TITLE

High residual vaccine-serotype *Streptococcus pneumoniae* carriage 4 to 6 years after the introduction of 13-valent pneumococcal conjugate vaccine in Malawi: a prospective serial cross-sectional study

AUTHORS

T.D. Swarthout (MSc)[1,2*], C. Fronterre (PhD)[3], J. Lourenço (PhD)[4], U. Obolski(PhD)[4], A. Gori (PhD)[5], N. Bar-Zeev (PhD)[1,6], D. Everett (PhD)[1,7], A.W. Kamng'ona (PhD)[8], T.S. Mwalukomo (MMed)[9], A.A. Mataya (MBBS)[1], C. Mwansambo (MBChB)[10], M. Banda[11], S. Gupta (PhD)[4], P. Diggle (PhD)[3], N. French (PhD)[1,12¥], R.S. Heyderman (PhD)[1,5¥]

*Correspondence to: Todd Swarthout, Malawi-Liverpool-Wellcome Trust Clinical Research Programme, P.O. Box 30096, Chichiri Blantyre 3, Malawi; Email: todd.swarthout@lstmed.ac.uk; Phone: +265 (0)1 874 628

¥: Joint last authors have contributed equally to this manuscript (French and Heyderman)

AFFILIATIONS & ADDRESSES

1. Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi
2. Clinical Sciences Department, Liverpool School of Tropical Medicine, Liverpool, United Kingdom
3. Medical School, Lancaster University, Lancaster, United Kingdom
4. Department of Zoology, University of Oxford, Oxford, United Kingdom
5. Division of Infection & Immunity, University College London, London, United Kingdom
6. Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA
7. The Queens Medical Research Institute, University of Edinburgh, Edinburgh

8. Department of Biomedical Sciences, College of Medicine, University of Malawi, Blantyre, Malawi
9. Department of Medicine, College of Medicine, University of Malawi, Blantyre, Malawi
10. Ministry of Health, Lilongwe, Malawi
11. Ministry of Education, Blantyre, Malawi
12. Centre for Global Vaccine Research, Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom

Keywords: *Streptococcus pneumoniae*; Pneumococcal carriage; Pneumococcal conjugate vaccine; Children; Adults; HIV; Africa; Indirect protection

ABSTRACT

Background: There are concerns that current pneumococcal conjugate vaccine (PCV) schedules in sub-Saharan Africa sub-optimally interrupt vaccine-serotype (VT) carriage and transmission, thus limiting indirect protection. We assessed pneumococcal carriage in vaccinated children and unvaccinated populations targeted for indirect vaccine protection, between 4 and 6 years after the 2011 introduction of a 13-valent PCV (PCV13) 3+0 schedule in Malawi.

Methods: We conducted four sequential prospective nasopharyngeal carriage surveys in urban Blantyre, from June, 2015, to April, 2017. We recruited healthy PCV13-vaccinated children 3-6 years old, children 5-10 years old born before PCV13 introduction, and HIV-infected adults 18-40 years old on antiretroviral therapy. Carriage risk by age was analysed by non-linear regression.

Findings: We sampled 1382 PCV13-vaccinated children, 889 PCV13-unvaccinated children, and 985 adults. VT carriage prevalence declined from 23% to 17% among vaccinated children (adjusted prevalence ratio [aPR] 0.75, 95% CI 0.56-1.01; p=0.062) and 27% to 15% among unvaccinated children (aPR 0.65, 95% CI 0.44-0.98; p=0.039). Adult prevalence remained 14% (aPR 0.92, 95% CI 0.59-1.44; p=0.72). VT carriage probability declined with age, with a decay half-life of 5.3 years (95% CI 3.2-9.0).

Interpretation: The PCV13 3+0 schedule in Malawi has not achieved optimal reduction in pneumococcal carriage prevalence, compared to high-income settings. This is likely due to recolonisation of vaccinated children with waning vaccine-induced immunity and suboptimal indirect protection of unvaccinated populations. Rigorous evaluation of strategies to augment vaccine-induced control, including alternative schedules and catch-up campaigns among children under 5 years old is required.

Funding: Bill & Melinda Gates Foundation, Wellcome Trust UK, Medical Research Council.

INTRODUCTION

Streptococcus pneumoniae is estimated to be responsible for over 500 000 deaths every year in children aged 1 to 59 months worldwide, with the highest burden among African children.[1] *S. pneumoniae* has over 90 immunological serotypes and is a common coloniser of the human nasopharynx, particularly in young children, resource-poor and HIV-affected populations.[1] Although most carriers are asymptomatic, pneumococcal colonisation is a necessary precursor for transmission and the development of pneumonia, meningitis, and bacteraemia.[2]

In Europe and North America, routine infant administration of pneumococcal conjugate vaccine (PCV) has rapidly reduced vaccine-serotype (VT) invasive pneumococcal disease (IPD) and carriage.[3–6] Importantly, this has occurred in vaccinated and unvaccinated age groups. Thus, indirect protection resulting from diminished carriage and transmission amplifies PCV impact and cost-effectiveness.[7] Pneumococcal epidemiology in sub-Saharan Africa is characterised by high rates of carriage and transmission, differing markedly from high-income settings.[8,9] Carriage studies pre-dating PCV introduction in Kenya,[8] Mozambique,[10] Malawi,[11] The Gambia,[12] and South Africa[13] reported VT carriage prevalences ranging from 49.7% to 28.2% in under 5s with colonisation occurring early in life,[14] consistent with higher transmission rates than those found in resource rich settings.

Vaccine trials and post-routine-introduction studies in Africa have demonstrated substantial direct effects of PCV against IPD, pneumonia, and all-cause mortality among young children.[15–18] Although Kenya,[19] The Gambia,[18] Mozambique[20], and South Africa[21] have reported VT carriage reductions, these prevalences are still higher than in industrialised countries,[22–24] and NVT replacement is emerging.[25] Thus, it is uncertain whether PCV introduction in sub-Saharan Africa will achieve the sustained direct or indirect protection necessary to reduce pneumococcal carriage to levels sufficient to interrupt transmission and disease. This is of particular concern in many sub-Saharan African countries where the 3+0 schedule has been implemented into infant expanded programmes on immunisation (EPI).[26]

In November 2011, Malawi (previously PCV-naïve) introduced 13-valent PCV as part of the national EPI using a 3+0 schedule (6, 10 and 14 weeks of age). A three-dose catch-up vaccination campaign included infants <1 year of age. Field studies among age-eligible children have reported an even higher PCV13 uptake (90–95%)[27,28] than the 83% previously reported by WHO/UNICEF.[29]

We hypothesised that despite evidence of PCV13 impact on IPD and pneumonia in Malawi,[30,31] there would be persistent VT carriage and that this would maintain transmission in both childhood and adult reservoirs. We have investigated this among PCV13-vaccinated children (in whom vaccine-induced immunity wanes after the first year of life[32]); children too old to have received PCV13; and HIV-infected adults on antiretroviral therapy (ART) who do not routinely receive pneumococcal vaccination (previously demonstrated to have a high carriage prevalence).[33,34]

METHODS

Study Design

This was a prospective cross-sectional observational study using stratified random sampling to measure pneumococcal nasopharyngeal carriage in Blantyre, Malawi. Sampling consisted of a time series profile from twice-annual surveys over 2 years.

Study population and recruitment

Blantyre is located in Southern Malawi with a population of approximately 1·3 million. Recruitment included three groups: i) randomly sampled healthy children 3–6 years old (3–6yrs) who received PCV13 as part of EPI or the catch-up campaign, ii) randomly sampled healthy children 5–10 years old (5–10yrs) who were age-ineligible (born on or before 11 November 2010 and therefore too old to receive PCV13 as part of EPI or the catch-up campaign), recruited from households and Blantyre schools; and iii) HIV-infected adults (18–

40yrs) on ART, recruited from Blantyre's Queen Elizabeth Central Hospital ART Clinic using systematic sampling. Exclusion criteria for all participants included current TB treatment, pneumonia hospitalisation ≤14 days before study enrolment or terminal illness. Exclusion criteria for children included reported immunocompromising illness (including HIV), having received antibiotics ≤14 days prior to screening, having received PCV13 if age-ineligible or not having received PCV13 if age-eligible. Individuals were not purposely resampled but were eligible if randomly re-selected in subsequent surveys.

