (VT) disease, also in young infants (10, 11).

PCV-7	diphtheria CRM197	4	6B	9V	14	18C	19F	23F						
PCV-10	ntHi protein D	4	6B	9V	14	18C	19F	23F	1	5	7F			
PCV-13	diphtheria CRM197	4	6B	9V	14	18C	19F	23F	1	5	7F	3	6A	19A

Figure 1.1 Serotypes included in 7-valent, 10-valent and 13-valent pneumococcal conjugate vaccines.
ntHi: non-typeable Haemophilus Influenza

PCV has been introduced in the infant vaccination schedule of numerous high-income countries and is being rolled out in middle- and low-income countries with the support of the Global Alliance for Vaccines and Immunisations (GAVI). The World Health Organisation (WHO) recommends a vaccination schedule of three primary doses ("3+0") given at 6, 10, 14 weeks or 2, 4, 6 months, or a schedule of two primary doses plus a booster ("2+1") given between 9 and 15 months (12). An early schedule (6, 10, 14 weeks) is favoured in low-income countries where incidence of pneumococcal disease in the first year of life is high. Low uptake of measles vaccination, given routinely in low-income countries at 9 months, can be another reason for preference of a 3+0 versus a 2+1 schedule. Use of a 2+1 schedule may be preferred in settings where duration of protection may be a concern, although it has not been confirmed yet how much longer the duration of protection is with a schedule including a booster dose (13).

PCV have been shown effective against IPD caused by VT (14-16). An important feature of PCV is its ability to reduce nasopharyngeal carriage in vaccinated individuals (17). A herd effect, whereby disease incidence is also decreased in non-vaccinated individuals as a result of reduced transmission from vaccinated children, has been observed in various settings (18-20). Many studies performed in high-income countries have shown a decline in pneumococcal disease in unvaccinated individuals after the introduction of PCV (21-23). Limited results are available for the more recent introduction of PCV in low-income countries. A randomised controlled trial in The Gambia reported PCV-9 vaccine efficacy of 77% (95% CI 51-90) for VT IPD, and 50% (95% CI 21-69) for IPD caused by all serotypes, when administered to children 6-51 weeks of age (16). In South Africa, where PCV-7 was introduced in 2009, substantial declines in VT IPD were observed in both children and adults, suggesting a herd effect (24).

To accelerate the development of herd protection, a catch-up campaign can be offered on introduction of PCV, whereby PCV is offered to children too old to be vaccinated in the routine immunisation schedule (12). In Kenya, a catch-up campaign was held for all children <5 years living in Kilifi County but not the rest of the country (25). Targeting those age groups most involved in transmission is expected to obtain the largest herd effect, although a systematic evaluation of the added benefit of catch-up campaigns on the establishment of herd protection has not yet been done (13). Catch-up campaigns also provide direct protection for vaccinated children. Catch-up campaigns were held on introduction of PCV in the UK (26) and the US (27) for children <2 years to reduce the high rates of IPD in children <2 years in these countries. No catch-up campaign

was held in South Africa where the main burden of pneumococcal disease is in infants <1 year (28).

A concern regarding the introduction of PCV is that vaccination against a selected number of VT is likely to result in an increase in IPD caused by non vaccine types (NVT), diminishing the advantage of vaccination (29). Several studies performed in high-income countries showed an increase in disease caused by NVT IPD after PCV-7 introduction (30). Introduction of PCV in low-income countries can be expected to result in a higher rate of serotype replacement due to high background rate of pneumococcal carriage. Also the burden of human immunodeficiency virus (HIV) in many countries could have an effect on the rate by which serotype replacement will occur after vaccine introduction. To decrease the extent of serotype replacement, pneumococcal conjugate vaccines covering a wider range of serotypes, PCV-10 and PCV-13, are currently recommended by the World Health Organisation (WHO) (31).

1.3 Pneumococcal carriage and transmission

Worldwide surveillance on the spread and serotype distribution of pneumococci is required to study the impact of vaccination and the extent of serotype replacement. Understanding the drivers of pneumococcal transmission in different settings is vital to understand and predict VT and NVT incidence in vaccinated and unvaccinated individuals and thus to inform vaccination policies. Given the impressive impact of PCVs on disease burden in high-income settings, there is an expectation that this will also manifest in low-income country settings. If pneumococcal transmission is similar then there will be every possibility of the successes seen in the US and Europe. But if as seems likely transmission varies under different demographic circumstances then understanding these differences will allow consideration of alternate vaccine schedules and uses to deliver herd protection.

The exact mechanisms whereby PCV influences carriage are unclear (32). Vaccination may reduce an individual's susceptibility to pneumococcal carriage acquisition. It is also possible that vaccination increases clearance of pneumococci from the nasopharynx, thereby reducing the duration of carriage. Lastly, vaccination may influence the density of carriage, the quantitative bacterial load in the nasopharynx.

Because of its importance for indirect vaccine effects, pneumococcal carriage is currently being introduced as an endpoint in vaccine licensing (32). Additional

advantages of using carriage as well as disease or immunological assays as an endpoint are the easy detection of carriage and its common occurrence, allowing for smaller sample sizes. The main drawback of using carriage as an endpoint in clinical trials is its poor prediction as a surrogate of disease: some serotypes that frequently cause disease are rarely found in carriage (e.g. serotypes 1 and 5), and many serotypes frequently carried are rarely causing invasive disease.

1.4 Pneumococcal carriage and disease and HIV/AIDS

HIV-positive adults and children are at 20-100 fold higher risk of IPD (33-35). In sub-Saharan Africa, IPD in adults is strongly associated with HIV infection and has a high mortality rate (36-39). Antiretroviral therapy (ART) reduces the incidence of IPD in HIV-positive adults (33) and there is a strong temporal relationship between large-scale ART introduction and declines in IPD in Malawi (40, 41). This population impact will be driven by immune reconstitution and/or immune maintenance at the individual level. However, HIV-positive individuals established on ART remain at much higher risk of IPD as compared to HIV-uninfected individuals (42). This suggests immune reconstitution is incomplete; a finding supported by earlier work in Malawi suggesting that ART did not alter the risk of recurrent IPD events (43).

Many high-income countries including the UK and US recommend vaccination for HIV-positive adults. UK guidelines recommend a single dose of PCV-13 irrespective of CD4 cell count, ART use and viral load. An additional single dose of PPV-23 is recommended for HIV-positive adults who meet the indications for PPV-23 vaccination (aged >65 years or with co-morbidity other than HIV) (44). US guidelines recommend a single dose of PCV-13 followed by a dose of PPV-23 for all adults with immunocompromising conditions (45). A booster dose of PPV-23 is recommended after five years. Vaccination of HIV-positive adults is uncommon in low-income countries. A randomized, controlled trial conducted in Malawi showed that PCV would be beneficial for HIV-positive adults: PCV-7 was found to protect HIV-positive adults from recurrent vaccine-type pneumococcal disease (43). An earlier trial in Uganda showed that PPV-23 was ineffective in HIV-positive adults and even found higher rates of disease in vaccinated individuals (46).

Pneumococcal carriage is also more common in HIV-positive than HIV-uninfected individuals (47-49), which could lead to increased transmission in HIV-affected populations. It is possible that HIV-positive individuals represent a large reservoir of *S. pneumoniae* that could have an impact on the effect of the development of herd

immunity after introduction of pneumococcal vaccines in infant immunisation schedules in HIV-affected countries.

1.5 Situation in Malawi

Malawi is a land-locked country in Southern Africa. It is one of the poorest and most underdeveloped countries worldwide (50). Malawi has a high burden of malnutrition, HIV/AIDS, malaria, diarrhoea and acute respiratory infections (51). HIV-prevalence nationwide was estimated at 10% in adults aged 15-49 years in 2013 (50). Life expectancy at birth is 55 years. Under-five mortality has decreased in recent years from 245 per 1,000 life births in 1990 to 68 per 1,000 life births in 2013 (50). With this decrease Malawi is well on track to meet the fourth Millennium Development Goal of reducing child mortality by two-thirds between 1990 and 2015 (52).

The early adoption of PCV-13, introduced in November 2011, has been part of the strategy to meet the Millennium Development Goals. PCV-13 is given according to the WHO recommended schedule at 6, 10 and 14 weeks, along with Pentavalent vaccine (diphtheria, pertussis, tetanus, haemophilus influenza type B and hepatitis B), oral polio and oral monovalent rotavirus vaccine (introduced in October 2012). Initial catch-up for PCV-13 occurred among children <1 year of age at the time of introduction.

In Malawi, rates of IPD in children under 5 years old were estimated at 62 per 100,000 child years before introduction of pneumococcal vaccination, among the highest rates recorded globally. Pneumococcal meningitis incidence had been declining in all age groups prior to introduction of PCV-13, making it difficult to assess whether a direct and indirect vaccine impact has taken place. The proportion of pneumococcal meningitis attributable to vaccine types has declined in children <5 years two years post introduction of PCV-13 (58.3% vs. 29.8%, p=0.003), but not in children 5-14 years (61.4% vs. 62.1%, p=0.952) and adults (53.4% vs. 58.7%, p=0.548). These findings provide evidence for a direct vaccine effect, but suggest absence of vaccine indirect effect (53).

The two groups that would be at most benefit from any herd protection effect in Malawi are HIV-positive individuals and infants too young to be fully vaccinated who also suffer a high burden of disease. If herd protection is obtained with PCV-13 provided in the infant immunisation programme only, this will provide advocacy to continue the vaccination programme in Malawi, and for other countries in similar settings to also

introduce PCV-13. If insufficient herd protection is currently obtained, other vaccination strategies need to be considered, including the vaccination of pregnant women and/or HIV-positive adults to protect these vulnerable groups directly from pneumococcal disease. Another strategy could be to implement catch-up vaccination campaigns in older children to further reduce transmission and thereby increase the potential herd protection effect.

This thesis describes pneumococcal carriage and transmission in Karonga District, Malawi, before and after introduction of PCV-13. The studies described in this thesis can be divided into three parts: studies conducted before introduction of PCV-13 in the Malawi infant immunisation schedule (Part I), studies conducted after introduction of PCV-13 (Part II) and mathematical modelling studies (Part III). The main focus of this thesis is on infants and HIV-positive adults, both high-risk groups for developing IPD.

2. Aims and objectives

The overall aims of this thesis were to study pneumococcal carriage and transmission in Karonga District, Malawi, before and after introduction of PCV-13, in order to review the effect of the current pneumococcal vaccination programme on carriage, and to give recommendations on the implementation of different vaccination strategies in Malawi.

Specific objectives were:

1. To determine what proportion of pneumococcal carriage can be explained as a result of transmission from household members or extra-household transmission in different geographical settings.
2. To study pneumococcal carriage in infants and HIV-positive adults, two high-risk groups for IPD, in the pre-vaccination period;
   - 2.1. To compare infant pneumococcal acquisition by maternal HIV-status.
   - 2.2. To examine serotype-specific associations between infant carriage and carriage in their households.
   - 2.3. To investigate the impact of ART on pneumococcal carriage in Malawian adults infected with HIV.
3. To study pneumococcal carriage in infants and HIV-positive adults, two high-risk groups for IPD, in the post-vaccination period;
   - 3.1. To investigate socio-demographic and programmatic factors affecting vaccination uptake and timeliness during the introduction of PCV-13.
   - 3.2. To measure the direct impact of PCV-13 vaccination on pneumococcal carriage in vaccinated individuals.
   - 3.3. To measure the indirect impact (herd effect) of PCV-13 vaccination on pneumococcal carriage in unvaccinated individuals.
   - 3.4. To examine serotype-specific associations between infant carriage and carriage in their households.

4. To describe pneumococcal carriage and transmission before and after introduction of PCV-13 using a mathematical model.
   4.1. To build a mathematical model on pneumococcal carriage and fit this to the Karonga population structure and observed pneumococcal carriage data.
   4.2. To compare household transmission and community transmission of pneumococcal carriage.
   4.3. To investigate what level of vaccination coverage in the infant immunisation schedule would have to be achieved before a herd effect is observed in pneumococcal carriage in unvaccinated individuals.
   4.4. To compare the effect of different waning immunity half-lives after vaccination and determine which scenario is most likely given the post-vaccination prevalence data.
   4.5. To assess the effect of different vaccination strategies on pneumococcal carriage in vaccinated and unvaccinated individuals.

3. Methodology

This chapter describes methods used in more than one study. Methods specific to individual studies are described in their respective chapters.

3.1 Overview of included studies

Table 3.1 provides an overview of studies included in this thesis. The studies' main focus is on infants and HIV-positive adults, both high-risk groups for developing IPD. In the infant studies, samples were also collected from mothers and other household members, allowing for epidemiological analyses of household transmission and mathematical modelling of household and community-wide transmission.

3.2 Study location and population

All studies were conducted at the Karonga Health and Demographic Surveillance System (KHDSS) site in northern Malawi (54). Established in 2002, the KHDSS covers an area of 135km² where the population is under continuous surveillance through which all births, deaths and migrations are recorded (54, 55). The area has an annual birth cohort of about 1350. Population growth is approximately 2.5% per year: the population increased from 34,111 people on 1st January 2009 (start of the pneumococcal household study) to 39,786 people on 11th September 2015 (end of data used for mathematical modelling study).

Table 3.1 Overview of studies included in this thesis

Chapter	Study name	Before / after introduction PCV-13 (data collection period)	Study participants	Longitudinal / cross-sectional / modelling study	Data collection	Aims of study
4	Systematic literature review	Both	NA	Literature review	NA	• To determine what proportion of pneumococcal carriage can be explained as a result of transmission from household members or extra-household transmission in different geographical settings.
5	Pneumococcal carriage and vaccine study – before PCV-13 introduction	Before (2009-2011)	Infants Mothers Household members	Longitudinal	Nasopharyngeal samples	• To compare infant pneumococcal acquisition by maternal HIV-status. • To examine serotype-specific associations between infant carriage and carriage in their households.
8	Pneumococcal carriage and vaccine study – after PCV-13 introduction	After (2014)	Infants Mothers Household members	Longitudinal & cross-sectional	Nasopharyngeal samples	• To measure the direct impact of PCV-13 vaccination on pneumococcal carriage in vaccinated individuals. • To measure the indirect impact (herd effect) of PCV-13 vaccination on pneumococcal carriage in unvaccinated individuals. • To examine serotype-specific associations between infant carriage and carriage in their household
6	ART cohort study	Before (2008-2010)	HIV-positive adults in ART clinic	Longitudinal	Nasopharyngeal samples	• To investigate the impact of ART on pneumococcal carriage in Malawian adults infected with HIV.

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Chapter	Study name	Before / after introduction PCV-13 (data collection period)	Study participants	Longitudinal / cross-sectional / modelling study	Data collection	Aims of study
8	Pneumococcal carriage and vaccine study – ART arm	After (2014)	HIV-positive adults in ART clinic	Cross-sectional	Nasopharyngeal samples	• To measure the indirect impact (herd effect) of PCV-13 vaccination on pneumococcal carriage in unvaccinated individuals.
7	Vaccine uptake study	After (2011-2014)	Vaccine age-eligible children	Longitudinal	Vaccine records Socio-demographic indicators	• To investigate socio-demographic and programmatic factors affecting vaccination uptake and timeliness during the introduction of PCV-13.
9	Mathematical modelling study	Before	Whole population	Modelling	Model fitted to prevalence estimations from all available studies	• To describe pneumococcal carriage and transmission before and after introduction of PCV-13 using a mathematical model. • To compare household transmission and community transmission of pneumococcal carriage.
10	Mathematical modelling study	After	Whole population	Modelling	Model fitted to prevalence estimations from all available	• To investigate what level of vaccination coverage in the infant immunisation schedule would have to be achieved before a herd effect is

Table 3.1 Overview of studies included in this thesis

Chapter	Study name	Before / after introduction PCV-13 (data collection period)	Study participants	Longitudinal / cross-sectional / modelling study	Data collection	Aims of study
					studies	observed in pneumococcal carriage in unvaccinated individuals. • To investigate what the effect would be of different vaccination strategies on pneumococcal carriage in vaccinated and unvaccinated individuals.

The population growth is the result of a net difference between the annual birth rate (37.3/1000 in 2010) and death rate (6.1/1000 in 2010). Population dynamics are further influenced by the in-migration (73.2/1000 per year) and out-migration rates (76.7/1000 per year). Life expectancy at birth was 69.4 years in 2010. Infant and under-five mortality rates were 35.0 and 59.1 per 1000 live births in 2010, respectively (54).

The population pyramid of the KHDSS area shows a highly triangular shape, reflecting the high childhood mortality, mortality in adults as a result of the HIV epidemic and out-migration of young adults from this rural area (Fig 3.1) (54).

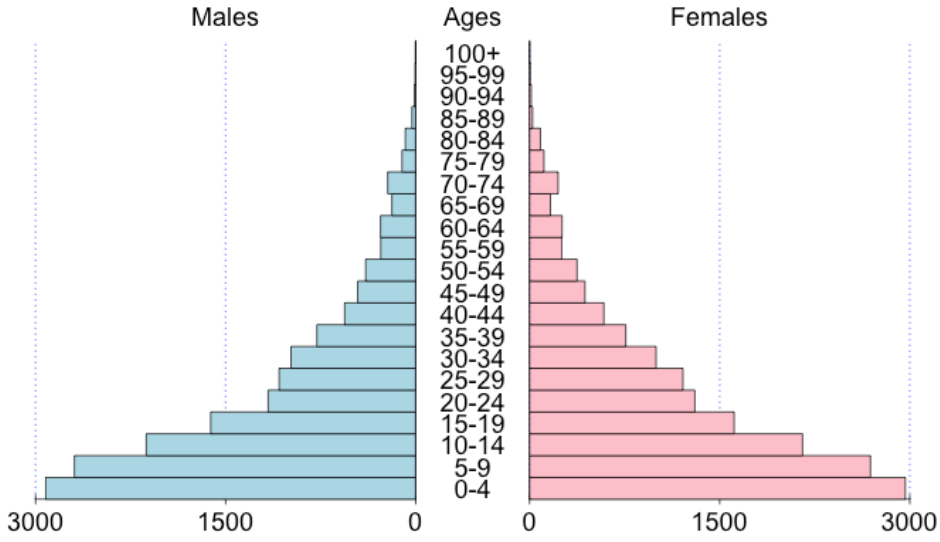


Figure 3.1 Population pyramid for Karonga Health and Demographic Surveillance System
Obtained from census data from 01/01/2010.

HIV prevalence in the area was estimated at 7.1% in men and 9.2% in women in 2008/2009 (56). HIV-prevalence in women of childbearing age ranged between 3% in women 15-24 years and 16% in women 30-39 years (56). ART has been available in the clinic located within the study site since 2006.

The Karonga Health and Demographic Surveillance System (KHDSS) site is bordered by the hills of the Nyika national park in the west and by Lake Malawi in the east (Fig 3.2). The surveillance area includes one rural hospital, five health centres and 23 outreach vaccination clinics. The area has three distinct seasons: rainy from December to April, cool and dry from May to August and hot and dry from September to

November. Main sources of income in the KHDSS area are subsistence farming, fishing and small scale trading.

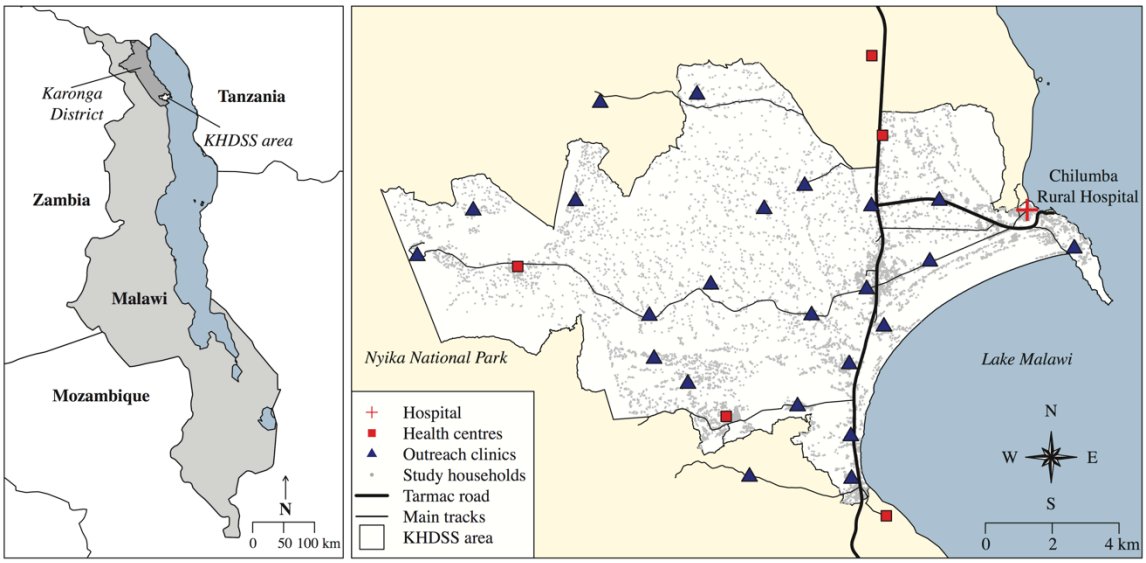


Figure 3.2 Map of the Karonga Health and Demographic Surveillance System

3.3 Recruitment and data collection

Recruitment for the studies took place at local hospitals. For the infant studies, recruitment took place during antenatal clinics or on the postnatal ward. HIV-positive adults were recruited from ART clinics. See the methods sections in individual chapters for more details on recruitment criteria.

Pneumococcal carriage was detected using nasopharyngeal samples. Trained nurses and field workers collected samples at the household for the infant studies, or at the ART clinic for the studies on HIV-positive adults.

Data on household characteristics, socio-demographics and vaccination status were retrieved from the KHDSS records. Data were collected during the KHDSS annual census at which all households are visited and individual and household socio-demographic data are collected by trained interviewers. Vaccine status and date of vaccination were transcribed from parent-held booklets (“health passports”) issued free by the government to all children at birth or first clinic visit. Absent vaccine documentation, parent/guardian reported vaccination status was recorded.

3.4 Laboratory procedures

3.4.1 Description of sample collection, growth and serotyping procedures

Nasopharyngeal samples were collected and analysed according to standard procedures (57). A pernasal swab (Medical Wire & Equipment, Corsham, UK) was inserted into the posterior nasopharynx. In 2008-2011 a calcium alginate swab was used, in 2014 a flocked swab was used. The swab was transported in skim milk-tryptone-glucose-glycerol medium. Inoculated vials were stored at -20^{o}C within 6 hours of collection, and were frozen at -80^{o}C until tested. Samples were cultured on gentamicin (5 µg/mL) sheep blood agar plates and incubated overnight at 37^{o}C with 5% carbon dioxide. Pneumococci were identified by morphology and sensitivity for optochin.

In studies conducted before introduction of PCV-13, one colony was isolated and cultured in Todd-Hewitt broth. Pneumococci were serogrouped using Latex agglutination and serotyped by Quellung reaction using standard antisera (Statens Serum Institute, Copenhagen, Denmark). Reagents were available to type 48 of the potentially 92 serotypes, including the PCV-13 vaccine (VT) serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F).

Samples collected after introduction of PCV-13 were analysed using two different methods: 1) LytA and serotyping polymerase chain reactions (PCR), and 2) Latex agglutination.

For the PCR methods, a sweep of pneumococcal growth was taken of the positive primary culture plates using an inoculation loop and placed in 500µl nuclease free water. The bacterial suspension was boiled for 10 minutes at 98^{o}C and 5µl of the supernatant containing bacterial genomic DNA was used in subsequent real-time polymerase chain reactions (qPCR). Pneumococcal DNA was detected using a qPCR assay for the autolysin-A-encoding gene (LytA). Cycle threshold (Ct) values, defined as the number of cycles required for the fluorescent signal to cross the threshold and exceed the background fluorescence level, were used to evaluate density of pneumococcal carriage. Isolates were serotyped using seven sequential triplex qPCR reactions, designed to detect 37 serotypes, including the PCV-13 serotypes. Samples were considered positive for a specific serotype if the serotype-specific Ct-value was less than 40. The PCR method did not allow a distinction between VT and NVT

serotypes for serogroups 9A/V, 7A/F, and 18A/B/C/F. For these serotypes, additional latex agglutination was performed to make the distinction.

For Latex agglutination, a single colony was selected from the primary plate and a secondary plate was grown to obtain pure growth. Both the primary and secondary plates were tested for optochin sensitivity. In case of an indeterminate or non-susceptible result, a bile solubility test was used for confirmation. Latex agglutination was performed on pure pneumococcal growth with use of a 13-valent Latex kit (Staten Serum Institut, Copenhagen, Denmark) (58).

The advantage of PCR methodology is that it allows for detection of multiple serotypes per sample and gives an indication of the abundance of specific serotypes in the sample. The disadvantage of PCR methodology is that it was a newly installed method at the Malawi Liverpool Wellcome Trust Clinical Research Programme Laboratories where analysis took place. At time of the study the comparability between serotyping of PCR and Quellung/Latex had yet to be evaluated (see section 3.4.3). The advantage of serotyping by Latex agglutination is that it is comparable to the Quellung reaction whilst being less labour intensive (58). The disadvantage of Latex agglutination is that is has the same drawbacks as Quellung serotyping: in practice only one serotype can be detected per sample and there is no quantification of the abundance of the serotype. Another disadvantage of Latex agglutination with the Statens Serum Institut 13-valent latex kit is that only the PCV-13 serotypes and a few non-vaccine serotypes (NVT) can be determined. Conventional Quellung would have to be used to differentiate the NVT serotypes. The majority of NVT serotypes were reported as “NVT” with no further discrimination available.

3.4.2 Repeated measures of growth data and evaluation of LytA PCR

Samples collected after introduction of PCV-13 were assessed for pneumococcal presence up to five times: 1. Culture for LytA and serotyping PCR, 2. LytA PCR to confirm pneumococcal growth, 3. Culture for Latex serotyping to confirm PCR ambiguous VT/NVT results, 4. Culture for Latex serotyping, 5. Culture for Quality control (QC) testing (Figure 3.3). Optochin sensitivity testing was not systematically done when culture was performed for LytA and serotyping PCR (Step 1). Optochin sensitivity testing was performed on all samples when culture was performed for Latex serotyping (Step 4).

Of 1337 samples, 584 (43.7%) tested positive on culture (Step 1): 554 (95%) of those were tested positive on LytA PCR, 24 (4%) tested negative on LytA PCR (Step 2). Of

584 samples tested positive on culture (Step 1), 450 (85%) tested positive and 102 (18%) tested negative when the culture-step was repeated for Latex-serotyping (Step 4). Quality control testing (Step 5) was done for 37 culture-positive samples and 20 culture-negative samples: all 37 (100%) positive samples were found to be culture-positive on repeat testing; 12 out of 20 (60%) negative samples were found to be culture-negative on repeat testing.

Pneumococcal growth on culture was found to have inconsistent results on repeat testing. Only 85% of samples initially identified with pneumococcal growth and positive on LytA PCR were found to have pneumococcal growth on re-culturing for the Latex analysis. It is possible that these results are a result of the different use of optochin susceptibility testing in both steps. Optochin susceptibility testing was not performed on all samples initially: it is likely that samples containing alpha-haemolytic streptococci other than *Streptococcus pneumoniae* were incorrectly assigned to be culture-positive. Although LytA PCR should have only identified *Streptococcus pneumoniae,* there are concerns over the specificity of LytA PCR and the possibility that it may not discriminate between *Streptococcus pneumoniae* and viridans streptococci, resulting in false positive results (59, 60). LytA PCR has high sensitivity and specificity for the detection of *Streptococcus pneumoniae* from clinical isolates (61), but specificity is lower comparing the detection of pneumococcal carriage with culture: 76% in an experimental human pneumococcal carriage trial conducted on adults in the UK (59), and 46%, 46% and 19% in samples from children <5 years, HIV-positive and HIV-negative adults in Kenya (60). Apart from the concern of cross-reactivity with other streptococci, lower specificity can be the result of the inability of PCR to distinguish between live and dead bacteria, and the ability of PCR to detect low-density culture which can be missed on culture (59). It is likely that at least part of our negative culture results can be explained by the different use of optochin and suboptimal specificity of LytA PCR, but we cannot rule out that the pneumococci collected in our study had lost viability on repeat testing. Samples containing *Streptococcus pneumoniae* from throat swabs collected from children in Tanzania were found to be viable after four freeze-thaw cycles when stored in STGG at -70C (62), providing reassurance for the viability of our samples which were stored under the same conditions (62).

3.4.3 Comparison between PCR and Latex agglutination serotyping methods

We compared the serotyping results of the PCR and Latex agglutination methods. We compared the Latex serotyping results to all PCR results (detection of multiple serotypes), and to the most abundant serotype detected on PCR. The most abundant

serotype was defined as the serotype with the lowest CT-value. However, if the CT-values of the LytA PCR (detecting total pneumococcal DNA) and the most abundant serotype PCR differed by 9.9 or more (equivalent to a 1000x concentration difference), we hypothesised that there was another serotype present that was undetectable on the serotyping PCR. We summarize our analyses for the most abundant serotype on PCR before and after adjustment of this difference in CT-value.

In total, 473 samples were serotyped using PCR and Latex agglutination methods. Tables 3.2 shows the sensitivity and specificity of PCR serotyping to identify VT serotypes: using all PCR results (A), only the most abundant PCR results (B) or using only the most abundant PCR results and adjusting for a difference in PCR and serotyping CT-value (C). In this analysis, Latex agglutination is assumed to be the gold standard for serotyping.

Sensitivity to identify a VT serotype was 66% when including all PCR results, 62% when only including the most abundant PCR result and 50% when adjusting the PCR result for a difference in CT-value. The specificity of PCR to correctly identify an NVT serotype as most abundant was 87%, or 96% after adjustment for CT value.

Table 3.3 shows the results of table 3.2b by serotype. Amongst the 64 samples that were identified as having a most abundant VT serotype on PCR and Latex, the same serotype was identified on both methods for 60 samples (94%). Serotype-specific sensitivity was particularly low for serotypes 19F (6/21, 29%), 23F (2/7, 29%) and 19A (6/13, 46%). Of seven samples identified as 9V on Latex serotyping, 6 were identified as “9VA” on PCR, but only one was subsequently identified as 9V when the factortype was confirmed by Latex.

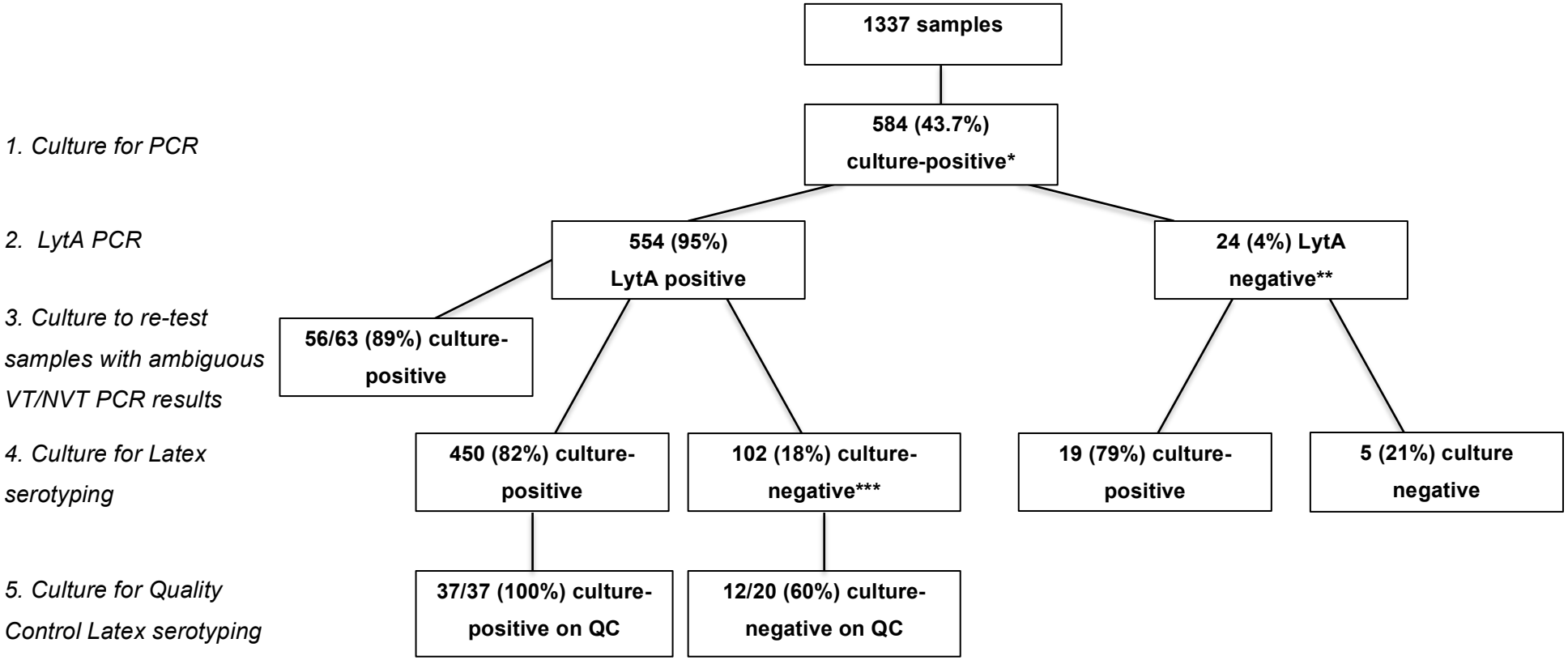


Figure 3.3 Flowchart with culture and LytA PCR results performed for the post-vaccination studies

* Optochin testing not systematically done for first culture tests

** LytA not done for 6 samples

*** Culture not done for 2 samples

Table 3.2a Comparison between PCR and Latex serotyping methods including all PCR results

		Latex		
		+ (VT)	- (NVT)	Total
PCR	+ (VT)	68	79	147
	- (No VT)	35	291	326
	Total	103	370	473

Sensitivity: 68/103 = 66%

Not possible to calculate specificity from this table: NVT on Latex and VT result on PCR are not necessary inconsistent results as PCR result could be a lesser abundant serotype.

Table 3.2b Comparison between PCR and Latex serotyping methods including only most abundant PCR result (not corrected for CT value difference)

		Latex		
		+ (VT)	- (NVT)	Total
PCR (most abundant result)	+ (VT)	64	48	112
	- (NVT)	39	322	361
	Total	103	370	473

Sensitivity: 64/103 = 62%

Specificity: 322/370 = 87%

Table 3.2c Comparison between PCR and Latex serotyping methods including the only most abundant PCR result (adjusted for CT value difference)

		Latex		
		+ (VT)	- (NVT)	Total
PCR (most abundant result)	+ (VT)	51	16	67
	- (NVT)	52	354	406
	Total	103	370	473

Sensitivity: 51/103 = 50%

Specificity: 354/370 = 96%

Table 3.3 Comparison of serotypes detected by Latex / PCR serotyping methods

		Latex													
		1	3	4	5	6A	6B	7F	9V	14	18C	19A	19F	23F	NVT
PCR	1	0													
	3		6												3
	4			0									1		
	5				1										3
	6AB					12	19					2			16
	7F							0							
	9V								1						
	14									5				1	12
	18C										2				1
	19A											6			8
	19F												6		5
	23F													2	
	NVT					5	4		6*	1		5	14	4	322

* 5/6 samples were identified as 9VA on PCR, but latex confirmed as 9A.

3.4.4 Quality Control of PCR and Latex agglutination serotyping methods

Quality control (QC) testing was performed by repeat testing of samples using the same serotyping method. Lab technicians performing the repeat testing were blinded to the original test results. QC testing of PCR was performed on 40 samples with pneumococcal growth. PCR results on repeat testing were the same for 26 of 40 samples (65%). QC testing of Latex agglutination serotyping was performed on 34 samples with pneumococcal growth. Concordance for VT/NVT results was 85% (29/34 concordantly identified as VT/NVT). Four out of five discordant VT/NVT results were a result of a difference in factor type (e.g. 19F vs. 19B/19C). Of 10 samples with VT serotypes on both the original testing and the QC, 9 had identical serotypes on both occasions (90%), 1 sample (10%) had a different factor type on QC (6A vs. 6B). Of 19 samples with NVT serotypes on both the original testing and the QC, 16 had identical serotyping results (84%), 1 had different serotype results, and two were non-typeable "NVT" on the original test, but had a typeable NVT serotype on QC.

3.4.5 Conclusion on pneumococcal serotyping methods

The sensitivity of the PCR serotyping method to identify a VT pneumococcus was found to be too low (66% when including all PCR results) to allow for an accurate comparison of the post-vaccination data to the pre-vaccination data. Also QC testing provided better results for Latex serotyping than for PCR serotyping. Validation of the PCR method is ongoing at the Malawi Liverpool Wellcome Trust Clinical Research Programme Laboratories, but optimisation had not yet been completed at the time of this PhD thesis. Based on the results presented in this section, we decided to use the culture results where optochin testing was done consistently (Step 4 in Fig 3.3) and the Latex serotyping results to report findings of the post-vaccination data (Chapter 8).

3.5 Statistical analysis

Statistical analyses were performed using R 3.0.1 (R Foundation for Statistical Computing, Vienna) and Stata 12.1 (Statacorp, Texas). Comparisons between categorical data, including proportions of pneumococcal carriage, were made using the Pearson's Chi-square test and Fisher exact test as appropriate. Log-binomial regression or Poisson regression with robust standard errors was used to report rate ratios (RR) for risk factors of pneumococcal carriage (Chapters 4,5,7) and vaccine uptake (Chapter 6). Cox proportional hazard regression was used to examine time to first carriage in infants (Chapter 4) and vaccine timeliness (Chapter 6). Multivariable

models included initially only covariates achieving *p*-value <0.2 in univariable analysis. We retained in the final multivariable model those covariates achieving a likelihood ratio test *p*-value <0.05. Mixed-effect models (Chapter 4) and generalized estimated equations models (Chapter 5) were used to study within-person clustering of the data.

Seasonality was studied in the carriage studies conducted prior to vaccine introduction (Chapter 4, 5). For seasonality, parametric functions with different numbers of sin-cosine waves were examined. For instance, a model with two sin-cosine waves was specified as:

$$y_{i,t} = \alpha + \beta_1 \sin\left(\frac{2\pi t}{365}\right) + \beta_2 \cos\left(\frac{2\pi t}{365}\right) + \beta_3 \sin\left(\frac{4\pi t}{365}\right) + \beta_4 \cos\left(\frac{4\pi t}{365}\right) + \beta_5 \, covariates_{i,t}$$

where $y_{i,t}$ is the carriage in individual i on day of the year t, β_{1-4} terms are regression coefficients for each sine and cosine function, and $covariates$ are other factors studied.

3.6 Ethics

Informed written consent was obtained from participants of the studies. In the infant carriage studies, informed written consent was obtained from mothers and/or heads of participating households. Ethical approval was granted by the National Health Sciences Research Committee in Malawi and the London School of Hygiene and Tropical Medicine or University of Liverpool ethics committee.

4. Transmission of *Streptococcus pneumoniae* to and from children under five years of age: a systematic literature review

4.1 Introduction

Worldwide surveillance on the spread and serotype distribution of pneumococci is required to study the impact of vaccination and the extent of serotype replacement. Understanding the drivers of pneumococcal transmission in different settings is vital to understand and predict VT and NVT incidence and herd protection and thus to inform vaccination policies. Given the impressive impact of PCVs on disease burden in developed world settings, there is expectation that this will also manifest in developing country settings. If pneumococcal transmission is similar then there will be every possibility of the successes seen in the US and Europe. But if transmission varies under different demographic circumstances then the extent of herd protection may be lower than observed in western societies. Understanding differences in transmission dynamics will allow consideration of alternate vaccine schedules and uses to deliver herd protection.

This chapter reviews the available data on pneumococcal transmission in developed and developing countries. This literature research focuses on transmission of pneumococci to and from children under five years of age, the age group targeted for routine vaccination, and where the largest burden of IPD and otitis media can be found. Understanding transmission to and from this age group will be essential to understand the success of PCV-13 introduction in Malawi. Besides reviewing literature on pneumococcal transmission dynamics, the different study designs and definitions used

to study pneumococcal transmission will be reviewed, with the aim of informing the study design and analysis for our setting in rural Malawi.

4.2 Research questions

Research questions related to pneumococcal transmission

1. What are the pneumococcal transmission dynamics to and from children under five?
   - 1.1 What is the evidence for pneumococcal transmission to and from children under five within the household?
   - 1.2 What is the evidence for pneumococcal transmission to and from children under five outside the household?
   - 1.3 Is there any evidence that pneumococcal transmission dynamics differ by geographical and cultural setting?
   - 1.4 Is there any evidence that pneumococcal transmission dynamics differ by serotype?
   - 1.5 What is the evidence for altered pneumococcal transmission dynamics post introduction of pneumococcal conjugate vaccination?

Research questions related to study designs

2. Which study designs are used to study pneumococcal transmission dynamics?
   - 2.1 What are the strengths and weaknesses of each study design?
   - 2.2 In which settings has pneumococcal transmission to and from children under five years of age been studied?
   - 2.3 What definitions are used to extrapolate pneumococcal transmission from epidemiological data?

4.3 Methodology

A systematic search of English literature published up to February 2015 was performed on the following databases: Scopus, Medline (PubMed), Web of Knowledge, World Health Organization Library (WHOLIS). We searched the databases on the following keywords in any field of the articles: (pneumococc* OR "strep* pneumo*" OR "s. pneumo*") AND (child* OR infant* OR preschool OR newborn* OR "under five*" OR family OR families OR household* OR "day care" OR mother* OR parent*) AND (transmission OR cluster* OR transmit* OR spread* OR longitudinal OR "cross-sectional") AND (epidemiology OR "carrier state" OR "time factors" OR model*). To account for unpublished research, we searched the abstract books of the 2012 and 2014 ‘International Symposium on Pneumococci and Pneumococcal Diseases’.

Firstly, abstracts were screened and excluded from further assessment if they only included results from non-human samples or laboratory studies, or if they did not include any results on *Streptococcus pneumoniae.* Secondly, articles were assessed for eligibility: articles were included if they discussed human-to-human transmission of *Streptococcus pneumoniae*. Outbreak investigations, cross-sectional studies, case-control studies, longitudinal studies and modelling papers were included. A study was defined as longitudinal if there were at least two samples collected from the same study population with a sample interval of less than three months. Studies were included only if they reported *Streptococcus pneumoniae* culture results. Modelling studies were included only if they studied person-to-person transmission specifically. Outbreak investigations were included if they reported on contact tracing amongst asymptomatic contacts of the index case(s).

4.4 Literature search results

A total number of 2948 records were retrieved through database searches (figure 1). Searches through unpublished sources yielded 34 conference abstracts. After duplicates were removed, 1081 abstracts were screened. We assessed 250 records for eligibility. We excluded 145 records, mostly because results on transmission were not reported (n=113 for cross-sectional and longitudinal studies, n=12 for mathematical models). One conference abstract was excluded because the study used the same results as reported in chapter 5 of this PhD thesis. Finally, we included 103 records, from 34 cross-sectional studies (22, 63-95), 20 outbreak reports (96-115), 1 case-control study (116), 30 longitudinal studies (7, 8, 117-144) and 18 mathematical modelling papers (145-162).

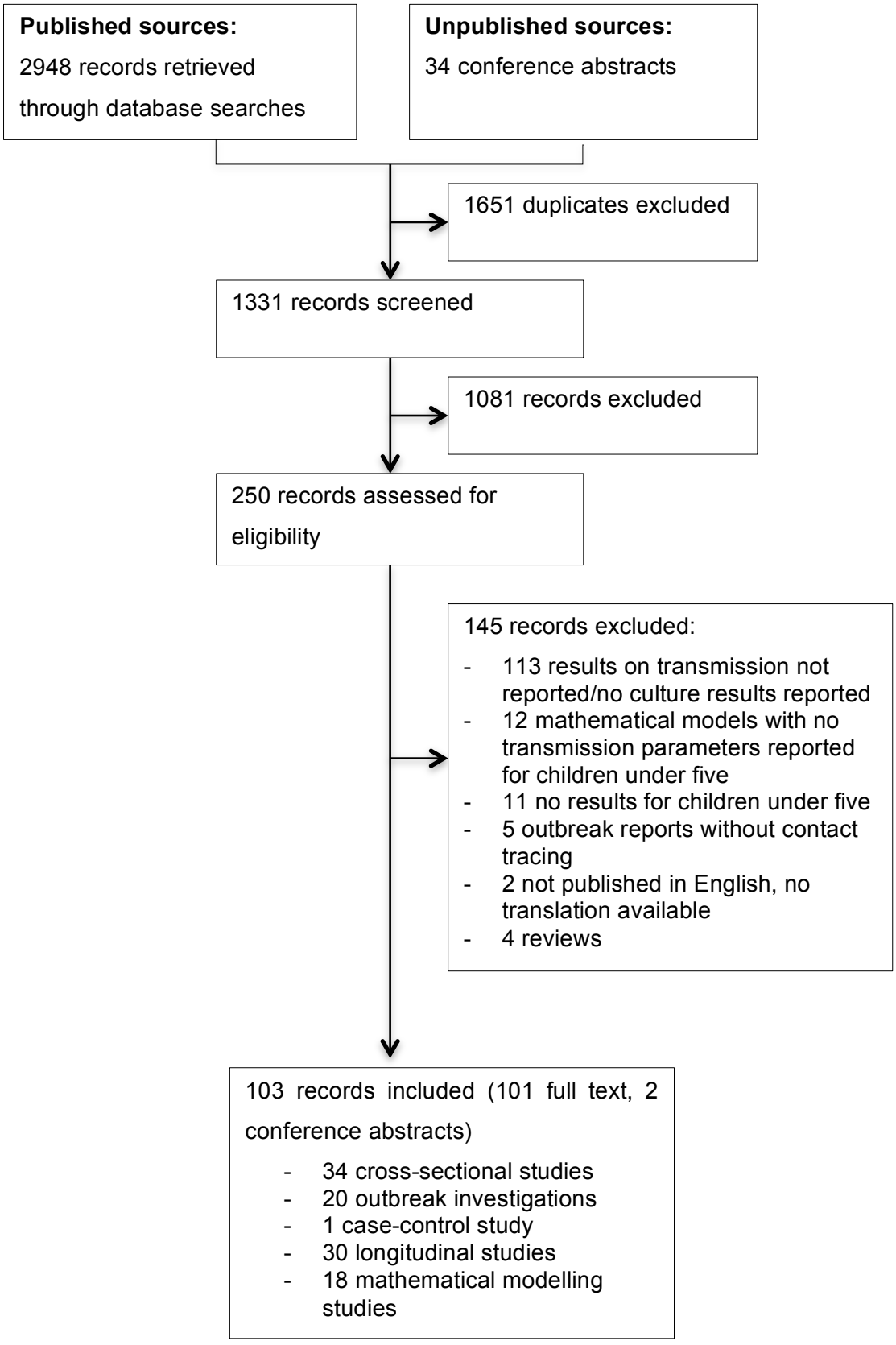


Figure 4.1 Search results and selection of included studies

4.5 Cross-sectional studies and outbreak investigations

Settings

In total, 54 studies were included: 34 cross-sectional studies and 20 outbreak investigations. The cross-sectional studies investigated pneumococcal transmission in day care centres (DCCs) (n=17) (63-67, 69, 71, 73, 75-77, 87, 88, 91-94), households (n=10) (22, 68, 72, 74, 78, 82-84, 86, 89), ethnic communities (n=5) (70, 79, 81, 85, 90), a post-tsunami camp (n=1) (95), or various settings (n=1) (80). In seventeen out of the twenty outbreak investigations, contacts at the DCC or school of the index children were screened (97-113). Two investigations involved screening of mothers after diagnosis of IPD in neonates (96, 114). The last outbreak investigation combined an outbreak report of serotype 12F with information from carriage surveillance studies in the same area (115).

More than half of the studies were based in the USA or Canada (n=9) (22, 65, 73, 89, 97-99, 112, 115) or in European countries (n=23, of which 8 from Sweden) (63, 64, 69, 71, 75, 77, 80, 87, 88, 91-94, 100-104, 108-111, 114). Seven studies took places in Middle-Eastern countries (66, 76, 79, 81-83, 113), four in Asia (67, 72, 95, 107), four in Africa (68, 74, 86, 105), four in South-America (84, 85, 96, 106) and three in Australia and Papua New Guinea (70, 78, 90).

Study design

Many cross-sectional studies had a large sample size, resulting in a median sample size of 380 children under five (range 29-8330[1]). Individual outbreak investigations involved contact tracing in up to 372 contacts (median 70, range 6-372).

Twenty-six (76%) cross-sectional studies used molecular typing methods to determine clusters of pneumococci, with pulsed-field gel electrophoresis (PFGE) (n=25) used by most studies (64-67, 69, 72, 74-77, 79-91, 94, 95). Other methods included multilocus sequence typing (MLST) (n=7), (63, 75, 79, 81, 84, 90, 94), polymerase chain reaction (PCR) (n=4) (72, 79, 81, 84, 90), restriction fragment end labeling (RFEL) (n=2) (64, 85) and hybridization methods (n=2) (88, 89). Four studies did not use molecular typing methods, but used antibiotic resistance patterns to infer pneumococcal clustering (71, 73, 92, 93). Molecular typing methods were also used in eleven (55%) of the twenty

[1] Maximum estimate (8330) combines multiple studies reported in one publication that were not reported separately. One study with 12,793 hospital samples excluded.

outbreak investigations (96, 99, 103-108, 111, 113, 115). Six studies did not use typing methods, but inferred pneumococcal clustering from antibiotic resistance patterns (98, 100, 101, 109, 110, 112).

Findings – cross-sectional studies in DCCs

There were sixteen cross-sectional studies conducted in DCCs (Table 4.1). Carriage prevalence differed widely between the different studies conducted in different geographical settings (range 5%-78%). Thirteen (81%) studies reported pneumococcal transmission within DCCs (63-67, 69, 73, 87, 88, 91-94). An average cluster size of approximately 3.5 (range 2-13) individuals was reported. The remaining three studies reported no or very little evidence of within DCC transmission (76, 77, 163).

Different conclusions were drawn on whether spread between DCCs and/or within the community is likely to have occurred. Eight studies report that different DCCs had different PFGE profiles (65, 69, 88, 91, 94), or that specific serotypes or antibiotic resistance profiles tended to be overrepresented in certain DCCs (66, 71, 93), supporting the hypothesis that each DCC is an “autonomous epidemiological unit” (88). Six studies reported that pneumococci with identical PFGE clones or antibiotic resistance profiles were found in several DCCs (73, 75, 87, 91-93). Two studies identified isolates obtained from DCCs to be representative of internationally recognized strains (63, 87).

Table 4.1 Cross-sectional studies on pneumococcal transmission in DCCs

Author, year (ref)	Country	Study population / samples	Molecular method*	Key findings
Almeida, 2013 (63)	Portugal	Retrospective study on carriage samples of serotypes 1 (n=21), 5 (n=7), 7(n=42) in DCCs	MLST	Although serotypes 1, 5 and 7 were detected sporadically, there was evidence of cross-transmission in some DCCs. Isolates were associated with international clones.
Bogaert, 2001 (64)	The Netherlands	Isolates from 244 children in DCC and 61 not in DCC	RFEL	Higher risk for horizontal spread of pneumococci in DCCs than in the general population
Boken, 1995 (65)	USA	Carriage in 32/54 (59%) children in one DCC	PFGE	Single predominant strain in each of the five DCC rooms
Borer, 2001 (66)	Israel	Carriage in 29/50 (58%) children in six DCCs	PFGE	Pneumococci more frequently acquired from DCC attendees than from family members.
Chen, 2007 (67)	Taiwan	Carriage in 25/94 (27%) children in 6 classes at one DCC	PFGE	Evidence for intraclass transmission
Gudnason, 2014 (71)	Iceland	Before/after study on hygiene intervention in 2399 children in 15 intervention and 15 control DCCs	-	Hygiene intervention associated with reduced risk of carriage. Pneumococcal carriage differed significantly between DCCs
de Lencastre, 1999 (69)	Portugal	277/586 (47%) children in 7 DCCs	PFGE	Each DCC had a unique microbiological profile
Kellner, 1999 (73)	Canada	586/1322 (44%) children in 39 DCCs	-	Overall prevalence of antibiotic resistance and specific serotypes was not significantly different between DCCs
Nunes, 2008 (75)	Portugal	1973/2888 (68%) children attending DCCs	PFGE, MLST	Cross-transmission of serotype 1 in one DCC, but same clone also in four other DCCs.
Percin, 2011 (76)	Turkey	11/212 (5%) children in 3 DCCs	PFGE	No evidence for spread in DCC
Petrosillo, 2002 (77)	Italy	91/610 (15%) children in 19 DCCs	PFGE	Clonal relationship only in three pairs of strains: one within one DCC, two in different DCCs
Sa-Leao, 2000 (87) (88)	Portugal	1096/2111 (52%) children in 16 DCCs	PFGE	45 of 57 PFGE types each in a single DCC. 8 PFGE types in several DCCs, and in international samples

Table 4.1 Cross-sectional studies on pneumococcal transmission in DCCs

Author, year (ref)	Country	Study population / samples	Molecular method*	Key findings
Souli, 2007 (91)	Greece	461/1451 (32%) children in 49 DCCs: 74 samples PFGE	PFGE	Extensive spread within and amongst DCCs.
Syrogiannopoulos, 1997 (92)	Greece	132/338 (39%) children in 7 DCCs	-	Strains with common serotypes, resistance patterns, and penicillin MICs were noted in up to 4 DCCs
Tsolia, 1999 (93)	Greece	136/382 (36.5%) in 8 DCCs	-	Strains of the same serogroup/serotype and identical susceptibility patterns appeared to cluster in DCCs
Vestrheim, 2008 (94)	Norway	449/573 children (78.4%) in 29 DDCs	MLST	Clonal distribution of pneumococci was unique for each DCC

* MLST: multi-locus sequence typing, PFGE: pulsed-field gel electrophoresis, RFEL: restriction fragment end labelling,

Findings – cross-sectional studies in households

No consistent evidence was found for the importance of within-household transmission in ten cross-sectional studies (Table 4.2). In a study performed in Japan, sixteen out of 29 children with IPD and their parents carried identical pneumococcal strains, leading to the conclusion that a high rate of transmission occurred between children and their parents (72). In Papua New Guinea, 12 out of 61 mother/infant pairs carried the same serotype (78). No evidence for simultaneous carriage of the parent being a risk factor for pneumococcal acquisition was found in a study in the Gaza strip aOR = 1.31 (95%CI 0.59–2.94), whereas a positive association was found for DCC attendance aOR = 3.72 (95% CI (0.98–14.17), leading to the conclusion that spread in DCCs is more important than intrafamilial spread (82). The same conclusion was drawn from another study in Israel by the same authors: of 151 child/parent pairs, only one carried an identical strain. Having siblings <6 years was found to be positively associated with carriage of pneumococci (any strain) (OR, 2.3; 95% CI, 0.95–5.6; p=0.06), but only four out of 32 sibling pairs (12.5%) carried identical strains. A highly positive association was found for DCC attendance (OR, 4.7, 95% CI, 2.5–8.6; *P* <0.001) (83). There is one other study which reported one child/parent pair and one sibling pair with identical pneumococcal strains, but no conclusions can be drawn from this result since no denominator was reported (74).

Evidence for intra-sibling spread was found in a study performed in Utah, USA, both for any (aOR 3.3, 95%CI 2.2–5.0) and resistant (aOR 7.5, 95%OR 2.7–20.7) pneumococcal carriage. The results for resistant pneumococci were confirmed by molecular analysis: in all eight sibling pairs, identical strains were found (89). DCC attendance was found to be positively associated with any pneumococcal carriage (aOR 2.4, 95%CI 1.6-5.0), but not with carriage of resistant strains 0.5 (0.1–2.1) (89), possibly a result of a small number of children attending DCC in this community.

If spread to younger siblings is important, an indirect effect of vaccination would be expected to occur: pneumococcal carriage of VT would be expected to drop not only in PCV-vaccinated children, but also in their younger unvaccinated siblings as a result of reduced spread (herd effect). This is exactly what was observed in a randomized-controlled trail in the USA: VT carriage was lower amongst non-vaccinated siblings in PCV-7 randomized households (OR, 0.57; 95% CI, 0.26–0.98) (22). However, no

differences in VT carriage were seen amongst non-vaccinated siblings in PCV-7 randomized households in The Gambia. This is likely to have been a result of the observed high transmission and widespread carriage across age groups, and suggests that indirect effects as seen in the USA may not be as profound in settings with different transmission dynamics (68).

Simultaneous pneumococcal carriage of children under five and older children (5-17yrs) or adult household members was found in 11.9% and 31.6% in a slum community in Brazil. No positive association for household carriage was found when the results of all household members were combined (OR 1.2, 95%CI 0.5-3.1). DCC attendance was not associated with carriage (OR 1.3, 95%CI 0.3-5.3), probably due to low attendance in this community. School attendance was found to be associated with carriage (aOR 2.7, 95%CI 1.2-6.0).

Also no convincing evidence for the importance of the size of household was found. One study found that the risk of pneumococcal carriage did increase with the number of members in the household (aOR 1.08 per member (95%CI 1.01–1.14) (82), whereas another study did not find an association with the size of household (84).

Only one study looked at the association between pneumococcal carriage and HIV-exposure by the mother in 207 mother-infant pairs. No differences in colonization rates were found between HIV-positive, uninfected but HIV-exposed and HIV-unexposed children when the children were asymptomatic. A significant different in carriage rates between the HIV-positive and HIV-exposed children and the controls was found when the children had respiratory symptoms (86).

Table 4.2 Cross-sectional studies on pneumococcal transmission in households

Author, year (ref)	Country	Study population / samples	Molecular method*	Key findings
Cheung, 2009 (68)	The Gambia	2342 children (9-27 mo) participating in a randomized controlled trial PCV-9, 675 non-vaccinated younger siblings (median 3 mo)	-	No significant differences in the patterns of carriage of VT or NVT were seen among the younger siblings of the trial participants who had received PCV-9 or placebo.
Hoshino, 2002 (72)	Japan	29 children (mean 1.7 yrs) with IPD and their parents	PFGE, PCR	18 (62%) pairs with identical serotypes, suggesting high rate of transmission between children and their parents
Medina, 2005 (74)	Kenya	104 HIV-positive adults and 46 children <5yrs	PFGE	Intrafamilial spread in two families (no denominator)
Millar, 2008 (22)	USA	Randomized controlled PCV-7 trial in children <5. 241 unvaccinated siblings in PCV group, 99 unvaccinated siblings in control group	-	VT pneumococcal carriage was lower among adults and unvaccinated children living with a PCV-7 vaccinee.
Pickering, 1988 (78)	Papua New Guinea	61 mother/infant pairs and 12 older siblings	-	12 of 61 mothers carried the same serotype as their infant. From 14 sibling/infant pairs, 6 carried the same serotype.
Regev-Yochay, 2012 (82)	Gaza strip	379 children (3wks-5.5yrs) and one of their parents	-	No evidence for simultaneous carriage of parent being a risk factor for pneumococcal acquisition aOR = 1.31 (95%CI 0.59–2.94), positive association for DCC attendance aOR = 3.72 (95% CI (.98–14.17),
Regev-Yochay, 2004 (83)	Israel	404 children (3wks-6yrs, median 2 yrs), 32 siblings and 151 parents	PFGE	Only 1 child-parent pair carrying an identical strain. Only 4 (12.5%) of sibling pairs carried identical strains
Reis, 2008 (84)	Brazil	39 households, including 50 children <5 yrs	PCR, PFGE, MLST	13 (33%) households and 9 housholds (23%) had more than one member who was colonized with pneumococci of the same serotype and clonal group, respectively
Rusen, 1997 (86)	Kenya	207 children <5 yrs born to 122 HIV positive and 85 HIV negative mothers	-	No differences in colonization rates found between HIV-positive, uninfected but HIV-exposed and HIV-unexposed children when the children were asymptomatic.
Samore, 2001 (89)	USA	351 children <5 yrs in two communities	PFGE	Transmission within households suggested by risk factor analysis (aOR 3.3 (2.2–5.0) for siblings with simultaneous carriage and molecular analysis of isolates from pairs of siblings (8 identical strains found in sibling pairs).

* MLST: multi-locus sequence typing, PCR: polymerase chain reaction, PFGE: pulsed-field gel electrophoresis, RFEL: restriction fragment end labelling,

Findings – cross-sectional studies in other settings

Table 4.3 lists the included cross-sectional studies conducted in other settings. Two studies showed that when two geographical close, but distinct communities were compared, differences in serotypes (70) or clonal distributions (79, 81) could be found, possibly as a result of low contact patterns between the communities. Two other studies compared a larger number of indigenous communities in Venezuela (9 communities) (85) and Australia (3-19 communities) (90) and found that transmission occurred across the communities. The Australian study reports on widespread circulation of serotype 1, a potentially highly invasive serotype which is rarely reported in carriage from other settings (90). Some transmission was also detected in a post-tsunami camp in Sri Lanka. A maximum cluster size of 3 was reported (95).

One study in Romania compared pneumococcal strains across children living in an institution and children presenting to the hospital for pneumococcal infection or for elective surgery. An identical PFGE type was recovered from 24% of children with pneumococcal infections and 25% from children living in an institution. The same type was not recovered from healthy children admitted for elective surgery (80).

Table 4.3 Cross-sectional studies on pneumococcal transmission in other settings

Author, year (ref)	Setting, Country	Study population / samples	Molecular method*	Key findings
Gratten, 1981 (70)	Villages, Papua New Guinea	25 adults and 37 children (<15 yrs) from 9 households in 8 hamlets	-	Significant clustering of the serotypes by hamlets
Porat, 2000 (80)	Children living in an institution, Romania	574 children (1-156 mo): 193 closed community; 209 hospital-elective surgery; 84 hospital-pneumococcal infection; 88 orphanage (HIVpositive)	PFGE	Large fraction of strains recovered from children with pneumococcal infections and from those living in an institution (24% and 25%, respectively) were represented by a single PFGE type, which was absent from healthy children admitted for elective surgery
Porat, 2010 (79, 81)	Hospital, Israel	Middle ear fluid cultures obtained from 12,793 children <5 yrs (Jewish and Bedouin) with acute otitis media	PFGE, PCR, MLST	Clonal distributions between Jewish and Bedouin children were different due to the rarity of contact between them.
Rivera-Olivero, 2007 (85)	Indigenous communities in Venezuela	356 Warao children <6 yrs from 9 communities	RFEL	High degree of horizontal transfer between the Warao children
Smith-Vaughan, 2009 (90)	Indigenous communities in Australia	Various studies.	PCR, MLST	Detected serotype 1 strains circulating in carriage in indigenous children living in remote communities
Watanabe, 2007 (95)	Post-tsunami camp in Sri Lanka	324 people, including 48 children <5 yrs in 3 camps	PFGE	Potential person-to-person transmission with a maximum cluster size of 3.

* MLST: multi-locus sequence typing, PCR: polymerase chain reaction, PFGE: pulsed-field gel electrophoresis, RFEL: restriction fragment end labelling

Findings – outbreak investigations

Table 4.4 lists the outbreak investigations included in this literature review. Much of what we know about the spread of penicillin-non-susceptible pneumococci (PNSP) is due to the South Swedish Pneumococcal Intervention Project, an intervention introduced in Southern Sweden in the 1995 to control the spread of PNSP (100, 101, 103, 104, 108-111). After an increase in PNSP was noticed the early 1990s, infection and carriage with PNSP was made notifiable in Southern Sweden. Whenever a person with PNSP was notified, extensive contact tracing was performed amongst family members and close contacts. If an index case was found to attend DCC, swabs were obtained from all children and staff in the same group. PNSP carrying children were restricted from attending DCC until they were found to be PNSP-negative (109). An overview paper on 2269 PNSP cases (40% index, 60% contact) reported that the average number of PNSP-positive contact cases per index case 0-6 years old was 1.6, but that this was 3.46 within the DCCs. The number of positive contacts in DCCs ranged between 0 (24% DCCs) and 25, with a median of 2 DCC positive contacts per index child. Only 20 DCC staff at 227 screened DCCs were found positive (109). One internationally recognized stable 9V clone was identified in 26 geographically dispersed areas (108). Apparently continuous spread of the 9V clone was identified from one area to the neighbouring one, with the clone disappearing and reappearing in the same community within a couple of months (108). In contrast, various DNA types and resistance patterns were found amongst other serotypes. Most variants (73%) were found only in a single DCC, supporting the hypothesis that DCCs are unique microenvironments (111). One study compared the incidence of PNSP in an area where the South Swedish Pneumococcal Intervention Project was implemented and PNSP-carries were excluded from DCC with an area where this had not occurred. At the end of the intervention period, the incidence of PNSP was 2.9% in the intervention area, and 18.4% in the non-intervention area (RR 6.4, 95%CI 2.0-20.7). Eighty-four percent (95%CI 49-95%) of cases occurring in the non-intervention area were estimated to be attributed to the lack of intervention. Twenty-one cases were estimated to have been averted in the intervention area. Each prevented case was estimated to have demanded the exclusion of two PNSP-carriers from DCC for approximately 4 weeks each.

Within-DCC spread of the outbreak strain was also reported in all outbreaks reported in other countries were the index case(s) attended DCC (97-99, 102, 105-107, 112, 113). Reported attack rates within the DCC (including index case(s), excluding staff) were between 5% (4/75) (97) and 100% (6/6) (99) (median 21%), with a cluster size

between 4 (97), and 53 (112) (median 6). Intrafamilial spread was reported in three of six outbreaks including contract tracing of family members.

Two studies reported on cases of neonatal sepsis with evidence of vertical transmission (96, 114).

One community outbreak investigation in Alaska, USA, used information from repeated carriage studies in the outbreak area to investigate spread of serotype 12F through the population. The study suggested that outbreaks in rural Alaska occur in the following steps: (1) introduction of the organism into an immunologically naïve population, (2) widespread transmission of the organism, with the appearance of IPD cases, (3) rapid development of widespread mucosal immunity, with the elimination of carriage and transmission, and then the disappearance of IPD, and (4) regrowth, through incoming birth cohorts, of an immunologically naïve population, which sets the stage for re-initiation of the cycle with the next introduction of 12F, most likely from larger populations in which its circulation was sustained (115). In between outbreaks, carriage was found mostly in adult populations in the main city in the area, suggesting adult carriage can act as a reservoir (115).

