Pneumococcal Nasopharyngeal Carriage following Reduced Doses of a 7-Valent Pneumococcal Conjugate Vaccine and a 23-Valent Pneumococcal Polysaccharide Vaccine Booster

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This study was conducted to evaluate the effect of a reduced-dose 7-valent pneumococcal conjugate vaccine (PCV) primary series followed by a 23-valent pneumococcal polysaccharide vaccine (23vPPS) booster on nasopharyngeal (NP) pneumococcal carriage. For this purpose, Fijian infants aged 6 weeks were randomized to receive 0, 1, 2, or 3 PCV doses. Within each group, half received 23vPPS at 12 months. NP swabs were taken at 6, 9, 12, and 17 months and were cultured for Streptococcus pneumoniae. Isolates were serotyped by multiplex PCR and a reverse line blot assay. There were no significant differences in PCV vaccine type (VT) carriage between the 3- and 2-dose groups at 12 months. NP VT carriage was significantly higher (P < 0.01) in the unvaccinated group than in the 3-dose group at the age of 9 months. There appeared to be a PCV dose effect in the cumulative proportion of infants carrying the VT, with less VT carriage occurring with more doses of PCV. Non-PCV serotype (NVT) carriage rates were similar for all PCV groups. When groups were pooled by receipt or nonreceipt of 23vPPS at 12 months, there were no differences in pneumococcal, VT, or NVT carriage rates between the 2 groups at the age of 17 months. In conclusion, there appeared to be a PCV dose effect on VT carriage, with less VT carriage occurring with more doses of PCV. By the age of 17 months, NVT carriage rates were similar for all groups. 23vPPS had no impact on carriage, despite the substantial boosts in antibody levels.

A recent review estimated that in the year 2000, more than 14 million episodes of serious pneumococcal disease occurred worldwide, with more than 800,000 deaths of children under the age of 5 years (30). Since the introduction of the 7-valent pneumococcal conjugate vaccine (PCV) in the United States, there has been a dramatic reduction in vaccine type (VT) invasive pneumococcal disease (IPD) and a modest increase in non-vaccine type (NVT) IPD (43), particularly due to serotype 19A (32). Since nasopharyngeal (NP) carriage is an antecedent event in IPD, the reduction or prevention of NP carriage may reduce the transmission of pneumococci. This has occurred in the United States, where the widespread use of the infant PCV has resulted in significant protection of unimmunized individuals (12, 32), presumably mediated by reduced NP carriage interrupting the transmission of pneumococci (12, 25).

Clinical trials using 5-, 7-, or 9-valent pneumococcal conjugate vaccines have shown a reduction in VT carriage compared with that for unvaccinated infants (21, 23, 27) or toddlers (5, 6, 42). However, the overall rate of NP pneumococcal carriage remained essentially unchanged, due to serotype replacement with NVT pneumococci (5, 10, 23, 24, 27). Since the routine introduction of PCV into infant national immunization schedules, a number of carriage surveys have documented the effect of PCV on NP pneumococcal carriage. Like the clinical trials, all these studies have found that there has been a reduction in VT carriage (4, 8, 11, 15, 17, 18, 26, 34). NVT colonization has increased following vaccination, with serogroups 11 and 15 being commonly reported (4, 8, 11, 15, 18, 34), and more recently, the newly identified serotype 6C has been reported (31).

In the United Kingdom and some Scandinavian countries, a 2-PCV-dose schedule in infancy, followed by a PCV booster toward the end of the first year of life, is routinely given. Little is known about the effects of reduced-dose PCV schedules on carriage and the consequent effects on herd immunity. Only one other randomized controlled trial that evaluated the effects of reduced-dose pneumococcal conjugate vaccine schedules on NP carriage has been published (40). Since serotype replacement of NVT has occurred in both NP carriage and IPD with the routine use of the infant PCV, it is important to improve our understanding of this fine balance in order to...
determine the number of PCV doses that is likely to provide protection from serious pneumococcal disease but may have a lesser impact on carriage, in an attempt to minimize serotype replacement.

In Fiji a vaccine trial with the aim of finding an optimal pneumococcal vaccination strategy for resource-poor countries has been completed. A phase II study was undertaken documenting the safety, immunogenicity, and impact on carriage of various schedules combining 1, 2, or 3 doses of PCV in infancy, followed by a booster consisting of the 23-valent pneumococcal polysaccharide vaccine (23vPPS). In this report, the effects of the different vaccination schedules on overall NP pneumococcal carriage rates are presented, along with rates of VT and NVT carriage at the ages of 6, 9, 12, and 17 months.