Site selection

Households and schools were selected from within three non-administrative zones representative of urban Blantyre's socioeconomic spectrum in high-density townships. These zones were further divided into clusters, allowing for approximately 25 000 adults per zone and 1 200 adults per cluster. Within each zone, two to three clusters were randomly selected per survey. Clusters were not purposely resampled but eligible if randomly selected in subsequent surveys. Within each cluster, a handheld GPS device guided study teams to a randomly selected starting point. After the first house was chosen randomly, teams moved systematically, recruiting one eligible child per household until the required number of children were recruited from each cluster. Individual schoolgoers were randomly selected from school registers, and letters sent home inviting parents or legal guardians to discuss the study and consider consenting to their child's participation.

Determining PCV13 vaccination status

A child was considered "PCV13-vaccinated" if s/he had received at least one dose of PCV13 prior to screening. Vaccination status and inclusion/exclusion criteria were further assessed from subject-held medical records (known as health passports). If a child was reported by the parent/guardian to be PCV13-vaccinated but no health passport was available, a questionnaire was applied. The questionnaire was developed by identifying in a subset of 60 participants, the four questions most commonly answered correctly by parents/guardians of

children with proof of PCV vaccination. The questions included child's age when vaccinated, vaccine type (oral or injectable), anatomical site of vaccination, and which other (if any) vaccines were received at time of PCV13 vaccination. If all four questions were answered correctly, the child was recruited as "PCV13-vaccinated."

Ethics

The study protocol was approved by the College of Medicine Research and Ethics Committee, University of Malawi (P.02/15/1677) and the Liverpool School of Tropical Medicine Research Ethics Committee (14.056). Adult participants and parents/guardians of child participants provided written informed consent, children 8-10 years old provided informed assent.

Sample size

The sample size strategy was a pragmatic approach to allow for adequate precision of the carriage prevalence estimates. Using VT carriage as the primary endpoint, the sample size was calculated based on the precision of the prevalence estimation, assuming an infinite sampling population. Among children 3–6yrs (vaccinated), an absolute VT prevalence up to 10% was expected, with a sample of 300/survey providing a 95% confidence interval (CI) of 6·6–13·4%. Among children 5–10yrs (unvaccinated) and HIV-infected adults, an absolute VT prevalence of 20% was expected, with a sample of 200/survey providing a 95% CI of 14·5–25·5%.

Nasopharyngeal swab collection

A nasopharyngeal swab (NPS) was collected from each participant using a nylon flocked swab (FLOQSwabs™, Copan Diagnostics, Murrieta, CA, USA) and then placed into 1·5mL skim milk-tryptone-glucose-glycerol (STGG) medium and processed at the Malawi–Liverpool–Wellcome Trust (MLW) laboratory in Blantyre, according to WHO recommendations.[35] Samples were frozen on the same day at −80°C.

Pneumococcal identification and latex serotyping

After being thawed and vortexed, 30 µL NPS–STGG was plated directly on gentamicin-sheep blood agar (SBG; 7% sheep blood agar, 5 µl gentamicin/mL) and incubated overnight at 37°C in 5% CO_2. Plates showing no *S. pneumoniae* growth were incubated overnight a second time before being reported as negative*. S. pneumoniae* was identified by colony morphology and optochin disc (Oxoid, Basingstoke, UK) susceptibility. The bile solubility test was used on isolates with no or intermediate (zone diameter <14mm) optochin susceptibility. A single colony of confirmed pneumococcus was selected and grown on a new SBG plate as before. Growth from these secondary plates was used for serotyping by latex agglutination (ImmuLex™ 7-10-13-valent Pneumotest; Statens Serum Institute, Denmark). This kit allows for differential identification of each PCV13 VT but not for differential identification of NVT serotypes; NVT and non-typeable isolates are therefore reported as NVT. Samples were batch tested on a weekly basis, blinded to the sample source. Latex serotyping results showed good concordance with whole genome sequence and DNA microarray serotyping.[36] There were no changes to protocols over the duration of the study.

Statistical analysis

Participant demographic characteristics were summarised using means, standard deviations, medians, and ranges for continuous variables and frequency distributions for categorical variables. Non-ordinal categorical variables were assessed as indicators. Carriage prevalence ratios (PR) were calculated over the study duration by log-binomial regression using months (30.4 days) between study start and participant recruitment, coded as a single time variable, allowing an estimate of prevalence ratio per month. Comparisons between surveys included estimates of PR per survey. Potential confounders were identified by testing the association between variables and included in the multivariable models when $p<0.1$. Adjusted prevalence ratios (aPR) were calculated using log-binomial regression. Confidence intervals are binomial exact. Statistical significance was inferred from two-sided $p<0{\cdot}05$. Statistical analyses were completed using Stata 13.1 (StataCorp, College Station, TX, USA).

Development of non-linear regression analysis for decay rate in VT carriage

To better understand the rate at which VT carriage prevalence was decreasing, we performed non-linear regression analysis. Using empirical study data from children 3–10 years of age, a non-linear model was developed to describe the variation in risk of VT carriage with age, adjusted for baseline characteristics (crowding, number of children <5 years old in the household, gender, socioeconomic status [SES], time since vaccination [i.e. time between first dose PCV13 and date of recruitment], and date of recruitment). The analysis was left-censored at age 6 months. The analysis did not include a seasonality effect because no significant effect was detected. The population-level half-life (i.e. time in years for the carriage in the sampled cohort to reduce to one-half of its peak) was $\log(2)/\delta$), where δ = rate of decay of VT carriage prevalence with age. Model parameters were estimated by maximum likelihood, and hypothesis tests were conducted using generalized likelihood ratio tests. This analysis used R open-source software (www.r-project.org). Details of the analysis framework are in appendix 1.

Role of the funding source

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

RESULTS

Between June 19, 2015 and April 12, 2017, four cross-sectional surveys were completed: survey 1 from June–August, 2015; survey 2 from October, 2015–April, 2016; survey 3 from May–October, 2016; and survey November, 2016–April, 2017. 3430 individuals were screened (figure 1), including 1435 children 3–6yrs old (PCV13 vaccinated), 966 children 5–

10yrs old (PCV13 unvaccinated), and 1029 HIV-infected adults 18–40yrs old and on ART (PCV13 unvaccinated). Among these, 49 (3·4%) children age-eligible for PCV13, 74 (7·7%) children age-ineligible for PCV13, and 41 (4·0%) adults were excluded (figure 1). Ten participants (seven children, three adults) did not allow a swab to be collected. The final analysis included 3256 participants: 1382 children 3–6yrs (vaccinated), 889 children 5–10yrs (unvaccinated), and 985 HIV-infected adults on ART. Among the children, 1483 were recruited from households and 788 from schools.

Demographics and vaccination history

The three surveyed groups had similar demographics (Table 1). However, a higher proportion of children 3–6yrs (vaccinated) lived in houses with some lower infrastructure standards (walls, floors, and latrine facilities), relied more on shared communal water sources, and scored lower on the aggregate index of household possessions. Among those screened and age-eligible for PCV13 vaccination, 1424 (99%) reported being PCV13-vaccinated.

Among the 1386 PCV13-vaccinated children recruited and providing an NPS, 648 (46·8%) had documented (health passport) vaccination status and dates of vaccination; median (IQR) ages at first, second, and third dose of PCV13 were 6·7 (3·7), 11·6 (5·3), and 17·0 (8·5) weeks, respectively. Among those with health passports confirming dates of vaccination, 614 (94·8%) received three doses PCV13, 12 (1·8%) only two doses and 22 (3·4%) only one dose.

