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Immunogenicity and serotype-specific efficacy of a 9-valent pneumococcal conjugate vaccine (PCV-9) determined during an efficacy trial in The Gambia

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ABSTRACT

This study aimed to determine the immunogenicity of a 9-valent pneumococcal conjugate vaccine (PCV-9) in a subgroup of Gambian children enrolled in a large vaccine efficacy trial. To place the antibody results in context, in this paper we also report previously unpublished data on serotype-specific clinical vaccine efficacy from the main trial. In the sub-study, a single 2–4 ml venous blood specimen was collected from 212 Gambian children 4–6 weeks after the administration of a third dose of PCV-9 or placebo. IgG antibodies to pneumococcal serotype 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F polysaccharides were measured by ELISA. The proportions of infants with antibody concentrations above 0.2, 0.35 and 1.0 μg/ml, and the geometric mean concentrations (GMCs) of anti-pneumococcal polysaccharide antibodies were substantially higher for each serotype in children who received three doses of PCV-9 than those in the placebo group. Among PCV-9 recipients, GMCs ranged between 2.61 and 11.09 μg/ml with the highest being against serotype 14 and the lowest against 9V polysaccharide. The estimated overall protective antibody level for all nine serotypes, based on the vaccine efficacy against vaccine-type invasive pneumococcal disease (IPD) of 77% (95% CI: 51, 90) observed in the trial, was 2.3 μg/ml (95% CI: 1.0, 5.0). The PCV-9 studied was immunogenic in a Gambian population where it was also found to be efficacious.

1. Introduction

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide, particularly in young children and individuals with specific risk factors [1,2]. The annual incidence of invasive pneumococcal disease (IPD) among children aged <2 years in industrialized countries can be as high as 27 cases per 100,000 [3,4] and several-fold higher in developing countries, where the pneumococcus causes an estimated 1.2 million deaths among young children annually, mostly due to pneumonia [5,6]. Of special public health concern in both industrialized and developing world settings, has been the rapid rise in the proportion of pneumococcal strains exhibiting intermediate or high levels of antibiotic resistance [7,8].

In The Gambia, *S. pneumoniae* is the most prevalent bacterial pathogen isolated from children with pneumonia [9,10] and this bacterium is also responsible for about 50% of cases of pyogenic meningitis [11]. In rural parts of The Gambia, about 50% of cases of invasive pneumococcal disease in children are seen before the age of 1 year and 20% before the age of 6 months [9]. Approximately 80% of the serogroups responsible for invasive disease in young children are contained in the 9-valent vaccine [9,12,13]. This vaccine contains conjugates of serotypes 1 and 5 which accounted for about 33% of cases of invasive pneumococcal disease in a Gambian population with the mean age of 15.3 years (median 4 years) [13]. Pneumococcal conjugate vaccines offer a realistic prospect of reducing mortality and morbidity from pneumococcal infections [14–16].

We have reported previously [16] that in the most easterly part of The Gambia, a 9-valent pneumococcal conjugate vaccine (PCV-9) gave 37% protection against radiological pneumonia, 77% protection against vaccine serotype-specific pneumococcal disease, 50% protection against all invasive pneumococcal disease and 15% and 16% protection against hospital admission and overall mortality,

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respectively, when analysed per-protocol (with minor reductions in efficacy in intent-to-treat analyses). Here, we report, the immunogenicity of the PCV-9 in a sub-study of 212 of the 17,437 children recruited into the efficacy trial, the estimated protective antibody concentrations, and serotype-specific clinical vaccine efficacy in the main trial.

2. Methods

This immunogenicity sub-study was nested within the Gambian pneumococcal vaccine trial (PVT), a large efficacy trial carried out in a rural area of Gambia where the prevalence of HIV-1 infection among antenatal clinic attendees has remained stable at about 1% [17]. The climate of the study area is highly seasonal. Malaria transmission during the rainy season of July–November and immediately afterwards is moderately high. *Plasmodium falciparum* is the dominant malaria parasite. The PVT was a double blind, placebo-controlled, randomized trial undertaken between August 2000 and April 2004. The study design and methods of the trial have been described in detail previously [16]. Briefly, 17,437 children were enrolled, half of whom were randomized to receive PCV-9 (reconstituted with Diphtheria–Pertussis–Tetanus–*Haemophilus influenzae* type b (DPT–Hib) vaccine) and half placebo mixed with DPT–Hib; all received concurrent oral polio and hepatitis B vaccines according to the national immunization schedule. The PCV-9 (Wyeth Vaccines, Collegeville, PA, USA) contains 2 μg of types 1, 4, 5, 9V, 14, 19F and 23F polysaccharides, 4 μg of type 6B polysaccharide, and 2 μg of type 18C oligosaccharide linked to the mutant diphtheria CRM_{197} protein. Children were eligible to receive three doses of PCV-9 or placebo at approximately 6, 10 and 14 weeks of age according to the Gambian EPI schedule. Details of clinical follow-up and investigation for cases of potential invasive pneumococcal diseases have been reported previously [16,18].