MATERIALS AND METHODS

**Study design.** Details of the study design and randomization procedures have been reported elsewhere (16, 37). In brief, following informed consent, healthy infants in Suva, Fiji, were stratified by ethnicity and, within each stratum, were randomized to receive either no vaccine or 1, 2, or 3 doses of PCV (Prevenar, containing serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F at 2 μg/serotype except for serotype 6B [4 μg]; Wyeth Vaccines) at the ages(s) of 6 weeks, 6 and 14 weeks, or 6, 10, and 14 weeks. The vaccines administered at the ages of 6, 10, and 14 weeks were Trilance-HepB, Hiberts, and oral polio vaccine. At the age of 12 months, half the infants were randomized to receive 23vPPS (Pneumovax, 25 μg/serotype; Merck & Co., Inc.), and all infants received a microdose (5 μg/serotype) of 23vPPS (mPPS) at the age of 17 months. All children received a measles-rubella vaccine at the age of 12 months. The children randomized to receive no PCV or 1 PCV dose in infancy had a single dose of PCV administered at the age of 2 years. Table 1 shows the vaccination and blood collection schedules for the eight groups.

The study was approved by the Fiji National Research Ethics Review Committee and the University of Melbourne’s Human Research Ethics Committee. Parents or legal guardians signed a written document giving informed consent for their children to participate in the study. The study was conducted and monitored according to good clinical practice.

**Nasopharyngeal swabs.** Buffered cotton NP swabs (aluminum shaft-buffered; Sarstedt, Australia) were taken at the ages of 6, 9, 12, and 17 months by horizontal insertion into the nares by trained personnel. The swab was left in situ for 5 s and was rotated, after which it was immediately placed in a sterile cryovial (Simport, Canada) containing 1 ml of skim milk-tryptone-glucose-glycerol (STGG) transport medium (28). This was transported in a chilled carrier to the Colonial War Memorial Hospital Laboratory, Suva, Fiji, on the same day. The swabs were processed according to the consensus guidelines of a World Health Organization working group (29). The swabs were vortexed and either were stored at −70°C until plating or were plated upon receipt in the laboratory. Fifty microliters was inoculated onto a 2.5-mglitter gentamicin-5% citrated sheep blood (36) Columbia agar (Oxoid) plate. Plates were incubated at 37°C under 5% CO₂ for 18 to 24 h. Pneumococcal isolates were initially identified by alpha-hemolysis, colony morphology, and optochin (Difco) sensitivity. Isolates with intermediate optochin sensitivity were confirmed as pneumococci by bile solubility testing. Single colonies were subcultured, and pure colonies were sent to the Pneumococcal Reference Laboratory, Centre for Infectious Diseases and Microbiology, ICPMR, Westmead, NSW, Australia, where they were serotyped by multiplex PCR and a reverse line blot assay (20, 45). Ten percent were also serotyped by a Quellung reaction using specific antisera (Statens Serum Institute, Copenhagen, Denmark). Any discrepancy in serotype between the 2 methods was resolved by a Quellung reaction. Laboratory staff members were blinded to the group allocation for each isolate.

**Questionnaires.** At each of the 4 NP swab study visits, the following information was collected by the study nurse in a parent/guardian interview: the number of children in the household who were ≤5 years old, family income, exposure to household cigarette smoking, breastfeeding status, whether the child had symptoms of an upper respiratory tract infection (coryza or cough) at the time of the visit, and whether the child had received antimicrobials in the preceding 2 weeks. The nurse did not ask about attendance at child care, since this is uncommon in Fiji.

**Statistical analysis.** All case reporting forms were monitored prior to data entry. Double data entry was performed on all case report forms. Cleaned data were exported to Stata, version 9.0 (Stata Corporation, College Station, TX) for analysis. Serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F were classified as VT; all other viable isolates, including those that were nontypeable, were classified as NVT. Rates of NP carriage were calculated using the number of total pneumococcal, VT, or NVT isolates in each group at each time point divided by the total number of children who had an NP swab taken in each group at each time point. Analyses comparing the proportions of infants with NP carriage of any pneumococcus, VT pneumococci, and NVT pneumococci were performed using Fisher’s exact test. For each group, the cumulative proportion of children carrying VT pneumococci was calculated. Due to the multiple comparisons, a P value of <0.01 was considered statistically significant.