Table 1: Demographic and household characteristics of child and adult participants

	Children 3-6 years PCV13-vaccinated (n=1382)	Children 5-10 years PCV13-unvaccinated (n=889)	Adults 18-40 years PCV13-unvaccinated HIV-infected on ART (n=985)
Demographics			
Age, median, (SD)	4·1 (0·70)	8·1 (1·6)	32·7 (5·6)
[range]	[3·0-6·3]	[5·0-10·9]	[18·0-40·9]
Gender, male % (n)	703 (50·9)	468 (52·6)	294 (29·9) [1]
Household/crowding			
Crowding index[2], mean (median)	2·7 (2·5)	3·0 (2·5)	2·2 (2·0)
Smoker in household[3]			
Yes, % (n)	40 (2·9)	50 (5·6)	16 (1·6)
House structure, % (n)			
Walls			
Burnt brick & concrete	498 (36·0)	587 (66·0)	666 (67·6)
Unburnt brick	884 (64·0)	297 (33·4)	183 (18·6)
Mud, thick/thin	0 (0)	5 (0·6)	136 (13·8)
Floor			
Tiles	4 (0·3)	3 (0·35)	11 (1·1)
Concrete	1151 (83·3)	816 (91·8)	879 (89·3)
Mud	227 (16·4)	70 (7·9)	95 (9·6)
Latrine			
Water toilet	30 (2·2)	157 (17·7)	155 (15·8)
Simple pit latrine	1338 (96·8)	727 (81·8)	828 (84·2)
Other	14 (1·0)	5 (0·5)	2 (0·2)
Water			
Tap to house	134 (9·7)	285 (32·1)	340 (34·5)
Shared communal tap	1242 (89·9)	565 (63·5)	507 (51·5)
Bore hole	6 (0·4)	29 (3·3)	109 (11·1)
Well (covered or open)	0	10 (1·2)	29 (3·0)
Durable items possessions[4]			
Possessions index, mean (SD)	6·8 (3·2)	8·2 (3·3)	8.2 (3·3)

ART=antiretroviral therapy. SD=standard deviation.
[1]The gender distribution among adults recruited from ART Clinic is representative of the gender distribution among those attending the clinic.
[2]Crowding index: Calculated as number of persons residing in main house divided by number of bedrooms in main house
[3]Smoker in household: reports the percentage of households with at least one household member who smokes tobacco
[4]Possession index: calculated as a sum of positive responses for household ownership of each of fifteen different functioning items: watch, radio, bank account, iron (charcoal), sewing machine (electric), mobile phone, CD player, fan (electric), bednet, mattress, bed, bicycle, motorcycle, car, television

Pneumococcal carriage

The aggregated (survey 1 through survey 4) prevalences of VT and NVT carriage were, respectively, 20·1% (95% CI 18·0–22·3) and 55·9% (95% CI 53·3–58·6) among children 3–6yrs (vaccinated), 20·1% (95% CI 17·5–22·9) and 36·8% (95% CI 33·6–40·0) among children

5–10yrs (unvaccinated), and 13·9% (95% CI 11·8–16·2) and 29·5% (95% CI: 26·7, 32·5) among HIV-infected adults on ART. All 13 VTs were identified in each of the study groups, with serotype 3 the predominant VT in each (figure 2; appendix 2). The proportion of VT carriage in each age group accounted for by serotype 1 (a common cause of IPD in Africa[37,38]) were 2·9%, 8·4%, and 5·1%, respectively.

Among children 3–6yrs (vaccinated), there was a 26·7% relative reduction in VT carriage, from 23·2% (95% CI 17·9–27·1) in survey 1 to 17·0% (95% CI 13·4–21·2) in survey 4 (figure 3; table 2). When adjusted for age at recruitment, the aPR over the study duration was 0·75 (95% CI 0·56–1·01; p=0·062) (see also appendix 3). A sensitivity analysis for this age group showed neither the overall VT prevalence nor the VT distribution changed significantly when limiting these analyses to children i) who received only one, only two, or all three doses PCV13; ii) with document-confirmed PCV13 vaccination or iii) who adhered to the vaccination schedule to within 2 weeks of each scheduled dose (data not shown). Among children 3–6yrs (vaccinated), there was an 18·7% relative reduction in NVT carriage during the study period, from 61·1% (95% CI 55·7–66·4) to 49·7% (95% CI 44·6–54·9) (aPR: 0·84, 95% CI 0·74–0·96); p=0·011).

Among children 5–10yrs (unvaccinated), there was a 44·4% relative reduction in VT carriage, from 26·6% (95% CI 21·1–32·6) to 14·8% (95% CI 10·1–20·6) over the 2 years (aPR 0·65, 95% CI 0·44–0·98; p=0·039). In the same age group there was a 43·4% relative reduction in NVT carriage, from 41·5% (95% CI 35·2–48·0) to 23·5% (95% CI 17·7–30·0) (aPR 0·62, 95% CI 0·46–0·83; p=0·001).

Among HIV-infected adults on ART, VT prevalence remained largely unchanged, with a 2·8% relative reduction, from 14·1% (95% CI 9·6–19·8) to 13·7% (95% CI 10·0–18·0) (aPR 0·92, 95% CI 0·59–1·44; p=0·72). There was a non-statistically significant 15·8% relative increase

in NVT carriage, from 25·3% (95% CI 19·3–31·9) to 29·3% (95% CI 24·3–34·8) (aPR 1·15, 95% CI 0·85–1·54; p=0·36).

Table 2: Vaccine and non-vaccine serotype *S. pneumoniae* prevalence in each study group per survey with prevalence ratios and relative change

Children 3–6 years (vaccinated)	**Survey 1 (n=332)**	**Survey 2 (n=306)**	**Survey 3 (n=362)**	**Survey 4 (n=382)**	**Total (n=1382)**		**cPR¥ (95% CI) p-value Survey 1 thru Survey 4**	**aPR¥ (95% CI) p-value Survey 1 thru Survey 4**	**Relative change in carriage prevalence**
No carriage									
% (n)	15·7 (52)	23·9 (73)	21·8 (79)	33·3 (127)	24·0 (331)				
95% CI	11·9–20·0	19·2–29·0	17·7–26·4	28·5–38·2	21·7–26·3				
VT									
% (n)	23·2 (77)	20·9 (64)	19·9 (72)	17·0 (65)	20·1 (278)		0·73 (0·55, 0·99)	0·75 (0·56, 1·01)	−26·7%
95% CI	17·9–27·1	16·5–25·9	15·9–24·4	13·4–21·2	18·0–22·3		0·040	p=0·062	
NVT									
% (n)	61·1 (203)	55·2 (169)	58·3 (211)	49·7 (190)	55·9 (773)		0·81 (0·71, 0·93)	0·84 (0·74, 0·96)	−18·7%
95% CI	55·7–66·4	49·5–60·9	53·0–63·4	44·6–54·9	53·3–58·6		0·002	0·011	
Children 5–10 years (unvaccinated)	**Survey 1 (n=241)**	**Survey 2 (n=224)**	**Survey 3 (n=228)**	**Survey 4 (n=196)**	**Total (n=889)**				
No carriage									
% (n)	32·0 (77)	38·4 (86)	43·4 (99)	61·7 (121)	43·1 (383)				
95% CI	26·1–38·2	32·0–45·1	36·9–50·1	54·5–68·6	39·8–46·4				
VT									
% (n)	26·6 (64)	20·5 (46)	17·5 (40)	14·8 (29)	20·1 (179)		0·55 (0·37, 0·83)	0·65 (0·44, 0·98)	−44·4%
95% CI	21·1–32·6	15·4–26·4	12·8–23·1	10·1–20·6	17·5–22·9		p=0·004	p=0·039	
NVT									
% (n)	41·5 (100)	41·1 (92)	39·0 (89)	23·5 (46)	36·8 (327)		0·57 (0·42, 0·76)	0·62 (0·46, 0·83)	−43·4%
95% CI	35·2–48·0	34·6–47·8	32·7–45·7	17·7–30·0	33·6–40·0		<0·000	0·001	
Adults 18-40 years	**Survey 1 (n=198)**	**Survey 2 (n=201)**	**Survey 3 (n=279)**	**Survey 4 (n=307)**	**Total (n=985)**				
No carriage									
% (n)	60·6 (120)	52·7 (106)	55·9 (156)	57·0 (175)	56·6 (557)				
95% CI	53·4–67·5	45·6–59·8	49·9–61·8	51·3–62·6	53·4–59·7				
VT									
% (n)	14·1 (28)	14·4 (29)	13·6 (38)	13·7 (42)	13·9 (137)		0·97 (0·62, 1·51)	0·92 (0·59, 1·44)	−2·8%
95% CI	9·6–19·8	9·9–20·1	9·8–18·2	10·0–18·0	11·8–16·2		0·88	0·72	
NVT									
% (n)	25·3 (50)	32·8 (66)	30·5 (85)	29·3 (90)	29·5 (291)		1·16 (0·86, 1·56)	1·15 (0·85, 1·54)	+15·8%
95% CI	19·3–31·9	26·4–39·8	25·1–36·2	24·3–34·8	26·7–32·5		0·32	0·36	

cPR=crude prevalence ratio. aPR= adjusted prevalence ratio. CI=confidence interval. VT=vaccine serotype. NVT=non-vaccine serotype. n=total number recruited.

¥Carriage prevalence ratios (PR) were calculated over the study duration by log-binomial regression using months (30·4 days) between study start and participant recruitment, coded as a single time variable, allowing an estimate of prevalence ratio per month.