The immunogenicity sub-study was conducted towards the end of the vaccination phase of the PVT among children recruited at Basse, an urban health centre, or at the outreach clinics of Basse or Gambisara health centres in surrounding rural villages. In the PVT, exclusion criteria were non-residence in Upper and Central River Divisions; intent to move out within 16 weeks; previous receipt of DPT–Hib or DPT vaccine or uncertainty about receipt of such a vaccine; age younger than 40 days or older than 364 days; inclusion in a previous vaccine trial; serious chronic illness. Children who attended Basse health centre or its outreach clinics or outreach clinics of the nearby Gambisara health centre from July 2002 onwards were invited to participate in this sub-study, and specific consent was sought for a blood sample to be taken. If children joined the sub-study, home visits were conducted to remind children to come on time for the second and third doses of vaccine and for venepuncture after the third dose. If parents did not want a blood sample to be taken post-vaccination, they were still eligible to join the main trial.

Previous study of the immunogenicity of a PCV-9 in The Gambia showed that 78–90% of infants fully vaccinated per protocol developed serum anti-capsular IgG concentrations ≥1.00 μg/ml [19]. The sample size of 212 infants in this immunogenicity sub-study provided 80% power to allow measurement of a prevalence of 78% of infants with ≥1.00 μg/ml of antibody to a specific serotype with a lower 95% confidence limit of 70% on that estimate.

Approximately 2–4 ml of blood was collected by venepuncture from each child 4 weeks after the third dose of PCV-9 or placebo had been given (median 30 days, interquartile range 30, 36 days). Blood was taken in a cold box to the Basse MRC laboratory where serum samples were separated after centrifugation and stored at −70 °C until analysis.

2.1. Serology

Purified pneumococcal polysaccharides of vaccine serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, 22F and 23F were obtained from ATCC (Manassas, VA, USA). Pneumococcal cell wall polysaccharides (CWPS) were obtained from Statens Seruminstitut (Copenhagen, Denmark). The US Reference Pneumococcal Serum standard, Lot 89SF-3 was obtained from the FDA/CBER (Bethesda, MD, USA). Goat anti-human IgG conjugated to alkaline phosphatase, *p*-nitrophenyl phosphate and diethanolamine were all procured from Sigma (Dorset, England). Ninety-six-well, flat bottom, medium binding microlon ELISA plates were obtained from Greiner Bio-One GmbH (Frickenhausen, Germany).

Test samples and controls were tested by ELISA for type-specific IgG to pneumococci as previously described [20]. Briefly, each of the sera, at an initial dilution, was absorbed with non-type-specific cell-wall polysaccharides and type 22F polysaccharide, both at a concentration of 10 μg/ml, for 30 min at room temperature. Sera were then diluted serially and allowed to bind to type-specific capsular polysaccharide (CPS) antigen-coated microtitre plates. The bound antibodies were detected using goat anti-human alkaline phosphatase conjugate, which reacted with *p*-nitrophenyl phosphate in diethanolamine buffer (pH 9.80) to yield a coloured product. Absorbance was read at a wavelength of 405 nm, and the concentration measured against the pneumococcal reference standard 89-SF-3 (Center for Biological Evaluations Rockville, Maryland). The lower limit of detection of the assay was 0.001 μg/ml for the serotypes tested, apart from serotype 14, which was 0.002 μg/ml.

High and low concentration internal quality control samples were incorporated in all assays and results were accepted only if the controls produced values within twofold of the standard deviation of the mean. Additionally, 12 WHO-recommended reference standards from the NIBSC (UK) for evaluating performance of pneumococcal ELISAs were obtained (courtesy of Professor David Goldblatt, WHO reference laboratory for pneumococcal ELISAs, UCL UK) and incorporated into the assay to help to improve the quality of the results. The 12 sera were used during the set up of the assay to confirm agreement between The Gambia assay and the reference laboratory.

Twenty-five samples were tested at the WHO reference laboratory for pneumococcal ELISAs, UCL Institute of Child Health, UK and at the MRC laboratories in The Gambia. Measurements were made at both laboratories on 198 of the 225 possible pairs (25×9 serotypes). The overall correlation between measurements made at each laboratory was 0.87. The Spearman's correlation coefficient ranged from 0.815 for 6B polysaccharide to 0.964 for 9V polysaccharide. Bland Altman plots showed that that there were no substantial differences between results obtained in the two laboratories.