**RESULTS**

A total of 552 children were enrolled in the study. The characteristics of the study children are shown in Table 2. Age at enrollment was comparable across the groups, but there were minor differences in some of the characteristics, such as higher exposure to cigarette smoking in the 2-PCV-dose group. Of the 2,208 NP swabs planned, 90.8% were taken; of these, 47.8% were positive for Streptococcus pneumoniae. There were 959 pneumococcal isolates, of which 33 (3.4%) were nontypeable and 13 (1.4%) were no longer viable for serotyping.

Table 3 shows the impacts of the different vaccination schedules on overall NP carriage in the first 12 months of life. There were no significant differences in NP pneumococcal carriage between any of the groups at any time point. There was a trend toward higher NP pneumococcal carriage rates in the single-PCV-dose group than in the unvaccinated group at the age of...
6 months (55% versus 42%; \( P, 0.04 \)). For NP VT carriage, the rates were significantly higher in the unvaccinated group at 9 months (16% versus 3%; \( P, <0.01 \)) than in the 3-dose group. There was a trend toward higher NP VT carriage rates (10% versus 3%; \( P, 0.03 \)) for the 2-PCV-dose group compared with the 3-PCV-dose group and for the unvaccinated group compared with the single-dose group (16% versus 7%; \( P, 0.03 \)) at the age of 9 months, as well as for the unvaccinated group compared with the 3-dose group at 12 months (16% versus 7%; \( P, 0.03 \)). Figure 1 shows little difference in the cumulative proportions of infants carrying VT pneumococci at the age of 6 months. However, there were substantial differences between the PCV groups and the unvaccinated group in the cumulative proportions of children carrying VT pneumococci at the ages of 9 and 12 months. Moreover, there appeared to be a PCV dose effect on the cumulative proportions of infants carrying VT pneumococci at the ages of 9 and 12 months, with the 3-PCV-dose group having the lowest cumulative proportion compared with the 2-dose and single-dose groups.

For NP NVT carriage, there were no significant differences between any groups at any time point. Still, there was a trend toward higher NP NVT carriage in the 3-dose group compared with the unvaccinated group at the ages of 6 (40% versus 24%; \( P, 0.03 \)) and 9 (43% versus 27%; \( P, 0.01 \)) months and in the single-dose group compared with the unvaccinated group at the ages of 6 (43% versus 24%; \( P, 0.01 \)), 9 (42% versus 27%; \( P, 0.03 \)), and 12 (43% versus 29%; \( P, 0.01 \)) months.

At the age of 17 months, there were no significant differences in total pneumococcal and VT carriage rates between those groups that had or had not received 23vPPS or by number of PCV doses (Fig. 2). There was a trend toward higher VT carriage among those that had received no PCV or 1 PCV dose than in the 2- or 3-PCV-dose groups (Fig. 2). Figure 1 shows the cumulative proportion of VT carriage at 17 months by PCV and 23vPPS group allocation. Minimal differences can be seen in VT carriage within each PCV group, whether individuals had or had not received 23vPPS at the age of 12 months. However, those who had received 23vPPS alone had the highest cumulative proportion of VT carriage at the age of 17 months (\( P, <0.001 \)) compared to the group receiving 3 PCV doses only. There were no significant differences in NVT carriage rates between those groups that had or had not received 23vPPS at the age of 12 months.

Serotypes 19F and 23F were the most frequently carried PCV serotypes in all groups in the first 12 months, and 6B was
the most frequently carried serotype by the age of 17 months.

Of the non-PCV serotypes, 6A and 19A were the most frequently carried serotypes throughout the study.

**DISCUSSION**

Our findings suggested a PCV dose effect on VT carriage, with 3 PCV doses appearing to have a greater effect on VT carriage than fewer PCV doses. Unfortunately, the sample size was too small to evaluate the impact of the different vaccination groups on the carriage of individual serotypes. We found that a single PCV dose results in some initial reduction in VT carriage (though not statistically significant), which, along with

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**TABLE 3.** Nasopharyngeal (NP) carriage of all pneumococcal serotypes, 7-valent pneumococcal conjugate vaccine (PCV) serotypes (VT), and non-PCV serotypes (NVT) at the ages of 6, 9, and 12 mo following no vaccination or the administration of 1, 2, or 3 doses of PCV as a primary series.