Reduction in risk of VT carriage with age (VT carriage decay rate)

Using non-linear regression analysis to investigate the risk of VT carriage by age among children 0·5–11 years old, the probability of VT carriage was found to decline with age (figure 4). The population-averaged immediate effect of vaccination reduced VT carriage prevalence to an estimated fraction, β=0·52 (95% CI 0·33–0·71) at 6 months of age, compared to the pre-vaccination baseline. Thereafter, the rate of decay in VT carriage translates to an estimated (decay) half-life of 5·3 years (95% CI 3·2–9·0), irrespective of vaccination status. Baseline characteristics describing gender, household crowding, and SES did not show a significant association with probability of carriage. The effect of calendar time on the probability of being a VT carrier did not reach the conventional 5% level of significance (odds ratio [OR] 0·68, 95% CI 0·45–1·02; p=0·06). Nevertheless, we included calendar time in the analysis because excluding it led to numerical instability. Assessment of the goodness-of-fit indicates a good fit, with no discernible relationship between the residual and the predicted values and the range of residuals compatible with the theoretical mean and the standard deviations of 0 and 1, respectively. (appendix 1)

Discussion

In this community-based assessment of pneumococcal carriage we surveyed potential reservoir populations between 4 and 6 years after the routine introduction of PCV13 in Malawi. We found only a modest decline in VT carriage in vaccinated children 3–6yrs (26·7%). The residual VT carriage prevalence (17%) among these children was lower than that previously observed among 1–4 year olds in northern Malawi before vaccine introduction (28·2%),[11] but did not reach the levels reported in high-income low carriage prevalence settings (<5%) that have been associated with control of carriage and transmission.[22–24] We found a more marked decline among unvaccinated age-ineligible children 5–10yrs (44·4%), and no significant change among HIV-infected adults on ART. All 13 VTs were found among the three study groups, despite high vaccine uptake and good adherence to the three-dose schedule among vaccine-eligible children. In the light of the recent WHO Technical Expert Consultation Report

on Optimization of PCV Impact,[39] these data start to address the paucity of information on the long-term impact of the widely implemented 3+0 vaccine schedules on serotype-specific disease and carriage. These findings also highlight the critical need for surveillance post-vaccine introduction in high-burden resource-poor countries.

To achieve herd protection in settings with high carriage prevalences, such as Malawi, we need to effectively interrupt person-to-person transmission. In Finland, a microsimulation model suggested that the transmission potential of pneumococcal carriage is moderate, predicting the elimination of VT type carriage among those vaccinated within 5–10 years of PCV introduction, with high (90%) coverage of vaccination and moderate (50%) vaccine efficacy against acquisition of carriage.[40] Our non-linear statistical analysis shows that the probability of VT carriage beyond 6 months of age in Malawi decreases, with a half-life of approximately 5·3 years, independently of vaccination status. This suggests that much of the vaccine-induced protection against carriage occurs in the first 6 months of life. We postulate that in older vaccinated and unvaccinated children, the reductions in carriage prevalence predicted in the statistical model are due to the indirect benefits of vaccination augmented by naturally acquired immunity to subcapsular protein antigens.[41,42] Thus, the impact of vaccine predicted by transmission models from countries with low carriage prevalences may not translate to settings with high carriage prevalences. Although it has previously been assumed that PCVs would eliminate VT carriage in mature PCV programmes,[43] our data bring into question the potential for either a sustained direct or indirect effect on carriage using the current 3+0 strategy. This schedule has been widely rolled out across high-pneumococcal-carriage-prevalence and high-disease-burden sub-Saharan African countries.

In Malawi, the vaccine impact on carriage prevalence has been less than that observed in Kenya, The Gambia and South Africa which have used different vaccination strategies. Kenya reported a reduction from 34% to 9% VT carriage among PCV-vaccinated children under 5 years of age, 6 years after introduction of 10-valent PCV.[19] The Gambia reported a reduction

from 50% to 13% VT carriage among children 2–5 years old, 20 months after introducing the 7-valent PCV.[44] Likewise, a study from South Africa showed a decrease of PCV13-serotype colonisation from 37% to 13% within 1 year of transitioning from PCV7 to PCV13.[45] However, none of these countries has achieved the low carriage rates seen in Europe and North America within 2 to 3 years of vaccine introduction.[3,46] We propose that the high force of infection (FOI) in Malawi and other similar settings limits a 3+0 schedule to achieving only a short duration of VT carriage control in infants. While a 2+1 schedule, as deployed in South Africa, may improve colonisation control, this is as yet unproven in other parts of Africa. Given the likely importance of an early reduction in transmission intensity to maintain a reduced carriage prevalence, a catch-up-campaign with a broader age range (ie, <2 years or <5 years of age) may also be required. Although the Global Alliance for Vaccines and Immunization (GAVI) has considerably reduced PCV costs for low-income countries,[47,48] it is also important for financial sustainability that vaccine impact be optimised (particularly the indirect effects). Indeed, the FOI and the determinants of transmission between and within age groups need to be considered as new approaches to improving vaccine-induced carriage reduction are proposed and tested.

Unlike what has been observed in low-transmission settings,[49] as well as The Gambia[25] and South Africa,[45] we observed an unexplained decrease in NVT carriage among children in Malawi. We implemented a rigorous quality assurance programme, including routine onsite supportive supervision and repeated rigorous retraining of field staff, to avoid surveillance fatigue. It is possible that serotype replacement and redistribution had already occurred before the start of this study, and that as part of a stochastic secular trend, we are now observing an overall decrease in pneumococcal carriage prevalence which may be sustained or may reverse. It is plausible that overall improvement in living conditions (improved nutrition, sanitation and disease control efforts, improved socioeconomic status) and improvements in health care (antiretroviral roll-out, improved measles and rotavirus vaccination) have resulted in a sustained overall drop in pneumococcal carriage as a result of improved health, evidenced

by falling under 5 mortality in recent years.[50] However, this decrease in overall prevalence has not been observed in countries with transitioning economies such as Kenya and South Africa. Either way, the importance of these trends in NVT carriage will become clearer as the trends in NVT invasive disease become available from these different settings.

We have previously shown incomplete pneumococcal protein antigen-specific reconstitution of natural immunity and high levels of pneumococcal colonisation in HIV-infected Malawian adults on ART.[33] We now show that this adult population has not benefitted from indirect protection against carriage following routine infant PVC13 introduction and indeed may represent a reservoir of VT carriage and transmission. Previous studies in Malawi and South Africa have suggested that despite a higher risk of VT pneumococcal colonisation among HIV-infected women, they are still unlikely to be a significant source of transmission to their children.[14,51] However, in the context of routine infant PCV13 and rapid waning of vaccine-induced immunity, the balance of transmission may now be different. Given the higher risk of IPD, ongoing burden of pneumococcal pneumonia,[52,53] and the evidence that PCV protects HIV-infected adults from recurrent VT pneumococcal infections,[54] targeted vaccination benefitting this at-risk population may help reduce overall carriage and disease prevalence.

Limitations

This work provides a robust community-based estimate of VT and NVT pneumococcal carriage in Blantyre. The study was conducted over a relatively short timeframe for understanding long-term temporal trends. For this reason, the statistical analysis is limited in its ability to disentangle the effects of calendar time and age-since-vaccination, given the small overlap in ages of vaccinated and unvaccinated children in our data. The analysis is also limited in that including calendar time in the model adjusts for, but does not explain, a secular trend in carriage. Rather, time acts as a proxy for unmeasured risk factors that themselves would show secular trends, leading to a reduction in the residual variance and, consequently, more efficient parameter estimation. In the context of high adult HIV seroprevalence (~10%),[55]

the small number of children excluded because they were reported to be HIV-infected could have been due to misclassification, and this could have introduced bias. However, the 2011 adoption of Option B+ in Malawi, whereby all HIV-positive pregnant or breastfeeding women commence lifelong ART regardless of clinical or immunological stage, has dramatically reduced mother-to-child-transmission.[56] Although there are pre-vaccine-introduction data from elsewhere in Malawi, there are no equivalent historical carriage data for urban Blantyre using the same sampling frame. However, this does not detract from the finding of high levels of residual VT carriage in these reservoir populations. Finally, given evidence that more sensitive serotyping methods that detect multiple serotype carriage (e.g. DNA microarray) will increase VT carriage estimates,[57–59] our carriage prevalence data likely underestimate the true residual VT prevalence levels.