Table 4.4 Outbreak investigations on pneumococcal transmission

Author, year (ref)	Country	Setting	Molecular method*	Key findings
South Swedish Pneumococcal Intervention Project				
Christenson, 1998 (100)	Sweden	DCCs, family	-	25 index cases of which 13 in DCCs. Same drug resistant serogroup in 12/424 DCC attendees, 0/128 DCC staff, 5 family members.
Ekdahl, 1998 (101)	Sweden	Community	-	429 index cases (all ages) with PRP, 609 contact cases (no denominators reported)
Henriqus Normak, 2003 (103)	Sweden	DCCs	PFGE, MLST	36 index cases, of which 16 in DCC. Within DCC spread recorded in 5/16 DCCs in 13/611 children.
Högberg, 2004 (104)	Sweden	DCCs	PFGE	Intervention study in 2 areas where index children carrying PNSP were (area A) or were not (area B) excluded from DCCs until negative. Incidence from 9% (26/292) to 2.9% (4/139) in area A, 15% (10/67) to 18.4% (7/38) in study area B. Same clonal type as index case found in 11/20 DCCs in area A, 3/5 DCCs in area B.
Melander 1998, (108)	Sweden	Community	PCR	22/26 geographically dispersed drug resistant 9V isolates were identical, suggesting community spread.
Melander, 1998 (110)	Sweden	DCCs	-	30 DCCs with index child: drug resistant pneumococci in 2%- 42% (median 8.9%) of DCCs attendees
Melander, 2004 (109)	Sweden	DCCs	-	Average 1.6 contact cases per index case 1-6 yrs old, 3.46 in DCCs. Number of contacts in DCC between 0 (24% DCCs) and 25 (median 2).
Nilsson, 2001 (111)	Sweden	DCCs	PCR	Internationally recognized 9V clone found very stable. Other serogroups had diverse DNA types and resistance patterns, supporting the hypothesis that DCCs have a unique microenvironment

Table 4.4 Outbreak investigations on pneumococcal transmission

Author, year (ref)	Country	Setting	Molecular method*	Key findings
Other outbreak investigations				
Cane, 2014 (96)	Argentina	Neonatal care	PFGE	Report of two cases of neonatal sepsis with genetically identical pneumococci identified from the mother's genital tract.
CDC, 1995 (97)	USA	DCC	-	Two index children (4-7mo) in DCC. Same serogroup 19 in 3/38 DCC attendees.
CDC, 2002 (98)	USA	DCC	-	Index child (11mo) in DCC. Same serogroup 14 in 10/22 DCC attendees.
Cherian, 1994 (99)	USA	DCC, family	ribotyping	Four index children (8-19mo). Identical 12F strain in 2/2 DCC attendees, 0/9 family members.
Gupta, 2008 (102)	UK	School, family	-	Five index childen (4-5 yrs) in school with serotype 1. Serotype 1 found in 1/81 contacts
Klugman, 1994 (105)	South Africa	DCC, family	MLEE	Index child (3yrs) in DCC. Identical 23F isolates in sibling, mother, 2/28 DCC attendees and their 2 siblings.
Lamaro-Cardoso, 2012 (106)	Brazil	School, DCC, family	MLST	Index child (10yrs) linked to younger brothers DCC. Identical 22F/A strain in 8/111 DCC attendees.
Lauderdale, 2005 (107)	Taiwan	Kindergarten, family	PFGE	Index child (5 yrs) in DCC. Identical 19F strain in 10/78 DCC attendees, not found in family of index case.
Reichler, 1992 (112)	USA	DCC, family	-	Index child (17mo) in DCC. Identical MDR 23F strain in 52/250 DCC attendees and 17/119 family members of DCC attendees. No carriers found in 121 children in two nearby DCCs.
Shouval, 2010 (113)	Israel	DCC	PFGE	Index child (20mo) in DCC. Identical 19A clone in 3/5 DCC attendees.
Simpson, 1995 (114)	UK	Neonatal care	-	11 neonatal cases in 11 years. Evidence of vertical transmission from 6/7 mothers sampled.

Table 4.4 Outbreak investigations on pneumococcal transmission

Author, year (ref)	Country	Setting	Molecular method*	Key findings
Zulz, 2013 (115)	USA	Community	PFGE, MLST	Outbreak of 12F in two neighbouring rural Alaska regions. Different 12F clones appear to have been introduced episodically into rural populations, spread widely in young, immunologically naïve populations and then disappeared rapidly from the population. After initial introduction in children, carriage was predominantly in adults.

* MLEE: multi-locus enzyme electrophoresis, MLST: multi-locus sequence typing, PFGE: pulsed-field gel electrophoresis

4.6 Longitudinal studies and case-control study

Settings

Thirty longitudinal studies were included (29 full text, 1 conference abstract), which studied pneumococcal carriage and transmission in DCCs (n=7) (117, 123, 136, 138, 139, 143, 144), households (n=21) (7, 8, 116, 118-122, 125-129, 131-135, 140-142), orphanages (n=1) (137), or a combination of these settings (n=2) (124, 130). Amongst the household studies, eight studies (119, 121, 122, 131, 133, 134, 141, 142) looked at mother-infant pairs specifically, of which two studies (122, 133) looked at the difference between HIV-exposed and HIV-unexposed infants. The only case-control study (116) compared serotype distributions between children with IPD and healthy age-matched controls. Family exposure was only studied for the children with IPD and included a baseline and one month follow-up visit; this study will therefore be discussed alongside the longitudinal studies for the rest of this chapter.

The included studies used data from a wide range of geographical locations. About half of the studies used data from a European country (n=8) (128-130, 135-139) or the USA (n=7) (117, 118, 120, 123, 126, 132, 140). Eight studies were conducted in African countries (8, 116, 119, 122, 127, 131, 133, 134). Other countries studied included Israel (124, 143), Bangladesh (7, 125), Papua New Guinea (121), Japan (144), Costa-Rica (142) and the Thailand-Myanmar border (141).

Study design

The study interval ranged between twice weekly and trimonthly swabs. There were eight studies with a study interval of less than four weeks (8, 120, 121, 126, 127, 134, 143, 144), 18 studies with a study interval of four weeks or more (116-119, 122, 124, 128-133, 135, 136, 138-141), and five study which combined both study intervals over the total study time (7, 8, 125, 137, 142). The total follow-up period ranged between one month and 6.5 years.

All studies collected nasopharyngeal samples. Other sample types included pharyngeal samples (120, 126) and saline nasal lavage (117). Serogroup data was available for five studies (116, 120, 126, 129, 138), serotype information was available for twenty studies (7, 8, 117, 119, 122-125, 127, 128, 130, 132-134, 136, 137, 139-141, 143). Six studies did not use serogroup or serotype data to study transmission (118, 121, 131, 135, 142, 144). Ten studies performed one or more additional microbiological

analyses on the collected samples, including PFGE (117, 118, 124, 137, 139, 144), ribotyping (123, 143), electrophoresis (117, 136), MLST (127) and PCR (143).

The study size ranged between 6 and 1358 children under five years of age, with a mean of 217 and median of 134 children. On average, studies conducted in household settings (including on mother-infant pairs) were larger than studies conducted in DCCs or orphanages, or studies combining both settings (median size 171, 81.5, 72 respectively).

All but one study (133) on mother-infant pairs recruited the infants at birth. Also four of the thirteen other household studies started at birth of the index child (7, 8, 125, 129). First samples from infants were taken at birth (119, 134), within the first week (7, 8, 121, 125), 6 weeks (122, 131), 1 month (141, 142), 2 months (129) or 4 months (133). The other household studies had an enrolment age between 4 months and 17 years. Age on enrolment was between birth and 4 years in the DCC, orphanage or mixed settings.

Two studies were conducted post-introduction of pneumococcal conjugate vaccination (132, 140), the remainder of the studies involved vaccine-naïve children.

Exposure definitions

The included studies used different methods to define pneumococcal transmission or exposure. Eight studies defined transmission on molecular typing results, mostly by PFGE (117, 118, 123, 136, 137, 139, 143, 144): they defined transmission as occurring when two or more identical strains were isolated from the same DCC or household. The remainder of studies base their conclusions on serogrouping or serotyping results. Definitions using different timings for exposure have been used. Six studies defined pneumococcal exposure as occurring when household or DCC members carry the same serotype at the same sample time (127-129, 133, 134, 142). A disadvantage of this definition is that the direction of transmission cannot be identified: it is unclear whether transmission occurred from the household member to the index child or from the index child to the household member. Also simultaneous acquisition from a third person is possible. To overcome this problem, six studies only consider previous carriage as exposure: carriage with the same serogroup/serotype in the previous 30 days (8, 130, 132), one or two months (125), 3 months (138) or 12 weeks (120). Two studies compared carriage of any serotype: in the previous month (135) or at time of delivery in a mother-infant study (121). Three studies combined both definitions, and defined exposure as carriage by a DCC or household member on the same or previous

sample time (119, 124, 141). There were three studies which considered exposure by a DCC or household member, but did not specify which time period was applied (126, 131, 140).

Findings – DCCs and orphanage

Several studies indicate that DCCs can play a major role in the spread of pneumococci (Table 4.5). In two studies involving multiple DCCs was reported that each DCC had a distinct pattern of pneumococcal serotypes (123, 130), suggesting that DCCs are "independent microenvironments" (123). On the other hand, no significant differences between DDCs could be detected in a study performed in Sweden (138).

Spread of pneumococcal serotypes in DCCs is reported in all studies, reporting colonization up to 68-76% (137, 139, 143). The most common serogroups/types reported to spread in DCCs were 6/6B (123, 137, 138, 143), 14 (123, 137, 138), 19/19F (123, 130, 137-139) and 23/23F (123, 136-138, 143). Two studies mention that it is also likely that horizontal transmission of genetic information has occurred in the studied DCCs (117, 136). Only one study reported that just one episode of child-to-child transmission was recognized and that clusters of infection could not be measured, but this could be a result of the small study size (n=6) (144).

A measure of association for carriage in index child and carriage in the DCC and/or the household on the previous time point was only reported by one study: this was estimated to be RR 5.3 (95%CI 2.9-10.0) for carriage in both household and DCC and RR 5.4 (95%CI 3.6-8.2) for carriage in DCC only (130).

Pneumococci can spread from DCCs into households, and become a risk for young siblings: when 262 children from 8 DCCs and their 46 younger sibling were studied in Israel, the younger siblings were colonized with an identical strain that was present in the older sibling's DCC in 76% vs. another DCC in 32-63% (RR 1.47, 95%CI: 1.34-1.62) (124).

Table 4.5 Longitudinal studies on pneumococcal transmission in DCCs

Author, year (ref)	Country	Population, setting	Sample interval	Exposure definition	Findings (95%CI)
Barnes, 1995 (117)	USA	92 children in 1 DCC	Monthly	Identical strain on electrophoresis, PFGE	Multi drug resistant clone 23F in 14 children over 20 months. Horizontal transmission of genetic information likely.
Givon-Lavi, 1999 (123)	USA	264 children in 8 DCCs	Monthly	Identical strain on ribotyping	Each day care center had a unique pattern of the carried pneumococci.
Givon-Lavi, 2002 (124)	Israel	262 children in 8 DCCs, 46 younger siblings	Monthly, bimonthly	Identical strain on PFGE	Identical strain from older sibling in 38%, from older sibling's DCC 76%, from other DCC 32-63%.
Leino, 2008 (130)	Finland	59 children in 3 DCCs and their family	Monthly	Same serotype, previous time point(s)	Each DCC dominated by one serotype. Relative rate 5.3 (2.9-10.0) for carriage in both family and DCC, 5.4 (3.6-8.2) for carriage in DCC only. Carriage in family only not possible to assess. Index child responsible for 66% of new introductions to family.
Pons, 1996 (136)	France	116 children in one DCC	Bimonthly	Identical strain on electrophoresis	Evidence of within DCC spread of resistant strains, no evidence for susceptible strains.
Raymond, 2000 (137)	France	71 children in one orphanage	Monthly, biweekly	Identical strain on PFGE	Clones rapidly spread in the orphanage and were then eliminated. Transmission of 4 main clones (serotype 6B, 14, 19F, 23F). Serotype 23F colonized 76% of the children.
Rosen, 1984 (138)	Sweden	405 children in 66 DCCs	Monthly	Number of children carrying a newly introduced type	Group 6 isolated in average 3.6 children in the first month after initial appearance: 2.6, 2.8, 3.2 for groups 14, 19 and 23, respectively. No significant differences between DCCs.

Table 4.5 Longitudinal studies on pneumococcal transmission in DCCs

Author, year (ref)	Country	Population, setting	Sample interval	Exposure definition	Findings (95%CI)
Sa-Leao, 2008 (139)	Portugal	47 children in one DCC	3-9 weekly	Identical strain on PFGE	21 clones detected. One 19F clone colonised 68% of children.
Yagupsky, 1998 (143)	Israel	48 children in one DCC	Biweekly	Identical strain on ribotyping/ PCR	One 23F clone colonised 11 (37%) and 13 (72%) children in the two cohorts. One 6B close in 14 (47%) children of one cohort and 2 (11%) in other cohort.
Yano, 2000 (144)	Japan	6 children (7mo-6yrs) in one DCC	Irregular	Identical strain on PFGE	One episode of child-to-child transmission recognized. Clusters of infection could not be measured. Strains changed with time in each child.

Findings – Mother-infant pairs

Eight studies (119, 121, 122, 133, 134, 141, 142) looked at pneumococcal acquisition in mother-infant pairs (Table 4.6). Two studies discussed the effect of colonisation of the mother at time of delivery. One study found that maternal carriage at delivery was a risk factor for earlier onset of pneumococcal carriage in the first four weeks of life (aHR 2.60; 95% CI 1.25-5.39; p=0.01) (121). These results were not repeated in another study which looked at earlier onset of pneumococcal carriage in the first 24 months of life (aHR 1.19, 95% CI 0.84-1.69) (141).

Three studies report that infants carried different pneumococcal serotypes than their mothers. In a study performed in The Gambia, 80.6% of infants had a discordant serotype with their mothers (134). Also at the Thailand-Myanmar border, only in 5.9% of mother-infant swabs a common serotype was identified. Concordant serotype carriage was found to be less common as infant age increased (p=0.02), which could indicate that transmission occurred more frequently from the mother in the early months of life. (141). In Costa Rica, only 2 out of 30 (7%) infants colonised at 6 months and 9 out of 37 (24%) infants colonised at 12 months had simultaneous carriage (any type) with their mother. Further microbiological and epidemiological investigations suggested that siblings were the main source of pneumococcal acquisition in the infants (142).

In a study in The Gambia, carrying a specific serotype if the mother carried this type at any previous point was associated with an OR of 9.1 (95% CI 6.4-13.6). Still, the population attributable fraction (PAF) of maternal carriage, the contribution of mother-to-child transmission to infant carriage of a specify serotype was only 9.5% (95% CI 7.4-11.6%), suggesting that reducing mother-infant transmission would have a minimal effect on infant carriage (119). There was no evidence that this effect differed by infant age. A smaller effect was found for PCV-13 serotypes (OR=5.4, 95%CI: 3.1-9.6, PAF=7.0%, 95% CI: 4.4-9.6%) than for non-PCV-13 serotypes (OR=14.1, 95%CI: 8.4-23.5, PAF=13.3, 95%CI 9.5-17.3%) (119). Simultaneous maternal pneumococcal colonization (aOR 1.54, 95%CI 1.10-2.14) was also associated with pneumococcal carriage in the child in a study conducted in South Africa, but in contrast to the Gambian study, a larger effect for PCV-13 serotypes was reported (aOR 2.36, 95%CI 1.55-3.57), with similar results for both HIV-exposed and HIV-unexposed infants (133). Weak evidence for maternal carriage at any time point being a risk factor for infant carriage at any time point was found in a study in Tanzania (131).

Two studies specifically looked at the difference in pneumococcal acquisition between infants born to HIV-positive and HIV-negative mothers in Zambia (122) and South Africa (133). One study did not find a difference the incidence of new pneumococcal acquisition; 272 new events/554 swabs (49.1%) in HIV-exposed vs. 302 new events/ 581 swabs (52.0%) in HIV-unexposed infants (133), although a difference in prevalence of pneumococcal colonization in HIV-exposed children at 4-monhts of age was found (65% in HIV-exposed vs. 52% in HIV-unexposed children, p=0.04) (133). The other study also found that HIV-exposed infants were more likely to be colonized than HIV-unexposed infants (25.3% versus 18.1%, RR: 1.4; 95% CI: 1.0–1.9, p=0.04) (122).

Table 4.6 Longitudinal studies on pneumococcal transmission in mother/infant pairs

Author, year (ref)	Country	n	Sample interval	Exposure definition	Findings
Darboe, 2010 (119)	The Gambia	196	Birth, 2,5, 12 months	Same serotype, any time points	Ever carrying serotype if mother carried: OR=9.1 (6.4-13.6) PAF = 9.5% (7.4-11.6): maternal carriage accounts for small percentage infant carriage.
Francis, 2009 (121)	Papua New Guinea	89	Weekly	Maternal carriage at delivery	Maternal pneumococcal carriage at the time of delivery risk factor for early acquisition (HR 1.97; 95%CI 1.01-3.85; p=0.046).
Gill, 2008 (122)	Zambia	260	Trimonthly	Difference HIV-exposed vs HIV-unexposed infants	HIV-exposed infants colonized more frequently than HIV-unexposed infants (RR: 1.4; 95% CI: 1.0–1.9, p=0.04)
Kinabo, 2013 (131)	Tanzania	338	3 wks, 3mo, 6mo (mother only once)	Any type, any time point	OR 2.6 (95%CI 0.9-7.5) for carriage in the infant when the mother carried any type pneumococci at any time point
Nunes, 2013 (133)	South Africa	243	4, 7, 9, 12, 16 months	Any type, simultaneous carriage	Maternal colonisation associated with pneumococcal acquisition in child (aOR 1.54). New acquisition of PCV-7 or PCV-13 serotype in mother associated with colonization in child (aOR 2.01, aOR 2.04).
Ogundare, 2012 (134)	The Gambia	36	Biweekly	Same type, simultaneous carriage	80.6% of infants had a discordant serotype with their mothers
Turner, 2012 (141)	Thailand/ Myanmar	234	Monthly	Simultaneous carriage. Mother colonized at birth.	Concordant serotype at same time in 5.9%. Less common as infant age increased (p=0.02). Mother colonized at birth associated with infant carriage: aOR 1.19 (0.84-1.69)
Vives, 1997 (142)	Costa Rica	95	1,3,6,12 months	Simultaneous carriage	2 mother-infant pairs colonised at 6 months, 9 pairs colonised at 12 month. Infants with siblings had a higher carriage than children without siblings.

Findings – Household studies

The first included longitudinal studies on household spread of pneumococci date back to the 1970s (120, 126), with Gwaltney *et al.* reporting on 25 episodes of spread from one family member in a study of fifteen families with children observed for one year (126), and Dowling *et al.* reporting age-specific risks from community and household exposure ($p<0.001$, actual risk estimates not reported) (120).

Four studies concluded that new serotypes were most frequently introduced in the household by children (127, 128, 130, 132). In 66% (61/92) of new events the pneumococcus was introduced by an index child attending DCC in Finland (130) and in 76% (81/106) of households in The Gambia carriage was first observed in a child <15 years (127). Age-specific estimates for introduction were reported in a study in the UK: 25% of pneumococci types were introduced by children under one year of age, 20% by children aged 1 year, 19% by children aged 2 years, 5.5% by 3-4 year-olds and 1% by 5-17 year-olds (128). A study conducted in the USA after routine introduction of pneumococcal conjugate vaccination concluded that the rate of new household introduction was 3–5 times higher in children <9 years as compared to adults (132).

Four studies compared the serotype distribution amongst households (127) or amongst family members (7, 116, 118). Strong evidence of non-random distribution of serotypes among households was found, suggesting that within-household transmission is important (127). Two studies conclude that the serotype distribution of the index children is most similar to their siblings < 5yrs old, suggesting sibling-to-sibling spread (7, 116). No evidence of intrafamilial spread was found in the study considering 30 cystic fibrosis patients and their families in the USA (118).

Eight studies report risk estimates for acquisition of carriage in children <5 years by family exposure status (8, 125, 127-129, 132, 135, 140) (Table 4.7). Seven studies report a positive association between pneumococcal carriage in the index child and pneumococcal carriage in other household members (8, 125, 127-129, 132, 135, 140). Age-specific results are reported for two studies, with higher estimates reported for children ≤ 6 months (OR 3.8, 95%CI: 2.1-6.9), as compared to children >6 months (OR 0.7, 95%CI: 0.3-2.1) (129), and higher estimates reported for 0-2 year-olds (RR 1.42 (95%CI: 1.26-1.61) as compared to 3-4 year-olds (RR 1.25, 95%CI: 0.95-1.64) (128).

A study performed in Bangladesh reported profound differences between family exposure to the same, or a different serotype in the previous two (biweekly or monthly) study visits: a large positive association (HR 7.97, 95% CI: 4.89-13.00) was reported

for exposure to homologous serotypes, whereas a protective effect (HR 0.66, 95%CI: 0.49-0.89) was reported for exposure to heterologous carriage in the household, indicating that competitive interaction between serotypes occurred (125).

A large study conducted in Kenya, including 1358 infants and their family members, reported that mothers and siblings were equally effective in transmitting pneumococci to the infants (hazard rate per 30-day period: 0.27, 95%CI 0.19-0.40 (mother), 0.27, 95%CI: 0.23-0.32 (sibling)). No difference was observed for the age of siblings 0-9 years (8).

Carriage by another family member at the previous visit was not associated with carriage in the index child in an multivariable analysis performed in the UK (aOR 0.89 (95% CI: 0.69–1.14). However, in their multilevel model with individual- and family-level random effects, the family-level random effect was of similar size to the individual-level random effect, indicating that variability not accounted for in the model could be explained by family transmission (135).

Table 4.7 Longitudinal studies on pneumococcal transmission in households

Author, year (ref)	Country	Child population	Sample interval	Exposure definition	Findings (95%CI)
Studies reporting measure of association for exposure of household members to acquisition in children <5 years					
Granat, 2009 (125)	Bangladesh	99 infants	Biweekly, monthly	Same type, simultaneous	Family exposure to carriage of the same serotype: HR 7.97(4.89-13.00) Family exposure to carriage of another serotype: HR 0.66 (0.49-0.89)
Hill, 2010 (127)	The Gambia	56 children <5 yrs	Biweekly	Same type, simultaneous	Serotype specific results, ranging between OR 0.7 (0.4-1.2) for serotype 23F and OR 2.7 (1.3-5.6) for serotype 19A for child carriers in family. Effect adult carriers ranging between OR 0.7 (0.3-1.4) for serotype 11 and 5.5 (0.9-33.1) for serotype 23B.
Hussain, 2005 (128)	UK	151 children <3 yrs	Monthly	Same type, simultaneous	Family exposure: RR 1.42 (1.26-1.61) for 0-2-year-olds, RR 1.25 (0.95-1.64) for 3-4 year olds
Leino, 2001 (129)	Finland	100 infants	1-6 monthly	Same group, simultaneous	Family exposure: OR 3.8 (2.1-6.9) for children ≤ 6 months, OR 0.7 (0.3-2.1) for children >6 months.
Mosser, 2014 (132)	USA	545 children <5 yrs	Monthly	Same type, previous time point	Exposure by child 2-8 years OR 3.80 (1.66-8.70) for serotype 19A, OR 6.93 (2.16-22.19) for serotype 22F. Non-significant results for exposure by children <2 years or adults
Pebody, 2009 (135)	UK	181 children <5 yrs	Monthly	Same type, previous time point(s)	Family exposure: aOR 0.89 (95% CI: 0.69–1.14)
Scott, 2012 (140)	USA	459 children <5 years	Monthly	Unclear	Exposure to a colonized child aged <9 years: aOR = 3.6 (2.90-4.51)

Table 4.7 Longitudinal studies on pneumococcal transmission in households

Author, year (ref)	Country	Child population	Sample interval	Exposure definition	Findings (95%CI)
Tigoi, 2012 (8)	Kenya	1358 newborns	Twice weekly, weekly	Same type, previous time point(s)	Family exposure: hazard rate per 30-day period: 0.26 (0.23-0.31), exposure by mother: 0.27 (0.19-0.40), father: 0.10 (0.03-0.56), siblings: 0.27 (0.23-0.32). No difference for age of siblings 0-9 years. Serotype-specific hazard rates per 30-day period, ranging between 0.04 (0.01-0.39) for serotype 3 and 0.58 (0.20-1.98) for serotype 19B.
Studies not reporting measure of association for exposure of household members to acquisition in children <5 years					
Chidekel, 2000 (118)	USA	30 CF patients 1-17 yrs	Three-monthly	Determined by PFGE	No evidence of intrafamilial spread
Dowling, 1971 (120)	USA	97 children <5 years	Biweekly	Same type, previous time point(s)	Age-specific risk of acquisition from household and community was significantly different from zero for each serotype.
Granat, 2007 (7)	Bangladesh	99 infants	Biweekly, monthly	Serotype distribution	Serotype distribution similar in <1 year and 1-4 year-old children but different from adults.
Gwaltney, 1975 (126)	USA	18 children <5 yrs, 105 children (age unspecified)	Biweekly, weekly	Same type	25 episodes of spread from one family member to another noted.

Table 4.7 Longitudinal studies on pneumococcal transmission in households

Author, year (ref)	Country	Child population	Sample interval	Exposure definition	Findings (95%CI)
LloydEvans 1996 (116)	The Gambia	81 children with IPD (age unspecified)	Within two days of IPD diagnosis, one month later	Same type	8.5% (initial), 9.4% (follow-up) of family members of index cases carried same serotype. 65.3% of carriers same type were siblings <5 yrs, 2.8% mothers.

4.7 Mathematical modelling studies

Settings

Eighteen mathematical modelling studies were included in this review (Table 4.5). Six studied the transmission of pneumococci in DCCs (145, 147, 150, 155, 159, 160), two in schools (148, 149), six in households (146, 152, 157, 158, 161, 162), three in both DCCs or schools and households (152, 154, 156), and one selected their study participants from the community (151). Intervention strategies were modelled in two studies, looking at the impact of excluding carriers of penicillin-resistant pneumococci from DCC (145, 156), reducing DCC group size (156) and reducing the proportion of the population attending DCC (156). None of the included studies modelled the impact of vaccination on transmission in households, schools or DCCs.

The models included empirical data from, or simulated data based on Scandinavian countries (n=9) (145-147, 151, 152, 154, 156, 159, 160), UK (n=2) (157, 158), USA (n=2) (155, 161), France (n=3) (148-150), South Africa (n=1) (162) and Bangladesh (n=2) (152, 153).

Methods - Model structure

Most studies adopted a “susceptible-infectious-susceptible” (SIS) structure, assuming an individual does not gain immunity from pneumococcal colonisation, and not taking into account symptomatic infection by pneumococci (146-160, 162). Only one study assumed that some individuals were immune to carriage of the study serotype (161), and only one study took infection by pneumococci into account (145). There were eight age-structured models, with different parameters for children and adults (152-154, 157, 158, 162), or age-groups within the child population (146, 151, 156). The studies that did not have an age-structure included young children only (145, 147-150, 155, 159-161).

Most models used a stochastic approach to model pneumococcal transmission, taking into account the role of chance in transmission patterns (145, 146, 148, 149, 151, 152, 154, 156-162). Deterministic models were used by three studies (147, 150, 155). Fifteen studies used an individual-based approach, keeping track of individual acquisition and clearance of pneumococci (146-149, 151-154, 156-162). Three studies used aggregate data in a compartmental model (145, 150, 155).

Thirteen models included pneumococcal serotypes in their model structure (146-150, 152-154, 158-162), including between one (161) and 33 (159) different serotypes. One study distinguished between PCV-7 and non-PCV-7 serotypes (149), the remainder of studies included the most prevalent serotypes. Five studies did not consider serotype-specific data in their models (145, 151, 155-157). Only two studies allowed for co-colonization with multiple serotypes (147, 159).

Methods - Definitions and assumptions made

All studies had to make several assumptions to simplify reality in a mathematical model. Assumptions were for instance made on the possibility of carriage or clearance events occurring within a certain order or time span: one study assumed that two carriages of different serotypes must be separated by a period of non-carriage (146) and one study assumed that maximum one acquisition per susceptible occurred during a one month period (149). Two studies using the same dataset broke the data down in small time intervals of one day, and assumed that only one household member would change status in this period (157, 158).

Assumptions about serotype-specific behaviour were made in all studies including serotype-specific results. All but two studies (147, 159) assumed that simultaneous carriage of multiple serotypes is not possible. Five studies assumed that acquisition and clearance rates are the same for all serotypes studied, for the total study population (146, 154, 159, 160), or for adults only (153). One study set parameters for within-DCC transmission as serotype-specific, but assumed the same parameter for transmission outside the DCC for all serotypes (150).

Methods - Model fitting to data

Two studies fitted models to simulated data (155, 156), the remainder of model used empirical data. Fifteen studies used data from longitudinal studies (145-154, 157, 158, 160-162), one study used cross-sectional data (159). Goodness of fit was assessed by maximum likelihood estimation (145, 150, 151, 157, 158, 161) or Bayesian Markov Chain Monte Carlo (MCMC) methods (146-149, 152-154, 160, 162). One study used approximate Bayesian computation (ABC), in which summary statistics are used to approximate the likelihood (159).

All studies involving empirical longitudinal data encounter the problem of unobserved transitions: most longitudinal studies had a monthly sample interval, implicating that the exact time of acquisition or clearance of a pneumococcal serotype could not be determined. Also, scenarios are plausible, in which a pneumococcal serotype was

acquired, and cleared again within the same month, thus never showing in the actual samples. Likewise, carried pneumococci could be cleared, and reacquired within the same month. To overcome this problem of unobserved transitions, five studies used MCMC data augmentation to impute unknown intermediate states (146, 148, 149, 152, 153).

Methods - Sensitivity analysis and validation

All mathematical models inherently include some degree of uncertainty about the data, the parameters and model structure used. To deal with uncertainty about unobserved transitions in the data, MCMC data augmentation was used, as discussed above. Sensitivity analysis, in which the effect of different values of the included parameters is studied, was used by five studies (148, 155, 156, 158, 162). Six Bayesian studies performed a predictive check using simulated data (148, 152-154, 159, 160). Different model structures were tested in five studies, looking at density-dependency (146), heterogeneity in community-acquisition rates across families (146) or schools (149), serotype-specific results (147) or different combinations of regression and association models (151).

Findings - Transmission

Transmission of pneumococci occurred in all three settings investigated in the included studies; at DCC, household and community level. Children are most likely to introduce pneumococci into the household, especially when they are DCC attendees (154, 157, 158, 162). In Finnish families, 82% of new serotypes were introduced by DCC attendees, and in 71% this acquisition could be traced back to a fellow DCC attendee (154). Once a serotype has been introduced, within-household transmission frequently occurs, with reported risk ratios for carriage in the family as high as 8.7 for 0-2 yrs and 4.2 for 2-5 yrs (146). The presence of one carrier in a family of four resulted in a conditional acquisition rate roughly 12-fold as compared to a situation with no carriers in the family (152, 153).

Surprisingly, one UK modelling study found that within the household, the highest daily transmission rate was from adults to children, not amongst siblings (158). This could be a result of the small family sizes studied (mostly 3 to 4 members). Indeed, the expected prevalence in children increased by 3-10% for each additional child in the household (158). In young children in South Africa, child-to-child transmission was found to be more important than mother-to-child transmission, accounting for 51-67% versus 4-17% of all transmissions, respectively (162). Other studies did not report on the relative importance of pneumococcal transmission by parents or siblings.

One study found no carriage in 40 out of 97 families with newborn infants, concluding that transmission within families dominates over transmission from the population (146). Yet, other studies dispute this conclusion, and reported that the majority of acquisitions in children under five come from outside the household: 50-60% in the UK (157), 62% in the USA (161) and 90% in Finland (of which 65% from the child's DCC and 25% from the community) (154). The Finnish study reported slightly higher transmission rates for within-DCC as compared to within-household transmission (0.53/month (90%CI 0.38-0.71) and 0.37/month (90%CI 0.23-0.52)), but a large difference in the average time it takes for a carrying child to infect another DCC or family member (1.9 versus 4.5 months).

The relative rate for exposure in DCC was estimated to be 2.7 (95% CI: 1.7, 4.4) in Danish DCCs (147). In three Finnish DCCs, a mean number of 7.5 serotypes was reported, with an average of 2.7 new serotypes introduced into the DCC per month (154). A simulation study based on USA data reported that children attending DCC had 2.2-2.4 times higher odds to carry pneumococci as compared to children not attending DCC (155). Also a profound indirect effect of DCC attendance was reported: children not attending DCC but living in an area in which a fraction of children attended DCC had 3.7-5.8 higher odds of carrying pneumococci as compared to children in a hypothetical community where no DCCs were present. These effects were highly dependent on the proportion of attending children and the hours per week spent in DCC. Combining both indirect and direct effects, the total OR was 7.3 in a community where 44% of children attend DCC.

Comparing DCCs in Finland and Portugal, within-room and community transmission were found to be higher in Portugal, resulting in higher pneumococcal carriage prevalence (160).

Only one study reported that household contacts with siblings were more important to understand pneumococcal transmission than DCC attendance: for index children without siblings, DCC attendance increased the odds for carriage almost three times, but for children with siblings the effect of day-care was negligible (151).

Findings - Interventions

Two studies looked at the impact of the South Swedish Pneumococcal Intervention Project, in which carriers of penicillin-resistant pneumococci were excluded from DCC until a negative swab was obtained (145, 156). This approach was found most effective

in larger groups and in the second half of the year, due to seasonal variation with a peak in September (145). One of the studies compared this approach to two different interventions: reducing DCC group size and reducing the proportion of the population attending DCC (156). Reducing DCC group size was found to be most efficient to reduce spread of penicillin-resistant pneumococci: reducing the average group size from 16.7 to 13.4 was estimated to result in a decline of transmission events by 82%. Increasing average group size from 16.7 to 17.2 resulted in a mean increase of 20%. The same 82% reduction in transmission events was also reached by excluding 15% of penicillin-resistant pneumococci carriers from DCC. This approach was deemed less favourable, however, because of the economical and social costs associated with having parents staying away from work for an unknown period of time.

Findings - Serotype-specific results

Four out of five studies which compared transmissibility amongst serotypes reported that serotypes differed in transmissibility, either from the community (148, 153), within-household (157) or within-DCC (150). Serotypes 6A (148, 150, 153, 157), 19A (148, 150), 19F (high in (148, 153), medium in (157)) and 3 (148, 150) were consistently reported to be more transmissible than other serotypes. Serotype 9V was consistently reported to be less transmissible than other serotypes (148, 150). Conflicting results were reported for serotype 14 (high (148, 157), low in (150)), serotype 6B (high in (148, 153), low in (157)) and 23F (high in (148), medium in (157), low in (150, 153)). One study reported that antibiotic susceptibility can affect the transmissibility of strains within some serotype, with susceptible strains reported to be more epidemic than resistant strains for serotypes 6A and 19F (150). No difference between the transmissibility of serotypes was reported between PCV-7 and non-PCV-7 serotypes (149), which could be explained by the serotype-specific results reported above: the transmissibility of individual PCV-7 serotypes was reported to be high (19F), low (9V) or inconsistent (23F, 6B).

There is evidence for between-serotype competition, with current pneumococcal carriage reducing acquisition of other serotypes (153, 154, 159, 160). One study found that in particular serotype 6B reduced susceptibility to other serotypes, resulting in longer period of carriage (157). One study including multiple carriage showed that in individuals carrying multiple serotypes did not clear carriage quicker than single carriers (RR 0.81 95%CI 0.48-1.26), indicating that the presence of another serotype does not affect clearance (147).

Table 4.8 Mathematical modelling studies on pneumococcal transmission

Author, year (ref)	Country	Setting	Findings (95%CI)
Andersson, 2005 (145)	Sweden	DCCs with control strategy	DDC group size important for likelihood and size of an outbreak. Intervention measures have impact, mostly in large groups.
Auranen, 2000 (146)	Sweden	Households	No carriage in 40 of 97 families: carriage is clustered in some families. Association carriage in family RR 8.7 for 0-2 yrs, RR 4.2 for 2-5 yrs. Temporal clustering of pneumococcal carriage within families: transmission within families dominates transmission from population
Auranen, 2010(147)	Denmark	DCCs	Exposure in DCC: RR 2.7 (1.7-4.4)
Cauchemez, 2006 (148) (149)	France	Schools	No difference between VT and NVT for child-child transmission rate
De Celles 2011 (150)	France	DCC	Transmissibility differs by serotype: 3, 6A, 19A most epidemic, 23F,9V, 14 least epidemic
Ekholm, 2002 (151)	Finland	Community	Interaction between siblings/DCC: if the child does not have siblings at home, then the effect of DCC increases the odds for carriage by a factor of 3, but if the child has siblings at home, the effect of DCC is negligible.
Erasto 2010 (153) (152)	Bangladesh	Households	Within-family transmission rates similar between different serotypes. Acquisition rate in presence of household exposure was roughly 12-fold the community force of infection. Evidence for between-serotype competition: current pneumococcal carriage reduced acquisition of other serotypes
Hoti, 2009 (154)	Finland	DCC and households	Transmission in DCC driving force. Role of families minimal, because small size and lower susceptibility of adults
Huang, 2005 (155)	USA	DCC	Children in communities with higher fractions of DCC attendees have higher prevalence.
Karlsson, 2008 (156)	Sweden	DCC, school, households	Reducing DCC group size most efficient intervention to reduce pneumococcal transmission.

Table 4.8 Mathematical modelling studies on pneumococcal transmission

Author, year (ref)	Country	Setting	Findings (95%CI)
Melegaro, 2004 (158)	UK	Households	Children most likely to introduce pneumococci into household Within-household highest daily transmission rate is from adults to children
Melegaro, 2007 (157)	UK	Households	For all serotypes, acquisition rates from the community significantly higher in preschool children than older individuals.
Numminen, 2013 (159)	Norway	DCC	Evidence for strong between-strain competition.
Pessoa, 2013 (160)	Portugal, Finland	DCC	Significant higher within-room and community acquisition in Portugal as compared to Finland
Sheehe, 1969 (161)	USA	Households	Of 50 acquisitions among the cohort of pre-school children, 62% came from outside household, 38% from within household.
Shiri, 2013 (162)	South Africa	Mother-infant pairs	Children infected their mothers more frequently than vice versa. Mothers acquire pneumococci more frequently from their children than from the community.

4.8 Discussion

Comparison of study designs

We compared the methodology and results of 103 studies: 34 cross-sectional studies, 20 outbreak investigations, 1 case-control study, 30 longitudinal studies and 18 mathematical modelling studies. The different study designs pose different advantages and disadvantages. Cross-sectional studies have as advantage that they are generally easier to conduct than longitudinal studies, and therefore allow for a larger sample size. In the included studies, the cross-sectional studies had a median sample size of 380, versus a median sample size of 134 children amongst the longitudinal studies.

The main disadvantage of cross-sectional studies is that limited conclusions can be made on transmission. Although most studies included in this review assume that 'transmission' occurred when the same pneumococcal serotype or clone was found within a DCC or household, it would be more accurate to speak of 'co-localisation'. Assuming that transmission occurs when the same serotype or clone is detected, another disadvantage of cross-sectional studies is that the direction of transmission cannot be identified. Especially in household studies, it is unclear whether transmission occurred from the household member to the index child or from the index child to the household member. Even simultaneous acquisition from a third person cannot be ruled out. The same problem holds true for longitudinal studies with definitions of exposure based on simultaneous carriage. Preferred is a serotype specific definition for exposure based on previous time points to allow for conclusions of the direction of transmission.

The study interval used in longitudinal studies has direct implications on the conclusions made. If the interval between swabs is long, episodes of carriage can be missed, resulting in an underestimation of transmission. Especially in the adult population, known to carry pneumococci for shorter periods of time, episodes of carriage can be overlooked. To overcome this problem, MCMC data augmentation can be used to impute unobserved transitions – an advantage of mathematical modelling studies. Another advantage of mathematical modelling studies is that the effect of interventions to reduce transmission can be studied.

Stochastic, individual-based SIS mathematical models fitted to empirical longitudinal data were most commonly used. It is important to include stochasticity in a mathematical model designed to study household or DCC transmission: group sizes

are often small, making it more important to acknowledge the role of chance in transmission.

Comparison of different settings

Even though the included studies represent a reasonable geographical cover, with data from 30 different countries included, the majority of studies are from high-income countries, and the conclusions of this review may therefore represent a non-representative view. There is a wide variation in social mixing patterns between different geographical regions and wide variation in family sizes and structures, which will have resulted in differences in pneumococcal carriage. Also differences in laboratory capacity and performance could have led to the differences in findings observed from different geographical settings.

Most studies took place in DCCs or households or combination of both. Studies conducted in DCCs were almost solely from countries in high-income countries. All but one of the included African studies focused on household transmission only. With an official DCC structure lacking in many low-income societies, it would be interesting to see what the proportional attribution is of household versus child-to-child transmission. How do children under five interact with each other? How many child contacts does a child under five have in a society without a formal day care structure? What impact does this have on pneumococcal spread? A mixture of anthropological and epidemiological studies may be required to answer these questions, which are unfortunately outside the scope of this PhD.

Community transmission is more difficult to study directly than transmission in closed settings, such as a DCC or household where contact patterns are better defined. When studies report on community transmission, it is usually inferred from pneumococcal acquisition that cannot be explained by the transmission in the household or DCC. Other evidence for community transmission comes from cross-sectional studies or outbreak investigations, which have found the same pneumococcal clone in different DCCs or distinct communities. International recognized strains with identical MLST types are proving evidence for international spread.

Comparison of typing methods

The majority of included studies presented serotyping results. Distinguishing between different serotypes is important to evaluate the strain's potential invasiveness, and to evaluate the impact of pneumococcal conjugate vaccines, which target only specific serotypes. Distinguishing between different isolates of the same serotype can be done

with molecular typing methods, such as PFGE and MLST. Molecular typing methods were frequently used in the included cross-sectional studies (76%) and outbreak investigations (55%), but were less common in the included longitudinal studies (26%). None of the included mathematical modelling studies used molecular typing data.

Conclusions

Despite the different study designs, generic conclusions can be made (Table 4.9). Both transmission in DCCs and households are important to explain the spread of pneumococci. There is strong evidence that DDCs act as an amplifier of pneumococcal spread in the community. The average cluster size seems to be 3-4. There is some evidence that the cluster size is larger in outbreak situations with known invasive serotypes (median 6), but this could also be a result of reporting bias, with larger outbreaks being reported more frequently than small outbreaks.

Strong evidence for transmission within households also exists, although on population level this seems to have a smaller impact than transmission within DCCs or the wider community. Young children most frequently introduce new serotypes into the household. Within households, sibling-to-sibling spread is reported more commonly than parent-to-child transmission. Child-to-parent transmission is also frequently reported. Younger infants seem to be affected most by intrafamilial spread, which is a plausible result of increased contact rate with other toddlers at older age, either in a DCC in developed countries, or in the neighbourhood in some African countries.

The role of the mother in pneumococcal transmission to the infant remains uncertain. Almost all studies report an association between maternal and infant carriage, but the relative importance to sibling-to-sibling spread is doubtful. More studies on mother-infants pairs including results for other siblings in the household are required to get a better picture of household transmission to young infants. HIV-exposure by the mother does not seem to have an effect on the incidence of new pneumococcal acquisition, although differences in prevalence of pneumococci in infants have been reported. Contradictory results have been reported for differences in maternal transmission of PCV-13 and non-PCV-13 serotypes.

There is evidence for different transmissibility of serotypes, with serotypes 6A, 19F, 3 consistently being reported as being more transmissible than other serotypes. Some caution needs to be taken in this statement, however: serotypes 6A and 19F are carried for longer duration as compared to other serotypes; frequent detection of

serotypes 6A and 19F in clusters may be a function of their long carriage duration, rather than with transmissibility. There is also evidence that competition between serotypes occurs: current carriage seems to reduce acquisition of other serotypes when exposed.

Based on the results from this literature review, most acquisition in children <5 years is explained by transmission from other children <5 years; in DCCs and amongst siblings within the household. Within the household, children <5 years transmit to both their siblings and parents or other adults. Within household transmission from adults to children seems to be less important to explain pneumococcal transmission dynamics, even amongst mother-infant pairs. The best strategy to target effective transmitters and obtain herd immunity would be to focus on vaccination of all children under the age of five. Catch-up campaigns may be required for countries newly introducing the vaccination in the childhood immunisation programme, especially in high-incidence areas were transmission dynamics are rapid and children seem to be active transmitters until a higher age than in developed countries. If only targeting infants through childhood immunisation programmes, it could take many years for herd protection to be established. Randomised controlled trials in the Gambia seem to confirm this concern: no indirect benefits were seen for non-vaccinated siblings in a PCV-7 randomised controlled trial (68). In Kilifi District in Kenya, where a catch-up campaign was held for children <5 years, two-thirds reductions in VT were observed two years after vaccine introduction in children <5 years and unvaccinated older individuals, suggesting that a substantial herd effect is taking place in this setting (25). Surveillance and evaluation, through various study designs, will remain important to study the impact of the newly introduced vaccines, and the impact this has on transmission dynamics worldwide.

Table 4.9 Summary of systematic literature review findings

Findings	Evidence*
1.1 What is the evidence for pneumococcal transmission to and from children under five within the household?	
Children most frequently introduce serotypes into the household, especially when attending DCC.	strong
Carriage by another household member is a risk factor for pneumococcal acquisition.	strong
Intrafamilial spread is responsible for <50% of pneumococcal acquisitions in the index child.	strong
Younger children are more at risk of intrafamilial spread than older children.	strong
Siblings are important in intrafamilial spread to index children.	intermediate
HIV-exposure by the mother does not influences the rate of pneumococcal acquisition in the index child.	intermediate
Parents are important in intrafamilial spread to index children.	inconsistent
Mothers are important in spread to young infants.	inconsistent
The risk of pneumococcal acquisition increases within a larger household.	inconsistent
1.2 What is the evidence for pneumococcal transmission to and from children under five outside the household?	
DCCs are important amplifiers of pneumococcal spread.	strong
The average cluster size in DCCs is 3-4 individuals.	strong
The cluster size is higher for serotypes in outbreak situations (median 6).	strong (bias?)
DDCs are “autonomous epidemiological units” with different serotypes/clonal types circulating.	intermediate
The risk of pneumococcal acquisition increases within a larger DCC.	strong
International spread occurs as can be concluded from internationally recognized clones	strong
1.3 Is there any evidence that pneumococcal transmission dynamics differ by geographical and cultural setting?	
Transmission in high-income countries is largely driven by DCCs. We don’t know how important child-to-child contact is in settings with low DCC attendance	incomplete
Children most commonly introduce new serotypes into the household. Because household composition differs worldwide, pneumococcal transmission dynamics within-household will differ between different geographical and cultural settings.	incomplete
1.4 Is there any evidence that pneumococcal transmission dynamics differ by serotype?	
Serotypes differ in transmissibility: 6A, 19F, 3 are more transmissible than other serotypes.	strong
Competition between serotypes occurs: current carriage reduces acquisition of other serotypes.	strong

Table 4.9 Summary of systematic literature review findings

Findings	Evidence*
1.5 What is the evidence for altered pneumococcal transmission dynamics post introduction of pneumococcal conjugate vaccination?	
Children remain the main drivers of NVT pneumococcal transmission within the household post introduction of PCV	strong

* Level of evidence based on proportion of studies reporting on topic in favour of hypothesis: strong = >80%, intermediate = 60-80%, inconsistent = 40-60%, no = <40%, incomplete = 1 or no studies

RESULTS

Part I: Pre-vaccination period

5. Pneumococcal acquisition in HIV-exposed and HIV-unexposed infants

5.1 Background

Our literature review (Chapter 4) provided inconclusive evidence for the role of the mother in pneumococcal transmission. Almost all studies reported an association between maternal and infant carriage, but the relative importance to sibling-to-sibling spread was doubtful. In this chapter we show the results of a study which investigated pneumococcal acquisition in HIV-exposed and HIV-unexposed infants, and looked into the relative contribution of exposure by the mother and other children in the household. A longitudinal study design was adopted, shown in the previous chapter to be most effective for the investigation of pneumococcal transmission.

Children born to HIV-positive mothers have been shown to experience higher rates of respiratory illness (164). Whilst part of this association is explained by HIV-infection in the offspring, HIV-exposed uninfected infants also appear to be at increased risk of morbidity and mortality from invasive pneumococcal disease (165). In countries of sub-Saharan Africa with generalized HIV epidemics, HIV-exposure occurs in up to 25% of pregnancies. We hypothesized that infants born to HIV-positive mothers ("HIV-exposed") would have higher rates of pneumococcal acquisition than infants born to HIV-uninfected mothers ("HIV-unexposed") and that this may lead to increased rates of disease in the HIV-exposed. There are two rationales for this hypothesis: 1) HIV-positive adults have higher rates of pneumococcal carriage than HIV-uninfected adults, resulting in an increased risk of exposure by HIV-positive parents to their infants (133), and 2) differences in the immune system of HIV-exposed and HIV-unexposed children, in particular altered transplacental and breast milk transfer of antibody may lead to different susceptibility to pneumococci (166). We conducted a longitudinal household

study to compare pneumococcal acquisition rates between HIV-exposed and HIV-unexposed infants in Malawi. We examined serotype-specific associations between infant carriage and carriage in their mothers and household members <5 years, both hypothesized to be key transmitters to infants.

5.2 Methods

5.2.1 Study population and design

Recruitment of HIV-positive pregnant women took place in antenatal clinics in two rural hospitals between January 2009 and December 2010. Attendance at antenatal clinics was very high: 99.7% among pregnant women in the HDSS area in 2009 and 2010. HIV-testing was offered to all women attending antenatal clinic. All HIV-positive pregnant women living in the HDSS area were eligible for inclusion. For each HIV-positive woman, up to three HIV-uninfected pregnant women were recruited from the HDSS area, frequency-matched on the number of children <10 years in the household. Nasopharyngeal swabs were collected from the infant, mother and other household members willing to participate at 6, 10, 14, 18, 22, 26, 30, 34, 40, 46, and 52 weeks of the infant's age. Follow-up ceased if the index infant died, the mother-infant pair moved outside the study area, consent was withdrawn or there was a failure to sample on two sequential visits. Available data for infants lost to follow up were included in the analyses.

HIV-exposed infants were tested for HIV DNA at 6 and 26 weeks and for HIV antibodies at 12 months of age or when visiting the local clinic as part of another affiliated study. Infants with a positive antibody test at 12 months were followed up to confirm HIV-status. HIV-positive mothers and infants were referred for appropriate care. Data on antiretroviral therapy and cotrimoxazole prophylaxis were available from databases linked to the HDSS. A retrospective questionnaire was used for verification and to complete missing data.

5.2.3 Definitions

An episode of carriage was defined as isolation of a pneumococcus from one or more consecutive samples. An episode was deemed to terminate if the serotype was not detected in two consecutive samples. A new acquisition event was defined as the

identification of a serotype when the same serotype was not identified at the previous two sampling times. We stratified the analyses by PCV-13 (VT) serotypes and by the major serotypes associated with colonization and IPD in children ("pediatric serotypes"; 4, 6A, 6B, 9V, 14, 18C, 19A, 19F, and 23F). Pneumococcal isolates identified in the first sample were regarded as new acquisitions. The date of acquisition was defined as the midpoint between the last negative and the first positive result. The date of termination was defined as the midpoint between the last positive and the first of two negative results. We defined pneumococcal exposure as carriage by another household member at any of the previous two sample times. We defined concordance in carriage as simultaneous carriage of the same serotype by the infant and household members. Negative and non-typeable results were excluded from the concordance analyses.

5.2.4 Statistical analysis

Statistical analyses were performed using R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) (167). Comparisons between categorical data were made using the Pearson's χ^2 test and Fisher exact test as appropriate. We used survival analysis including Kaplan-Meier plots and log-rank tests to study differences in the mean time to first pneumococcal acquisition (168). Crude and adjusted risk ratios (RR and aRR) for risk factors associated with pneumococcal acquisition of any serotype were obtained by log-binomial regression (169).

Rates of infant pneumococcal acquisition were studied using counting process (Anderson-Gill) models. The counting process model is a simple extension of the Cox proportional hazards model where a subject is at continuous risk for an event and has the same baseline hazards function (170). We considered our study subjects to be at continuous risk of acquiring a new serotype because of the possibility of multi serotype carriage. Time at risk was calculated as the number of days between sampling dates. If an infant was missed on a sampling appointment, we estimated the start of the next period at risk as the midpoint between the two samples for which a time in days was available. We examined the proportional hazards assumption by testing the correlation coefficient between transformed survival time and the scaled Schoenfeld residuals.

The effects of infant age and seasonal and secular trends were studied with fitted generalized additive mixed models. Secular trends were further studied by serotype using generalized additive models and the χ^2 test for trend in proportions. For seasonal

trend, parametric functions with different numbers of sin-cosine waves were examined. For instance, a model with three sin-cosine waves and fitted splines for infant age in days and secular trend (days since study onset) was specified as:

$$y_{i,t} = \alpha 0 + \alpha 1 \sin(\frac{2\pi t}{365}) + \beta 2 \cos(\frac{2\pi t}{365}) + \alpha 2 \sin(\frac{4\pi t}{365}) +$$
$$\beta 2 \cos(\frac{4\pi t}{365}) + \alpha 3 \sin(\frac{6\pi t}{365}) + \beta 3 \cos(\frac{6\pi t}{365}) + f(age) + f(trend) + u_{i,t}$$

where $y_{i,t}$ is the carriage in infant *i* for each day of the year *t,* α and β terms are the regression coefficients for each sine and cosine function, *f1* and *f2* are smooth functions for infant age in days and secular trend (days since study onset), and $u_{i,t}$ is the infant-specific random effect. Log-binomial regression was used to report rate ratios. In addition, generalized linear and additive mixed models with individuals-level random effects were fitted to examine the extent of within-person clustering (171, 172).

Serotype-specific analyses on household exposure were performed for the six commonest serotypes for infants, mothers and children <5 years. Acquisition and exposure was assessed at each sample with the results pooled to give a summary estimate. Crude and adjusted population attributable fractions (PAF and aPAF) of exposure from the mother/infants/children <5 years -the proportion of new acquisitions in the population that is attributable to exposure- were calculated using the formula: PAF = $P_e(RR-1) / (1+(P_e(RR-1))$, where P_e is the proportion of individuals exposed and RR is the risk ratio (risk in exposed/risk in unexposed). The PAF was adjusted using the aRR obtained in the multivariable log-binomial model.

Duration of carriage was estimated for infants, mothers and other children <5 years. Duration of carriage could not be estimated for other adult household members, because the response rate was low. Episodes starting at the end of the follow up period were excluded, because duration of carriage could not be accurately determined. For mothers and children <5 years also events starting at the first observation were excluded, because duration of carriage could not be accurately determined. Differences in duration of carriage between infants, mothers, and other children <5 years, between serotypes, and between HIV-status of the mother were calculated using the Mann-Whitney *U* test.

5.3 Results

5.3.1 Study participants and samples

In total, 54 HIV-exposed infants and 131 HIV-unexposed infants were recruited between January 2009 and December 2010. Follow up ceased in November 2011. Follow-up was terminated prematurely for 24 infants (13.0%). Nine infants departed from the study area, six were lost to follow-up, one was withdrawn from the study and one left for other reasons. Four HIV-exposed infants (7.4%) and three HIV-unexposed infants (2.3%) died within the first year of life (p=0.22). HIV-results were available for 44 HIV-exposed infants (81.5%) of which seven (15.9%) tested HIV-positive. Information on cotrimoxazole prophylaxis and antiretroviral treatment was available for 46 HIV-exposed infants (85.2%), of which 18 infants (39.1%) received cotrimoxazole and 3 infants (6.5%) received antiretroviral treatment during the study.

At least one pernasal swab was available for 140 of 168 (83.3%) children <5 years, 198 of 288 (68.8%) children aged between 5-14 years and 95 of 287 (33.3%) adults other than the mother. In total, 1721 results (90.2% of scheduled visits) were available for index infants, 1763 (92.4%) for mothers, 806 (46.3%) for children <5 years, 718 (24.1%) for children 5-14 years and 200 (6.8%) for other adult household members.

Data on cotrimoxazole and antiretroviral treatment use was available for 50 HIV-positive mothers (92.6%): 39 (78.0%) mothers received cotrimoxazole during all (n=31) or part (n=8) of the study period and 28 (56.0%) mothers received antiretroviral treatment during all (n=24) or part (n=4) of the study period.

5.3.2 Pneumococcal prevalence

Pneumococcal carriage prevalence in the index infants increased from 30.6% at week 6 to 60.6% at week 18 (p<0.001) and subsequently decreased to 25.0% at week 52 (p<0.001) (Figure 5.1). Overall pneumococcal carriage prevalence was higher in HIV-positive mothers than in HIV-uninfected mothers (24.8% vs. 14.5%, p<0.001). Pneumococcal carriage prevalence did not significantly differ between HIV-positive mothers using antiretroviral treatment or not (26.2% vs. 25.5%, p=0.94) or using cotrimoxazole or not (27.1% vs. 23.2%, p=0.42). Overall pneumococcal carriage prevalence was 51.2% in children <5 years, 40.3% in children 5-14 years and 17.5% in adults. Carriage in other children in the household mirrored the differences by week

observed for index infants with higher carriage observed in earlier than later sampling weeks (64.7% vs. 27.1%, p<0.001 for children <5 years and 50% vs. 15.2%, p<0.001 for children 5-14 years at week 14 vs. week 52). Carriage in mothers and other adult household members did not significantly differ by sampling week.

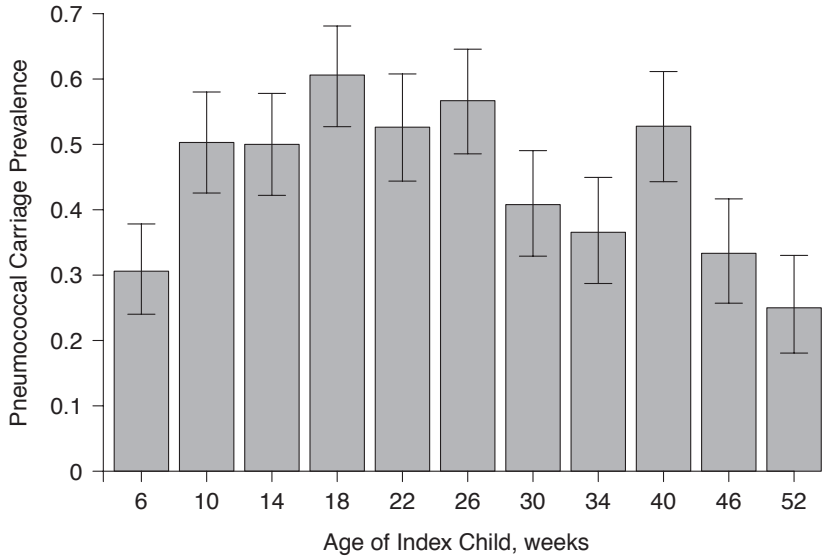


Figure 5.1 Pneumococcal carriage prevalence by age of index infant in weeks
Bars represent 95% confidence intervals.

The HIV-status of the mother did not result in different pneumococcal carriage prevalence in index infants (44.1% HIV-unexposed vs. 46.2% HIV-exposed, p=0.44) and other children (50.7% HIV-unexposed vs. 53.4% HIV-exposed, p=0.60 for children <5 years and 41.5% HIV-unexposed vs. 36.9% HIV-exposed, p=0.30 for children 5-14 years).

5.3.3 Serotype distribution

In total, 46 different serotypes were isolated from the infants. The commonest serotypes were 19F, 19A, 6B, 23F, 6A and 15B, together accounting for 47.6% of isolates. VT serotypes accounted for 54.7%, 48.4%, 40.8%, 34.4% and 25.7% in index infants, children <5 years, children 5-14 years, mothers and other adults respectively. Paediatric serotypes accounted for 52.4%, 45.0%, 34.6%, 31.2% and 20.0% in index infants, children <5 years, children 5-14 years, mothers and other adults respectively. There were no significant differences in the proportion VT or pediatric serotypes between HIV-exposed and HIV-unexposed infants or children. HIV-positive mothers

carried lower proportions of paediatric serotypes than HIV-uninfected mothers (24.4% vs. 35.9%, p=0.04). A large proportion of isolates could not be fully typed with the available reagents: 22.6%, 33.7%, 32.5%, 43.2% and 48.6% in index infants, children <5 years, children 5-14 years, mothers and other adults respectively.

Concordance between serotypes simultaneously carried by mothers and infants was low: only in 39 of 586 (6.7%) instances when the infant carried a typeable pneumococcus was the same serotype found in the mother. Concordance with maternal carriage was 9.0% (15/165) in infants less than 3 months of age. Concordance with infant serotype carriage at any age was 17.4% (49/281) for children <5 years, 9.1% (19/209) for children 5-14 years and 4.5% (3/66) for adults.

5.3.4 Pneumococcal acquisition in infants

There were 553 new acquisitions observed in index infants during 59,365 days at risk, resulting in an acquisition rate of 0.0093 per day (95%CI 0.0086-0.0101). The observed median time to first acquisition was 59 days, with no difference observed between HIV-exposed and HIV-unexposed infants (median time 58.5 and 59.0 days, p=0.99) (Figure 5.2). Infants living with children <5 years acquired pneumococci faster than infants without children <5 years in the household (median time to first acquisition 56.5 and 83.0 days, p=0.001) (Figure 5.3).

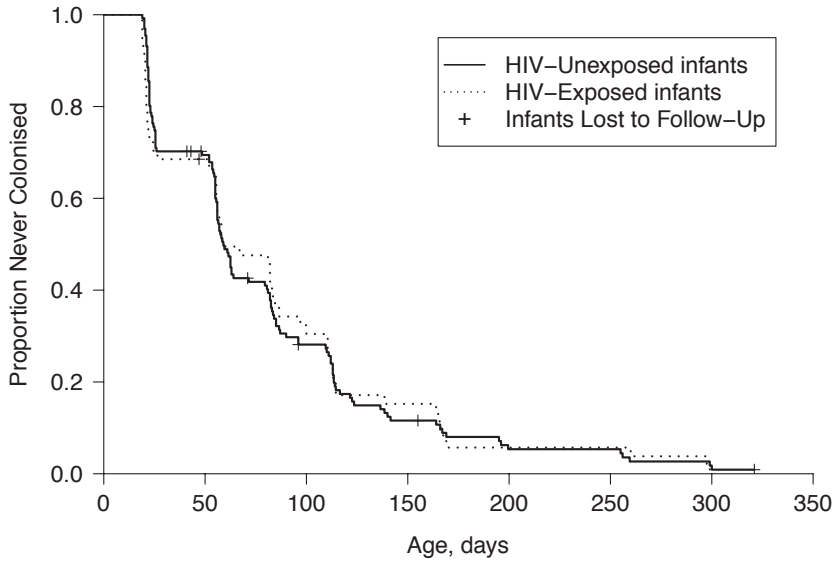


Figure 5.2 Kaplan-Meier plot for time to first pneumococcal carriage acquisition in infants by human immunodeficiency virus (HIV) exposure status

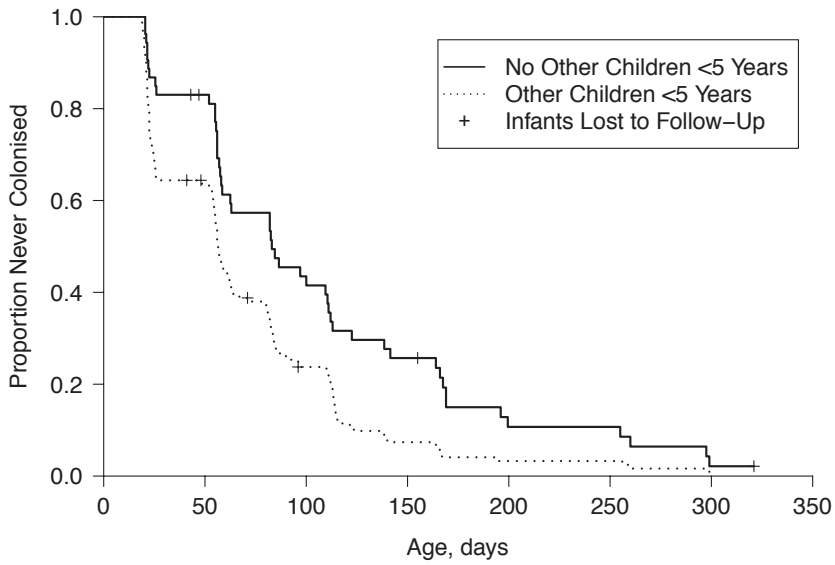


Figure 5.3 Kaplan-Meier plot for time to first pneumococcal carriage acquisition in infants by presence or absence of other children <5 years in the household

Table 5.1 presents the univariable and multivariable risk factor analysis for pneumococcal carriage acquisition in infants. The individual-level variance was found to be negligible for infants ($\sigma 2<0.01$); hence results from the non-mixed model are reported. There was no significant difference in pneumococcal acquisition by maternal HIV-status (aRR 1.00, 95%CI: 0.87-1.15) or infant HIV-status (aRR 1.00, 95%CI: 0.71-1.42). Infant age was not found to be associated with pneumococcal acquisition. There was weak evidence that cotrimoxazole prophylaxis reduced pneumococcal carriage acquisition amongst HIV-exposed infants (aRR 0.77, 95%CI: 0.59-1.02). Results from the Cox proportional hazards model were similar to the log-binomial regression model. The proportional hazard assumptions were met by all variables apart from infant age, which has been omitted from the model presented in Table 5.1.

5.3.5 Household exposure

In the pooled analysis for the six commonest serotypes, both serotype-specific exposure by the mother (aRR 3.09, 95%CI: 1.47-6.50) and exposure by other children <5 years (aRR 4.30, 95%CI: 2.80-6.60) were found to be associated with infant pneumococcal acquisition (Table 2). The aPAF was low for infant pneumococcal acquisition: only 1.9% (95%CI: 0.0-4.3) and 8.8% (95%CI: 4.0-13.4) of acquisitions were attributable to exposure by the mother and other children <5 years respectively (table 2). Extreme case sensitivity analysis changing all missing exposure data to carriage or to non-carriage had limited impact on the calculated PAFs suggesting data were missing at random (data not shown).

Our results suggest that pneumococcal transmission occurs between infants, other children and mothers in all directions (Table 2). Only the association between pneumococcal acquisition in other children <5 years and exposure by the mother could not be established. For all groups, exposure by children <5 years resulted in the highest aRR and aPAFs, identifying them as main transmitters in the household.

