

Effect of Reduced-Dose Schedules With 7-Valent Pneumococcal Conjugate Vaccine on Nasopharyngeal Pneumococcal Carriage in Children

A Randomized Controlled Trial

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THE SUCCESS OF THE INTRODUCTION in 2000 in the United States of routine infant vaccination with the licensed 7-valent pneumococcal conjugate vaccine (PCV-7) is based on direct protection against vaccine serotype pneumococcal disease among vaccinees but also on the observed and unexpectedly large and widespread reduction in invasive and respiratory (eg, pneumonia and otitis media) vaccine serotype pneumococcal disease in nonimmunized individuals (indirect effect or herd protection).¹⁻⁶ This herd effect has been attributed to reduced carriage of vaccine serotype pneumococci in vaccinated infants and subsequent transmission to (household) adult contacts and spread in the community. The resulting decreased circulation of the 7 serotypes and herd effects have contributed substantially to the public health benefit and cost-effectiveness of PCV-7 programs.^{7,8}

Increasingly crowded infant vaccine schedules and less favorable cost-

Context The effects of reduced-dose schedules of 7-valent pneumococcal conjugate vaccine (PCV-7) on pneumococcal carriage in children are largely unknown, although highly relevant in the context of subsequent herd effects.

Objective To examine the effects of a 2-dose and 2 + 1-dose PCV-7 schedule on nasopharyngeal pneumococcal carriage in young children compared with controls.

Design, Setting, and Patients A randomized controlled trial of nasopharyngeal carriage of *Streptococcus pneumoniae* enrolling 1003 healthy newborns and 1 of their parents in a general community in the Netherlands, with follow-up to age 24 months and conducted between July 7, 2005, and February 14, 2008.

Intervention Infants were randomly assigned to receive 2 doses of PCV-7 at 2 and 4 months; 2 + 1 doses of PCV-7 at 2, 4, and 11 months; or no dosage (control group).

Main Outcome Measure Vaccine serotype pneumococcal carriage rates in infants in the second year of life.

Results At 12 months, vaccine serotype pneumococcal carriage was significantly decreased after both PCV-7 schedules, with vaccine serotype pneumococcal carriage rates of 25% (95% confidence interval [CI], 20%-30%) and 20% (95% CI, 16%-25%) in the 2-dose and 2 + 1-dose schedule groups, respectively, vs 38% (95% CI, 33%-44%) in the control group (both $P < .001$). At 18 months, in the 2 + 1-dose schedule group, vaccine serotype pneumococcal carriage had further decreased to 16% (95% CI, 12%-20%) and, at 24 months, to 14% (95% CI, 11%-18%; both $P < .001$); whereas in the 2-dose schedule group, vaccine serotype pneumococcal carriage had remained stable at 18 months (24%; 95% CI, 20%-29%), but at 24 months had further decreased to 15% (95% CI, 11%-19%; both $P < .001$). In the control group, vaccine serotype pneumococcal carriage remained around 36% to 38% until 24 months.

Conclusion Compared with no pneumococcal vaccination, a 2 + 1-dose and 2-dose schedule of PCV-7 resulted in significant reductions of vaccine serotype pneumococcal carriage in the second year of life.

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effectiveness calculations have prompted exploration of reduced-dose vaccine schedules other than the currently recommended 3 + 1-dose schedule of PCV-7, comprising 3 primary doses before age 6 months followed by a booster vaccination in the second year of life. Clinically, protection against invasive pneumococcal disease (IPD) after less than 4 doses was observed in the Northern California Kaiser Permanente study.¹ In this study, clinical efficacy against IPD in the intention-to-treat analysis was high (93.9%), even though only 58% of the children had received the full PCV-7 schedule. Furthermore, the association between use of reduced-dose schedules and prevention of vaccine serotype IPD in vaccinees was demonstrated in a large case-control study from the United States showing high reductions associated with a 2 + 1-dose schedule (98%; 95% confidence interval [CI], 75%-100%) and even a 2-dose schedule (96%; 95% CI, 88%-99%) during a period of vaccine shortage.⁹ A recent immunogenicity study from the United Kingdom also supported introduction of a reduced-dose schedule.¹⁰ Consequently, several European countries such as the United Kingdom and Norway have recently implemented a 2 + 1-dose schedule. Norway has reported high direct protection against vaccine serotype IPD in the first 2 years following national implementation of PCV-7 vaccination at 3, 5, and 12 months.¹¹

Difficulty in implementing the 3 + 1-dose schedule in developing countries is another reason for exploring reduced schedules. Although *Streptococcus pneumoniae* is still the leading cause of meningitis, bacteremia, and pneumonia worldwide with an estimated annual death rate of 1 million children younger than 5 years, the overwhelming majority of deaths are due to pneumonia and occur in developing countries.¹² Despite the World Health Organization's recommendations of global implementation of pneumococcal vaccine in national immunization programs for infants, only a few countries have actually introduced PCV-7.¹² Poor resources and programmatic differences (eg, the cur-

rent expanded program on immunization for developing countries lacks a health visit for a booster in the second year of life) are among reasons for the low implementation rates.