2.2. Analysis

Serotype-specific vaccine efficacy was estimated in the main trial using per-protocol and intention to treat analyses, as described previously [16]. Efficacy was calculated using standard methods for each individual serotype and for serotypes grouped according to vaccine serotype, vaccine-related serotype (caused by another serotype within a serogroup included in the vaccine) or non-vaccine serotype. For cases of vaccine-type invasive disease in fully vaccinated children according to protocol, we examined the median age at diagnosis and interval after the third dose of vaccine was received.

In the immunogenicity sub-study, antibody data were analysed in children who received three doses of PCV-9 or placebo according to protocol. GMCs and their confidence limits were calculated using

Table 1a
Vaccine efficacy against invasive pneumococcal disease caused by pneumococci of vaccine serotypes

PCV-9 serotypes	Per protocol (PP)					Intent to treat (ITT)				
	PCV-9 group		Placebo group		Efficacy[a]	PCV-9 group		Placebo group		Efficacy[a]
	Cases	Rate[b]	Cases	Rate[b]	VE (95% CI)	Cases	Rate[b]	Cases	Rate[b]	VE (95% CI)
1	4	0.26	2	0.13	−98 (−2090, 72)	4	0.24	2	0.12	−99 (−2100, 71)
4	0	–	1	0.065	100 (−3760, 100)	0	–	2	0.12	100 (−430, 100)
5	1	0.064	8	0.52	88 (8, 100)	1	0.061	9	0.55	89 (20, 100)
6B	1	0.064	4	0.26	75 (−150, 99)	1	0.061	4	0.24	75 (−151, 99)
9V	2	0.13	2	0.13	1 (−1266, 93)	2	0.12	2	0.12	0.46 (−1273, 93)
14	1	0.064	12	0.78	92 (44, 100)	3	0.18	15	0.92	80 (30, 96)
18C	0	–	1	0.065	100 (−3760, 100)	0	–	1	0.061	100 (−3780, 100)
19F	0	–	1	0.065	100 (−3760, 100)	0	–	2	0.12	100 (−430, 100)
23F	0	–	7	0.45	100 (31, 100)	2	0.12	8	0.49	75 (−25, 97)

[a] VE = [1 − (rate in PCV − group-rate in placebo group) × 100], discrepancies between VE and rates shown are due to rounding error.
[b] Rate: per 1000 child-years.

the assumption of a log-normal distribution. Confidence limits on proportions were calculated using exact procedures in the program StatExact.

Results have been categorised according to percentages of subjects with GMCs of ≥0.2, ≥0.35 and ≥1.0 mcg/ml. Subgroup analysis was done to examine the effect of season of vaccination. All analyses were done using log transformed data because the immunogenicity data had a log-normal distribution.

The vaccine efficacy estimates from the main trial for efficacy against invasive disease of vaccine serotypes were used to derive estimates and confidence limits of the protective antibody concentrations in the Gambian study population as described previously [21,22].

We used our composite estimate of vaccine efficacy to derive the protective antibody concentration, as done previously [21,22], because the confidence intervals around our serotype-specific efficacy estimates were too wide to allow meaningful serotype-specific estimates of protective antibody concentrations.

The Gambia PVT and this sub-study were approved by The Gambia Government/MRC Joint Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee.

3. Results

3.1. Background characteristics of children in the immunogenicity sub-study

Two hundred and thirty of the 262 children who were invited to participate (87.7%) agreed and a blood sample was obtained from 212 (81%) children. One hundred and ninety-six of these children (92.5%) met the criteria for per-protocol vaccination and had adequate volumes of sera for assay of antibodies to any of the nine pneumococcal serotypes; 103 samples from infants who had received PCV-9 and 93 from the placebo group. There were no gender or demographic differences between PCV-9 and placebo groups. The median age at receipt of the third dose of PCV-9 or placebo in the whole trial was 24 weeks (IQR 19–32), and in the sub-study this was 18 weeks (IQR 16, 21 weeks), with no significant difference between the groups. Over 90% of subjects in each group had received all three doses of PCV-9 in the rainy season, reflecting the period of recruitment for this sub-study.

