ORIGINAL ARTICLE

Pneumococcal nasopharyngeal carriage in children following heptavalent pneumococcal conjugate vaccination in infancy

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Aims: To ascertain whether the reduction in nasopharyngeal carriage of vaccine serotypes induced by pneumococcal conjugate vaccine (PnCV) administered to infants persists beyond the age of 2 years.
Methods: Non-randomised, unblinded controlled study of 2–5 year old children who had received three doses of heptavalent PnCV (7VPnCV) in infancy and 23-valent pneumococcal polysaccharide vaccine at 13 months, and unimmunised controls. Nasopharyngeal swabs were taken in summer (150 vaccinated subjects, 126 controls) and winter (143 vaccinated subjects, 188 controls). The swabs were cultured and serotyped for *Streptococcus pneumoniae*.
Results: Carriage rates (vaccinated subjects: 24.7% and 43.4%; controls: 27.0% and 41.0%, in summer and winter respectively) and carriage of vaccine serotypes (subjects: 10.0% and 30.0%; controls: 13.5% and 31.5%, in summer and winter respectively) were similar in the two groups.
Conclusions: Effects of vaccination in infancy on rates of nasal carriage of pneumococcus and serotype replacement in children living in a largely unvaccinated population are no longer evident by 2–5 years of age.

Streptococcus pneumoniae is a major cause of bacterial otitis media, pneumonia, bacteraemia, and meningitis among infants worldwide. The main reservoir of pneumococci is the human nasopharynx. The mean age of first acquisition is 6 months and carriage rates peak in the preschool age group.[1] Carriers usually remain asymptomatic but may transmit the organism to other individuals.

Vaccines that prevent carriage in immunised subjects could lead to herd immunity—the protection of unvaccinated individuals by reducing the risk of transmission in the community. The introduction of *Haemophilus influenzae* type b (Hib) vaccine into the universal immunisation schedule has led to a dramatic reduction in the incidence of Hib disease. In addition there has been a decrease in carriage of this organism with no replacement by other serotypes.[2]

One might therefore expect immunisation with pneumococcal conjugate vaccine (PnCV) to have a similar effect on pneumococcal carriage. However, factors affecting carriage and serotype replacement are complex and it is likely that the effect of vaccination on carriage of different organisms will vary. Heptavalent pneumococcal conjugate vaccine (7VPnCV) given in infancy has been shown to be immunogenic and effective in preventing invasive pneumococcal disease caused by vaccine serotypes in the first two years of life.[3] A recent study reported that the number of episodes of acute otitis media, attributable to vaccine serotypes in infants immunised with a 7VPnCV in infancy, was reduced by 57%, but those attributed to other serotypes increased by 33%.[4] These findings are in agreement with other studies which have also shown that immunisation with PnCVs in infancy reduces nasopharyngeal carriage of vaccine serotypes,[5 6] but increases carriage of non-vaccine serotypes.[6–8]

This effect of immunisation on pneumococcal carriage may be a result of the generation of local mucosal immune responses against vaccine serotypes. However, these studies have only examined carriage within the first two years following immunisation of infants and young children. This study explores the effects of 7VPnCV on pneumococcal nasopharyngeal carriage in children aged 2–5 years who were immunised as infants.

MATERIALS AND METHODS

Study design

The study protocol was approved by the South Sheffield Local Research Ethics Committee. Two groups of children were newly recruited to this study between June and August 2000 after obtaining written informed consent from parents or guardians. The first group were healthy children who had received three doses of Wyeth-Lederle 7VPnCV (containing saccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F coupled to the protein carrier CRM197) in infancy aged 2, 3, and 4 months followed by a 23-valent pneumococcal polysaccharide vaccine (Wyeth-Lederle) booster at 13 months of age as part of a previous study conducted in Sheffield.[9] The families of all the children who had taken part in that study were contacted. The second group were healthy children who had not received any immunisation against *Streptococcus pneumoniae*. The families of these children were contacted through nurseries attended by the vaccinated children and through their primary care doctors and nurses. Information regarding number of siblings, antibiotic administration during the month preceding the visit, exposure to cigarette smoke, and day care attendance was recorded prior to sample collection. If the subject had received antibiotics in the preceding week, the collection was deferred.

Sample collection

Nasopharyngeal (NP) samples were collected in summer (June–August 2000) and again in winter (January–March 2001) by trained research nurses through a nostril with a flexible calcium alginate tipped swab (Medical Wire and Equipment Co Ltd, Bath, UK). Swabs were also collected from one or both parents where consent was obtained. NP swabs were immediately inoculated into 1 ml of skimmed milk

Abbreviations: 7VPnCV, heptavalent pneumococcal conjugate vaccine; Hib, *Haemophilus influenzae* type b vaccine; NP, nasopharyngeal; STGG, skimmed milk powder-tryptone-glycerol-glucose