Receiving fewer primary doses or missing a booster dose may, however, affect the size and duration of reduction in vaccine serotype carriage and subsequent herd effects.¹³ Evaluation of herd effects after widespread introduction of conjugate vaccines needs long-term and high-quality disease surveillance. Investigating vaccine effects on nasopharyngeal pneumococcal carriage provides an important surrogate in exploring alternative vaccine schedules for potential herd effects on pneumococcal disease.^{14,15}

We assessed the effects of a 2-dose and a 2 + 1-dose schedule of PCV-7 on vaccine serotype pneumococcal carriage in children and unvaccinated household adult contacts in a large randomized controlled trial in the Netherlands before nationwide implementation of PCV-7 for all infants.

METHODS

Study Population

The study area covered 5 participating well-baby clinic organizations (birth cohort of approximately 16 000 per year) in the western region of the Netherlands. All parents living in this region were informed about the study by written information in their newborn's first weeks of life and asked to participate. Infants younger than 12 weeks, not yet having received any infant vaccination and living in the study region, were eligible for inclusion. Exclusion criteria were known immunodeficiency, craniofacial or chromosomal abnormalities, language barrier, or expected relocation within the follow-up period. Enrollment started on July 7, 2005, and was completed on February 9, 2006, before the introduction of PCV-7 in the Dutch National Immunization Program for infants born after March 31, 2006. Follow-up ended February 14, 2008. Participants did not receive any financial compensation.

Study Design

We conducted a randomized controlled trial to assess the effect of reduced-dose schedules with PCV-7 on vaccine serotype nasopharyngeal carriage of *S pneumoniae*. After written informed consent had been obtained from both parents or guardians, infants were randomly allocated by simple randomization via a computer randomization interface during the first home-visit to receive (1) PCV-7 at 2 and 4 months (2-dose schedule group); (2) PCV-7 at 2, 4, and 11 months (2 + 1-dose schedule group); or (3) no dosage (control group). Children in the control group were offered a PCV-7 vaccination free of charge after completing the study. Parents were aware of the child's vaccine schedule. Laboratory personnel assessing pneumococcal carriage were unaware of treatment allocation and the randomization key was not disclosed until after the study was completed.

The intervention vaccine was the 7-valent pneumococcal polysaccharide-CRM197 protein conjugate vaccine (CRM197-PCV-7; Wyeth Pharmaceuticals). Each 0.5-mL dose contained 2 µg each of serotypes 4, 9V, 14, 19F, and 23F polysaccharides, 2 µg of serotype 18C oligosaccharide, and 4 µg of serotype 6B polysaccharide, conjugated individually to the CRM197 protein, and 0.5 mg of aluminum phosphate as an adjuvant. Vaccinations were administered intramuscularly in the leg or upper arm during regular well-baby clinic visits, together with routine immunizations according to the Dutch National Immunization Program (DTaP-IPV-Hib [diphtheria and tetanus toxoids and acellular pertussis; inactivated polio vaccine; *Haemophilus influenzae* type b]; DTaP-IPV-Hib-Hep-B or DTaP-IPV-Hib and Hep-B for children at high risk for hepatitis B [n=54]). During the first home-visit at age 6 weeks old and 4 follow-up visits at ages 6, 12, 18, and 24 months, a nasopharyngeal sample was obtained from the children and, at ages 12 and 24 months, also from 1 of the parents. With each nasopharyngeal swab, a questionnaire on risk factors for pneumococcal carriage in children and parents was ob-

tained from the parents. Because pneumococcal yield in adults is known to be higher when taking a transnasal and transoral nasopharyngeal swab, both swabs were collected from parents.¹⁶

An acknowledged national ethics committee from the Netherlands (Stichting Therapeutische Evaluatie Geneesmiddelen, <http://www.stegmetc.org>) approved the study protocol. The trial was undertaken in accordance with the European Statements for Good Clinical Practice, which includes the provisions of the Declaration of Helsinki of 1989. An external committee was appointed to review progress and advise on data eligibility for analysis.

Nasopharyngeal Swabs

Deep nasopharyngeal samples were taken transnasally with a flexible, sterile, dry cotton-wool swab (Transwab Pernasal Plain, Medical Wire and Equipment Co, Corsham, Wiltshire, England) by trained study nurses according to World Health Organization standard procedures.¹⁷ Transoral nasopharyngeal swabs were taken under direct observation of the posterior pharynx with a rigid, sterile, dry cotton-wool swab (Transwab Plain). After sampling, swabs were immediately inoculated in Transwab (modified Amies) transport medium, stored at room temperature, and plated within 24 hours onto two 5% sheep-blood agar plates, with and without 5-mg/L gentamicin and incubated aerobically at 35°C for 48 hours (the gentamicin plate with increased carbon dioxide levels). Identification of *S pneumoniae* was based on colony morphology and conventional methods of determination (optochin susceptibility and bile solubility assays). One *S pneumoniae* colony per plate was then subcultured, harvested, and kept frozen at -70°C for further testing. Pneumococcal serotyping was performed by capsular swelling method (Quellung reaction) using type-specific antisera from the Statens Seruminstitut (Copenhagen, Denmark). Pneumococcal isolates were defined nontypeable when optochin susceptible and bile soluble but negative with

the Quellung reaction. Validation of the typing procedures was performed in collaboration with the National Reference Laboratory for Bacterial Meningitis (Amsterdam, the Netherlands).

