



Timing of bacterial carriage sampling in vaccine trials: A modelling study



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ABSTRACT

Background: Pathogenic bacteria are often asymptotically carried in the nasopharynx. Bacterial carriage can be reduced by vaccination and has been used as an alternative endpoint to clinical disease in randomised controlled trials (RCTs). Vaccine efficacy (VE) is usually calculated as 1 minus a measure of effect. Estimates of vaccine efficacy from cross-sectional carriage data collected in RCTs are usually based on prevalence odds ratios (PORs) and prevalence ratios (PRs), but it is unclear when these should be measured.

Methods: We developed dynamic compartmental transmission models simulating RCTs of a vaccine against a carried pathogen to investigate how VE can best be estimated from cross-sectional carriage data, at which time carriage should optimally be assessed, and to which factors this timing is most sensitive. In the models, vaccine could change carriage acquisition and clearance rates (leaky vaccine); values for these effects were explicitly defined (f_{acq} , $1/f_{dur}$). POR and PR were calculated from model outputs. Models differed in infection source: other participants or external sources unaffected by the trial. Simulations using multiple vaccine doses were compared to empirical data.

Results: The combined VE against acquisition and duration calculated using $POR(\widehat{VE}_{acq,dur}, (1 - POR) \times 100)$ best estimates the true VE ($VE_{acq,dur}, (1 - f_{acq} \times f_{dur}) \times 100$) for leaky vaccines in most scenarios. The mean duration of carriage was the most important factor determining the time until $\widehat{VE}_{acq,dur}$ first approximates $VE_{acq,dur}$: if the mean duration of carriage is 1–1.5 months, up to 4 months are needed; if the mean duration is 2–3 months, up to 8 months are needed. Minor differences were seen between models with different infection sources. In RCTs with shorter intervals between vaccine doses it takes longer after the last dose until $\widehat{VE}_{acq,dur}$ approximates $VE_{acq,dur}$.

Conclusion: The timing of sample collection should be considered when interpreting vaccine efficacy against bacterial carriage measured in RCTs.

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1. Introduction

The estimation of vaccine efficacy (VE) from randomised controlled trials (RCTs) is complex because vaccines can act on different stages of the infection dynamics and disease, and because vaccination itself can affect the transmission of disease in the trial

population (Smith et al., 1984; Halloran et al., 1997, 1999). Much has been published about complexities in the estimation of VE against clinical disease (Smith et al., 1984; Halloran and Struchiner, 1995; Halloran et al., 1997, 1999), but less information is available for other outcomes such as asymptomatic colonisation of the nasopharynx by bacterial pathogens. Asymptomatic colonisation, or carriage, is often measured in RCTs of vaccines that aim to prevent disease caused by bacterial pathogens because carriage is a more common outcome than clinical disease and efficacy of vaccine against clinical disease can be mediated through carriage (Simell et al., 2012). Examples of such pathogens and vaccines include *Streptococcus pneumoniae* and pneumococcal conjugate vaccines

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Table 1
Model parameters describing the transmission of the pathogen, and the extent and effect of vaccination.

Parameter	Description	Baseline value	Sensitivity analyses (uni- and multivariate)	Restricted multivariate sensitivity analysis	Sensitivity analysis on steady state assumption	Multiple doses of vaccines	Model validation
		Baseline and constant FOI models	Baseline and constant FOI models	Baseline and constant FOI models	Baseline and constant FOI models	Baseline model	Constant FOI model
<i>Carriage parameters</i>							
$1/\gamma$	Mean duration of carriage in the absence of vaccination (γ , carriage clearance rate)	1.25m ^a	1 day–12m ^b	1 day–12m ^b	1.25m ^a	1.25m ^a	5d, 1.25m 4.3m ^c
P_B	Equilibrium prevalence of carriage in the absence of vaccination	0.25 ^a	0.001–0.99	0.001–0.5 ^d	0.001–0.99	0.25 ^a	VT 0.32 ^e VT + 6A 0.37 ^e
P_{init}	Prevalence at the start of the model run ($P_{init} = P_B$)	Steady state ($P_{init} = P_B$)	Steady state ($P_{init} = P_B$)	Steady state ($P_{init} = P_B$)	0.001–0.99	Steady state ($P_{init} = P_B$)	VT 0.15 ^f VT + 6A 0.18 ^f
β	Transmission parameter, $\gamma/(1-P_B)$	1.07 ^g	Changes with γ and P_B	Changes with γ and P_B	Changes with γ and P_B	Changes with γ and P_B	Changes with γ and P_B
<i>Vaccine parameters</i>							
VE_{acq}	Vaccine efficacy against acquisition of carriage, $(1 - f_{acq}) \times 100$	60%	0–99%	0–99%	60%	See Table 2	See Table 3
VE_{dur}	Vaccine efficacy against duration of carriage, $(1 - f_{dur}) \times 100$	0%	0–99%	0–20% ^h	0%	See Table 2	See Table 3
$VE_{acq,dur}$	Vaccine efficacy against acquisition and duration of carriage $(1 - (f_{acq} \times f_{dur})) \times 100$	60%	Calculated from f_{acq} and f_{dur}	Calculated from f_{acq} and f_{dur}	60%	Calculated from f_{acq} and f_{dur}	Calculated from f_{acq} and f_{dur}
q	Proportion vaccinated in the trial population	0.33	0.01–0.99	0.1–0.6	0.33	0.33	0.33 vaccinated with each schedule

FOI – force of infection; m – months; VT – 7-valent pneumococcal conjugate vaccine serotypes.

^a Based on combined estimate for all 7-valent PCV serotypes in 3–59 month olds in Kenya (Abdullahi et al., 2012). Other studies in Denmark, Finland and the United Kingdom did not provide prevalence or duration of carriage data for 7-valent PCV serotypes separately, but duration of carriage for all serotypes combined or individual serotypes were generally similar or longer than in the Kenya data (Auranen et al., 2000, 2010; Raymond et al., 2001; Melegaro et al., 2004).

^b Range explored in sensitivity analysis reflects a very short duration (1 day) and the longest estimates for the carriage of pneumococcus, *Haemophilus influenzae* type b and *Neisseria meningitidis* (Auranen et al., 1996; Trotter et al., 2006; Abdullahi et al., 2012).

^c Based on data for 7-valent PCV serotypes in 3–59 month olds in Kenya (Abdullahi et al., 2012). The minimum lower bound for the confidence interval around the mean duration of carriage for any vaccine serotype was 5 days, and the maximum upper bound for the confidence interval around the mean duration of carriage for any vaccine serotype was 130 days.

^d Carriage prevalence is generally below 50% although it can be higher in some populations (Simell et al., 2012).

^e Maximum prevalence amongst randomised unvaccinated individuals in the Israeli trial (calculated from individual patient data). These maximums occurred at 12 months of age (Dagan et al., 2012).