CONCLUSION

Despite success in achieving direct protection of infants against disease, a 3+0 PCV13 schedule in Malawi has not achieved the low universal VT carriage prevalence reported in high-income settings that is required to control carriage and transmission. We propose that although vaccine-induced immunity reduces the risk of VT carriage in children up to approximately 6 months of age, in the context of a high residual FOI, this impact is limited by rapid waning of vaccine-induced mucosal immunity and pneumococcal recolonisation (figure 5). Furthermore, we suggest that carriage reduction observed after 6 months of age largely relies on indirect vaccine protection and naturally-acquired immunity. Therefore, alternative schedules and vaccine introduction approaches in high pneumococcal carriage, high-disease-burden countries should be revisited through robust evaluation rather than through programmatic change without supporting evidence.

Authors' contributions

TDS, NBZ, DE, NF, and RSH designed the study. All contributed to the development or design of methodology. TDS, NF and RHS oversaw the study, data collection, and data management.

CF and PD developed the statistical regression analysis. TDS, NF, and RSH conducted the statistical analysis. TDS, NF, and RSH wrote the first draft of the paper, and all authors contributed to subsequent drafts. All read and approved the final version of the report.

Declaration of interests

Dr. Bar-Zeev reports investigator-initiated research grants from GlaxoSmithKline Biologicals and from Takeda Pharmaceuticals outside the submitted work. No other competing interests were reported by authors.

Acknowledgements

We thank the individuals who participated in this study and the local schools and authorities for their support. We are grateful to the study field teams (supported by Farouck Bonomali and Roseline Nyirenda) and the study laboratory team. We are grateful to the MLW laboratory management team (led by Brigitte Denis) and the MLW data management team (led by Clemens Masesa).

References

1 O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* 2009; **374**(9693): 893–902.

2 Simell B, Auranen K, Kayhty H, et al. The fundamental link between pneumococcal carriage and disease. *Expert review of vaccines* 2012; **11**(7): 841–55.

3 Flasche S, Van Hoek AJ, Sheasby E, et al. Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. *PLoS medicine* 2011; **8**(4): e1001017.

4 Waight PA, Andrews NJ, Ladhani NJ, Sheppard CL, Slack MP, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. *The Lancet Infectious diseases* 2015; **15**(6): 629.

5 Moore MR, Link-Gelles R, Schaffner W, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. *The Lancet Infectious diseases* 2015; **15**(3): 301–9.

6 Jayasinghe S, Menzies R, Chiu C, et al. Long-term Impact of a "3 + 0" Schedule for 7- and 13-Valent Pneumococcal Conjugate Vaccines on Invasive Pneumococcal Disease in Australia, 2002-2014. *Clinical infectious diseases* 2017; **64**(2): 175–83.

7 Melegaro A, Edmunds WJ. Cost-effectiveness analysis of pneumococcal conjugate vaccination in England and Wales. *Vaccine* 2004; **22**(31-32): 4203–14.

8 Kobayashi M, Conklin LM, Bigogo G, et al. Pneumococcal carriage and antibiotic susceptibility patterns from two cross-sectional colonization surveys among children aged <5 years prior to the introduction of 10-valent pneumococcal conjugate vaccine - Kenya, 2009-2010. *BMC infectious diseases* 2017; **17**(1): 25.

9 Hill PC, Cheung YB, Akisanya A, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian infants: a longitudinal study. *Clin Infect Dis* 2008; **46**(6): 807–14.

10 Verani JR, Massora S, Acácio S, et al. Nasopharyngeal carriage of Streptococcus pneumoniae among HIV-infected and -uninfected children <5 years of age before introduction of pneumococcal conjugate vaccine in Mozambique. *PLoS ONE* 2018; **13**(2): e0191113.

11 Heinsbroek E, Alaerts M, Phiri A, et al. Pneumococcal carriage in households in Karonga District Malawi, before and after introduction of pneumococcal conjugate vaccination. European Society for Paediatric Infectious Diseases. Brighton, UK; 2016

12 Usuf E, Badji H, Bojang A et al. Pneumococcal carriage in rural Gambia prior to the introduction of pneumococcal conjugate vaccine: a populationbased survey. *Trop Med Int Health* 2015; **20**(7) 871–9

13 Nunes MC, Shiri T, van Niekerk N, et al. Acquisition of Streptococcus pneumoniae in pneumococcal conjugate vaccine-naive South African children and their mothers. Pediatr Infect Dis J 2013;32:e192–205

14 Heinsbroek E, Tafatatha T, Chisambo C, et al. Pneumococcal Acquisition Among Infants Exposed to HIV in Rural Malawi: A Longitudinal Household Study. *American journal of epidemiology* 2016; **183**(1): 70–8.

15 Cutts FT, Zaman SM, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005; **365**(9465): 1139–46.

16 von Gottberg A, de Gouveia L, Tempia S, et al. Effects of vaccination on invasive pneumococcal disease in South Africa. *The New England journal of medicine* 2014; **371**(20): 1889–99.

17 Mackenzie GA, Hill PC, Jeffries DJ, et al. Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a

population-based surveillance study. *The Lancet Infectious diseases* 2016; **16**(6): 703–11.
18 Roca A, Bojang A, Bottomley C, et al. Effect on nasopharyngeal pneumococcal carriage of replacing PCV7 with PCV13 in the Expanded Programme of Immunization in The Gambia. *Vaccine* 2015; **33**(51): 7144–51.
19 Hammitt LL, Etyang AO, Morpeth SC, et al. Impact of 10-valent pneumococcal conjugate vaccine on invasive pneumococcal disease and nasopharyngeal carriage in Kenya. bioRxiv (2018); doi: https://doi.org/10.1101/369876
20 Adebanjo T, Lessa FC, Mucavele H, et al. Pneumococcal carriage and serotype distribution among children with and without pneumonia in Mozambique, 2014-2016. PLoS ONE 2018; **13**(6): e0199363
21 Nunes MC, Jones SA, Groome MJ, et al. Acquisition of *Streptococcus pneumoniae* in South African children vaccinated with 7-valent pneumococcal conjugate vaccine at 6, 14 and 40 weeks of age. *Vaccine* 2015; **33**(5): 628–34.
22 Southern J, Andrews N, Sandu P, et al. Pneumococcal carriage in children and their household contacts six years after introduction of the 13-valent pneumococcal conjugate vaccine in England. *PLoS ONE* 2018; **13**(5): e0195799.
23 Spijkerman J, van Gils EJM, Veenhoven RH, et al. Carriage of *Streptococcus pneumoniae* 3 Years after Start of Vaccination Program, the Netherlands. *Emerging Infectious Diseases* 2011*;* **17**(4)
24 Huang SS, Hinrichsen VL, Stevenson AE, et al. Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics* 2009; **124**:e1–11.
25 Kwambana-Adams B, Hanson B, Worwui A, et al. Rapid replacement by non-vaccine pneumococcal serotypes may mitigate the impact of the pneumococcal conjugate vaccine on nasopharyngeal bacterial ecology. *Sci Rep* 2017; **7**(1): 8127.
26 Pneumococcal Vaccines WHO Position Paper-2012. *Wkly Epidemiol Rec* 2012; **87**(14): 129–44.
27 Mvula H, Heinsbroek E, Chihana M, et al. 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; **11**(5): e0154997.
28 Tsega A, Hausi H, Chriwa G, Steinglass R, Smith D, Valle M. Vaccination coverage and timely vaccination with valid doses in Malawi. *Vaccine Reports* 2016; **6**: 8–12
29 UNICEF. Malawi: WHO and UNICEF estimates of immunization coverage: 2016 revision Geneva: World Health Organization, 2016. https://data.unicef.org/wpcontent/uploads/country_profiles/Malawi/immunization_country_profiles/immunization_mwi.pdf (accessed 30 June, 2018).
30 McCollum ED, Nambiar B, Deula R, et al. Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Clinical and Hypoxemic Childhood Pneumonia over Three Years in Central Malawi: An Observational Study. *PloS one* 2017; **12**(1): e0168209.
31 Bar-Zeev N, Swarthout TD, Alaerts M, et al. Direct and indirect impact of 13v-pneumococcal conjugate vaccine in invasive pneumococcal disease in Blantyre, Malawi. International Symposium on Pneumococci and Pneumococcal Disease 2018; Melbourne, Australia; 2018; ISPPD-0444.
32 Puumalainen T, Zeta-Capeding MR, Käyhty H, et al. Antibody response to an eleven valent diphtheria- and tetanus-conjugated pneumococcal conjugate vaccine in Filipino infants. *The Pediatric infectious disease journal* 2002; 21: 309-14
33 Glennie SJ, Sepako E, Mzinza D, et al. Impaired CD4 T cell memory response to *Streptococcus pneumoniae* precedes CD4 T cell depletion in HIV-infected Malawian adults. *PloS one* 2011; **6**(9): e25610.
34 Heinsbroek E, Tafatatha T, Phiri A, et al. Persisting high prevalence of pneumococcal carriage among HIV-infected adults receiving antiretroviral therapy in Malawi: a cohort study. *AIDS* 2015; **29**(14): 1837–44.
35 Satzke C, Turner P, Virolainen-Julkunen A, et al. Standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: updated recommendations from