Statistical Analyses

Pneumococcal vaccine serotypes are the serotypes included in PCV-7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and nonvaccine serotypes are all remaining serotypes (including nontypeables). The primary outcome measure was the proportion of children positive for vaccine serotype pneumococcal carriage in the second year of life. The sample size was calculated with the assumption of a vaccine serotype carriage rate of 35% in children in the second year of life based on previous experience.^{18,19} The smallest clinically significant difference to detect was an estimated 33% relative reduction in vaccine serotype carriage (25% vaccine serotype carriage rate) after a 2-dose schedule of PCV-7 compared with the control group, with 80% power at a 5% significance level. This resulted in a sample size of 330 infants per group, including a 10% dropout rate. All other outcomes are designated secondary outcomes for statistical analysis, including nonvaccine serotype carriage in children and vaccine serotype and nonvaccine serotype carriage in parents. The study was not adequately powered to evaluate nonvaccine serotype carriage, individual serotypes, or indirect effects on vaccine serotype and nonvaccine serotype carriage in adults.

Statistical analyses followed the intention-to-treat principle, meaning that all available data from all randomized participants were analyzed according to the assigned intervention. Because the dropout rate (<2%) and the amount of missing data for the primary analysis (<2%) were low, available data were analyzed without using imputation methods.²⁰ Per-protocol analyses yielded similar results due to the low number of protocol violations (n=20). Proportional differences in pneumococcal carriage between treatment groups and controls were analyzed by using χ^2 test or 2-sided Fisher exact test,

where appropriate. In a post-hoc analysis, we also compared vaccine serotype pneumococcal carriage between the 2-dose and 2 + 1-dose vaccine schedules. We verified the primary analyses using a repeated measurements model taking more than 1 measurement per child into account using generalized linear models in SAS version 9.1 (SAS Institute Inc, Cary, North Carolina) with an autoregressive correlation structure, with correlations becoming smaller over time.^{21,22} The generalized linear models results are reported for the primary and post-hoc analyses. Results were virtually the same indicating that potential within-person dependency was not substantially affecting the precision of our estimates. $P < .05$ was considered significant and all reported P values are 2-sided. We did not correct for multiple testing in the analysis of the secondary outcomes (eg, by using the Bonferroni method). Adjustments for multiple testing are mostly concerned with the general null hypothesis that all null hypotheses are true simultaneously, which was not true for the secondary outcome comparisons in our trial.²³

RESULTS

Parents of 9782 newborns were asked to participate. A total of 1003 children (including 15 twin pairs), representing 10.3% of the total birth cohort, were enrolled and assigned to the 3 study groups (FIGURE); 2 of the original 1005 were excluded because a parent did not provide consent. There were no major differences in demographics or distribution of risk factors (eg, number of siblings, day care attendance) between the 3 study groups (TABLE 1). A total of 4939 (98.5% of planned) nasopharyngeal swabs were collected from all children, of which 50% were positive for *S pneumoniae*. We were unable to determine the serotypes of 34 isolates (<1%) due to lack of growth on culture.