Table 5.1 Generalized linear model and Cox proportional hazard model describing maternal HIV status and other risk factors associated with infant pneumococcal carriage acquisition

Risk factor	No.	New acquisitions		Generalized linear model				Cox proportional hazard model			
		n	%	cRR	95%CI	aRR	95%CI	cHR	95%CI	aHR	95%CI
Age (weeks)											
6	183	56	30.6	-		-					
10	171	65	38.0	1.24	0.93, 1.66	1.24	0.93, 1.64				
14	168	55	32.7	1.07	0.79, 1.46	1.07	0.79, 1.44				
18	165	64	38.8	1.27	0.95, 1.69	1.26	0.95, 1.68				
22	152	47	30.9	1.01	0.73, 1.40	0.99	0.72, 1.37				
26	157	58	36.9	1.21	0.90, 1.63	1.24	0.92, 1.66				
30	152	49	32.2	1.05	0.77, 1.45	1.17	0.86, 1.59				
34	145	33	22.8	0.74	0.51, 1.08	0.82	0.57, 1.18				
40	144	64	44.4	1.45	1.09, 1.93	1.58	1.18, 2.11				
46	144	38	26.4	0.86	0.61, 1.22	1.05	0.74, 1.50				
52	140	24	17.1	0.56	0.37, 0.86	0.74	0.48, 1.14				
Sex											
Female	824	265	32.2	-		-		-		-	
Male	897	288	32.1	1.00	0.87, 1.15	0.99	0.87, 1.13	0.99	0.86, 1.12	1.00	0.88, 1.12
Maternal HIV-status											
HIV-uninfected	1219	381	31.3	-		-		-		-	
HIV-positive	502	172	34.3	1.10	0.95, 1.27	1.00	0.87, 1.15	1.10	0.97, 1.24	1.02	0.88, 1.16
HIV-status child[a]											
HIV-uninfected	363	127	35.0	-		-		-		-	
HIV-positive	69	23	33.3	0.95	0.66, 1.37	1.00	0.71, 1.42	0.94	0.72, 1.16	0.99	0.78, 1.20

Table 5.1 Generalized linear model and Cox proportional hazard model describing maternal HIV status and other risk factors associated with infant pneumococcal carriage acquisition

Risk factor	No.	New acquisitions		Generalized linear model				Cox proportional hazard model			
		n	%	cRR	95%CI	aRR	95%CI	cHR	95%CI	aHR	95%CI
Cotrimoxazole[a]											
No	311	112	36.0	-		-		-		-	
Yes	152	46	30.3	0.84	0.63, 1.12	0.77	0.59, 1.02	0.80	0.57, 1.03	0.78	0.54, 1.03
Maternal age											
<28	807	243	30.1	-		-		-		-	
≥28	914	310	33.9	1.13	0.98, 1.29	1.05	0.92, 1.20	1.13	1.00, 1.26	1.04	0.92, 1.17
Feeding											
Exclusively breastfed	740	255	34.5	-		-		-		-	
Mixed	910	276	30.3	0.88	0.77, 1.01	1.04	0.82, 1.30	0.81	0.53, 1.09	0.85	0.55, 1.14
Weaned	49	15	30.6	0.89	0.58, 1.37	1.04	0.66, 1.65	0.80	0.30, 1.30	0.82	0.35, 1.29
Seasonality											
Rain (Dec-April)	720	170	23.6	-		-		-		-	
Cold (May-Aug)	574	228	39.7	1.68	1.43, 1.99	1.59	1.35, 1.87	1.78	1.60, 1.95	1.68	1.50, 1.86
Hot (Sept-Nov)	427	155	36.3	1.54	1.28, 1.84	1.37	1.13, 1.64	1.57	1.38, 1.77	1.39	1.19, 1.60
Year											
2009	631	257	40.7	-		-		-		-	
2010	810	236	29.1	0.72	0.62, 0.83	0.79	0.68, 0.91	0.71	0.56, 0.86	0.76	0.60, 0.91
2011	280	60	21.4	0.53	0.41, 0.67	0.62	0.48, 0.80	0.52	0.29, 0.76	0.59	0.34, 0.83

Table 5.1 Generalized linear model and Cox proportional hazard model describing maternal HIV status and other risk factors associated with infant pneumococcal carriage acquisition

Risk factor	No.	New acquisitions		Generalized linear model				Cox proportional hazard model			
		n	%	cRR	95%CI	aRR	95%CI	cHR	95%CI	aHR	95%CI
Other household members <5 years											
No	515	149	28.9	-		-		-		-	
Yes	1206	404	33.5	1.16	0.99, 1.35	1.15	0.99, 1.33	1.16	1.00, 1.31	1.15	1.00, 1.29
Exposure by mother[b]											
No	1119	340	30.4	-		-		-		-	
Yes	410	155	37.8	1.24	1.07, 1.45	1.14	0.98, 1.33	1.20	1.02, 1.37	1.10	0.91, 1.28
Exposure by other children <5 years[b]											
No	762	227	29.8	-		-		-		-	
Yes	467	172	36.8	1.24	1.05, 1.45	1.07	0.90, 1.26	1.16	0.99, 1.32	1.05	0.87, 1.23

Abbreviations: aRR, adjusted rate ratio; CI, confidence interval; cRR, crude rate ratio; HIV, human immunodeficiency virus

[a] Only including HIV-exposed infants.

[b] Exposure to any serotype. Exposure unknown for all week 6 samples.

Table 5.2 Serotype-specific acquisition of infants, mothers and other children <5yrs, stratified by exposure to other household members

Acquiring category, Exposing category, and Exposure[a]	No. with events	No. without events	Total no.[b]	cRR	aRR[c]	95%CI	cPAF (%)	aPAF[b] (%)	95%CI
Infant									
Mother									
Yes	8	69	77	3.95	3.09	1.47, 6.50	2.6	1.9	0.0, 4.3
No	225	8335	8560						
Infant									
Other child									
Yes	22	173	195	4.70	4.30	2.80, 6.60	9.4	8.8	4.0, 13.4
No	162	6593	6755						
Mother									
Infant									
Yes	13	474	487	4.83	3.89	1.98, 7.65	15.2	12.9	1.5, 23.1
No	55	9894	9949						
Mother									
Other child									
Yes	9	236	245	5.75	3.99	1.82, 8.76	13.5	9.0	0.0, 17.6
No	46	7154	7300						

Table 5.2 Serotype-specific acquisition of infants, mothers and other children <5yrs, stratified by exposure to other household members

Acquiring category, Exposing category, and Exposure[a]	No. with events	No. without events	Total no.[b]	cRR	aRR[c]	95%CI	cPAF (%)	aPAF[b] (%)	95%CI
Other child									
Infant									
Yes	15	228	243	2.22	1.86	1.06, 3.29	6.1	5.1	0.0, 11.2
No	121	4227	4348						
Other child									
Mother									
Yes	1	52	53	0.65	0.33	0.04, 2.45	0.0	0.0	0.0, 0.4
No	113	3777	3890						
Other child									
Other child									
Yes	7	57	64	3.99	3.17	1.50, 6.70	4.6	3.7	0.0, 7.9
No	108	3835	3943						

Abbreviations: aRR, adjusted rate ratio; aPAF, adjusted population attributable fraction; CI, confidence interval; cRR, crude rate ratio; cPAF, crude population attributable fraction.

[a] Exposure was defined as carriage by another household member at any of the previous two sample times. Analysis was limited to those samples for which exposure data for at least one of the two previous sample times was available. [b] The six most common serotypes (19F, 19A, 6B, 23F, 6A, 15B) were assessed for acquisition and exposure at each sample with the results pooled to give a summary estimate. [c] Adjusted analysis for exposure by index child, exposure by children <5 yrs, exposure by mother, seasonality stratified by year and within-person clustering (mother only). Using a generalized linear mixed model, there was negligible individual-level variance for index infants ($\sigma 2 < 0.01$) and other children <5 years of age ($\sigma 2 < 0.01$), hence results from a (non-mixed) generalized linear model were reported. Using a generalized linear mixed model, the individual-level variance for mothers was 0.51.

5.3.6 Seasonal and secular trends

A model with three sin-cosine waves and fitted splines for infant age in days and secular trend (days since study onset) was found to provide the best fit. The individual-level variance was found to be negligible for infants ($\sigma^2<0.01$); hence results from the non-mixed model are reported.

A significant seasonal trend was observed, with the highest incidence in August, corresponding with the cold season, and the lowest incidence in March, corresponding with the end of the rainy season ($p<0.001$) (Table 5.3, Figure 5.4). Over the two-year study period, pneumococcal incidence showed a decreasing trend ($p<0.001$) (Table 5.3, Figure 5.6). Acquisition of NVT carriage showed a greater decrease over the study period ($p<0.001$), whilst no decrease in VT carriage was observed ($p=0.82$). For serotype 19A an increase was observed over the study period ($p<0.001$).

The generalized additive mixed model provided further evidence that pneumococcal acquisition was not associated with infant age (Table 5.3, Figure 5.5).

Table 5.3 Generalized additive mixed model results for pneumococcal incidence in infants

Parametric coefficients				
Coefficient	Estimate	Standard error	z value	*P*-value
Intercept	-1.215486	0.038901	-31.246	< 2e-16
α1	-0.399873	0.069015	-5.794	6.87e-09
β1	-0.154872	0.061535	-2.517	0.01184
α2	0.047957	0.052210	0.919	0.35833
β2	0.161008	0.052797	3.050	0.00229
α3	-0.122454	0.049683	-2.465	0.01371
β3	-0.005179	0.050301	0.103	0.91800
Smooth terms				
Coefficient	edf	Ref. df	Chi-square	*P*-value
f1 (infant age in days)	2.068	2.068	3.425	0.189
f2 (secular trend; days since study onset)	3.751	3.751	27.525	1.5e-05

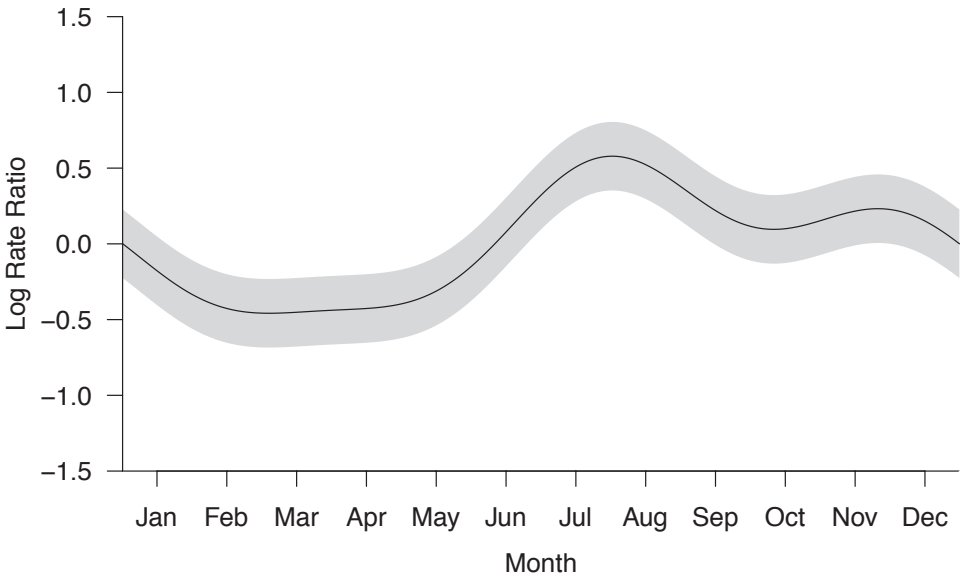


Figure 5.4 Fitted parametric seasonal trend in pneumococcal carriage incidence in infants
Grey area represents 95% confidence interval.

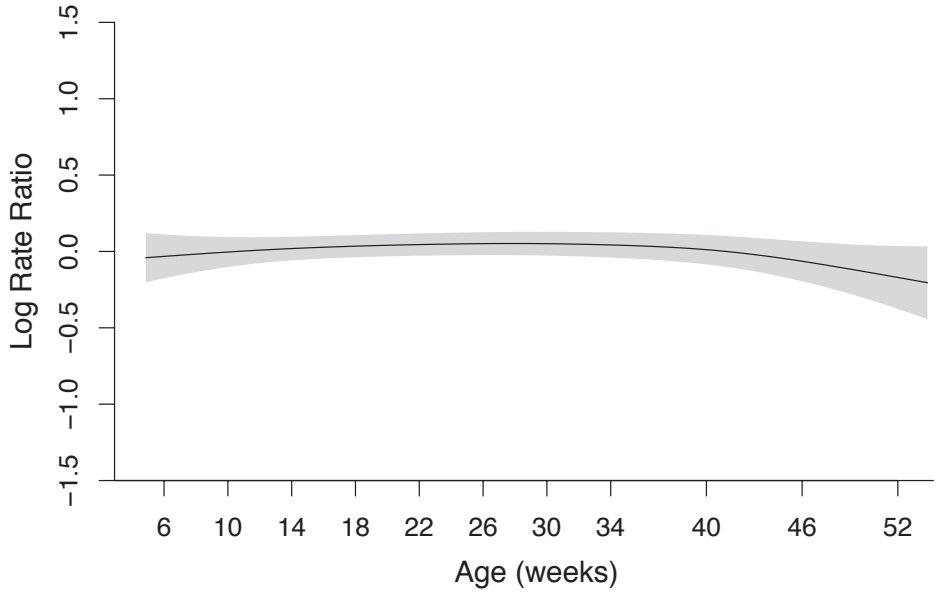


Figure 5.5 Non-parametric spline fit to infant age and pneumococcal incidence in infants
Grey area represents 95% confidence interval.

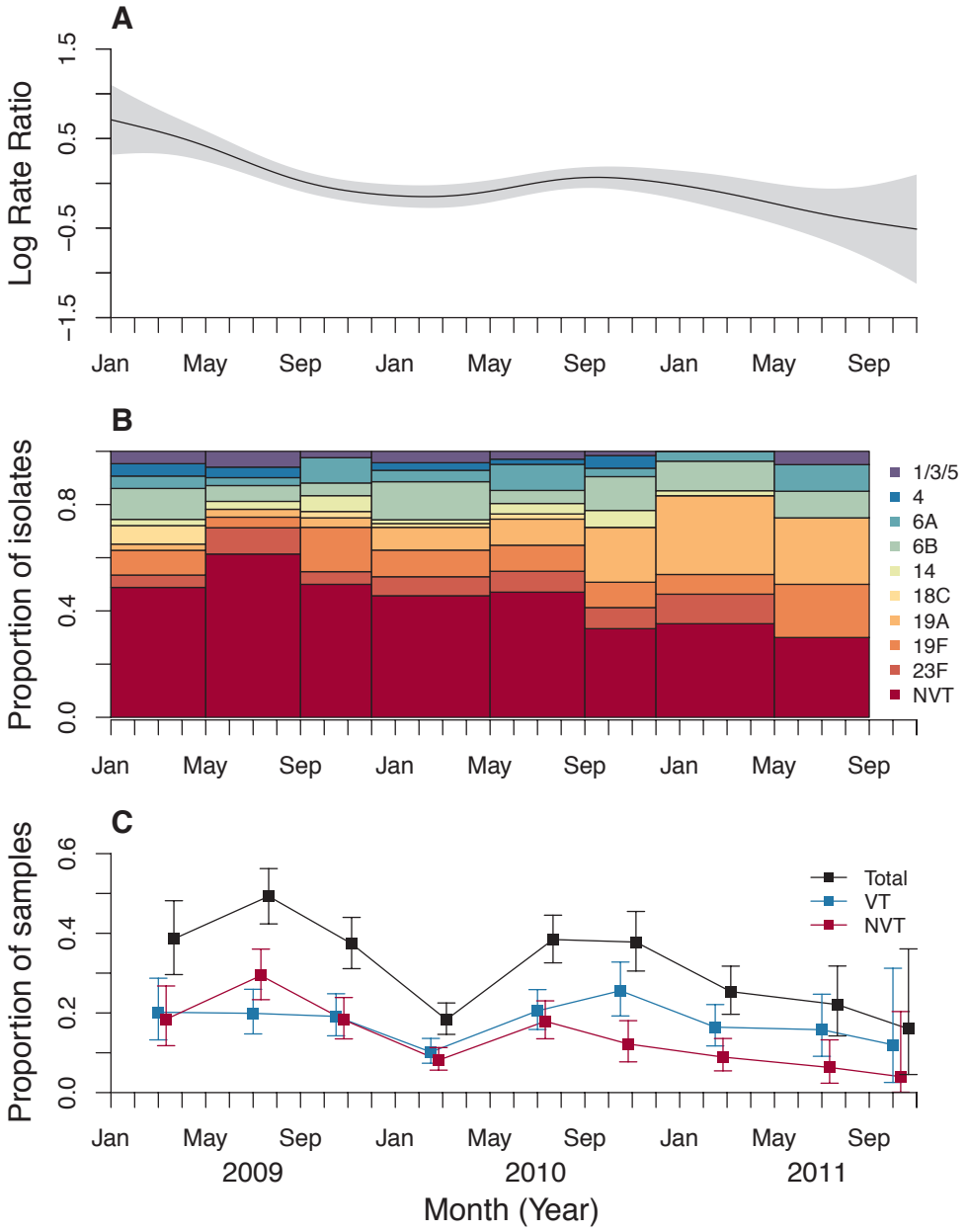


Figure 5.6 Serotype-specific trends of pneumococcal carriage incidence in infants

A. Non-parametric spline fit to secular trend in pneumococcal carriage incidence in infants. Grey area represents 95% confidence interval.

B. Relative abundance of serotypes. Data summarized by season; rain = December-April, cold = May-August, hot = September-November. Data for hot season 2011 omitted because of low number of isolates (n=5). Increase in serotype 19A: $p<0.001$, decrease in nonPCV-13: $p<0.001$ (χ^2 test for trend in proportions)

C. Absolute abundance of serotypes. Data summarized by season: rain = December-April, cold = May-August, hot = September-November. Bars depict 95% confidence intervals. Total decrease: $p<0.001$; VT decrease: $p=0.82$; NVT decrease: $p<0.001$ (χ^2 test for trend in proportions)

5.3.7 Pneumococcal carriage duration

Table 5.4 shows results for duration of carriage in infants, mothers and other children < 5 years. Infants carried pneumococci of the studied serotypes (19F, 19A, 6B, 23F, 6A and 15B) on average for 52.5 days (median 39.5 days). Duration of pneumococcal carriage was shorter in mothers (mean 37.2 days, median 28.5 days, p=0.008) and other children <5 years (mean 38.9 days, median 28.5 days, p<0.001). No significant differences were found between the duration of carriage of the studied serotypes. There were no significant differences in duration of carriage by maternal HIV-status for the infant, mother or other children <5 years.

Table 5.4 Duration of pneumococcal carriage in infants, mothers and other children <5 years

Serotype	Episodes (n)	Duration of carriage (days)	
		Median	Mean
Infants			
19F	47	41.0	58.7
19A	48	40.3	53.2
06B	46	41.5	50.7
23F	36	41.3	52.3
06A	31	35.0	42.7
15B	19	42.0	57.0
Total	227	39.5	52.5
HIV-exposed[a]	76	35.0	44.6
HIV-unexposed	151	41.5	56.5
Mothers			
19F	8	35.5	46.3
19A	11	28.0	32.4
06B	12	28.3	33.1
23F	3	28.0	36.5
06A	8	31.3	37.4
15B	9	35.5	40.7
Total[b]	51	28.5	37.2
HIV-positive[a]	18	31.5	40.6
HIV-negative	33	28.3	35.4
Other children <5 years			
19F	18	28.3	41.7
19A	10	28.3	37.0
06B	21	30.5	40.8
23F	10	28.8	42.6
06A	16	28.5	36.0
15B	8	27.8	31.4
Total[c]	83	28.5	38.9
HIV-exposed[a]	17	29.0	40.2
HIV-unexposed	66	28.5	38.6

[a] No significant differences in duration of carriage by maternal HIV-status for infant, mother or other children <5 years

[b] Difference duration infants and mothers; p=0.008

[c] Difference duration infants and children <5 years; p<0.001

5.4 Discussion

We report similar pneumococcal acquisition in HIV-exposed and HIV-unexposed infants in Malawi. Infant pneumococcal acquisition was associated more with carriage in children <5 years in the household than with maternal carriage, but exposure from both mothers and other children <5 years could only be linked to a limited proportion of acquisitions in the infants.

There is no evidence that pneumococcal acquisition in infants differs by maternal HIV-status, even though pneumococcal prevalence was higher in HIV-positive mothers than in HIV-uninfected mothers. Similar findings were reported from a South African study where no difference in pneumococcal acquisition was found between HIV-unexposed and HIV-exposed infants (133). The South African study reported an association between colonization of the mother and infant, but was not designed to report the direction of spread. A mathematical modelling study on the same data concluded that children transmit pneumococci more frequently to their mothers than vice versa (162). Our results also suggest that infant-to-mother transmission frequently occurs, indicating the importance of defining exposure by previous rather than simultaneous carriage.

Although large associations were found for serotype-specific exposure by the mother or other children <5 years, the proportion of acquisitions that could be explained by these exposures was small: only 1.9% and 8.8% respectively. It is likely that methodological limitations are responsible for the small PAFs, as discussed below, but the possibility of transmission from other household members, or from outside the household cannot be dismissed. Similar results were found in a longitudinal study in the Gambia: the probability of an infant ever carrying a particular serotype was much increased if their mother ever carried it (OR 9.1, 95%CI: 6.4-13.6), but maternal carriage accounted for only 9.5% of infant carriage (95%CI: 7.4-11.6) (119). Infants in Malawi and other African countries are frequently carried on their mother's back: it is plausible that their contact with other members of the community resembles the contact pattern of their mother, increasing the possibility of pneumococcal acquisition from outside the household.

Pneumococcal carriage prevalence was lower in older than in younger infants. Although at first hand this seems to be related to infant ageing, further analysis suggests this is not the case. Similar carriage prevalence trends were observed in older child household members, for whom the ageing hypothesis is less likely. In the

multivariable analysis including infant ageing, seasonal and secular trends as covariates, infant ageing was found not to be significantly associated with pneumococcal carriage acquisition, whereas a significant result was found for secular trends. This suggests the decrease in carriage at later sampling points is a result of a background drop in pneumococcal carriage. There is no obvious explanation for the overall decrease in pneumococcal acquisition over the study period: PCV-13 was not introduced in Malawi until November 2011. The observed reducing carriage incidence is contemporaneous with decreasing rates of invasive disease in Malawi (40). Other possible explanations are long-term ecological trends in pneumococcal disease, on the back of improved food security and nutrition in this population. Lastly, it is also possible that the decrease in pneumococcal carriage was linked to inadvertent changes in sample collection and analysis over time. Older infants are more likely to resist sample collection by moving their head, which could have negatively influenced the sample taken. The same holds true for older children: it is plausible that they showed more resistance to sampling on repeated sampling visits. We observed no changes in the method the study nurses used for sample collection, or the laboratory personnel used for analysis of the samples, but cannot rule out that 'study fatigue' occurred towards the end of the study.

Carriage prevalence in infants was highest in the cold season and lowest at the end of the rainy season. Several factors could underlie these seasonal patterns. Firstly, people spend more time indoors in the cold season, allowing for closer contact between household members, which could lead to enhanced transmission of pneumococci. Also exposure to indoor air pollution, linked to increased pneumococcal carriage (244), is likely to be higher in the cold season when people light more fires for warmth. Secondly, the occurrence of other seasonal respiratory infections such as influenza could have influenced pneumococcal carriage differences by year. Concurrent increases in other respiratory viruses and pneumococcal activity have been reported in other countries (242, 243). Lastly, the cold season in Karonga District corresponds with the school summer holidays: it is possible that older siblings spend more time around the index infants in this period, potentially increasing pneumococcal transmission in the household.

There are several limitations to our study design. Firstly, the sampling interval of 4-6 weeks may not have been short enough to detect all carriage episodes, especially in mothers who clear pneumococcal carriage faster than children (127, 152). Secondly, coverage of household members was incomplete, particularly among older children and adults other than the mother who were often absent during study visits or refused

to participate. This precludes analyses on the role of older children and adults in household transmission. Thirdly, our laboratory procedures did not allow for detection of simultaneous colonization with multiple serotypes. A sub-analysis on 64 samples from 16 infants detected multiple serotypes in 51% of the samples (unpublished results). The low estimated PAFs for exposure by the mother and children <5 years may be a result of this suboptimal detection of carriage. Another limitation of our laboratory procedures is that a large proportion of isolates could not be typed with available reagents, resulting in fewer serotype results available to study transmission within the household. A limitation of our Cox model is that failure to adjust for infant age could have led to residual confounding. Another limitation of the Cox proportional hazards model is that our sampling interval changed over time from four to six weeks. This could have led to an underestimation of hazard rates towards the end of the study period. A sub-analysis including only time points with sampling intervals of four weeks found similar results, suggesting the change in sample intervals did not affect the results found.

In conclusion, maternal HIV-status does not affect pneumococcal acquisition in infants in this African population. Infant pneumococcal acquisition is associated with carriage in other children and mothers, but this could only explain a limited proportion of acquisitions. Our findings suggest that maternal HIV-infection and infant exposure will not affect the impact of the introduction of PCV-13 into this population, although the extent to which PCV-13 effectiveness will mirror the success of this vaccine in high-income settings remains to be established. From our study we cannot conclude whether the majority of infant pneumococcal acquisitions is transmitted within the household or the community. Further understanding of pneumococcal transmission in all age groups is merited as it is vital to our understanding of both vaccine protection and the evolution of non-vaccine serotype replacement disease in the era of conjugate vaccine use.

6. Pneumococcal carriage among HIV-positive adults receiving antiretroviral therapy

6.1 Introduction

Besides young infants, the other group at high risk for IPD are HIV-positive adults. This chapter describes a cohort study investigating pneumococcal carriage in HIV-positive adults in the pre-vaccination period. Antiretroviral therapy (ART) reduces the incidence of IPD in HIV-positive adults (33) and there is a strong temporal relationship between large-scale ART introduction and declines in IPD in Malawi (40, 41). This population impact will be driven by immune reconstitution and/or immune maintenance at the individual level. However, HIV-positive individuals established on ART remain at much higher risk of IPD as compared to HIV-uninfected individuals (42). This suggests immune reconstitution is incomplete; a finding supported by earlier work in Malawi suggesting that ART did not alter the risk of recurrent IPD events (43).

There is limited information on the effect of ART on pneumococcal carriage in HIV-positive adults. Three studies have reported carriage prevalence in ART-treated adults with diverging results. In two immunological studies on HIV-positive Malawian adults, carriage was highest in those receiving ART for more than 12 months (47, 173). The third study in HIV-positive Brazilian adults reported a lower risk of colonization in ART-treated individuals (174). The major limitation in these three studies was the lack of control for potential confounders such as child contacts, whilst the studies in Malawi were not powered to investigate carriage epidemiology effectively. Based on the drop in IPD since introduction of ART in Malawi and the impact of immune reconstitution, we hypothesized that pneumococcal carriage in individuals would decrease when established on ART. Using a cohort design and recruiting attendees at a rural HIV clinic in northern Malawi we investigated the impact of ART on pneumococcal carriage in Malawian adults infected with HIV. The study was undertaken prior to introduction of PCV-13 in the infant immunisation schedule in October 2011.

6.2 Methods

6.2.1 Study design

This study is part of a cohort study on ART eligibility, adherence and outcomes, as described elsewhere (175). The study was set in a rural HIV clinic within the area covered by the Karonga Health and Demographic Surveillance System (HDSS) in northern Malawi (54). HIV prevalence in the area was estimated at 7.1% in men and 9.2% in women in 2008/2009 (56). ART was available in the study clinic since 2006. At the time of the study, individuals were eligible for ART if they had clinical features consistent with World Health Organization (WHO) disease stage 3 or 4, or had a CD4 count of <250 cells/mm3, as per government guidelines. All individuals attending ART clinic were offered cotrimoxazole prophylactic treatment. Pneumococcal vaccination was not provided to HIV-positive adults at the time of the study. All HIV-positive adults and adolescents (>15 years) living in the HDSS who newly attended the clinic and were not already taking ART were invited to participate in the cohort study from January 2008. Detailed clinical data were obtained at baseline and on follow-up visits every three months. CD4 counts were recorded at baseline and at six-monthly intervals. Viral loads were recorded at baseline and at six months after treatment initiation. Individuals not qualifying for ART at baseline were assessed on subsequent visits for qualification. Treatment failure was assessed as a CD4 count <100cells/mm^3 or a fall of CD4 count below pre-therapy baseline after at least 12 months on ART and/or a viral load >10,000 copies/ml after at least 6 months on ART despite good adherence. Poor adherence was defined as missing more than three days of therapy in three months.

This study on the effect of ART on pneumococcal carriage included all individuals recruited between February 2008 and May 2010. Nasopharyngeal samples were taken at baseline and at six-monthly intervals from individuals returning to the HIV clinic until February 2011. We included all individuals with a baseline and at least one follow-up result in the analysis.

The cohort was established with the primary objective of understanding outcomes of HIV care delivery in adults resident in the HDSS. We expected to recruit 500 participants of whom 300 would be commenced on ART. Under these circumstances we would have 80% power to detect a fall in nasopharyngeal carriage by 40% or more following ART initiation from a baseline prevalence of 25% (two-tailed alpha 0.05).

6.2.2 Data analysis

We compared pneumococcal carriage prevalence between individuals started on ART and individuals not started on ART. Individuals starting ART during the follow-up period contributed observation time to both non-ART and ART groups. Proportions of pneumococcal carriage were tested using the Chi-square or Fisher's exact test as appropriate.

Crude and adjusted risk ratios (RR and aRR) for pneumococcal carriage by ART status were obtained by log-binomial regression using generalized estimated equations (GEE) models with an exchangeable correlation structure and robust standard errors to account for any within-person clustering of the data (176). Other factors considered were; sex, age, CD4 count, cotrimoxazole use, WHO-defined clinical disease stage, and household composition. For seasonality, parametric functions with different numbers of sin-cosine waves were examined. For instance, a model with two sin-cosine waves was specified as:

$$y_{i,t} = \alpha + \beta_1 \sin\left(\frac{2\pi t}{365}\right) + \beta_2 \cos\left(\frac{2\pi t}{365}\right) + \beta_3 \sin\left(\frac{4\pi t}{365}\right) + \beta_4 \cos\left(\frac{4\pi t}{365}\right) + \beta_5 ART_{i,t} + \beta_6 covariates_{i,t}$$

where $y_{i,t}$ is the carriage in individual i on day of the year t, β_{1-4} terms are regression coefficients for each sine and cosine function, ART is a binomial variable of the individuals ART-status and $covariates$ are other factors studied.

6.2.3 Sensitivity analyses

Missing CD4 count data were estimated for individuals with at least two CD4 counts available by simple linear regression on two CD4 counts closest in time to the missing data point. Sensitivity analysis was performed excluding individuals with poor adherence or treatment failure. A separate sensitivity analysis was performed including only those individuals that started ART at some point during follow-up.

6.3 Results

6.3.1 Study participants and samples

In total, 468 individuals newly attended the ART clinic between February 2008 and May 2010. Seventy-two individuals (15.4%) did not re-visit the ART clinic because they died (n=22) or departed from the study area (n=23) within 6 months of recruitment or were lost to follow-up for unknown reasons (n=27). At least one follow-up visit was recorded for 396 individuals (84.6%). At least two nasopharyngeal specimens (one at baseline, one at follow-up) were taken from 363 individuals (91.6%). For 26 individuals (7.2%) sample results were not available. One individual was excluded from the analysis because of unknown ART start date.

In total, 336 individuals were included in the analysis, of which 233 individuals started ART during follow-up (Table 1). Individuals completed on average 15.7 months of ART during the follow-up period (range 5-31 months). On average, three nasopharyngeal samples were available per person. The average number of samples was slightly higher in individuals who started ART during follow-up as compared to those who did not (3.1 vs. 2.9 samples, p=0.06). More females (n=207) than males (n=129) attended the ART clinic and participated in the study (p<0.001). Poor adherence was observed for five individuals (2.2%). Treatment failure was observed for 23 of 209 (11.0%) individuals with good adherence and repeated CD4 count and/or viral load results available. Cotrimoxazole prophylactic treatment was reported to be taken during part or all of the follow-period by 94.8% (221/233) of individuals receiving ART during follow-up and 81.5% (84/103) of individuals who did not receive ART during follow-up.

Pneumococcal carriage was detected in 232 out of 1027 samples (22.6%). Pneumococcal carriage prevalence at baseline was similar in individuals who subsequently received ART compared to those who did not (21.0% vs. 21.4%, p=0.99).

Table 6.1 Baseline characteristics of HIV-positive adults who started ART during the follow-up period or not

	Started ART during follow-up (n=233)	Not started ART during follow-up (n=103)
Female sex	131 (56.2%)	76 (73.8%)
Age (mean, sd)	39.9, sd=10.3	37.8, sd=11.4
CD4 count (median, IQR)	174 cells/mm^3 (86-280)	462 cells/mm^3 (365-653)
WHO clinical disease stage	Stage 1: 31 (13.3%) Stage 2: 77 (33.0%) Stage 3: 101 (43.3%) Stage 4: 24 (10.3%)	Stage 1: 34 (33.0%) Stage 2: 69 (67.0%) Stage 3: 0 (0%) Stage 4: 0 (0%)
Pneumococcal carriage	49 (21.0%) - all types 26 (11.1%) - VT 23 (9.9%) - NVT	22 (21.4%) - all types 10 (9.7%) - VT 12 (11.7%) - NVT

6.3.2 Pneumococcal carriage by ART status

Median CD4 count increased over time in patients started on ART, but no decrease in pneumococcal carriage was observed (Figure 1). Individuals started on ART were more likely to have pneumococcal carriage than individuals not started on ART at all subsequent sampling points (25.6% vs. 17.9%, p=0.03) (Figure 1). Amongst individuals on ART, carriage did not differ between those who had undetectable or low (≤400 copies/ml) viral loads and those who had viral loads >400 copies/ml at 6 months after treatment initiation (26.1% vs. 23.3%, p=0.84). In the multivariable analysis including all samples, increased carriage was still observed for individuals on ART, but results were non-significant (aRR 1.22, 95%CI 0.95-1.56) (Table 2). Similar results were maintained when omitting results from individuals with treatment failure or poor adherence (aRR 1.25, 95%CI 0.96-1.63). In the sensitivity analysis with imputed CD4 count data a significant increase in carriage was observed for ART-treated individuals (aRR 1.29, 95%CI 1.03-1.62). Including only individuals who received ART during the follow-up period (n=233) resulted in a weaker association in the same direction (aRR 1.17, 0.86-1.60). There was no evidence that amongst individuals on ART pneumococcal carriage differed by duration of treatment (Table 2, Figure 1).

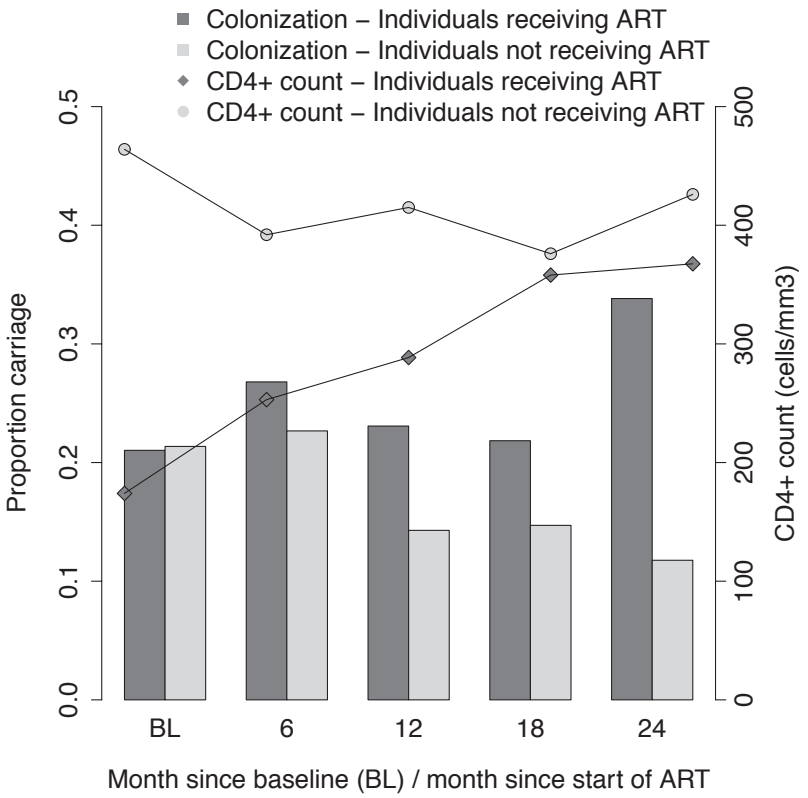


Figure 6.1 Pneumococcal colonization and median CD4 count on baseline and by month since ART/month since baseline in patients receiving ART or not.

6.3.3 Serotype distribution

Most common serotypes isolated were 3 (9.1% of isolates), 19F (6.5%), 6B (5.2%), 11 (4.7%) and 23F (4.7%) (Figure 2). Sixty-six isolates (28.4%) could not be fully typed with the available reagents. There was weak evidence that the diversity of serotypes carried was greater in the ART-treated group than in the ART-untreated groups (32 serotypes in 87 typable isolates vs. 25 serotypes in 79 typable isolates): a comparison of bootstrapped Shannon diversity indices gave a simulated p-value of 0.08. In a pooled analysis of all time points, VT carriage did not differ between individuals on ART or not (9.9% vs. 10.2%, p=0.94), but NVT carriage was higher in individuals on ART (16.1% vs. 9.6% p=0.003). NVT carriage remained significantly higher in the ART-treated group after adjustment in the multivariable analysis (aRR 1.72, 95%CI 1.13-2.62) (Table 2).

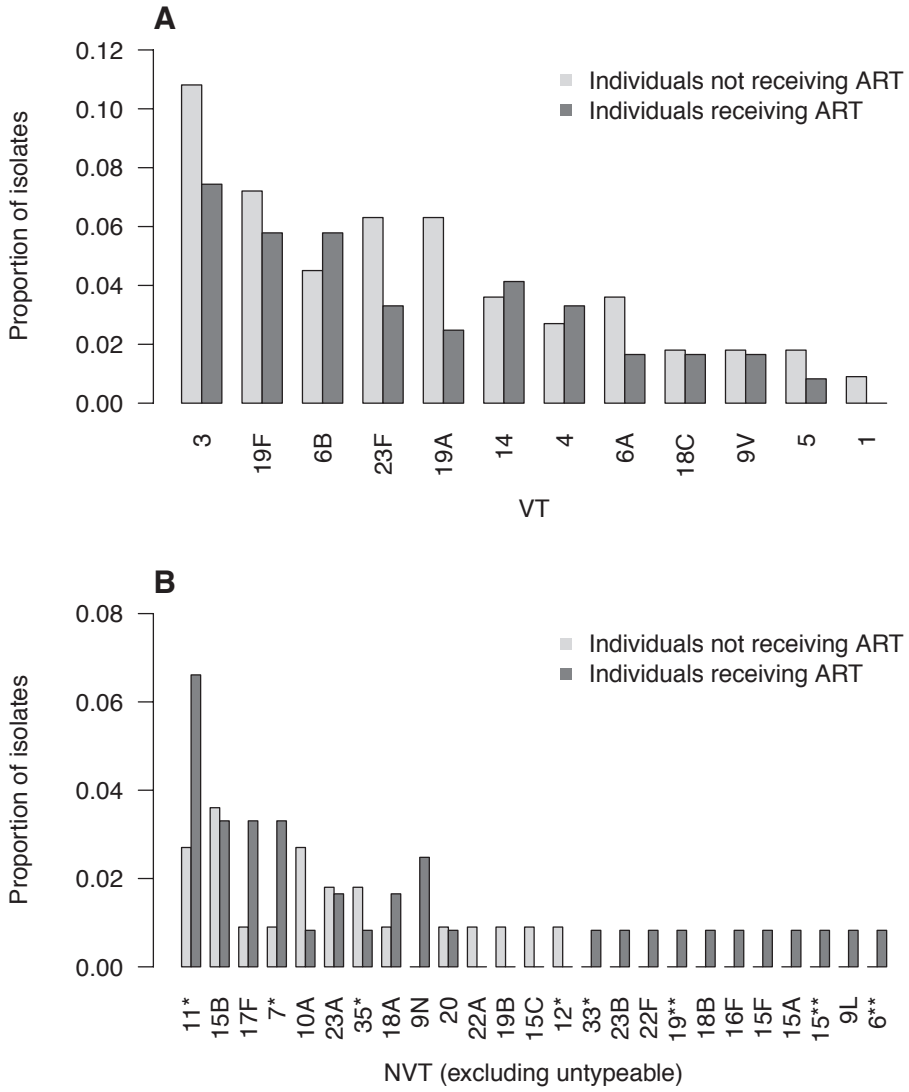


Figure 6.2 Carriage of serotypes including in PCV-13 (A) and not included in PCV-13 (B) by ART status.

* Factor typing not done, ** Not able to establish factor typing

Table 6.2. Risk ratios and 95% confidence intervals (CI) of risk factors associated with pneumococcal carriage in HIV-positive adults.

Risk factor	n	Carriage		Crude RR	95%CI	*P*	Adjusted RR	95%CI	*P*
		n	%						
Antiretroviral treatment (ART)									
Carriage of all serotypes									
Not on ART	560	111	19.8	-			-		
On ART	467	121	25.9	1.29	1.02-1.62	0.03	1.22	0.95-1.56	0.12
VT carriage									
Not on ART	560	57	10.2	-			-		
On ART	467	46	9.9	0.96	0.68-1.37	0.84	0.87	0.57-1.33	0.51
NVT carriage									
Not on ART	560	54	9.6	-			-		
On ART	467	75	16.1	1.64	1.19-2.27	0.003	1.72	1.13-2.62	0.01
Months on ART									
0 (at start ART)	233	53	22.7	-			-		
6	153	41	26.8	1.20	0.76-1.91	0.43	1.26	0.88-1.80	0.21
12	143	33	23.1	1.06	0.68-1.64	0.80	0.85	0.54-1.33	0.47
18	87	19	21.8	0.99	0.57-1.72	0.98	1.03	0.63-1.66	0.92
24	68	23	33.8	1.92	1.10-3.34	0.02	1.59	1.09-2.33	0.02
Sex									
Male	392	61	15.6	-			-		
Female	635	171	26.9	1.68	1.25-2.27	<0.001	1.74	1.26-2.40	<0.001

Table 6.2. Risk ratios and 95% confidence intervals (CI) of risk factors associated with pneumococcal carriage in HIV-positive adults.

Risk factor	n	Carriage		Crude RR	95%CI	*P*	Adjusted RR	95%CI	*P*
		n	%						
Age (years)									
<31	153	45	29.4	-			-		
31-40	424	99	23.3	0.80	0.58-1.10	0.18	0.84	0.60-1.17	0.30
41-50	262	56	21.4	0.76	0.53-1.08	0.13	0.85	0.60-1.21	0.38
>50	188	32	17.0	0.58	0.38-0.89	0.01	0.78	0.48-1.28	0.33
Cotrimoxazole prophylactic treatment (CPT)									
Not on CPT	418	84	20.1	-			-		
On CPT	609	148	24.3	1.22	0.96-1.56	0.11	0.95	0.68-1.34	0.77
CD4count (cells/mm^3)[a]									
> 250	549	105	19.1	-			-		
≤ 250	320	84	26.3	1.35	1.03-1.77	0.03	1.40	1.08-1.82	0.01
WHO stage									
1	563	130	23.1	-			-		
2	324	71	21.9	0.92	0.71-1.18	0.50	1.13	0.85-1.49	0.40
3	112	29	25.9	1.06	0.75-1.50	0.76	1.15	0.80-1.66	0.45
4	28	2	7.1	0.34	0.12-0.99	0.05	0.37	0.10-1.45	0.15

Table 6.2. Risk ratios and 95% confidence intervals (CI) of risk factors associated with pneumococcal carriage in HIV-positive adults.

Risk factor	n	Carriage		Crude RR	95%CI	*P*	Adjusted RR	95%CI	*P*
		n	%						
Children <5 years in household[b]									
No	480	105	21.9	-			-		
Yes	470	109	23.2	1.03	0.79-1.33	0.85	1.05	0.81-1.37	0.70
Number of household members[b]									
0-2	248	52	21.0	-			-		
3-7	611	132	21.6	1.07	0.78-1.47	0.69	1.07	0.77-1.49	0.68
8-15	91	30	33.0	1.50	0.93-2.41	0.10	1.52	0.95-2.41	0.08
Year									
2008	179	33	18.4	-			-		
2009	452	95	21.0	1.13	0.81-1.56	0.47	1.03	0.72-1.48	0.87
2010	395	103	26.1	1.38	0.98-1.95	0.06	1.29	0.85-1.95	0.23

Risk ratios were estimated from log-binomial regression models. Within-person clustering was adjusted for using the generalized estimating equations (GEE) method. ART, seasonality (parametric spline fit), CD4 count and sex were included in the multivariable analysis. Analysis on all serotypes unless specified otherwise. a. CD4 count missing for 158 observations (15.4%). Estimation by linear regression for 137 observations from individuals with at least two CD4 counts available resulted in an aRR for the association of ART with pneumococcal carriage of all serotypes of 1.29 (95%CI 1.03-1.62). b. Household information missing for 77 observations (7.5%).

6.3.4 Risk factors for pneumococcal carriage

Low CD4 count was associated with higher pneumococcal carriage prevalence (aRR 1.40, 95%CI 1.08-1.82 for ≤250 vs. >250 cells/mm3). There was no evidence for an association between pneumococcal carriage and cotrimoxazole prophylactic treatment (aRR 0.95, 95%CI 0.68-1.34). Female sex was associated with higher pneumococcal carriage prevalence (aRR 1.74, 95%CI 1.26-2.40). Individuals living in large households seemed to be more likely to carry a pneumococcus than individuals living in small households (aRR 1.52, 95%CI 0.95-2.41 for 0-2 vs 8-15 household members). There was no evidence for an association between pneumococcal carriage and living with children <5 years of age (aRR 1.05, 95%CI 0.81-1.37). Pneumococcal carriage showed strong seasonality with a model with two sine-cosine pairs providing the best fit (Figure 3). Carriage prevalence was higher in the cold (May-August) and hot (September-November) seasons as compared to the rainy season (December-April).

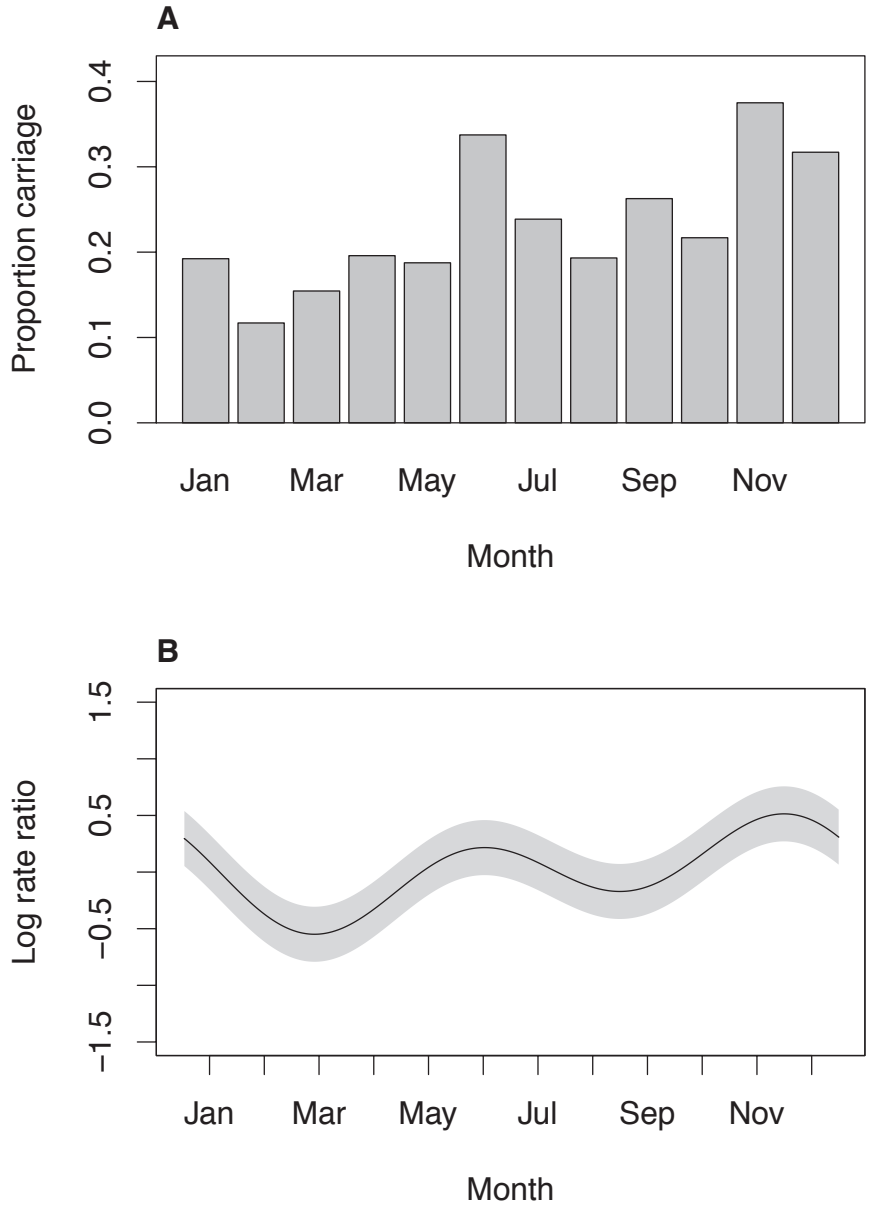


Figure 6.3. Seasonality of pneumococcal carriage.
A. Crude carriage prevalence; B. Fitted parametric spline from adjusted generalized estimated equations (GEE) model. Grey area represents 95% confidence intervals. Black horizontal bars represent months in the rainy season (December-April).

6.4 Discussion

This cohort study provides strong evidence that pneumococcal carriage in HIV-positive adults remains high during the first two years of ART use, with a tendency to increased NVT carriage. Two immunological studies from Malawi have reported consistent findings (47, 173), but this is the first cohort study that addresses important confounders for pneumococcal carriage and provides robust measures of association.

Several of our risk factors for carriage are well known. We found that females are at higher risk of pneumococcal carriage, consistent with their higher contact rates with young children in which pneumococcal carriage is greatest. However, household contact with children <5 years was not found to be independently associated with pneumococcal carriage in HIV-positive adults. A possible explanation for this inconsistency is that child contact may be universal amongst women and not determined by household contact only. Strong seasonality patterns were observed in this study, with lowest carriage observed in the rainy season. This finding is consistent with the household cohort study on pneumococcal carriage conducted in 2009-2011 (Chapter 5).

Our findings are consistent with two immunological studies of HIV-positive adults in Malawi, which reported continued high pneumococcal carriage over a period of 12-18 months on ART (47, 173). Both studies reported failure of mucosal immune responses to normalise after initiation of ART. Similar incomplete mucosal CD4 T-cell immune reconstitution has frequently been reported in gut-associated lymphoid tissue (177). Our findings differ from the two smaller Malawian studies in that we did not find a difference in pneumococcal carriage between symptomatic and asymptomatic HIV-positive adults. We did find evidence for higher NVT carriage, providing further evidence for the hypothesis of “loss of control” of pneumococcal carriage amongst ART-treated adults that was generated from the immunological studies (47, 173). A cross-sectional study from Brazil reported stable ART for one year or more was associated with lower odds of pneumococcal carriage (174). This study was highly male biased (70% in Brazil vs. 38% in our study) and reported low household contact with young children (18% in Brazil vs. 50% in our study), representing different demography and risk factors from African HIV-positive populations.

A decrease in IPD has been observed since roll-out of ART in Malawi (40), yet no decrease in pneumococcal carriage was observed in individuals established on ART. There are several possible explanations for this finding. At an individual level ART-

mediated immune reconstitution may have a different impact on anti-pneumococcal mucosal responses than on systemic responses necessary for the control of IPD. Incomplete recovery of both mucosal and systemic response to the pneumococcus have been measured (47, 173, 178) and a mechanism for differential recovery mediated by T_{reg} cells has been suggested from murine studies (179, 180). Behavioural changes as a result of improved well-being on ART may result in increased social mixing and thus more exposure to pneumococcal transmission. Extending this further, reduced use of antibiotics as a consequence of improved health may lead to less clearance of pneumococcal carriage. The latter explanation is unlikely given the lack of impact of cotrimoxazole on carriage observed in this study.

Several limitations can be identified for this study. Our two-tailed sample size calculations were limited by the geographical boundaries of the cohort and based on a hypothesized 40% change in pneumococcal carriage in ART-treated adults. We recruited less participants than anticipated: 336 were included in this study of which 233 started ART instead of an expected number of 500 participants of whom 300 would commence on ART. Despite this lower sample size than anticipated we can confidently refute our initial hypothesis and conclude that pneumococcal carriage does not decrease after ART initiation. Our study only included data from the first two years of ART initiation. It is possible that different results would be found for a prolonged time on ART. Despite adjusting for CD4 count, WHO-defined disease stage and other potential confounders, residual confounding must also be considered with individuals receiving ART differing from individuals not receiving ART during the study period. A self-controlled analysis including only the 233 individuals receiving ART during follow-up found similar results as reported for the full cohort, suggesting major confounders were not missed. Our laboratory procedures did not allow for detection of simultaneous colonization with multiple serotypes. This will not have impacted our comparison of pneumococcal carriage by ART status but may have affected our conclusions over serotype diversity. Six-month sampling intervals are too long to study carriage duration and investigate whether our findings can be explained by similar carriage incidence but a prolonged carriage in HIV-positive adults on ART. We used a self-reported definition for poor adherence. Underreporting of poor adherence as a result of social desirability could have led to misclassification of patients. The impact of misclassification will have been minimized, however, as patients with unreported poor adherence are likely to have been classified under treatment failure, for which the definition relied on CD4 count and viral load measurements only. In the sensitivity analysis, similar results were maintained when omitting results from individuals with treatment failure or poor adherence.

These findings have implications for HIV care and potentially broader public health. The results suggest a continued respiratory mucosal immune defect after up to two years of ART and are consistent with the continued increased risk of IPD in this population. How long and why this defect persists merits further investigation through follow-up studies. In the meantime it is clear that prophylactic measures to prevent pneumococcal disease remain a priority and a better understanding of the burden of non-invasive pneumococcal disease is required. Rates of IPD have fallen, but pneumonia remains a common and serious problem in the HIV clinic and requires a renewed focus of attention.

Pneumococcal conjugate vaccination is known to work in this population. It has not been recommended for use on the expectation of HIV-positive adults benefiting from herd protection following the introduction of infant pneumococcal vaccination into the Malawi EPI schedule. However the observed high rates of carriage in HIV-positive adults may have implications for pneumococcal transmission and consequently for the scale of herd protection. It is unclear how important HIV-positive adults are in pneumococcal transmission, but they represent a large reservoir of *S.pneumoniae* which is not reduced by ART in at least the first two years of treatment. Herd protection following infant PCV has to date been demonstrated in developed regions with low prevalence pneumococcal carriage in adults (181) and in African centres with low HIV prevalence (25, 182, 183). In South Africa, declines in vaccine-type carriage (184) and IPD (24) in unvaccinated adults suggest indirect effects of PCV in the context of high HIV-burden although it is difficult to distinguish vaccine effects from an ongoing background drop in pneumococcal carriage that predates the introduction of PCV. Surveillance of pneumococcal carriage as well as disease in HIV-positive adults during PCV roll-out would be a prudent measure in Malawi and countries with similar generalized HIV epidemics. Vaccination of HIV-positive adults could be considered to provide both direct protection and maximize herd protection.

In summary, pneumococcal carriage in HIV-positive adults in Malawi remained high despite two years of ART, with evidence of increased NVT carriage. Following PCV introduction monitoring of carriage in HIV-positive adults should be undertaken to determine whether they continue at risk from vaccine serotype pneumococcal disease and whether they constitute a reservoir for persisting vaccine serotype carriage and pneumococcal diversity.

Part II: Post-vaccination period

7. Predictors of uptake and timeliness of newly introduced pneumococcal and rotavirus vaccines, and of measles vaccine

7.1 Introduction

PCV-13 was introduced on 12th November 2011 as part of the infant immunisation schedule, with doses given at 6, 10, 14 weeks alongside Pentavalent vaccine (diphtheria, pertussis, tetanus, *Haemophilus influenza* type B and hepatitis B) and oral polio. Initial catch-up vaccination for PCV-13 in children <1 year of age was conducted at the time of introduction. Currently no PCV booster dose is scheduled.

To understand what effect this introduction has had on pneumococcal carriage and transmission, we need to know what level of vaccination coverage was reached in the birth and catch-up cohorts and whether any PCV-13 is given according to schedule or whether delays occur. High risk groups often achieve poorer coverage than the national average (185). Furthermore, vaccine coverage estimates do not reflect timeliness of vaccination, which may frequently be delayed (186, 187). Previous work in this setting prior to introduction of PCV-13 showed high uptake of vaccines but delays in schedule (188). Understanding any socio-demographic predictors for vaccine uptake and timeliness will help us understand any differences in pneumococcal carriage that may arise post vaccination introduction.

We analysed data from a population-based birth cohort study to investigate factors affecting vaccination coverage and timeliness in the KHDSS during the period of introduction of PCV-13. We took the opportunity to also examine predictors of

monovalent rotavirus vaccine (RV1) and measles vaccine (MV) coverage and timeliness. RV1 was introduced on 29th October 2012 and is given alongside PCV-13 at 6 and 10 weeks. Understanding predictors for RV1 coverage and timeliness will place our findings for PCV-13 into a wider perspective. Measles vaccine (MV) is currently given as a single dose at 9 months of age, but a number of African countries including Malawi are planning introduction of a second dose (189). Nationwide and district level MV campaigns are conducted when necessary. In our study site in Karonga district there have recently been four MV campaigns among infants <1 year of age; one in 2011 and three in 2014. Understanding predictors for MV coverage and timeliness is also relevant for PCV-13 vaccination: if Malawi were to change it's PCV-13 schedule to a "2+1" or "3+1" schedule, it is likely that the booster dose will be given alongside MV to keep the number of visits to the health centre at a minimum.

7.2 Methods

7.2.1 Study population and design

In this prospective population-based birth cohort study we followed up all children born in the KHDSS on or after: 11th November 2010 (thus eligible for PCV-13 catch-up), 30th September 2011 (eligible for routine PCV-13 schedule) and 17th September 2012 (eligible for RV1). Data were collected during the KDHSS annual census at which all households are visited and individual and household socio-demographic data are collected by trained interviewers. Individual socio-demographic data including vaccine status for children <5 years were available until August 2014. Vaccine status and date of vaccination were transcribed from parent-held booklets ("health passports") issued free by the government to all children at birth or first clinic visit. Absent vaccine documentation, parent/guardian reported vaccination status was recorded. Geographical Positioning System (GPS) coordinates were collected to calculate radial distance to nearest tarmac road or main track and to the nearest vaccination centre. We included in the analysis all children eligible for PCV-13 or RV1 who were at least 1 year old at time of interview. We excluded: (1) children who died within the first year of life; (2) children who had migrated into the study area after 6 weeks of age from the PCV-13/RV1 birth cohort or those migrating after start of the PCV-13 catch-up campaign from the catch-up cohort; (3) any child whose date of birth or vaccination status could not be verified by written record. Children with documented evidence of vaccine receipt but lacking date were included in the coverage analysis, but excluded from timeliness analysis. Sensitivity analyses were performed to define risk factors associated with lack of written document and to repeat the main analyses including children without written documentation.

7.2.2 Definitions

Individual uptake of vaccination was defined by the child's written record in the health passport. Population vaccination coverage was calculated as the number of children receiving vaccination by one year of age divided by age-eligible population for each respective vaccine. We were unable to distinguish measles vaccination as a result of mass campaign from routine doses given off schedule. Timeliness of vaccination for birth cohorts was calculated as number of days between the recommended vaccination age and the date vaccine was given, regardless of age. Children who never received the vaccine were right-truncated at one year of age. Delays in later doses subtracted any delays on account of prior doses. Total delay was defined as the number of days between completing the schedule for a particular vaccine (third dose for PCV-13, second dose for RV1, first dose for MV) and the recommended vaccination age. We presumed vaccine non-availability at local clinic when PCV-13 or RV1 were administered later than the corresponding dose of Pentavalent vaccine.

7.2.3 Statistical analysis

Potential individual level predictors of vaccine uptake and clinic level predictors of discordant delays (presumed non-availability) were separately examined using univariable and multivariable Poisson regression with robust standard errors (190, 191), and reported as risk ratios (RR) and adjusted RR (aRR) respectively ("risk" being probability of receiving a vaccine dose) (176). We report timeliness as median days delay (and interquartile range [IQR]). Predictors of vaccine timeliness (total delay in vaccination) were examined using univariable and multivariable Cox regression, and reported as crude and adjusted hazard ratio (HR and aHR) respectively, where HR<1 implies delayed vaccination compared with baseline group ("hazard" being probability of receiving a vaccine dose at time *t*). Although our analysis examined numerous covariates, we did not perform correction for multiple comparisons in univariable analysis (192), but for multivariable models included initially only covariates achieving *P*-value <0.2 in univariable analysis. We retained in the final multivariable model those covariates achieving a likelihood ratio test *P*-value <0.05.

7.3 Results

We visited 2616 vaccine-eligible children at home who were >1 year at time of interview. Of these 51 had no documented birth date, 428 had no written documentation to confirm vaccination status and 152 migrated into the study area after

vaccine-eligible age, leaving 1985 (75.8%) for analysis. Among these, 768 were eligible for PCV-13 catch-up, 820 for PCV-13 by routine schedule prior to RV1 introduction and 397 for both PCV-13 and RV1 (Figure 7.1).

7.3.1 Vaccination coverage

PCV-13 coverage in the catch-up cohort for dose 1, 2 and 3 was 70.2% (539/768), 61.6% (470/763) and 49.8% (378/759), respectively. Among children birth-eligible for PCV-13, coverage by one year of age for each of three doses was 95.4% (1161/1217), 94.4% (1149/1217) and 89.4% (1086/1215), respectively. Among those eligible for RV1, 90.2% (358/397) received one dose and 86.9% (344/396) received two doses. Coverage for MV by one year, regardless of routine dose or mass campaign, was 87.7% (1772/2020) (Figure 7.1).

7.3.2 Vaccine 'non-availability'

In the PCV-13 birth cohort there were 276 of 1007 (27.4%) children who received the third dose of PCV-13 later than the third dose of Pentavalent vaccine (Table 7.1). Of 336 children eligible for RV1, 122 (36.3%) children received second dose RV1 later than the respective Pentavalent dose (Supplementary Table 7.1). Assumed "vaccine non-availability" for PCV-13 decreased with time since national introduction, but vaccine non-availability for RV1 remained high and no clear association with time since introduction was observed. PCV-13 non-availability was higher in the rainy than in the dry season (dose 1: aRR 1.63, CI 1.12-2.37). Non-availability of vaccines was associated with further distance from the road for both PCV-13 dose 1 (aRR1.57, 95%CI 1.07-2.30) and RV1 dose 1 (aRR 2.65, CI 1.68-4.19). Children whose initial PCV-13 doses were delayed compared with Pentavalent, were less likely to complete their PCV-13 series (aRR 0.82, CI 0.76- 0.89 where dose 1 delayed; aRR 0.86, CI 0.81- 0.91 where dose 2 delayed) (Table 7.1).

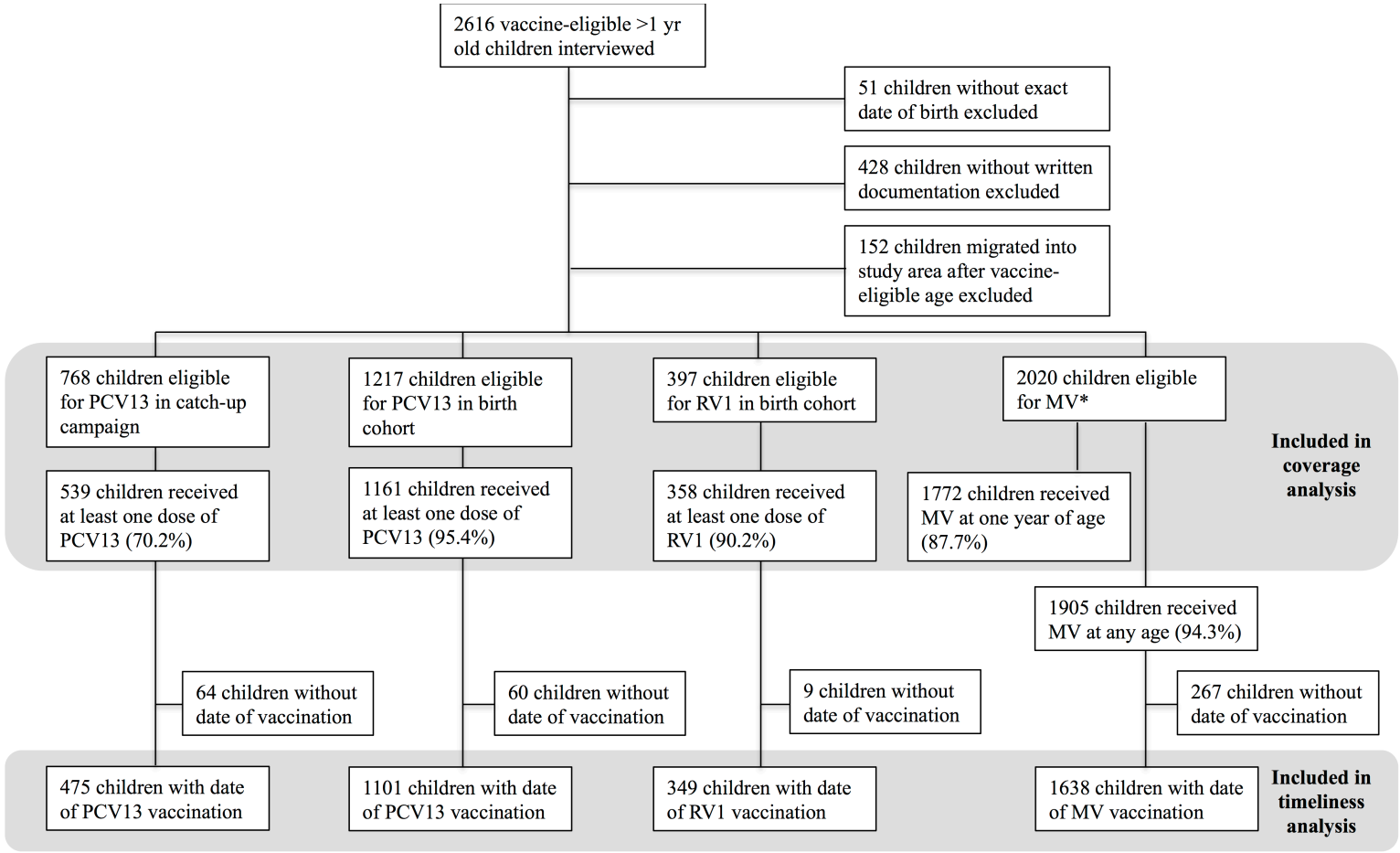


Figure 7.1 Flowchart of eligible children included in vaccination coverage and timeliness analyses.