3.2. Serotype-specific vaccine efficacy

Tables 1a and 1b show vaccine efficacies against invasive pneumococcal disease caused by pneumococci of vaccine-type or vaccine-related-type observed in the main trial. Both ITT and per protocol analyses showed statistically significant efficacy against invasive disease caused by pneumococci of serotypes 5 and 14. Although the efficacy result of PP was statistically significant for serotype 23F it was not in the ITT analysis. The numbers of cases of other vaccine serotypes were small and efficacy results were not statistically significant. For serotype 1, there were four cases in PCV-9 recipients and two in the placebo group, while for serotype 6B there were one and four, respectively (Table 1a). For serotypes such as 4, 9V, 18C and 19F, numbers of cases were very small. There were no major differences between results obtained by per protocol and ITT analyses. We include ITT analyses to facilitate future meta-analyses of results from all PCV trials.

The 9 cases of invasive disease of vaccine serotype in children who received 3 doses of PCV-9 according to protocol occurred at a median interval of 13 months (IQR 10, 22) after the third dose of vaccine, and all but one (a case due to serotype 5 in a 9-month-old child) occurred in the second or third year of age. The four cases of serotype-1 invasive disease occurred at ages 18, 21, 27 and 27 months.

Table 1b shows efficacy against invasive disease caused by pneumococci of vaccine-related serotypes. More cases caused by pneumococci of serotypes 9L and 18F were seen among the placebo than PCV-9 group but numbers are small. There was no indica-

Table 1b
Vaccine efficacy against invasive pneumococcal disease caused by pneumococci of vaccine related serotypes

PCV-9-related serotypes	Per protocol (PP)					Intent to treat (ITT)				
	PCV-9 group		Placebo group		Efficacy[a]	PCV-9 group		Placebo group		Efficacy[a]
	Cases	Rate[b]	Cases	Rate[b]	VE (95% CI)	Cases	Rate[b]	Cases	Rate[b]	VE (95% CI)
6A	1	0.064	2	0.13	51 (−851, 99)	3	0.18	3	0.18	0.31 (−644, 87)
9L	0	–	4	0.26	100 (−50, 100)	1	0.061	5	0.31	80 (−78, 100)
18F	0	–	1	0.065	100 (−3760, 100)	0	–	1	0.061	100 (−3787, 100)
19A	5	0.32	4	0.26	−24 (−524, 73)	6	0.37	7	0.43	15 (−197, 76)

[a] VE = [1 − (rate in PCV − group-rate in placebo group) × 100], discrepancies between VE and rates shown are due to rounding error.
[b] Rate: per 1000 child-years.

Table 2
Post-vaccination antibody concentrations (GMCs) in μg/ml (with 95% confidence intervals, CI) for PCV-9 and placebo groups by serotype

Serotype	Group			
	PCV-9		Placebo	
	n	GMC (95% CI) in μg/ml	*n*	GMC (95% CI) in μg/ml
1	99	5.79 (4.73, 7.09)	86	0.04 (0.03, 0.06)
4	97	4.88 (4.06, 5.87)	85	0.03 (0.02, 0.04)
5	94	4.67 (3.82, 5.70)	86	0.07 (0.05, 0.11)
6B	101	7.08 (5.38, 9.32)	93	0.03 (0.02, 0.04)
9V	93	2.61 (2.09, 3.27)	81	0.04 (0.03, 0.05)
14	88	11.09(8.37, 14.68)	82	0.08 (0.06, 0.12)
18C	97	3.00 (2.37, 3.80)	83	0.01 (0.01, 0.02)
19F	96	6.41 (5.08, 8.10)	89	0.08 (0.06, 0.12)
23F	99	3.20 (2.45, 4.17)	88	0.03 (0.02, 0.04)

tion of any protection against infections caused by pneumococci of serotype 19A.

3.3. Antibody concentrations following vaccination

GMCs attained by infants in the placebo and the PCV-9 groups to each of the serotypes contained in the PCV-9 are shown in Table 2. After three doses of PCV-9, GMCs in the PCV-9 group were substantially and significantly higher for each serotype than those in the placebo group. The GMC of PCV-9 recipients to individual polysaccharides ranged from 2.61 μg/ml for 9V polysaccharide to 11.09 μg/ml for serotype 14 polysaccharide.

Point estimates of the proportion of infants with post-vaccination antibody concentrations ≥0.2, ≥0.35 or ≥1.0 μg/ml ranged from 97.0 to 100%, 92.9 to 100% and 83.9 to 95.8%, respectively, for individual polysaccharides. In contrast, only 8.4–31.7%, 7.2–20.9% and 1.1–10.1% of infants in the placebo group attained these antibody concentrations (Table 3). Reverse cumulative distribution (RCD) curves for type-specific antibody responses in vaccinated and control infants are presented in Fig. 1.