Pneumococcal Carriage in PCV-7 Unvaccinated Control Children

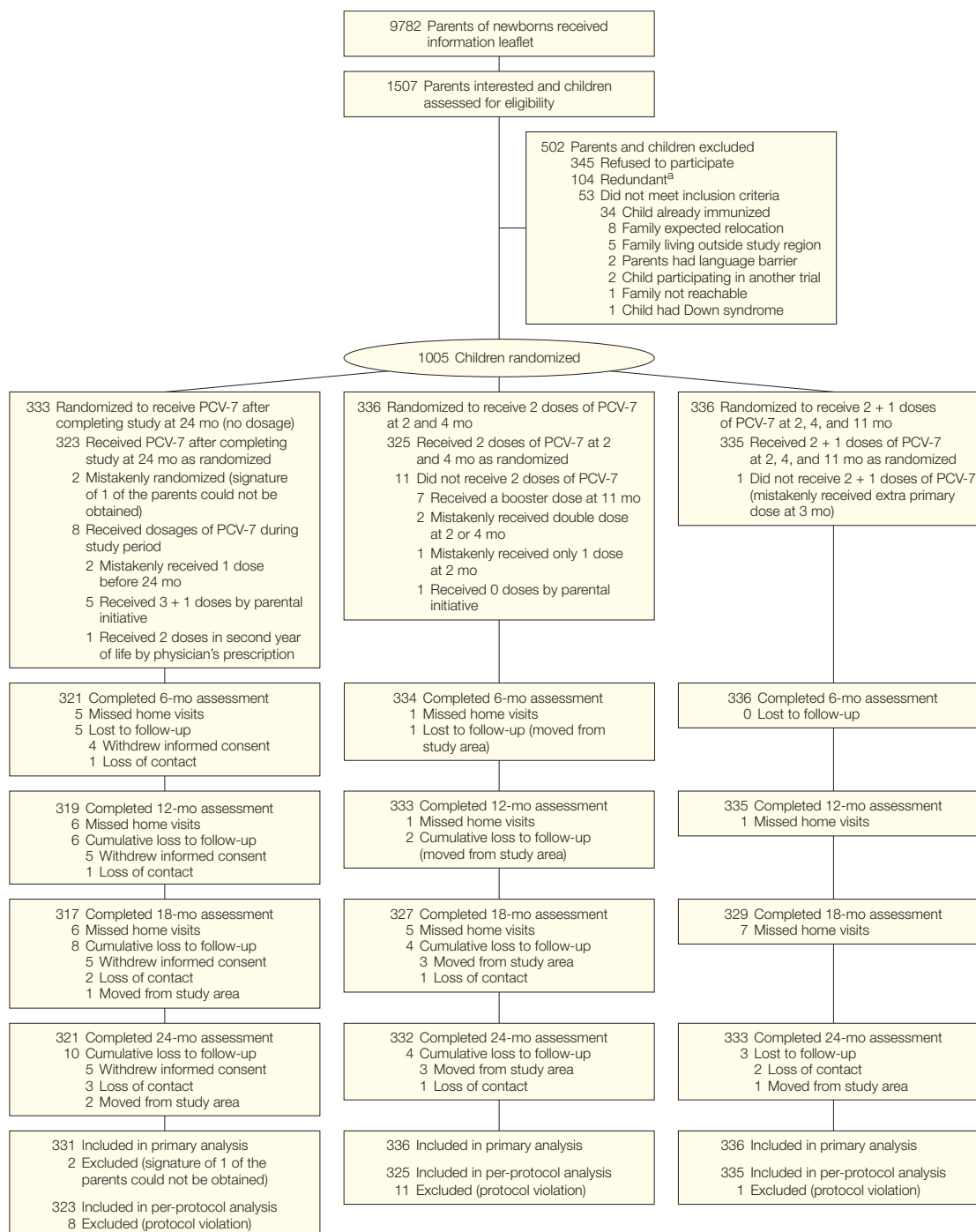
At 6 weeks and before the first vaccination, the overall pneumococcal carriage rate in children was 17% (95% CI,

15%-20%) in all groups. In unvaccinated control children, pneumococcal carriage increased to 49% (95% CI,

43%-54%) at 6 months and stabilized around 67% (95% CI, 62%-72%) between 12 and 24 months. The vaccine

serotype carriage rate gradually increased from 5% (95% CI, 3%-8%) to 23% (95% CI, 19%-28%) at 6 weeks and

Figure. Enrollment Flow Diagram



PCV-7 indicates 7-valent pneumococcal conjugate vaccine.

^aParents of children interested in participating in the study were redundant because they were still in the information process of the study after enrollment target had already been achieved and informed consent process was cancelled.

6 months, respectively, and reached its plateau at 12 months at 38% (95% CI, 33%-44%) (TABLE 2).

Effects of PCV-7 Vaccinations on Pneumococcal Carriage in Children

No significant differences in vaccine serotype, nonvaccine serotype, and overall pneumococcal carriage were observed at 6 months in both vaccine groups compared with the control group. At 12 months, vaccine serotype carriage rates were significantly lower in both vaccine groups compared with the control group, with 25% (95% CI, 20%-30%) in the 2-dose schedule group, 20% (95% CI, 16%-25%) in the 2 + 1-dose schedule group, and 38% (95% CI, 33%-44%) in the control group (Table 2). A further decrease of vaccine serotype carriage was found at 18 months after the 2 + 1-dose schedule and at 24 months after 2 primary doses compared with the control group (Table 2). In the post-hoc analysis comparing the 2-dose and 2 + 1-dose schedules, we observed a significant difference in vaccine serotype carriage at 18 months with 24% (95% CI, 20%-29%) vaccine serotype carriage in the 2-dose schedule group compared with 16% (95% CI, 12%-20%) in the 2 + 1-dose schedule group ($P=.01$). At 24 months, the point estimates for vaccine serotype carriage in both vaccine groups were at the same level with 15% (95% CI, 11%-19%) in the 2-dose schedule group and 14% (95% CI, 11%-18%) in the 2 + 1-dose schedule group, compared with 36% (95% CI, 30%-41%) in the control group.

Although this study was not powered for detecting differences in individual serotypes, both vaccine schedules showed lower point estimates for frequently carried serotype 23F at 12 and 18 months. Also, the point estimates for serotype 19F at 12 months and serotypes 6B and 19F at 18 months were lower in children having received a booster dose than in children in the 2-dose schedule group (TABLE 3). At 24 months, both vaccine schedules showed point estimates at the same level for all vaccine serotypes.