| Age | No. receiving 1 PCV dose (%) vs 2 PCV doses (%) | Comparison (1 PCV dose vs no vaccination) | OR (95% CI) | Comparison (1 PCV dose vs 3 PCV doses) | OR (95% CI) | Comparison (2 PCV doses vs 3 PCV doses) | OR (95% CI) |
|-----|-----------------------------------------------|----------------------------------------|------------|---------------------------------------|------------|---------------------------------------|------------|
| 6 m | 64/127 (50) vs 71/148 (48)                     | No. receiving 1 PCV dose (%)           | 0.72       | 1.1 (0.68–1.77)                       |            | 1.44 (0.87–2.36)                     | 0.17       |
| 9 m | 13/127 (10) vs 16/148 (11)                     | No. receiving 1 PCV dose (%)           | 0.84       | 0.88 (0.38–2.0)                       |            | 0.61 (0.26–1.42)                     | 0.29       |
| 12 m| 8/114 (7) vs 9/143 (6)                        | No. receiving 1 PCV dose (%)           | 1.0        | 1.04 (0.39–2.78)                      |            | 0.75 (0.29–1.98)                     | 0.63       |
|     | 51/127 (40) vs 53/148 (36)                    | No. receiving 1 PCV dose (%)           | 0.54       | 1.17 (0.72–1.9)                       |            | 0.9 (0.54–1.5)                       | 0.54       |
|     | 31/127 (24) vs 36/125 (29)                    | No. receiving 1 PCV dose (%)           | 0.03       | 1.78 (1.05–3.04)                      |            | 1.63 (0.97–2.75)                     | 0.07       |
immunogenicity data (35), provides supportive evidence that one dose may offer some early protection from IPD. Early differences (though not statistically significant) in NVT carriage rates between groups were no longer evident by the age of 17 months. Furthermore, the addition of 23vPPS at the age of 12 months had no impact on carriage, despite the substantial boosts in antibody levels observed (38).

One of the key questions is the duration of the effect of reduced-dose schedules on NP carriage. In this study we found that the reduction in VT carriage rates was sustained to the age of 17 months following a 3- or 2-PCV-dose schedule. There is only one other randomized and published study reporting the effect of reduced-dose PCV schedules on NP carriage. This study, from the Netherlands, compared carriage rates following 2 PCV doses at the ages of 2 and 4 months versus a 2-plus-1 schedule at the ages of 2, 4, and 11 months or no vaccination (control group). Both vaccinated groups had significantly lower rates of VT carriage in the second year of life than controls (40). The booster dose resulted in lower rates of VT carriage at 18 months than those seen with no booster dose (24% versus 16%). However, by the age of 2 years, the two vaccinated groups had similar VT carriage rates (15% each) (40). Similarly, in a case-control study, Gambian infants vaccinated with either 3 or 2 doses of a 5-valent pneumococcal conjugate vaccine in infancy, followed by 23vPPS at the age of 18 months, showed significantly lower VT carriage rates than unvaccinated matched controls at the age of 2 years (27).

There are a number of studies evaluating the duration of the effect of a 3-PCV-dose schedule on VT carriage. In one study, the effects on VT colonization were no longer evident by the ages of 2 to 5 years following 3 PCV doses in infancy and the 23vPPS at the age of 13 months (21). Similarly, a study in South Africa found no differences among non-HIV-infected children in VT colonization in the 3-PCV-dose group compared with the placebo group 5 years after vaccination in infancy (22a). In contrast, in the Gambia, the effects on carriage persisted at least 16 months postvaccination when 3 doses of an infant 9-valent pneumococcal conjugate vaccine were administered (2). In another study, following the vaccination of toddlers under the age of 2 years, the reduction in VT carriage continued for at least 1 year (6); in 2 other studies, it continued to the age of 4 years but not beyond (5, 42). Since the routine introduction of PCV into national infant immunization schedules, a number of carriage surveys have documented the effect of PCV on NP pneumococcal carriage. Like the clinical trials, all these studies have reported a reduction in VT carriage (4, 8, 11, 15, 17, 18, 26, 34), particularly in those children who are up to date with their immunizations (4, 17, 26), have had a PCV booster in the second year of life (4, 11), or have not had prolonged intervals between PCV doses (17).

One of the disadvantages of the currently available PCV is serotype replacement by NVT pneumococci filling the vacant ecological niche following vaccination (22). In our study, higher (though not statistically significant) NVT carriage rates were found in the PCV groups than in unvaccinated controls in the first 12 months of life. However, by the age of 17 months, this trend was no longer evident, suggesting that this initial effect was not sustained. Our NVT carriage rate (approximately 40%) is higher than those reported by the reduced-dose Netherlands study: 15 to 17% for the vaccinated groups and 8% for the unvaccinated control group (40). The NVT carriage rates reported in observational studies of children from industrialized countries following PCV tend to be lower (18% in the United States [11] and 15.5% in France [4]). However, NVT carriage rates following vaccination of children from nonindustrialized countries (77% in the Gambia [27] and 36% in South Africa [23]) and high-risk disadvantaged communities (39.2% for Navajo and White Mountain Apache children [24]) tend to be higher. This suggests that the impact of PCV on NVT carriage may be greater in geographical settings with high burdens of pneumococcal disease, since the direct and indirect effects of PCV differ with age and the presence of underlying conditions such as HIV (9). Ongoing surveillance is needed to detect changes in rates of NVT IPD, in addition to VT IPD, following the introduction of PCV.