* There were 35 children for whom written documentation was available for MV but missing for PCV-13/RV1

Table 7.1. Robust Poisson regression for factors associated with PCV-13 given at a later date than Pentavalent vaccine: "vaccine non-availability"

		PCV-13 dose 1				PCV-13 dose 3		
Variable	N	N (%) received dose later than Pentavalent	RR (95% CI)	aRR[1] (95% CI)	N	N (%) received dose later than Pentavalent	RR (95% CI)	aRR[2] (95% CI)
Time since vaccine introduction[3]								
0-3 months	214	47 (22.0%)	1	1	190	78 (41.1%)	1	1
4-6 months	186	32 (17.2%)	0.78 (0.52-1.17)	0.97 (0.63-1.49)	168	65 (38.7%)	0.94 (0.73-1.22)	0.94 (0.73-1.22)
7-9 months	170	27 (15.9%)	0.72 (0.47-1.11)	1.15 (0.65-2.04)	163	54 (33.1%)	0.81 (0.61-1.06)	0.81 (0.61-1.06)
> 9 months	517	57 (11.0%)	0.50 (0.35-0.71)	0.61 (0.41-0.90)	486	79 (16.3%)	0.40 (0.30-0.52)	0.40 (0.30-0.52)
Distance to road (km)								
<1	823	119 (14.5%)	1	1	766	202 (26.4%)	1	1
1-1.49	146	19 (13.0%)	0.90 (0.57-1.41)	0.89 (0.57-1.41)	138	48 (34.8%)	1.31 (1.02-1.71)	1.29 (1.00-1.66)
≥ 1.5	118	25 (21.2%)	1.47 (1.00-2.15)	1.57 (1.07-2.30)	103	26 (25.2%)	0.96 (0.67-1.36)	1.13 (0.80-1.59)
Season[4]								
Dry	566	71 (12.5%)	1	1	600	174 (29.0%)	1	1
Rainy	521	92 (17.7%)	1.60 (1.20-2.14)	1.63 (1.12-2.37)	407	102 (25.1%)	0.86 (0.70-1.07)	0.97 (0.75-1.25)

[1]Adjusted for distance to the main road, season and time since vaccine introduction

[2]Adjusted for time since vaccine introduction

[3]Time between vaccine introduction and PCV-13 due date

[4] Season at time of Pentavalent receipt: dry season = May-November, rain season = December-April

7.3.3 Predictors of vaccination coverage by catch-up campaign

Among children in the PCV-13 catch-up cohort, age at onset of campaign was strongly associated with uptake of third dose of PCV-13 with 77.5% vaccinated among those <4 months old versus 9.7% amongst those 10-12 months old at time of campaign onset (aRR 0.13, CI 0.08-0.22). Children born to mothers aged ≥30 years were less likely to be vaccinated with three doses (aRR 0.77, CI 0.64-0.92). Vaccine coverage with at least one dose of PCV-13 was higher amongst males (aRR 1.11, CI 1.02-1.20), but this effect was no longer observed for coverage with three doses (aRR 1.05, CI 0.93-1.18) (Table 7.2).

7.3.4 Predictors of vaccination coverage by routine schedule

Time since national introduction was an important predictor of vaccination coverage in the PCV-13 birth cohort, with higher coverage >9 months compared to 0-3 months since introduction (aRR 1.09, CI 1.05- 1.13; aRR 1.16, CI 1.09-1.23 for first and third dose respectively) (Table 7.3). Uptake was lower if vaccination was due in the rainy season than in the dry season (aRR 0.97, CI 0.95-1.00 for dose 1). Maternal farming was associated with a lower uptake of PCV-13 (aRR 0.96, CI 0.93-0.98 for dose 1, aRR 0.93, CI 0.89-0.98 for dose 3). Children from larger households or those with more children were more likely to receive PCV-13. Children were less likely to be fully vaccinated for the third dose of PCV-13 if distance to the nearest clinic was ≥1.5km (aRR 0.89, CI 0.81- 0.98).

For RV1 uptake was higher if the mother was not married (aRR 1.08, CI 1.01-1.15 for dose 1) or if maternal age was ≥40 years (aRR 1.10, CI 1.00- 1.21 for dose 2), but lower if distance to the nearest road was ≥1.5km (aRR 0.80, CI 0.69- 0.93 dose 1; aRR 0.75, CI 0.63- 0.90 dose 2). In contrast to the PCV-13 birth cohort, children were less likely to be vaccinated with RV1 if they lived in a household with more than three children <5 years of age (aRR 0.77, CI 0-59-1.00 dose 2) (Supplementary table 7.2).

Infants were less likely to receive MV if there were other children in the household (aRR 0.94, CI 0.91-0.97), if distance to the nearest clinic was ≥1.5km (aRR 0.91, CI 0.85-0.98) or if their mothers were farmers (aRR 0.92, CI 0.89-0.96). Children were more likely to be vaccinated if the mother attended post-primary education (aRR 1.09, CI 1.00-1.18) (Supplementary Table 7.3).

Table 7.2 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the catch-up campaign cohort

		Predictors of being vaccinated with one dose of PCV-13				Predictors of being vaccinated with three doses of PCV-13		
Variable	N	Coverage (%)	RR (95% CI)	a RR[1] (95% CI)	N	Coverage (%)	RR (95% CI)	a RR[2] (95% CI)
Gender								
Female	372	65.9	1	1	367	47.7	1	1
Male	396	74.2	1.13 (1.03 -1.24)	1.11 (1.02- 1.20)	392	51.8	1.09 (0.94 - 1.25)	1.05 (0.93- 1.18)
Child's age at campaign onset								
<4 months	148	85.8	1	1	147	77.5	1	1
4-6 months	235	86.0	1.00 (0.92 -1.09)	1.04 (0.57- 1.88)	231	63.6	0.82 (0.72 -0.94)	0.83 (0.73- 0.95)
7-9 months	238	71.4	0.83 (0.75 - 0.92)	0.42 (0.24- 0.72)	236	43.6	0.56 (0.48 - 0.67)	0.58 (0.49- 0.68)
10-12 months	147	27.2	0.32 (0.24 -0.42)	0.06 (0.03- 0.11)	145	9.7	0.12 (0.08 - 0.21)	0.13 (0.08- 0.22)
Mother's age (yrs)								
<20	125	75.2	1	1	121	63.6	1	1
20-29	408	71.6	0.95 (0.85- 1.07)	1.00 (0.90- 1.11)	406	50.3	0.79 (0.67- 0.93)	0.87 (0.75- 1.00)
30-39	209	68.4	0.91 (0.79- 1.04)	0.95 (0.84- 1.07)	205	44.4	0.70 (0.57- 0.86)	0.77 (0.64- 0.92)
≥ 40	26	38.5	0.51 (0.31- 0.84)	0.65 (0.41- 1.03)	27	22.2	0.35 (0.17- 0.72)	0.51 (0.28- 0.93)
Mother's education								
<5 years primary	63	65.1	1	1	63	47.6	1	1
>= 5 years primary	492	67.6	1.04 (0.85- 1.26)	0.97 (0.83- 1.13)	487	47.6	1.00 (0.75- 1.32)	0.82 (0.65- 1.02)
Secondary / tertiary	212	77.8	1.20 (0.98- 1.45)	1.11 (0.95- 1.30)	208	55.8	1.17 (0.88- 1.56)	0.97 (0.76- 1.23)
Mother's marital status								
Married	682	70.1	1	1	674	49.4	1	1
Unmarried[3]	84	71.4	1.02 (0.88- 1.18)	1.03 (0.91- 1.16)	84	53.6	1.18 (0.75- 1.86)	1.08 (0.90- 1.30)
Mother mobile phone								
No	490	71.8	1	1	485	49.5	1	1
Yes	75	68.0	0.95 (0.80- 1.12)	1.01 (0.87- 1.17)	73	42.5	0.86 (0.65- 1.14)	1.00 (0.76- 1.30)
Mother's occupation								
Farming	710	70.0	1	1	701	48.9	1	1
Other	45	75.6	1.08 (0.91- 1.28)	1.14 (0.95- 1.35)	45	50.1	0.98 (0.72- 1.33)	1.13 (0.82-1.56)
Orphanhood								
Both parents alive	749	70.4	1	1	740	49.9	1	1
Father died	12	58.3	0.83 (0.51- 1.34)	0.82 (0.55- 1.23)	12	50.0	1.00 (0.57- 1.77)	1.09 (0.68- 1.74)

Table 7.2 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the catch-up campaign cohort

		Predictors of being vaccinated with one dose of PCV-13				Predictors of being vaccinated with three doses of PCV-13		
Variable	N	Coverage (%)	RR (95% CI)	a RR[1] (95% CI)	N	Coverage (%)	RR (95% CI)	a RR[2] (95% CI)
Place of birth								
Health centre	668	71.3	1	1	662	50.6	1	1
Home / TBA / other	70	65.7	0.92 (0.77- 1.10)	0.91 (0.78- 1.07)	68	47.1	0.93 (0.71- 1.21)	0.94 (0.75- 1.19)
Housing standard								
1 (lowest)	127	68.5	1	1	126	50.0	1	1
2	280	68.9	1.01 (0.87- 1.16)	1.02 (0.91- 1.15)	275	50.6	1.01 (0.82- 1.25)	1.06 (0.87- 1.27)
3	151	68.2	1.00 (0.85- 1.17)	0.99 (0.87- 1.14)	150	46.7	0.93 (0.73- 1.19)	0.96 (0.77- 1.20)
4 (highest)	124	72.6	1.06 (0.90- 1.24)	1.07 (0.93- 1.22)	124	50.0	1.00 (0.78- 1.28)	1.00 (0.80- 1.26)
Household size								
<4	148	74.3	1	1	145	57.2	1	1
4-6	396	69.7	0.94 (0.84- 1.05)	1.00 (0.91- 1.11)	393	50.1	0.88 (0.74- 1.04)	1.05 (0.88- 1.25)
≥ 7	224	68.3	0.92 (0.81- 1.05)	0.96 (0.86- 1.08)	221	44.3	0.77 (0.63- 0.95)	0.95 (0.78- 1.17)
Number of children <5 years in household								
1	329	70.8	1	1	324	52.5	1	1
2	394	69.5	0.98 (0.89- 1.08)	0.96 (0.88- 1.05)	391	47.3	0.90 (0.78- 1.04)	0.95 (0.83- 1.09)
≥ 3	45	71.1	1.00 (0.82- 1.23)	0.96 (0.81- 1.14)	44	49.8	1.00 (0.74- 0.35)	0.99 (0.76- 1.31)
Distance to road (km)								
<1	579	71.0	1	1	571	49.6	1	1
1-1.5	125	67.2	0.95 (0.83- 1.08)	0.93 (0.84- 1.04)	124	52.4	1.06 (0.88- 1.28)	1.04 (0.89- 1.21)
≥ 1.5	64	68.8	0.97 (0.81- 1.15)	0.92 (0.77- 1.10)	64	46.9	0.95 (0.72- 1.24)	0.89 (0.68- 1.16)

Table 7.2 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the catch-up campaign cohort

		Predictors of being vaccinated with one dose of PCV-13				Predictors of being vaccinated with three doses of PCV-13		
Variable	N	Coverage (%)	RR (95% CI)	a RR[1] (95% CI)	N	Coverage (%)	RR (95% CI)	a RR[2] (95% CI)
Distance to clinic (km)								
<1	514	72.0	1	1	509	50.9	1	1
1-1.49	176	67.1	0.93 (0.83- 1.05)	0.93 (0.84- 1.03)	174	50.6	0.99 (0.84- 1.18)	0.98 (0.85- 1.13)
≥ 1.5	78	65.4	0.92 (0.77- 1.07)	0.94 (0.81- 1.08)	76	40.8	0.80 (0.60- 1.07)	0.85 (0.66- 1.09)
Moved house								
No	742	69.7	1	1	734	49.5	1	1
Yes	26	84.6	1.21 (1.02- 1.44)	1.14 (0.98- 1.31)	25	60.0	1.21 (0.87- 1.69)	1.10 (0.74- 1.38)

[1] Adjusted for child's age at onset of catch-up vaccination campaign and sex

[2] Adjusted for child's age at onset of catch-up vaccination campaign and maternal age

[3] Never married/divorced/widowed

Table 7.3 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the birth cohort

Variable	N	Predictors of being vaccinated with one dose of PCV-13			N	Predictors of being vaccinated with three doses of PCV-13		
		Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)		Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Gender								
Female	634	95.1	1	1	632	89.7	1	1
Male	583	95.7	1.01 (0.98- 1.03)	1.00 (0.98- 1.03)	583	89.0	1.00 (0.96- 1.03)	0.99 (0.95- 1.03)
First dose PCV-13 given later than Pentavalent								
No	-	-	-	-	922	96.2	1	1
Yes	-	-	-	-	163	79.1	0.82 (0.76- 0.89)	0.82 (0.76- 0.89)
Second dose PCV-13 given later than Pentavalent								
No	-	-	-	-	834	97.6	1	1
Yes	-	-	-	-	243	84.0	0.86 (0.81- 0.91)	0.86 (0.81- 0.91)
Time since vaccine introduction[4]								
0-3 months	268	90.7	1	1	268	81.7	1	1
4-6 months	220	92.7	1.02 (0.97- 1.08)	1.03 (0.97- 1.08)	220	86.4	1.06 (0.98- 1.14)	1.07 (0.99- 1.15)
7-9 months	194	94.3	1.04 (0.99- 1.10)	1.02 (0.97- 1.07)	194	89.7	1.10 (1.02- 1.18)	1.10 (1.02- 1.18)
> 9 months	535	99.3	1.09 (1.05- 1.14)	1.09 (1.05- 1.13)	533	94.4	1.15 (1.09- 1.23)	1.16 (1.09- 1.23)
Mother's age								
<20	205	94.2	1	1	205	88.8	1	1
20-29	662	94.7	1.10 (0.97- 1.05)	0.99 (0.95- 1.03)	661	88.5	1.00 (0.94- 1.05)	0.99 (0.94- 1.05)
30-39	312	97.4	1.03 (1.00- 1.08)	1.02 (0.98- 1.06)	312	91.0	1.03 (0.97- 1.09)	1.03 (0.97- 1.09)
≥ 40	36	97.2	1.03 (0.97- 1.10)	1.01 (0.95- 1.08)	35	94.3	1.06 (0.97- 1.17)	1.07 (0.98- 1.17)

Table 7.3 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the birth cohort

Variable	Predictors of being vaccinated with one dose of PCV-13				Predictors of being vaccinated with three doses of PCV-13			
	N	Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)	N	Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Mother's education								
<5 years primary	77	96.1	1	1	77	85.7	1	1
>= 5 years primary	791	95.5	0.99 (0.95- 1.04)	0.99 (0.95- 1.05)	790	89.0	1.04 (0.94- 1.14)	1.04 (0.95- 1.14)
Secondary / tertiary	348	95.1	0.99 (0.94- 1.04)	0.99 (0.94- 1.04)	347	91.1	1.06 (0.96- 1.17)	1.05 (0.95- 1.16)
Mother's marital status								
Married	1085	95.6	1	1	1083	89.6	1	1
Unmarried[5]	130	93.9	0.98 (0.94- 1.03)	0.97 (0.93- 1.02)	130	87.7	0.98 (0.92- 1.05)	0.97 (0.90- 1.03)
Mother mobile phone personal use								
No	926	96.1	1	1	962	90.1	1	1
Yes	155	93.9	0.98 (0.94- 1.02)	0.96 (0.92- 1.00)	165	90.9	1.01 (0.96- 1.06)	0.98 (0.93- 1.04)
Mother's occupation								
Farming	1113	95.2	1	1	1111	88.8	1	1
Other	82	98.8	1.04 (1.01- 1.07)	1.04 (1.02- 1.07)	82	95.1	1.07 (1.02- 1.13)	1.07 (1.02- 1.13)
Orphanhood								
Both parents alive	1194	95.3	1	1	1192	89.3	1	1
Father died	11	100	1.05 (1.04- 1.06)	1.05 (1.02- 1.08)	11	90.9	1.02 (0.15- 9.47)	1.02 (0.85- 1.21)
Mother died	5	100	1.05 (1.04- 1.06	1.01 (0.98- 1.05)	5	100	1.12 (1.09- 1.14)	1.08 (1.03- 1.13)
Place of birth								
Health centre	1091	95.4	1	1	1089	90.1	1	1
Home / TBA / other	114	94.7	0.99 (0.95- 1.04)	1.00 (0.96- 1.05)	114	83.3	0.93 (0.85- 1.01)	0.94 (0.87- 1.03)

Table 7.3 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the birth cohort

Variable	N	Predictors of being vaccinated with one dose of PCV-13			N	Predictors of being vaccinated with three doses of PCV-13		
		Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)		Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Housing standard								
1 (lowest)	158	93.0	1	1	157	82.8	1	1
2	401	95.5	1.03 (0.98- 1.08)	1.02 (0.97- 1.07)	400	90.0	1.09 (1.00- 1.18)	1.06 (0.98- 1.14)
3	168	96.4	1.04 (0.98- 1.09)	1.03 (0.97- 1.08)	168	88.7	1.07 (0.98- 1.17)	1.03 (0.95- 1.13)
4 (highest)	148	93.9	1.01 (0.95- 1.07)	1.00 (0.94- 1.06)	148	89.9	1.09 (0.99- 1.19)	1.04 (0.95- 1.13)
Household size (persons)								
<4	256	92.6	1	1	256	85.9	1	1
4-6	641	96.1	1.04 (1.00- 1.08)	1.04 (1.00- 1.08)	640	90.9	1.06 (1.00- 1.12)	1.05 (1.00- 1.11)
≥ 7	320	96.3	1.04 (1.00- 1.08)	1.04 (1.00- 1.09)	319	89.0	1.04 (0.97- 1.10)	1.04 (0.98- 1.10)
Number of children <5 years in household								
1	495	94.1	1	1	495	88.3	1	1
2	621	96.0	1.02 (0.99- 1.05)	1.00 (0.97- 1.03)	619	89.8	1.02 (0.98- 1.06)	1.02 (0.98- 1.07)
≥ 3	101	98.0	1.04 (1.00- 1.08)	1.03 (0.99- 1.08)	101	92.1	1.04 (0.98- 1.11)	1.07 (1.00- 1.13)
Distance to road (km)								
<1	922	95.3	1	1	921	89.8	1	1
1-1.49	164	95.1	1.00 (0.96- 1.04)	1.00 (0.96- 1.04)	163	92.0	1.02 (0.97- 1.08)	1.05 (1.00- 1.10)
≥ 1.5	131	96.2	1.00 (1.05- 1.05)	1.00 (0.96- 1.04)	131	83.2	0.93 (0.86- 1.00)	0.95 (0.87- 1.03)

Table 7.3 Univariable and multivariable analysis of predictors of pneumococcal vaccine uptake in the birth cohort

Variable	N	Predictors of being vaccinated with one dose of PCV-13			N	Predictors of being vaccinated with three doses of PCV-13		
		Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)		Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Distance to clinic (km)								
<1	856	96.1	1	1	854	90.8	1	1
1-1.49	249	94.8	0.99 (0.95- 1.02)	0.98 (0.95- 1.02)	249	88.8	0.98 (0.93- 1.03)	0.98 (0.93- 1.03)
≥ 1.5	112	91.1	0.95 (0.89- 1.01)	0.95 (0.90- 1.01)	112	80.4	0.86 (0.81- 0.97)	0.89 (0.81- 0.98)
Moved house								
No	1155	95.7	1	1	1153	89.9	1	1
Yes	62	90.3	0.94 (0.87- 1.03)	0.96 (0.88- 1.04)	62	80.7	0.90 (0.79- 1.02)	0.91 (0.80- 1.03)
Season[6]								
Dry	621	97.1	1	1	619	92.7	1	1
Rainy	596	93.6	0.96 (0.94- 0.99)	0.97 (0.95- 1.00)	596	85.9	0.97 (0.93- 1.00)	0.97 (0.93- 1.01)

PCV = Pneumococcal Conjugate Vaccine, TBA = Traditional Birth Attendant

[1] Coverage is percent vaccinated

[2] Adjusted for age at onset of vaccination, number of household members, season and maternal occupation

[3] Adjusted for age at onset of vaccination, distance to the nearest clinic and maternal occupation

[4] Time between vaccine introduction and due date of first dose PCV

[5] Never married/divorced/widowed

[6] Season at due date of PCV: dry season = May-November, rainy season = December-April

7.3.5 Timeliness of vaccination

Figure 7.2 shows the timeliness of vaccination in the eligible birth cohorts. Median time delay for each PCV-13 dose was 17 days (IQR 7-36), 7 days (IQR 0-28) and 7 days (IQR 0-35) respectively. For RV1 doses, median delay was 19 days (IQR 8-36) and 7 days (IQR 0-35) respectively. MV median delay was 20 days (IQR 3-46). Total delay in vaccination was associated with living further away from the road. Living with more under-five children was associated with delays for MV and RV1. Maternal farming and non-facility birth were associated with delays in PCV-13 and MV. Lower maternal education was associated with delayed MV. PCV-13 delay was associated with moving house in the vaccination period and vaccination due in the rainy season, but no such associations were observed for RV1 and MV (Table 7.4, Supplementary table 7.4 & 7.5).

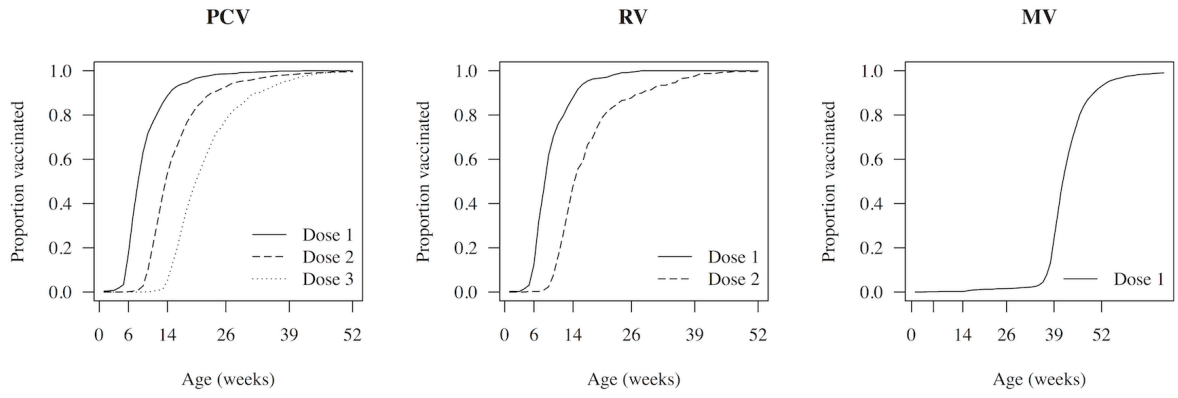


Figure 7.2 Timeliness of pneumococcal conjugate, rotavirus and measles vaccines, among vaccinated children in the Karonga Health and Demographic Surveillance Site

Table 7.4 Survival analysis of predictors of timeliness of pneumococcal vaccination

Variable	N	Median delay in days (IQR)	Crude hazard ratio (95%CI)	Adjusted hazard ratio[1] (95%CI)
Gender				
Female	601	51 (25-102)	1	1
Male	555	53 (23-110)	0.97 (0.86-1.10)	0.99 (0.87-1.13)
Mother's age (yrs)				
<20	186	54 (22-102)	1	1
20-29	629	57 (25-114)	0.97 (0.82-1.16)	0.96 (0.80-1.15)
30-39	304	46 (23-97)	1.10 (0.90-1.33)	1.09 (0.89-1.32)
≥ 40	35	40 (21-99)	1.27 (0.87-1.85)	1.25 (0.85-1.84)
Mother's education				
<5 years primary	74	72 (27-143)	-	-
>= 5 years primary	751	57 (25-114)	1.19 (0.92-1.54)	1.15 (0.88-1.50)
Secondary / tertiary	330	41 (19-92)	1.43 (1.09-1.87)	1.27 (0.96-1.68)
Mother's marital status				
Married	1028	54 (24-107)	1	1
Unmarried [2]	126	46 (19-119)	1.00 (0.82-1.21)	0.97 (0.79-1.19)
Mother mobile phone				
No	919	55 (24-105)	1	1
Yes	159	39 (19-79)	1.21 (1.01, 1.44)	1.08 (0.90-1.30)
Mother's occupation				
Farming	1056	55 (24-113)	1	1
Other	80	32 (18-49)	1.69 (1.33-2.14)	1.80 (1.41-2.28)
Orphanhood				
Both parents alive	1133	51 (24-109)	1	1
Father died	11	46 (13-113)	1.03 (0.55-1.92)	1.20 (0.64-2.24)
Mother died	5	42 (7-69)	1.66 (0.69-4.00)	2.64 (0.98-7.12)
Place of birth				
Health centre	1040	49 (23-100)	1	1
Home / TBA / other	104	71 (35-193)	0.71 (0.57-0.88)	0.77 (0.61-0.97)
Housing standard				
1 (lowest)	146	77 (34-166)	1	1
2	380	53 (24-112)	1.35 (1.09-1.66)	1.25 (1.00-1.55)
3	160	55 (24-107)	1.34 (1.05-1.71)	1.22 (0.95-1.57)
4 (highest)	146	41 (79-91)	1.58 (1.23-2.02)	1.27 (0.97-1.66)
Household size				
<4	235	52 (23-110)	1	1
4-6	611	50 (23-101)	1.10 (0.94-1.30)	1.10 (0.93-1.29)
≥ 7	310	56 (25-115)	1.01 (0.85-1.22)	1.01 (0.84-1.21)
Number of children <5 years in household				
1	469	46 (21-93)	1	1
2	593	58 (26-117)	0.91 (0.80-1.03)	0.95 (0.83-1.08)
≥ 3	94	48 (25-102)	1.01 (0.80-1.28)	1.19 (0.93-1.50)
Distance to road (km)				
<1	875	46 (22-98)	1	1
1-1.49	155	56 (31-92)	0.96 (0.81-1.15)	1.02 (0.85-1.22)
≥ 1.5	126	86 (47-156)	0.65 (0.53-0.79)	0.68 (0.55-0.84)

Table 7.4 Survival analysis of predictors of timeliness of pneumococcal vaccination

Variable	N	Median delay in days (IQR)	Crude hazard ratio (95%CI)	Adjusted hazard ratio[1] (95%CI)
Distance to clinic (km)				
<1	820	48 (22-99)	1	1
1-1.49	230	63 (27-111)	0.89 (0.76-1.04)	0.91 (0.78-1.07)
≥ 1.5	106	57 (30-160)	0.71 (0.57-0.89)	0.80 (0.63-1.01)
Moved house				
No	1102	49 (23-101)	1	1
Yes	54	96 (68-199)	0.54 (0.40-0.74)	0.50 (0.37-0.69)
Season[3]				
Dry	606	50 (25-99)	1	1
Rainy	550	55 (22-119)	0.90 (0.79- 1.02)	0.86 (0.76- 0.98)

TBA = Traditional Birth Attendant

[1] Adjusted for maternal occupation, place of birth, distance from the road, moved house, season.

[2] Never married/ divorced/widowed

[3] At due date of vaccination

7.3.6 Sensitivity analysis including children without written documentation

There were 428 children without written documentation of PCV-13 and RV1 vaccine status. Verbal report of PCV-13 vaccine status was available for 356 children, provided by the mother (44.9%), father (34.3%), a sibling (3.7%), other relative (16.9%) or a non-relative (0.3%). Coverage by verbal report amongst children without written documentation, excluded from the analyses reported above, was lower for the PCV-13 catch-up cohort (45.8% (87/190) dose 1, 45.3% (86/190) dose 2, 45.3% (86/190) dose 3), PCV-13 birth cohort (84.3% (140/166) dose 1, 84.3% (140/166) dose 2, 83.7% (139/166) dose 3) and RV1 (78.0% (39/50) dose 1, 76.0% (38/50) dose 2), but higher for coverage of MV (99.7% (361/362)). Moving house (aRR 0.89, CI 0.80-1.00) and being born to an unmarried mother (aRR 0.93, CI 0.87-1.00) were associated with lack of written documentation. Children born to farming mothers were found to be more likely to have written documentation available (aRR 1.17, CI 1.07-1.29). Including children without written documentation in the primary analyses did not change our main findings, although significance was lost for some risk factors, possibly as a result of misclassification of verbal report of vaccination status. No new risk factors for vaccine uptake were identified in analyses including only children without written documentation.

7.4 Discussion

Malawi has been proactive in the trialling, introduction and post-roll out evaluation of vaccines, and as our data show, has achieved high coverage and good timeliness, even in our remote study setting. As with all universal programmes some gaps remain. Studies that have examined factors associated with vaccination coverage and timeliness of vaccines in sub-Saharan Africa, have found that lower vaccine uptake and delayed vaccination were associated with low socioeconomic status, low maternal education, non-facility birth, and increased distance to a health facility (186-188, 193-209) (Supplementary Table 7.6), consistent with the findings of this study. No studies were conducted in the context of newly introduced PCV-13 and RV1. In this study, despite our communal context of homogenous cultural affiliation and of socioeconomic standing and remoteness, we found programmatic and socio-demographic characteristics that are associated with vaccination coverage and timeliness among individual infants in a rural region of Malawi. The recognition that there exist individual vulnerabilities even in an otherwise homogenous setting is important, and we return to this point in our recommendations.

We made the assumption that if Pentavalent vaccine was received on time but PCV-13 was delayed then this was due to local non-availability of the latter. At the time of PCV-13 introduction in late 2011 Malawi suffered major fuel shortages that impacted on distribution of newly introduced vaccines and many other societal functions. Although we are unable to verify this assumption, presumed non-availability reduced with time since national introduction and was associated with seasonality and distance from the road. More remote health centres generally serve a more scattered population residing away from dense centres, and often away from sealed roads. Such health centres are more difficult to access by the vaccine programme delivery mechanisms; we tried to capture this, albeit imperfectly by including in distance to sealed road. Non-availability of early doses predicted not only total vaccination delay, but also non-completion of the vaccine course. Recognising the challenges in place at the time of PCV-13 introduction, the Malawi National Immunisation Technical Advisory Group conducted several sessions to review lessons learnt from PCV-13 in planning for RV1 introduction. These reviews included wide representation from ministerial, non-government and academic health planners with vaccine programme experience, epidemiologists, procurement, media, finance and transport specialists. Separately conducted coverage estimates from southern Malawi do suggest rapid attainment of

RV1 population coverage (210), although in our more remote setting RV1 non-availability seemed to persist over time.

Despite the decision to provide catch-up vaccination to infants, the country was provided with doses adequate only for the birth cohort (211). Coverage achieved among infants was moderate, but was low amongst older infants (9.7% for dose 3 amongst those 10-12 months at time of campaign onset). It is likely that available PCV-13 doses were prioritised for younger infants, but alternative hypotheses for the lower uptake amongst older infants are that mothers were unaware of the programme for older infants, or that mothers were reluctant or could not afford to make an additional visit to the health centre, but accepted vaccination as part of the routine schedule for younger infants.

We identified several socio-demographic predictors of vaccine coverage and timeliness. Specific associations varied by vaccine and dose and should be interpreted with caution, particularly when results are counterintuitive, conflicting, of borderline significance or so small as to be not meaningful for example RV1 uptake being higher among unmarried mothers, or crowded households being associated with higher uptake of PCV-13 coverage but lower uptake of RV1 and MV. Despite these caveats, we did find that infants living further away from the road or clinic were consistently more likely to be vaccinated late and less likely to complete vaccination as previously found in the same setting (188). Both measures of distance capture slightly different challenges in reaching vaccine services. Distance from road is a measure of remoteness and in this setting a marker for socioeconomic standard. Distance to clinic more directly reflects accessibility. Lower maternal education was consistently associated with lower vaccine uptake and more delays, although significance on multivariable analysis was only retained for MV. This finding provides further evidence for the importance of maternal education in remote rural settings, and of community sensitisation methods other than printed media. Infants born to farming mothers were more likely to be vaccinated late and less likely to complete vaccination for PCV-13 and MV. It is plausible that farming mothers have fewer opportunities to bring their child to the clinic on time. With regards to the catch-up campaign for PCV-13, greater maternal age was associated with lower uptake. For both RV1 and MV living in a household with multiple children <5 years was a risk factor for both lower uptake and delayed timeliness. Infants born outside of health facilities were vaccinated later for PCV-13 and MV. For PCV-13, vaccination due in the rainy season was found to be associated with lower uptake and more delays. In rural Malawi health facilities are spaced approximately every 10km to make access possible by foot. However, during

the rains this is challenging, since torrents frequently wash away bridges and render footpaths impassable and farming communities are busy with planting and weeding. Noting the factors associated with MV coverage is important because if our coverage levels reflect those in the whole population <5 years herd protection against measles will not be achieved.

Vaccination timeliness is important for several reasons. Optimising vaccine schedules requires balancing benefits of delayed vaccination, such as prolonged immunity, against protection at an early age in the context of high force of infection (212, 213). Timeliness is also a marker of the functionality of the national vaccine delivery system so is of inherent interest to health service planners.

Limitations

Our study has limitations. Excluding children if written documentation of dates was unavailable may lead to an overestimation of vaccine coverage for PCV-13 and RV1 since reported coverage was lower among children without documentation. Coverage estimates for measles may have been underestimated if doses given during district-wide campaigns were unrecorded, consistent with higher coverage reported by parents than documented in health passport. A sensitivity analysis including children without written documentation did not alter the main findings of this study. Our data could not distinguish whether written documentation was missing because the health passport was truly lost, or because the mother, generally in charge of keeping the children's health passports, was not available at time of the interview. The low proportion of mothers available at time of interview for children for whom written documentation was not available (44.9% vs. 84.3% for children with documentation) suggests that both scenarios occur. We included only children surviving to one year of age. We hypothesise that children dying in infancy are more likely to be unvaccinated, and this is currently being investigated (214); results are anticipated in 2016. Our data come from an area under continuous demographic surveillance which may have an increased vaccine uptake. Although vaccine uptake may be higher in our Demographic Surveillance Site than in other areas, the risk factors we found for low uptake or poor timeliness of vaccination are likely to be relevant to other rural African settings not under continuous demographic surveillance. *A fortiori*, if in an area with relatively good service provision we identified children whose circumstances adversely affect vaccine coverage, then in other rural areas with similar socio-demographics but less functioning health systems, such children are very likely to be under-served. In the absence of reliable data on stock-outs, we made the assumption that delay in new vaccines when older vaccines were given on time was due to non-availability of new vaccines. The

validity of this assumption cannot be confirmed. Our definition would miss non-availability of all vaccines, however in practice initial post-introduction delivery to clinics of PCV-13 and RV1 occurred separately from routine vaccine delivery.

Conclusion

Although vaccination coverage is moderately high in this rural population of northern Malawi, we found that infants born to lower educated mothers or farming mothers and those living further away from the road or clinic were at greater risk of being not fully vaccinated and being vaccinated late. We also found delays in vaccination to be associated with non-facility birth. Vaccine stock-outs which were more likely during the rains resulted in both a delay in vaccine timeliness and in fewer infants being fully vaccinated. Countries introducing new vaccines should (i) ensure adequate stock and resources for planned catch-up campaigns and strengthen system required for rapid roll-out and delivery. (ii) Understand who remains most vulnerable so that focussed delivery to improve access to immunisation occurs. This is crucial for maximising the equitable benefits of universal vaccination programmes. (iii) Ensure culturally appropriate and understandable health information about vaccines is widely available together with a continued focus on making vaccines as accessible as possible to families on the social margins. These suggested recommendations are essential if the full benefits of vaccination programmes are to be realized among the most vulnerable.

Supplementary tables

Supplementary table 7.1: Robust Poisson regression for factors associated with rotavirus vaccine given at a later date than Pentavalent vaccine: "vaccine non-availability"

		RV1 dose 1				RV1 dose 2		
Variable	N	N (%) received dose later than Pentavalent	RR (95% CI)	aRR[1] (95% CI)	N	N (%) received dose later than Pentavalent	RR (95% CI)	aRR[2] (95% CI)
Time since vaccine introduction[3]								
0-3 months	79	18 (22.8%)	1	1	77	30 (39.0%)	1	1
4-6 months	90	23 (25.6%)	1.58 (0.97-2.56)	1.64 (1.02-2.64)	87	33 (37.9%)	1.11 (0.79-1.55)	1.14 (0.82-1.58)
> 6 months	179	29 (16.2%)	1.41 (0.83-2.38)	1.55 (0.91-2.64)	172	59 (34.3%)	1.14 (0.80-1.61)	1.27 (0.87-1.84)
Distance to road (km)								
<1	270	44 (16.3%)	1	1	265	86 (32.5%)	1	1
1-1.49	37	16 (43.2%)	2.65 (1.68-4.19)	2.65 (1.68-4.19)	34	23 (67.7%)	2.08 (1.56-2.79)	2.08 (1.56-2.79)
≥ 1.5	41	10 (24.4%)	1.50 (0.82-2.74)	1.50 (0.82-2.74)	37	13 (35.1%)	1.08 (0.68-1.73)	1.08 (0.68-1.73)
Season[4]								
Dry	154	34 (22.1%)	1	1	137	48 (35.0%)	1	1
Rainy	194	36 (18.6%)	0.84 (0.55-1.28)	0.86 (0.57-1.30)	199	74 (37.2%)	1.06 (0.79-1.42)	1.02 (0.76-1.37)

[1] Adjusted for distance to the main road and time since vaccine introduction

[1] Adjusted for distance to the main road

[2] Time between vaccine introduction and RV1 due date

[3] Season at time of Pentavalent receipt: dry season = May-November, rain season = December-April

Supplementary table 7.2. Univariable and multivariable analysis of predictors of rotavirus vaccine uptake

	Predictors of being vaccinated with one dose of Rotavirus vaccine				Predictors of being vaccinated with two doses of Rotavirus vaccine			
Variable	N	Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)	N	Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Gender								
Female	201	90.1	1	1	201	86.1	1	1
Male	196	90.3	1.00 (0.94- 1.07)	1.01 (0.50- 2.04)	195	87.7	1.02 (0.94- 1.10)	1.01 (0.94- 1.09)
First dose RV1 given later than Pentavalent								
No	-	-	-	-	278	96.4	1	1
Yes	-	-	-	-	69	94.3	0.99 (0.94- 1.05)	1.00 (0.95- 1.05)
Time since vaccine introduction[4]								
0-3 months	198	91.9	1	1	197	88.3	1	1
4-6 months	109	83.5	0.91 (0.83- 1.00)	0.92 (0.84- 1.00)	109	80.7	0.91 (0.82- 1.02)	0.93 (0.84- 1.02)
>7 months	90	94.4	1.03 (0.96- 1.10)	1.03 (0.96- 1.10)	90	91.1	1.03 (0.95- 1.12)	1.03 (0.94- 1.13)
Mother's age (years)								
<20	62	93.6	1	1	62	90.3	1	1
20-29	228	89.5	0.96 (0.88- 1.04)	1.00 (0.92- 1.07)	227	86.3	0.96 (0.87- 1,05)	1.00 (0.91- 1.09)
30-39	98	88.8	0.95 (0.86- 1.04)	0.98 (0.90- 1.07)	98	84.7	0.94 (0.83- 1.05)	0.97 (0.87- 1.08)
≥ 40	8	100	1.07 (1.00- 1.14)	1.07 (0.99- 1.16)	8	100	1.11 (1.02- 1.20)	1.10 (1.00- 1.21)
Mother's education								
<5 years primary	27	74.1	1	1	27	70.4	1	1
≥ 5 years primary	260	90.8	1.23 (0.98- 1.54)	1.19 (0.97- 1.45)	259	86.9	1.23 (0.96- 1.58)	1.19 (0.95- 1.49)
Secondary / tertiary	109	92.7	1.25 (0.99- 1.57)	1.19 (0.97- 1.46)	109	90.8	1.29 (1.00- 1.66)	1.22 (0.97- 1.53)

Supplementary table 7.2. Univariable and multivariable analysis of predictors of rotavirus vaccine uptake

	Predictors of being vaccinated with one dose of Rotavirus vaccine				Predictors of being vaccinated with two doses of Rotavirus vaccine			
Variable	N	Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)	N	Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Mother's marital status								
Married	354	89.6	1	1	353	86.4	1	1
Unmarried[5]	42	95.2	1.06 (0.99- 1.15)	1.08 (1.01- 1.15)	42	90.5	1.05 (0.94- 1.16)	1.05 (0.96- 1.15)
Mother mobile phone								
No	326	89.6	1	1	325	86.2	1	1
Yes	65	92.3	1.03 (0.95- 1.12)	1.02 (0.94- 1.10)	65	90.8	1.05 (0.96- 1.15)	1.04 (0.95- 1.13)
Mother's occupation								
Farming	365	90.1	1	1	364	86.8	1	1
Other	27	88.9	0.99 (0.85- 1.13)	0.96 (0.86- 1.07)	27	88.9	1.02 (0.89- 1.18)	0.98 (0.88- 1.10)
Orphanhood								
Both parents alive	389	90.0	1	1	388	86.7	1	1
Father died	4	100	1.11 (1.08- 1.15)	1.22 (1.02- 1.46)	4	75.0	0.86 (0.49- 1.52)	0.96 (0.64- 1.45)
Mother died	2	100	1.11 (1.08- 1.15)	1.12 (0.91- 1.38)	2	100	1.15 (1.11- 1.20)	1.20 (0.96- 1.49)
Place of birth								
Health centre	367	90.2	1	1	366	87.4	1	1
Home / TBA / other	25	88.0	0.98 (0.84- 1.13)	0.97 (0.91- 1.03)	25	76.0	0.87 (0.69- 1.09)	0.93 (0.74- 1.15)
Housing standard								
1 (lowest)	42	86.7	1	1	41	82.9	1	1
2	127	88.2	1.03 (0.90- 1.18)	0.95 (0.81- 1.10)	127	84.3	1.02 (0.87- 1.19)	0.93 (0.77- 1.11)
3	54	87.0	1.02 (0.86- 1.19)	0.95 (0.81- 1.12)	54	85.2	1.03 (0.86- 1.23)	0.95 (0.79- 1.14)
4 (highest)	38	94.7	1.11 (0.96- 1.28)	0.97 (0.83- 1.14)	38	89.5	1.08 (0.90- 1.29)	0.93 (0.76- 1.13)
Household size (persons)								
<4	70	92.9	1	1	70	90.0	1	1
4-6	220	88.6	0.95 (0.88- 1.03)	1.00 (0.91- 1.09)	219	85.8	0.95 (0.87- 1.04)	0.98 (0.89- 1.09)
≥ 7	107	91.6	0.99 (0.90- 1.08)	1.06 (0.97- 1.16)	107	86.9	0.97 (0.87- 1.08)	1.02 (0.92- 1.14)

Supplementary table 7.2. Univariable and multivariable analysis of predictors of rotavirus vaccine uptake

	Predictors of being vaccinated with one dose of Rotavirus vaccine				Predictors of being vaccinated with two doses of Rotavirus vaccine			
Variable	N	Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)	N	Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Number of children <5 years in household								
1	148	94.6	1	1	148	91.9	1	1
2	226	88.9	0.94 (0.89- 1.00)	0.97 (0.91- 1.03)	225	85.3	0.93 (0.86- 1.00)	0.96 (0.90- 1.03)
≥ 3	23	73.9	0.78 (0.61- 1.00)	0.81 (0.63- 1.03)	23	69.6	0.76 (0.58- 1.00)	0.77 (0.59- 1.00)
Distance to road (km)								
<1	297	93.9	1	1	297	91.6	1	1
1-1.49	45	84.4	0.90 (0.79- 1.02)	0.90 (0.80- 1.02)	45	77.8	0.85 (0.72- 1.00)	0.85 (0.73- 1.00)
≥ 1.5	55	74.6	0.79 (0.68- 0.93)	0.80 (0.69- 0.93)	54	68.5	0.75 (0.62- 0.90)	0.75 (0.63- 0.90)
Distance to clinic (km)								
<1	284	92.3	1	1	283	89.1	1	1
1-1.49	84	88.1	0.95 (0.88- 1.04)	0.98 (0.91- 1.07)	84	85.7	0.96 (0.87- 1.06)	1.00 (0.91- 1.10)
≥ 1.5	29	75.9	0.82 (0.67- 1.01)	0.90 (0.73- 1.11)	29	69.0	0.77 (0.60- 0.99)	0.85 (0.65- 1.10)
Moved house								
No	383	90.1	1	1	382	87.2	1	1
Yes	14	92.9	1.03 (0.89- 1.20)	1.07 (0.90- 1.28)	14	78.6	0.90 (0.68- 1.19)	0.96 (0.74- 1.24)

Supplementary table 7.2. Univariable and multivariable analysis of predictors of rotavirus vaccine uptake

	Predictors of being vaccinated with one dose of Rotavirus vaccine				Predictors of being vaccinated with two doses of Rotavirus vaccine			
Variable	N	Coverage[1] (%)	Crude RR (95% CI)	Adjusted RR[2] (95% CI)	N	Coverage (%)	Crude RR (95% CI)	Adjusted RR[3] (95% CI)
Season[6]								
Dry	178	91.6	1	1	178	89.3	1	1
Rainy	219	89.0	0.97 (0.88- 0.96)	1.01 (0.84- 1.10)	218	84.9	0.95 (0.88- 1.03)	0.95 (0.88- 1.03)

RV1 = Monovalent Rotavirus Vaccine, TBA = Traditional Birth Attendant

[1] Coverage is percent vaccinated

[2] Adjusted for age at onset of vaccination, distance to the nearest main road and number of children <5 years in the household

[2] Adjusted for distance to the nearest main road and number of children <5 years in the household

[4] Time between vaccine introduction and due date of first dose RV1

[5] Never married/divorced/widowed

[6] Season at due date of RV1 receipt: dry season = May-November, rainy season = December-April

Supplementary table 7.3. Univariable and multivariable analysis of measles vaccine uptake

Variable	N	Coverage (%)	RR (95% CI)	a RR[1] (95% CI)
Gender				
Female	1025	87.5	1	1
Male	995	87.9	1.00 (0.97-1.04)	1.00 (0.97-1.04)
Mother's age (yrs)				
<20	332	91.0	1	1
20-29	1093	88.0	0.97 (0.93-1.01)	0.99 (0.95-1.03)
30-39	530	85.9	0.94 (0.90-0.99)	0.96 (0.92-1.01)
≥ 40	63	82.5	0.91 (0.81-1.02)	0.91 (0.81-1.03)
Mother's education				
<5 years primary	146	80.8	1	1
>= 5 years primary	1304	87.4	1.08 (0.97-1.17)	1.08 (0.99-1.17)
Secondary / tertiary	568	90.1	1.12 (1.03-1.21)	1.09 (1.00-1.18)
Mother's marital status				
Married	1801	88.0	1	1
Unmarried[2]	215	85.6	0.97 (0.92-1.03)	0.95 (0.89-1.00)
Mother mobile phone				
No	1478	87.1	1	1
Yes	245	88.2	1.01 (0.96-1.06)	0.99 (0.95-1.05)
Mother's occupation				
Farming	1744	87.1	1	1
Other	130	96.2	1.10 (1.06-1.15)	1.09 (1.05-1.13)
Orphanhood				
Both parents alive	1976	87.6	1	1
Father died	23	87.0	0.99 (0.85-1.16)	1.00 (0.86-1.16)
Mother died	7	100.0	1.14 (1.12-1.16)	1.12 (1.09-1.16)
Place of birth				
Health centre	1787	88.3	1	1
Home / TBA / other	191	81.7	0.93 (0.86-0.99)	0.94 (0.88-1.01)
Housing standard				
1 (lowest)	293	84.0	1	1
2	694	86.7	1.03 (0.98-1.09)	1.02 (0.96-1.09)
3	325	88.3	1.05 (0.99-1.12)	1.03 (0.96-1.10)
4 (highest)	278	91.1	1.08 (1.02-1.15)	1.04 (0.97-1.11)
Household size (persons)				
<4	409	90.7	1	1
4-6	1057	88.0	0.97 (0.93-1.00)	1.01 (0.97-1.06)
≥ 7	554	85.0	0.94 (0.89-0.98)	0.98 (0.93-1.03)
Number of children <5y in household				
1	837	91.2	1	1
2	1035	84.8	0.93 (0.90-0.96)	0.94 (0.91-0.97)
≥ 3	148	88.5	0.97 (0.91-1.03)	0.99 (0.93-1.05)
Distance to road (km)				
<1	1526	88.7	1	1
1-1.49	295	85.8	0.97 (0.92-1.02)	1.00 (0.95-1.05)
≥ 1.5	199	83.4	0.94 (0.88-1.00)	0.98 (0.92-1.05)

Supplementary table 7.3. Univariable and multivariable analysis of measles vaccine uptake

Variable	N	Coverage (%)	RR (95% CI)	a RR[1] (95% CI)
Distance to clinic (km)				
<1	1397	89.3	1	1
1-1.49	431	85.9	0.96 (0.92-1.00)	0.97 (0.93-1.01)
≥ 1.5	192	80.7	0.90 (0.84-0.97)	0.91 (0.85-0.98)
Moved house				
No	1931	87.9	1	1
Yes	89	84.3	0.96 (0.88-1.05)	0.96 (0.88-1.05)
Season[3]				
Dry	1158	87.9	1	1
Rainy	862	87.5	1.00 (0.96- 1.03)	0.99 (0.96- 1.04)

[1] Adjusted for maternal occupation, children <5 years in household, distance to clinic
[2] Never married/divorced/widowed
[3] At time measles vaccine dose due

Supplementary table 7.4 Survival analysis of predictors of timeliness of rotavirus vaccination

Variable	N	Median delay in days (IQR)	Crude hazard ratio (95%CI)	Adjusted hazard ratio[1] (95%CI)
Gender				
Female	197	45 (20-101)	1	1
Male	190	41 (18-116)	1.05 (0.84-1.30)	1.06 (0.85-1.32)
Mother's age				
<20	58	33 (16-87)	1	1
20-29	222	46 (21-100)	0.87 (0.64-1.18)	1.04 (0.75-1.44)
30-39	98	44 (19-145)	0.81 (0.57-1.14)	0.97 (0.68-1.39)
≥ 40	8	6 (5-16)	2.91 (1.38-6.14)	3.47 (1.63-7.41)
Mother's education				
<5 years primary	27	46 (19-*)	1	1
>= 5 years primary	255	48 (22-113)	1.34 (0.84-2.14)	1.07 (0.66-1.72)
Secondary / tertiary	104	30 (11-68)	1.77 (1.08-2.90)	1.36 (0.82-2.24)
Mother's marital status				
Married	345	45 (19-110)	1	1
Unmarried[2]	41	31 (13-67)	1.24 (0.88-1.75)	1.22 (0.85-1.74)
Mother mobile phone personal use				
No	317	46 (20-113)	1	1
Yes	65	29 (13-73)	1.32 (0.99-1.75)	1.25 (0.94-1.66)
Mother's occupation				
Farming	356	45 (19-104)	-	-
Other	27	28 (12-67)	1.23 (0.81-1.86)	1.15 (0.76-1.77)
Orphanhood				
Both parents alive	379	42 (18-104)	1	1
Father died	4	61 (13-176)	0.62 (0.20-1.93)	0.71 (0.23-2.23)
Mother died	2	35 (35-86)	1.09 (0.27-4.37)	1.13 (0.27-4.65)
Both died	-		-	-

Supplementary table 7.4 Survival analysis of predictors of timeliness of rotavirus vaccination

Variable	N	Median delay in days (IQR)	Crude hazard ratio (95%CI)	Adjusted hazard ratio[1] (95%CI)
Place of birth				
Health centre	358	42 (18-101)	1	1
Home / TBA / other	24	37 (30-215)	0.76 (0.47-1.22)	0.92 (0.57-1.50)
Housing standard				
1 (lowest)	41	62 (29-169)	1	1
2	124	49 (22-145)	1.09 (0.74-1.60)	0.86 (0.58-1.29)
3	52	34 (16-101)	1.28 (0.82-2.01)	1.00 (0.62-1.59)
4 (highest)	38	26 (12-65)	1.73 (1.07-2.78)	1.22 (0.74-2.01)
Household size (persons)				
<4	66	35 (21-81)	1	1
4-6	215	49 (19-119)	0.85 (0.63-1.13)	1.09 (0.76-1.55)
≥ 7	106	31 (18-81)	1.01 (0.73-1.41)	1.49 (1.02-2.18)
Number of children <5 years in household				
1	144	31 (13-73)	1	1
2	220	47 (23-133)	0.72 (0.57-0.90)	0.80 (0.64-1.00)
≥ 3	23	64 (19-*)	0.52 (0.31-0.87)	0.55 (0.33-0.92)
Distance to road (km)				
<1	290	33 (16-77)	1	1
1-1.49	44	73 (44-215)	0.51 (0.36-0.74)	0.53 (0.37-0.75)
≥ 1.5	52	72 (36-*)	0.47 (0.33-0.67)	0.50 (0.35-0.71)
Distance to clinic (km)				
<1	277	44 (18-89)	1	1
1-1.49	82	38 (22-134)	0.88 (0.67-1.14)	0.95 (0.73-1.25)
≥ 1.5	28	46 (19-*)	0.61 (0.39-0.98)	0.71 (0.44-1.14)
Moved house				
No	374	41 (19-104)	1	1
Yes	13	57 (21-97)	0.80 (0.43-1.52)	0.96 (0.51-1.82)
Season[3]				
Dry	138	42 (18-97)	1	1
Rainy	249	41 (19-113)	0.92 (0.74- 1.15)	0.96 (0.77- 1.20)

TBA = Traditional Birth Attendant

[1]Adjusted for distance to road and number of children <5 in the household

[2] Never married/divorced/widowed

[3] At time of due date of vaccination

* No 75th percentile delay reported: >25% not vaccinated

Supplementary table 7.5 Survival analysis of predictors of timeliness of measles vaccination

Variable	N	Median delay in days (IQR)	HR (95%CI)	aHR[1] (95%CI)
Gender				
Female	898	23 (5-52)	1	1
Male	900	22 (4-53)	1.03 (0.94, 1.14)	1.00 (0.91, 1.11)
Mother's age				
<20	279	22 (3-49)	1	1
20-29	982	22 (4-50)	0.97 (0.85, 1.11)	1.03 (0.89, 1.19)
30-39	477	27 (6-60)	0.87 (0.75, 1.02)	0.95 (0.81, 1.12)
≥ 40	58	29 (10-56)	0.76 (0.56, 1.03)	0.83 (0.61, 1.13)
Mother's education				
<5 years primary	126	28 (6-83)	1	1
>= 5 years primary	1163	23 (5-54)	1.33 (1.09, 1.62)	1.30 (1.06, 1.59)
Secondary / tertiary	508	20 (4-48)	1.47 (1.19, 1.81)	1.34 (1.08, 1.67)
Mother's marital status				
Married	1605	23 (4-52)	1	1
Unmarried[2]	190	20 (6-55)	0.98 (0.84, 1.15)	0.91 (0.77, 1.07)
Mother mobile phone personal use				
No	1328	24 (5-54)	1	1
Yes	223	20 (4-54)	1.03 (0.89, 1.19)	0.96 (0.82, 1.12)
Mother's occupation				
Farming	1646	23 (5-55)	1	1
Other	123	16 (2-32)	1.45 (1.21, 1.75)	1.37 (1.12, 1.66)
Orphanhood				
Both parents alive	1760	23 (4-52)	1	1
Father died	20	16 (6-57)	1.04 (0.67, 1.62)	1.07 (0.68, 1.69)
Mother died	5	34 (22-44)	1.25 (0.52, 3.00)	1.18 (0.44, 3.17)
Both died	-		-	-
Place of birth				
Health centre	1604	22 (4-50)	1	1
Home / TBA / other	160	27 (4-74)	0.77 (0.65, 0.92)	0.82 (0.69, 0.98)
Housing standard				
1 (lowest)	258	27 (4-62)	1	1
2	617	26 (5-55)	1.04 (0.90, 1.21)	1.00 (0.86, 1.17)
3	283	22 (5-59)	1.09 (0.91, 1.30)	1.00 (0.84, 1.20)
4 (highest)	249	19 (4-45)	1.23 (1.03, 1.47)	1.06 (0.88, 1.29)
Household size				
<4	354	21 (4-48)	1	1
4-6	953	22 (4-50)	0.94 (0.83, 1.07)	1.05 (0.90-1.23)
≥ 7	491	28 (6-61)	0.82 (0.71, 0.94)	0.94 (0.79-1.11)
Number of children <5y in household				
1	742	20 (4-48)	1	1
2	925	25 (5-56)	0.86 (0.78, 0.95)	0.89 (0.80, 0.98)
≥ 3	131	26 (4-62)	0.82 (0.67, 0.99)	0.83 (0.68, 1.00)
Distance to road (km)				
<1	1362	21 (4-49)	1	1
1-1.49	259	23 (6-58)	0.89 (0.78, 1.03)	0.96 (0.83-1.10)
≥ 1.5	177	32 (11-73)	0.78 (0.66, 0.92)	0.85 (0.71-1.01)

Supplementary table 7.5 Survival analysis of predictors of timeliness of measles vaccination

Variable	N	Median delay in days (IQR)	HR (95%CI)	aHR[1] (95%CI)
Distance to clinic (km)				
<1	1251	21 (4-50)	1	1
1-1.49	375	25 (7-54)	0.92 (0.81, 1.03)	0.95 (0.84, 1.07)
≥ 1.5	172	27 (4-77)	0.80 (0.67, 0.94)	0.86 (0.72, 1.02)
Moved house				
No	1723	23 (4-52)	1	1
Yes	75	27 (6-74)	0.87 (0.68, 1.11)	0.88 (0.69, 1.13)
Season[3]				
Dry	1042	22 (4-55)	1	1
Rainy	756	24 (5-49)	0.97 (0.87- 1.07)	0.96 (0.87- 1.06)

[1] Adjusted for maternal education, maternal occupation, number of children <5 years in the household and place of birth.

[2] Never married/divorced/widowed

[3] At due date of measles vaccination

Supplementary table 7.6 Literature reporting risk factors for low vaccine uptake and/or late vaccination in sub-Saharan countries

Reference	Data source	Country	Rural/urban	Risk factors for low vaccine uptake and/or late vaccination
Abadura SA et al, 2015 (193)	DHS	Ethiopia	Both	-non-facility birth -low maternal education -low socio-economic status -resident in rural area -increasing number of children <5 years in the household
Babalola S, 2008 (194)	Household survey	Nigeria	Both	-non-facility birth -maternal age <20 years -low maternal education -low socio-economic status
Babirye JN et al, 2012 (195)	Cross-sectional survey	Kampala, Uganda	Urban	-non-facility birth -increasing number of children per woman -household poverty
Bosch-Capblanch X et al, 2012 (196)	Review of DHS	96 low & middle income countries including Malawi	Both	-household poverty -low education status of caregiver and caregiver's partner -caregivers not received tetanus toxoid vaccine
Canavan ME et al, 2014 (197)	Review of DHS in Africa	Burundi, Ethiopia, Kenya, Rwanda, Tanzania, and Uganda	Both	-non-facility birth
Fadness LT et al, 2011 (187)	Secondary analysis data from cluster randomised controlled trial	Uganda	Both	-low maternal education
Fadness LT et al, 2011 (198)	Secondary analysis data from cluster randomised controlled trial	South Africa	Both	-low maternal education -non-facility birth -resident in rural area
Favin M et al, 2012 (199)	Review of grey literature	Global (53.9% African)	Both	-long distances to health facilities -low socio-economic status -low parental educational status

Supplementary table 7.6 Literature reporting risk factors for low vaccine uptake and/or late vaccination in sub-Saharan countries

Reference	Data source	Country	Rural/urban	Risk factors for low vaccine uptake and/or late vaccination
Glatman-Freedman A & Nichols K, 2012 (200)	Review	Low, middle & high income countries	Both	-low socio-economic status -low parental education particularly maternal education. -long distances to health facilities
Gram L et al, 2014 (186)	Secondary analysis of HDSS	Ghana	Rural	-resident in rural area -low maternal education -low socio-economic status
Jahn A et al, 2008 (188)	HDSS	Malawi	Rural	-non-facility birth -low parental education -long distances to health facilities -low socio-economic status
Jani JV et al, 2007 (201)	Cross-sectional survey	Mozambique	Rural	-long distances to health facilities -low maternal education -non-facility birth
Le Polain de Waroux O et al, 2012 (202)	Cluster survey	Southern Tanzania	Rural	-long distances to health facilities -household poverty -low maternal education -rainy season
Malawi Demographic and Health Survey 2010 (204)	DHS	Malawi	90% of the population live in rural areas.	-resident in urban area -low maternal education -low socio-economic status
Munthali AA, 2007 (203)	DHS 1992, 1996, 2000, 2004	Malawi	Both	-low maternal education -resident in rural areas -increased child birth order

Supplementary table 7.6 Literature reporting risk factors for low vaccine uptake and/or late vaccination in sub-Saharan countries

Reference	Data source	Country	Rural/urban	Risk factors for low vaccine uptake and/or late vaccination
Odutola A et al, 2015 (205)	Cross sectional survey	Western Gambia	Both	-increased child birth order -non-facility birth
Payne S et al, 2014 (206)	HDSS	Gambia	Both	-resident in urban areas -ethnicity (Mandinka)
Rainey JJ et al, 2011 (207)	Systematic literature review	Global literature published between 1999-2009	Both	-low maternal education -low socio-economic status -long distances to health facilities -high costs (direct and indirect) -fear of adverse events
Schoeps A et al, 2013 (208)	HDSS	Burkina Faso	Both	-low maternal education -low socio-economic status -dry season at birth -long distances to health facilities
Wysonge CS et al, 2012 (209)	DHS	24 countries in sub-Saharan Africa	Both	-low maternal education -low paternal education -low socio-economic status -resident in urban areas -countries with high fertility rates

8. Pneumococcal carriage and transmission in the post-vaccination period

8.1 Background

In chapter 7 we discussed the introduction of PCV-13 in November 2011 and the levels of vaccine coverage and timeliness achieved by August 2014. This section of the thesis will discuss the impact of PCV-13 on pneumococcal carriage. With the introduction of PCV-13, VT carriage is expected to be reduced in vaccinated children. If a herd effect has taken place as a result of reduced transmission from vaccinated children, reduced carriage prevalence is also expected to occur in unvaccinated individuals.

This chapter includes the findings of a longitudinal and cross-sectional study performed in 2014, 2.5 years after introduction of PCV-13. We repeated sample collection of the same age groups as were studied in 2008-2011: infants, mothers and their household members, and HIV-positive adults attending a local ART clinic.

The objectives of the studies were:

1) To measure the direct impact and indirect impact of PCV-13 vaccination on pneumococcal carriage in households, by measuring changes in VT and NVT serotype carriage in vaccinated and unvaccinated individuals in households in Karonga District, comparing the pre-vaccination (2009-2011) and post-vaccination (2014) period.
2) To measure the indirect impact of PCV-13 vaccination on pneumococcal carriage in HIV-positive adults attending antiretroviral therapy (ART) clinic, by measuring changes in VT and NVT serotype carriage in HIV-positive adults

attending an ART clinic in Karonga District, comparing the pre-vaccination (2008-2010) and post-vaccination (2014) period.

3) To study characteristics of carriage in infants, other household members <5 years old and mothers using a 2-week study interval (instead of a 4-6 week study interval as was used before), to inform the analysis of longitudinal data collected in 2009-11.

8.2 Methods

8.2.1 Study design

This analysis combines the results of three related studies: a longitudinal household study on mother-infants pairs and any household members <5 years of age; a cross-sectional study on mother-infant pairs and any household members <15 year; and a cross-sectional study on HIV-positive adults attending a local ART clinic. The combination of a longitudinal and cross-sectional household study was adopted to increase sample size for infants 6 weeks of age, and to include older children. Because participation of older children in longitudinal sampling was found unsatisfactory in the earlier study conducted in 2009-2011, older children (5-15 years) were only included in the cross-sectional component of this study to focus sampling efforts.

Household studies

Recruitment of mother-infant pairs took place in the postnatal clinics of two rural hospitals between April and July 2014. All mother-infant pairs that were discharged within one week of delivery and were living within the KHDSS area were eligible for inclusion in the study. For the longitudinal study, nasopharyngeal swabs were collected at the household from the infant, mother and any other household members <5 years at 6, 8, 10, 12, 14, 16, 18 weeks of the index infant's age (Table 8.1). In addition, a sample was taken from the mother and any household members <5 years around the first week of the index infant's age to assess exposure in the first weeks of life. No sample from the index child was taken at this point: a pilot study performed in the cold (peak) season in 2010 on carriage acquisition in infants showed that all 24 infants included were negative for *Streptococcus pneumoniae* at week 1 (unpublished results). In the cross-sectional study, a nasopharyngeal sample was taken at 6 weeks of the infant's age. A nasopharyngeal sample was taken from all household members <15 years willing to participate.

HIV-status of the mother was transcribed from her health passport. If the health passport was unavailable the mother was asked for her HIV-status verbally. HIV-status reported verbally was crosschecked with the KHDSS database from the ART clinic.

Index infants were offered all routine vaccinations including PCV-13 at 6, 10, and 14 weeks of age. Vaccination status of other household members <5 years was obtained from the KHDSS records.

Table 8.1 Overview of household carriage studies conducted before (2009-2011) and after (2014) introduction of PCV-13

Year	Participants	Sampling weeks								
		1	6	8	10	12	14	16	18	… 52[a]
2009-2011 longitudinal	Infant, mother, all hh members		X		X		X		X	X
2014 longitudinal	Infant, mother, children <5yrs	X[b]	X	X	X	X	X	X	X	
2014 cross-sectional	Infant, mother, children <15yrs		X							

[a] Sampling continued at 22, 26, 30, 34, 40, 46, 52 weeks

[b] Week 1 sample not collected for infants

HIV-positive adults attending ART clinic

HIV-positive adults were recruited from the local ART clinic between May and August 2014. All HIV-positive adults attending the local ART clinic who were living in the KHDSS were eligible for participation in the study, regardless of ART status. A nasopharyngeal sample was taken at the ART clinic. Household characteristics and ART prescription history were obtained from the KHDSS records. Using unique identifiers we linked participation in this study to individual records of nasopharyngeal swabs taken in the study conducted in 2008-2010 (chapter 6).