the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* 2013; **32**(1): 165–79.
36 Swarthout TD, Gori A, Bar-Zeev N, et al. Pneumococcal serotyping of Malawi carriage samples by latex agglutination, whole genome sequencing (PneumoCat) and DNA microarray is highly concordant: Which should I choose? International Symposium on Pneumococci and Pneumococcal Disease 2018. Melbourne, Australia; 2018.
37 Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS medicine* 2010: **7**(10).
38 Cornick JE, Tastan Bishop O, Yalcin F, et al. The global distribution and diversity of protein vaccine candidate antigens in the highly virulent Streptococcus pnuemoniae serotype 1. *Vaccine* 2017; **35**(6): 972–80.
39 Andrejko K, Cohen O, Cohen AL, et al. WHO Technical Expert Consulation Report on Optimization of PCV Impact: review of evidence and programmatic considerations to inform policy: World Health Organization, 2017.
40 Nurhonen M, Cheng AC, Auranen K. Pneumococcal transmission and disease in silico: a microsimulation model of the indirect effects of vaccination. *PloS one* 2013; **8**(2): e56079.
41 Pido-Lopez J, Kwok WW, Mitchell TJ, Heyderman RS, Williams NA. Acquisition of Pneumococci Specific Effector and Regulatory Cd4+ T Cells Localising within Human Upper Respiratory-Tract Mucosal Lymphoid Tissue. *PLoS Pathog* 2011; **7**(12): e1002396.
42 Trzciński K, Thompson CM, Srivastava A, Basset A, Malley R, Lipsitch M. Protection against Nasopharyngeal Colonization by Streptococcus pneumoniae Is Mediated by Antigen-Specific CD4 T Cells Infection and immunity. *Infect Immun* 2008;**76**(6): 2678-84.
43 Flasche S, Le Polain de Waroux O, O'Brien KL, Edmunds WJ. The serotype distribution among healthy carriers before vaccination is essential for predicting the impact of pneumococcal conjugate vaccine on invasive disease. *PLoS Comput Biol* 2015; **11**(4): e1004173.
44 Roca A, Hill PC, Townend J, et al. Effects of community-wide vaccination with PCV-7 on pneumococcal nasopharyngeal carriage in the Gambia: a cluster-randomized trial. *PLoS medicine* 2011; **8**(10): e1001107.
45 Nzenze SA, von Gottberg A, Shiri T, et al. Temporal Changes in Pneumococcal Colonization in HIV-infected and HIV-uninfected Mother-Child Pairs Following Transitioning From 7-valent to 13-valent Pneumococcal Conjugate Vaccine, Soweto, South Africa. *The Journal of infectious diseases* 2015; **212**(7): 1082–92.
46 Hammitt LL, Bruden DL, Butler JC, et al. Indirect effect of conjugate vaccine on adult carriage of *Streptococcus pneumoniae*: an explanation of trends in invasive pneumococcal disease. *The Journal of infectious diseases* 2006; **193**(11): 1487–94.
47 Gavi welcomes new record low price for pneumococcal vaccine. Geneva; 2016.
48 Cernuschi T, Furrer E, Schwalbe N, Jones A, Berndt ER, McAdams S. Advance market commitment for pneumococcal vaccines: putting theory into practice. *Bulletin of the World Health Organization* 2011; **89**(12): 913–8.
49 Hanage WP, Finkelstein JA, Huang SS, et al. Evidence that pneumococcal serotype replacement in Massachusetts following conjugate vaccination is now complete. *Epidemics* 2010; **2**(2): 80–4.
50 National Statistics Office, Macro ICF. Malawi demographic & health survey2010. Zomba, Malawi: National Statistics Office, Macro ICF; 2011.
51 Madhi SA, Izu A, Nunes MC, et al. Longitudinal study on *Streptococcus pneumoniae*, Haemophilus influenzae and Staphylococcus aureus nasopharyngeal colonization in HIV-infected and -uninfected infants vaccinated with pneumococcal conjugate vaccine. *Vaccine* 2015; **33**(23): 2662–9.

52 Janoff EN, Breiman RF, Daley CL, Hopewell PC. Pneumococcal disease during HIV infection. Epidemiologic, clinical, and immunologic perspectives. *Ann Intern Med* 1992; **117**(4): 314–24.

53 Heffernan RT, Barrett NL, Gallagher KM, et al. Declining incidence of invasive *Streptococcus pneumoniae* infections among persons with AIDS in an era of highly active antiretroviral therapy, 1995-2000. *The Journal of infectious diseases* 2005; **191**(12): 2038–45.

54 French N, Gordon SB, Mwalukomo T, et al. A Trial of a 7-Valent Pneumococcal Conjugate Vaccine in HIV-Infected Adults. *N Engl J Med* 2010;**362**:812-22.

55 UNAIDS. Country Factsheet, Malawi 2017. Geneva http://www.unaids.org/en/regionscountries/countries/malawi/ (accessed 12 October 2018)

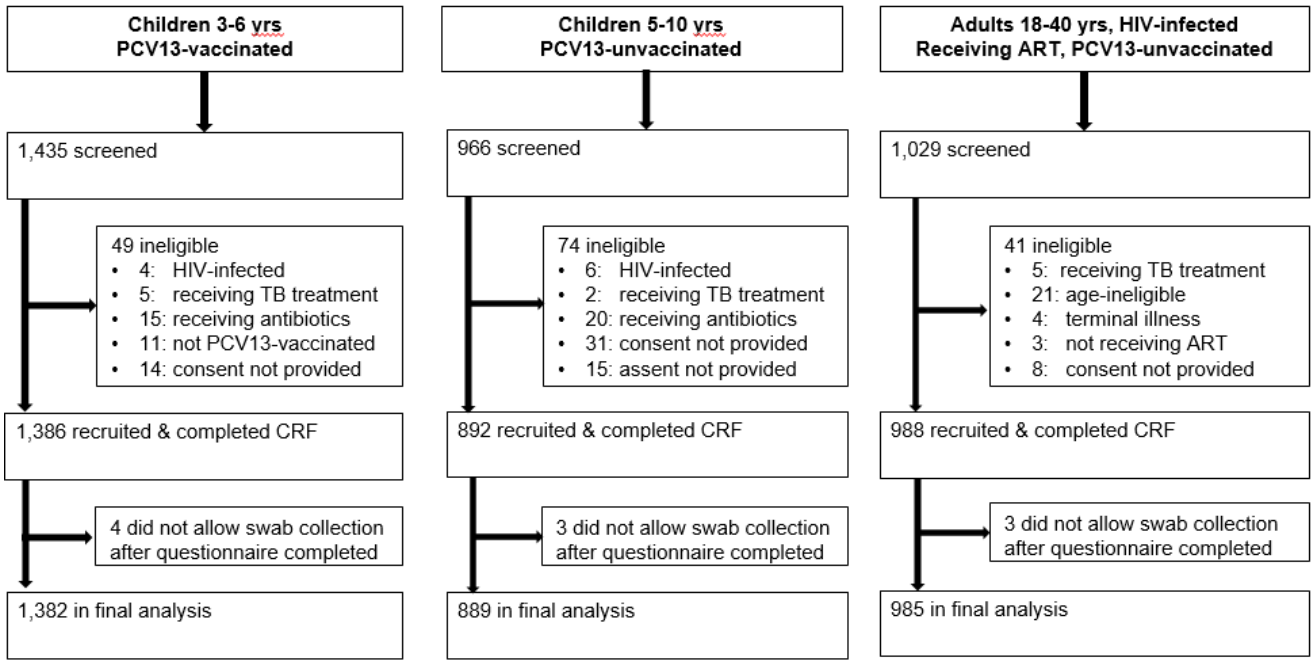
56 UNICEF. Malawi's Option B+ programme is helping to eliminate mother-to-child transmission of HIV. Geneva https://www.unicef.org/infobycountry/malawi_70997.html

57 Swarthout TD, Everett D, Bar-Zeev N, et al. Persisent vaccine-type carriage of *Streptococcus pneumoniae* four years after introducing PCV13 in a 3+0 schedule in Malawi. International Symposium on Pneumococci and Pneumococcal Disease 2016. Glasgow, U.K.; 2016.