^f Prevalence amongst randomised unvaccinated individuals in the Israeli trial at 2 months of age (calculated from individual patient data). In simulations, model runs were started at 2m of age.

^g The baseline value for β is calculated from the baseline values of γ and P_B .

^h Conjugate vaccines have not been observed to have a marked effect on duration of carriage (Barbour et al., 1995; Dagan et al., 2005; O'Brien et al., 2007).

(PCVs) (van Gils et al., 2009; Russell et al., 2010; Ota et al., 2011; Dagan et al., 2012), *Haemophilus influenzae* type B (Hib) and Hib conjugate vaccines (Adegbola et al., 1998), and *Neisseria meningitidis* and meningococcal conjugate vaccines (Daugla et al., 2014).

Vaccines might affect carriage through several mechanisms including reducing susceptibility to acquiring carriage, reducing the duration of carriage, or reducing the density of colonisation (Rinta-Kokko et al., 2009; Mina et al., 2013). In this article, the terms VE_{acq} and VE_{dur} refer to the vaccine efficacy against acquisition and duration respectively, while $VE_{acq,dur}$ captures the combined efficacy against acquisition and duration (Table 1). Some RCTs have attempted to directly estimate VE_{acq} using longitudinal data from repeated nasopharyngeal samples (Dagan et al., 2003, 2012). However, to ensure the detection of each new acquisition and consequently accurately measure the underlying acquisition rate, sampling would need to be more frequent than is feasible in most trials. Instead, carriage is usually assessed cross-sectionally, by sampling once or a few times after vaccination, typically starting one to two months after the last dose (Obaro et al., 2000; Dagan et al., 2003; van Gils et al., 2009; Russell et al., 2010).

Vaccine efficacy is usually estimated using 1 minus a measure of effect that is expressed as a ratio. For cross-sectional carriage

data, possible ratio measures are prevalence odds ratios (PORs) and prevalence ratios (PRs). The estimated VE against acquisition and duration ($\widehat{VE}_{acq,dur}$) in a trial can then be calculated as either $(1 - POR) \times 100$ or $(1 - PR) \times 100$. Previous studies have shown that $\widehat{VE}_{acq,dur}$ calculated using the POR, once stable, can be used to estimate the “true” $VE_{acq,dur}$ (Rinta-Kokko et al., 2009). The time until the POR, and therefore $\widehat{VE}_{acq,dur}$, becomes stable has not been thoroughly investigated (Auranen et al., 2013a) either for when single doses or for when multiple doses of vaccine are given in a vaccine schedule. Previous methods have also assumed that the force of infection (FOI) is constant during trials (Rinta-Kokko et al., 2009; Auranen et al., 2013a,b). The assumption of a constant FOI leads to greater analytical simplicity than allowing the FOI to change over time but it might be an over-simplification as it assumes that vaccination of the trial population has no effect on the FOI.

Many groups including vaccine trial investigators, epidemiologists, mathematical modellers, policy makers and systematic reviewers need to know which ratio measure and sampling points in time can be used to best estimate $VE_{acq,dur}$. We used a dynamic transmission model to investigate how values of $\widehat{VE}_{acq,dur}$ calculated from model outputs compared to the “true” values of $VE_{acq,dur}$ used to parameterise the model. We assessed the optimal time

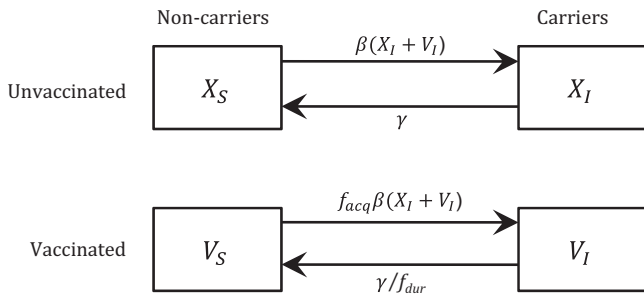


Fig. 1. Baseline model. X_I , X_S , V_I and V_S are the unvaccinated carrying, unvaccinated non-carrying, vaccinated carrying, and vaccinated non-carrying proportions of the trial population respectively. β – transmission parameter; f_{acq} – acquisition rate ratio; f_{dur} – carriage duration ratio; γ – carriage clearance rate.

at which carriage should be measured, and factors to which this timing is most sensitive. We considered different assumptions on transmission, simulated trials with multiple doses of vaccine, and validated findings with empirical data.

2. Methods

2.1. Dynamic transmission model (baseline model)

We developed a dynamic compartmental transmission model that simulates an RCT of a vaccine against a pathogen that can be asymptotically carried in the nasopharynx (Fig. 1). The baseline model represents an unvaccinated group (X) and a group that receives one dose of a vaccine (V). Vaccination occurs at the start of each simulation, with a percentage of participants (q) being assigned to the vaccinated group (V). In both groups, people can either carry the pathogen (I) or are free of carriage (S). X_I , X_S , V_I and V_S represent the unvaccinated carrying, unvaccinated non-carrying, vaccinated carrying, and vaccinated non-carrying proportions of the trial population, N ($N = X_I + X_S + V_I + V_S = 1$). Unvaccinated non-carrying individuals acquire carriage at rate $\beta(X_I + V_I)$, and after infection can clear carriage at a rate of γ , after which individuals become susceptible to acquiring carriage again. The transmission parameter, β , is calculated from the equilibrium prevalence of carriage in the absence of vaccination (P_B) and γ (Appendix A, Section 1.1). In the model, trial participants acquire carriage only from other trial participants, meaning that the FOI changes with prevalence in the trial population. The prevalence of carriage in the trial population is assumed to be in steady state at the start of the trial unless otherwise stated ($P_B = P_{mit}$, where P_{mit} is carriage prevalence at the start of the simulation). The model is described by a set of ordinary differential equations (Appendix A, Section 1.1). The baseline values of the parameters resemble those of serotypes of *S. pneumoniae* included in the 7-valent PCV and are based on empirical data where available (Table 1).

2.2. Vaccine effect

The vaccine was incorporated into the model as a leaky vaccine, meaning that those vaccinated are still susceptible to acquiring carriage but at a lower rate than the unvaccinated. We explored two ways in which a vaccine can provide protection against carriage of a pathogen. First, the vaccine can reduce the rate at which vaccinated individuals acquire carriage. In the model, this is defined by f_{acq} (where $f_{acq} = 1 - (\text{true}VE_{acq}/100)$). f_{acq} is also the acquisition rate ratio: the carriage acquisition rate in the vaccinated divided by the carriage acquisition rate in the unvaccinated. Second, the

vaccine can reduce the mean duration of carriage. This is defined by f_{dur} (where $f_{dur} = 1 - (\text{true}VE_{dur}/100)$). f_{dur} is also the carriage duration ratio: the mean duration of carriage in the vaccinated divided by the mean duration of carriage in the unvaccinated. $VE_{acq,dur}$, the “true” combined effect of the vaccine on acquisition and carriage, is defined as $(1 - f_{acq,dur}) \times 100$ where $f_{acq,dur} = f_{acq} \times f_{dur}$ (Rintakokko et al., 2009). $VE_{acq,dur}$ can be interpreted as the percentage reduction in time spent in the carrier state due to not acquiring carriage, more rapid clearance, or both in the vaccinated group when compared to the unvaccinated group. For simplicity, we do not consider a vaccine effect on the density of colonisation in the model. In all simulations, any effect against acquisition or duration of carriage was assumed to commence immediately after vaccination.