8.2.2 Sample size

Cross-sectional analysis

Sample size calculations by age group are presented in Table 8.1. Sample size calculations were based on the prevalence of VT serotypes measured in 2009-11. We hypothesized that a 70% reduction of VT carriage occurred in vaccine-eligible and non-vaccine-eligible age groups in frequent contact with vaccinated individuals (infants, mothers and children 5-14 years). We hypothesized that a 60% reduction of VT carriage occurred in HIV-positive adults attending the ART clinic. Sample sizes were calculated based on one sample per individual for the children and for HIV-positive individuals presenting to the ART clinic. Too few mothers (n=201) were recruited in the PVC study in 2009-11 to allow for a comparison based on one sample per individual, given the low baseline VT carriage of adults (6.0%) We therefore used all available

samples (multiple samples per individual) in the prevalence comparison. We calculated the design effect (Deff), the factor by which the sample would need to be inflated to adjust for this use of multiple samples per person. For this, we calculated the intraclass correlation coefficient (ICC) to determine the correlation between samples of the same individual, and between different individuals:

$ICC = \frac{\sigma b^2}{\sigma b^2 + \sigma w^2}$ with $\sigma b^2 =$ between-individual variance and

$\sigma w^2 =$ within-individual variance

$Deff = 1 + (m - 1) * ICC$ with m= number of samples per individual (m=8 in 2014)

Sample and power calculations were performed using the 'pwr' and 'ICC' packages in the statistical software programme R 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) (167, 215, 216).

Table 8.2 presents power calculations for the index infants at 6 weeks of age. Recruitment of at least 134 infants was required to allow a detection of 70% reduction in VT carriage with 80% power (α=0.05).

Longitudinal analysis

Table 8.3 shows the power calculations for a survival analysis, comparing VT acquisition in infants 2009-2011 (50% of 185 infants acquired a VT serotype at least once in 18 weeks) with VT acquisition in infants in 2014. Recruitment of at least 35 infants was required to allow detection of a 70% reduction in acquisition of VT pneumococcus in the first 18 weeks of life. Recruiting 40 mother/infant pairs would allow the detection of a 70% reduction with 86% power and a 60% reduction with 77% power.

Table 8.2 Sample size calculations by age group – cross-sectional study

Age group	Prevalence VT carriage pre-vaccination (2009-11)	Expected reduction	Expected prevalence VT carriage post-vaccination (2014)	Number of individuals (samples) 2009-11	Minimum sample size 2014*
Household study					
6 wks	11.4%	70%	3.4%	185 (185)	134
18 wks	32.1%	70%	9.6%	165 (165)	29
1-3 yrs (vaccine-eligible)	24.1%	70%	7.2%	104 (586)	51
4-14 yrs	18.3%	70%	5.5%	227 (889)	59
Mothers	6.0%	70%	1.8%	201 (1858)	276** (134 persons)
Study at ART clinic					
HIV-positive adults	10.3%	60%	4.1%	544 (1227)	171

* Power calculation for two proportions (different sample sizes), α=0.05, power = 0.80, two-sided.

** Using all available samples (n=1858) from 2009-11 ICC = 0.09, Deff = 1.63. Sample size for 2009-11 unadjusted = 169 (unadjusted) * 1.63 = 276 (adjusted).

Table 8.3 Power calculations for cross-sectional study on 6-week infants

(Hypothesized scenario highlighted)

2014 sample size (2009-11=185)	Reduction in VT serotype carriage (two-sided test)			
	50%	60%	70%	80%
100	0.38	0.54	0.72	0.87
110	0.40	0.57	0.75	0.89
120	0.42	0.59	0.77	0.91
130	0.44	0.61	0.79	0.92
140	0.45	0.63	0.81	0.93
150	0.47	0.65	0.82	0.94

Table 8.4 Power calculations for longitudinal study infants - survival analysis comparing 2009-11 and 2014

(Hypothesized scenario highlighted)

2014 sample size	Reduction in PCV-13 serotype acquisition (two-sided test)			
(2009-11=185)	50%	60%	70%	80%
30	0.54	0.67	0.77	0.85
35	0.59	0.72	0.82	0.89
40	0.64	0.77	0.86	0.92
45	0.68	0.81	0.89	0.94

8.2.3 Changes to analysis design post sample size calculations

Based on the above presented sample size calculations, we aimed to recruit at least 134 mother-infant pairs, of which at least 35 participated in the longitudinal study. We aimed to recruit at least 171 HIV-positive adults attending the ART clinic.

During the analysis phase of this study four changes to the original analysis design were made. Firstly, it became evident that matching the pre- and post-vaccination period on month of sampling was crucial for the household study: seasonality was shown in the pre-vaccination period to be a strong predictor of pneumococcal carriage in this population (results in chapters 5). Adjusting all results from both periods on sampling month, for instance using sine-cosine waves, proved difficult because sampling in the post-vaccination period was only performed during April-August, with no estimates available from September-March. It was therefore decided to only use samples collected in the same calendar months (April-August) when comparing both sampling periods in the household study. In the study on HIV-positive adults attending the local ART clinic in 2008-2010, no difference was found between carriage in May-August (study period 2014) and September-April. Hence, all samples from the pre-vaccination study were included for this analysis.

Secondly, only a few HIV-positive mothers (n=4) were recruited post-vaccination introduction. This is not surprising, given that mothers in the post-vaccination period were not selected based on their HIV-status. Because HIV-status was found to be associated with carriage amongst mothers in the pre-vaccination period, comparisons amongst mothers in the pre- and post-vaccination period included only HIV-negative mothers. For infants and other children, all samples were included because maternal

HIV-status was not found to be associated with their pneumococcal carriage (chapter 5).

Thirdly, the above sample size calculations for children 1-14 years and HIV-positive adults on ART were based on a single sample per person. Multiple samples per person were included in the analysis: within-person clustering was tested for using random effect models.

Lastly, the above sample size calculations for children 1-3 years were based on vaccine eligibility, not on vaccine receipt. Because information on vaccine receipt was available during the analysis, this group was further divided into fully vaccinated and non-vaccinated children.

8.2.4 Statistical analysis

Carriage prevalence ratios (PR) were calculated for the periods before and after vaccine introduction. Potential confounders were identified by testing the association between variables and the vaccine period: any potential confounding variable with a p-value of < 0.1 that was *a priori* thought to be associated with pneumococcal carriage was included in the multivariable models. Adjusted prevalence ratios (APR) were calculated using log-binomial regression, or Poisson regression with robust standard errors if the log-binomial regression failed to converge (169).

Acquisition of first pneumococcal carriage in infants was assessed by survival analysis including Kaplan-Meier plots and log-rank tests (168). For the comparison of mean time to first acquisition of any or VT carriage between 2009-2011 and 2014, only samples from weeks 6, 10, 14 and 18 were used, to allow for direct comparison. All available samples were used for analyses including samples collected in 2014 only.

Vaccine effectiveness (VE) against new acquisition of VT carriage was calculated as: $VE_c = 1 - RR$, with RR being the rate ratio of new acquisition of VT carriage in the post-vaccination period as compared to the pre-vaccination period. Only samples from weeks 6, 10, 14 and 18 collected in 2009-2011 and 2014 were used, to allow for direct comparison. A new acquisition event was defined as the identification of a serotype when the same serotype was not identified at the previous two sampling times.

Serotype-specific analyses on household exposure and duration of carriage were conducted using the same definitions as in chapter 5. Serotyping using latex

agglutination did not allow for the distinction between the factortypes of certain NVT, e.g. 18A/B or 7A/7B/7C. For the serotype-specific analyses we included those serogroups where no further distinction was possible and regarded them to be the same serotype. Samples with result “NVT” without further detail available were excluded from analysis on carriage duration, but included on the serotype-specific analysis on household exposure for mothers and children <5 years, because too few typeable isolates were available to exclude “NVT” results in this analysis.

8.3 Results: household study

8.3.1 Study participants and samples in the post-vaccination period

There were 156 mother-infant pairs approached to participate in the study (Figure 8.1). Two mothers refused to take part in the study, one infant died before participation, and one infant was excluded from the study because of a prolonged stay in hospital. Four recruited mother-infant pairs were lost to follow-up after recruitment. In total, 148 mother-infant pairs participated in the study: the first 44 in the longitudinal study, the latter 104 in the cross-sectional study. One mother withdrew consent for her own participation, but allowed her infant to continue in the longitudinal study. Documented HIV test results were available for 88.5% (131/148) mothers. Four (2.7%) mothers were HIV-positive (two with documented, two with verbal report): two in the longitudinal and two in the cross-sectional study. Of the HIV-negative mothers, 97.9% (141/144) reported to have been tested within the last year. In the longitudinal study there were 23 other household members <5 years; at least one nasopharyngeal sample was collected from all of them. In the cross-sectional study there were 54 household members <5 years and 121 household members 5-15 years: a nasopharyngeal sample was collected from 51 (94.4%) and 89 (73.6%) respectively.

All infants participating in the study received PCV-13. Amongst the 77 household members <5 years, 25 (32.5%) were age-eligible for vaccination in the birth cohort: all had received full vaccination with PCV-13 at time of the study. Twenty-eight children (36.4%) were age-eligible for vaccination in the catch-up cohort; 15 (53.6%) had received full vaccination, 4 (14.3%) had received partial vaccination and 9 (32.1%) had received no vaccination. Twenty-three children (29.9%) were not age-eligible for PCV-13 and had not received the vaccine. One child’s birth date and hence vaccine eligibility was unknown: this child had not received PCV-13 vaccination.

In total, 1140 samples were collected: 792 in the longitudinal and 348 in the cross-sectional arm of the study. Six samples were lost (3 on initial storage, 3 during laboratory analysis), leaving 1334 samples for analysis on pneumococcal carriage.

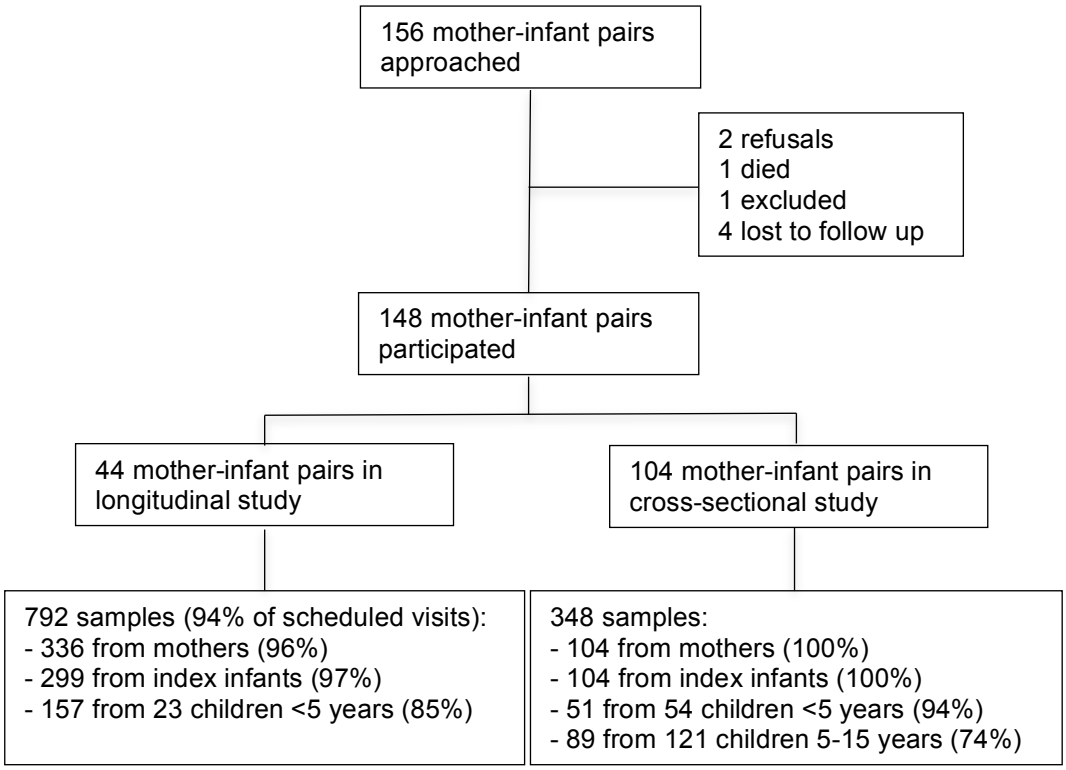


Figure 8.1 Flowchart of recruitment of mother-infant pairs and other children in the household in the post-vaccination period

8.3.2 Comparing characteristics of participants in the pre- and post-vaccination periods

Table 8.5 shows the characteristics of participants in the pre- and post-vaccination periods. For the seasonality-matched analysis, samples from the pre-vaccination period were included from 70 infants 6-week old, 71 infants 18-week old, 109 children 1-4 years, 144 children 5-15 years and 135 HIV-negative mothers. There were seven mothers who participated in both the pre- and post-vaccination period. Four children were included as index infant in the pre-vaccination and as children 1-4 years in the post-vaccination period. Mean age of all children 1-4 years was the same in the pre- and post-vaccination period (2.7 years vs. 2.7 years), but differed when considering the children's vaccination status in the post-vaccination years (3.5 years for unvaccinated children, 2.1 years for vaccinated children; $p<0.001$). Participating children in the age group 5-15 years were older in the post-vaccination period (mean 7.8 vs. 8.5 years, $p=0.002$). HIV-negative mothers were younger in the post-vaccination period (mean 26.2 vs. 24.2 years, $p<0.001$). In the pre-vaccination period, there were more households with one or two children <5 years in the household other than the index

infant. Participant's sex did not differ between the two vaccination periods, with the exception of children 1-4 years (60.6% vs. 36.5% females, p=0.002). All pneumococcal prevalence ratios were adjusted for age and number of children <5 years in the household, known to be associated with pneumococcal carriage.

Table 8.5 Characteristics of participants in the pre- and post-vaccination period

	Pre-vaccination	Post-vaccination	p-value
Female sex			
Infants 6 weeks	35/70 (50.0%)	75/146 (51.4%)	0.97
Infants 18 weeks	33/71 (46.5%)	26/44 (59.1%)	0.26
Children 1-4 years	66/109 (60.6%)	12/29 (41.4%) (unvaccinated)	0.10
		15/38 (39.5%) (vaccinated)	0.04
		27/74 (36.5%) (all)	0.002
Children 5-15 years	72/144 (50.0%)	40/89 (44.9%)	0.54
HIV-negative mothers	135/135 (100%)	144/144 (100%)	-
Age in years (mean, sd)			
Children 1-4 years	2.7, sd = 0.9	3.5, sd=0.6 (unvaccinated)	<0.001
		2.1, sd=0.7 (vaccinated)	<0.001
		2.7, sd=1.0 (all)	0.99
Children 5-15 years	7.8, sd=2.6	8.5, sd=3.0	0.002
HIV-negative mothers	26.2, sd=6.9	24.2, sd=6.3	<0.001
Number of children <5 years other than the index infant (households)	0: 45 (27.1%) 1: 92 (55.4%) 2: 29 (17.5%)	0: 79 (53.4%) 1: 61 (41.2%) 2: 8 (5.4%)	<0.001

sd: standard deviation

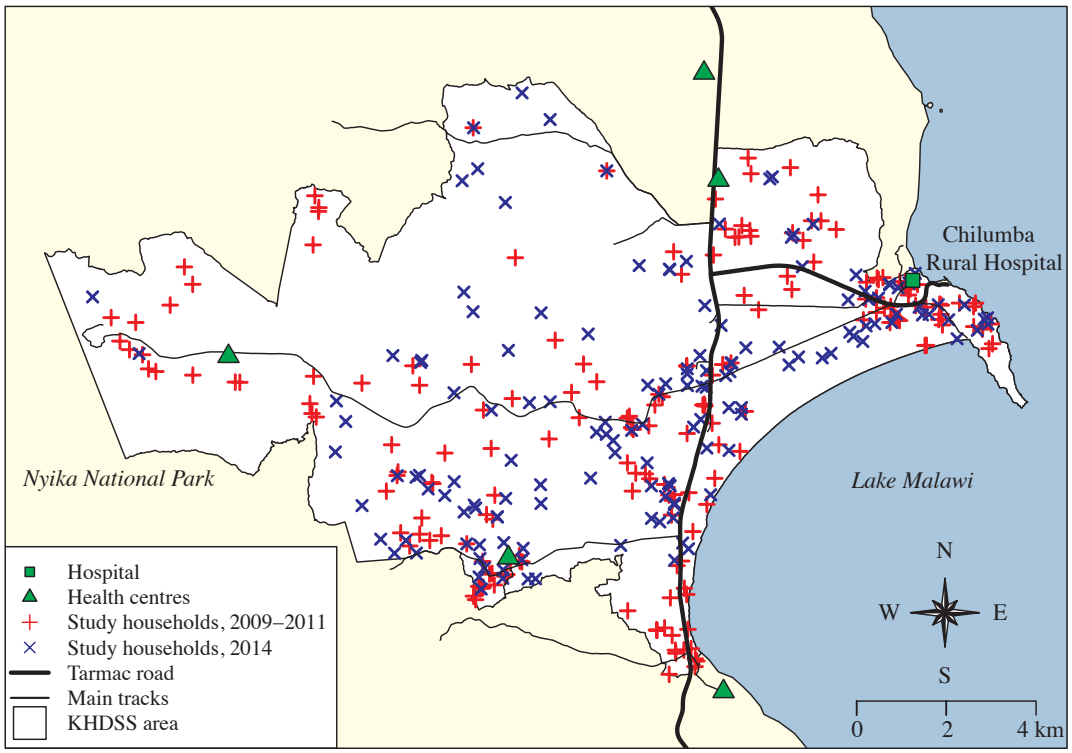


Figure 8.2 Map of the study area depicting study households in 2009-2011 and 2014

Figure 8.2 shows the geographical spread of households participating in the pre-vaccination (2009-2011) and post-vaccination (2014) period. In both periods, good geographical coverage of the KHDSS area was achieved.

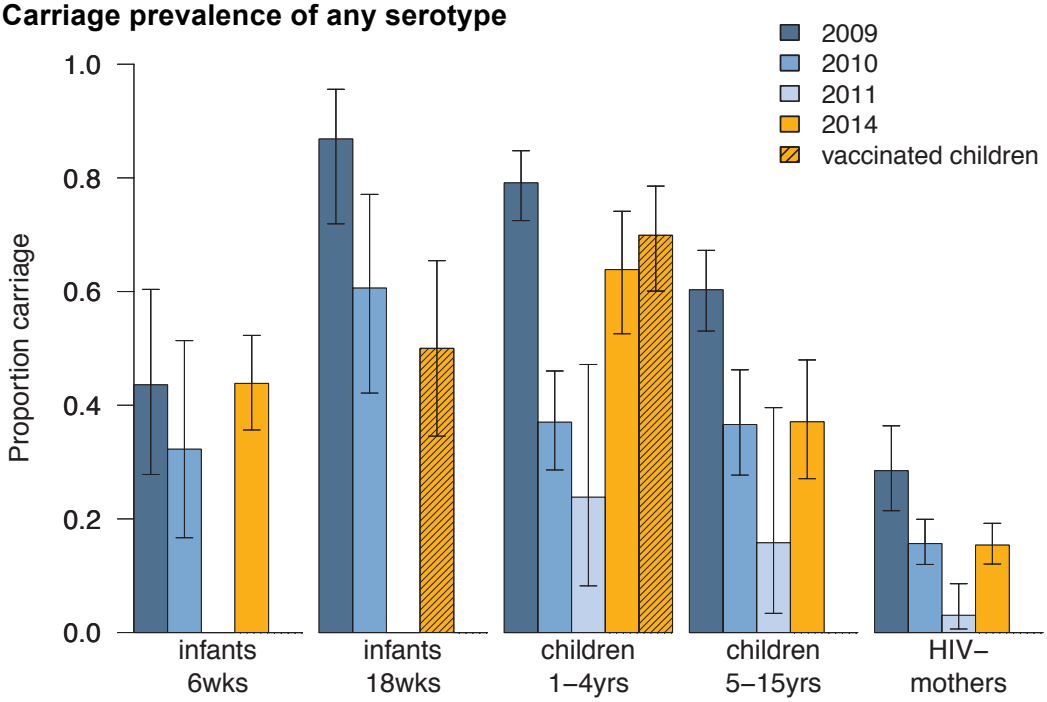
8.3.3 Pneumococcal carriage prevalence in the pre- and post-vaccination period

Figure 8.3 shows the prevalence of pneumococcal carriage of any serotype (a), VT serotypes (b) and NVT serotypes (c) for each of the age groups in the household study by year. Fluctuations in carriage prevalence by year can be observed for all age groups. There is no evidence that the decreasing trend in pneumococcal carriage of any serotype that was reported for the period 2009-2011 (Chapter 5) continued in 2014: carriage prevalence in 2014 is as high or higher than carriage prevalence in 2010 for all age groups. Figure 8.4 shows the proportion of VT isolates amongst pneumococcal carriers by age group by year. Estimates for the proportion of VT isolates are lower in 2014 than in 2009-2010 for all age groups, with the exception of 6-week old infants.

In figure 8.5 and 8.6 the same information is summarized for the pre- and post-vaccination period. Table 8.6 shows the crude and adjusted prevalence ratios for the pre- and post-vaccination period. After vaccine introduction, VT carriage decreased amongst 18-week old infants (APR 0.24, 95CI% 0.08-0.75) and vaccinated children 1-4 years (APR 0.54, 95%CI 0.33-0.88). VT carriage also decreased in children 5-15 years and mothers: APR 0.37 (95%CI 0.17-0.78) and APR 0.34 (95%CI 0.15-0.79) respectively. No decrease in VT carriage was observed for 6-week infants (APR 1.07, 95% CI 0.38-3.02) and unvaccinated children 1-4 years (APR 0.84, 95%CI 0.53-1.33). NVT carriage increased amongst vaccinated children 1-4 years (APR 1.58, 95%CI 1.21-2.06). No significant increase or decrease in NVT carriage was observed for the other age groups.

In the post-vaccination period, the most common VT isolated were 19F, 6B and 6A, isolated from 14, 13 and 10 individuals respectively. Other isolated VT were 19A (7 individuals), 9V (5), 14 (5), 3 (4), 18C (2) and 5(1). Serotypes 1, 4 and 7F were not detected in this study population. The most common NVT that we were able to type were 19B/19C, 23A/23B and 7A/7B/7C isolated from 19, 14 and 9 individuals respectively.

A. Carriage prevalence of any serotype



B. VT carriage prevalence

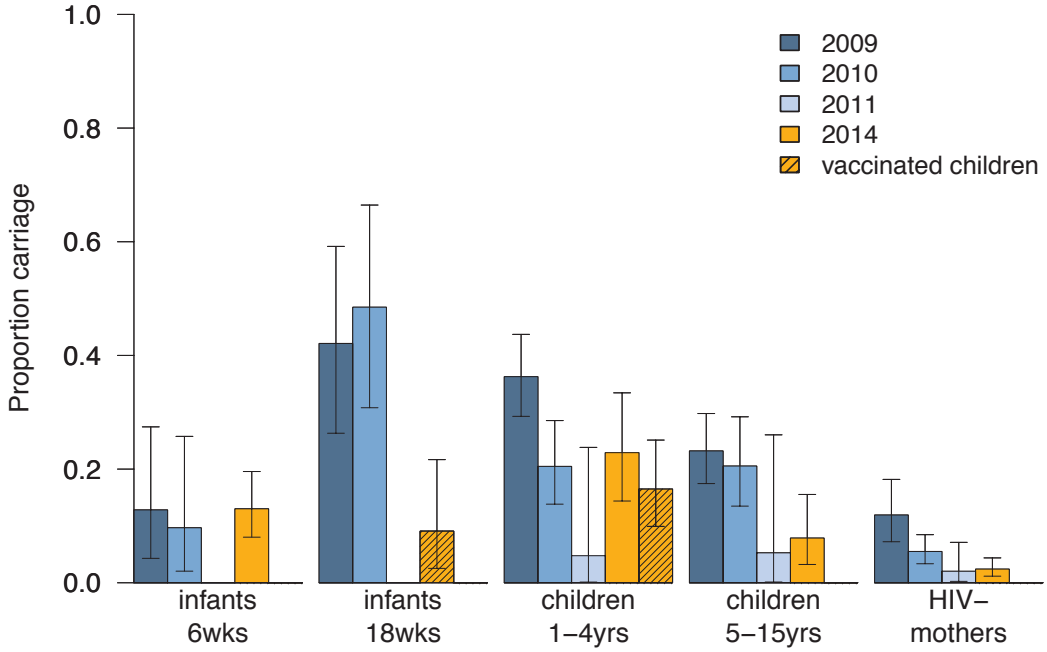


Figure 8.3 Prevalence of carriage of any serotype (A), VT carriage (B) or NVT carriage (C) by age group by year.

Estimates for pre-vaccination period matched by month of sample collection. No samples from infants collected in 2011

C. NVT carriage prevalence

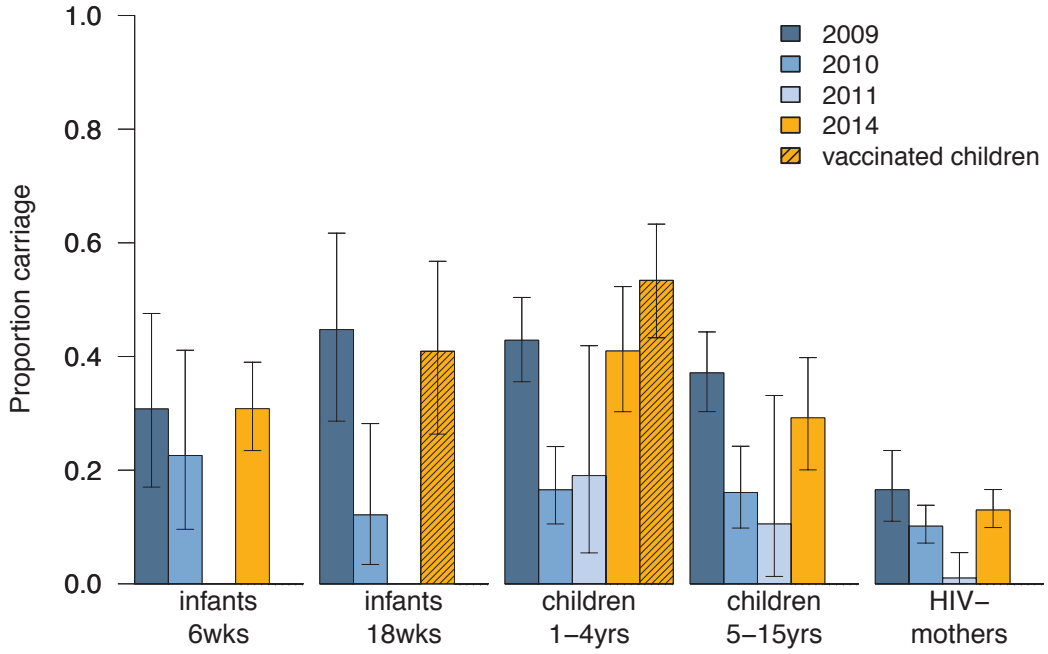


Figure 8.3 continued

Estimates for pre-vaccination period matched by month of sample collection. No samples from infants collected in 2011

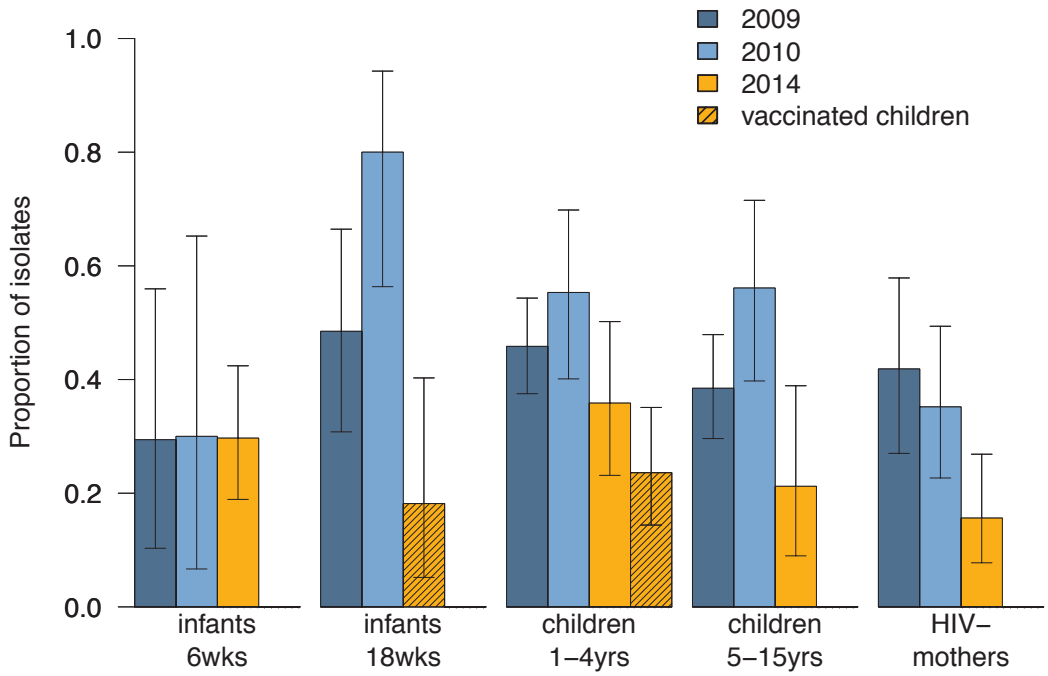
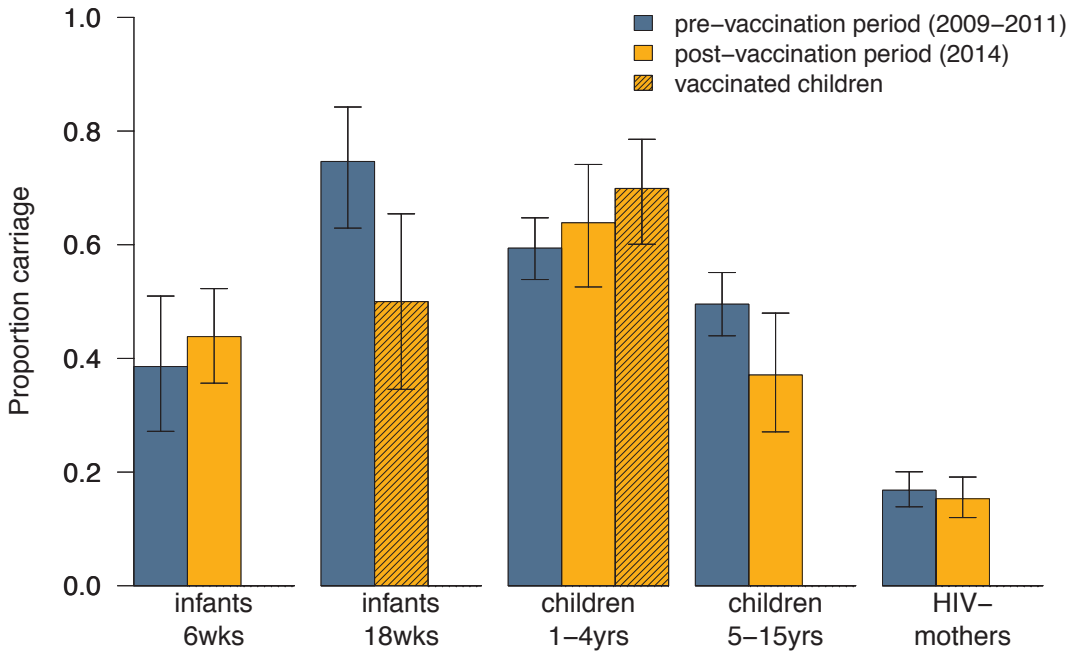


Figure 8.4 Proportion of VT isolates amongst pneumococcal carriers by age group by year

Not enough samples collected in 2011 to report proportion of VT isolates.

A. Carriage of any serotype



B. Carriage of PCV-13 serotypes

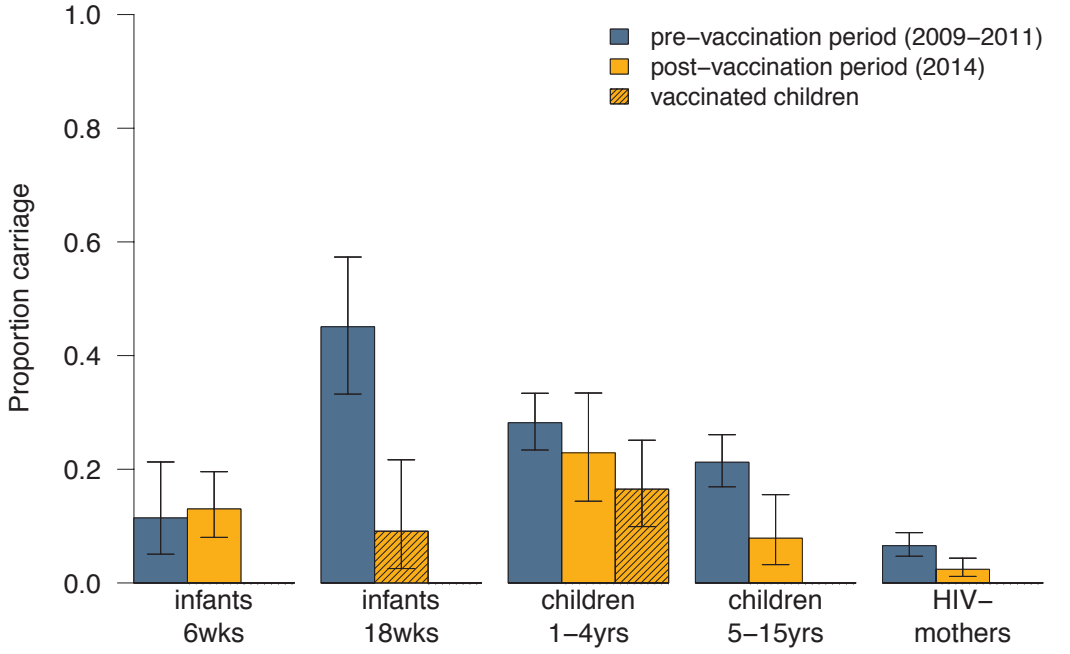


Figure 8.5 Prevalence of carriage of any serotype (A) or VT carriage (B) or NVT carriage (C) by age group in the pre- and post-vaccination periods.
Estimates for pre-vaccination period matched by month of sample collection.

C. Carriage of NVT serotypes

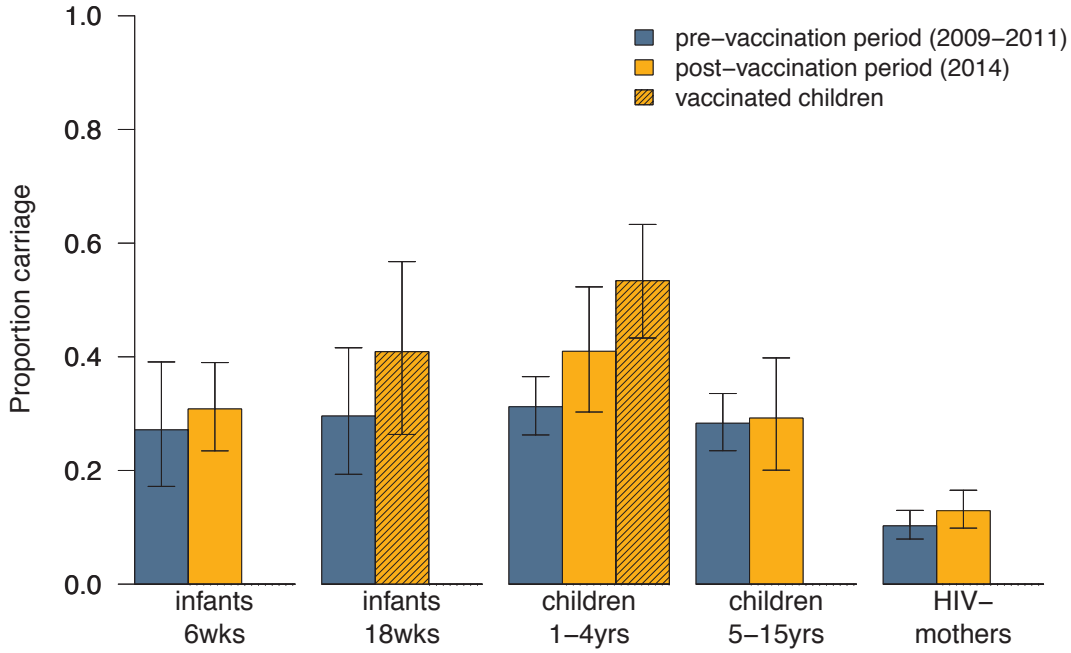


Figure 8.5 continued. Estimates for pre-vaccination period matched by month of sample collection.

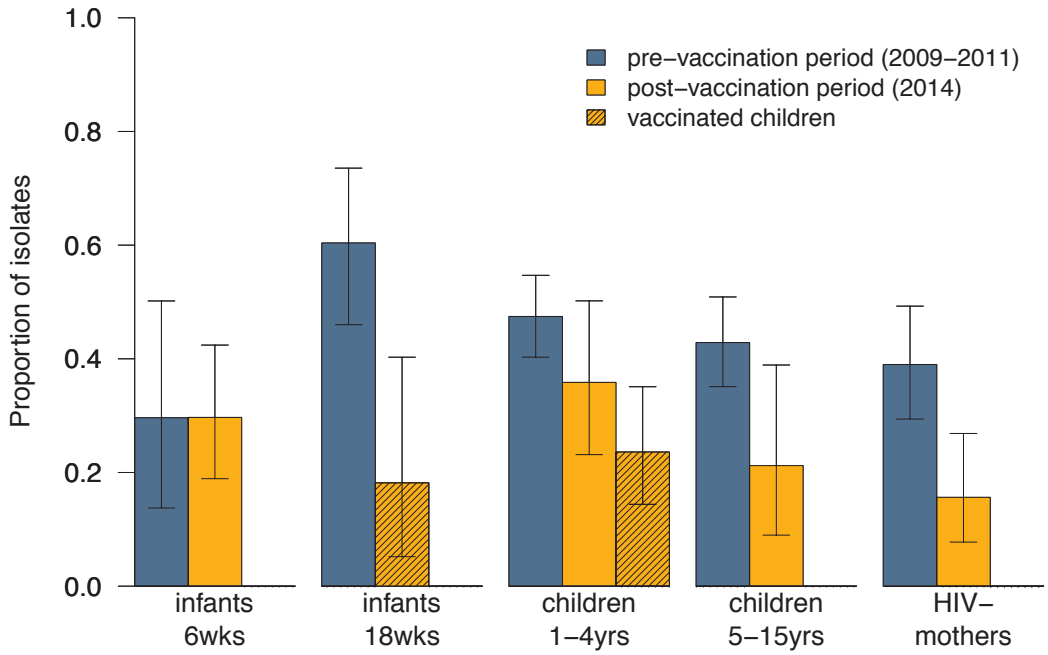


Figure 8.6 Proportion of PCV-13 serotype isolates amongst pneumococcal carriers by age group in the pre- and post-vaccination periods.

Table 8.6 Carriage prevalence and prevalence ratio for pneumococcal carriage by age group

	Carriage prevalence pre-vaccination	Carriage prevalence post-vaccination	Crude prevalence ratio (95%CI)	Adjusted prevalence ratio (95%CI)[2]
All serotypes				
Infants, 6 wks	27/70 (38.6%)	64/146 (43.8%)	1.14 (0.80-1.61)	0.98 (0.66-1.47)
Infants, 18 wks	53/71 (74.7%)	22/44 (50.0%)	0.67 (0.48-0.93)	0.60 (0.42-0.86)[3]
Children 1-4 yrs				
- unvaccinated	196/330 (59.4%)	53/83 (63.9%)	1.08 (0.89-1.29)	1.06 (0.86-1.30)[3]
- vaccinated		72/103 (70.0%)	1.18 (1.01-1.37)	1.08 (0.91-1.28)[3]
- all[1]		141/207 (68.1%)	1.15 (1.01-1.30)	1.10 (0.97-1.25)[3]
Children 5-15 yrs	161/325 (49.5%)	33/89 (37.1%)	0.75 (0.56-1.00)	0.73 (0.55-0.97)[3]
HIV- mothers	100/595 (16.8%)	64/418 (15.3%)	0.92 (0.69-1.22)	0.75 (0.47-1.17)
PCV-13 serotypes				
Infants, 6 wks	8/70 (11.4%)	19/146 (13.0%)	1.14 (0.52-2.47)	1.07 (0.38-3.02)
Infants, 18 wks	32/71 (45.1%)	4/44 (9.1%)	0.20 (0.08-0.53)	0.24 (0.08-0.75)[3]
Children 1-4 yrs				
- unvaccinated	93/330 (28.2%)	19/83 (22.9%)	0.81 (0.53-1.25)	0.84 (0.53-1.33)[3]
- vaccinated		17/103 (16.5%)	0.59 (0.37-0.93)	0.54 (0.33-0.88)[3]
- all[1]		37/207 (17.9%)	0.63 (0.45-0.89)	0.63 (0.45-0.90)[3]
Children 5-15 yrs	69/325 (21.2%)	7/89 (7.9%)	0.37 (0.18-0.78)	0.37 (0.17-0.78)
HIV- mothers	39/595 (6.6%)	10/418 (2.4%)	0.37 (0.19-0.73)	0.34 (0.15-0.79)

Table 8.6 Carriage prevalence and prevalence ratio for pneumococcal carriage by age group

	Carriage prevalence pre-vaccination	Carriage prevalence post-vaccination	Crude prevalence ratio (95%CI)	Adjusted prevalence ratio (95%CI)[2]
Non-PCV-13 serotypes				
Infants, 6 wks	19/70 (27.1%)	45/146 (30.8%)	1.14 (0.72-1.79)	0.95 (0.57-1.56)
Infants, 18 wks	21/71 (29.6%)	18/44 (40.9%)	1.38 (0.83-2.29)	0.91 (0.47-1.77)
Children 1-4 yrs				
- unvaccinated	103/330 (31.2%)	34/83 (41.0%)	1.31 (0.97-1.78)	1.24 (0.88-1.74)[3]
- vaccinated		55/103 (53.4%)	1.71 (1.34-2.18)	1.58 (1.21-2.06)[3]
- all[1]		104/207 (50.2%)	1.61 (1.30-1.99)	1.50 (1.22-1.86)
Children 5-15 yrs	92/325 (28.3%)	26/89 (29.2%)	1.03 (0.72-1.49)	1.03 (0.71-1.49)
HIV- mothers	61/595 (10.3%)	54/418 (12.9%)	1.27 (0.90-1.79)	0.99 (0.57-1.73)

[1]Including children with unknown vaccination status

[2]Adjusted for month of sample collection, number of children <5 years in the household, age and within-person clustering (mother only). Using a generalized linear mixed model, there was negligible individual-level variance for children 1-4 and 5-5 years of age ($\sigma 2 < 0.01$), hence results from a (non-mixed) generalized linear model were reported. Using a generalized linear mixed model, the individual-level variance for mothers was 0.68 (all serotypes) / 1.25 (PCV-13) / 1.03 (non-PCV-13).

[3]Log-binomial regression model data did not to converge so results of a Poisson model with robust standard errors are presented.

8.3.4 Pneumococcal acquisition in infants

Figure 8.7 and 8.8 show the time to first pneumococcal carriage acquisition in the pre- and post-vaccination periods for any serotype and VT, respectively. For acquisition of pneumococcal carriage of any serotype, no difference was observed between the pre- and post-vaccination period (median time 55.5 vs. 56.5 days, p= 0.71).

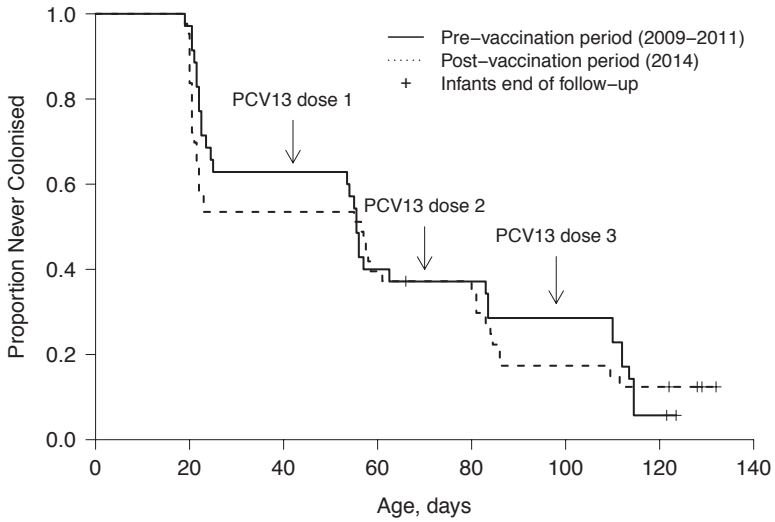


Figure 8.7 Kaplan-Meier plot for time to first pneumococcal carriage acquisition in infants pre and post introduction of PCV-13

Observations in the pre-vaccination period right-truncated at 18 weeks (maximum observation period post-vaccination period). Individuals matched on month of birth.

For VT carriage, a difference was observed between the pre- and post-vaccination period, with 62.9% (22/35) vs. 27.9% (12/43) of infants acquiring a VT pneumococcus at least once within the first 18 weeks of life (p=0.008). The Kaplan-Meier estimates for VT acquisition in the pre-vaccination and post-vaccination period diverge after each dose of PCV-13 receipt, providing evidence for a direct vaccine effect.

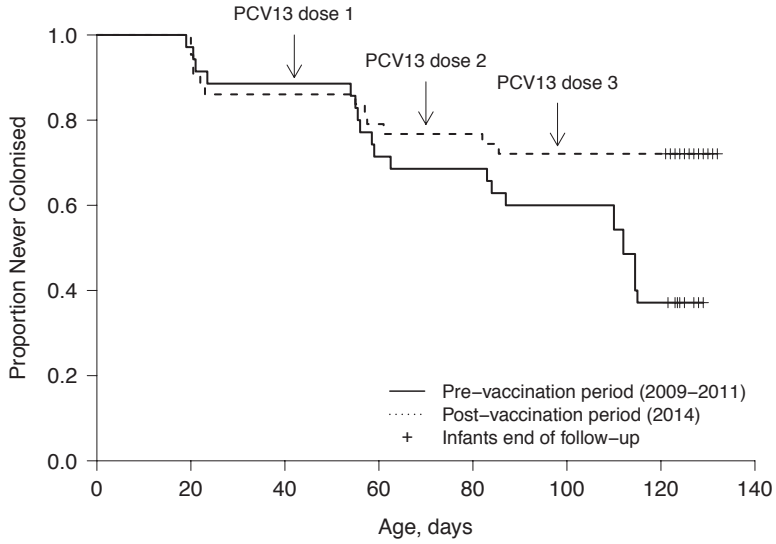


Figure 8.8 Kaplan-Meier plot for time to first acquisition of a vaccine type pneumococcus in infants pre and post introduction of PCV-13
Observations in the pre-vaccination period right-truncated at 18 weeks (maximum observation period post-vaccination period). Individuals matched on month of birth.

Incidence of VT carriage acquisition in weeks 6-10 among infants was 22.2% (38/171) in the pre-vaccination period and 9.3% (4/43) in the post-vaccination period, implying a vaccine efficacy (VE) of 58% for one dose of PCV-13. Incidence in weeks 10-14 was 14.9% (25/168) in the pre-vaccination period and 4.9% (2/41) in the post-vaccination period, implying a VE of 67% for two doses of PCV-13. Incidence in weeks 14-18 was 20.0% (33/165) in the pre-vaccination period and 2.3% (1/44) in the post-vaccination period, implying a VE of 89% for three doses of PCV-13. Combining the results for all weeks, VE for at least one dose of PCV-13 was 71%.

In the post-vaccination period, infants living with children <5 years acquired pneumococci faster than infants without children <5 years in the household, both for pneumococci of any type (p=0.03) (Figure 8.9) and for VT pneumococci (p=0.13) (Figure 8.10). No evidence was found for a difference in first VT acquisition between infants living with vaccinated or unvaccinated children <5 years (p=0.73, results not shown).

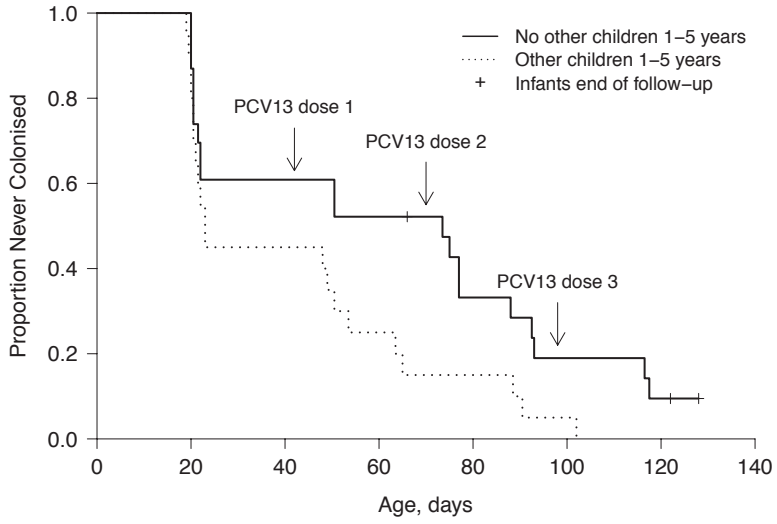


Figure 8.9 Kaplan-Meier plot for time to first pneumococcal carriage acquisition in infants by presence or absence of other children <5 years in the household

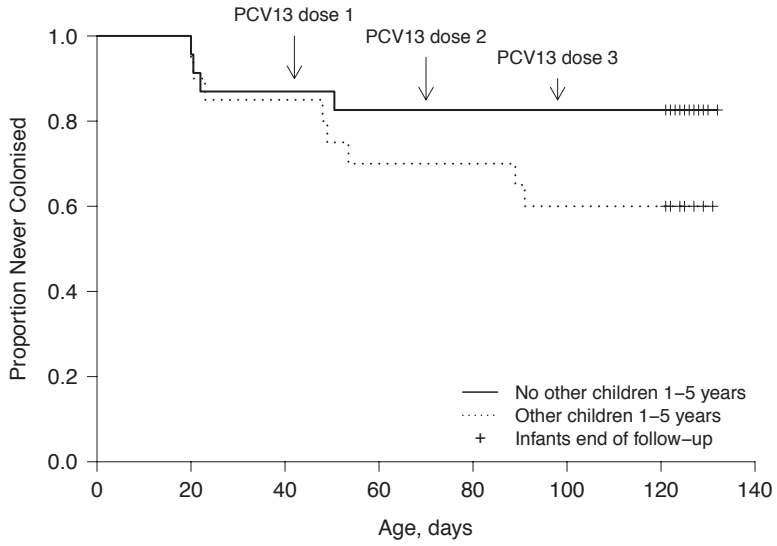


Figure 8.10 Kaplan-Meier plot for time to first acquisition of a vaccine type pneumococcus in infants by presence or absence of other children <5 years in the household

8.3.5 Duration of pneumococcal carriage

Table 8.7 shows results for duration of carriage in infants, mothers and other children <5 years. There was some evidence that duration of VT carriage was longer than duration of NVT carriage for infants (p=0.08) and other children <5 years (p=0.15), although the longest carriage episodes observed were for NVT: an infant carried NVT "7A/7B/7C" for an estimated 104 days and a vaccinated child <5 years carried NVT "23A/23B" for an estimated 88 days. There was no evidence for a difference in carriage duration between vaccinated and unvaccinated children <5 years (p=0.54). Carriage episodes of a single sample occurred in 11/26 (42.3%) of episodes for infants, 2/5 (40%) of episodes for mothers and 14/22 (63.6%) of episodes for children <5 years, indicating carriage duration of 14 days or less occurred frequently.

Table 8.7 Duration of Pneumococcal Carriage in Infants, Mothers and Other Children < 5 Years in Karonga District, Malawi, 2014

Serotype	Episodes (n)	Duration of carriage (days)		
		Median	Mean	Range
Infants				
PCV-13	13	36	47	14-97
non-PCV-13	13	16	32	14-104
Total	26	32	39	14-104
Mothers				
Total	5	26	32	14-39.5
Other children <5 years: fully vaccinated				
PCV-13	4	37	34	13-52
non-PCV-13	6	20	35	13-88
Total	10	24	35	13-88
Other children <5 years: unvaccinated				
PCV-13	6	32	38	14-86
non-PCV-13	5	14	14	13-16
Total	11	16	27	13-86

Excluding episodes of NVT which were not further serotyped.

8.3.6 Household exposure

Concordance between serotypes simultaneously carried by mothers and infants was low: only in 5 of 84 (6.0%) instances when an infant in the longitudinal or cross-sectional study carried a typeable pneumococcus was the same serotype found in the mother. Concordance with infant serotype carriage was 28.9% (11/38) for children <5 years and 28.6% (2/7) for children 5-14 years.

In the pooled analysis for serotype-specific exposure, both exposure by the mother (aRR 9.35, 95%CI 4.51, 20.11) and exposure by other children <5 years (aRR 8.24, 95%CI 4.22, 16.09) were found to be associated with infant pneumococcal acquisition (Table 8.8). Exposure by the infant was associated with pneumococcal acquisition in the mother (aRR 18.14, 95%CI 7.22-45.58) and other children in the household (aRR 11.00, 95%CI 4.51-26.80). Only the association between pneumococcal acquisition in other children <5 years and exposure by the mother was not significant – the same result as was found in the pre-vaccination period (chapter 5).

Of all acquisitions in the infant, 8.8% (95%CI 0.3-16.6) were attributable to exposure by the mother and 15.3% (95%CI 4.1-25.2) were attributable to exposure by another child <5 years in the household. The largest aPAFs were seen for exposure by the infant: 18.5% (95%CI 0.1-33.4) of acquisitions in mothers and 31.2% (95%CI 5.8-49.8) of acquisitions in other children <5 years were attributable to exposure by the infant.

Table 8.8 Acquisition of infants, mothers and other children <5yrs, stratified by exposure to other household members

Acquiring category, Exposing category, and Exposure[a]	No. with events	No. without events	Total no.[b]	cRR	aRR[c]	95%CI	cPAF (%)	aPAF[b] (%)	95%CI
Infant									
Mother									
Yes	11	38	49	18.55	9.53	4.51, 20.11	16.8	8.8	0.3, 16.6
No	51	4163	4214						
Infant									
Other child									
Yes	15	89	104	13.49	8.24	4.22, 16.09	23.54	15.3	4.1, 25.2
No	44	4071	4115						
Mother									
Infant									
Yes	6	52	58	21.45	18.14	7.22, 45.58	19.7	18.5	0.1, 33.4
No	23	4746	4769						
Mother									
Other child									
Yes	5	122	127	9.09	5.83	2.20,15.45	18.5	11.7	0, 25.3
No	19	4366	4385						
Other child									
Infant									
Yes	9	45	54	8.43	11.00	4.51, 26.80	20.9	31.2	5.8, 49.8
No	29	1438	1467						

Table 8.8 Acquisition of infants, mothers and other children <5yrs, stratified by exposure to other household members

Acquiring category, Exposing category, and Exposure[a]	No. with events	No. without events	Total no.[b]	cRR	aRR[c]	95%CI	cPAF (%)	aPAF[b] (%)	95%CI
Other child									
Mother									
Yes	2	5	7	15.8	3.23	0.88, 11.88	5.1	1.3	0, 6.8
No	35	1903	1938						
Other child									
Other child									
Yes	4	18	22	8.74	4.87	1.44, 16.40	7.2	4.1	0,12.9
No	45	2118	2163						

Abbreviations: aRR, adjusted rate ratio; aPAF, adjusted population attributable fraction; CI, confidence interval; cRR, crude rate ratio; cPAF, crude population attributable fraction.[a] Exposure was defined as carriage by another household member at any of the previous two sample times. Analysis was limited to those samples for which exposure data for at least one of the two previous sample times was available. [b] Adjusted analysis for exposure by index child, exposure by children <5 yrs, exposure by mother.

8.4 Results: HIV-positive adults on ART

8.4.1 Study participants and samples

In 2008-10 545 participants (contributing 1248 samples) were recruited, in 2014 199 participants were recruited. Individuals participating in the post-vaccination period were on average older than individuals participating in the pre-vaccination period, more likely to be female, and less likely to live with one or more children <5 years in the household (Table 8.9). The study conducted in 2008-2010 recruited only individuals newly attending the ART clinic, whereas in the study conducted in 2014 all individuals attending the ART clinic were included, resulting in a difference in time on ART treatment. Also a difference in cotrimoxazole prophylactic treatment and tuberculosis treatment was observed between the two time periods.

Table 8.9 Characteristics of participants in the pre- and post-vaccination period

	Pre-vaccination	Post-vaccination	p-value
Female sex	326/545 (59.8%)	136/199 (68.3%)	0.04
Age in years (mean, sd)	39.1, sd=10.9	45.3, sd = 10.2	<0.001
Number of children <5 years in the household	0: 232 (47.6%) 1: 180 (37.0%) 2: 62 (12.7%) 3-4: 13 (2.7%)	0: 111 (69.8%) 1: 40 (25.2%) 2: 7 (4.4%) 3: 1 (0.6%)	<0.001
Years on ART	0-2: 545/545 (100%)	0-2: 38 (19.1%) 3-6: 99 (49.7%) 7+: 62 (31.2%)	<0.001
Received cotrimoxazole prophylactic treatment[1]	307/545 (56.3%)	199/199 (100%)	<0.001
Received tuberculosis[1] treatment	11/545 (2.0%)	0/193 (0%)	0.10

[1]For pre-vaccination period: received treatment at least once during the study period

8.4.2 Pneumococcal carriage amongst HIV-positive adults in the pre and post-vaccination period

Pneumococcal carriage prevalence amongst HIV-positive adults attending the local ART clinic was 10.1% (20/199) in the post-vaccination period. A vaccine serotype was detected in only one sample (0.5%). There was some evidence that carriage of any serotype was higher in females (aPR 2.18, 95%CI 0.76-6.23) and individuals living with children <5 years (aPR 2.19, 95%CI 0.98-4.91) (Table 8.10).

Table 8.10. Risk factors associated with pneumococcal carriage in HIV-positive adults sampled in the post-vaccination period.

Risk factor	n	Carriage		Crude PR	95%CI	*P*	Adjusted PR	95%CI	*P*
		n	%						
Sex									
Male	63	4	6.4%	-			-		
Female	136	16	11.8%	1.85	0.65, 5.32	0.25	2.18	0.76, 6.23	0.15
Age (years)									
<40	59	6	10.2%	-					
≥40	140	14	10.0%	0.98	0.40, 2.43	0.97	1.23	0.49, 3.10	0.66
Years on ART									
0-2.5	38	5	13.2%	-					
>2.5	161	15	9.3%	0.71	0.27, 1.83	0.48	0.54	0.22, 1.32	0.18
Children <5 years in household[b]									
No	111	11	9.9%	-					
Yes	48	9	18.8%	1.89	0.84, 4.27	0.12	2.19	0.98, 4.91	0.06
Number of household members[b]									
0-2	29	4	13.8%	-					
3-6	106	13	12.3%	0.89	0.31, 2.52	0.83	0.65	0.23, 1.88	0.43
7-11	24	3	12.5%	0.91	0.22, 3.66	0.89	0.52	0.11, 2.42	0.40

Table 8.11 Carriage prevalence and prevalence ratio for pneumococcal carriage amongst HIV-positive adults on ART

	Carriage prevalence pre-vaccination	Carriage prevalence post-vaccination	Crude prevalence ratio (95%CI)	Adjusted prevalence ratio (95%CI)[1]
Any serotype	284/1248 (22.8%)	20/199 (10.1%)	0.44 (0.29-0.68)	0.79 (0.36-1.74)
VT	130/1248 (10.4%)	1/199 (0.5%)	0.05 (0.01-0.34)	0.06 (0.01-0.40)
NVT	154/1248 (12.3%)	19/199 (9.6%)	0.77 (0.49-1.22)	1.17 (0.46-2.95)

[1] Adjusted for sex, number of children <5 years in the household, month of sampling and ART duration (any serotypes and NVT only)

Pneumococcal carriage prevalence in 2014 was lower than in 2008-2010 for VT, NVT and any serotype (Table 8.11), although in the adjusted analysis only the prevalence ratio for VT carriage remained significant (aPR 0.06, 95%CI 0.01-0.40).

Table 8.12 shows the different comparisons done to investigate the association between pneumococcal carriage prevalence and ART duration in the pre- and post vaccination period. Carriage prevalence in individuals 0-2.5 years on ART in 2008-2010 was 22.8% (284/1248) for any serotype. In 2014, carriage prevalence of any serotype was 13.2% (5/38) and 9.3% (15/161) for individuals 0-2.5 years and >2.5 years on ART respectively (aPR 0.54, 95%CI 0.22-1.32). Combining both periods, the crude prevalence ratio was 0.41 (95%CI 0.25-0.68) for >2.5 vs. 0-2.5 years ART and the crude prevalence ratio was 0.44 (0.29-0.68) for the comparison between the pre- and post-vaccination period. In the multivariable model including month of sampling, sex and the presence of children <5 years in the household, the adjusted prevalence ratio for ART duration was 0.57 (95%CI 0.22-1.41) for >2.5 vs. 0-2.5 years of ART and the adjusted prevalence ratio for vaccination period was 0.79 (95%CI 0.36-1.74) for the post-vaccination vs. pre-vaccination period. No evidence was found for a difference in carriage by cotrimoxazole prophylactic treatment (aPR 1.03, 95%CI 0.84-1.28). Lower carriage was observed for individuals on tuberculosis treatment, but numbers were too small to draw any conclusions from this observation (aPR 0.47, 95%CI 0.08-2.87).

Table 8.12 Comparisons to assess the association with ART duration and vaccination period in HIV-positive adults on ART

Comparison group	n	Carriage		cPR	95%CI	aPR	95%CI
		n	%				
Comparison between pre-and post vaccination period amongst individuals 0-2.5 years on ART							
Pre-vaccination	1248	248	22.8%				
Post-vaccination	38	5	13.2%	0.58	0.25, 1.32	0.78	0.35, 1.73
Comparison between pre-and post vaccination period including all samples							
Pre-vaccination	1248	284	22.8%				
Post-vaccination	199	20	10.1%	0.44	0.29, 0.68	0.79	0.36, 1.74
Comparison between 0-2.5 and >2.5 years ART within the post-vaccination period							
0-2.5 years on ART	38	5	13.2%				
>2.5 years on ART	161	15	9.3%	0.71	0.27, 1.83	0.54	0.22, 1.32
Combining the pre- and post-vaccination period, assessing duration of ART							
0-2.5 years on ART	1286	253	19.7%				
> 2.5 years on ART	161	15	9.3%	0.41	0.25-0.68	0.57	0.23, 1.41

8.4.3 Analyses tot assess survival bias

The possibility of survival bias should be taken into account in our analysis on prolonged ART duration. Survival bias would occur if individuals on prolonged ART are different from those 0-2.5 years on ART because they have survived for >2.5 years: i.e. that pneumococcal carriage could be an indicator for adverse survival outcome and those that have died before 2.5 years on ART may have had higher carriage in 2008-2010. To investigate this, we compared carriage from 2008-2010 by survivorship to 2014. We found that pneumococcal carriage in 2008-2010 was higher in those who died in the next years: carriage was 27.4% (34/124 samples) in 68 individuals who died before August 2014 versus 23.0% (202/877 samples) in 346 individuals who were still alive in August 2014. Survivorship was unknown for 131 individuals because they moved out of the study area (n=99) or where lost to follow-up for other reasons (n=32). After adjusting for sex, ART use and CD4 count at time of sampling, the risk ratio for carriage associated with death in the following four years was 1.36 (95%CI 0.98-1.89). Another sub-analysis done to avoid survival bias was to only include those individuals who were sampled in both 2008-2010 and 2014. Amongst this subgroup of 65 individuals, pneumococcal carriage of any serotype decreased from 24.9% (45/181 samples) in 2008-2010 to 9.2% (6/65) in 2014.

8.5 Discussion

We provide evidence for a reduction in carriage of VT pneumococcus three years after vaccine rollout in this rural Malawi population. Reductions in VT carriage in unvaccinated individuals yet no change in NVT carriage suggest herd protection is taking place. Six-week old infants and unvaccinated children <5 years continued to carry at a high rate.

PCV is being rolled out worldwide: in December 2015 there were 125 countries with a nationwide pneumococcal infant vaccination policy (217). This is the first study on pneumococcal carriage prevalence after routine introduction of PCV-13 in a vaccine-naive Sub-Saharan African country using a 3+0 schedule. Previous studies were conducted in Kenya, The Gambia and South Africa, but different vaccines and/or schedules were used (Table 8.12). In all three countries a decrease in VT carriage was observed in vaccinated age groups. In Kenya and South Africa, also a decrease in VT carriage was observed in unvaccinated age groups, suggesting an indirect vaccine effect. No decrease in carriage was observed in mothers in The Gambia. An increase in NVT carriage is observed in vaccinated age groups in Kenya and South Africa. In South Africa, a decrease in NVT carriage is also observed in unvaccinated age groups, suggesting that overall reduction of pneumococcal carriage may have occurred due to reasons other than PCV introduction.

Cross-sectional carriage surveys are also being undertaken in Blantyre in Southern Malawi since June 2015, with nasopharyngeal samples being collected and analysed using the same techniques as in this study. Higher VT carriage was observed in Blantyre than in Karonga: VT carriage was 22.5% (95%CI 18.3-27.1), 25.7% (20.9-31.1), 13.9% (9.8-18.8) in 476 vaccinated children 3-4 years, 375 unvaccinated children 5-10 years, and 318 adults receiving ART respectively. Serotype-1 was detected in 7.3%, 18.0% and 9.1% of VT carriage; a particularly worrying finding given serotype-1's potential for outbreaks of invasive disease. No serotype-1 carriage was detected in this study in Karonga. NVT carriage was 62.2%, 41.3% and 28.6% in vaccinated children, unvaccinated children and adults, also higher than reported in this study.