58 Turner P, Hinds J, Turner C, et al. Improved detection of nasopharyngeal cocolonization by multiple pneumococcal serotypes by use of latex agglutination or molecular serotyping by microarray. *Journal of clinical microbiology* 2011; **49**(5): 1784–89.

59 Kamng'ona AW, Hinds J, Bar-Zeev N, et al. High multiple carriage and emergence of *Streptococcus pneumoniae* vaccine serotype variants in Malawian children. *BMC infectious diseases* 2015; **15**: 234.

Figure 1. Screening, reasons for exclusion and recruitment



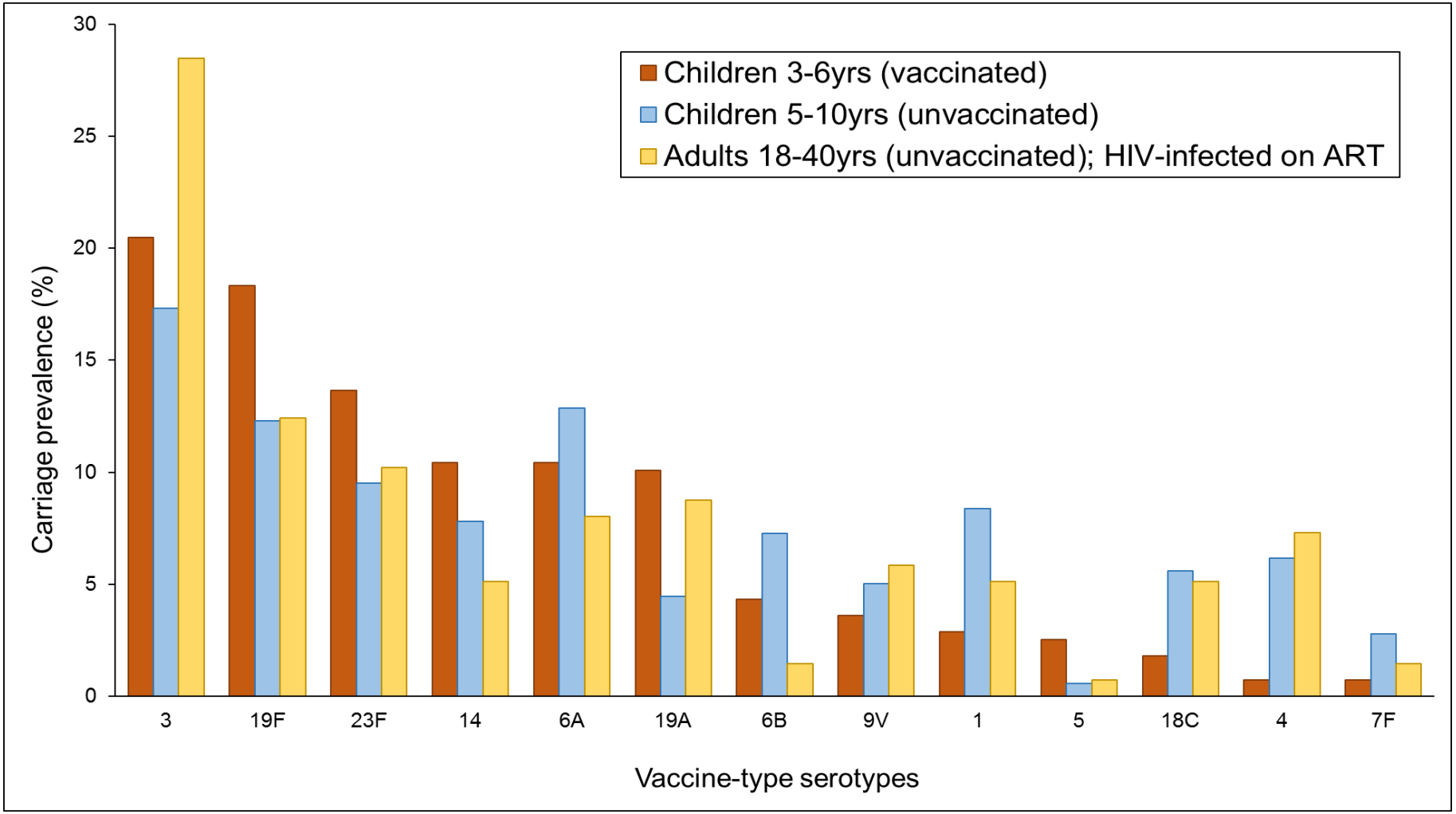


Figure 2. Distribution of vaccine-type pneumococcal carriage across all surveys, stratified by study group
Proportion of vaccine-serotype (VT) carriage attributed to individual vaccine serotypes across all of the surveys, stratified by study group. Serotype 3 was the predominate VT in all strata, representing 20·5%, 17·3%, and 28·5% of VT carriage respectively within children 3–6 years old (vaccinated), children 5–10 years old (unvaccinated) and HIV-infected adults 18-40 years old on ART (unvaccinated).