To our knowledge, this is the first published randomized study to show the impact of a single PCV dose on NP carriage. There appeared to be a PCV dose effect on VT carriage, with a single PCV dose having less effect on VT carriage than a 2- or 3-dose schedule. A single PCV dose initially reduced VT carriage (though not statistically significantly), but this effect was not sustained, and by the age of 12 months, there was no difference in rates between the single-dose group and the unvaccinated controls. The single-dose group had a higher (though not statistically significantly) NVT carriage rate than unvaccinated controls up to the age of 12 months, but there were no significant differences in NVT carriage rates between any of the groups by the age of 17 months. In contrast, the results from a series of prevalence surveys in Canada showed that children who had received no vaccination or 1 or 2 PCV doses were less likely to be colonized with NVT pneumococci than those who had received 3 PCV doses (18). In a small observational study coinciding with a shortfall of PCV supply in the United States, NVT carriage rates were similar for those children who had received 1 or 2 doses (22 versus 27%) but higher for the 3-dose group (47%) (17). The data from our study, combined with our previously reported immunogenicity data, in which a single PCV dose elicited significant responses for all serotypes post-primary series compared with the unvaccinated group (35), provide additional evidence that a single PCV dose in infancy would offer some protection in the first 12 months of life. Moreover, memory responses were most profound for children who had received only a single dose of PCV previously, compared with the 2- or 3-dose groups (38). The results from this study and previously published immunogenicity data (35, 38) raise the intriguing possibility that a schedule

| Pneumococcus | No. (%) of children with NP pneumococcal carriage: | P | OR (95% CI)* |
|--------------|---------------------------------|---|-------------|
|              | Boosted with 23vPPS | Not boosted with 23vPPS |       |            |
| All pneumococci | (n = 232) | (n = 240) |       |            |
| NVT          | 109 (47) | 111 (46) | 1.000 | 0.99 (0.71–1.4) |

* OR, unadjusted odds ratio; 95% CI, 95% confidence interval.
based on a single dose of vaccine early in infancy with an early booster might provide adequate protection while avoiding the problems of serotype replacement.

The 12-month 23vPPS booster had no additional effect on pneumococcal carriage rates and no statistically significant effect on VT or NVT carriage rates despite significant boosts in antibody levels (38). Similarly, studies using other pneumococcal polysaccharide vaccines have shown no effect on pneumococcal carriage (3, 7, 14, 34, 44). PCV followed by 23vPPS given to 1- to 7-year-old children with recurrent acute otitis media in the Netherlands showed no beneficial effect from the booster vaccine (41). In addition to having no effect on carriage, 23vPPS may have deleterious immunological effects. In this study, there was a suggestion that the VT carriage rate was higher at the age of 17 months in the group that received 23vPPS alone than in all other groups. Despite our study previously demonstrating the potential value of a PCV/23vPPS schedule (38), immunological hyporesponsiveness to a small rechallenge dose of 23vPPS has been demonstrated (37). These findings suggested that additional immunization with 23vPPS following a primary series of PCV does not provide an added benefit for antibody production and instead results in impaired immune responses following a subsequent pneumococcal polysaccharide antigen challenge.

Although 2 or 3 doses have an impact on VT carriage, the overall pneumococcal carriage rates did not change, due to the increase in VT carriage. This has been consistently found in many other studies in different settings (5, 8, 10, 11, 15, 18, 23, 24, 26, 33, 42). Considering this effect and the potential loss of natural boosting after widespread implementation of reduced doses, ongoing surveillance of the impact on carriage and IPD is important, since a booster dose may be necessary for long-term protection (13, 19).

In conclusion, although the sample size was small, there appeared to be a PCV dose effect on VT carriage, with less VT carriage occurring with more doses of PCV. A single PCV dose resulted in some initial reduction in VT carriage rates, and along with supportive immunogenicity data, this finding provides further evidence that a single dose would offer some protection from IPD. There was no significant difference in NVT carriage among the groups. The addition of 23vPPS at the age of 12 months had no impact on carriage.

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