Table 1 Demographic data

Group	Season	Mean age (months)	Day care		Siblings	Daycare and siblings			Nasal carriage of pneumococcus in parents	
			Number (%) attending	Mean hours of attenders per week	Number (%) with 1 or more siblings	Number (%) with a sib, or daycare or both	Number (%) exposed to cigarette smoke	Number (%) using antibiotics in previous 4 weeks	Father, number (%) positive	Mother, number (%) positive
Control (n=126)	Summer	36.4	105 (83)	13.3	91 (72)	122 (97)	40 (32)	11 (8.7)	1/23 (4.3)	5/115 (4.3)
Vaccinated (n=150)	Summer	33	73 (49)	13.2	100 (67)	126 (84)	43 (29)	8 (5.3)	2/36 (5.6)	3/138 (2.2)
Control (n=188)	Winter	39.9	152 (81)	14.7	139 (74)	181 (96)	59 (31)	16 (8.5)	1/32 (3.1)	4/171 (2.3)
Vaccinated (n=143)	Winter	40.3	121 (85)	16.6	101 (71)	139 (97)	36 (25)	14 (9.8)	1/26 (3.8)	4/122 (3.3)

Daycare included any setting outside the family home where the child spent time on a regular basis and came in contact with two or more unrelated children. For the purpose of this study, a child was considered to be attending daycare if they spent at least four hours per week outside the family home in such settings.

powder-tryptone-glycerol-glucose (STGG) broth and transported in a cool box (2–8°C) to the laboratory where they were stored at −80°C for batch processing.

Bacteriology

NP samples were thawed to room temperature, 50 µl of broth plated onto blood agar plates and incubated for 16–18 hours at 37°C in an atmosphere containing 5% CO_2. *S pneumoniae* was identified by colony morphology, susceptibility to optochin, and bile solubility. Positive cultures were serotyped using the Quellung reaction with antiserum obtained from the Statens Serum Institut, Denmark. Samples were analysed blind.

Statistical analysis

Mean ages of the groups of children were compared using *t* tests; percentages of carriage of *S pneumoniae* (all serotypes and only vaccine serotypes), antibiotic use, and exposure to cigarette smoke were calculated for each season and compared using the χ^2 test. Day care attendance was defined as a setting outside the family home where the child spent at least four hours per week on a regular basis and came in contact with two or more unrelated children. Percentages of children meeting this definition for day care attendance were calculated for both groups at each phase of the study and compared using the χ^2 test; $p < 0.05$ was considered significant for all comparisons. The average number of hours of day care attendance was also calculated for each group, including only those children who attended day care for more than four hours a week

RESULTS

Description of study groups

Swabs were obtained from 150 of 267 vaccinated children in the summer and from 143 of them in the winter, and from 126 healthy controls in the summer and 188 in the winter. In the summer, swabs were obtained from 91.3% and 92% of mothers and from 18.3% and 24% of fathers in the control and study groups respectively. In the winter, swabs were obtained from 91% and 85.3% of mothers and from 17% and 18.2% of fathers in the control and study groups respectively.

Table 1 shows demographic characteristics of the children in the two groups. Mean age was 36.4 months (range 24.6–59.5) in the control group and 33.0 months (range 26.8–38.1) in the study group for the first (summer) phase, and 39.9 months (range 26.0–65.6) and 40.3 months (range 33.6–46.6) in the winter for the two groups respectively ($p = 0.5$, no significant difference, for both phases). In the summer only, significantly more children in the control group attended day care (83% *v* 49%, $p < 0.05$). For the children attending day care in the two groups, the mean hours attended per week were similar (13.3 hours in control group, 13.2 hours in study group). In the winter, the percentage attending day care was similar in the two groups (81% of controls and 85% of study subjects, $p = 0.5$); average attendance was 14.7 hours in controls and 16.6 hours in study subjects. There were no significant differences ($p > 0.05$) between the groups when comparing exposure to cigarette smoke, antibiotic use in the four weeks prior to swabbing, average number of siblings per subject, and number of subjects with one or more siblings.

Nasopharyngeal carriage rates

The NP carriage rate of *S pneumoniae* in summer was 24.7% and 27% in vaccinated subjects and controls respectively ($p = 0.7$, no significant difference; fig 1). Carriage rates with vaccine serotypes were similar in the two groups (vaccinated subjects = 10%, controls = 13.5%, $p = 0.45$). Total carriage rates increased significantly in winter in both groups (vaccinated subjects = 43.4%, controls = 41%; $p = 0.01$ and $p = 0.05$ relative to summer, respectively, $p = 0.8$ between groups), and again carriage rates with vaccine serotypes were