2.3. Analyses

We first simulated one vaccine dose using baseline values of all parameters (Table 1). We calculated the POR and PR at each time step in the simulation from the proportion of the study population in each of the four compartments ($POR = V_I X_S / V_S X_I$, and $PR = (V_I (X_S + X_I)) / ((V_S + V_I) X_I)$). An estimate of $VE_{acq,dur}$ ($\widehat{VE}_{acq,dur}$) was then calculated at each time step as $(1 - POR) \times 100$ or $(1 - PR) \times 100$. We plotted the $\widehat{VE}_{acq,dur}$ against time and examined whether $VE_{acq,dur}$ was more closely estimated by using POR or PR . We determined the time after vaccination at which $\widehat{VE}_{acq,dur}$ first came within an absolute 5% of the baseline value of $VE_{acq,dur}$ (i.e. the time when $\widehat{VE}_{acq,dur}$ reached 55% for the baseline $VE_{acq,dur}$ of 60%). We use the term “first approximates” throughout this article to describe the point in time when $\widehat{VE}_{acq,dur}$ reaches a value that is a set distance below $VE_{acq,dur}$.

To assess whether patterns in $\widehat{VE}_{acq,dur}$ over time depend on the mechanism of vaccine action, we explored three different scenarios. In the first scenario, the vaccine only affected the acquisition rate (controlled by f_{acq}), with f_{acq} set at 0.4 and f_{dur} at 1. This resulted in $f_{acq} \times f_{dur}$ of 0.4 and consequently a $VE_{acq,dur}$ of 60%. In the second scenario, the vaccine had an effect only on the duration of carriage (controlled by f_{dur}), with f_{acq} set to 1 and f_{dur} to 0.4, again resulting in $f_{acq} \times f_{dur}$ of 0.4 and $VE_{acq,dur}$ of 60%. The third scenario examined the combined effect on duration and acquisition. The same values of $f_{acq} \times f_{dur}$ and $VE_{acq,dur}$ were maintained by setting f_{acq} at 0.6 and f_{dur} at 0.67. We explored each of these scenarios for when the reproduction number after vaccination (the number of secondary infections caused by one infected individual introduced into a hypothetical population in which there was previously no carriage) was above 1, where R was exactly 1, and where R was below 1 (Appendix A, Section 3). We changed the value of R by setting P_B to 0.25 (baseline value), 0.2, and 0.15 respectively.

2.4. Sensitivity analysis

We performed an analysis on the sensitivity of the time after vaccination at which $\widehat{VE}_{acq,dur}$ first approximates the $VE_{acq,dur}$ to parameter values. We defined the time until $\widehat{VE}_{acq,dur}$ approximated $VE_{acq,dur}$ to be the time until $\widehat{VE}_{acq,dur}$ first reached a value of $(11/12) \times VE_{acq,dur}$. Analyses were also performed using an alternative definition of when $\widehat{VE}_{acq,dur}$ first came within an absolute 5% of $VE_{acq,dur}$. If $VE_{acq,dur}$ is 60% then the value of $\widehat{VE}_{acq,dur}$ to be reached is 55% for both of these definitions. All sensitivity analyses use $\widehat{VE}_{acq,dur}$ based on POR , unless otherwise stated. First, using the baseline model, we changed the parameters one at a time over broad ranges (Table 1, fourth column). Then we performed multivariate sensitivity analyses by creating 4000 parameter sets through uniform sampling of parameter values over the broad

Table 2
Scenarios of vaccine effects explored for multiple doses of vaccines.

Scenario, trial	Comparison	$VE_{acq, \%}$				$VE_{dur, \%}$
		2m dose	3m dose	4m dose	6m dose	
<i>Scenario 1: Each dose increases the effect on acquisition, and the extent is not affected by the age at which the dose is given</i>						
Trial 1	No doses	0	0	0	–	0
	vs.					
	2, 3, 4m	20	40	60	–	0
Trial 2	No doses	0	–	0	0	0
	vs.					
	2, 4, 6m	20	–	40	60	0
<i>Scenario 2: Third dose has no additional effect, regardless of when given</i>						
Trial 1	No doses	0	0	0	–	0
	vs.					
	2, 3, 4m	30	60	60	–	0
Trial 2	No doses	0	–	0	0	0
	vs.					
	2, 4, 6m	30	–	60	60	0
<i>Scenario 3: First dose has no effect</i>						
Trial 1	No doses	0	0	0	–	0
	vs.					
	2, 3, 4m	0	30	60	–	0
Trial 2	No doses	0	–	0	0	0
	vs.					
	2, 4, 6m	0	–	30	60	0

m – months.

ranges defined in Table 1. We repeated the multivariate sensitivity analysis, restricting parameters to values commonly observed, or most likely given available evidence (Table 1, fifth column).

Second, we explored the possibility that the prevalence of carriage in the trial population is not in steady state at the start of the trial. We did this by first changing the initial prevalence in the trial population (P_{init}) while maintaining the baseline value of the equilibrium prevalence of carriage in the absence of vaccination (P_B). We then explored whether these findings were sensitive to the equilibrium prevalence of carriage by performing the same analysis over a range of values of P_B .

Third, we explored the effect of the assumption in the baseline model of a changing FOI. We simplified the model to the case where the study population acquires carriage only from outside the study population and vaccination in the trial does not affect carriage outside the study population (Appendix A, Section 1.2). These simplifications mean that the model is no longer a dynamic model because the force of infection is constant over time. The model now takes on assumptions similar to those in previous studies (Rinta-Kokko et al., 2009; Auranen et al., 2013a,b). We then performed all analyses and sensitivity analyses described above and compared the results to those from the baseline model.

2.5. Simulated trials with multiple doses of vaccines

To simulate more realistic trial scenarios, we extended the baseline model to include multiple doses of the vaccine (Appendix A, Section 1.3). We varied both the interval between doses and the effect of each dose on $VE_{acq, dur}$. The unvaccinated group remained unchanged. We simulated two separate trials, each comparing a single vaccinated group receiving three vaccine doses to an unvaccinated (or placebo) group. The effect of each dose of vaccine did not differ between trials (Table 2). The trials differed only in the vaccine schedule: 2, 3 and 4 months in trial 1 and 2, 4 and 6 months in trial 2. We explored whether the interval between doses could lead to apparent differences in trial results even when the value of VE_{acq} after the last dose was the same. We used three scenarios for the value of VE_{acq} for each dose (Table 2).