Table 8.13 Studies comparing pneumococcal carriage pre- and post introduction of PCV in Sub-Saharan African countries

Country	Vaccine introduction	Schedule	Catch-up	Comparison	Number sampled	Age group	Result VT: adjusted prevalence or risk ratio (95%CI)	Result NVT: adjusted prevalence or risk ratio (95%CI)
Kenya (25)	PCV-10: 2011	3+0	Kilifi Region: <5 years received up to two doses	2009-2010 vs. 2011-2012	623 1408	<5 yrs ≥5 yrs	0.36 (0.26-0.51) 0.34 (0.18-0.62) (estimates for PCV-10)	1.37 (1.13-1.65) 1.13 (0.92-1.38)
South Africa (184)	PCV-7: 2009 PCV-13: 2011	2+1	None	2009 vs. 2011	1307 695 794 443 1821 609	<2 yrs 2-5 yrs 6-12 yrs 13-18 yrs 19-45 yrs >45 yrs	0.50 (0.42-0.59) 0.79 (0.64-0.99) 0.66 (0.48-0.92) 0.49 (0.17-1.39) 0.36 (0.18-0.74) 0.63 (0.16-2.49) (estimates for PCV-7)	1.35 (1.17-1.56) 0.99 (0.84-1.18) 0.87 (0.73-1.04) 0.55 (0.34-0.89) 0.46 (0.31-0.68) 1.15 (0.48-2.72)
The Gambia (218)	PCV-7: 2009 PCV-13: 2011	3+0	No formal catch-up, but most <2 years received one dose PCV-7	2011 vs. 2012	689 689	6-11 mo mothers	0.52 (0.39-0.69) PCV-13 0.52 (0.28-0.97) PCV-7 1.14 (0.65-2.01) PCV-13 0.85 (0.31-2.30) PCV-7	

There is no clear explanation for the difference in carriage observed between Karonga and Blantyre. PCV-13 coverage is high (>85%) in both settings. It is possible that a difference in social mixing patterns between the rural community in Karonga and the urban community in Blantyre led to different pneumococcal transmission and hence a difference in indirect vaccine effect. It is also likely that seasonal and secular changes explain part of the variation in carriage prevalence observed. In Karonga we observed large variation of pneumococcal carriage by season and by year before the introduction of PCV-13. The results from the Blantyre study are from a different year and from the dry (peak) season. Surveillance of pneumococcal carriage prevalence in Blantyre and Karonga is ongoing and will be closely monitored to assess whether a change in vaccination schedule is required to obtain and sustain a direct and indirect vaccine effect.

Using a 2-week sampling interval rather than a 4-6 week interval as was used before gave us a better understanding of the duration of carriage and household exposure. In 40% of carriage episodes in infants, a pneumococcal serotype was only identified in a single sample, indicating that duration of carriage of 14 days or less is common. Using a shorter sampling interval also increased the proportion of carriage episodes that could be attributed to carriage in the household. Using a 4-6 week interval we found an aPAF of 1.9% (95%CI 0.0-4.3) for infant acquisition for exposure by the mother and an aPAF of 8.8% (95%CI 4.0-13.4) for exposure by children <5 years in the household (chapter 5). Using a 2-week interval we found an aPAF of 8.8% (95%CI 0.3-16.6) for exposure by the mother and an aPAF of 15.3% (95%CI 4.1-25.2) for exposure by children <5 years in the household. Our study also provided further evidence that infants themselves contribute to pneumococcal transmission in the household: 18.5% (95%CI 0.1-33.4) of carriage episodes in the mother and 31.2% (95%CI 5.8-49.8) of carriage episodes in other children <5 years could be attributed to exposure by the index infants. A limitation of the household exposure analysis is that results for which no serotyping result was available other than “NVT” had to be included. Most results from the household exposure analysis involved “NVT” acquisition and household exposure. It is likely that some “NVT” acquisitions were wrongly attributed to household exposure. In the pre-vaccination period we relied on serotype-specific results only: we had a large enough sample size and observed enough VT carriage to be able to include the five most carried serotypes.

In the study on HIV-positive adults on ART a remarkable decrease in VT carriage was observed, with only one VT isolated in 2014. The reduction in VT carriage suggests an indirect effect of the vaccine, but should be considered with caution: although the

results from the household study also suggest an indirect vaccine effect, smaller reductions are seen in the household study. We hypothesised that a larger indirect effect would occur in the household study, where all unvaccinated participants are in contact with at least one vaccinated child. Also a decrease in carriage of any serotype was observed for HIV-positive adults on ART, mostly attributable to the decrease in VT. Thus it is difficult to fully reconcile these findings. We provide some evidence that pneumococcal carriage prevalence is lower in HIV-positive adults on prolonged ART. It is likely that the combination of prolonged duration of ART and PCV-13 introduction both attributed to the decrease in any serotype and VT pneumococcal carriage in HIV-positive adults. Our results need to be considered with caution: no difference in pneumococcal carriage by ART duration was observed in the cross-sectional conducted study in Blantyre. Our study also suggests that pneumococcal dynamics are still driven by children <5 years: individuals living with children <5 years were more than two times as likely to carry a pneumococcus than individuals living without children <5 years. No evidence for this association was found in the study conducted in 2008-2010 (aRR 1.05, 95%CI 0.81-1.37); we previously had assumed that the increased risk of carriage in women was attributable to universal child contact and not determined by household contact only.

Several limitations can be identified for this study. Firstly, we recruited mother-infant pairs at the local hospital, thus missing infants born at home. During the study period, 97% of infants were born in a health centre, indicating that our recruitment strategy included most infants. We also recruited HIV-positive adults from the ART clinic only, potentially missing HIV-positive adults not attending the clinic. A cross-sectional analysis conducted in 2007-2008 showed that of 837 HIV-positive adults, 788 (94%) knew their status, but only 209 (25%) completed screening for ART. Of 202 eligible for ART, 194 (96%) received ART at time of the study. Delays between HIV-diagnosis and ART screening were shorter in men and decreased with increasing age (219). Secondly, we did not perform HIV-testing on participating mothers, but relied on the HIV-status in their health passport or verbal report. Documented HIV status was available for the majority of mothers and 98% of mothers were HIV-tested within the last year, making HIV testing for the purpose of this study unnecessary. Thirdly, adult household members other than the mother were not included in this study and children 5-15 years were included in the cross-sectional arm of the study only, prohibiting the investigation of their role in household dynamics. The cross-sectional analysis suggests that concordance of same serotype carriage does occur (2/7: 28.6%), but numbers are small and we cannot determine the direction of spread. Fourthly, the sample size calculations for the household study were based on including samples

from all months in the pre-vaccination period. Using only seasonality-matched samples was important for the comparison between the pre- and post-vaccination period in the household study, but this will have reduced the power to detect a difference between the two periods. Fifthly, our results suggest that some survival bias is occurring in our study on HIV-positive adults: we found that pneumococcal carriage in 2008-2010 was higher in those who died in the next years. However, because carriage in 2008-2010 amongst those that survived is still higher than carriage in 2014 (23.0% vs. 10.1%), this does not change our conclusion on the association of reduced carriage with prolonged ART. Lastly, our laboratory procedures did not allow for detection of simultaneous colonization with multiple serotypes. A subset of samples from Blantyre was assessed for multiple serotype carriage using microarray technique. Multiple serotype carriage was found to be 54.1% in vaccinated and 59.3% in unvaccinated children. Serotyping using microarray increased detectable VT carriage by 35.6%. Our comparisons between the pre- and post-vaccination period are still valid, because multiple serotype assessment was performed in neither period, but will have decreased the accuracy of our estimates of carriage duration and household transmission.

In conclusion, we provide evidence for a reduction in carriage of VT pneumococcus three years after vaccine rollout in this rural Malawi population. Reductions in VT carriage in unvaccinated individuals yet no change in non-VT carriage suggest herd protection is taking place. However, high VT carriage including carriage of serotype-1 is observed in carriage prevalence surveys conducted in Blantyre, implicating that ongoing surveillance is required to evaluate whether a change in vaccination schedule is required to optimise the indirect vaccine effect from PCV-13. We also provide some evidence that pneumococcal carriage prevalence is lower in HIV-positive adults on prolonged ART. Ongoing work on HIV-positive adults on ART in Blantyre will be analysed to assess whether the same finding can be observed in this population.

Part III: Mathematical modelling

9. A mathematical model to study pneumococcal transmission in the pre-vaccination period

9.1 Introduction

The longitudinal household studies conducted in the pre-vaccination and post-vaccination period (chapter 5+8) showed that infant pneumococcal acquisition is associated with carriage in other children <5 years and mothers, but that this could only explain a limited proportion of acquisitions. With the results of the longitudinal studies, we could not study the transmission in the household as a whole, because of the low response rate from household members older than 5 years. Also, the results of the longitudinal studies did not allow for studying pneumococcal dynamics outside the household. Most importantly, the exact timing and direction of transmission events cannot be observed from longitudinal studies. Investigating transmission, including the inferring of unobserved transmission events, in the whole household, including older children and adults, and in the wider population, was possible with the use of a mathematical model parameterised with data established in the field studies.

The study on HIV-positive adults attending an ART clinic (chapter 6) showed that pneumococcal carriage in HIV-positive adults remained high during the first two years of ART use and raised the question whether HIV-positive adults could remain a reservoir for pneumococcal carriage and diversity in the post-vaccination period. Understanding the dynamics of pneumococcal carriage in this group will help us understand their importance in pneumococcal transmission in the population. A mathematical model which allows for characteristics of carriage to differ between HIV-

positive and HIV-negative adults would provide us with more information on the differences and similarities between the two groups.

This chapter describes the mathematical model set up to study pneumococcal transmission in the pre-vaccination period. The model was fitted to pneumococcal carriage prevalence data collected in 2009-2011 (chapters 5+6). We developed a model based on the known household and population structure in the KHDSS with the aim of defining the importance of household versus community transmission of pneumococci in this population. The model included five different age/HIV groups (infants, children 1-4 years, children 5-15 years, HIV-positive and HIV-negative adults) to allow for further characterisation of pneumococcal dynamics for each age/HIV group. The model was then applied to investigate the impact of different vaccination strategies: results of the mathematical model for the post-vaccination period can be found in chapter 10.

Figure 9.1 gives an overview of the different steps taken in the mathematical modelling process: 1. Design of the model (section 9.2-4), 2. Fit to dummy data (section 9.5), 3. Fit to observations from Karonga District (section 9.6) and 4. Simulate different vaccination scenarios (chapter 10). A glossary for mathematical modelling terms used in this chapter is provided in table 9.1.

Table 9.1 Glossary for mathematical modelling terms	
Term	**Explanation**
acceptance rate	Proportion of particles accepted by applying a specific threshold.
approximate Bayesian computation	Computational method based on Bayesian statistics that bypasses the need for the calculation of a likelihood function by using summary statistics to assess the fit of a model to observations.
force of infection (λ)	Rate at which susceptible individuals become infected per unit of time.
individual-based model	Model which keeps track on the infection status of each individual in the population.
likelihood	Measurement of the support provided by the data for each possible value of the parameter.
particle	Set of parameter values, e.g. the combination of parameter values for duration of infection and transmission rate.
particle filtering method (sequential Monte Carlo - SMC)	Method to fit a model to observations. Assessing a set of parameter values (particle) in a step-wise approach. In each step the best fitting particles are kept and assigned a weight with which they are resampled in the next step.
perturbation	Randomly changing a particle along a particular distribution.
prior distribution	Probability distribution that expresses one's beliefs about a variable before some evidence is taken into account.
posterior distribution	Distribution of unobserved observations conditional on observed data.
stochastic model	Model allowing for random variation in one or more inputs.
Susceptible-Infected-Susceptible model	Model in which individuals can belong to one of two states: susceptible or infected. After infection, the individual returns to a susceptible state.
tolerance threshold	Defined threshold, e.g. based on a statistical test value, that determines whether a particle gets accepted or not.
Distributions	
beta distribution	Continuous distribution with two positive parameters, α and β that determine its shape. Defined on interval [0,1].
gamma distribution	Continuous distribution with shape parameter k and scale parameter θ. Defined on interval [0,∞].
log-normal distribution	Continuous distribution in which the logarithm of a variable has a normal distribution. Contains parameters μ (mean) and σ (standard deviation). Defined on interval [0,∞].
logit-normal distribution	Continuous distribution in which the logit (=log(p/1-p)) of a variable has a normal distribution. Contains parameters μ (mean) and σ (standard deviation). Defined on interval [0,1].
uniform distribution	Continuous distribution with constant probability. Contains parameters *a* and *b* which are its minimum and maximum value. Defined on interval [-∞, ∞].

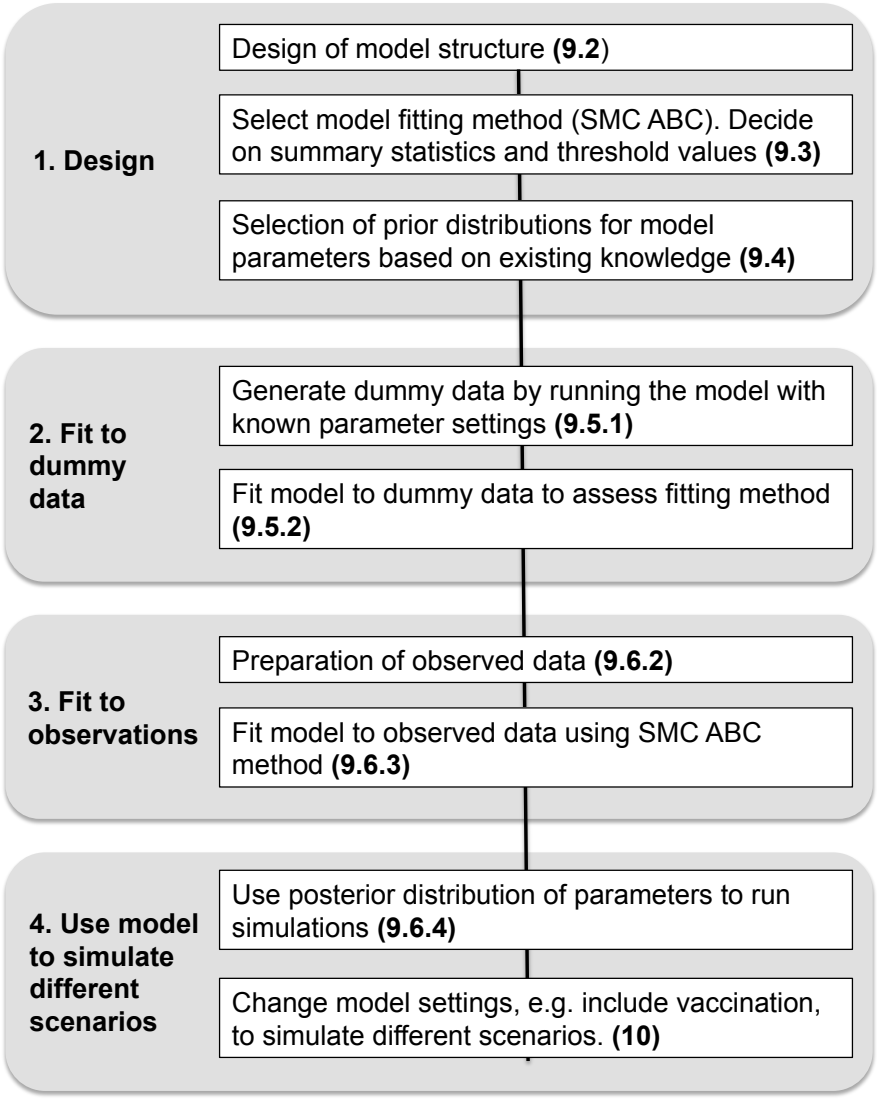


Figure 9.1 Flowchart describing steps in mathematical modelling process
Thesis chapters describing the steps in brackets.

9.2 Description of the model

Based on the literature review (chapter 4) we identified the following key characteristics for our model:

- Susceptible-Infected-Susceptible (SIS) infection dynamics
- Individual-based model
- Stochastic infection and recovery transitions
- Two levels of transmission: within-household and community (random mixing)
- Explicit, dynamic household membership
- Explicit, dynamic population structure

We developed an individual-based stochastic transmission model, based on the model used by Melegaro et al. to describe pneumococcal transmission in the UK (220) (Figure 2). This is a Susceptible-Infected-Susceptible (SIS) type model: individuals can be non-carriers (S), carriers of vaccine serotype pneumococci (VT), carriers of non-vaccine serotype pneumococci (NVT) or carriers of both (B). Carriers revert to the susceptible status when carriage is lost. The model includes the possibility of simultaneous VT and NVT carriage (B status) to reflect the findings of two studies conducted in Malawi which found that multiple serotype carriage was common amongst samples from both children and adults (47, 221) (see section 9.3.2 for details).

Acquisition and loss of carriage occur as stochastic processes, with probability of acquisition determined by the force of infection and probability of loss determined by an age and HIV-infection status specific duration of carriage. Non-carriers (S) become infected according to the force of infection of vaccine type (λ_{vt}) and non-vaccine type (λ_{nvt}) pneumococci, which consists of: 1) transmission in the household as determined by β_{hh} and 2) transmission in the community as determined by β_{com}. Individuals carrying vaccine type (VT) or non-vaccine type (NVT) pneumococci can become carriers of both (B) at the rate $c\lambda$ where c is a competition parameter which defines how much carriage of one type (VT or NVT) protects against acquisition of the other type (VT or NVT) where 1 is no competition and 0 is complete competition. The model assumes there is no natural immunity following infection, as has been proposed by all but one modelling studies included in the literature review (chapter 4) (145-158, 222). To still reflect different susceptibilities by age and HIV group, parameter $\sigma_{a,s}$ was introduced. This parameter also distinguishes between susceptibility to VT and NVT

serotypes: it is a relative rate with susceptibility to VT serotypes in infants <1 year as the reference group ($\sigma_{0yrs,VT}$ =1). Individuals loose pneumococcal carriage at an age/HIV-specific recovery rate γ_a. Recovery rates do not differ between VT/NVT or B states, in accordance with a study from Denmark reporting that inhibition of colonization is the main mechanism of competition, and that the rate of clearance is not altered in multiple serotype carriage (147). Susceptibility σ_a and recovery γ_a were set for five different age/HIV groups: infants <1 year, children 1-4 years, children 5-15 years, HIV-positive adults and HIV-negative adults.

The transmission rates for our stochastic model are summarized in the following set of differential equations:

$$\frac{dS}{dt} = VT(t) \cdot \gamma_a + NVT(t) \cdot \gamma_a - S(t) \cdot \sigma_{a,VT} \cdot \lambda_{VT}(t) - S(t) \cdot \sigma_{a,NVT} \cdot \lambda_{NVT}(t) \quad (1)$$

$$\frac{dVT}{dt} = B(t) \cdot \gamma_a + S(t) \cdot \sigma_{a,VT} \cdot \lambda_{VT}(t) - VT(t) \cdot \sigma_{a,NVT} \cdot c \cdot \lambda_{NVT}(t) \quad (2)$$

$$\frac{dNVT}{dt} = B(t) \cdot \gamma_a + S(t) \cdot \sigma_{a,NVT} \cdot \lambda_{NVT}(t) - NVT(t) \cdot \sigma_{a,VT} \cdot c \cdot \lambda_{VT}(t) \quad (3)$$

$$\frac{dB}{dt} = VT(t) \cdot c \cdot \sigma_{a,NVT} \cdot \lambda_{NVT}(t) + NVT(t) \cdot c \cdot \sigma_{a,VT} \cdot \lambda_{VT}(t) - B(t) \cdot \gamma_a \quad (4)$$

where $\sigma_{a,s}$ is the age/HIV group and VT/NVT specific susceptibility parameter, γ_a is the age/HIV group specific recovery rate and λ_{VT} and λ_{NVT} are the force of infection for VT and NVT respectively.

The force of infection is described by the following equations:

$$\lambda_{NVT}(t) = \left(\frac{\beta_{hh} \cdot \left(NVT_{hh}(t) + B_{hh}(t)\right)}{n_{hh}(t) - 1} + \frac{\beta_{com} \cdot \left(NVT_{com}(t) + B_{com}(t)\right)}{n_{com}(t) - 1} \right) \quad (5)$$

$$\lambda_{VT}(t) = \left(\frac{\beta_{hh} \cdot \left(VT_{hh}(t) + B_{hh}(t)\right)}{n_{hh}(t) - 1} + \frac{\beta_{com}(t) \cdot \left(VT_{com}(t) + B_{com}(t)\right)}{n_{com}(t) - 1} \right) \quad (6)$$

where $VT_{hh}, NVT_{hh}, B_{hh}, VT_{com}, NVT_{com}, B_{com}$ are the total number of individuals carrying VT, NVT or both within the household and the community at time t.

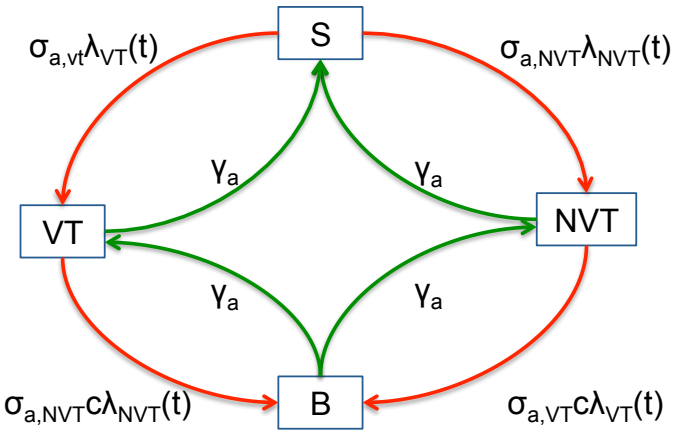


Figure 9.2. Model structure.
Adapted from Melegaro et al, 2010 (220). Model describes the transmission to different states: S (susceptible), VT (carrier of vaccine type), NVT (carrier of non-vaccine type), B (carrier of both VT and NVT), where λ_{VT} and λ_{NVT} are the forces of infection for VT and NVT, γ_a is the age-specific recovery rate, c is the competition parameter, and $\sigma_{a,s}$ is the age/HIV and serotype-specific susceptibility parameter.

9.3 Description of fitting process

The model's steady-state pneumococcal carriage prevalence for status VT, NVT and B by age group was fitted to the cross-sectional data available from nasopharyngeal swabs collected in the pre-vaccination period (see section 9.3.2). Sequential Monte Carlo approximate Bayesian computation (SMC ABC) was used for the fitting process (223). ABC combines a particle (see below) filtering method with summary statistics, allowing for the fitting of complex stochastic models such as ours for which the likelihood is difficult to define (224-226). The algorithm for ABC rejection sampling, the simplest form of ABC, is as follows:

1. A candidate set of parameter values θ^*, called a particle, is drawn from their respective prior distributions $\pi(\theta)$.
2. The model is run with the drawn parameter values, simulating a dataset x^*.
3. The simulated dataset x^* is compared to the observed data x_0 with summary statistics, creating distance function $d(x_0, x^*)$
4. If the distance function $d(x_0, x^*)$ is lower than a predefined tolerance threshold ε, the parameter set is accepted, otherwise the parameter set is rejected.

If ε is sufficiently small, the distribution of accepted particles will be a good approximation of the posterior distribution (223). The disadvantage of ABC rejection sampling is that the acceptance rate is very small if the prior distribution is different from the posterior distribution. To overcome this problem, SMC ABC is performed in multiple rounds r to obtain intermediate distributions with decreasing tolerance thresholds $(\varepsilon_1 > \varepsilon_2 > \cdots > \varepsilon_r)$, until the target distribution is reached which will approximate the posterior distribution.

In our fitting process, SMC ABC was performed in the following steps (223), depicted in figure 9.3:

Preparation

1. A pre-defined series of tolerance values was set with decreasing values for each round *r*: $\varepsilon_1 > \varepsilon_2 > \cdots > \varepsilon_r$. We used the step-wise linear series of ε = 8000,6000,4000,2000,1000,800,600,400,200,100,80,60,40,20.

Round 1

2. Parameter values were sampled from their respective prior distributions $\pi(\theta)$ (see 9.2.5) to form a candidate parameter set θ^* (called a particle) (Fig. 9.2A).
3. A simulation was run with the sampled parameter values until time T. We used one-day time steps for a simulation period of two years (730 days).

4. The simulated steady-state pneumococcal carriage prevalence for status VT, NVT and B by age group was fitted to the cross-sectional data from nasopharyngeal swabs collected in the pre-vaccination period. We used the Chi-squared test on observed vs. model counts of individuals in age group a with status VT, NVT and B as the summary statistic.
5. If the z-value calculated by the Chi-square test was lower than the pre-defined tolerance value ε_1, the set of parameter values was accepted (Fig. 9.2B).
6. Steps 1-5 were repeated until the number of accepted values reached the pre-defined threshold N when round 2 was started. We used N=200 in our fitting algorithm.

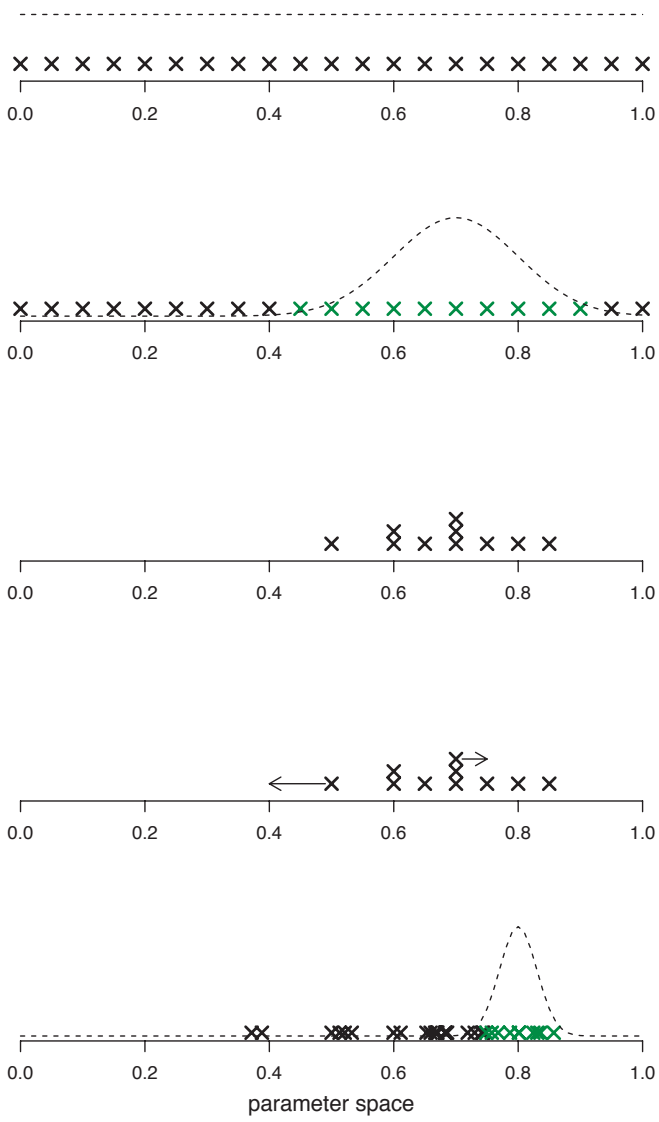
Subsequent rounds

7. A candidate particle was sampled from the accepted particles of the previous round with weights w_{r-1} as defined in step 11 (Fig. 9.2C). Accepted particles of round 1 were all given equal weight.
8. The sampled particle was perturbed using a perturbation kernel (Fig 9.2D). For the parameter β_{hh} log-normal perturbation was used to avoid negative parameter values. For all other parameters logit-normal perturbation was applied to restrain parameter values between 0 and 1. A standard deviation of 0.5 was used in all perturbations.
9. A data set was simulated and evaluated as in step 4 described above.
10. If the z-value calculated by the Chi-square test was lower than the pre-defined tolerance value ε_r, the set of parameter values was accepted.
11. Accepted particles' weight was calculated using the formula

$$w_t^{(i)} = \frac{\pi(\theta_r^{(i)})}{\sum_{j=1}^{N} w_{r-1}^{(i)} K_r(\theta_{r-1}^{(j)}, \theta_r^{(i)}))}$$

where the particle i in round r, $\theta_r^{(i)}$, was assessed according to its prior distribution $\pi(\theta)$ and to each of the particles j accepted in the previous round $r-1$ by assessing it's weight $w_{r-1}^{(j)}$ and perturbation kernel $K_r(\theta_{r-1}^{(j)}, \theta_r^{(i)})$.

12. Steps 7-11 were repeated until the number of accepted values reached the pre-defined threshold N=200. When threshold was reached, all weights for the round were normalized and the next round was started.
13. Approximate posterior distribution was obtained after the final round (Fig 9.2E). The simulation was stopped when additional rounds only provide marginal changes in the distribution and when the acceptance rate was very low (<1%) causing an increase in the time to reach N.



A. Particles are drawn from the prior distribution (uniform between 0 and 1 in this example)

B. A dataset is simulated with each of the drawn particles. Summary statistic is calculated. If distance function < ε, particle is accepted (shown in green). Intermediate distribution shown in dashed line.

C. In the next round, the accepted particles from the previous round are resampled with replacement

D. Each of sampled particles is perturbed using a perturbation kernel.

E. A dataset is simulated with each of the perturbed particles. Summary statistic is calculated. If distance function < ε, particle is accepted (shown in green). After final round is finished, the approximate posterior distribution can be obtained (shown in dashed line)

Figure 9.3 Schematic overview of sequential Monte Carlo approximate Bayesian computation (SMC ABC)

The main advantage of using ABC in contrast to more conventional optimization methods such as MCMC is that that the likelihood does not need to be theoretically derived (223-226). This allows for the fitting of more complex models. ABC is relatively simple and generalizable, and can be thus applied to deterministic and stochastic models (223). Moreover, by using different rounds in the fitting process, information on the sensitivity of the model to different parameters can be gathered from the shape of intermediate distributions (223). If the model is sensitive to a parameter, this parameter will be inferred in earlier rounds and have narrow credible intervals. If on the other hand the distribution for a parameter does not change much in subsequent SMC ABC rounds, it can be concluded that the parameters is not inferable given the available

data. ABC methods can also be used to compare alternative model structures (223, 225). Lastly, a major advantage of SMC ABC is that it is computationally efficient and can be run in parallel, allowing its application to complex models and large datasets without being too computationally demanding (225).

The main drawback of using ABC is that it is a relatively new method and that does not yet have the statistical rigor of more conventional optimization methods. Studies have been published showing the competitiveness of ABC with full like-likelihood methods, but more studies are needed to better evaluate the degree of approximation (226). These studies, however, are restricted to models where explicit likelihoods functions are tractable. Another disadvantage of ABC methods is that they are reliant on the user-choice of appropriate summary statistics. Different summary statistics are found to influence how closely the posterior distribution is approximated (227). There are no well-defined rules as to which and how many summary statistics should be used (226), although the academic field is rapidly moving towards standardization of methods (227, 228). The choice of the acceptance threshold is another area that similarly requires standardization (227). For the purpose of this PhD, the choice of summary statistics and the acceptance thresholds were set in advance and were not evaluated in a dynamic process. This is one area that could be improved in follow-up work.

9.4 Selection of prior distributions

We searched household modelling studies included in the systematic literature review (chapter 4) for estimates on which to base our prior distributions for each parameter requiring estimation. A summary of the prior distributions is shown in Table 9.6 at the end of this section.

Transmission rates

Table 9.2 shows household transmission parameters reported from mathematical studies in the literature. Some studies reported separate parameters by age group, but we decided to fit one parameter for within-household transmission only (β_{hh}). For our model we chose a gamma prior distribution with a mean of 0.10 and a variance of 0.10. This mean is higher than the mean reported in the literature (table 9.2), because training simulations showed that a lower β_{hh} did not sustain transmission.

Table 9.2. Household transmission parameters and community force of infection reported in literature

Setting (ref)	Household transmission rate per day (95%CI)		Community force of infection per day (95% CI)	
Bangladesh (153)	family to child	0.0241 (0.0229-0.0258)	community to child	0.0054 (0.0049–0.0059)
Finland (154)	family to child	0.012 (0.017-0.033)	community to child	0.0002 (0.0001-0.0003)
UK (157, 158, 220)	adult to adult	0.048 (0.010-0.180)	community to adult	0.004 (0.002-0.005)
	adult to child	0.106 (0.020-0.450)	community to child	0.012 (0.008-0.016)
	child to adult	0.005 (0.000-0.018)		
	child to child	0.047 (0.008-0.200)		
South Africa (162)	child to mother	0.0027 (0.0013-0.004)	community to child	0.0051 (0.0044-0.0056)
	mother to child	0.00044 (0.000-0.001)	community to mother	0.0013 (0.0008-0.0019)

All studies identified in the literature reported the community force of infection as a constant, rather than a community transmission parameter which indicates the chance of effective transmission upon contact in the community. Because our model keeps track of individual infection status for the whole community, a community transmission parameter was used. We decided to use a uniform prior from 0 to 1 for the ratio of household and community transmission. This makes the assumption that β_{com} is smaller or identical to β_{hh}: that the rate at which two individuals come into "effective" contact (contact sufficient to lead to infection if it occurs between a susceptive and infective person) is smaller in the community than amongst household members. Justification for this assumption is that household contact is more intimate and of longer duration than contact in the community. Support for our assumption comes from studies on influenza showing that susceptible individuals were more likely to be infected by their household members than by members of the community (229).

Susceptibility

Table 9.3. Susceptibility parameters reported in literature

Setting (ref)	Age groups comparison	Susceptibility parameter (95% CI)
Bangladesh (153)	≥5 vs. <5 yrs	0.46 (0.45-0.49)
Finland (154)	≥7 vs. <7 yrs	0.41 (0.28-0.58)

Two studies used a relative rate to estimate differences in susceptibility between age groups (Table 9.3). In our model, we used an age/HIV group and serotype (VT vs. NVT) specific parameter. We made the assumption that infants <1 year were most susceptible for pneumococcal acquisition, followed by children 1-4 years, 5-15 years and adults. We also assumed that infants <1 year were equally or more susceptible to VT than to NVT serotypes. The susceptibility parameter, defined as a relative rate of the force of infection, was set at 1 for infants <1 year for VT transmission (reference group). We used a prior beta distribution with a mean of 0.75 to describe susceptibility of infants <1 year for NVT as compared to VT serotypes. For age groups 1-4 years, 5-15 years and adults a prior beta distribution was chosen for both VT and NVT transmission, with means of 0.75, 0.50 and 0.25 respectively. This is in line with the estimates from the studies in Table 9.3, which used a different age structure (our relative rate for ≥ 5 years vs. <5 years = 0.43). A wide variance (σ^2 =0.03) was used on all susceptibility priors, allowing for parameter values on the whole range between 0 and 1 to be selected. The priors for susceptibility of HIV-positive and HIV-negative

adults were set the same, but the parameter values were allowed to differ between HIV-positive and HIV-negative adults in the model.

Competition

Table 9.4. Competition parameters reported in literature

Setting (ref)	Parameter for VT (c_v), NVT (c_n) or both (c)	Competition parameter (95% CI)
Bangladesh (153)	c	0.83 (0.73-0.93)
UK / USA (157, 158, 220)	c_v	0.5
	c_n	0.85
Denmark (147)	c	0.09 (0.05-0.15)
Finland (154)	c	0.68 (0.35-1.10)
Norway (159)	c	0.10 (0.06-0.14)

Estimates of the competition parameter, defined as the relative rate of acquisition for non-carriers as compared to carriers of another serotype, differed in the literature with estimates ranging from 0.09 to 0.85 (Table 9.4). We therefore decided to use a uniform prior distribution from 0 to 1 for our model.

Recovery rates

Training simulations showed poor performance when both susceptibility parameters and recovery rates were fitted. We therefore decided to fix the recovery rates during our model fitting. Table 9.5 shows the estimated days of carriage reported from longitudinal or modelling studies in the literature. Four studies reported days of carriage for infants <1 years separately: a modelling study in the UK reported an average carriage duration of 71 days, a longitudinal study in Thailand-Myanmar reported 60 days, a longitudinal study in Kenya reported 48.5 days and a modelling study in Sweden reported 30 days. For our model we choose carriage duration of 48.5 days, since we assumed that the situation in Kenya most closely resembled the situation in Malawi.

For children 1-4 years, reported days of carriage ranged between 22 and 42.6 days. For our model we choose a mean duration of 31 days, again reflecting the Kenyan situation. Two studies included children 5-15 years, both reporting similar estimates of 15 and 18 days. We used an estimate of 18 days for our model. For adults, studies reported carriage of 14 (Sweden), 17 (UK) or 31 (Thailand-Myanmar) days. The longitudinal study from Thailand-Myanmar used monthly swabbing intervals, however, and could therefore not detect carriage duration shorter than one month. We chose the UK estimate of 17 days for our model.

Table 9.5. Days of carriage by age group reported in literature

Setting (ref)	Age group	Days carriage (95% CI*)
Bangladesh (153)	<5 yrs	42.6 (40.4 - 45.5)
	≥5 yrs	38
Denmark (147)	1-3 yrs	24
Finland (154)	≤7 yrs	44 (41-48)
	>7 yrs	36
France (149)	3-6 yrs	22 (20 - 24)
Kenya (230)	6-11m	48.5 (30.8 - 76.4)
	3-59m	31.3 (29.7 - 33.6)
Sweden (231)	<1 yrs	30
	1-4	21
	5-6	13
	7-18	15
	18+	14
Thailand-Myanmar (141)	0-2 yrs	60 (57.0 - 60.5)
	Mothers	31 (31.0 - 31.5)
UK (157, 158, 220)	0-1 yrs	71
	2-4 yrs	28
	5-17 yrs	18
	18+ yrs	17

*90% confidence intervals reported for study in Bangladesh.

Table 9.6. Prior distributions chosen for mathematical model in pre-vaccination period

Parameter: meaning		Prior / value	Mean	Variance
Fitted parameters				
β_{hh}: transmission rate household		$\Gamma(0.1,1)$	0.10	0.10
ratio β_{hh}/β_{com}		$U(0,1)$	0.5	0.083
σ_a: susceptibility	0 yr – VT	1	1	-
age groups	0 yr - NVT	B(3.9375, 1.3125)	0.75	0.03
	1-4 yr (VT & NVT)	B(3.9375, 1.3125)	0.75	0.03
	5-15 yr (VT & NVT)	B(3.6667, 3.6667)	0.5	0.03
	16+ yr HIV+ (VT & NVT)	B(1.3125, 3.9375)	0.25	0.03
	16+ yr HIV- (VT & NVT)	B(1.3125, 3.9375)	0.25	0.03
c: competition		$U(0,1)$	0.5	0.083
Fixed parameters				
γ_a: recovery rate	0 yr	48.5 days		
(1/days carriage)	1-4 yr	31 days		
	5-15 yr	18 days		
	16+ yr HIV+	17 days		
	16+ yr HIV-	17 days		

9.5 Assessment of fitting process

9.5.1 Generating dummy data

We assessed the fitting process by generating dummy data with known parameter settings. Fitting the model with prior settings as described in section 9.4 allowed us to assess how well the fitted parameters resembled the parameters set for this dummy data. The dummy data was fitted to an earlier version of the final model: the susceptibility parameter σ_a did not distinguish between VT and NVT serotypes in the dummy model.

Parameter settings were defined as follows: β_{hh} and c were fixed at 0.15 and 0.85 respectively to ensure steady state transmission was achieved for all statuses (VT, NVT, B) in all age groups; for the ratio between β_{hh} and β_{com} and σ_a a random parameter was chosen between 0.5 and 2 times the mean of the prior as specified in Table 9.6; γ_a was fixed as described in section 9.3. The model was run with one-day time steps for a period of two years. Dummy data was generated by taking the mean values of 100 simulations. Table 9.8 shows the parameter settings for generating the dummy data and the results of the fitting process.

9.5.2 Results of fitting to dummy data

We fitted the model to dummy data using the SMC ABC algorithm described in section 9.4.1. In the first round, in which particles were obtained from the prior distribution, the acceptance was 7.8% (Table 9.7). This was increased to 51.5% in round 2 and thereafter decreased with each round until we terminated the procedure after round 10, when the acceptance was 0.6%.

Table 9.7 Acceptance for SMC ABC fitting to dummy data

round	tolerance ε	acceptance	# simulations required to reach N = 200
1	8000	7.8%	2564
2	6000	51.5%	388
3	4000	35.5%	563
4	2000	16.7%	1198
5	1000	12.3%	1626
6	800	9.6%	2083
7	600	7.9%	2532
8	400	4.9%	4082
9	200	1.5%	13333
10	100	0.6%	33333

Figure 9.4 shows the prior and (intermediate) posterior distributions for rounds 1, 4, 7 and 10. Credible intervals were increasingly narrowing around the 'true' value with each round for all parameters, apart from the ratio β_{hh} / β_{com}. Table 9.8 shows the 'true' value that the dummy data was fitted to and the weighted mean of the posterior after round 10.

Table 9.8 Parameter settings for creating dummy data to assess fitting process and SMC ABC posterior distribution mean

Parameter: meaning		**Value**	**Weighted mean posterior (95% credible interval)**
β_{hh}: transmission rate household		0.150	0.172 (0.102-0.326)
ratio β_{hh}/β_{com}		0.602	0.535 (0.106-0.948)
σ_a: susceptibility	0 yr	1	-
age groups	1-4 yr	0.782	0.802 (0.591-0.963)
	5-15 yr	0.454	0.468 (0.338-0.622)
	16+ yr HIV+	0.167	0.172 (0.091-0.266)
	16+ yr HIV-	0.375	0.380 (0.275-0.515)
c: competition		0.850	0.828 (0.670-0.978)
γ_a: recovery rate	0 yr	48.5 days	-
(1/days carriage)	1-4 yr	31 days	-
(fixed)	5-15 yr	18 days	-
	16+ yr HIV+	17 days	-
	16+ yr HIV-	17 days	-

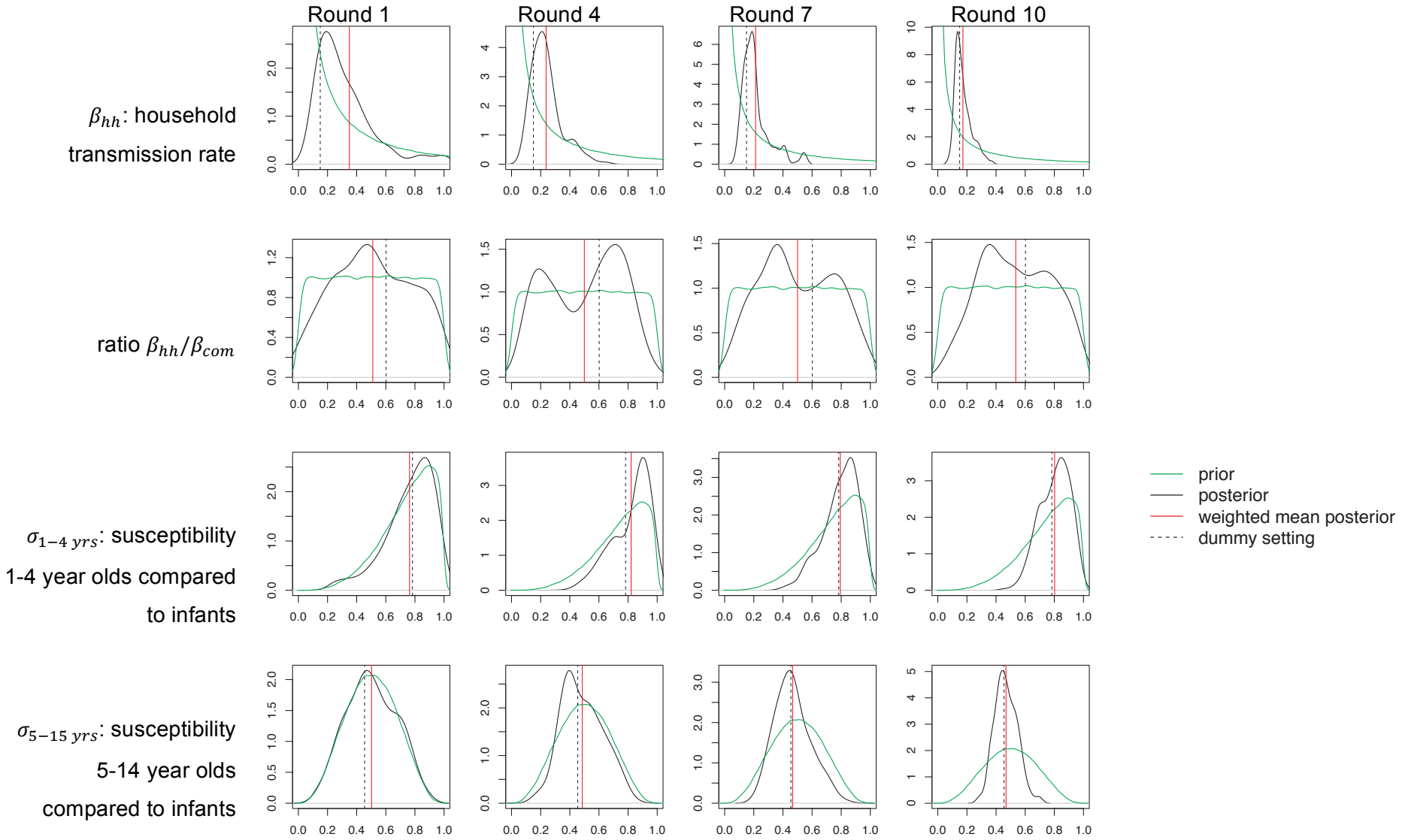


Figure 9.4 Prior and posterior distribution of several rounds of SMC ABC fitting to dummy data

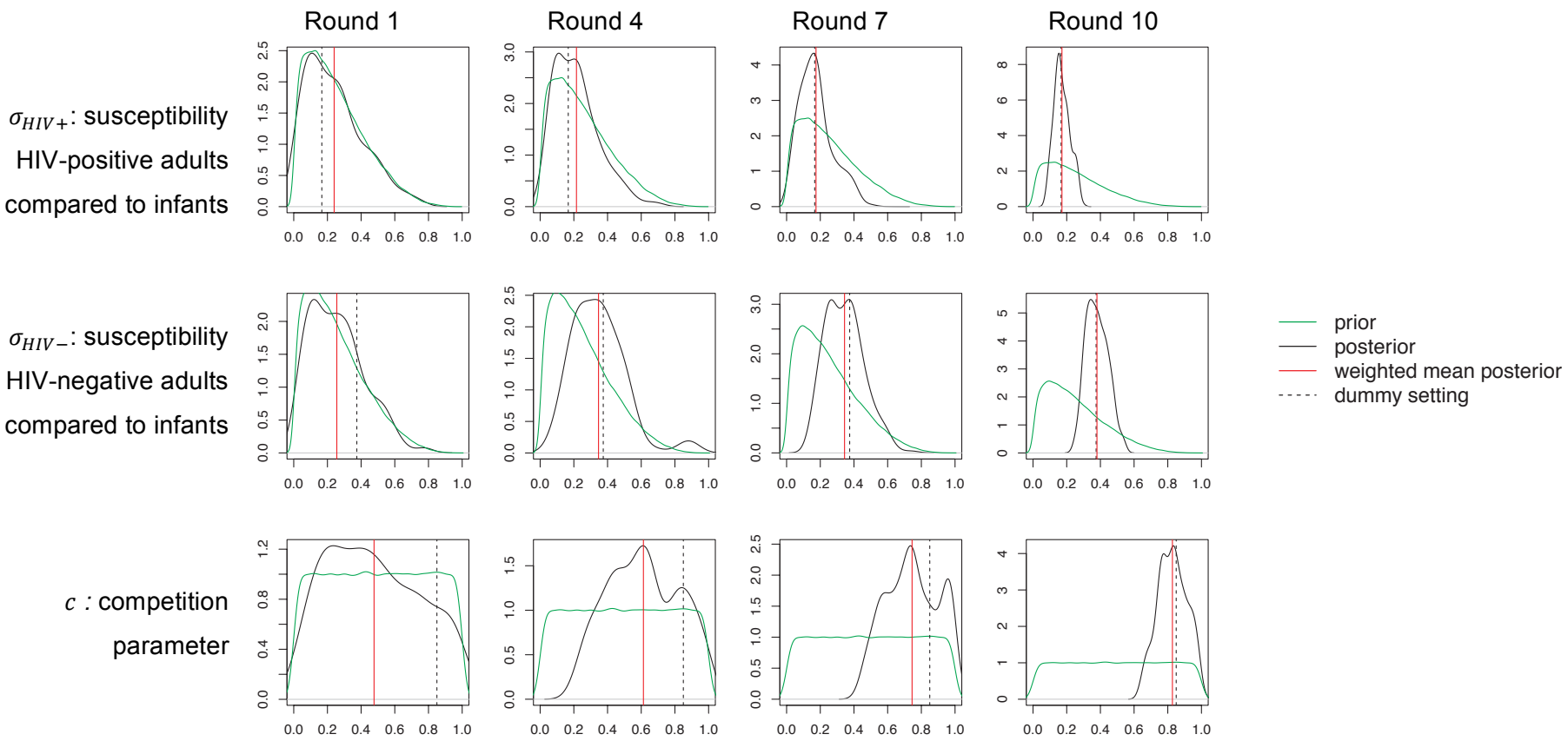


Figure 9.4 *cont.* Prior and posterior distribution of several rounds of SMC ABC fitting to dummy data

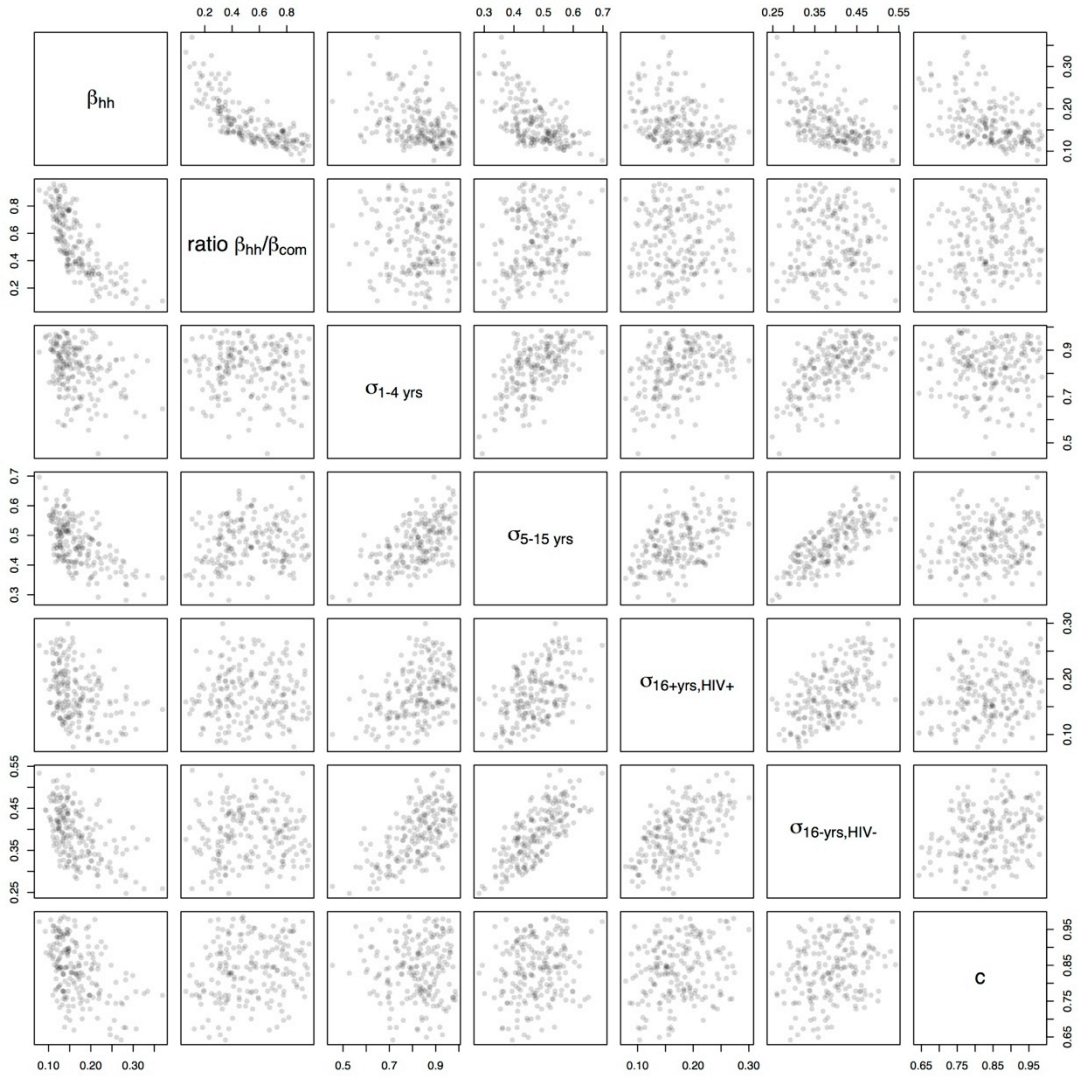


Figure 9.5 Correlation between the posterior distribution of parameters fitted to dummy data.

Figure 9.5 shows the correlation between the posterior distribution of parameters that were fitted to dummy data. A strong negative non-linear correlation was observed between β_{hh} and the ratio β_{hh}/β_{com}, showing a trade-off between household and community transmission. Positive correlations were observed for the four susceptibility parameters. The susceptibility parameters were negatively correlated with β_{hh}, especially those for children 5-14 years and HIV-negative adults.

9.6 Fitting the model to observed data from Karonga

9.6.1 Population demography

The model uses the known population and household structure in the KHDSS area, captured through the demographic surveillance system (54). The model uses census data from the population from 1st January 2009 to 30th June 2015. On 1st January 2009 there were 34,111 individuals living in 7,742 households. There were 718 (2.1%) individuals living in more than one household (mostly polygamous heads of households); they were randomly assigned to one of the households they belong to for the purpose of this mathematical model. On 11th September 2015 there were 39,721 individuals living in 9,602 households. There were 889 (2.2%) individuals who belonged to more than one household. HIV-status for individuals was obtained from the KHDSS records. There were 5,616 of 21,693 (25.9%) adults with unknown HIV-status. For individuals with unknown HIV-status, HIV-status was imputed according to their sex and age, based on HIV prevalence as reported for this population by Floyd et al. (56). We also imputed an HIV-status for older children who were included in the "5-15 years" age group at the start of the simulation period, but matured into the adult age group during the simulation period. HIV-status for this group was missing for 5807 of 8437 (68.8%) adolescents.

9.6.2 Observed proportions of carriage

The model was fit to observed prevalence of pneumococcal carriage in the pre-vaccination period (2009-2011). In short, nasopharyngeal sample results were available from two longitudinal studies: a household study on pneumococcal carriage in HIV-exposed and HIV-unexposed infants (chapter 5), and a longitudinal study on HIV-positive adults attending ART clinic (chapter 6). For the purpose of this modelling study, a cross-sectional estimate of pneumococcal carriage prevalence was taken from all samples collected in both longitudinal studies, for the five different age/HIV groups: infants <1 year, children 1-4 years, children 5-15 years, HIV-positive adults (16+ years) and HIV-negative adults (16+ years). The estimates for children 0-15 years were age-standardized based on the census data from 2010.

The laboratory methods used in both longitudinal studies conducted in the pre-vaccination period allowed only for the detected of a single serotype per sample. Because the model involved the state "B" (carriers of both VT and NVT), we adjusted our prevalence estimates according to estimates from studies conducted in Malawi which used microarrays for serotyping, allowing the detection of multiple serotypes per sample (47, 221) (Table 9.9). Multiple serotype carriage was identified amongst 40% of

samples from children 0-13 years positive for *Streptococcus pneumoniae*, with 61% carrying a VT and NVT serotype simultaneously. Multiple serotype carriage was higher in children 0-2 years (48%) than in children 3-13 years (32%). Although this difference was not statistically significant (p=0.08) (221), we used these proportions for the age groups 0-1 years and 5-15 years in our modelling study. For the age group 1-4 years old we took the overall estimate (40%). Multiple serotype carriage was found to be 16% in HIV-negative adults and 20% in HIV-positive adults (232). In the study on adults no estimate was given on the proportion of samples with multiple carriage in with both a VT and NVT serotype was detected. For the purpose of this study we assumed this proportion of VT and NVT carriage to be the same as for the children (61%). For calculation of the proportion of samples with VT carriage, NVT carriage or carriage of both, we assumed that VT and NVT samples were misclassified as single carriage in equal proportions.

Table 9.9 Observed proportion of VT and NVT carriage and estimated proportion of multiple carriage of VT and NVT by age group

	Proportion multiple carriage amongst positive samples (47, 221)	Observed carriage* (%)			Estimated carriage (%)			
Age group	**VT and NVT**	**VT**	**NVT**	**Total**	**B**	**VT**	**NVT**	**Total**
<1 years	0.48 · 0.61 = 0.293	23.9	18.4	42.3	0.293 · 42.3 = 12.4	23.9 – (12.4/2) = 17.7	18.4 – (12.4/2) = 12.2	42.3
1-4 years	0.40 · 0.61 = 0.244	25.9	25.7	51.6	0.244 · 51.6 = 12.6	25.9 – (12.6/2) = 19.6	25.7 – (12.6/2) = 19.4	51.6
5-15 years	0.32 · 0.61 = 0.195	14.9	22.0	36.9	0.195 · 36.9 = 7.2	14.9 – (7.2/2) = 11.3	22.0 – (7.2/2) = 18.4	36.9
16+, HIV+	0.20 · 0.61 = 0.122	10.0	12.6	22.6	0.122 · 22.6 = 2.8	10.0 – (2.8/2) = 8.6	12.6 – (2.8/2) = 11.2	22.6
16+, HIV-	0.16 · 0.61 = 0.098	5.6	10.4	16.0	0.098 · 16.0 = 1.6	5.6 – (1.6/2) = 4.8	10.4 – (1.6/2) = 9.6	16.0

* age-standardized for children 0-15 years to 2010 KPS population

9.6.3 Results of fitting to observed data

We fitted the model to the observed Karonga carriage prevalence data (section 9.3.2) using the SMC ABC algorithm described in section 9.4.1. In the first round, in which particles were obtained from the prior distribution, the acceptance was 2.6% (Table 9.10). This was increased to 16.1% in round 2 and thereafter decreased with each round. We terminated the procedure after round 5, when the acceptance was 0.4%.

Table 9.10 Acceptance for SMC ABC fitting to Karonga data

round	tolerance ε	acceptance	# simulations required to reach N = 200
1	8000	2.6%	7745
2	6000	16.1%	1244
3	4000	10.7%	1878
4	2000	1.9%	10300
5	1000	0.4%	48500

Figure 9.6 shows the observed carriage prevalence and the model carriage estimate for VT, NVT and B carriage in the five age/HIV groups. The 95% credible intervals for the observed and model estimate prevalence overlap for all carriage types (VT/NVT/B) and age/HIV groups, apart from NVT carriage in infants which is overestimated in the model.

Figure 9.7 shows the prior and (intermediate) posterior distributions for rounds 1-5. For most parameters, credible intervals were increasingly narrowing with each fitting round. Round 5 was only marginally different from round 4, and saw the introduction of multimodal distributions. A narrow credible interval was observed for the household transmission rate β_{hh}, and competition parameter c, identifying those two parameters as most important for the model fit. The peak of the distribution β_{hh} lies around 0.1; this is higher than estimated from other studies (Table 9.2). The competition parameter defines how much carriage of one type (VT or NVT) protects against acquisition of the other type (VT or NVT) where 1 is no competition and 0 is complete competition. We estimate the competition parameter to be between 0.8 and 1, meaning that very little competition occurs in our setting.

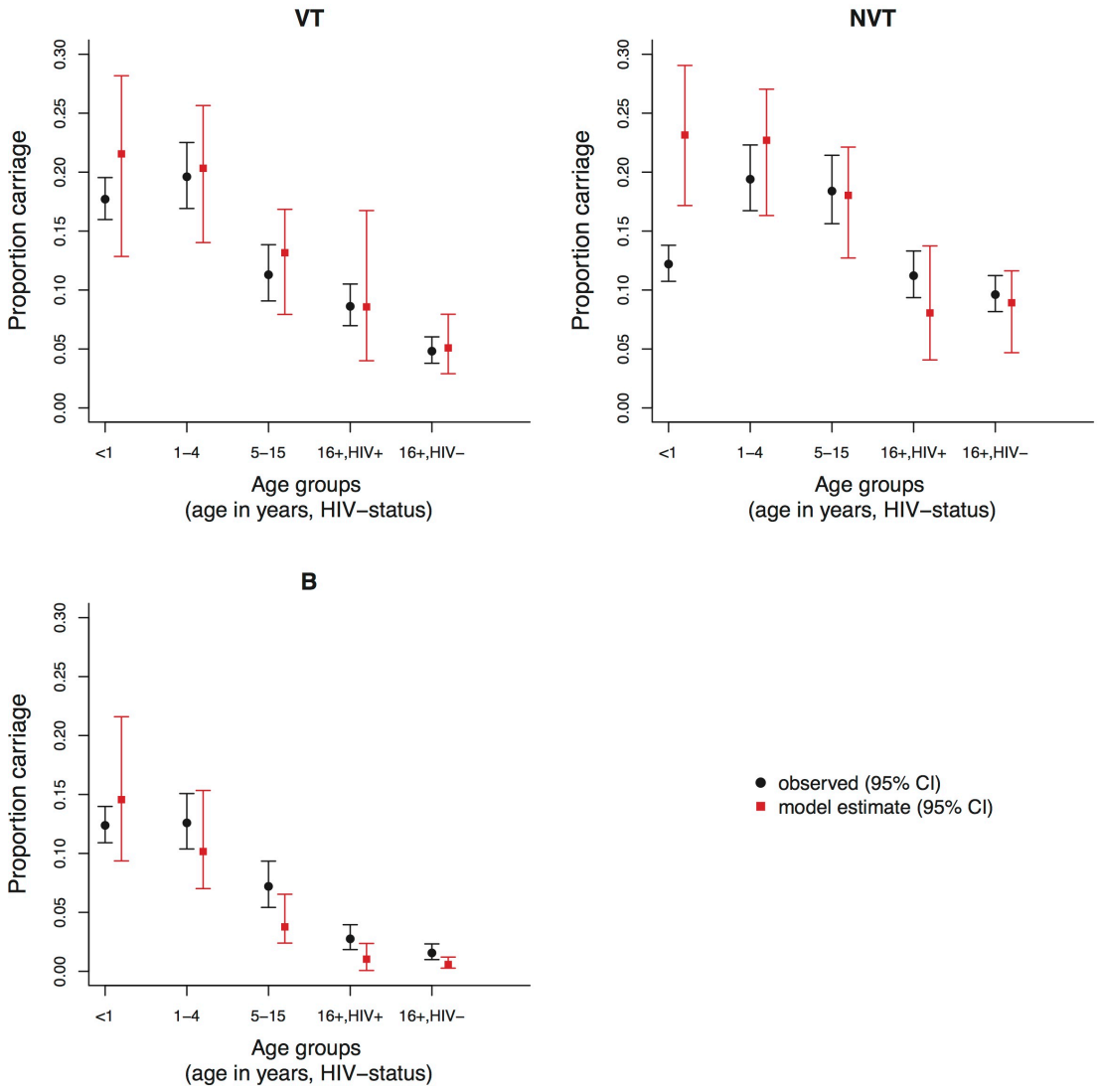


Figure 9.6 Observed and model carriage prevalence for data of Karonga District.
Model results from 200 simulations with parameters drawn from ABC SMC round 5 with weighted sampling. Model carriage prevalence was assessed at 2 years (730 days).

For the susceptibility parameters, we made the assumption that infants were most susceptible for pneumococcal acquisition and that infants were equally or more susceptible to VT than to NVT serotypes. The susceptibility parameter, defined as a relative rate of the force of infection, was set at 1 for infants for VT transmission (reference group). Figure 9.7 shows that the model fit for $\sigma_{1-4\ yrs,VT}$ tends to move towards 1, indicating that there is not much difference between the susceptibility of children 1-4 years for VT carriage as compared to infants. Also the model fit for $\sigma_{<1\ yrs,NVT}$ tends to move towards 1, although the credible interval is wide. Poor fitting of this parameter is likely to have resulted in the overestimate of NVT carriage in infants in the model (Figure 9.6).