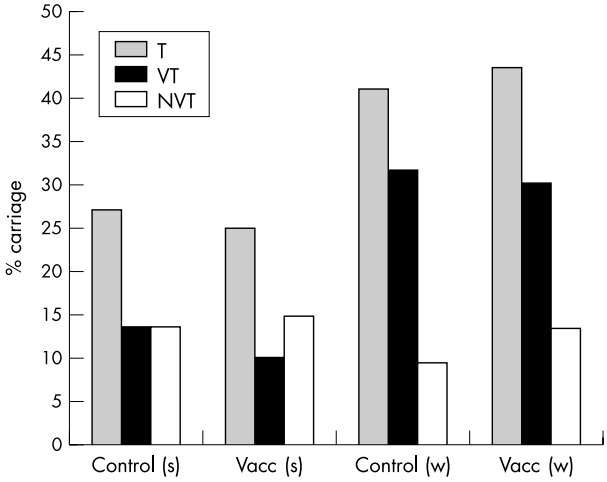

Figure 1 Pneumococcal carriage in summer (s) and winter (w) in control and vaccinated (vacc) subjects. T, total carriage; VT, carriage of vaccine serotypes; NVT, carriage of non-vaccine serotypes.

similar in the two groups (vaccinated subjects = 30%, controls = 31.5%, p = 0.8). Carriage rates in parents were low in all groups (see table 1).

DISCUSSION

The two groups of children were well matched for age, sex, exposure to cigarette smoke, and administration of antibiotics in the preceding four weeks. In the summer phase, a significantly greater percentage of the control group children attended day care—a difference which, if anything, would be expected to exaggerate any apparent vaccine induced reduction in carriage; but in the winter, day care attendance rates were similar in the two groups. There was no clustering of study or control children in the same families or the same day care settings, which could have influenced the carriage rate. The methodology and laboratory processing used was identical for both groups. While previous studies have tended to culture swabs directly onto blood agar plates, storage of swabs in STGG broth at −80°C and batch processing has been reported to be equally sensitive with little loss of bacterial viability.[10] The overall carriage rate (34.2%) is similar to those reported in previous studies for developed countries.[2 11] In agreement with some previous studies[12] and in contrast to others,[13] we found carriage rates to increase significantly during the winter months. The increase in total carriage with season was predominantly caused by increased carriage of the commoner vaccine serotypes (20% increase in study subjects and 18% increase in controls in winter).

Several studies have shown that vaccination with PnCV reduces carriage of vaccine serotypes in the first 1–2 years after immunisation.[5–8] Serotype replacement has been shown in some studies[6 7] but not in others.[5 8] Dagan and colleagues[14] reported that vaccination of toddlers aged 12–35 months with PnCV reduces pneumococcal carriage in their younger siblings. An effect of PnCV immunisation on nasopharyngeal carriage could lead to herd immunity and reduced colonisation and transmission of common virulent and antibiotic resistant strains. Accordingly, provided there was no significant serotype replacement, such immunisation could reduce the incidence of mucosal infections as well as invasive disease.

Young children aged 2–3 years have significant carriage rates. Day care attendance may be important for the transmission of the organism to the susceptible young and indirectly to the elderly in the community. This study suggests that immunisation in infancy with PnCV reduces pneumococcal carriage little or not at all and does not affect its seasonal fluctuation in this preschool age group. The absence of differences in the carriage rates of vaccine serotypes seen in the two groups in this study may reflect a waning of the systemic or mucosal immune responses with time in the vaccinated children[15]; these responses may be most prominent only for the early months following immunisation. However, there is no evidence that invasive pneumococcal disease increases with any such waning of mucosal immunity. Additionally, differences may become less prominent as mucosal immune responses develop naturally because of pneumococcal carriage. A maturation phenomenon leading to a reduction in pneumococcal carriage and specifically a reduction in colonisation with many of the serotypes found in the vaccine with increasing age has been suggested,[16] and was corroborated by the unusually low carriage rates we showed in the parents of these children.[17] However, there were no significant differences observed in the rates of carriage of this group of children, when divided into two groups around the median age, either in summer (younger 27.5%, older 23.9%) or in winter (younger 41.5%, older 44.2%).

Absence of increased carriage rates by non-vaccine serotypes in the vaccinated group suggests that, while in the months immediately after immunisation such effects may be seen, in the long term they do not persist. However, our study reflects the results of vaccination of a small group of subjects living in an unvaccinated population; the effects on carriage in children of this age when the majority of the population is vaccinated may be different, as in the case of Hib vaccine,[18] particularly if there is a catch up programme of immunisation in preschool children when the vaccine goes into general use. Likewise serotype replacement may become a general issue for pneumococcus even though this has not been shown with Hib vaccination.[2] It is also uncertain what differences may result from boosting with 23 valent polysaccharide vaccine in the second year, as reported in this study group, as opposed to using a fourth dose of 7VPnCV as is routine in the USA. One study has suggested that seroresponses to some serotypes following a second dose of pneumococcal polysaccharide vaccine can be poorer than in children receiving a first dose,[19] so it is conceivable that the strategy we used could be relatively disadvantageous if there are parallels between parenteral challenge with vaccine and mucosal challenge with live encapsulated organisms.

In conclusion, this study examined the effect of PnCV on pneumococcal carriage in young children aged 2–5 years immunised as infants in the UK. It suggests that the effects of such vaccination on pneumococcal carriage may be less clinically important in this age group than in infants, at least in this setting.

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