2.6. Model validation: comparison with trial data

We compared patterns in outcome measures produced by the model to empirical trial data. We obtained individual participant data from a 7-valent PCV trial conducted in Israel (Dagan et al., 2012; Givon-Lavi et al., 2010 and additional data from Dagan). We used data from three groups in the trial: a study arm that received 3 doses of the vaccine at 4, 6 and 12 months of age (two primary doses and a booster, a $2p+1$ schedule, “schedule a”), a study arm that received 3 doses of the vaccine at 2, 4 and 6 months (three primary doses and no booster, a $3p+0$ schedule, “schedule b”), and a study arm that received no vaccination until 12 months of age (“no dose” group). Children were enrolled from health centres and were not in close contact with each other. We therefore used the constant FOI model for these simulations. We adapted the model to include the comparison between schedules in the trial by adding a second vaccinated group (Appendix A, Section 1.4). In the model, we examined four scenarios of the effect of vaccine on acquisition (Table 3). The values used for other parameters in these simulations are shown in Table 1. The system was not assumed to be in steady state at the start of simulations. Values of P_{init} were based on prevalence in the trial at 2 months of age (when simulation runs were started), prior to vaccination. Values of P_B were based on prevalence in unvaccinated individuals in the trial at 12 months of age (Dagan et al., 2012). For both the trial data and the model, we compared the vaccinated groups to the unvaccinated group and then to each other. In the model we calculated, for each scenario, the *POR* for the $2p+1$ and $3p$ schedules (compared to the unvaccinated group) and the relative *POR* ($RPOR, (odds_{(2p+1\ schedule)}/odds_{(3p\ schedule)})$). We used these values to calculate $\widehat{VE}_{acq, dur}$ and the relative $\widehat{VE}_{acq, dur}$ ($\widehat{RVE}_{acq, dur}$, equals $(1 - RPOR) \times 100$). We then plotted these values beside the $\widehat{VE}_{acq, dur}$, the $\widehat{RVE}_{acq, dur}$ and corresponding 95% confidence intervals from carriage data collected in the vaccine trial. We conducted these analyses both excluding and including serotype 6A in the definition of vaccine serotypes (denoted by VT and VT+6A, respectively), because of the potential cross-reactivity between vaccine serotype 6B and non-vaccine serotype 6A (Vakevainen et al., 2001).

Table 3
Scenarios of vaccine effects explored in model validation.

Scenario	comparison	$VE_{acq,dur}$, %				VE_{dur} , %
		2m dose	4m dose	6m dose	12m dose	
<i>Scenario 1a</i> : Each dose increases the effect on acquisition, and the extent is not affected by the age at which the dose is given						
Trial 3	No doses	0	0	0	0	0
	vs.					
	2, 4, 6m	20	40	60	–	0
	vs.					
	4, 6, 12m	0	20	40	60	0
<i>Scenario 1b</i> : Each dose increases the effect on acquisition, and the third dose has more effect if given at 12m than if given at 6m						
Trial 3	No doses	0	0	0	0	0
	vs.					
	2, 4, 6m	20	40	60	–	0
	vs.					
	4, 6, 12m	0	20	40	80	0
<i>Scenario 2a</i> : Third dose has no additional effect, regardless of when given						
Trial 3	No doses	0	0	0	0	0
	vs.					
	2, 4, 6m	30	60	60	–	0
	vs.					
	4, 6, 12m	0	30	60	60	0
<i>Scenario 2b</i> : Third dose has no additional effect if given at 6m, but increases the effect on acquisition if given at 12m						
Trial 3	No doses	0	0	0	0	0
	vs.					
	2, 4, 6m	30	60	60	–	0
	vs.					
	4, 6, 12m	0	30	60	80	0

m – months.

Competition between vaccine serotypes and non-vaccine serotypes is not included in the model. We did not fit the model to the data, but instead examined whether predicted patterns in $\widehat{VE}_{acq,dur}$ and $\widehat{RVE}_{acq,dur}$ were consistent with those in the trial data.

3. Results

The estimated vaccine efficacy against acquisition and duration ($\widehat{VE}_{acq,dur}$) increases from 0% at the moment of vaccination, towards $VE_{acq,dur}$, whether calculated using *POR* or *PR* (Fig. 2). When the vaccine only affects the acquisition of carriage (Fig. 2, panel A), the $\widehat{VE}_{acq,dur}$ calculated using *POR* approximates the $VE_{acq,dur}$ sooner than the $\widehat{VE}_{acq,dur}$ calculated using *PR*. The delay after vaccination before the $\widehat{VE}_{acq,dur}$ calculated using *POR* first approximates $VE_{acq,dur}$ is around three months in this scenario. The $\widehat{VE}_{acq,dur}$ calculated using *PR* underestimates $VE_{acq,dur}$ for the whole trial period. These findings vary only slightly when *R* is 1 or below (Appendix B, Fig. SB1, panels D and G).

When the vaccine only has an effect on duration of carriage (Fig. 2, panel B), $\widehat{VE}_{acq,dur}$ calculated using *POR* rapidly increases from 0% to an overestimate of $VE_{acq,dur}$ soon after vaccination. The extent of overestimation is small in the scenario shown (maximum $\widehat{VE}_{acq,dur}$ is 63% and $VE_{acq,dur}$ is 60%). The $\widehat{VE}_{acq,dur}$ calculated using *POR* returns towards $VE_{acq,dur}$ if *R* is more than 1, but continues to overestimate $VE_{acq,dur}$ if *R* is less than 1 (Fig. SB1, panel H). $\widehat{VE}_{acq,dur}$ calculated using *PR* underestimates, equals or overestimates $VE_{acq,dur}$ depending on the value of *R* (Fig. SB1, panels B, E and H, mathematical background given in Appendix A, Section 2).

When the vaccine has an effect on both the acquisition and duration of carriage (Fig. 2, panel C), the results lie between those where only VE_{acq} or VE_{dur} is affected.

3.1. Sensitivity analyses

Sensitivity analyses on model structure and parameter values are shown in Figs. SB2–SB9. In all analyses the time until $\widehat{VE}_{acq,dur}$

first approximates $VE_{acq,dur}$ is most sensitive to the mean duration of carriage. Longer mean durations require longer times until $VE_{acq,dur}$ is first approximated (Figs. SB2 and SB2a, panels A, Fig. SB3, panels A, B, E and F, and Figs. SB4–SB7a, panel A). For example, if the mean duration of carriage is 1–1.5 m, up to 4 months are needed for the $VE_{acq,dur}$ to be approximated (Fig. SB6, panel A), for mean duration of 1.5–2 months up to 6 months are needed, and for a mean duration of 2–3 months up to 8 months are needed in the baseline model. The maximum time was around 40 months after trial start assuming a mean duration of carriage of 11–12 m (Figs. SB4, SB4a, SB6, SB6a, panel A). When vaccination increases the rate of clearance of carriage (i.e. shortens the duration of carriage), less time is required until $\widehat{VE}_{acq,dur}$ first approximates $VE_{acq,dur}$ (Figs. SB2 and SB2a panel E, SB3 and SB3a panels D and H, and panels SB4–SB5a panel E).