GMC antibody concentrations were generally higher in infants who received all three doses of PCV-9 in the rainy season than those who received at least one dose in the dry season (Table 4), and this effect was statistically significant for serotypes 1 and 14 and marginally so for 9V. Among placebo recipients, antibody concentrations tended to be higher in infants who received at least one dose in the cooler dry season, differences being statistically significant for serotypes 4, 9V and 18C, and marginally so for 6B. Fig. 2 shows a plot of findings for serotypes 1, 9V and 14. There were differences in GMC by month of sampling, especially in the placebo group in whom GMCs tended to fall in the cool dry season (December–January) and rise again in the hot dry season (March).

3.4. Protective antibody concentrations

With our overall vaccine efficacy of 77% (95% CI: 51, 90) against vaccine-type invasive pneumococcal disease, the estimated protective antibody concentration (GMC) for all 9 serotypes combined was 2.3 μg/ml (95% CI: 1.0, 5.0). We also estimated the protective antibody concentration for the seven serotypes in Prevenar™ (i.e. excluding serotypes 1 and 5). The estimated vaccine efficacy against invasive disease caused by pneumococci of serotypes contained in Prevenar™ was 87%; the protective antibody concentration (GMC) obtained for these seven serotypes combined was 1.24 μg/ml (95% CI: 0.3, 3.4). The vaccine efficacy for serotype 5 was 88 % (95% CI: 8, 100) and the corresponding estimated protective antibody concentration was 1.2 μg/ml (95% CI: 0.35, 15.0). Corresponding values for serotype 14 were a protective efficacy of 92% (95% CI: 44, 100) and an estimated protective antibody concentration of 0.9 μg/ml (95% CI: 0.13, 15.5). A point estimate of 100% efficacy for serotype 23F, the other individual serotype for which efficacy was significant in the main trial in per protocol analyses, precluded the calculation of antibody protective concentrations for that serotype.

4. Discussion

In this study we have presented the serotype-specific efficacy of a 9-valent pneumococcal conjugate vaccine in Gambian infants who participated in a large clinical trial, measured the immunogenicity of the vaccine in a sub-study of infants who participated in this trial and used these data to estimate protective antibody concentrations. The overall efficacy of PCV-9 against invasive pneumococcal disease of vaccine serotype was 77% and we showed significant serotype-specific efficacy against invasive disease caused by pneumococci of serotypes 5, 14 and 23, as previously reported [16]. Numbers were too small to allow precise calculations of efficacy for other serotypes but there was no suggestion of any efficacy against infections caused by pneumococci of serotype 1 or against the vaccine-related serotype 19A.

One month after three doses of PCV-9 given in a 6–10–14 weeks schedule, GMCs in the vaccine group were significantly higher for each serotype than those in the placebo group including serotype 1 for which the GMC was 5.79 (95% CI 4.73, 7.09) μg/ml. A good antibody response to the 19F conjugate was seen but this was not associated with any apparent cross-protection against serotype 19A invasive disease.

As was observed in other PCV-9 trials [23–25] and in places where other pneumococcal conjugate vaccines have been studied [26–29], we found high post-vaccination GMCs to all the nine polysaccharides contained in the study vaccine. There are, however, differences in post-vaccination antibody profiles between our

Table 3
Percentage of subjects attaining antibody concentrations above a defined value after vaccination by serotype

Serotype	IgG concentration cut off levels											
	≥0.2 mcg/ml				≥0.35 mcg/ml				≥1.0 mcg/ml			
	PCV-9		Placebo		PCV-9		placebo		PCV-9		Placebo	
	N	95% CI	*N*	95% CI	*N*	95% CI	*N*	95% CI	*N*	95% CI	*N*	95% CI
1	98	99.0 (94.5, 100.0)	15	17.4 (10.1, 27.1)	98	98.0 (92.9, 99.7)	11	12.8 (6.6, 21.7)	94	95.0 (88.6, 98.3)	7	8.1 (3.3, 16.1)
4	97	100.0 (96.3, 100.0)	14	16.5 (9.3, 26.1)	97	100.0 (96.3, 100.0)	7	8.2 (3.4, 16.2)	93	95.9 (89.8, 98.9)	3	3.5 (0.7, 10.0)
5	94	100.0 (96.1, 100.0)	23	26.7 (17.8, 37.4)	94	100.0 (96.1, 100.0)	18	20.9 (12.9, 31.1)	88	93.6 (86.6, 97.6)	4	4.7 (1.3, 11.5)
6B	99	98.0 (93.0, 99.8)	17	18.3 (11.0, 27.7)	99	98.0 (93.0, 99.8)	14	15.0 (8.5, 24.0)	90	89.1 (81.3, 94.4)	2	2.1 (0.3, 7.5)
9V	91	97.9 (92.5, 99.7)	12	14.8 (7.9, 24.5)	91	94.6 (87.9, 98.2)	7	8.6 (3.5, 17.0)	78	83.9 (74.8, 90.7)	3	3.7 (0.8, 10.4)
14	87	98.9 (93.8, 100.0)	26	31.7 (21.9, 42.9)	87	98.9 (93.8, 100.0)	11	13.4 (6.9, 22.7)	81	92.1 (84.3, 96.7)	1	1.2 (0.0, 6.6)
18C	95	97.9 (92.7, 99.7)	7	8.4 (3.5, 16.6)	95	95.9 (89.8, 98.9)	6	7.2 (2.7, 15.1)	83	85.6 (77.0, 91.9)	1	1.2 (0.0, 6.5)
19F	96	100.0 (96.2, 100.0)	23	25.8 (17.1, 36.2)	96	97.9 (92.7, 99.7)	19	21.3 (13.4, 31.3)	88	91.7 (84.2, 96.3)	9	10.1 (4.7, 18.3)
23F	96	97.0 (91.4, 99.4)	15	17.1 (9.9, 26.5)	98	92.9 (86.0, 97.1)	14	15.9 (9.0, 25.2)	87	87.9 (79.8, 93.6)	1	1.1 (0.0, 6.2)

