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Nasopharyngeal carriage of otitis media pathogens in infants receiving 10-valent non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10), 13-valent pneumococcal conjugate vaccine (PCV13) or a mixed primary schedule of both vaccines: A randomised controlled trial

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ABSTRACT

Background: Aboriginal children in Northern Australia have a high burden of otitis media, driven by early and persistent nasopharyngeal carriage of otopathogens, including non-typeable *Haemophilus influenzae* (NTHi) and *Streptococcus pneumoniae* (Spn). In this context, does a combined mixed primary series of Synflorix and Prevenar13 provide better protection against nasopharyngeal carriage of NTHi and Spn serotypes 3, 6A and 19A than either vaccine alone?

Methods: Aboriginal infants (n = 425) were randomised to receive Synflorix™ (S, PHiD-CV10) or Prevenar13™ (P, PCV13) at 2, 4 and 6 months (_SSS or _PPP, respectively), or a 4-dose early mixed primary series of PHiD-CV10 at 1, 2 and 4 months and PCV13 at 6 months of age (SSSP). Nasopharyngeal swabs were collected at 1, 2, 4, 6 and 7 months of age. Swabs of ear discharge were collected from tympanic membrane perforations.

Findings: At the primary endpoint at 7 months of age, the proportion of nasopharyngeal (Np) swabs positive for PCV13-only serotypes 3, 6A, or 19A was 0%, 0.8%, and 1.5% in the _PPP, _SSS, and SSSP groups respectively, and NTHi 55%, 52%, and 52% respectively, and no statistically significant vaccine group differences in other otopathogens at any age. The most common serotypes (in order) were 16F, 11A, 10A, 7B, 15A, 6C, 35B, 23B, 13, and 15B, accounting for 65% of carriage. Ear discharge swabs (n = 108) were culture positive for NTHi (52%), *S. aureus* (32%), and pneumococcus (20%).

Conclusions: Aboriginal infants experience nasopharyngeal colonisation and tympanic membrane perforations associated with NTHi, non-PCV13 pneumococcal serotypes and *S. aureus* in the first months of life. Nasopharyngeal carriage of pneumococcus or NTHi was not significantly reduced in the early 4-dose combined SSSP group compared to standard _PPP or _SSS schedules at any time point. Current pneumococcal conjugate vaccine formulations do not offer protection from early onset NTHi and pneumococcal colonisation in this high-risk population.

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1. Background

Remote-dwelling Aboriginal and Torres Strait Islander (hereafter respectfully termed Indigenous) children in Australia have a high burden of infectious disease. Otitis media (OM), which commences at a younger age, is more likely to progress to complications (perforations, chronicity and hearing loss) in this population [1]. On average almost 20% children have chronic sup-

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purative otitis media (CSOM) or acute otitis media with perforation (AOMwiP) at 18 months of age [2–4]. The predominant bacterial pathogens associated with early onset of persistent OM in this population are non-typeable *Haemophilus influenzae* (NTHi) and *Streptococcus pneumoniae* (Spn) [5]. Colonisation of the nasopharynx by Spn, NTHi and other potentially pathogenic bacteria occurs within the first weeks of life [5,6]. NTHi is the dominant bacterial pathogen cultured from discharging ears [7].

A pneumococcal conjugate vaccine (PCV) program commenced in Australia in 2001 for Indigenous infants and in 2005 for non-Indigenous infants with Prevenar7™ (PCV7). The Northern Territory (NT) then moved to Synflorix™ (PHiD-CV10) in 2009 and Prevenar 13™ (PCV13) in 2011. In the NT [8–11], more than 90% of infants have completed their 3 dose primary series by 12–15 months of age.

During the two year PHiD-CV10 era in the NT, there was a significant reduction in NTHi cultured from ear discharge (compared to PCV7 era), with no corresponding reduction in Np NTHi carriage [12]. International trials had previously shown that vaccine efficacy against tympanocentesis-confirmed NTHi-AOM in the 11PnPd precursor vaccine trial (POET) in Czech and Slovakian infants was 35%. The effect on carriage was small and a non-significant trend of reduced carriage compared to the control group carriage became significant only post booster dose, for any *H. influenzae* (18/177, 10.2% vs 31/175, 17.7%, p = 0.046) [13,14]. In the more recent COMPAS trial, there was lower Np carriage than predicted (4–6% over all age groups 12–27 months), and no reduction in carriage was detected, including post-booster dose [15].

Post-PCV7 introduction in Australia, whilst there was no change in overall carriage, there was a reduction in PCV7 serotype carriage and disease. Due to serotype replacement, an increased prevalence of pneumococcal serotype 19A was reported in carriage in Western Australian Aboriginal children [16], and nationwide a four-fold increase in 19A-invasive pneumococcal disease in children under 5 years old [17], likely preceded by carriage. As only PCV13 includes 19A, a study was designed that would use the two current pneumococcal conjugate vaccines in combination, to derive benefit from both formulations.