Both the baseline and constant FOI models were sensitive to the assumptions of steady state at the trial start, but only at extreme values. High background prevalence (P_B) and low initial prevalence in the trial population (P_{init}), result in $VE_{acq,dur}$ first approximating $VE_{acq,dur}$ rapidly (Fig. SB8). $\widehat{VE}_{acq,dur}$ can overestimate $VE_{acq,dur}$ when P_B is markedly higher than P_{init} . The extent of overestimation was small except for at extreme values of P_B and P_{init} .

The constant FOI model behaves slightly differently from the baseline model. In general the baseline model takes longer until $VE_{acq,dur}$ is first approximated by $\widehat{VE}_{acq,dur}$ (Figs. SB2–SB7a), although the difference between the models is usually small. The overestimation of $VE_{acq,dur}$ when vaccine affects the duration of carriage in the baseline model is not seen in the constant FOI model (Fig. SB10). It should be noted that the reproduction number within the trial is 0 in the constant FOI model. This is because trial participants do not infect each other and consequently there are no secondary infections within the trial population.

General patterns observed did not change when using the alternative definition of when $\widehat{VE}_{acq,dur}$ first approximates $VE_{acq,dur}$ ($\widehat{VE}_{acq,dur}$ within an absolute 5% of $VE_{acq,dur}$, Figs. SB2a, SB3a, SB4a, SB5a, SB6a, SB7a).

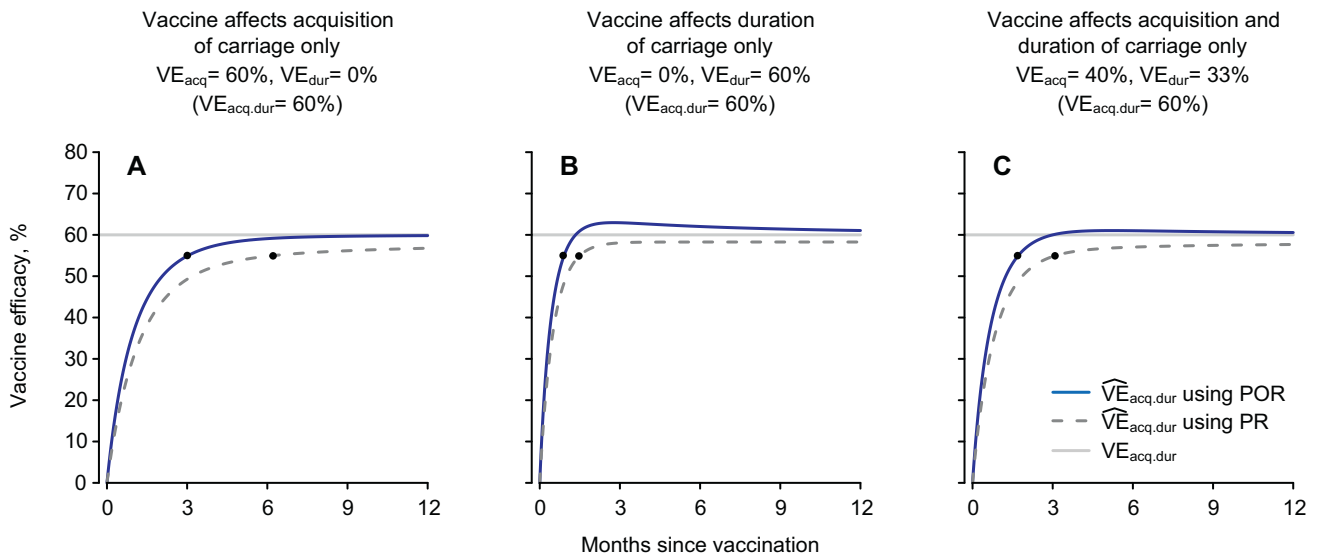


Fig. 2. Baseline model: Vaccine efficacy ($\widehat{VE}_{acq,dur}$) calculated using the prevalence odds ratio (POR) or prevalence ratio (PR) for different values of VE_{acq} and VE_{dur} , using baseline parameter values. VE_{acq} – “true” vaccine efficacy against acquisition of carriage; VE_{dur} – “true” vaccine efficacy against acquisition of carriage; $VE_{acq,dur}$ – “true” vaccine efficacy against acquisition and duration of carriage; $\widehat{VE}_{acq,dur}$ – calculated vaccine efficacy against acquisition and duration of carriage. Baseline parameter values are used except for VE_{acq} and VE_{dur} . The black dots indicate when values of $\widehat{VE}_{acq,dur}$ reach 55%.

3.2. Simulated trials comparing vaccine given as a multiple dose schedule to no doses of vaccine

Fig. 3 shows $\widehat{VE}_{acq,dur}$ for placebo controlled trials with different intervals between doses. All scenarios shown have the same $VE_{acq,dur}$ after the last dose. In the trial with shorter intervals between doses, $\widehat{VE}_{acq,dur}$ not surprisingly first approximates $VE_{acq,dur}$ sooner after the first dose of vaccination than for a schedule with longer intervals (Fig. 3, panels A–C). Until $\widehat{VE}_{acq,dur}$ becomes stable in both trials, the shorter interval schedule will appear more effective. This is seen regardless of assumptions about the effect of each dose of vaccine. If trial results are aligned at the time of last vaccination (Fig. 3, panels D–F), the two month interval schedule will look more effective than the one month interval schedule for a period after the last dose of vaccine in some scenarios (Fig. 3, panel E).

3.3. Model validation with trial data comparing multiple schedules in a single trial

The patterns over time in simulated trials were consistent with patterns in empirical data for $\widehat{VE}_{acq,dur}$ for the $2p+1$ schedule (Figs. 4 and SB11, panels A–D,) and the $3p$ schedule (panels E–H), as well as for $\widehat{RVE}_{acq,dur}$ comparing the $2p+1$ and $3p$ schedules (panels I–L). Empirical data for $\widehat{VE}_{acq,dur}$ were not available after 12 months of age in the 7-valent PCV trial, when the control group received a dose of vaccine. The point estimates for $\widehat{RVE}_{acq,dur}$ comparing the $2p+1$ and $3p$ from the 7-valent PCV trial follow trends that are most similar to the simulations in scenarios where the third dose of vaccine has an additional effect on carriage acquisition over the previous two doses (Figs. 4 and SB11, panels I and J).