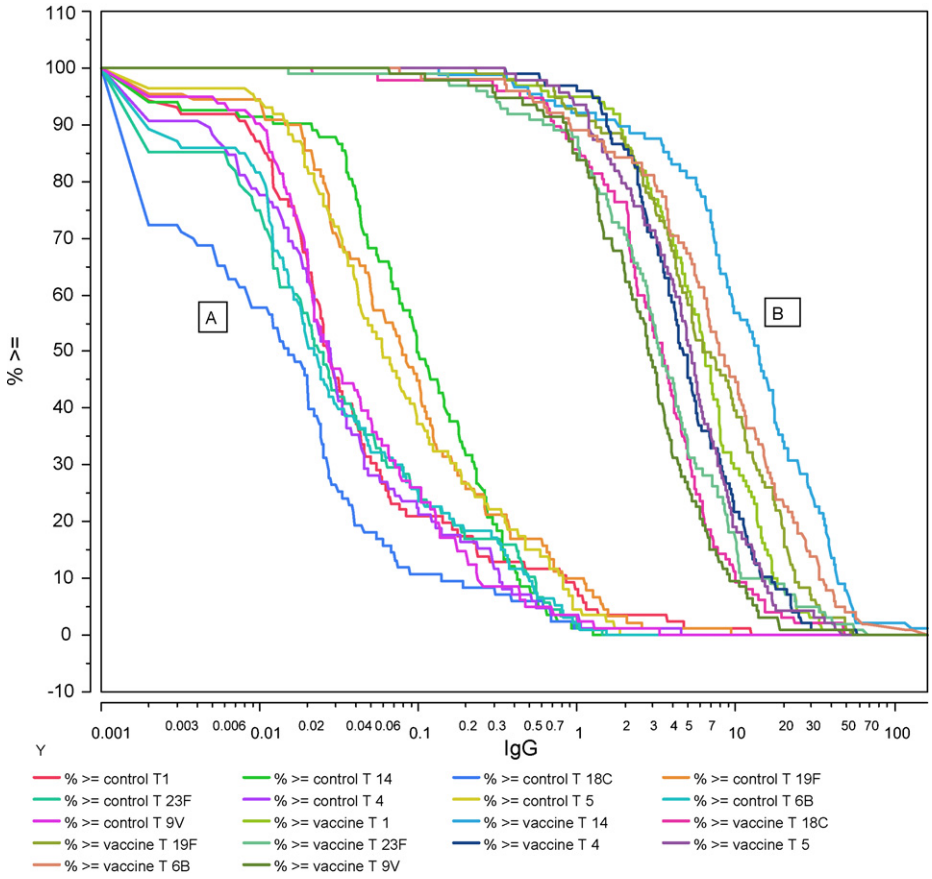

Fig. 1. Reverse cumulative distribution curves demonstrating the percentages of children achieving varying serum antibody concentration to each vaccine serotype after three doses of PCV-9 (A, placebo group and B, PCV-9 group).

Gambian and other study populations. For instance, antibody concentrations in vaccinated Gambian infants were higher for each serotype than those observed in Soweto [23,24] and than concentrations observed when other pneumococcal conjugate vaccines were used in other settings [27,28] but lower than GMCs reported from the Philippines where PCV-11 with different conjugate protein, was used [26]. Notably, GMCs for serotypes 6B and 14 were high among the PCV-9 vaccine recipients in our study unlike findings in some studies in South Africa [23,24].