Coinciding with the reduction in vaccine serotype carriage in vaccinees, we observed a significant increase in non-vaccine serotype carriage during the second year of life, with 38% (95% CI, 33%-43%), 38% (95% CI, 33%-43%),

and 29% (95% CI, 24%-34%) at 12 months and 40% (95% CI, 35%-45%), 43% (95% CI, 38%-49%), and 30% (95% CI, 25%-35%) at 24 months for the 2-dose schedule, 2 + 1-dose schedule, and control groups, respectively

Table 1. Characteristics of the Children and Parents at Time of Enrollment and During Follow-up

Characteristics	No. (%) of Children and Parents		
	Control Group	2-Dose Schedule Group	2 + 1-Dose Schedule Group
Infants/children	(n = 331)	(n = 336)	(n = 336)
Male sex	160 (48)	176 (52)	171 (51)
Gestational age, mean (SD), wk	39.7 (1.9)	39.8 (1.8)	39.8 (1.7)
Premature birth (gestational age <37 wk at birth)	21 (6)	22 (7)	21 (6)
At home delivery	94 (28)	101 (30)	97 (29)
Birth weight, mean (SD), g	3492 (532)	3476 (581)	3512 (544)
Feeding from birth			
(Partial) breastfed at 6 wk	256 (77)	268 (80)	272 (81)
(Partial) breastfed >3 mo	97 (30)	106 (32)	108 (32)
(Partial) breastfed >6 mo	59 (18)	65 (20)	63 (19)
No. of siblings, median (interquartile range)	0 (0-1)	1 (0-1)	0 (0-1)
Day care attendance ^a			
At 12 mo ^b	192 (60)	210 (63)	204 (61)
At 24 mo ^c	221 (68)	228 (69)	235 (70)
Use of oral or intravenous antibiotics during mo before swab			
At 12 mo ^b	20 (6)	25 (8)	23 (7)
At 24 mo ^c	10 (3)	18 (5)	11 (3)
Passive tobacco smoke exposure indoors			
At 12 mo ^b	21 (7)	26 (8)	20 (6)
At 24 mo ^c	26 (8)	28 (8)	21 (6)
Age at vaccination, mean (SD), wk			
First primary dose	NA	8.9 (1.5)	8.7 (1.1)
Second primary dose	NA	18.5 (2.5)	18.3 (1.7)
Booster dose	NA	NA	48.5 (2.0)
Parents ^d	(n = 305)	(n = 319)	(n = 329)
Male sex	55 (18)	48 (15)	57 (17)
Age, mean (SD), y	33.3 (4.8)	33.3 (4.3)	32.9 (4.7)
Active smoking			
At index child's age 12 mo	41 (13)	49 (15)	45 (14)
At index child's age 24 mo ^e	40 (14)	43 (14)	37 (12)
Passive tobacco smoke exposure indoors			
At index child's age 12 mo	21 (7)	23 (7)	25 (8)
At index child's age 24 mo ^e	23 (8)	16 (6)	20 (6)
Use of oral or intravenous antibiotics 1 mo before swab			
At index child's age 12 mo	9 (3)	8 (3)	15 (5)
At index child's age 24 mo ^e	9 (3)	13 (4)	14 (4)

Abbreviation: NA, not applicable.

^aDefined as more than 4 hours per week with at least 1 child from a different family.

^bFor control group, n=319; 2-dose schedule group, n=333; and 2 + 1-dose schedule group, n=335.

^cFor control group, n=321; 2-dose schedule group, n=323; and 2 + 1-dose schedule group, n=333.

^dNumbers of participating parents at child's age of 12 months is shown in parentheses. Data from parents were gathered at the index child's age of 12 and 24 months with data missing due to loss to follow-up or incidentally missed home visits (eg, illness, parent not present at visit). Also, parents of twins (n=15) were excluded.

^eFor control group, n=296; 2-dose schedule group, n=309; and 2 + 1-dose schedule group, n=321.

Table 2. Frequencies of Pneumococcal Nasopharyngeal Carriage in Vaccinated Children After a 2-Dose and 2 + 1-Dose PCV-7 Schedule and Unvaccinated Control Children at 12, 18, and 24 Months^a

	Control Group	2-Dose Schedule Group		2 + 1-Dose Schedule Group	
	No. (%)	No. (%)	Relative Risk (95% CI)	No. (%)	Relative Risk (95% CI)
Age 12 mo	(n = 319)	(n = 333)		(n = 335)	
Vaccine serotype	122 (38)	82 (25)	0.64 (0.51-0.81)	67 (20)	0.52 (0.41-0.68)
Nonvaccine serotype	92 (29)	125 (38)	1.30 (1.04-1.62)	126 (38)	1.30 (1.05-1.63)
All pneumococcal serotypes	214 (67)	207 (62)	0.93 (0.83-1.04)	193 (58)	0.86 (0.76-0.97)
Age 18 mo	(n = 317)	(n = 327)		(n = 329)	
Vaccine serotype	119 (38)	79 (24)	0.64 (0.51-0.82)	51 (16)	0.41 (0.31-0.55)
Nonvaccine serotype	96 (30)	129 (39)	1.30 (1.05-1.61)	134 (41)	1.34 (1.09-1.66)
All pneumococcal serotypes	215 (68)	208 (64)	0.94 (0.84-1.05)	185 (56)	0.83 (0.73-0.94)
Age 24 mo	(n = 321)	(n = 332)		(n = 333)	
Vaccine serotype	114 (36)	49 (15)	0.42 (0.31-0.56)	47 (14)	0.40 (0.29-0.54)
Nonvaccine serotype	97 (30)	132 (40)	1.32 (1.06-1.63)	144 (43)	1.43 (1.16-1.76)
All pneumococcal serotypes	211 (66)	181 (55)	0.83 (0.73-0.94)	191 (57)	0.87 (0.77-0.99)

Abbreviations: CI, confidence interval; PCV-7, 7-valent pneumococcal conjugate vaccine.