There is a slow decline in $\widehat{RVE}_{acq,dur}$ calculated from both simulated and empirical data towards a minimum value, which is maintained until the $2p+1$ group receives the booster dose. After this dose, $\widehat{RVE}_{acq,dur}$ in both simulated and empirical data change direction and do not immediately stabilise. The $\widehat{RVE}_{acq,dur}$ from trial data become relatively stable after 19 months of age (7 months after

the last vaccine dose) for both VT (Fig. 4, panels I–L) and VT+6A (Fig. SB11, panels I–L) data. In the simulated data, the $\widehat{RVE}_{acq,dur}$ moves to above 0% in the scenario where the booster dose at 12 m has more effect on acquisition than a third primary dose at 6m (Figs. 4 and SB11, panel J).

In simulations where a third dose of vaccine at 6m has no additional effect on carriage acquisition over the previous two doses (i.e. marginal effect of a third dose at 6m is 0, Figs. 4 and SB11, panels K and L) there is a change in $\widehat{RVE}_{acq,dur}$ towards 0% after the 6 month dose that is not reflected in the trial data. The confidence intervals around the trial data are, however, wide and include the simulated $\widehat{RVE}_{acq,dur}$.

4. Discussion

Our study found that in simulated vaccine trials there is a period after vaccination during which $\widehat{VE}_{acq,dur}$ calculated using POR will underestimate the true $VE_{acq,dur}$, and that the length of this period is most affected by the mean duration of carriage. Overestimation of $VE_{acq,dur}$ occurs in some uncommon circumstances. In simulated trials where multiple doses of vaccine are given, the schedule of vaccination can affect length of time during which $VE_{acq,dur}$ is underestimated. Furthermore, comparisons of multiple schedules simulated within a single trial also have periods in which $\widehat{RVE}_{acq,dur}$ (relative vaccine efficacy calculated from POR) do not reflect the true $RVE_{acq,dur}$. The patterns predicted by the model for such comparisons were also observed in empirical data from a vaccine trial. This study also confirms that estimates based on POR are better than those based on PR for assessing vaccine efficacy against acquisition and duration of carriage in individually randomised trials of leaky vaccines.

Simplicity and straightforward interpretation of outputs are advantages of the model. Different values for the effects of a vaccine on carriage can be defined and it can be determined when and how best to measure these effects. Strong assumptions made in previous investigations of the interpretation of carriage outcomes in vaccine trials can be relaxed (Rinta-Kokko et al., 2009; Auranen et al., 2013a,b), and changes in the estimated vaccine

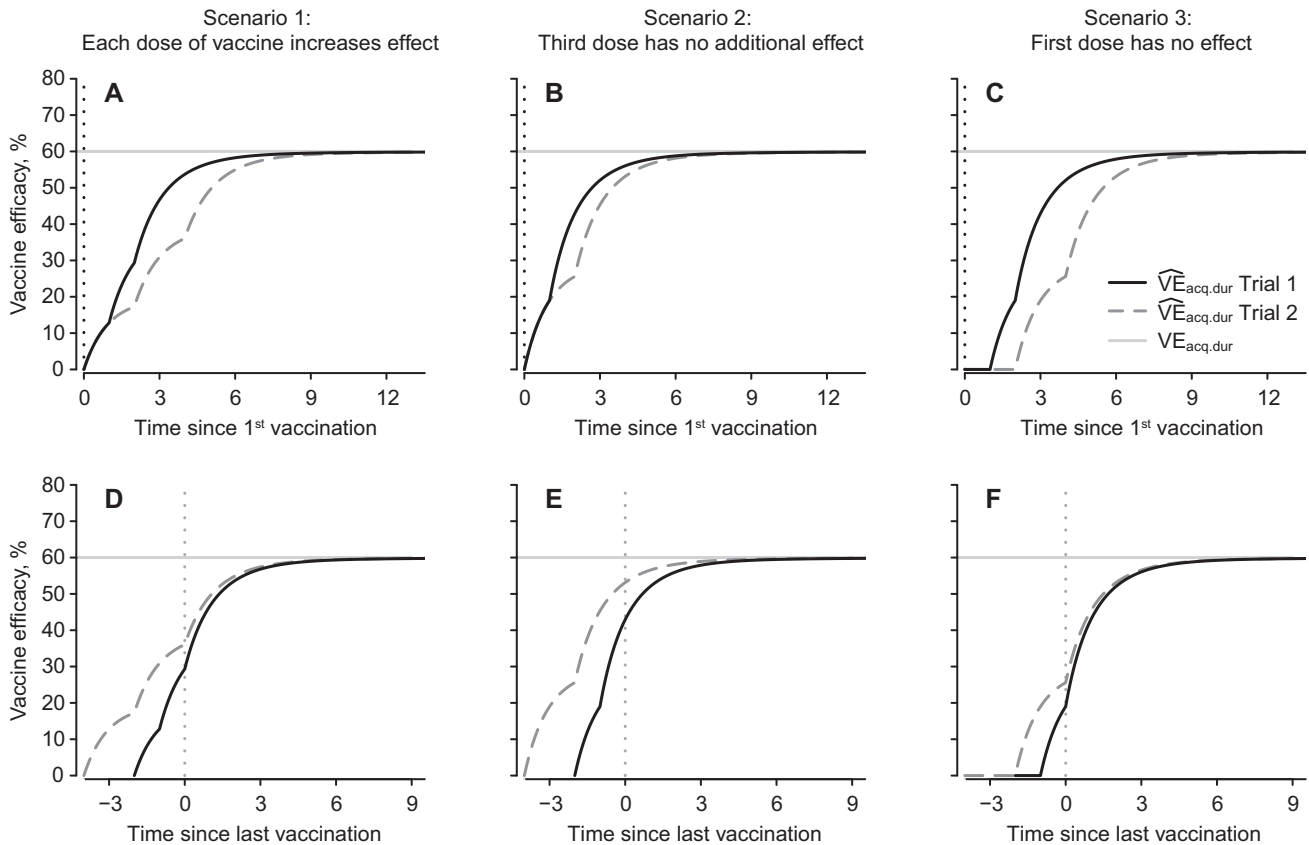


Fig. 3. Multiple doses of vaccines, baseline model. The effect of interval between three primary vaccine doses on $\widehat{VE}_{acq,dur}$ when comparing independent vaccine trials. $VE_{acq,dur}$ – “true” vaccine efficacy against acquisition and duration of carriage; $\widehat{VE}_{acq,dur}$ – calculated vaccine efficacy against acquisition and duration of carriage. Simulated results from two trials are shown in each panel; the black solid line shows $\widehat{VE}_{acq,dur}$ for a trial comparing vaccination at 2, 3, and 4 months to no vaccination (Trial 1), and the grey dashed line shows $\widehat{VE}_{acq,dur}$ for a trial comparing vaccination at 2, 4, and 6 months to no vaccination (Trial 2). The horizontal grey line indicates $VE_{acq,dur}$ in both trials after 3 doses of vaccine. The vaccine parameters used in the three scenarios are shown in Table 2. Results from simulations are the same in the upper row and the lower row except for the alignment of trials. The vertical black dotted line indicates the point at which first vaccination occurs in panels A–C, and the vertical grey dotted line indicates the point at which last vaccination occurs in panels D–F.