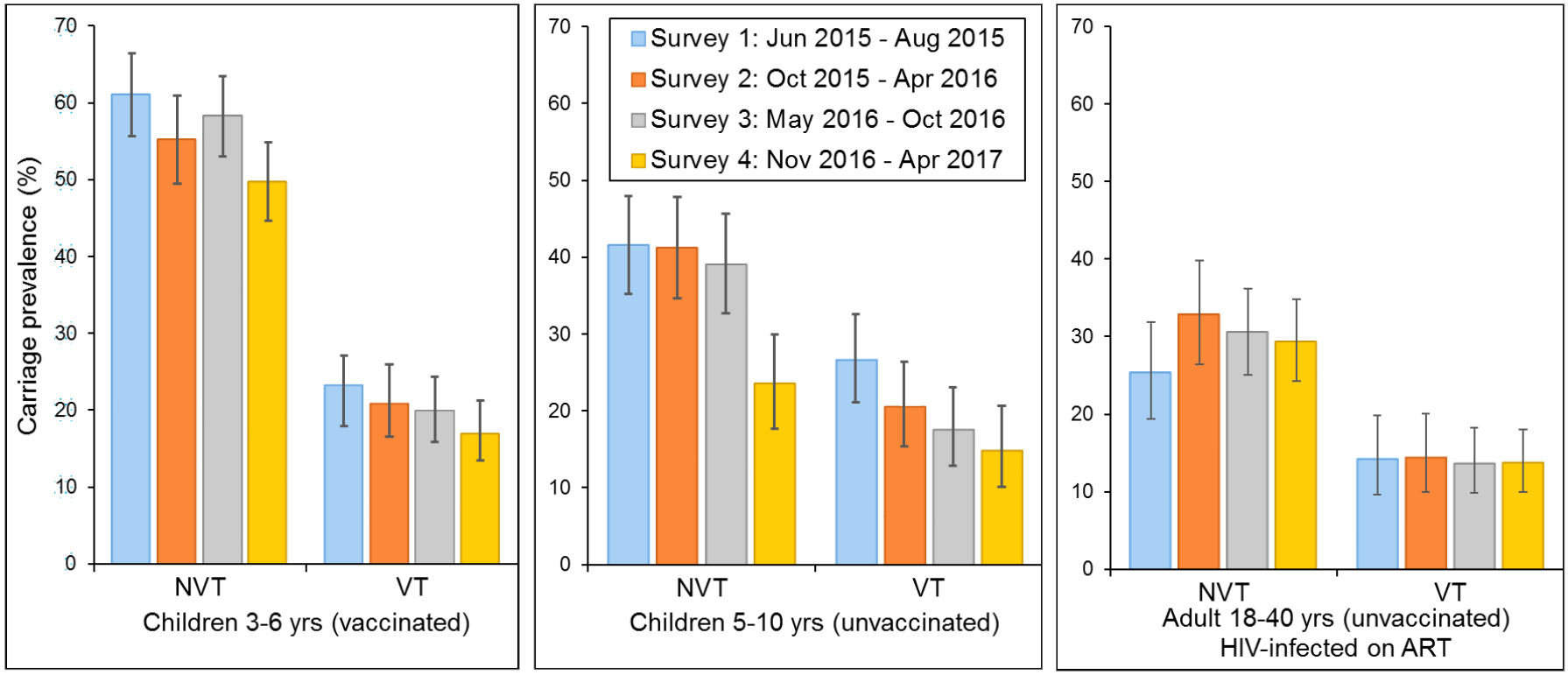


Figure 3. Prevalence of *Streptococcus pneumoniae* carriage per survey, stratified by age and PCV13 vaccination status.
The duration of each survey was 3–5 months, with 2–3 weeks between surveys. 95% confidence interval error bars are shown. Prevalence of non-carriers is calculated by 1−(NVT+VT)**.** Among children 3−6yrs old (vaccinated), there was a modest decrease in VT carriage, from 23·2% (95% CI 17·9–27·1) in survey 1 to 17·0% (95% CI 13·4–21·2) in survey 4. When adjusted for age at recruitment, the adjusted prevalence ratio (aPR) over the study duration was 0·75 (95% CI 0·56–1·01; p=0·062). Among children 5−10yrs old (unvaccinated), VT carriage decreased from 27·4% (95% CI 21·1–32·6) to 14·8% (95% CI 10·1–20·6) over the 2 years (aPR 0·65, 95% CI 0·44–0·98; p=0·039). Among HIV-infected adults on ART, VT prevalence remained unchanged, at 14·1% (95% CI 9·6–19·8) after survey 1 and 13·7% (95% CI 10·0–18·0) after survey-4 (aPR 0·92, 95% CI 0·59–1·44; p=0·72). Refer to appendix 3 for aPR and VT & NVT prevalence stratified by survey.

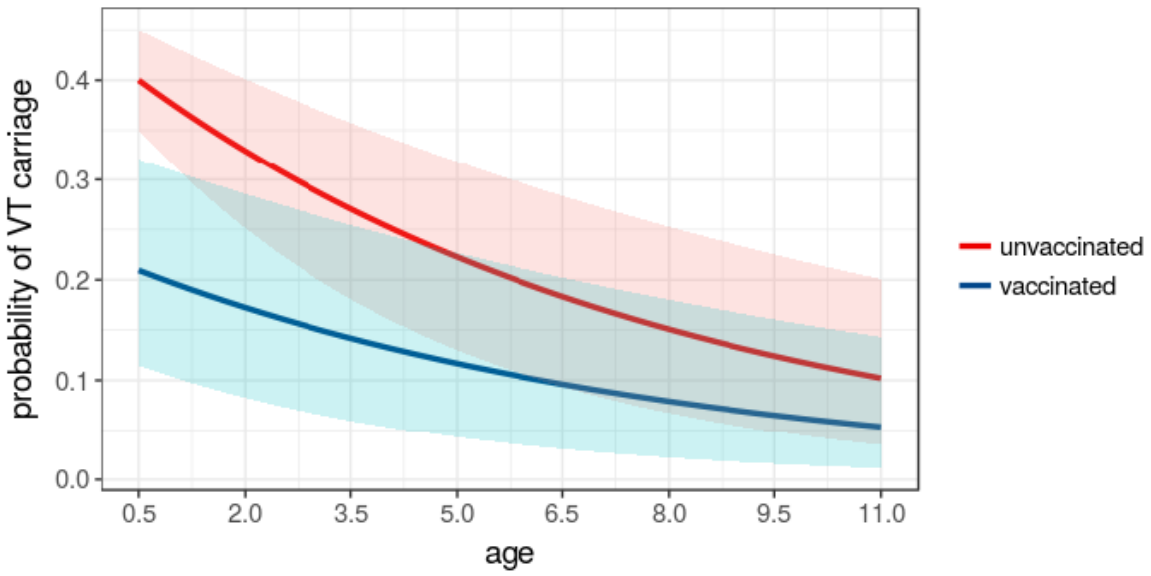


Figure 4. Non-linear modelling of the probability of *S. pneumoniae* vaccine-serotype (VT) carriage risk by age in children.

Estimated individual probabilities (solid lines) and pointwise 95% confidence intervals (shaded regions) of VT carriage as a function of a child's age (years), for an unvaccinated child whose baseline characteristics translate to a VT carriage prevalence of 0·4 at age 6 months (red line) and for a vaccinated child with the same baseline characteristics (blue line). Both fitted lines include extrapolations beyond the range of the empiric data. The population-averaged effect of vaccination reduces VT prevalence to an estimated fraction β=0·52 (95% CI 0·33–0·71) by 6 months of age, compared to the pre-vaccination baseline. This translates to an estimated half-life of 5·3 years (95% CI 3·2–9·0), irrespective of vaccination status. For the non-linear modelling framework, see appendix 2.

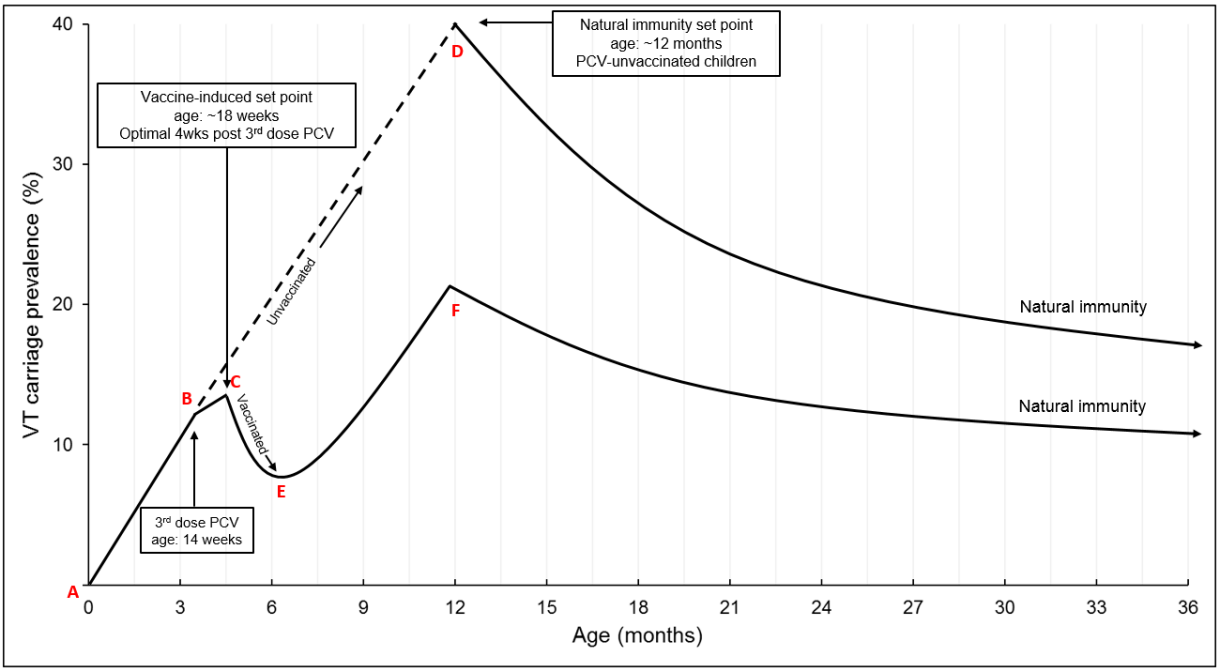


Figure 5: A hypothesis – the role of pneumococcal conjugate vaccine-induced and natural anti-pneumococcal immunity in determining the prevalence of colonisation by *S. pneumoniae* among children in a setting with high carriage prevalence

A: Children born uncolonised with *S. pneumoniae*. **A–B:** Soon after birth, children are colonised with VT and NVT pneumococcus via contact with family and community members. **B:** At 14 weeks of age, vaccine-eligible children have received 3 doses PCV13, with an optimal immunogenic response for vaccine-induced mucosal immunity 4 weeks later (**C**). Among PCV-vaccinated children, 18-weeks is the approximate vaccine-induced set point, with a rapid decrease in VT prevalence (**C–E**) until 6 months of age (**E**). At 6 months of age, there is an increase in risk of VT carriage (**E–F**), driven by increased force of infection in the context of waning vaccine-induced immunity, the former due partly to increased contact with other young children in the household and community. VT carriage prevalence increases until naturally acquired immunity starts to impact on colonisation (**F**), reducing pneumococcal carriage prevalence. Among PCV-unvaccinated children, risk of VT carriage continues to increase largely unchecked (**B–D**) until naturally acquired immunity starts to impact on colonisation (**D**), reducing pneumococcal carriage prevalence. Among these unvaccinated children, 12 months is the approximate set-point induced by naturally-acquired immunity. Indirect vaccine effects will impact on the height of C–F and B–D, as well as the rate of decline in VT carriage prevalence.