^aAnalyses were based on intention-to-treat and included all available data from all randomized infants in the assigned groups; missing data were due to loss to follow-up or incidentally missed home visits (eg, illness).

(Table 2). No cross-protection against vaccine-related serotype 6A for either vaccine schedule was observed (Table 3). Vaccine-related serotype 19A carriage was found increased at 12 months in the 2-dose schedule and 2 + 1-dose schedule groups and at 24 months in the 2 + 1-dose schedule group, becoming the second most prevalent nonvaccine serotype after serotype 6A (Table 3).

The decrease in vaccine serotype carriage together with the smaller increase in nonvaccine serotype carriage resulted in a significant decrease in overall pneumococcal carriage for the 2 + 1-dose schedule group from 12 months onward and for the 2-dose schedule group at 24 months (Table 3).

Effect of PCV-7 Vaccinations on Pneumococcal Carriage in Parents

A total of 3823 (96.7% of planned) nasopharyngeal swabs were collected from parents, of which 13.7% were positive for *S pneumoniae*. Results from parents of twins were excluded (n=15), because the effect of different randomized vaccine schedules could compromise the analyses. Nine parents (9%)

with both a positive transoral and transnasal swab had different serotypes isolated from the transoral and transnasal swabs. All collected serotypes were included in the analysis. No statistically significant differences in vaccine serotype and overall pneumococcal carriage were observed between parents of vaccinees and parents of controls at the index child's age of 12 and 24 months. However, at the index child's age of 24 months, nonvaccine serotype carriage in parents of vaccinees in the 2-dose and 2 + 1-dose schedule groups had increased by 80% and 102%, respectively (TABLE 4). Numbers were too small to evaluate serotype-specific results. The most frequent serotypes identified in parents of controls (n ≥ 5) at the child's age of 24 months were serotypes 19F, 6B, 14, and 3, and in parents of vaccinees (n ≥ 10) were 19A, 19F, 11A, and 6A.

COMMENT

This is to our knowledge the first large, randomized controlled trial investigating the effects of reduced-dose PCV-7 schedules on nasopharyngeal pneumococcal carriage in a

PCV-7 unvaccinated population. We have shown that a PCV-7 schedule with only 2 primary doses results in significantly decreased vaccine serotype carriage in immunized children from 12 months onward compared with unvaccinated controls. The booster dose resulted in an earlier further reduction of vaccine serotype carriage at 18 months compared with no booster dose. At 24 months, both vaccine schedules produced a similar reduction in vaccine serotype carriage. The observed reduction in vaccine serotype carriage after the 2 + 1-dose schedule is furthermore comparable with the reductions after 3 primary doses with or without a later booster dose as reported by others, despite variation in vaccination intervals, study population, and study vaccines.^{24,25} Only in 1 previous nonrandomized, case-control study²⁶ of 2 doses of 5-valent pneumococcal conjugate vaccine administered to infants younger than 6 months with a 1-month interval between doses was vaccine serotype carriage reduction at 24 months reported less than what we observed (26% reduction in vaccine serotype carriage vs 58% in our study). This may be due to the shorter 1-month interval between vaccinations.²⁶

The administration of a booster dose has been suggested as important for the magnitude and duration of vaccine serotype carriage reduction.^{14,27} The difference in vaccine serotype carriage reduction between both schedules at 18 months was primarily due to an earlier significant reduction of serotype 6B and 19F after administration of the booster dose compared with no booster dose. In our study, the temporary advantage of the booster on vaccine serotype carriage reduction observed at 18 months was not observed at 24 months when both schemes showed a reduction of approximately 60% vaccine serotype carriage. This suggests that the booster dose may contribute to an earlier reduction of vaccine serotype carriage in particular for lower immunogenic serotypes like serotype 6B

or for serotypes requiring high antibody levels such as serotype 19F,^{10,28} but may not be necessary for long-term carriage reduction. However, the effect of natural boosting of the immune system by circulating vaccine serotype strains in the population in this trial setting may differ after widespread PCV-7 implementation with disappearance of circulating vaccine serotype pneumococci and making a booster dose necessary.

The reduction in serotype 6B carriage was not observed until 18 months

in our study, which is late compared with other studies.^{24,25} Lower point estimates for serotype 6B carriage in children at 9 and 12 months following 3 priming doses with a 1- and 2-month interval but without a booster dose have been observed.^{24,25} Three priming doses may be more efficient in eliciting adequate antibody levels at an early age^{10,28} and may be required for early serotype 6B carriage reduction. For early protection against invasive disease caused by serotype 6B, however, 2 primary doses may still be sufficient.⁹ Although

threshold protective antibody levels are not well understood yet and seem to differ by serotype, the levels needed to prevent carriage²⁹ are likely higher than what is needed for invasive disease^{1,30} and possibly also for pneumonia and otitis media.³¹ Effect of reduced schedules on disease, in particular respiratory disease like pneumonia and otitis, thus needs to be evaluated.