We undertook a 3-arm randomised controlled trial to compare immunogenicity, safety, clinical and microbiological outcomes following standard 2-4-6 month schedules of PHiD-CV10, PCV13, or a novel early start mixed primary schedule of PHiD-CV10 at 1, 2, and 4 months followed by PCV13 at 6 months [18]. In the primary outcome paper we demonstrated that the combination schedule was immunogenic against all vaccine serotypes including the PCV13-only serotypes 3, 6A, and 19A and the PHiD-CV10-only non-typeable *Haemophilus influenzae* protein D at 7 months of age. These responses in the combination group were not significantly lower than the responses to either vaccine alone [19].

In this publication we report the priority Np and ear discharge microbiological outcomes by vaccine randomisation group. These primary microbiological objectives were to determine whether the combination schedule provides i) significant difference in Np carriage of PCV13-vaccine-only serotypes 3, 6A, and 19A compared to PHiD-CV10 alone, ii) significant difference in Np carriage of NTHi compared to PCV13 alone, one month post primary schedule, at 7 months of age, and iii) describe early life Np carriage and ear discharge respiratory pathogen bacteriology and antimicrobial resistance.

2. Methods

2.1. Study design and participants

This assessor blinded randomised controlled trial enrolled 425 Australian Indigenous (1:1:1) infants from 5 remote Aboriginal communities in the Northern Territory and Western Australia to receive either PHiD-CV10 (S) or PCV13 (P) at 2, 4 and 6 months (_SSS or _PPP), or an early start novel combination schedule of PHiD-CV10 at 1, 2 and 4 months and PCV13 at 6 months (SSSP) [18].

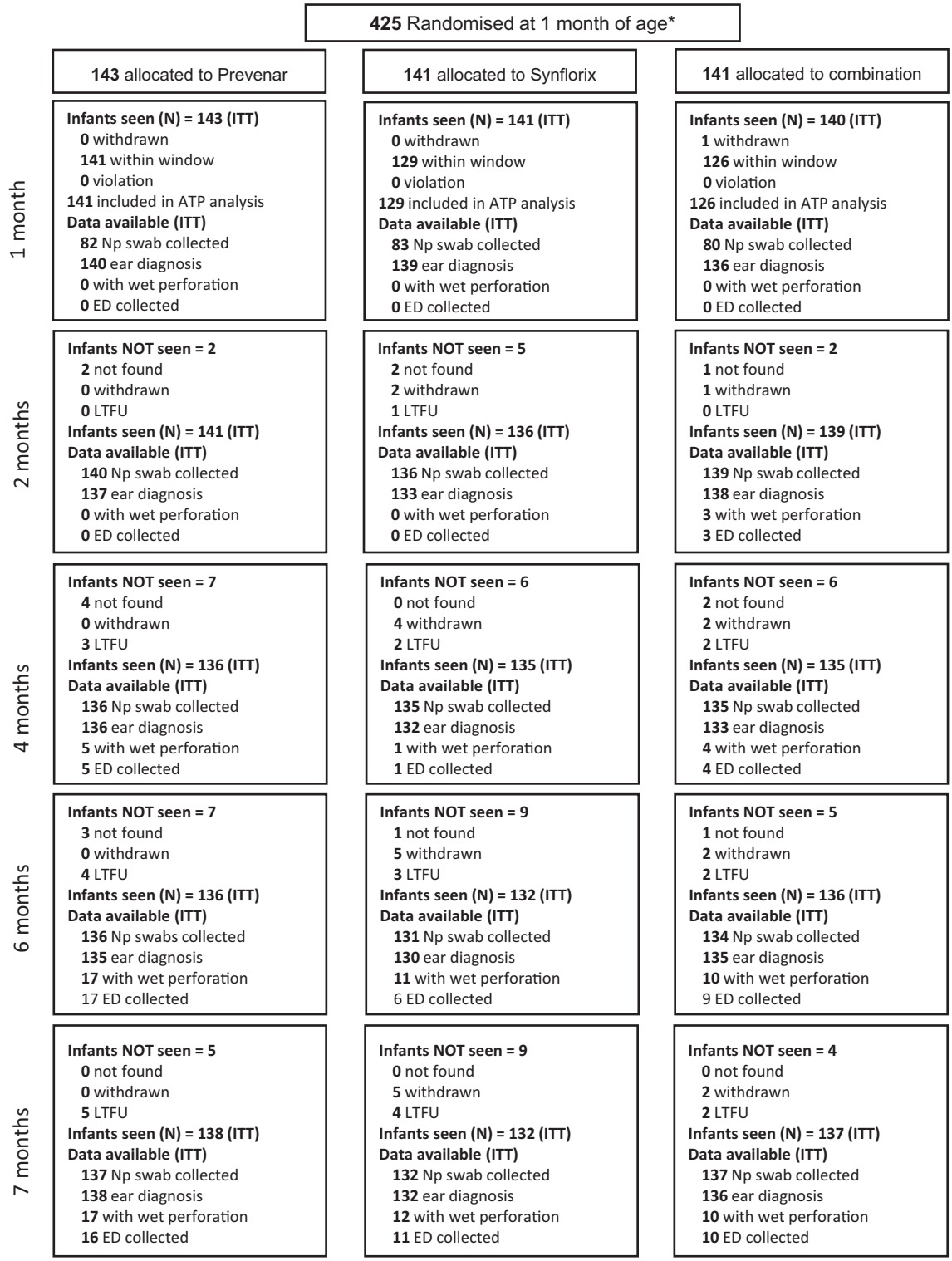
This trial was registered with ANZCTR (ACTRN12610000544077) and ClinicalTrials.gov (NCT01174849). Ethical approval for the trial was provided by the Human Research Ethics Committee of the Northern Territory Department of Health & Families and the Menzies School of Health Research (HREC - EC00153), ref: HREC-2010-1395, Central Australian Human Research Ethics Committee (CAHREC - EC00155), ref: 2010.06.02, West Australian Aboriginal Health Information Ethics Committee (WAAHIEC) ref: 377-12/2011, and the Western Australia Country Health Service Ethics (WACHS), ref: 2012:17.