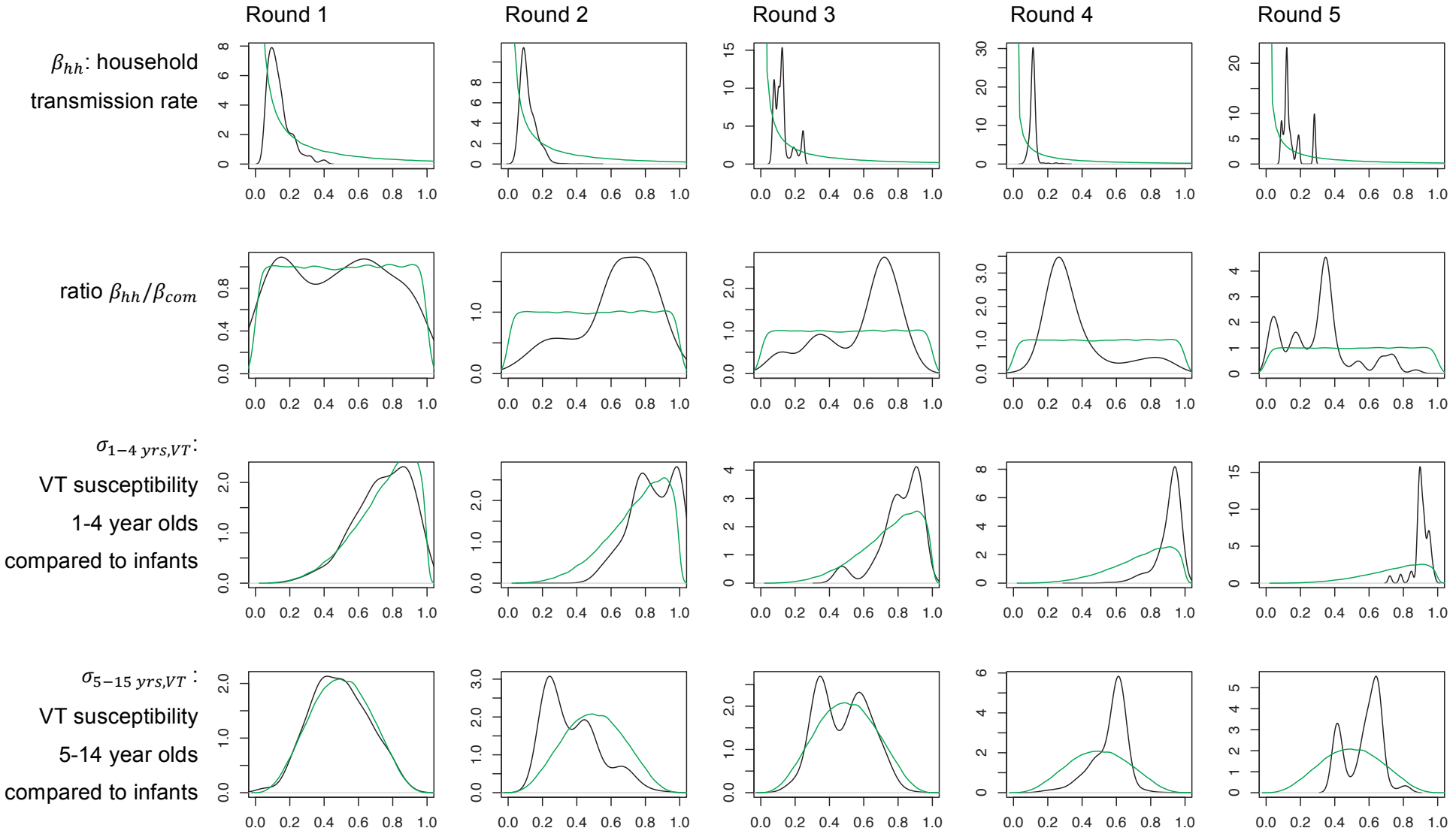


Figure 9.7 Prior and posterior distribution of several rounds of SMC ABC fitting to data from Karonga District

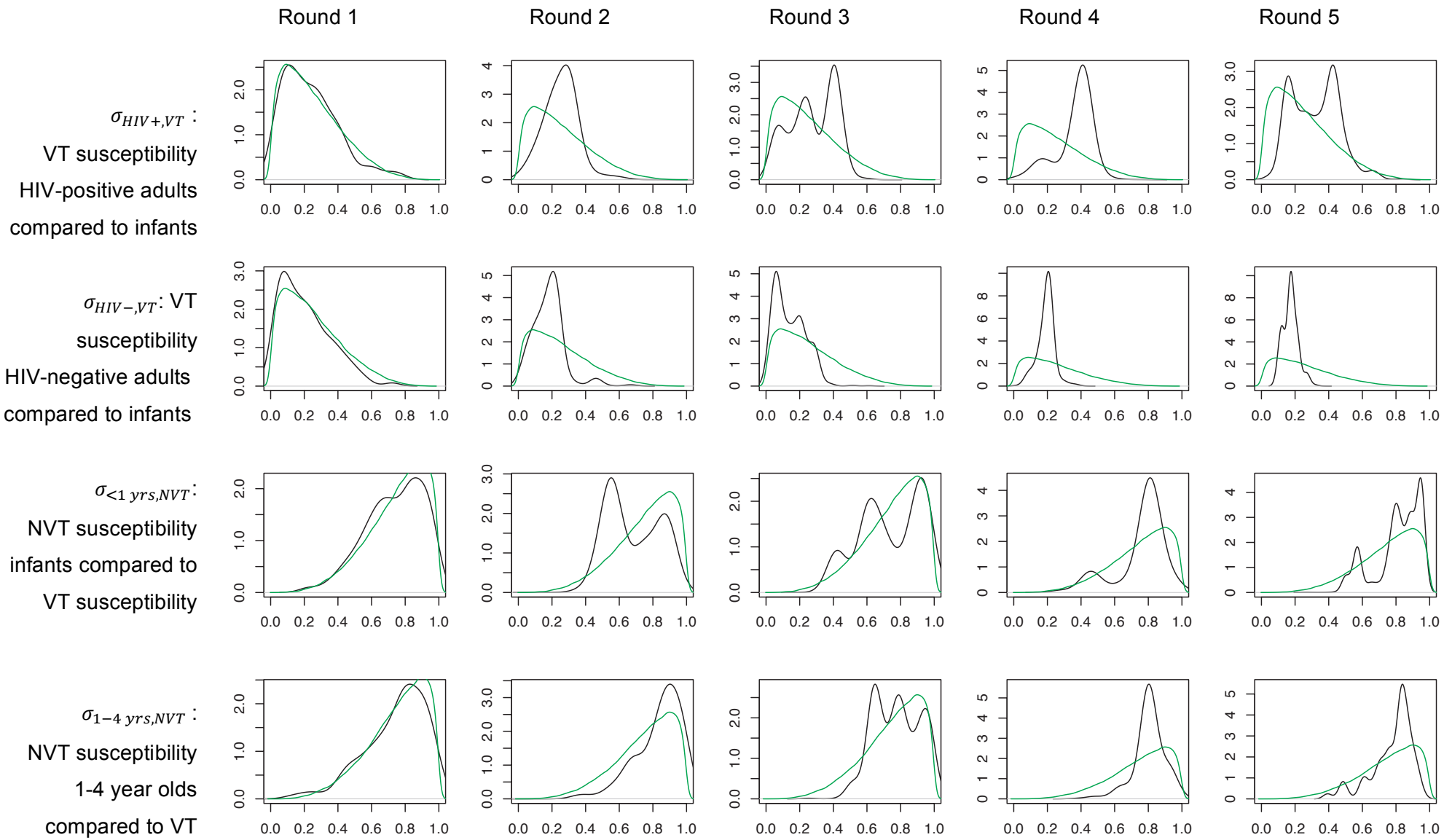


Figure 9.7 *cont.* Prior and posterior distribution of several rounds of SMC ABC fitting to data from Karonga District

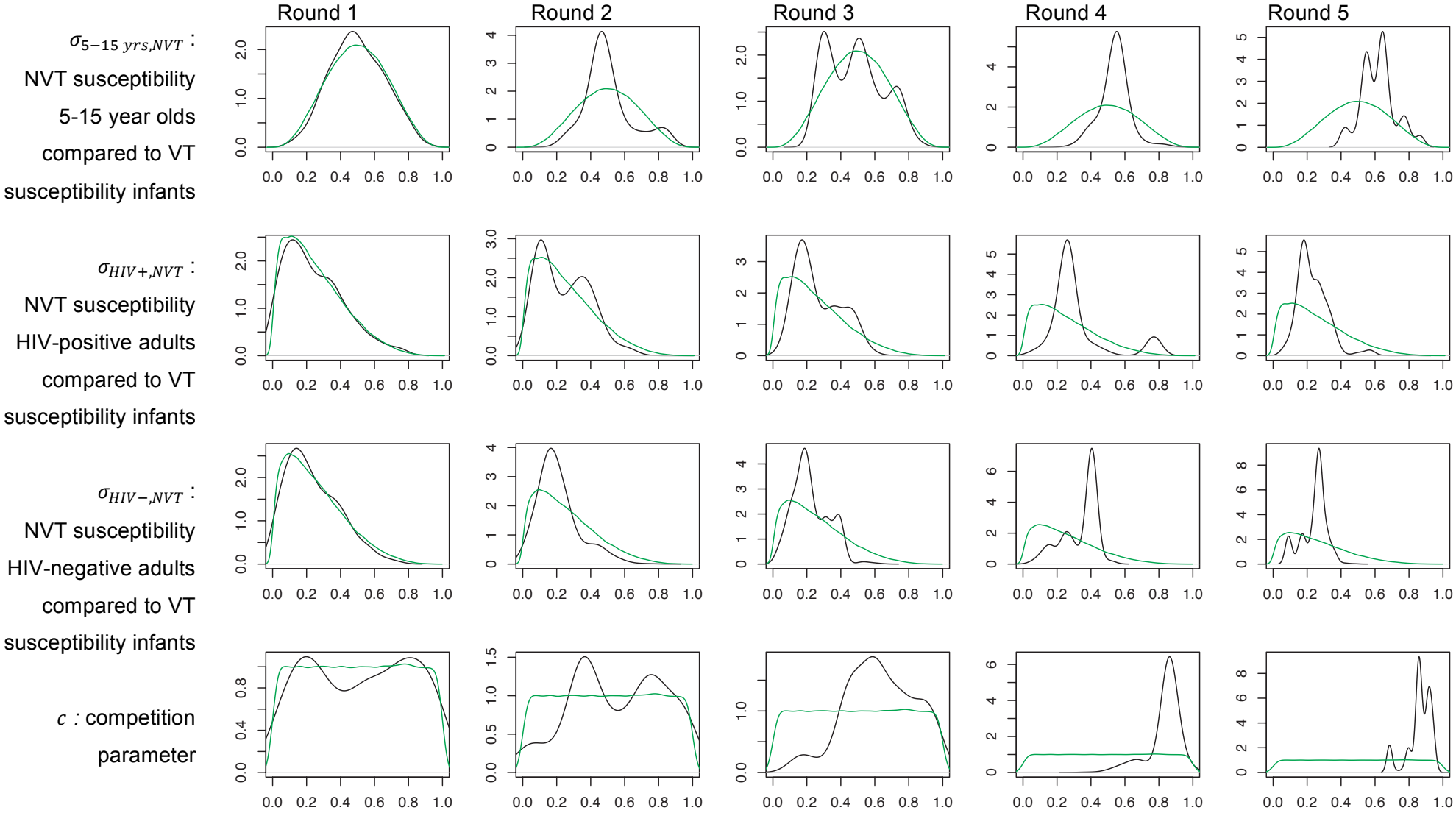


Figure 9.7 *cont.* Prior and posterior distribution of several rounds of SMC ABC fitting to data from Karonga District

There is some evidence from the model fit that HIV-positive adults are more susceptible for VT carriage than HIV-negative adults, although the credible estimates of the model fit were wide. No convincing difference by HIV-status is observed for susceptibility of NVT carriage. Figure 9.8 shows the correlation between the posterior distributions of parameters that were fitted the data established in the field studies. Positive correlations were observed for the correlation between the VT and NVT parameters for $\sigma_{5-15\,yrs}$ and σ_{HIV-}; the susceptibility parameters for children 5-15 years and HIV-negative adults, respectively. This suggests that for those age/HIV-groups separate parameters for susceptibility of VT and NVT carriage were not required.

A multimodal distribution is observed for the ratio β_{hh}/β_{com}. In round 5, a peak was observed around 0.35, which would indicate that β_{com} is approximately 0.035 given our estimate of β_{hh} of 0.10. Caution needs to be taken in reporting this finding, however, as smaller peaks are observed for estimates as small as 0.05 and as large as 0.7. The ratio β_{hh}/β_{com} was also found to have the poorest fit in the fitting to dummy data, indicating that this parameter is difficult to fit with the current model structure. Figure 9.8 shows that a strong negative non-linear correlation was observed between β_{hh} and the ratio β_{hh} / β_{com} , indicating a trade-off between household and community transmission. This trade-off could have resulted in the poor fitting of the ratio β_{hh}/β_{com}. Fitting the ratio β_{hh}/β_{com} was also complicated by the uninformative uniform prior; it is possible that this parameter would have had a better fit with a more informative prior.

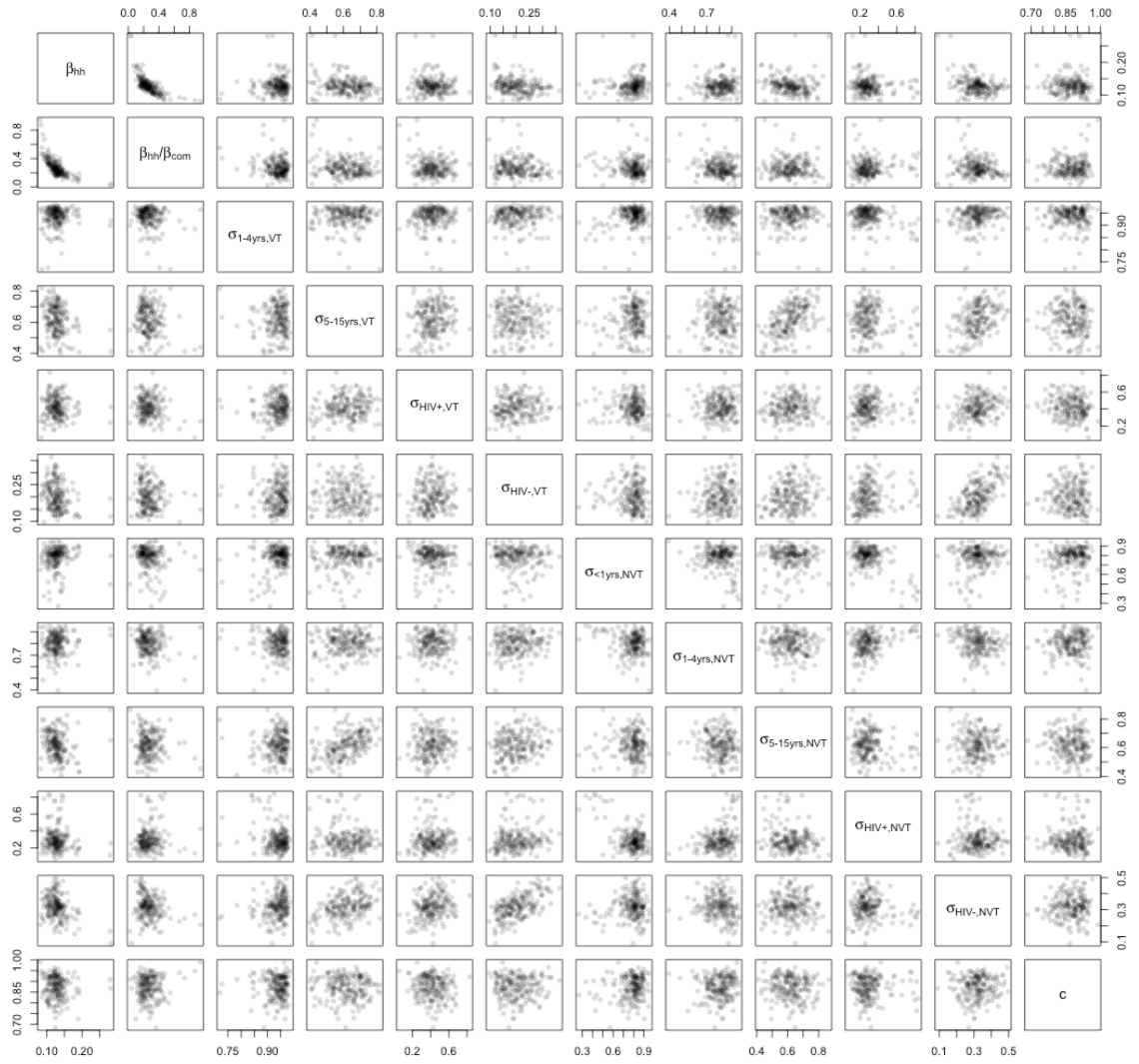


Figure 9.8 Correlation between the posterior distributions of parameters fitted to data from Karonga District.

9.6.4 Simulations of pneumococcal carriage without vaccination strategy.

Figure 9.9 shows the results from 200 simulations with parameters drawn from the posterior distribution (ABC SMC round 5) with weighted sampling. All simulations were started at the observed carriage prevalence by age/HIV group, in figure 9.9 depicted by the dashed horizontal line. The simulations were run from 1st January 2009 to 30th June 2015 (2371 days). The vertical dashed line in figure 9.9 shows the date at which the model was assessed for the model fit (section 6.5.2): 1st January 2011, two years (730 days) after the start of the simulation.

Pneumococcal carriage prevalence decreases over the simulation time, particularly for VT and B carriage. Mean VT carriage prevalence in infants was highest at 23.8% at

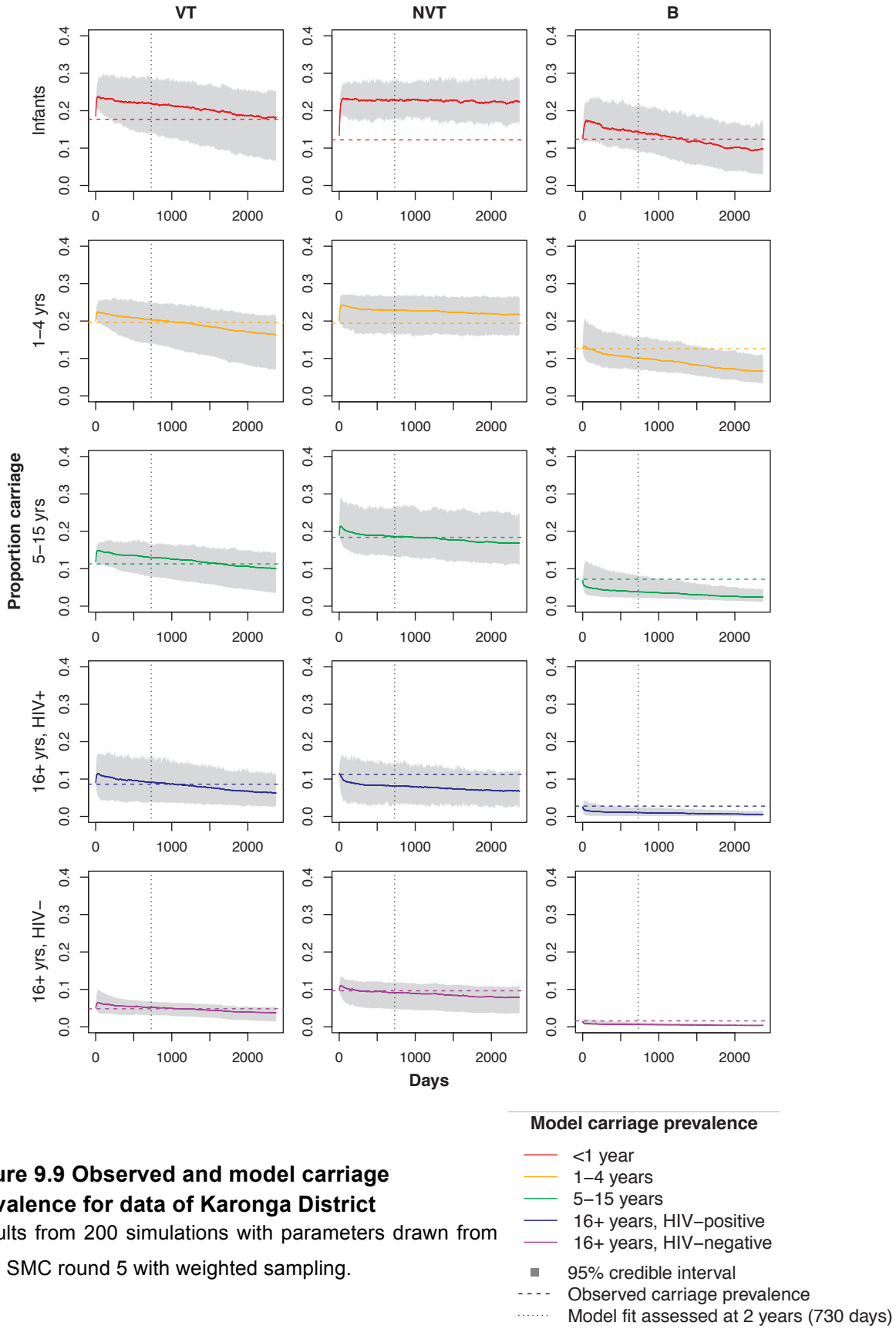


Figure 9.9 Observed and model carriage prevalence for data of Karonga District

Results from 200 simulations with parameters drawn from ABC SMC round 5 with weighted sampling.

day 35 after start of the simulation and lowest at 17.8% towards the end of the simulation. Mean carriage prevalence of both VT and NVT ("B status") decreased in infants from 17.4% to 9.3% over the simulation period.

9.7 Discussion

Findings

We developed a stochastic individual-based model with SIS infection dynamics and explicit, dynamic household and population structure, which allowed for the investigating of within-household and community transmission in five different age/HIV groups. We used SMC ABC to fit our model to observed data: a relatively new method for inference without the need of likelihood calculations, which was found to perform well in simulations using dummy data. Sequential ABC methodology has previously been used to estimate transmission dynamics of *Streptococcus pneumoniae* from strain prevalence data in a study on day-care centres in Finland (159).

Whilst the model fitting to dummy data reached SMC ABC round 10 (ε = z<100) before reaching the threshold of <1% acceptance, the model fitting to the observed carriage prevalence only reached SMC ABC round 5 (ε = z<1000) before reaching the threshold of <1% acceptance. It was expected that the model fitting to dummy data performed better than the model fitting to observed data; in theory a 'perfect fit' is possible for the dummy data given it has been created using exactly the same model. A 'perfect fit' is almost impossible for the model fitting to observed data.

The posterior distribution of the model fitted to observed data allows us to draw several conclusions. The peak of the distribution for household transmission (β_{hh}) lies around 0.1; this is higher than estimated from other studies (Table 9.2), indicating that household transmission frequently occurs in this population. We estimated the competition parameter to be between 0.8 and 1, meaning that very little competition occurs in our setting. Our competition parameter estimate is similar to the parameter fit from Bangladesh (c=0.83) (153). Low competition between VT and NVT is a likely reason for the high multiple serotype carriage estimates observed in children in this area. The latest study on multiple serotype carriage amongst carriers in Blantyre, Southern Malawi estimates that multiple serotype carriage occurs in 54.1% of vaccinated and 59.3% of unvaccinated children aged 5-6 years. These estimates (which only became available after model fitting had been completed) are higher than the 32% we used as an estimate for our model presented here. If we would have used

the latest estimate from Blantyre for our fitting, it is likely that we would have concluded that even less competition occurs between VT and NVT. There is some evidence from the model fit that HIV-positive adults are more susceptible for VT carriage than HIV-negative adults, although the credible estimates of the model fit were wide. Increased susceptibility could explain the higher prevalence of carriage observed in HIV-positive as compared to HIV-negative adults. It is also possible that HIV-positive adults carry a pneumococcus for a longer period of time as hypothesized in chapter 6, but unfortunately we could not test this hypothesis in our model.

When using a longer simulation time, a decreasing trend in pneumococcal carriage prevalence was observed. Most likely, a change in population structure is the result of this decreasing trend observed in the model; none of the model parameters were changed over the simulation period. When looking at the population dynamics over the simulation period, a decrease was observed for the proportion of infants and children <5 years in the population, whilst an increase was observed for the proportion of children 5-15 years and HIV-negative adults in the population. Also a decrease in the proportion of HIV-positive adults in the population is observed over the simulation period. This decrease in HIV-positive adults is a limitation of our model: we did not include HIV-transmission in the model, so no new HIV-infections in adults were included over the simulation period. Incidence of HIV decreased in Malawi in this period as a consequence of reduced transmission after introduction of ART (233), so although some new HIV-infections will have occurred, we do not expect our omission of implicit HIV-transmission will have had a large effect on the model. We did include an HIV-status for 5-15 year olds in the model: this (very low) HIV-prevalence was not taken into account for children 5-15 years, but did come into play when they aged to become HIV-positive or HIV-negative adults. Together, the shift in population structure will have affected the pneumococcal carriage prevalence dynamics over the simulation period. The decrease in carriage prevalence will need to be taken into account when using this model to predict the impact of different vaccination strategies in this population.

Limitations and future improvements

Several limitations can be identified for the model presented here which could be targets for future improvements. There are limitations related to the method used for model fitting, the design of the model, and the data used.

We already highlighted the strengths and limitations of the ABC SMC method in section 9.4. The main drawback of using ABC is that it is a relatively new method and

that does not yet have the statistical rigor of more conventional optimization methods. Another disadvantage of ABC methods is that they are reliant on the user-choice of appropriate summary statistics and the choice of the acceptance threshold. In the current model we only used one summary statistic; the z-value calculated by the Chi-square test performed on counts in the different VT/NVT/B statuses for the five age/HIV groups. Future work could explore using different summary statistics, e.g. the proportion of households infected. Similarly, the effect of using a dynamic process to set the acceptance threshold ε could be explored. We used a pre-defined step-wise linear series of acceptance thresholds, but a dynamic evaluation was used by Numminen *et al.* (159).

There are three main limitations to the design of the model: related to the assumed mixing pattern, duration of carriage and assumptions about immunity. We assumed random mixing with the household and community, whereas in reality some degree of assortative mixing will take place, e.g. between children of the same age in schools. Similarly, mixing within the household can be expected to be non-random; for instance more contact can be expected to take place between infants and mothers than between infants and other adults in the household. Fitting the model using an assortative mixing matrix or using different transmission parameters for mother-infant contacts could be explored. SMC ABC can be applied to compare different model structures (223). We could apply this strategy to compare models with or without assortative mixing. The comparison could similarly be used on the current model: we use this to compare our current model with a model without explicit household transmission, or look at the difference between a model with or without separate characteristics for HIV-positive adults. Limitations regarding the duration of carriage stem from our decision to fix the recovery rates during our model fitting, because training simulations showed poor performance when both susceptibility parameters and recovery rates were fitted. Fixing the recovery rates in our model meant that we could not study differences in carriage duration between the different age/HIV groups. Also, under or overestimation of carriage duration could have influenced our model fit, and hence our conclusions regarding the other parameters fitted. Lastly, our model involves a simplification of the dynamics involved in pneumococcal carriage and transmission, a limitation inherent to mathematical modelling. We adopted Susceptible-Infected-Susceptible dynamics for our model, consistent with other modelling studies in the literature (145-158, 222). Evidence is provided in the literature, however, for both serotype-specific (234) and serotype-independent (125) immunity. We did not include immunity as a result of previous carriage in the model. We did include a parameter reflecting different susceptibilities by age and HIV group.

Thirdly, there are limitations regarding the data we used to fit the model to and how this fitting was done. The data we used for the fitting of our model largely comes from households selected because they involved a newborn infant. We do not have any observations for HIV-negative adults living without young children. It is possible that this selection of data has led to an overestimation of carriage in HIV-negative adults. Our estimate for HIV-positive adults is generated from data collection within the household study (chapter 5) and the ART clinic (chapter 6): we expect this has given a good estimate of carriage among HIV-positive adults in the population. We fitted our model to the observed prevalence of pneumococcal carriage in the different age-HIV groups. More detailed use of the longitudinal household data from which the prevalence estimates were obtained could be used to study household transmission in more detail. Lastly, we did not include any variation in carriage by season in the model. Seasonality was found to be strongly associated with carriage in chapter 5 and 6. Including a parameter to model seasonality, fitted to carriage estimates by seasonality, would make the model more in line with reality.

Conclusion

In conclusion, we developed a stochastic individual-based model with SIS infection dynamics and explicit, dynamic household and population structure, which allowed for the investigating of within-household and community transmission in five different age/HIV groups. SMC ABC was found to perform well when fitting the model to dummy data. We used SMC ABC to fit the model to observed carriage prevalence in five age/HIV groups. From the model fit we can conclude that the household transmission rate is approximately 0.10 per effective contact. There was found to be little competition between VT and NVT serotype carriage, resulting in high prevalence of multiple serotype carriage in young children. There is some evidence from the model fit that HIV-positive adults are more susceptible for VT carriage than HIV-negative adults, although the credible estimates of the model fit were wide. Drawbacks of the current model fit are that the model overestimates the NVT carriage prevalence in infants, and that a decrease in VT and B carriage is observed in simulations without the implementation of a vaccination programme. Those two limitations will be taken into account when using this model to predict the impact of different vaccination strategies in this population (chapter 10).

10. A mathematical model to study pneumococcal transmission in the post-vaccination period

In chapter 9 we designed a stochastic individual-based mathematical model to describe pneumococcal carriage and transmission, and fitted this to the observed pre-vaccination carriage prevalence in the pre-vaccination period (chapter 5+6). In this chapter we will use this fitted mathematical model to simulate the effect of PCV-13 introduction in the population. We will validate our model with pneumococcal carriage prevalence data from the post-vaccination period (chapter 8). Using the observed carriage prevalence data and the known vaccination history of the population under surveillance in the KHDSS, we will run simulations with different assumptions about waning immunity, and report which duration for waning immunity fits best with the observed data. We will then use this finding to explore the effect of different vaccination schedules.

10.1 Description of the model

Figure 10.1 shows the adapted model including vaccination of the population. Vaccinated individuals gain partial protection against VT pneumococci acquisition by a degree of protection π, that ranges from π=1 (full protection) to π=0 (no protection) and increases with the number of doses of PCV-13 received. Vaccination does not affect acquisition of NVT pneumococci. Vaccine immunity wanes over time by waning rate ω, which is described by exponential decay:

$$N(t) = N_0 \left(\frac{1}{2}\right)^{\frac{t}{t\frac{1}{2}}} \quad (1)$$

where N_0 is the initial protection of the vaccine, $t\frac{1}{2}$ is the half-life of the vaccine protection (waning immunity), and N_t is the vaccine protection after time t.

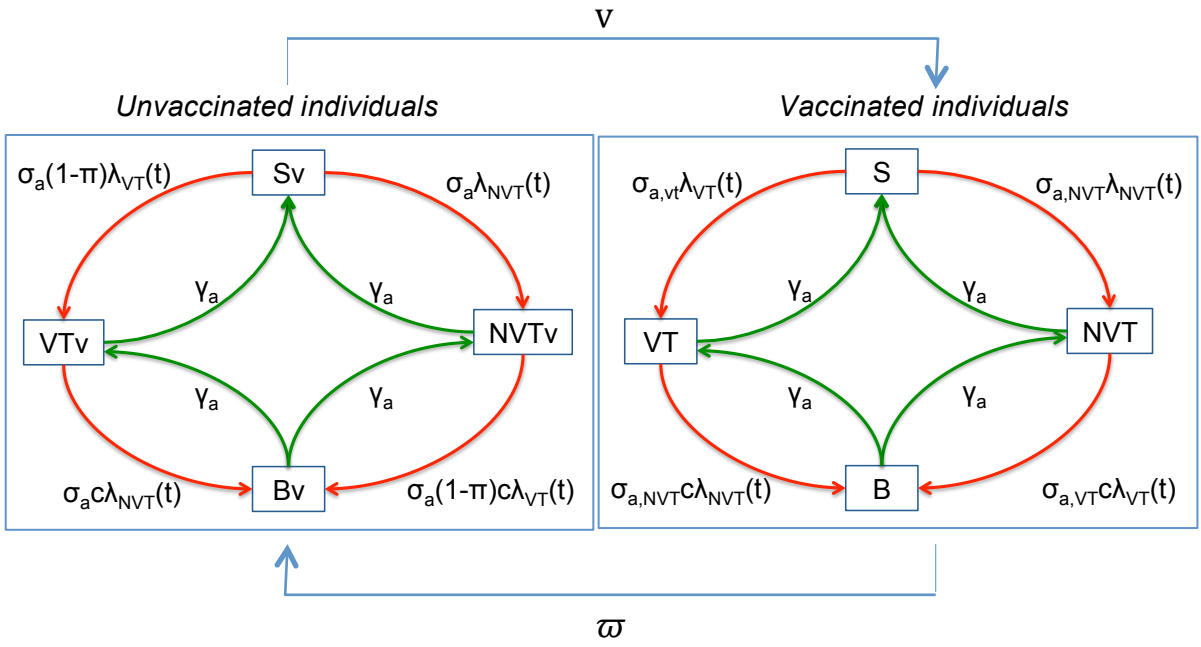


Figure 10.1 Model structure

Adapted from Melegaro et al, 2010 (220). Model describes the transmission to different states: S (susceptible), VT (carrier of vaccine type), NVT (carrier of non-vaccine type), B (carrier of both VT and NVT), where λ_{VT} and λ_{NVT} are the forces of infection for VT and NVT, γ_a is the age-specific recovery rate, c is the competition parameter, and σ_a is the age-specific susceptibility parameter. In vaccinated individuals, acquisition of a VT pneumococcus is modified by vaccine protection π. v describes vaccination of susceptible individuals, ϖ waning of immunity in vaccinated individuals.

The transmission states for our stochastic model are summarized in the following set of differential equations:

$$\frac{dS}{dt} = VT(t)\cdot\gamma_a + NVT(t)\cdot\gamma_a - S(t)\cdot\sigma_a\cdot\lambda_{VT}(t) - S(t)\cdot\sigma_a\cdot\lambda_{NVT}(t) - v\cdot Sv(t) + \varpi\cdot Sv(t) \quad (2)$$

$$\frac{dVT}{dt} = B(t)\cdot\gamma_a + S(t)\cdot\sigma_a\cdot\lambda_{VT}(t) - VT(t)\cdot\sigma_a\cdot c\cdot\lambda_{NVT}(t) - v\cdot VT(t) + \varpi\cdot VTv(t) \quad (3)$$

$$\frac{dNVT}{dt} = B(t)\cdot\gamma_a + S(t)\cdot\sigma_a\cdot\lambda_{NVT}(t) - NVT(t)\cdot\sigma_a\cdot c\cdot\lambda_{VT}(t) - v\cdot NVT(t) + \varpi\cdot NVTv(t) \quad (4)$$

$$\frac{dB}{dt} = VT(t) \cdot c \cdot \sigma_a \cdot \lambda_{NVT}(t) + NVT(t) \cdot c \cdot \sigma \cdot \lambda_{VT}(t) - B(t) \cdot \gamma_a - v \cdot B(t) + \varpi \cdot Bv(t) \quad (5)$$

$$\frac{dSv}{dt} = VTv(t) \cdot \gamma_a + NVTv(t) \cdot \gamma_a - Sv(t) \cdot \sigma_a \cdot (1-\pi) \cdot \lambda_{VT}(t) - Sv(t) \cdot \sigma_a \cdot \lambda_{NVT}(t) + v \cdot S(t) - \varpi \cdot Sv(t) \quad (6)$$

$$\frac{dVTv}{dt} = Bv(t) \cdot \gamma_a + Sv(t) \cdot \sigma_a \cdot (1-\pi) \cdot \lambda_{VT}(t) - VTv(t) \cdot \sigma_a \cdot c \cdot \lambda_{NVT}(t) + v \cdot VT(t) - \varpi \cdot VTv(t) \quad (7)$$

$$\frac{dNVTv}{dt} = Bv(t) \cdot \gamma_a + Sv(t) \cdot \sigma_a \cdot \lambda_{NVT}(t) - NVTv(t) \cdot \sigma_a \cdot c \cdot (1-\pi) \cdot \lambda_{VT}(t) + v \cdot NVT(t) - \varpi \cdot NVTv(t) \quad (8)$$

$$\frac{dBv}{dt} = VTv(t) \cdot c \cdot \sigma_a \cdot \lambda_{NVT}(t) + NVTv(t) \cdot c \cdot \sigma_a \cdot (1-\pi) \cdot \lambda_{VT}(t) - Bv(t) \cdot \gamma_a + v \cdot B(t) - \varpi \cdot Bv(t) \quad (9)$$

where $\sigma_{a,s}$ is the age/HIV group and VT/NVT specific susceptibility parameter, γ_a is the age/HIV group specific recovery rate and λ_{VT} and λ_{NVT} are the force of infection for VT and NVT respectively (described in chapter 9). In vaccinated individuals, acquisition of a VT is modified by protection π. v describes vaccination of susceptible individuals, ϖ waning of immunity in vaccinated individuals.

10.2 Observed data

10.2.1 Pneumococcal prevalence data in the post-vaccination period

Table 10.1 shows the observed prevalence of VT and NVT carriage by age group, as well as the estimated proportion of VT, NVT and B carriage. To estimate the proportion of multiple carriage amongst carriers in the post-vaccination period, we assumed that VT carriage decreased equally within the VT and B groups post introduction of PCV-13. We therefore decreased the proportion multiple carriage amongst positive samples observed in samples from Karonga (47, 221) (section 9.3.2) by the adjusted prevalence ratio reported in chapter 8 (Table 8.6).

Table 10.1. Observed proportion of VT and NVT carriage and estimated proportion of multiple carriage of VT and NVT by age group

	Proportion multiple carriage amongst positive samples (47, 221)	Observed carriage[1] (%)			Estimated carriage (%)			
Age group	**VT and NVT**	**VT**	**NVT**	**Total**	**B**	**VT**	**NVT**	**Total[2]**
<1 years	0.48 · 0.61 · 0.24 = 0.070	11.9	44.0	55.9	0.070 · 55.9 = 3.9	11.9 – (3.9/2) = 10.0	44.0 – (3.9/2) = 42.1	56.0
1-4 years	0.40 · 0.61 · 0.63 = 0.154	19.7	50.1	69.8	0.154 · 69.8 = 10.7	19.7 – (10.7/2) = 14.4	50.1 – (10.7/2) = 44.8	69.9
5-15 years	0.32 · 0.61 · 0.37 = 0.072	5.7	22.5	28.2	0.072 · 28.2= 2.0	5.7 – (2.0/2) = 4.7	22.5 – (2.0/2) = 21.5	28.2
16+, HIV+	0.20 · 0.61 · 0.06 = 0.007	0.5	9.6	10.1	0.007 · 10.1= 0.1	0.5 – (0.1/2) = 0.5	9.6 – (0.1/2) = 9.6	10.2
16+, HIV-	0.16 · 0.61 · 0.34 = 0.033	2.4	12.9	15.3	0.033 · 15.3= 0.5	2.4 – (0.5/2) = 2.2	12.9 – (0.5/2) = 12.7	15.4

[1] Age-standardized to 2010 KPS population for children 1-4 years and 5-15 years

[2] Total observed and estimated carriage different due to rounding

10.2.2 Vaccination uptake data

To include vaccination in our stochastic individual-based models, we used the vaccine uptake data as recorded in the KHDSS annual census. Recorded vaccination dates were used to assign onset of vaccine protection: if the model reached the date associated with an individual's vaccination date, this individual was assigned protection by vaccination π (see section 10.3).

Table 10.2 shows the vaccine uptake and the median age at vaccination amongst birth cohorts since introduction of PCV-13. This table includes any children born between 10th November 2010 (oldest eligible for the PCV-13 catch-up campaign) and 30th June 2015 (end of data included), including those that died and those that migrated in or out of the KHDSS area during this period. As described previously in chapter 7, PCV-13 vaccination coverage increased since introduction of PCV-13, with 86.4% of age-eligible infants receiving three doses of PCV-13 in 2011-2012, and 93.8% in 2013-2014. Median age to vaccination decreased since introduction of PCV-13, with 20.2 weeks in 2011-2012 and 18.4 weeks in 2013-2014. We included vaccine uptake data of measles vaccination (MV) in Table 10.2, because this data was used in simulations with vaccination strategies including a booster does: if a booster dose of PCV-13 were to be introduced, it is likely that it would be given at the same time as MV.

A written or verbal history of dose 1 PCV-13 vaccination receipt was missing for 430 of 6833 (6.3%) PCV-13 catch-up or birth cohort age-eligible children. Date of dose 1 PCV-13 vaccination was missing for 1292 of 5991 (21.6%) age-eligible children who were recorded (written or by verbal history) to have received the first dose of PCV-13. In the model, explicit vaccination status and vaccination dates in case of vaccine receipt were required. Vaccination status and vaccination date were therefore imputed for individuals with missing status or dates, matched by month of birth, in four stages:

1. Vaccination status for those who were interviewed before they were age-eligible was set to missing if the vaccine was not received at time of interview. This was done for all three doses of PCV-13 and MV.
2. Each individual with missing vaccination status was randomly matched to an individual with recorded vaccination status (including written recorded and verbal history) by month of birth. The matched vaccination status was assigned to the individual with missing vaccination status.
3. If vaccination date was available for two out of the three PCV-13 doses and the individual was said to have received all three doses (n=42), the third date was

estimated using the median time between PCV-13 dose 1 and PCV-13 dose 2 in the population (35 days) or the median time between PCV-13 dose 2 and PCV-13 dose 3 (35 days). If only one date was available and the individual was said to have received all three doses (n=6), this date was disregarded and new dates were imputed (see step 4)

4. Each individual with one or more missing vaccination dates was randomly matched to an individual with vaccination dates available. Matching was done by month of birth and number of vaccines received. Vaccination dates were calculated based on the age of vaccination of the matched individual.

Imputation was done matched by month of birth, because time since introduction of PCV-13 was found to be strongly associated with PCV-13 receipt and vaccination age in chapter 7 and in Table 10.2.

Table 10.2 Vaccine uptake and age at vaccination amongst birth cohorts since introduction of PCV-13

	Birth cohorts[1]				
	2010-2011 (catch-up)	**2011-2012**	**2012-2013**	**2013-2014**	**2014-2015[2]**
Total	1447	1573	1386	1427	1000
Vaccine uptake					
Vaccination status available[3]	1387 (95.9%)	1490 (94.7%)	1325 (95.6%)	1336 (93.6%)	865 (86.5%)
Uptake[4]					
PCV (6wk)	68.5%	92.5%	98.4%	99.2%	91.2%
PCV (10wk)	61.4%	91.2%	96.6%	97.4%	88.4%
PCV (14wk)	51.5%	87.4%	92.9%	94.5%	78.2%
MV (9mo)	96.5%	94.3%	93.0%	91.1%	68.6%
Age at vaccination					
Vaccination date available[5]	658 (75.0%)	1134 (85.1%)	1140 (88.0%)	1136 (86.3%)	631 (85.3%)
Median age at vaccination					
PCV (6wk)	31.0 wks	8.7 wks	8.4 wks	7.9 wks	7.9 wks
PCV (10wk)	36.8 wks	14.3 wks	13.7 wks	13.0 wks	13.1 wks
PCV (14wk)	40.7 wks	20.2 wks	19.6 wks	18.4 wks	18.0 wks
MV (9mo)	9.7 mo	9.6 mo	9.6 mo	9.6 mo	9.4 mo

[1] Birth cohorts from 29th November – 28th November to reflect time since introduction of PCV-13. Catch-up birth cohort = 10th November 2010 – 28th November 2011. Birth cohort 2014-2015 is 29th November – 30th June 2015.

[2] Uptake in 2014-2015 is expected to be lower than in previous years, because this cohort will include children that received vaccination late and have been interviewed after vaccine-eligible age, but before receiving vaccination.

[3] Availability of PCV-13 dose 1 receipt by written documentation or verbal history.

[4] Uptake at any age amongst age-eligible children

[5] Availability of date PCV-13 dose 1 receipt amongst age-eligible children who are recorded (written or verbal history) to have received PCV-13 dose 1

Including imputed data, vaccination coverage in all children <5 years (including age in-eligible children) was 82.0% for one dose of PCV-13 and 70.9% for three doses of PCV-13 on 30th June 2015 (end of model simulations) (Fig 10.2). Vaccination coverage in all infants (including infants too young to be vaccinated) was 82.6% for one dose of PCV-13 and 56.4% for three doses of PCV-13.

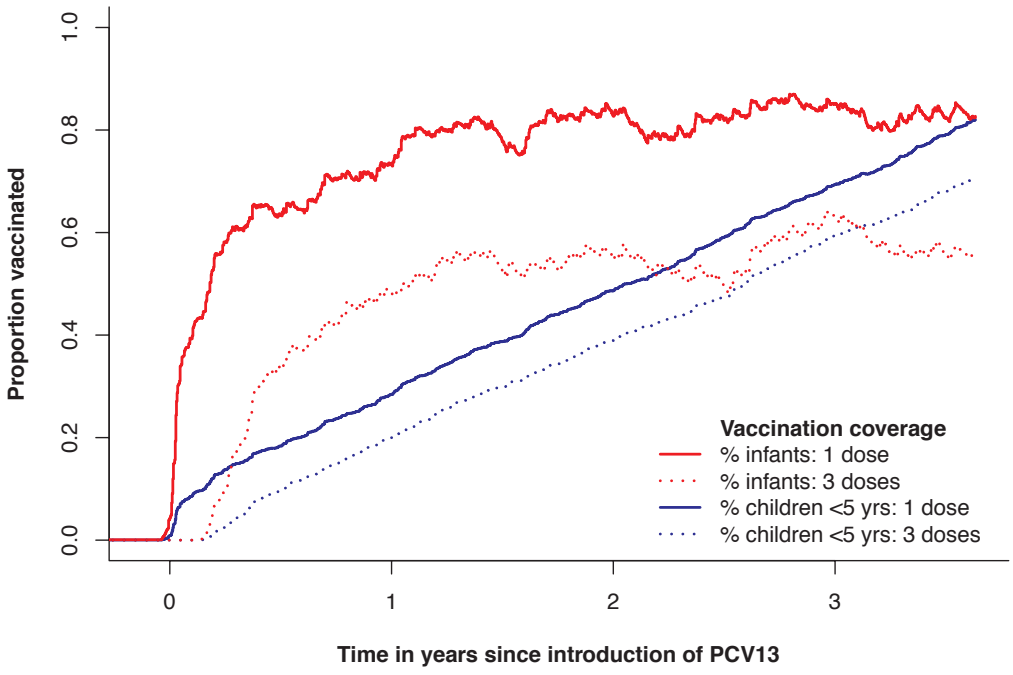


Figure 10.2 Vaccination coverage in infants and children <5 years (including age in-eligible children).

Data includes imputation of vaccination dates for children with missing vaccination dates.

On 30th June 2015, 40.6% of all households included at least one fully vaccinated child (Fig 10.3).

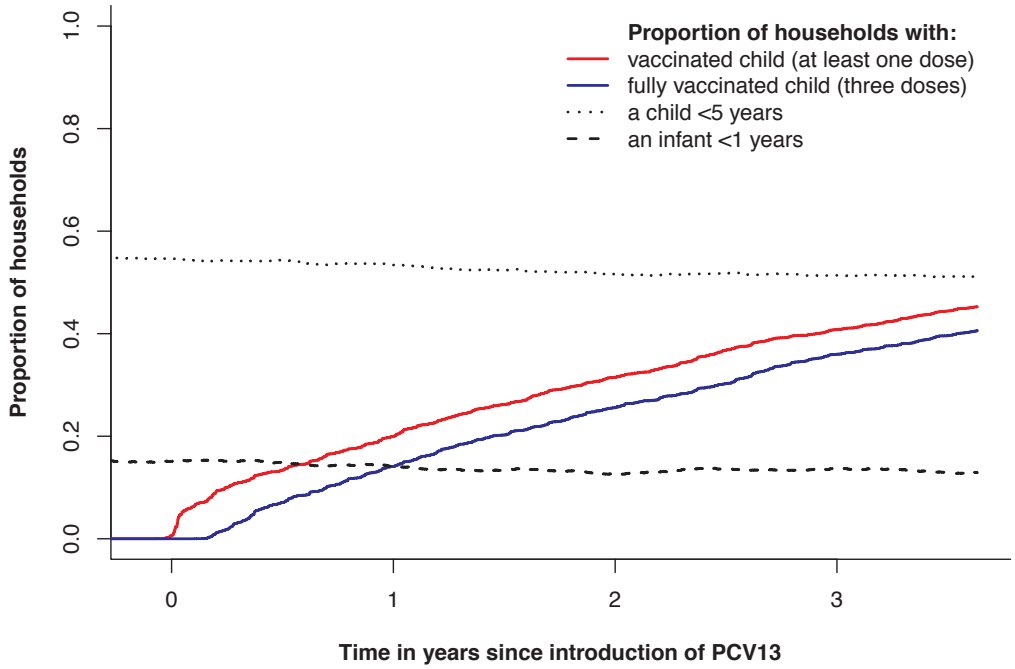


Figure 10.3 Vaccination coverage at household level

Data includes imputation of vaccination dates for children with missing vaccination dates. A slight decrease in the proportion of households with at least one infant/at least one child <5 years can be observed.

10.3 Models with different waning immunity rates

Table 10.3 shows the parameter settings for modelled scenarios including vaccination. We set the degree of vaccine protection at 0.58 for dose 1, 0.67 for dose 2 and 0.89 for dose 3, to reflect the VE observed in the post-vaccination carriage study (chapter 8.3.4). We ran simulations with four different levels of immunity half-life: 6 months, 1 year, 2 years and 3 years. Each scenario was realised 200 times with parameters drawn from the posterior distribution as described in chapter 9: mean results are presented in figures 10.4-10.7.

Table 10.3 Parameters describing vaccination

Parameter	Meaning	Value
v	vaccination rate	observed vaccination in KHDSS (see paragraph 10.2.3)
π	degree of protection by vaccine dose against acquisition of a VT pneumococcus	- dose 1: 0.58 - dose 2: 0.67 - dose 3: 0.89
ϖ	waning immunity (half-life)	models with different half-lives: - 6 months - 1 year - 2 years - 3 years

Figure 10.4 shows the observed post-vaccination carriage prevalence and model estimates for simulations with different waning immunity half-lives. Models including a waning immunity of 6 months or 1 year provided a good fit to VT and B carriage for all age/HIV groups. NVT carriage in infants and children 1-4 years was underestimated using models with all different settings for waning immunity.

Model fit to the observed post-vaccination carriage estimates was assessed by the Chi-squared test on observed vs. model counts of individuals in the different age/HIV groups with status VT, NVT and B at the mid-point of post-vaccination sample collection (5th May 2014). An immunity half-life of 1 year provided the best overall fit to the observed carriage data, taking into account VT, NVT and B carriage in all age/HIV groups. An immunity half-life of 6 months provided the fit to the observed carriage data for VT carriage in infants.

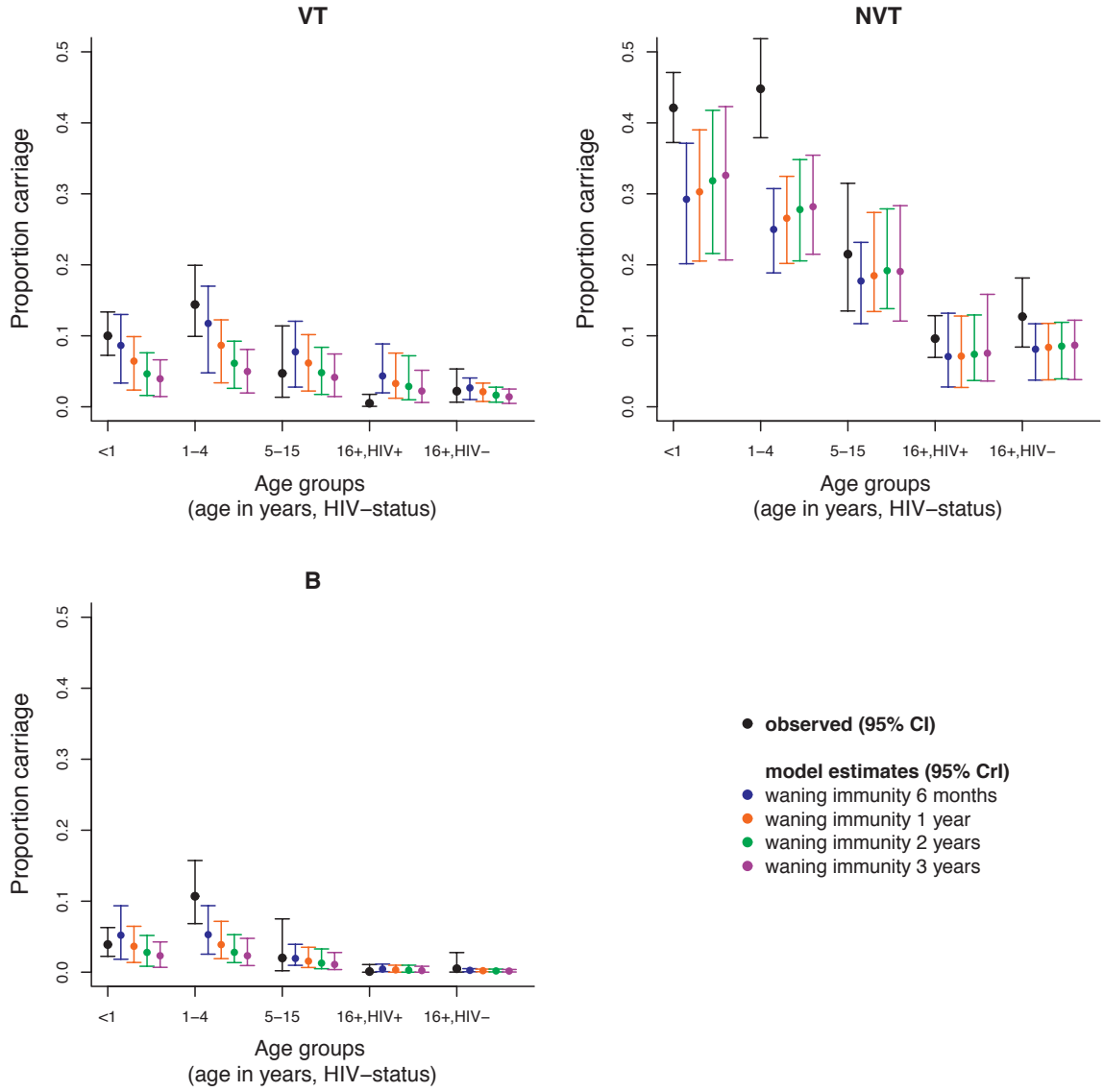


Figure 10.4 Observed post-vaccination carriage prevalence and model estimates for simulations with different waning immunity half-lives.
Mean results and 95% credible intervals of 200 realisations.

Figure 10.5 shows the different model simulations for VT carriage in the different age/HIV groups. A decrease in VT carriage was observed in all age/HIV groups, starting soon after introduction of PCV-13 on 12th November 2011 (indicated by an arrow in Figure 10.5). A large difference in carriage by the end of the model simulation (30th June 2015) was observed between models with waning immunity half-lives of 6 months, 1 year or 2 years. Only a marginal difference was observed between models with waning immunity half-lives of 2 year or 3 years. Model simulations with different waning immunity half-lives for NVT and B carriage can be found in Figures 10.6 and 10.7. NVT carriage was found to increase in all models simulations, with larger increases in NVT carriage observed in models with longer duration of protection against VT acquisition.

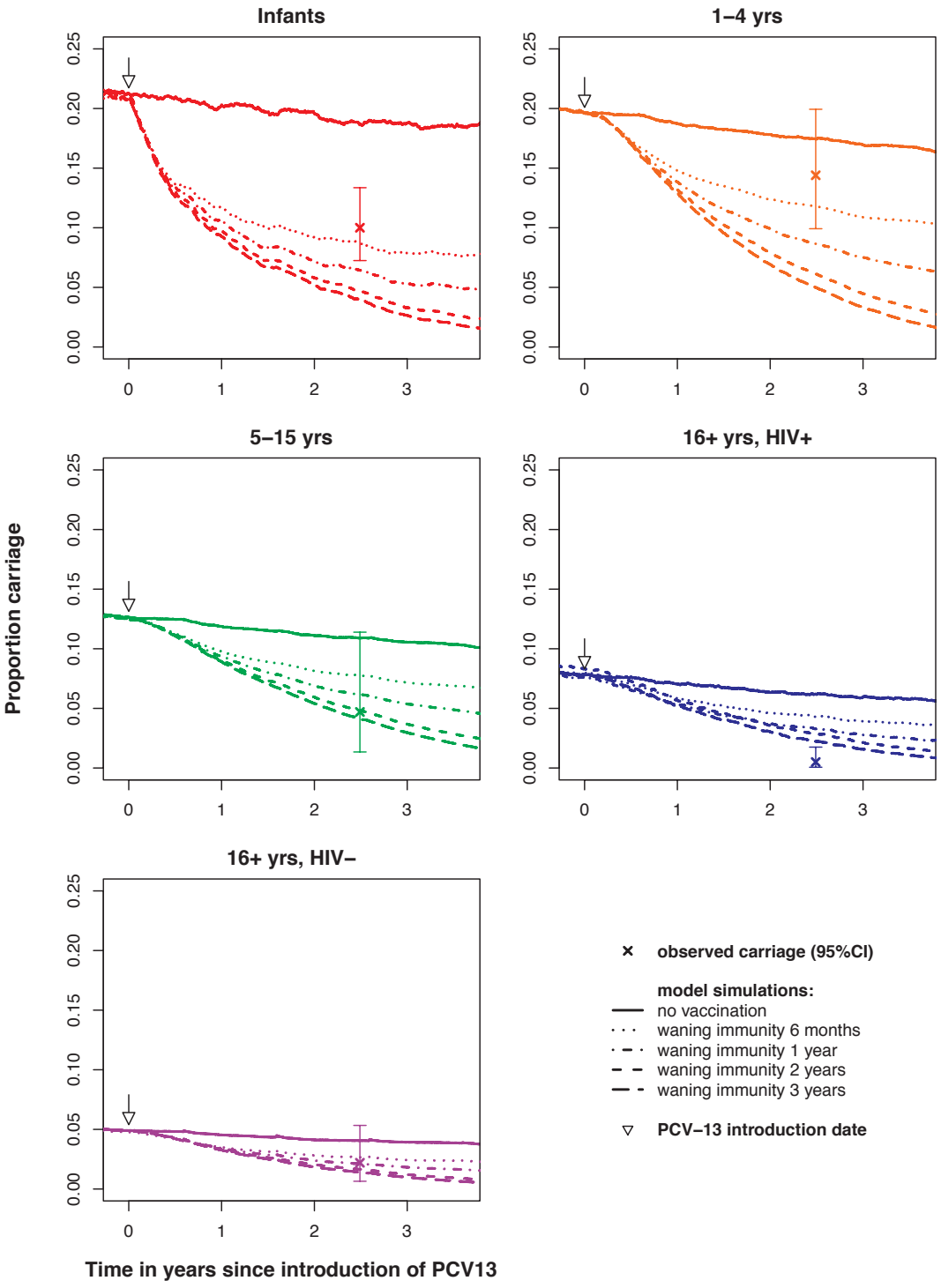


Figure 10.5 Simulated scenarios with different waning immunity half-lives: result for vaccine type carriage.

Mean results of 200 realisations.

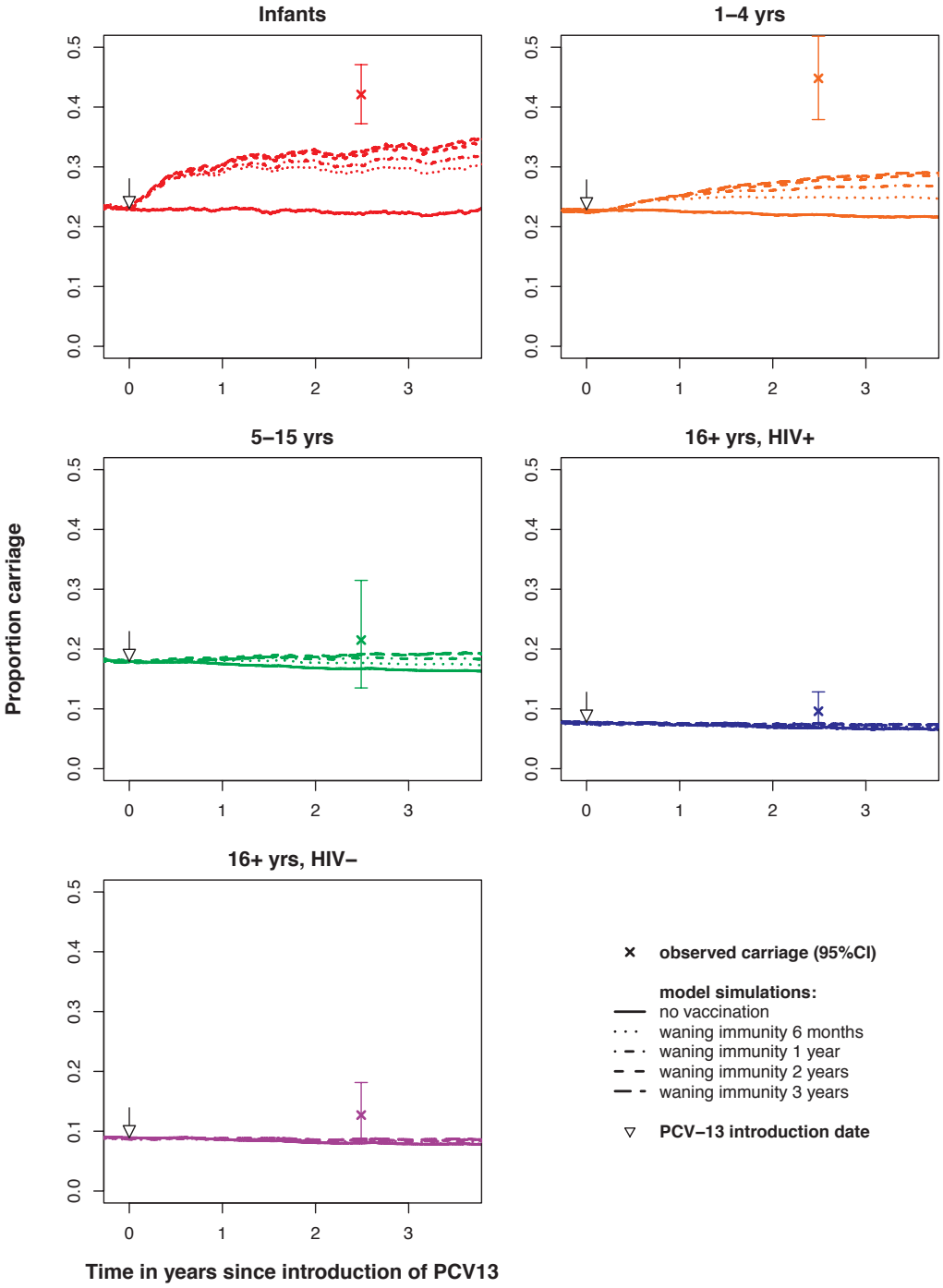


Figure 10.6 Simulations with different waning immunity half-lives: result for non-vaccine type carriage.

Mean results of 200 realisations.

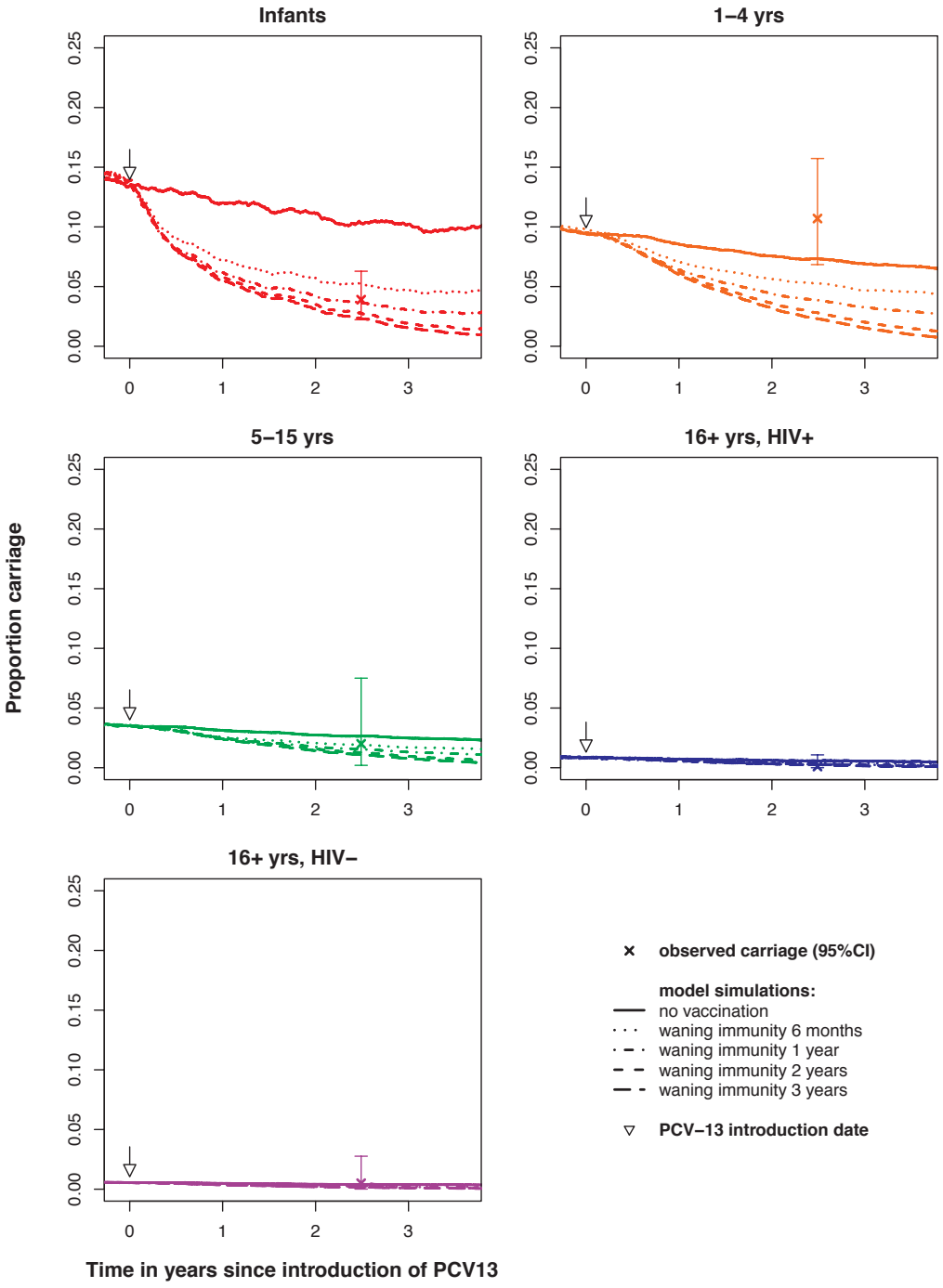


Figure 10.7 Simulations with different waning immunity half-lives: result for multiple carriage of both vaccine and non-vaccine types.
Mean results of 200 realisations.

10.4 Models with different vaccination strategies

10.4.1 Description of vaccination strategies

Table 10.4 describes the different vaccination strategies that were simulated. We simulated the currently implemented 3+0 vaccination schedule with a catch-up campaign for infants <1 year of age. The impact of a booster dose given at 9 months of age, at the same time as MW, was assessed in a 3+1 schedule and in a 2+1 schedule, with primary doses given at 6 and 14 weeks. All scenarios were run twice: with the parameter for waning immunity set to a half-life of 6 months or 1 year.

We also looked at the impact of different catch-up campaigns. The status quo scenario includes a catch-up campaign with 3 doses offered 4 weeks apart in infants <1 year, as was implemented in Karonga District in November 2011. We ran the same scenario without catch-up campaign to assess the contribution the catch-up campaign has made to the post-vaccination pneumococcal carriage dynamics. We also modelled the impact of a more intense catch-up scenario, as was implemented in Kilifi Region in Kenya (25). In Kilifi, all infants were offered three doses of PCV-10 given 4 weeks apart. In addition, two doses of PCV-10 were given to children aged 12-59 months in two 1-2 week outreach campaigns. For our model, we assumed that coverage of 80%, 75% and 70% would be reached with an enhanced 2-week catch-up campaign for the receipt of one, two and three doses PCV. In the catch-up campaign held in Karonga District in 2011, lower coverage was achieved, but this could be partially attributed to vaccine non-availability (chapter 7.3.2). We assumed that higher coverage could be achieved with enhanced outreach efforts and a better supply of vaccines. We assumed that vaccine protection in 12-59 month olds was better than in infants and that only two doses were required to reach the same level of protection.