One of the drawbacks of current pneumococcal conjugate vaccination with limited serotype coverage is serotype replacement, with nonvaccine se-

Table 3. Frequencies of Nasopharyngeal Carriage of Individual Pneumococcal Vaccine and Nonvaccine Serotypes in Vaccinated Children After a 2-Dose and 2 + 1-Dose PCV-7 Schedule and Unvaccinated Control Children at 12, 18, and 24 Months^a

Age 12 mo						Age 18 mo						Age 24 mo					
Control Group (n = 319)		2-Dose Schedule Group (n = 333)		2 + 1-Dose Schedule Group (n = 335)		Control Group (n = 317)		2-Dose Schedule Group (n = 327)		2 + 1-Dose Schedule Group (n = 329)		Control Group (n = 321)		2-Dose Schedule Group (n = 332)		2 + 1-Dose Schedule Group (n = 333)	
No. (%)	No. (%)	P Value	No. (%)	P Value	No. (%)	No. (%)	P Value	No. (%)	P Value	No. (%)	P Value	No. (%)	P Value	No. (%)	P Value	No. (%)	P Value
Vaccine Serotypes																	
4	1 (0)		0 (0)	.49	0 (0)	2 (1)		1 (0)	.62	1 (0)	.62	1 (0)		0 (0)	.49	0 (0)	.49
6B	26 (8)		24 (7)	.65	24 (7)	43 (14)		32 (10)	.14	15 (5)	<.001	43 (13)		18 (5)	<.001	9 (3)	<.001
9V	9 (3)		4 (1)	.14	3 (1)	8 (3)		4 (1)	.22	2 (1)	.06	6 (2)		1 (0)	.07	3 (1)	.33
14	10 (3)		9 (3)	.74	7 (2)	14 (4)		6 (2)	.06	5 (2)	.03	8 (3)		4 (1)	.22	5 (2)	.36
18C	6 (2)		2 (1)	.17	2 (1)	3 (1)		2 (1)	.68	2 (1)	.68	4 (1)		2 (1)	.44	3 (1)	.72
19F	36 (11)		26 (8)	.13	18 (5)	25 (8)		18 (6)	.23	13 (4)	.03	24 (8)		18 (5)	.29	22 (7)	.66
23F	34 (11)		17 (5)	.008	13 (4)	24 (8)		16 (5)	.16	13 (4)	.05	28 (9)		6 (2)	<.001	5 (2)	<.001
Nonvaccine Serotypes^b																	
6A	24 (8)		34 (10)	.23	23 (7)	25 (8)		24 (7)	.79	22 (7)	.56	25 (8)		23 (7)	.67	22 (7)	.56
19A	5 (2)		16 (5)	.02	17 (5)	11 (4)		14 (4)	.59	19 (6)	.16	9 (3)		10 (3)	.87	21 (6)	.03
11A	11 (3)		13 (4)	.76	9 (3)	11 (4)		15 (5)	.47	12 (4)	.90	10 (3)		18 (5)	.15	14 (4)	.46
23B	5 (2)		3 (1)	.50	8 (2)	5 (2)		6 (2)	.80	6 (2)	.81	12 (4)		7 (2)	.22	9 (3)	.45
NT	1 (0)		7 (2)	.07	6 (2)	2 (1)		6 (2)	.29	10 (3)	.02	3 (1)		7 (2)	.34	12 (4)	.02

Abbreviations: NT, nontypeables; PCV-7, 7-valent pneumococcal conjugate vaccine.

^a All *P* values are for comparison with control group and calculated with χ^2 or 2-tailed Fisher exact test where appropriate. Analyses were based on intention-to-treat and include all available data from parents of randomized infants in the assigned groups; missing data are due to loss to follow-up or incidentally missed home visits (eg, illness).

^b All individual nonvaccine serotypes for which at least 20 or more isolates were retrieved at 12, 18, or 24 months.

Table 4. Frequencies of Nasopharyngeal Pneumococcal Carriage in Parents of Children Vaccinated With a 2-Dose or 2 + 1-Dose PCV-7 Schedule and Parents of Unvaccinated Controls at the Child's Age of 12 and 24 Months^a

Child's Age at 12 mo						Child's Age at 24 mo					
Control Group (n = 305)		2-Dose Schedule Group (n = 319)		2 + 1-Dose Schedule Group (n = 328)		Control Group (n = 296)		2-Dose Schedule Group (n = 309)		2 + 1-Dose Schedule Group (n = 321)	
No. (%)	No. (%)	Relative Risk (95% CI)	No. (%)	Relative Risk (95% CI)	No. (%)	No. (%)	Relative Risk (95% CI)	No. (%)	Relative Risk (95% CI)	No. (%)	Relative Risk (95% CI)
VT	29 (10)		26 (8)	0.86 (0.52-1.42)	29 (9)	25 (8)		16 (5)	0.61 (0.33-1.12)	18 (6)	0.66 (0.37-1.19)
NVT	55 (18)		50 (16)	0.87 (0.61-1.23)	48 (15)	25 (8)		47 (15)	1.80 (1.14-2.85)	55 (17)	2.03 (1.30-3.17)
ALL	84 (28)		74 (23)	0.84 (0.64-1.10)	75 (23)	50 (17)		63 (20)	1.21 (0.86-1.69)	71 (22)	1.31 (0.95-1.81)

Abbreviations: ALL, all pneumococcal serotypes; CI, confidence interval; NVT, nonvaccine serotype; PCV-7, 7-valent pneumococcal conjugate vaccine; VT, vaccine serotype.