efficacy over time explicitly modelled. Nevertheless, we made several simplifying assumptions. The model only considered single serotype pathogens. For pathogens such as *S. pneumoniae* where multiple serotypes compete with each other for colonisation of the nasopharynx, the calculation of the *POR* may need to be adjusted for the time of being at risk of carriage acquisition (Auranen et al., 2013b). Despite this, and the possibility of changes to the time until $\widehat{VE}_{acq,dur}$ measures $VE_{acq,dur}$ well, the conclusions about the best estimator for calculating vaccine efficacy for leaky vaccines ($(1 - POR) \times 100$), and the presence of a delay before $VE_{acq,dur}$ can be accurately measured, are likely to remain the same.

Further research should elaborate the model to incorporate multiple serotypes, cluster-randomised trials and vaccines that result in complete protection for some recipients (all-or-nothing vaccines), rather than partial protection for all. For example, it is possible that different estimators (i.e. not $(1 - POR) \times 100$) will be needed for all-or-nothing vaccines, or for vaccines that completely protect some individuals and partially protect others. It would also be useful to investigate whether effects of a vaccine on acquisition and duration of carriage can be separately estimated from multiple cross-sectional samples. Previous work has examined some of these aspects, but in these analyses a different odds ratio was calculated to represent that used in the indirect cohort method (ratio of odds of vaccination in those carrying VT and those carrying non-vaccine serotypes (Omori et al., 2012)). Further studies could incorporate an effect of vaccine on colonisation density into

the model. Such an effect might reduce the transmissibility of carriage from vaccinated individuals and increase the time required to obtain a good estimate of vaccine efficacy. However, an effect on colonisation density could also reduce the sensitivity of nasal swabbing for detecting carriage, which would add additional complexity to the estimation of vaccine efficacy in trials.

In our models, we assumed that all trial participants receive vaccination simultaneously and that the mean duration of carriage remains the same over time in unvaccinated individuals. These assumptions are also unlikely to change our conclusions, but it is possible for confounding to occur when trial participants do not receive vaccination simultaneously. It is also possible that a *POR* calculated among those vaccinated late in the trial would take longer to stabilise than that among those vaccinated early due to a decreasing FOI. Last, we assumed that the effect of the vaccine does not wane over time. This is a reasonable assumption, since we only look at the effect of the vaccine over the time scale of a vaccine trial.

An important message from our study is that trials of vaccines against pathogens with long carriage durations would need to extend follow-up times for carriage in order to obtain accurate estimates of $VE_{acq,dur}$ from cross-sectional data. Our results show that the time until $\widehat{VE}_{acq,dur}$ first approximates $VE_{acq,dur}$ is most sensitive to the mean duration of carriage. Additionally, our models assume that vaccine effects on carriage begin immediately after vaccination and if this is not the case then even longer follow up