Possible reasons for variation in antibody responses between populations could be the boosting effect of early pneumococcal carriage common in developing countries, age at immunization, differences in the number of doses administered, and concomitant administration of other vaccines [26], although differences in laboratory methods and type of vaccines cannot be ruled out. A previous safety and immunogenicity study conducted in The Gambia with the same vaccine did not demonstrate any significant interference between different vaccine antigens, rather enhanced immunogenicity was seen when the pneumococcal conjugate vaccine was administered concomitantly with other routine vaccines [30]. Although overall antibody concentration is likely to be important for invasive disease prevention, other factors such as the functionality of antibody and T-cell immune response involvement [31] must also be taken into consideration. For instance the apparent lack of protection against serotype 1 invasive disease observed in our study despite a high GMC of serotype 1 specific antibody measured by ELISA, and a high proportion of children with a concentration above 0.35 μg/ml, could be due to some deficiency in the ability of these antibodies to promote phagocytosis or killing of pneumococci of this serotype, and study of functional antibody

Table 4
Variation in antibody concentrations ((GMCs) in μg/ml) among subjects vaccinated in the rainy season or in the dry and rainy seasons

Serotype	Control group					Vaccine group				
	Rainy season alone	*n*	Both[a]	*n*	*p*-Value	Rainy season alone	*n*	Both[a]	*n*	*p*-Value
1	0.03	76	0.11	9	0.07	6.16	90	2.22	7	0.01
4	0.03	75	0.10	9	0.02	4.93	89	3.35	5	0.37
5	0.07	77	0.10	8	0.53	4.86	85	2.62	6	0.14
6B	0.03	82	0.09	10	0.05	7.66	91	3.29	7	0.12
9V	0.03	70	0.10	10	0.02	2.78	84	1.12	6	0.05
14	0.08	73	0.08	8	0.95	11.91	79	2.40	6	0.004
18C	0.01	74	0.05	8	0.02	2.97	88	2.21	6	0.56
19F	0.07	78	0.17	10	0.18	6.59	88	2.74	5	0.10
23F	0.02	77	0.05	10	0.30	3.32	89	1.58	7	0.16

[a] Doses of vaccine spread across rainy and dry seasons.

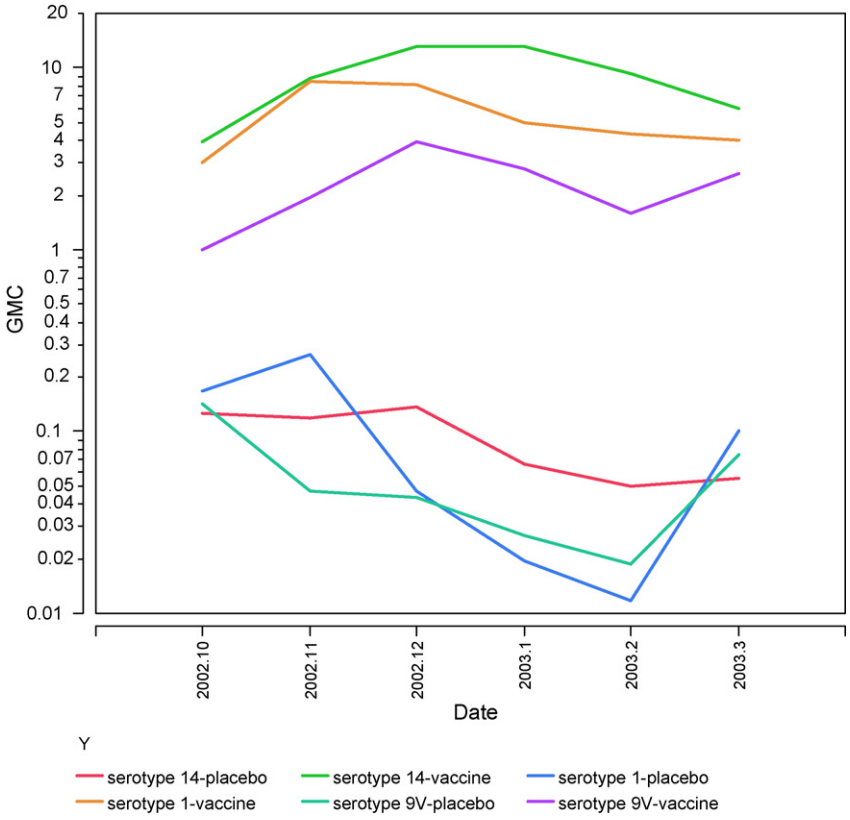

Fig. 2. GMC of serotypes 1, 9V, and 14 among PCV-9 recipients and placebo group by month of blood sampling.

responses to this and other serotypes is important. This may also be due to heterogeneity in the population structure of this serotype 1 isolates, which will require further molecular characterization. The only other trial of PCV-9 [32,31] found one case of serotype 1 in vaccinated and three in control non-HIV-infected children, and thus there are no strong data on which to estimate efficacy or protective antibody levels for this serotype. Therefore this apparent lack of protection against serotype 1 invasive disease observed in our study should be interpreted cautiously because of the limited number of isolates.