^a Analyses were based on intention-to-treat and include all available data from parents of randomized infants in the assigned groups; missing data are due to loss to follow-up or incidentally missed home visits (eg, illness, parent not present at visit). Parents of twins (n = 15) were excluded. Parents with a transnasal, transoral, or both nasopharyngeal swabs positive for *Streptococcus pneumoniae* were considered positive.

rototype pneumococci filling the ecological vacant nasopharyngeal niche and counterbalancing the reduction in vaccine serotype carriage, thereby potentially causing increased nonvaccine serotype disease.^{24,25,32} This replacement phenomenon seems to be more common in otitis media with a direct connection to the nasopharynx via the Eustachian tube but much less in IPD.³³ For IPD, a discrete increase of nonvaccine serotype-associated episodes has been reported but particularly among high-risk populations and elderly persons.^{2,34-36} In our study, we also observed nasopharyngeal serotype replacement in vaccinated children, but the reduction in vaccine serotype carriage still resulted in a net decrease in overall pneumococcal carriage in children. The observed net decrease in our study may however disappear with time after widespread PCV-7 implementation when nonvaccine serotypes may become more frequent colonizers of the nasopharynx in the community.

The sample size in our study was not adequately powered for detecting significant changes in vaccine serotype carriage in parents. However, we observed an increase of nonvaccine serotype carriage in adult contacts of vaccinees over time. This suggests that serotype replacement in vaccinated children leads to a prolonged period of increased colonization in parents because of increased exposure of these adults to a higher diversity of pneumococcal serotypes. Our observation is in line with studies from Alaska, where an increase in nonvaccine serotype carriage and IPD was observed in Alaskan Native adults, who are highly susceptible to IPD, upon PCV-7 vaccination of children.^{34,37} Furthermore, parents of 12-month-old children in the control group showed high pneumococcal carriage rates (28%) compared with adults from the general population in a previous study in the Netherlands,¹⁹ but these higher carriage rates decreased over time in parents of children in the control group.

Considering the increase of nonvaccine serotype carriage in vaccinated

children and adult contacts and the potential loss of natural boosting after widespread implementation of reduced-dose PCV-7 schedules, monitoring of pneumococcal carriage and disease remains mandatory in all age groups. A booster dose may be necessary for long-term protection.^{38,39} Surveillance will provide us with timely information on vaccine efficacy and potential shifts in serotype distribution that may require adjusting vaccine strategies such as extending vaccine-valency. Considering the observed increase in serotype 19A and the reported disease potential, serotype 19A seems an important future vaccine candidate.⁴⁰

To appreciate our results, some potential limitations should be addressed. First, we used a single colony-method for serotyping and other simultaneously carried serotypes may have been missed. Considering the reported low rate (1%-8%) of multiple serotype carriage by others, the use of multiple colony serotyping would not have substantially affected our results.^{24,41} Second, our study was not powered to detect changes in children at 6 months, when pneumococcal carriage rates are still relatively low in the Netherlands, similar to most western countries. The study by O'Brien et al²⁴ observed a significant reduction in vaccine serotype carriage in vaccinated children at 7 months, but this study was performed in a high-risk population already at its peak vaccine serotype carriage rate, in contrast with our study where the peak was later at 12 months. Third, when extrapolating our results to other countries, especially nonwestern countries, geographical differences need to be taken into account (eg, carriage dynamics, serotype distribution). Finally, we chose not to correct for multiple testing in the statistical analysis of our secondary outcomes, because such multiple testing in our trial with different hypotheses and dependency between data are too conservative. However, the results of the significant secondary outcomes need to be cautiously interpreted and we need to be aware that significant results may

have occurred by chance. Therefore, to confirm these results, the corresponding hypotheses have to be tested in confirmatory studies.

Strengths of our study include the randomized controlled study design with an adequate sample size, virtual absence of loss to follow-up, high sampling rates (99%), and relatively high carriage rates compared with other western countries. The level of antibiotic resistance that may result in selection of multiresistant serotypes (eg, serotype 19A) in the United States is very low in the Netherlands.⁴² Finally, the study ended well before potential herd effects of the introduction of PCV-7 in the Dutch National Immunization Program could have affected our results.

In conclusion, both 2-dose and 2 + 1-dose schedules of PCV-7 significantly reduce vaccine serotype pneumococcal carriage in children. This study supports future implementation of reduced-dose PCV-7 schedules.

Author Contributions: Drs van Gils and Sanders had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data: van Gils, Veenhoven, Hak, Bogaert, van Alphen, Sanders.

Drafting of the manuscript: van Gils, Veenhoven, Hak, Bogaert, van Alphen, Sanders.

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Study supervision: Veenhoven, Sanders.

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