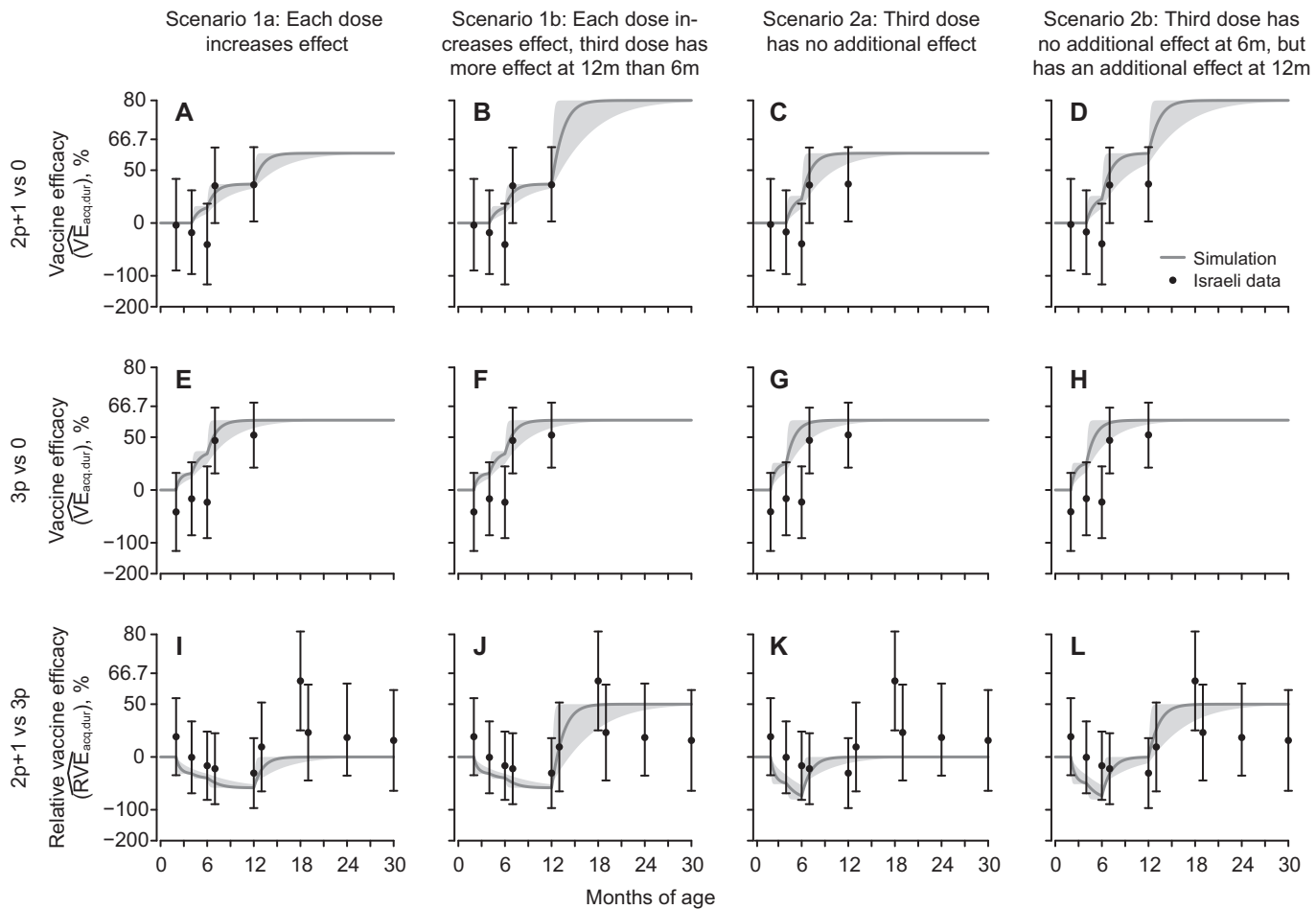


Fig. 4. Model validation, constant FOI model. Simulations are compared with empirical data from a 7-valent PCV trial in Israel. Comparisons are made between three trial groups. Groups receiving vaccine at 4, 6, 12 months ($2p+1$ schedule) or 2, 4, 6 months ($3p$ schedule) are compared to a group receiving no vaccination to calculate $\widehat{VE}_{acq,dur}$ and groups receiving vaccine at 4, 6, 12 months and 2, 4, 6 months are compared to calculate $\widehat{RVE}_{acq,dur}$. A $VE_{acq,dur}$ of 50% can be interpreted as halving the time spent in the carrier state when measured over everyone in the vaccinated group compared to everyone in the unvaccinated group. The black points and vertical lines represent point estimates and 95% confidence intervals for $\widehat{VE}_{acq,dur}$ and $\widehat{RVE}_{acq,dur}$ at each of the carriage sampling points in the trial data. The unvaccinated group in the trial received vaccination at 12m so $\widehat{VE}_{acq,dur}$ could not be calculated after this time. The parameters used in simulation of the four scenarios of vaccine effect are shown in Tables 1 and 3. Simulations using the constant FOI model are represented by the dark grey line and grey band. The dark grey line shows the simulated $\widehat{VE}_{acq,dur}$ and $\widehat{RVE}_{acq,dur}$ for a mean duration of carriage of 1.25m (baseline value). The outer limits of the grey band show $\widehat{VE}_{acq,dur}$ and $\widehat{RVE}_{acq,dur}$ for simulations with mean durations of carriage of 5 days and 3.4 months (Table 3). Simulation runs started at 2m of age using values of P_{mit} observed in the empirical data at this age. Prior to 2m of age the simulated $\widehat{VE}_{acq,dur}$ and $\widehat{RVE}_{acq,dur}$ take the value of 1 because no vaccinations have been given before this time. These values have been added between 0 and 2m for illustrative purposes.

times will be needed to accurately estimate $VE_{acq,dur}$. General statements about when carriage should be measured in trials cannot be made and there can be considerable uncertainty about when $VE_{acq,dur}$ should be estimated in trials of vaccines against pathogens for which the mean duration of carriage is not well established. It is clear, however, that unless mean duration of carriage is very short, cross-sectional samples taken soon after vaccination are unlikely to provide accurate estimates of $VE_{acq,dur}$. The exception to this would be if vaccination caused rapid clearance of carriage, which would allow estimates to be obtained faster. However, no increase in clearance rate has been found for several vaccines (Barbour et al., 1995; Dagan et al., 2005; O'Brien et al., 2007).

When results from several trials are being compared it should be recognised that low estimates of vaccine efficacy against carriage obtained soon after vaccination are not necessarily inconsistent with higher estimates obtained more distant from vaccination. Furthermore, when deciding if it is appropriate to combine data statistically using meta-analysis, systematic reviewers should consider whether there is any reason to believe that the mean duration of carriage may differ between populations in the different trials

and whether the POR is likely to have become stable in each trial. For *S. pneumoniae*, where there is variation in the mean duration of carriage with serotype and age, as well as uncertainty around each estimate (Abdullahi et al., 2012), both simulations and empirical trial data suggest that $\widehat{RVE}_{acq,dur}$ might become stable only 7 months after the last dose of vaccine. Several trials included in a systematic review of PCV took the last sample 6 months or less after the last dose of pneumococcal vaccine (Scott et al., 2011). Mean durations of carriage for other bacterial pathogens have been estimated to be similar to or longer than that for *S. pneumoniae*. The mean duration of *N. meningitidis* carriage has been estimated at 3 months for serogroup C and 9 months for other serogroups (Trotter et al., 2006), and the mean duration of Hib carriage has been estimated at over 5 months, depending on age (Auranen et al., 1996).

Assumptions about patterns of carriage transmission can also affect the time until $VE_{acq,dur}$ is first approximated. The baseline model, in which carriage is acquired only from other trial participants, generally takes longer to approximate $VE_{acq,dur}$ than in the constant FOI model in which trial participants are only infected by those outside the trial. Although these scenarios represent two

extremes that might not occur in reality, trial settings might more closely resemble one or the other scenario. For example, a trial that takes place in a day-care centre where children spend substantial amounts of time in close contact with each other more closely resembles the baseline model and $VE_{acq.dur}$ might be accurately measured later than in a trial where participating children are distributed throughout the community with little direct contact with each other.

The findings from this study have implications for the planning and interpretation of RCTs with carriage outcomes, and for the interpretation of results from multiple trials. Investigators designing individually randomised trials should consider explicitly when to measure carriage after vaccination. A modelling exercise, using available data to inform parameter values, could help to inform the process. At a minimum, efforts should be made to obtain data about the mean duration of carriage of the pathogen.

When synthesising the results of multiple trials, for example in a systematic review and meta-analysis, the *POR* extracted from each trial to be used in meta-analysis should be from a time point after the *POR* has become stable. Carriage results might be misinterpreted if $VE_{acq.dur}$ is estimated before the *POR* becomes stable in all trials in a systematic review. The results of simulated RCTs in this study using vaccine schedules with different intervals between doses show that, even when the effect of vaccine has been set at the same value in each trial, one schedule can appear better than another. Heterogeneity between trial results in this situation might be falsely attributed to differences in $VE_{acq.dur}$ rather than differences in trial design and timing of outcome assessment. Meta-regression or stratification of results by timing of outcome assessment could be used to explore heterogeneity between trials. There are also situations in which results across multiple trials appear to be consistent, but $\widehat{VE}_{acq.dur}$ does not approximate $VE_{acq.dur}$ well; for example, in trials of similar design, but in which samples have been taken before the *POR* becomes stable.

In conclusion, vaccine trial investigators, policy makers, and others using carriage data from vaccine trials should consider the timing of sample collection in trial design and in the interpretation of reported vaccine efficacy against carriage.

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Conflict of interest

In the last 5 years, Prof. Ron Dagan has received grants/research support from Pfizer, Berna/Crucell and MSD; he has been a scientific consultant for Pfizer, Berna/Crucell, GlaxoSmithKline, Novartis, MSD and a speaker for Pfizer, Berna/Crucell and GlaxoSmithKline. All other authors have no commercial or other associations that might pose a conflict of interest.

Author contributions

PS, SH, JH, NL, KA, and ME contributed to study design. PS, SH, and JH performed analyses. RD collected trial data used for model validation. All authors contributed to interpretation, critically edited the manuscript, and approved the final draft.

Appendices A and B. Supplementary data

Supplementary material related to this article can be found, in the online version, at [doi:10.1016/j.epidem.2014.08.003](https://doi.org/10.1016/j.epidem.2014.08.003).

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