The estimated protective antibody concentration for the nine serotypes combined in the Gambian population is much higher than that found in previous pneumococcal conjugate vaccine trials [32–34] and higher than the 0.35 μg/ml proposed by WHO [35]. Possible reasons for differences in different studies include differences in immunization schedule especially with respect to receipt or not of a booster dose; or variation in the pressure and type of infection. The first and largest trial of a PCV-7 vaccine at Northern California, on which licensure of Prevenar™ in the US was based, had the highest VE (97.3%) and the lowest estimated protective antibody concentration (0.20 μg/ml). In that trial, a primary vaccination schedule of 2, 4, and 6 months was followed and children received a booster dose at 12–15 months of age [34]. Most of the cases of vaccine-type invasive disease in fully vaccinated children in The Gambia study occurred in the second or third year of life, after children would have been eligible to receive a booster dose under the American schedule. We have recently completed a follow-up study of antibody persistence and the long-term effect of PCV-9 on nasopharyngeal carriage and results should help to determine whether waning immunity is important in The Gambia setting. The trial of PCV-9 in South Africa [32], which showed 90% vaccine efficacy against invasive disease due to the seven serotypes included in Prevenar™ in non-HIV-infected children after a 6, 10, and 14 weeks schedule with no booster, had an estimated protective antibody concentration of 0.68 μg/ml [22], intermediate between results in California and The Gambia for these seven serotypes. Estimates in each site have such variability, however, that differences are not statistically significant. Long-term follow-up of the South Africa study did not show any evidence of waning immunity in non-HIV-infected children, as only one additional case of vaccine-type invasive pneumococcal disease in non-HIV-infected children was identified between 2.3 and 6.1 years of follow-up [36]. It is possible that populations who are at high risk for pneumococcal diseases and early nasopharyngeal colonization, such as those in The Gambia and the South Africa, may require higher antibody concentrations to achieve an equivalent protective efficacy to American infants. Lastly, in the California study, most cases of invasive disease were relatively mild cases of occult bacteraemia, while in The Gambia, most cases had clinical or radiological pneumonia and many had signs of severe illness [18]. These differences in the study outcome under consideration could also contribute to differences in the reported 'correlates' of protection.

The analysis in this study, as well as those done previously, has pooled across serotypes to obtain a protective level. We made the simplifying assumption that protective antibody concentrations were similar for all serotypes, although it is plausible that some serotypes require higher protective antibody concentrations than others. But the absence of precise efficacy estimates for some of the serotypes, due to small numbers of cases for any particular serotype, makes type-specific thresholds difficult to define. Besides, Jodar et al. [21] has highlighted other difficulties which are associated with establishing serological criteria that predict protection. Importantly, these include the difficulty in defining pro-

tective concentrations for pneumonia, for which serotype-specific vaccine-efficacy estimates are unavailable.

We found some evidence of seasonal variation in GMCs by month of blood sampling, especially in the placebo group, whose GMCs seemed to be lower after the rainy season and to rise again in the dry season. GMCs post-vaccination were generally higher among infants who had received all three doses of vaccines in the rainy season than in those whose doses had been spread over dry and wet seasons, and this difference was significant for serotypes 1 and 14. These may have been chance findings although they were consistent across serogroups. In contrast to our antibody results, in the main trial, we found a suggestion of lower clinical efficacy against radiological pneumonia among children who received all three doses of vaccine in the rainy season [16]. Although previous studies have shown variation in antibody responses to pneumococcal polysaccharide vaccine by month, with higher responses during and for several months after the rainy season for some serotypes such as 14 and 23 [37,38], the antibody findings from this sub-study, in which only a small number of children received a dose outside the rainy season, should be viewed with caution. Further research is required to elucidate the precise mechanisms explaining these observations.

Overall, our results provide further evidence that the PCV-9 was immunogenic in this Gambian population. Evidences from this immunogenicity sub-study and the main efficacy trial suggest that use of this vaccine will be a valuable intervention to reduce pneumococcal disease burden in African children. Although a very high proportion of our study population who received PCV-9 achieved the 0.35 μg/ml minimum protective concentration recommended by WHO, a much higher protective antibody level was estimated in The Gambia population, suggesting that more data need to be pooled from other trials.

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