We also simulated vaccination of HIV-positive or all mothers, with one dose of PCV-13 given at time of delivery. We assumed the same vaccine protection as in infants (0.58 for dose 1, and 0.67 for dose 2 and 0.89 for dose 3 in the event of multiple births within the simulation period). We assumed an immunity half-life of one year in the mothers, regardless of HIV-status. Each scenario was realised 200 times with parameters drawn from the posterior distribution as described in chapter 9: mean results are presented in figures 10.8-10.12)

Table 10.4 Description of vaccination strategies

Infant vaccination strategies			
#	**Description**	**Schedule in birth-cohort**	**Schedule in catch-up cohort**
0	3+0 (status quo)	6,10,14 wks	3 doses 4 weeks apart in infants <1 yr
1	2+1	6,14 wks, 9 mo	3 doses 4 weeks apart in infants <1 yr
2	3+1	6,10,14 wks, 9 mo	3 doses 4 weeks apart in infants <1 yr
3	No catch-up	6,10,14 wks	-
4	Intense catch-up (Kilifi)	6,10,14 wks	3 doses 4 weeks apart in infants <1 yr 2 doses 4 weeks apart in children 12-59 mo

Adult vaccination strategies (simulated alongside current 3+0 infant schedule)		
#	**Description**	**Schedule**
5	HIV-positive mothers only	One dose at time of delivery
6	All mothers	One dose at time of delivery

10.4.2 Model results

Figure 10.8 shows the model results for VT carriage for simulations with different infant vaccination schedules. Only marginal differences were observed between the different vaccination schedules, with widely overlapping confidence intervals (not shown in Figure 10.8 for clarity). The 3+1 schedule was associated with lower carriage than the current schedule for all age/HIV groups. Adoption of a 2+1 schedule was initially associated with a less rapid decrease in VT carriage, but towards the end of the simulation time the gradient of VT carriage reduction was steeper in children 1-4 years than in simulations using the current 3+0 vaccination schedule, particularly when assuming an immunity half-life of 6 months (Figure 10.9). NVT carriage in infants and children 1-4 years increased after PCV-13 introduction in all simulations, but was not found to differ between the different vaccination schedules (Figure 10.10).

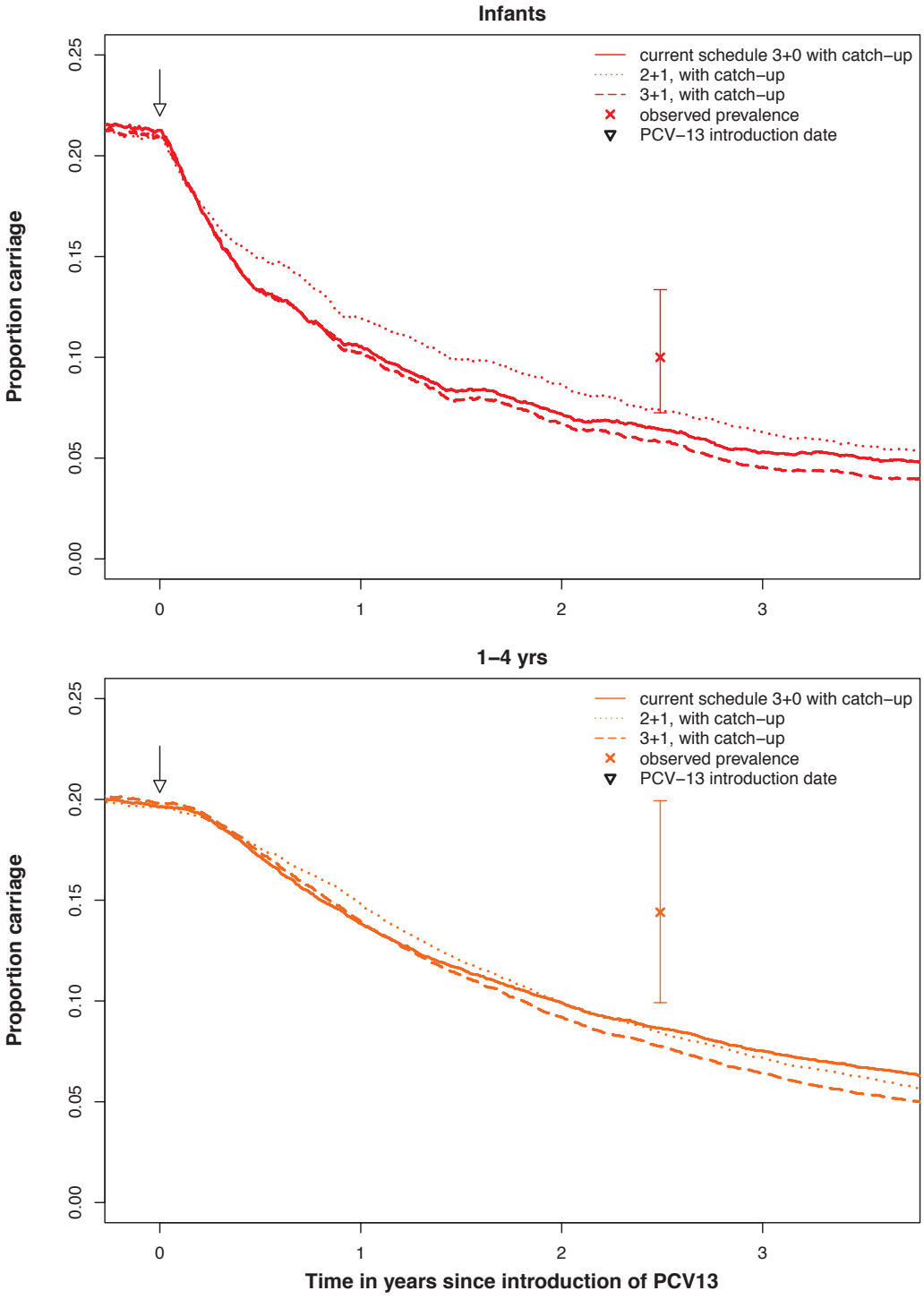


Figure 10.8 Simulated scenarios with different infant vaccination schedules: result for vaccine type carriage.

Mean results of 200 realisations. Waning immunity of 1 year. Note different y-axis scale for infants and children 1-4 years, and for children 5-15 years and adults.

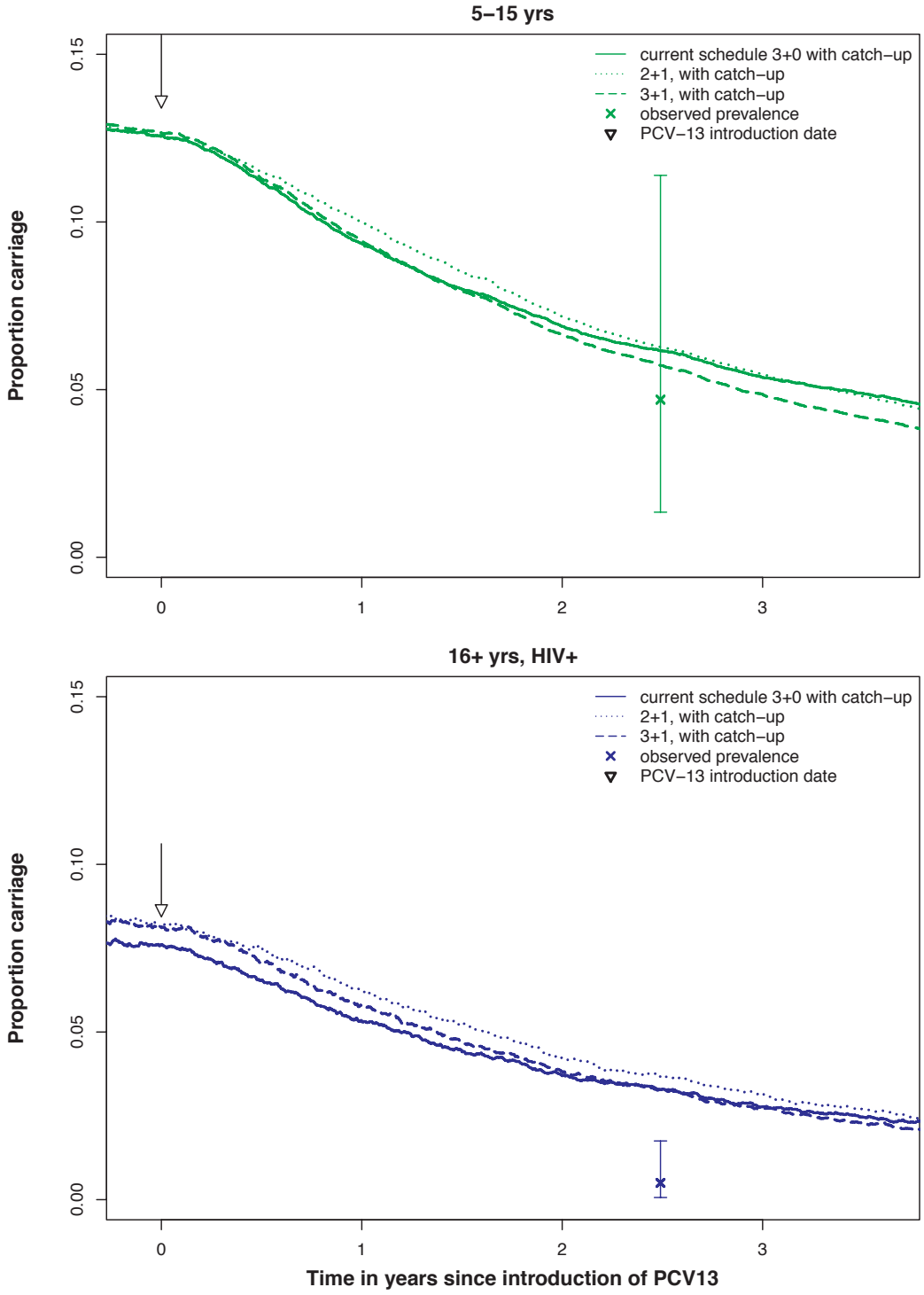


Figure 10.8 *cont.* Model results for simulations with different infant vaccination schedules: result for vaccine type carriage.

Mean results of 200 realisations. Waning immunity of 1 year. Note different y-axis scale for infants and children 1-4 years, and for children 5-15 years and adults.

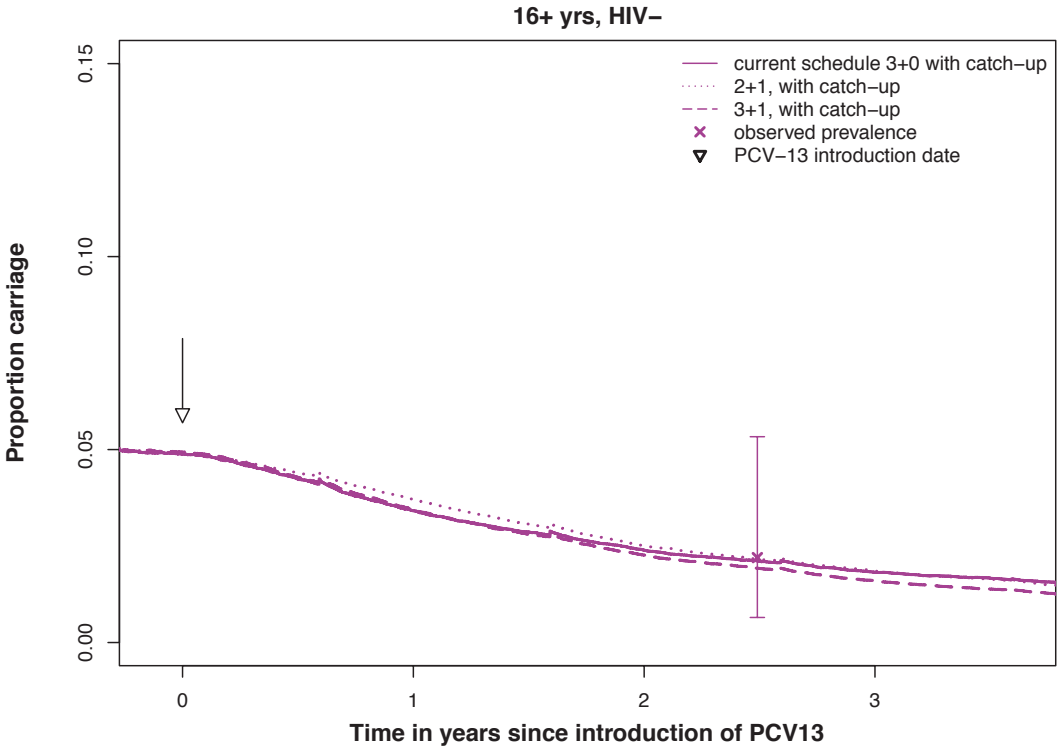


Figure 10.8 *cont.* Model results for simulations with different infant vaccination schedules: result for vaccine type carriage.

Mean results of 200 realisations. Immunity half-life of 1 year. Note different y-axis scale for infants and children 1-4 years, and for children 5-15 years and adults.

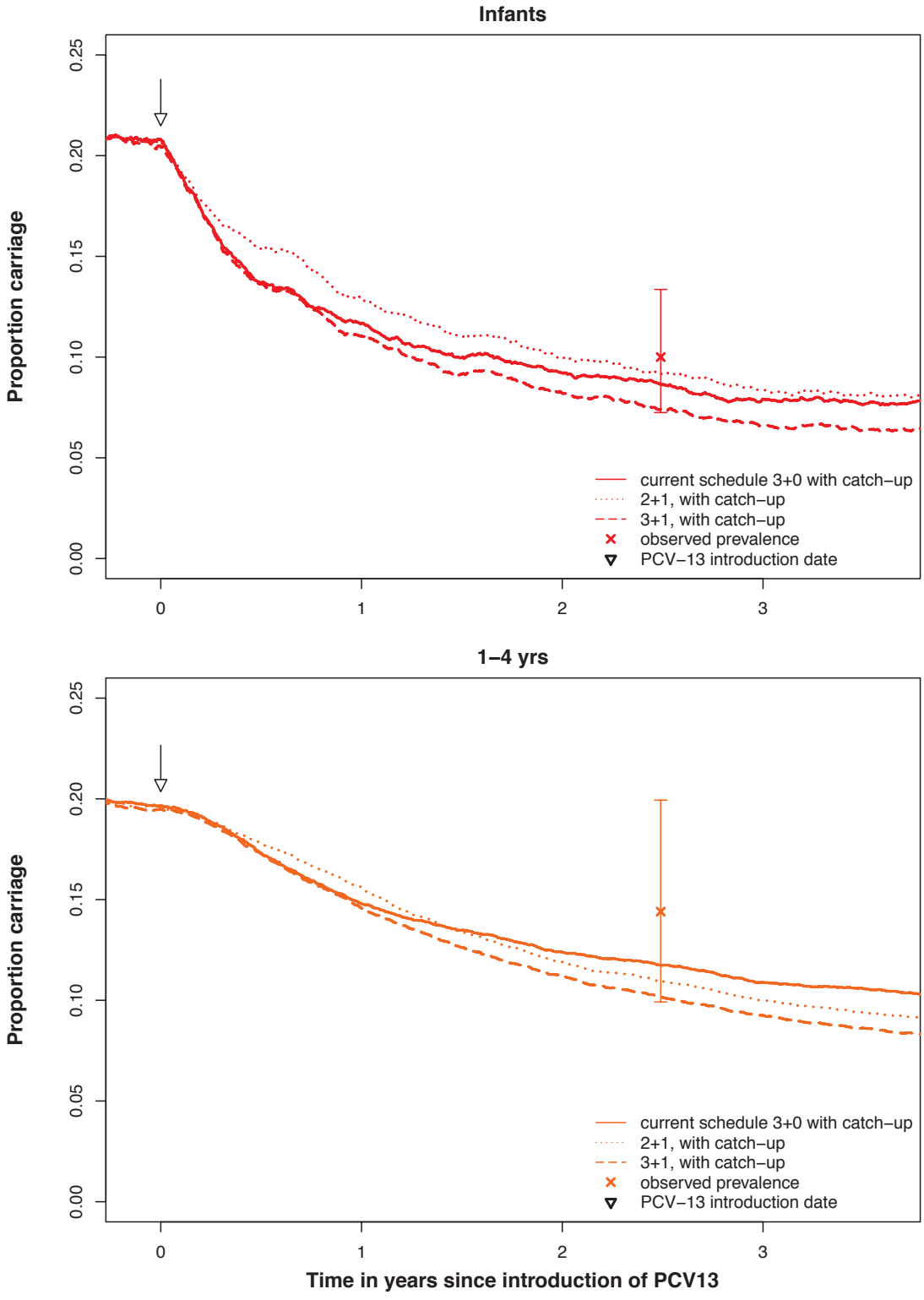


Figure 10.9 Simulations with different infant vaccination schedules assuming an immunity half-life of 6 months: result for vaccine type carriage

Mean results of 200 realisations. Results shown for infants and children 1-4 years only.

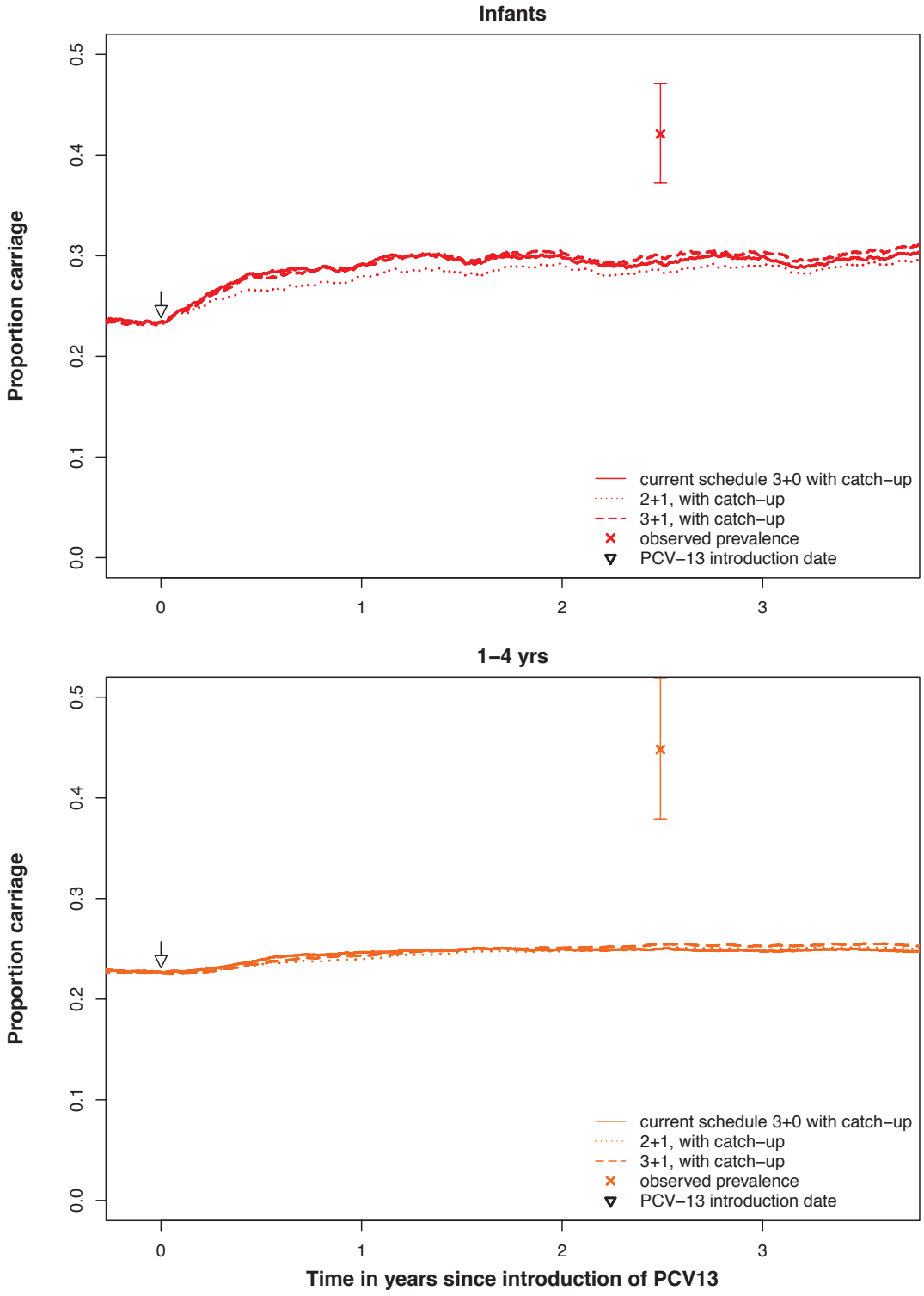


Figure 10.10 Simulations with different infant vaccination schedules: result for non-vaccine type carriage

Mean results of 200 realisations. Assuming an immunity half-life of 6 months. Results shown for infants and children 1-4 years only.

Figure 10.11 shows the results for the scenarios with different catch-up campaigns: vaccination as currently implemented in Karonga District, with or without catch-up campaign, or with an enhanced outreach campaign as was implemented in Kilifi, Kenya. Beneficial effects of the catch-up campaigns could be seen for all age/HIV groups, particularly for children 1-4 years old, with a more rapid decline in VT carriage prevalence observed when a catch-up campaign was implemented. Fifty per cent reduction in VT carriage in children 1-4 years old (from 19.5% to 9.8%) was achieved approximately 6 months later without a catch-up campaign, as compared to the current immunisation implemented in Karonga District. At the end of the simulation time only minimal differences in VT carriage were observed between the 3+0 strategy with or without catch-up as currently implemented in Karonga District.

An enhanced catch-up campaign with better coverage than currently achieved in Karonga District showed additional benefits: a very rapid decline in carriage was observed in all age/HIV groups. The enhanced catch-up campaign did not lead to a completed removal of VT carriage from the population: persistent low-level steady state VT carriage was observed in all age/HIV groups.

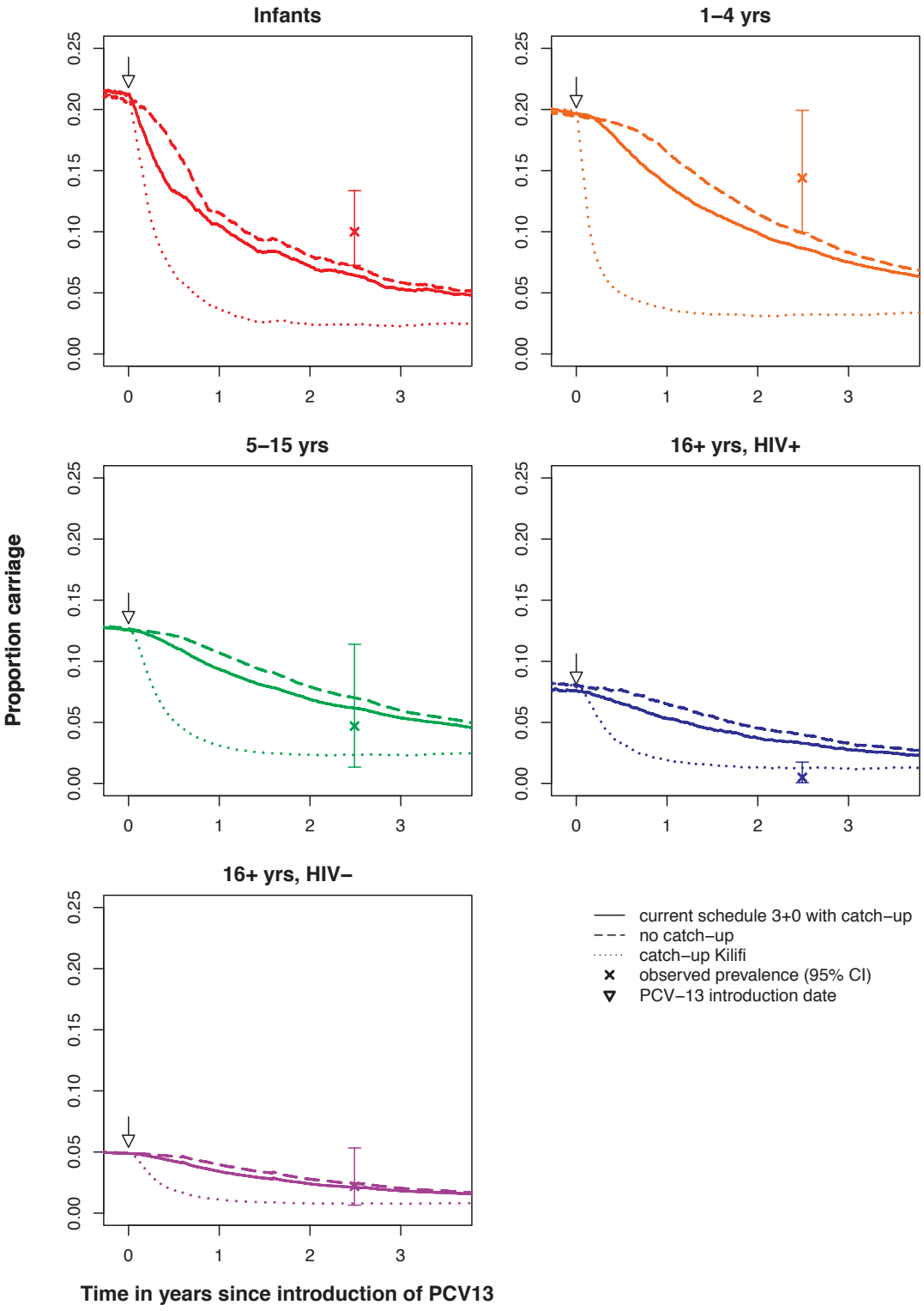


Figure 10.11 Simulations with and without catch-up campaign: result for vaccine type carriage.

Mean results of 200 realisations. Assuming an immunity half-life of 1 year.

Models with vaccination in all HIV-positive mothers at time of delivery did not result in additional VT carriage reduction in the infant. Vaccination of all mothers led to minimal reductions in VT carriage in the infant only (Fig 10.12).

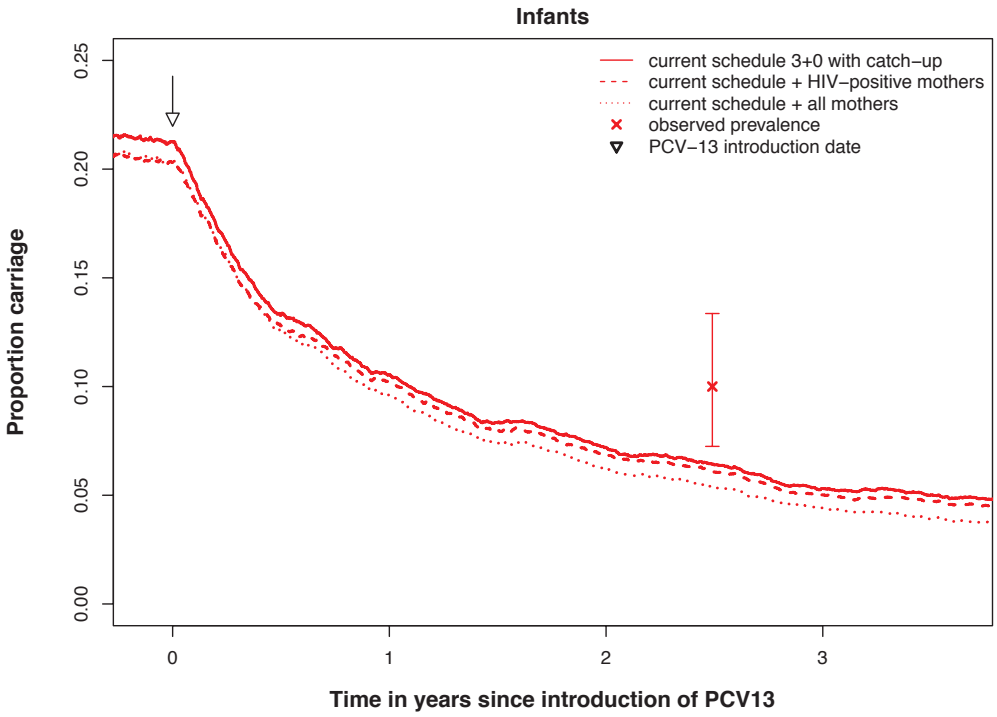


Figure 10.12 Model results for simulations including vaccination of HIV-positive or all mothers: result for vaccine type carriage.
Mean results of 200 realisations. Immunity half-life of 1 year in both mothers and infants.

10.5 Discussion

We used the model described in chapter 9 to simulate the effect of PCV-13 introduction in the population, including different assumptions about the immunity half-life. We evaluated our model with pneumococcal carriage prevalence data from the post-vaccination period and explored the effect of different vaccination schedules.

PCV-13 introduction was found to have an immediate direct and indirect effect: the proportion of VT carriage reduced immediately after PCV-13 introduction in all age-HIV groups. This finding seems to be at odds with the simple threshold theory of herd immunity, which states that the incidence of infection would decline if the proportion immune in the population exceeds $(1 - 1 / R_0)$, with R_0 being the average number of individuals that get infected by one individual in a fully susceptible population (235, 236). It is important to realise, however, that the simple threshold theory of herd immunity assumes vaccination delivered at random and random mixing in the population, whereas in reality targeted vaccination and non-random mixing occurs (235). Household transmission was explicitly included in our model, based on the real household structure observed in the KHDSS. At time of introduction of the vaccine, approximately 15% of households included at least one infant, and 55% of household included at least one child <5 years of age (Fig 10.3). At the end of the simulation period, 82% and 71% of all children <5 years had received one or three doses PCV-13, respectively. A large proportion of the population in contact with young children, together with good vaccine uptake, implied that a reduction in VT carriage in unvaccinated individuals could be observed before the herd immunity threshold was reached.

Different assumptions about the duration of the vaccine protection had a large effect on the reduction in VT carriage, both for vaccinated and unvaccinated individuals. Models with an immunity half-life of 6 months were found to fit best to the observed VT carriage in infants. Models with an immunity half-life of 1 year were found to have the best overall fit (including VT, NVT and B carriage in all age/HIV groups). Increasing the immunity half-life to 2 years had a large impact on the reduction in VT carriage in all age/HIV groups. A meta-regression study performed on 22 carriage studies found an aggregate vaccine efficacy of 57% (95%CI 50-65%) 6 months after completion of the vaccination schedule (237). This finding is consistent with our estimate of an immunity half-life between 6 months and 1 year, given initial vaccine efficacy of 87% immediately after completion of the schedule. The meta-regression study also found evidence of sustained vaccine protection at 5 years, with an aggregate vaccine efficacy of 42%.

This is much higher than what would be expected after 5 years given our estimate of an immunity half-life between 6 months and 1 year. Our results are consistent with a study from South Africa and a study from the UK, which found no difference in VT carriage between PCV and placebo recipients 2-5 or 5 years after vaccination, respectively (238, 239).

Unsurprisingly, a 3+1 schedule was found to have the largest reduction in VT carriage in all age/HIV groups. The 2+1 schedule yielded mixed results: reduction of VT carriage was found to be initially delayed as compared to the 3+0 schedule, but a steeper decline of VT carriage was observed towards the end of the observation time for children 1-4 years, particularly when assuming an immunity half-life of 6 months (Fig 10.9). A systematic literature review on the effect of PCV dosing schedules concluded that three-dose schedules are more effective in reducing VT carriage than two-dose schedules, but could not provide evidence on whether a 3+0 or 2+1 schedule reduced VT carriage more (240). Direct comparison between results from 3+0 and 2+1 schedules reported in the literature proved difficult, because data from 2+1 schedules came from studies in Europe only, whereas data from 3+0 schedules can from clinical trials in middle income and developing countries. Our model provides an opportunity to evaluate different vaccination schedules within the same setting, and show the trade-off between faster reduction of VT carriage after introduction (obtained in a 3+0 schedule) and longer-term gains in further reduction of VT carriage in 1-4 year-olds (obtained in a 2+1 schedule). Vaccination of HIV-positive or even all mothers was found to have no additional benefit to the current infant immunisation schedule. Our model could only estimate the reduction in VT carriage in infants as a result of reduced transmission from the mothers: it did not include any possible reduction in VT carriage as a result of maternal antibody transfer. Despite moderate uptake of PCV-13 in the catch-up campaign with only 51.5% of age-eligible infants receiving three doses, the catch-up campaign implemented in Malawi was found to have an effect on VT carriage reduction in all age/HIV groups, with faster decline of VT carriage observed when a catch-up campaign was introduced. Towards the end of the simulation period, however, the same VT carriage prevalence was obtained in models with or without catch-up campaign. Further decreases in carriage in all age/HIV groups were observed with an enhanced catch-up campaign with better coverage in infants (70% receiving three doses) and a two-dose schedule offered to children 12-59 months. Despite a rapid fall in carriage, persistent low-level VT carriage was observed in all age/HIV-groups. Carriage studies from Kilifi conducted 5 years after PCV-10 introduction, also show low-level persistence in VT carriage in all age groups (241), consistent with the findings from our models.

Strengths of the model are that it adequately predicted post-vaccination carriage prevalence in the VT and B groups. A unique feature of our model is that it includes observed vaccination data in combination with observed population data, including household structure. Other than in a model where the population and its vaccination is simulated, our model includes empiric data on vaccine uptake and timeliness. Also any 'hotspots' of low or delayed vaccine receipt, e.g. amongst children in the same household, will be included in this model. Some vaccination data had to be imputed, but information on vaccination dates was available for the majority of children.

A limitation of our model is that it did not provide a good fit to the observed NVT carriage. NVT carriage was already difficult to describe accurately when fitting to pre-vaccination data (chapter 9) and this remains a drawback in the model including vaccination. Another limitation of our model is that the simulation time is limited, because it is dependent on the available population census data. We currently used data up to 30th June 2015, but it would be possible to extend this model to the current date in the future. Forecasting population dynamics beyond data availability will be difficult, because one would have to incorporate births, deaths, migration; and changes in household composition which are often driven by those key events. We relied on vaccination data from the KHDSS yearly census and imputed vaccination receipt and vaccination dates for those for whom no information was available. We included report of vaccination, regardless of whether written documentation was observed or not. In chapter 7 we could exclude infants without written documentation from our analysis on risk factors for low vaccine uptake, but in this model we had to explicitly state the vaccination status for each individual. We therefore chose to include all data available, and impute vaccination status where no record was available. It is possible that we have overestimated or underestimated vaccination coverage by also including verbal report.

There are also limitations in the post-vaccination data collection that will have influenced the results of the validation of our model. We included the entire population in our model, but only have carriage prevalence estimates for particular sub-groups: children <15 years and mothers in households with a newborn infant, and HIV-positive adults attending the local ART clinic. In the post-vaccination period, we had no other estimate for HIV-negative adults than the carriage prevalence observed in mothers. It is likely that this estimate has been an overestimation of carriage prevalence in all HIV-negative adults, given the mothers' frequent contact with young children, including the index infant. Also sample collection from infants was sub-optimal for the purpose of this

model: we only collected samples from infants between 6 and 18 weeks of age, whereas the model includes infants at all ages. If the true prevalence of infants were lower or higher than estimated by our sample collection, this would have favoured an immunity half-life of 6 months or an immunity half-life of 1 year or more, respectively. We don't know enough about carriage dynamics in the first year of life after vaccine receipt to make a definite conclusion about which of those conditions is correct, hence our cautious approach of including both possibilities whilst exploring vaccination strategies in this chapter.

In conclusion, our model adequately simulated VT carriage in the post-vaccination period. The immunity half-life in infants is estimated to be between 6 months and 1 year. Increasing the immunity half-life to 2 years would be associated with a large reduction in VT carriage in all age/HIV groups. An enhanced catch-up campaign was associated with a more rapid and greater fall in VT carriage, but persisting low-level VT carriage was observed. A 3+1 schedule was found to be most efficient in reducing VT carriage. In choosing between a 2+1 and 3+0 schedule, the trade-off between faster reduction of VT carriage after introduction (obtained in a 3+0 schedule) and longer-term gains in further reduction of VT carriage in 1-4 year-olds (obtained in a 2+1 schedule) needs to be carefully considered.

11. Discussion

11.1 Summary of findings

In this thesis we have combined epidemiogical and mathematical modelling studies to describe pneumococcal carriage and transmission in Karonga District, Malawi, a rural sub-Saharan setting, before and after introduction of PCV-13. Our studies had a main focus on infants and HIV-positive adults, both high-risk groups for developing IPD. The main findings from our work are:

1) Pneumococcal acquisition did not differ between HIV-exposed and HIV-unexposed infants, even though pneumococcal prevalence was higher in HIV-positive mothers than in HIV-uninfected mothers.
2) Pneumococcal acquisition in infants was associated more with carriage in other young children in the household than with carriage in the mother.
3) Pneumococcal carriage in HIV-infected adults remained high despite two years of ART, with evidence of increased carriage of non-PCV-13 serotypes.
4) Vaccine coverage was moderately high in Karonga District, but infants born to lower educated mothers or farming mothers and those living further away from the road or clinic were at greater risk of being not fully vaccinated and being vaccinated late.
5) There is evidence for a direct and indirect vaccine effect with decreased VT carriage observed in vaccinated and unvaccinated older individuals, but no effect is observed in unvaccinated children <5 years.
6) SMC ABC was found to be a useful and reliable method to estimate model parameters.
7) Competition between VT and NVT pneumococci was estimated by our model to be limited
8) There is evidence for waning immunity in vaccinated infants, our model suggests an immunity half-life between 6 months and 1 year.

9) Increasing the immunity half-life to 2 years or more would have a large impact on the reduction of VT carriage.
10) An enhanced catch-up campaign including children up to 5 years of age would have resulted in a more rapid and greater decline in VT carriage, but would not have cleared all VT carriage in the population: a low-level steady-state of persistent VT carriage would have been observed.
11) Changing the vaccination schedule from 3+0 to 2+1 is associated with a slower decline in VT carriage in infants initially, but seems to involve a steeper decline in VT carriage in 1-4 years old towards the end of the simulation period (4 years).

We will discuss the findings and implications for future work in this chapter.

11.2 Pneumococcal carriage and transmission dynamics

In our systematic literature review we found that most acquisition in children <5 years was explained by transmission from other children <5 years; in DCCs and amongst siblings within the household. Within the household, children <5 years transmit to both their siblings and parents or other adults. Household transmission from adults to children appears to account for less pneumococcal transmission dynamics, even amongst mother-infant pairs. Almost all studies report on some association between maternal and infant carriage, but its relative importance to sibling-to-sibling spread is doubted. The results from our studies provide evidence that acquisition of pneumococci in infants is more strongly associated with carriage in other children <5 years in the household than with carriage in the mothers. No evidence was found for an effect of maternal HIV-status on pneumococcal acquisition in infants. If mother-to-infant transmission were a main driver for pneumococcal acquisition in infants, we would have expected to see increased carriage in HIV-exposed infants as a result of the higher carriage prevalence in HIV-positive mothers. Also our mathematical modelling studies showed that vaccinating HIV-positive, or even all mothers at time of delivery, did not result in additional VT carriage reduction in the infant. Our model could only estimate the reduction in VT carriage in infants as a result of reduced transmission from the mothers: it did not include any possible reduction in VT carriage as a result of maternal antibody transfer.

In the pre-vaccination period, a decrease in pneumococcal carriage prevalence was observed between 2009 and 2011. No parameter was included to reflect this decline in the mathematical models, yet a decreasing trend was also observed when simulating

pneumococcal carriage over a time period of five years. Looking more closely at the population dynamics provides us with a hypothesis to understand this decline: the proportion of children under five years within the whole population steadily declined over the study period. In 2009, 58.6% of all households included at least one child under five years old; in 2015 this had declined to 51.1%. Given the key influence of young children in transmitting pneumococci in the household, it is possible that the reduction in the proportion of children under five in the population led to a reduction in pneumococcal carriage. Conflicting with this hypothesis, however, is that no evidence was found for a sustained decrease in total carriage (including VT and NVT) in the post-vaccination period: total carriage levels in 2014 were similar to those observed in 2009 and 2010. Carriage levels seem to fluctuate between years, for reasons not understood. A factor that could have influenced pneumococcal carriage is seasonal variation by year such as the time of rainy season onset. Historical meteorological data could be obtained to look into this relationship further. The occurrence of other seasonal respiratory infections such as influenza may also have influenced pneumococcal carriage differences by year. Concurrent increases in other respiratory viruses and pneumococcal activity have previously reported in other countries (242, 243). The nasopharyngeal samples from our studies could be investigated for other bacterial and viral pathogens to give further insight into this relationship. Another hypothesis proposed to explain pneumococcal carriage differences by year is that nutrition and food availability fluctuates by year, and that poor nutritional status could lead to increased carriage. Although food security is a major problem in other parts of Malawi, this is less of a concern in the area under observation in the KHDSS: people in this area do not depend on one particular staple food, but produce maize, cassava and rice, which gave different harvesting times throughout the year. Fish from the lake is also available all year round.

Pneumococcal carriage in HIV-infected adults in Malawi remained high despite two years of ART, with evidence of increased carriage of non-PCV-13 serotypes. Prior to conducting the post-vaccination studies, we hypothesised that HIV-infected adults could constitute a reservoir for persisting VT carriage and pneumococcal diversity post vaccine introduction. No evidence for this was found in the post-vaccination studies. VT carriage in HIV-positive adults on ART had decreased, and there was no evidence for a change in NVT carriage. There was some evidence that pneumococcal carriage prevalence is lower in HIV-positive adults on prolonged ART, even after taking survival bias into account. Survival bias occurred, because carriage in individuals surviving >2.5 years after initiation of ART was lower as compared to carriage in those who died

within a couple of years after ART initiation. This finding suggests that pneumococcal carriage could be an indicator for adverse survival outcome in HIV-positive adults.

11.3 Impact of PCV-13 introduction on pneumococcal carriage

Malawi has achieved high coverage since the introduction of PCV-13 in November 2011, even in our remote study setting. In June 2015 vaccination coverage in all children <5 years (including age ineligible children) was 82.0% for one dose of PCV-13 and 70.9% for three doses of PCV-13. Vaccination coverage increased since introduction, from 87.4% in 2011-2012 to 94.5% in 2013-2014 for three doses of PCV-13. Lower coverage was observed during the catch-up campaign (51.5%). Our results suggest that the initial lower coverage achieved was attributable to vaccine non-availability. Despite the decision to provide catch-up vaccination to infants, the country was provided with doses adequate only for the birth cohort (211). Countries introducing new vaccines should ensure adequate stock and resources for planned catch-up campaigns and strengthen system required for rapid roll-out and delivery. Our model results show that a more rapid and greater fall in VT carriage could have been achieved with an enhanced catch-up campaign in children up to 5 years of age as was implemented in Kilifi, Kenya (25), but that the enhanced campaign would not clear VT carriage from the population: a low-level steady state of persistent VT carriage was observed in all age/HIV groups.

We provide evidence for a reduction in carriage of VT pneumococcus three years after vaccine rollout in this rural Malawi population. Reduction in VT carriage in unvaccinated individuals without change in non-VT carriage suggests herd protection is taking place. Herd protection, however, is found to be lower than in other countries, and does not seem to extend to infants too young to be vaccinated. One hypothesis to explain why an indirect effect is seen in older unvaccinated individuals, but not in younger unvaccinated individuals, is that natural birth spacing decreases the likelihood that young infants are in household contact with newly vaccinated children. Assuming that household contact is most important to explain pneumococcal transmission, and assuming that immunity in vaccinated infants wanes substantially within the first year, we can expect that those benefitting most from an indirect effect are those in contact with newly vaccinated infants. If infants too young to be vaccinated have vaccinated household contacts, the contacts are most likely to be siblings who are at least 9 months older and in whom immunity has already waned.

Surveillance of pneumococcal carriage is also undertaken in Blantyre, southern Malawi. High VT carriage was observed; 22.5% in vaccinated children aged 3-4 years, 25.7% in unvaccinated children aged 5-10 years, and 13.9% in adults receiving ART. Serotype 1 represented respectively 7.3%, 18.0% and 9.1% of VT carriage. This is higher than in our study, where VT carriage was 16.5% in vaccinated children, 7.9% in unvaccinated children 5-15 years, and 0.5% in adults receiving ART. No carriage of serotype 1 was detected in our study. The difference in VT carriage is unlikely to be a result of lower vaccination coverage: uptake of vaccination in Blantyre is high, with >85% coverage amongst age-eligible children. It is possible that population structure including household composition is significantly different in urban Blantyre and rural Karonga and that this explains the difference in VT dynamics, but further studies would be required to investigate his. Estimates of VT carriage in the community are lacking from Blantyre for the pre-vaccination period: it is possible that VT carriage in Blantyre was higher than in Karonga in the pre-vaccination period, and that the current estimates reflect an equitable decrease as compared to the pre-vaccination period. The occurrence of serotype-1 is unnerving: this serotype is rarely detected in nasopharyngeal carriage worldwide, but is associated with clinical disease and has the potential to cause community-wide outbreaks of pneumococcal meningitis (219). Ongoing surveillance is required to monitor the presence of VT carriage, particularly serotype 1 in this vulnerable population.

Immunity half-life in infants was modelled to be between 6 months and 1 year. Increasing the immunity half-life to 2 years would be associated with a large reduction in VT carriage in all age/HIV groups. A 3+1 schedule was found to be most efficient in reducing VT carriage, but the addition of an extra dose to the Malawi infant immunisation schedule is unlikely given the high costs of PCV-13. Countries newly introducing PCV need to decide between a 3+0 or a 2+1 schedule and carefully consider the trade-off between faster reduction of VT carriage after introduction (obtained in a 3+0 schedule) and longer-term gains in further reduction of VT carriage in 1-4 year-olds (obtained in a 2+1 schedule).

We provided some evidence that pneumococcal carriage prevalence is lower in HIV-positive adults on prolonged ART. It is likely that the combination of prolonged duration of ART and PCV-13 introduction both contributed to the decrease in any serotype and VT pneumococcal carriage in HIV-positive adults. Our results need to be considered with caution: no difference in pneumococcal carriage by ART duration was observed in the cross-sectional conducted study in Blantyre. Ongoing work on HIV-positive adults

on ART in Blantyre will be analysed to assess whether the same finding can be observed in this population.

11.4 Limitations and strengths of the included studies

The limitations of individual studies have been discussed in the discussion paragraphs of individual chapters. Some recurring themes can be identified, relating to the study designs, selection of participants, sample collection, and laboratory analysis.

All epidemiological studies presented here are observational studies: no randomized controlled trials (RCTs) were conducted as part of this PhD thesis. Observational studies are more prone to bias than RCTs, because treatment is not provided at random. RCTs could have been used to give us a more robust estimate of the impact of PCV-13 vaccination or ART on pneumococcal carriage and transmission. For instance, a cluster-RCT design could have been implemented before national rollout of PCV-13 vaccination to study changes in pneumococcal carriage in vaccinated individuals and their unvaccinated households, including younger infant siblings. An RCT on ART initiation could have given us a more robust measure of change in pneumococcal carriage since ART onset, but of course this would not have been possible because of the ethical issues in delaying ART in eligible individuals.

Selection of participants for our studies occurred in a non-random way. We recruited mother-infant pairs at the local hospital, thus missing infants born at home. During the study period, 97% of infants were born in a health centre, indicating that our recruitment strategy included most infants born in this population. Coverage of household members was incomplete, particularly for older children and adults other than the mother who were often absent during study visits or refused to participate in the pre-vaccination period, and were not included in the post-vaccination period. This precluded analyses on the role of older children and adults in household transmission. We recruited HIV-positive adults from the ART clinic only, thus missing HIV-positive adults not attending the clinic. A cross-sectional analysis conducted in 2007-2008 showed only about 25% of HIV-positive adults completed screening for ART, suggesting that our sample at the ART clinic missed a large proportion of HIV-positive adults in the population.

No formal assessment of the optimal sampling interval was made. We changed the sampling interval of 4-6 weeks in the pre-vaccination period to a 2-week sampling

interval in the post-vaccination period. We did not, however, calculate the likely optimal sampling interval for our studies based on estimates from other settings. Calculations on how our sampling interval would have likely impacted on our estimates for different serotypes and age groups would have helped us with the interpretation of our results.

Our laboratory procedures did not allow for detection of simultaneous colonization with multiple serotypes. A subset of samples from Blantyre was assessed for multiple serotype carriage using microarray technique. Multiple serotype carriage was found to be 54.1% in vaccinated and 59.3% in unvaccinated children. Serotyping using microarray increased detectable VT carriage by 35.6%. Our comparisons between the pre- and post-vaccination period are still valid, because multiple serotype assessment was not performed in either period. Not allowing for detection of simultaneous colonization with multiple serotypes will have decreased the accuracy of our estimates of carriage duration and household transmission.

Any limitations in our earlier studies will have had a 'knock-on' effect on the later studies and mathematical models. If our estimates of pneumococcal carriage in the pre-vaccination period were under- or overestimated, this will have influenced our conclusions in the before-after-comparison, and will have influenced the parameterization and inference of our mathematical models. In the pre-vaccination period, we observed a decreasing secular trend in pneumococcal carriage. The observed reducing carriage incidence was contemporaneous with decreasing rates of invasive disease in Malawi (40), suggesting long-term ecological trends in pneumococcal carriage and disease occur, possibly on the back of improved food security and nutrition in this population. However, we cannot rule out that the decrease in pneumococcal carriage was linked to inadvertent changes in sample collection and analysis over time. Enhanced quality control during the sample collection and analysis in the pre- and post-vaccination period would have given us more robust findings. In addition, sensitivity/specificity analyses on the different swabs (flocked vs. calcium alginate) and serotyping methods (Quellung vs. Latex agglutination) used would have provided us with a more robust comparison of samples collected before and after vaccine introduction.

The major strength of this PhD thesis is its breadth: with the inclusion of pre-vaccination carriage data, post-vaccination carriage data, vaccine uptake data, and mathematical modelling studies we provide an extensive overview of pneumococcal carriage during a time of vaccine introduction. Another strength of this PhD thesis is the extensive use of data from the Karonga HDSS. We used HDSS data in our

epidemiological studies to obtain information on an individual's household status, vaccination status, HIV status and ART status. We also used HDSS data extensively in our mathematical model: a unique feature of our model is that it includes observed vaccination data in combination with observed population data, including household structure. Other than in a model where the population and its vaccination is simulated, our model included empiric data on vaccine uptake and timeliness. Also any 'hotspots' of low or delayed vaccine receipt, e.g. amongst children in the same household, will have been included in this model. The continued sampling in the same population is also a strength of our studies: some adults participating in the ART study in 2008-2010 were sampled again in 2014, allowing for a comparison of pneumococcal carriage in the same study participants.

11.5 Future directions

Ongoing surveillance on pneumococcal carriage and disease is required to monitor the long-term impact of PCV-13 introduction. Further work is needed to confirm the existence of waning immunity in older vaccinated children, and how this impacts on herd immunity. A series of eight cross-sectional surveys conducted over four years is currently underway in Blantyre, southern Malawi. Recruitment includes PCV-13-vaccinated and unvaccinated children aged 3-10 years, using stratified random sampling of households and schools; and adults receiving ART.

An area that has not been explored in this PhD thesis is the impact of environmental factors on pneumococcal carriage and disease. Household air pollution from biomass fuel (animal or plant material) is known to be a risk factor for acute lower respiratory infection, with an estimated two-fold risk in exposed children (244). Very little is known about the impact of reducing household air pollution on reducing pneumococcal carriage. A study associated to this PhD thesis is underway to assess pneumococcal carriage in infants born into households taking part in a large randomized cluster trial on the impact of a Philips cookstove on pneumonia. Nasopharyngeal samples are collected at 6 weeks and 6 months of the infant's age and will be serotyped using microarray. An estimate of air pollution exposure will be available from personal monitors are kept close to the infant for 48 hours. Results for this study are expected late 2016 and will, in addition to the analysis on air pollution, be used to assess pneumococcal carriage in infants five years after introduction of PCV-13.

Nasopharyngeal samples collected for this PhD thesis are being used in several collaborations with national and international partners. Whole genome sequencing was performed on samples collected in the pre- and post-vaccination. Using sequencing results was deemed to be outside the scope of this PhD thesis, but future analyses could be conducted to inform our studies on household transmission. Another area of molecular work following from this PhD thesis will be investigations into the nasopharyngeal microbiome. The cohort study design adapted in studies described in this PhD thesis will provide an opportunity to study the nasopharyngeal microbiome in young infants, before and after PCV-13 receipt, and to compare the nasopharyngeal micriobiota of household members.

A review of vaccination schedules is required to determine whether the immunity half-life can be extended in vaccinated children, and whether in a larger herd impact can be achieved, extending to infants too young to be vaccinated and unvaccinated children <5 years. Negotiations regarding a change in vaccination schedule are ongoing with the Malawi Ministry of Health and potential funders. If vaccination were to be changed to a '2+1' schedule, a large cluster randomised controlled cluster trial design would be recommended to evaluate the impact of a booster dose on waning immunity in vaccinated children, and on herd immunity in unvaccinated individuals.

11.6 Ongoing vaccine developments

Global efforts are undertaken to develop new or enhanced conjugate vaccines, and to develop new generation pneumococcal vaccines. Current efforts to improve existing conjugate vaccines are focused on 1) decreasing the costs of PCVs, 2) increasing the number of serotypes included in the PCVs, and 3) increasing the effectiveness of PCVs.

Decreasing the costs of PCVs is essential for their continued success in low- and middle-income countries. Many countries, including Malawi, are currently funded through GAVI, but are expected to increasingly contribute to the purchase of vaccines in the years after vaccine roll-out. The cost to GAVI per dose of PCV is approximately $3.10-$3.40 per dose, or $9.30-$10.20 per vaccinated child (245). The cost per dose in developed countries is much higher: around $120 per dose of PCV-13 in the USA (246), and £50 in the UK (247, 248). PCVs require a complicated manufacturing process, resulting in their relatively high price per dose. The difficulty of manufacturing also makes technology transfer to developing countries difficult. The most advanced

candidates in a developing country are 10- and 13-valent vaccines being developed by the Serum Institute of India and Panacea Biotech, another Indian pharmaceutical company (245). The candidate vaccines will have a different set of serotypes to the existing 10- and 13-valent vaccines to better represent the distribution of invasive serotypes in India. Another strategy to reduce costs of PCVs would be to develop multi-dose vials, thereby potentially reducing packaging and transport costs. Lastly, manufacturing processes are being optimised to become more efficient, resulting in reduced overall costs (245).

The number of serotypes that can be included in conjugate vaccines is limited. The reason for this is two-fold: only a limited amount of serotypes can be conjugated to the carrier proteins currently in use, and the addition of extra serotypes seems to diminish the antibody response. Although protective levels (immunoglobulin type G (IgG) antibody concentration of >0.35 µg/l) were achieved by both vaccines for the seven common serotypes, lower geometric mean IgG antibody concentrations were obtained for the same serotypes when comparing PCV-13 with PCV-7 (249). In addition, diminishing results are expected with the inclusion of additional serotypes. After introduction of PCV-7, serotype-replacement disease was caused by a small number of serotypes, with serotype 19A in particular being a large contributor to NVT disease (18, 250, 251). The switch from PCV-7 to PCV-13 led to significant further reductions in disease (26). Invasive disease caused by NVT serotypes in the post-PCV-13 era is more diverse, with multiple serotypes causing invasive disease (26). The addition of one or two extra serotypes to create a 14-valent or 15-valent vaccine will likely have a minor impact on the total burden of NVT disease.

Also the efficacy and immune duration of PCVs could be improved. Our model suggests that increasing the immunity half-life has a large impact on the level of residual VT carriage in the population. A longer-lasting immune response could be achieved by the use of novel adjuvants. Adjuvants stimulating a toll-like receptor response on antigen-presenting cells have been found to enhance vaccine immune responses in vulnerable individuals, including preterm neonates (252) and HIV-infected adults (253). It is possible that a better immune response with an increased immunity half-life can be elicited with the use of adjuvants. In countries where the response to PCVs is suboptimal, adding an adjuvant to the vaccine, either as part of the vaccine formulation or as a stand-alone injection, could enhance its effect on carriage and disease. However, safety of newly developed adjuvants would need to be carefully monitored. Despite ongoing adjuvant research and development, there remain only a relatively small number of adjuvants used clinically as a result of safety concerns (254).

Numerous institutions worldwide are working on the development of a new serotype-independent pneumococcal vaccine. Several strategies are investigated, including whole cell vaccines, protein-based vaccines, and live attenuated vaccines (245, 255). Also the possibility of adding serotype-independent proteins to the existing PCVs is being investigated (245). Common proteins that are a target of new generation vaccines are; pneumolysin, pneumococcal surface proteins A & C, pneumococcal surface antigen A, neuraminidase enzymes, and histidine-triad proteins. The omnipresence of those proteins should elicit a serotype-independent response. Mice studies have been promising, and the most advanced protein vaccine candidates are currently undergoing phase 2 clinical trials (245).

New generation vaccines will face several challenges to licensure (245). PCV-13 was licensed based on non-inferiority immunogenicity studies, in which serotype-specific IgG antibody concentrations and functional antibody levels were measured (249). New PCVs in development, including those manufactured by Indian pharmaceuticals, may follow the same licensure pathway, but the requirements for new generation vaccines (whole-cell, live attenuated or protein-based vaccines) are unclear. Other than with serotype-specific vaccines, predictors of clinical outcome that can be used in immunogenicity studies for serotype-nonspecific are not yet well-defined (245). It is likely that large vaccine efficacy trials would be required to demonstrate efficacy of new generation vaccines. The outcome of those trials will also need to be defined: it is not yet clear whether a vaccine can be licensed based on its effect on nasopharyngeal carriage or otitis media, or whether a trial with pneumonia or IPD as outcome measure is required (245). It is also not clear what the role would be of a vaccine which protects against IPD, but has no or very little effect on carriage. It is possible that such a vaccine would be introduced alongside PCVs to allow for a reduction in serotype-nonspecific disease, yet sustain the herd effect acquired by PCVs.

This PhD thesis described the pneumococcal carriage and transmission in Karonga district, Malawi, before and after introduction of 13-valent pneumococcal conjugate vaccination. There is some evidence for a direct and indirect vaccine effect, but no effect was observed in unvaccinated children <5 years. VT carriage remains common in this population, and an increase in NVT carriage was observed in vaccinated individuals. It is yet unclear what the impact of new generation vaccines will be on pneumococcal carriage. Even if reduction of carriage with new vaccines is modest, an indirect vaccine effect could occur in this setting, given the high proportion of household contact with children <5 years. The shadow side of this dynamic is that if VT

carriage were to re-increase as a result of a future change from PCV-13 to serotype-independent vaccines with lower impact of carriage, this could result in reintroduction of increased circulation of current VT carriage. If any change in vaccine were to be made, careful monitoring of carriage and disease is warranted.

In conclusion, there is need for cheaper, more effective, serotype-independent pneumococcal vaccines. Potentially cheaper PCVs produced in India are in the pipeline, with results expected in the next couple of years. The pneumococcal research community is also eagerly awaiting the results from a couple of new generation vaccines that are currently in phase 2 clinical trials. The next decade is promising to be an exciting time for pneumococcal researchers and policy makers worldwide, as new vaccines will pose interesting possibilities to further decrease pneumococcal carriage and disease in settings worldwide.

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Appendix

Ethical approval - National Health Sciences Research Committee

Telephone: + 265 789 400
Facsimile: + 265 789 431
e-mail doccentre@malawi.net
All Communications should be addressed to:
The Secretary for Health

In reply please quote No. MED/4/36c
MINISTRY OF HEALTH
P.O. BOX 30377
LILONGWE 3
MALAWI

14th February 2014

Neil French
Karonga Prevention Study

Dear Sir/Madam,

RE: Protocol # 1232: Pneumococcal carriage and transmission in Karonga district, Malawi two years after the introduction of pneumococcal conjugate vaccine into the infant immunization programme
Thank you for the above titled proposal that you submitted to the National Health Sciences Research Committee (NHSRC) for review. Please be advised that the NHSRC has **reviewed** and **approved** your application to conduct the above titled study.

- **APPROVAL NUMBER** **: NHSRC # 1226**
  The above details should be used on all correspondence, consent forms and documents as appropriate.
- **APPROVAL DATE** **: 14/02/2014**
- **EXPIRATION DATE** :This approval expires **on 14/02/2015**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the NHSRC secretariat should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING** :All serious problems having to do with subject safety must be reported to the National Health Sciences Research Committee within 10 working days using standard forms obtainable from the NHSRC Secretariat.
- **MODIFICATIONS**: Prior NHSRC approval using standard forms obtainable from the NHSRC Secretariat is required before implementing any changes in the Protocol (including changes in the consent documents). You may not use any other consent documents besides those approved by the NHSRC.
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the NHSRC using standard forms obtainable from the NHSRC Secretariat.
- **QUESTIONS**: Please contact the NHSRC on Telephone No. (01) 724418, 0888344443 or by e-mail on mohdocentre@gmail.com
- **Other:**
  Please be reminded to send in copies of your final research results for our records as well as for the Health Research Database.

Kind regards from the NHSRC Secretariat.

.....
FOR CHAIRMAN, NATIONAL HEALTH SCIENCES RESEARCH COMMITTEE

Ethical approval - University of Liverpool

From: **Ethics** ethics@liverpool.ac.uk
Subject: RE: RETH000670 application for ethical approval
Date: February 27, 2014 at 8:55
To: Heinsbroek, Ellen heinsbro@liverpool.ac.uk, French, Neil [french] french@liverpool.ac.uk

Dear Professor French and Ellen

I am pleased to inform you that the Sub-Committee has approved your application for ethical approval for your study. Details and conditions of the approval can be found below.

Ref: RETH000670
Sub-Committee: Physical Interventions
Review type: Full committee review
Supervisor: Professor Neil French
Student Investigator: Ellen Heinsbroek
School: Department of Clinical Infection, Microbiology, and Immunology
Title: Pneumocchial carriage and transmission in Malawi two years after the introduction of pneumocchial conjugate vaccine into the infant immunisation programme
First Reviewer: Professor David Lalloo
Second Reviewer: n/a
Date of initial review: 13/12/13
Date of Approval: 27/02/14

The application was APPROVED subject to the following conditions:

Conditions

All serious adverse events must be reported to the Sub-Committee within 24 hours of their occurrence, via the Research Integrity and Governance Officer (ethics@liv.ac.uk).

This approval applies for the duration of the research. If it is proposed to extend the duration of the study as specified in the application form, the Sub-Committee should be notified. If it is proposed to make an amendment to the research, you should notify the Sub-Committee by following the Notice of Amendment procedure outlined at http://www.liv.ac.uk/media/livacuk/researchethics/notice%20of%20amendment.doc. If the named PI / Supervisor leaves the employment of the University during the course of this approval, the approval will lapse. Therefore please contact the Research Integrity and Governance Officer at ethics@liverpool.ac.uk in order to notify them of a change in PI / Supervisor.

Best wishes

Matthew

Matthew Billington
Research Integrity and Governance Officer

Research Support Office
University of Liverpool
Waterhouse Building (2nd Floor, Block D)
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Liverpool
L69 3GL

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Data collection tools

Data collection tools specific to this study were used in conjunction with generic data tools, used for e.g. birth registration, specimen collection, and HIV/AIDS status confirmation.

Version 2a 02/04/2014

PVCR – registration form (round 3)

Fill this form if willing to receive follow-up visit for consent

1	Date			intdate
2	Name of Mother			ident
3	Date of birth Birth decade estimate	1950-59 1960-69 1970-79 1980-89 1990-99 G H I J K		birthest birthmn birthyea
4	Village of birth			bvil
5	Name of mother			idmoth
6	if alive elsewhere, name head of HH and village	HH: vge.:	**A**live **D**ied	mothv
7	Name of father			idfath
8	if alive elsewhere, name head of HH and village	HH: vge.:	**A**live **D**ied	fathvit
9	**Have you been known by any other names in the past?**			
10	Name HH & village or HC where last seen:			

Household and Residence

11	Current household			idhdhs
12	Current village			curvil

13	List siblings alive in Karonga If no siblings are known alive in Kga.: List other members of the HH where last seen	*tick if same:* mother	father
14	*(1)*		
15	*(2)*		
16	*(3)*		

	Included in **L**ongitudinal (samples 1, 6,8,10,12,14,18, wks) or **C**ross-sectional study (1 sample at 6 wks)	**L**	**C** longcros

17	Interviewer			rcdr
18	Coder		Checker	codr chkr

This section does not need to be entered in the data office

Date of birth infant	
Firstborn or are there other children? What are their ages?	
Residence after delivery	
Where will you go in the weeks after delivery?	**C**urrent / **M**other's / **O**ther household
If mother's/other: How many weeks do you plan to stay there?	
If current / mother's: check details above are complete and correct	
If other: head of household	
If other: village	
Instructions to find the house / other comments:	

Version 5 (11/02/2014)

PVCF - follow-up pre-printed ticket – Post-vaccination (Round 3)

1	Week		Village			Week
2	CRSHSE		Head of house			repgp, cluste hhlistn
3	ID headhse					idhdhs
4	Mothers STID					mstid
5	Childs STID					cstid

If child no longer present fill exit form

6	Date of visit (DD/MM/YYYY)		intdate
7	Household Seen **If N then go to Q 24 staffcode**	**Y N**	hseseen
8	Does the child currently receive cotrimoxazole?	**Y N**	cotricurr
9	Has the child received cotrimoxazole in the last 4 weeks?	**Y N**	cotripast
10	If yes, how long was/is the treatment? **L**ess / **M**ore than 2 weeks	**L M**	
	If this is a 6,10 or 14 week visit vaccine is due		
11	Vaccine visit	**Y N**	
12	If yes, vaccine given?	**Y N**	vacc
13	If no, reason: U**n**well **R**efusal **A**lready given **O**ther	**N R A O**	novacc
14	If other describe		

15	Any change in household members	**Y N**	members

Mother,child and siblings <15 years old (cross-sectional) or < 5 (longitudinal)

	Ident	Name	Sex	Bthyr	Status*	Swab	STID	CNO
16					**P M D**	**Y N R**		orange label
17					**P M D**	**Y N R**		orange label
18					**P M D**	**Y N R**		orange label
19					**P M D**	**Y N R**		orange label
20					**P M D**	**Y N R**		orange label
21					**P M D**	**Y N R**		orange label
22					**P M D**	**Y N R**		orange label
23					**P M D**	**Y N R**		orange label
24					**P M D**	**Y N R**		orange label
25					**P M D**	**Y N R**		orange label

* for status **P**resent, **M**issing, **D**ied for swab **Y**es **N**o **R**efused

26	Staffcode		rcdr
27	Level 2 Checker		chkr

Version 1a (27/05/2014)

PVCA – Pneumococcal Carriage in ART clinic

1	Date			intdate
2	ART number *(hand copy from card)*			artno
3	ART STID and CNO	hand-copy register	orange label	stid,cno
4	Consent given – if NO finish		**Y** **N**	con
5	Currently taking cotrimoxazole prophylaxis?		**Y** **N**	cotrim
6	If yes, cotrimoxazole started at the same time as ART?		**Y** **N**	cotrst
	If no, since when? (dd/mm/yyyy or descriptive)			cotrdd cotrmm cotryr

	Comments		
9	Interviewer staffcode		rcdr