2.2. Procedures

Data collection began in September 2011 and was completed in May 2018. The published study protocol [18] outlines all study procedures. Briefly, Np swabs were collected at 1, 2, 4, 6 and 7 months (Fig. 1). Primary endpoint samples were collected at least 28 days post 6-month dose. Ear discharge swabs were collected when discharge was observed during the otoscopic examination or at tympanometry, from both AOMwiP (defined as discharge of pus through a small perforation (hole) in the eardrum within the last 6 weeks) and CSOM (persistent ear discharge for at least 6 weeks through a perforation of >2% of the pars tensa). All samples were collected and stored as per WHO guidelines for pneumococcal trials [20]. In brief, a 10 μl aliquot of the Np swab in STGGB was plated using a semi-quantitative method onto selective and non-selective media to isolate Spn, NTHi, *Moraxella catarrhalis* (Mc) and *Staphylococcus aureus* (Sa). Swabs collected from infants at ages 1, 2, and 4 months had two additional aliquots of 50 μl plated on Spn and NTHi selective media for the retrieval of low density carriage. Swabs were coded as positive if the bacteria of interest were grown from either the 50 μl spread plates or the semi-quantitative plates. Capsular Spn were identified by morphology, optochin resistance and a positive Quellung reaction (Statens Serum Institute of Copenhagen, Denmark). Throughout the manuscript 'Spn' refers to capsular Spn only. Presumptive NTHi were identified by morphology, X and V dependence (Oxoid) and coagglutination negative (Haemophilus Phadebact, Remel). NTHi isolated from ear discharge samples were confirmed as *H. influenzae* by PCR to differentiate them from non-haemolytic *H. haemolyticus*. Np NTHi isolates were not speciated by molecular methods, as only 0.34% of Np NTHi isolates from this population are misidentified Hh [21]. Mc were identified by morphology, oxidase production and Gram stain. Sa were identified by colony morphology and coagulation by latex agglutination (Staphaurex, Remel). βetalactamase production by NTHi and Mc was determined using nitrocephin (Oxoid, Australia). Spn (oxacillin, penicillin, tetracycline, erythromycin, sulphamethoxazole trimethoprim, chloramphenicol) and Sa (oxacillin, penicillin, erythromycin, cefoxitin, gentamicin, ciprofloxacin) had antimicrobial susceptibility screening by Calibrated Dichotomous Sensitivity (CDS) [22]. If Spn isolates were non-susceptible to penicillin or erythromycin, the MIC was determined by Etest (bioMerieux, Australia). Resistance breakpoints for penicillin and azithromycin are according to EUCAST guidelines (penicillin sensitive $\leq$ 0.06 mg/l, intermediate > 0.06 and $\leq$ 2 mg/l, resistant > 2 mg/l; azithromycin sensitive $\leq$ 0.25 mg/l, intermediate > 0.25 and $\leq$ 0.5 mg/l, resistant > 0.5 mg/l).

For NTHi, Mc and Sa, one colony was arbitrarily selected from the primary culture, plus any morphologically distinct colony types. Two arbitrarily colonies of the dominant morphotype of Spn were selected, plus any morphologically distinct colony types.

The guardian of the child, (usually the biological mother) was interviewed using a standardised risk-factor questionnaire when the child was 1 and 6 months of age.



*1018 mothers assessed for eligibility, 593 excluded [326 ineligible (gestation<32 weeks, baby >38 days, non-study residence, infant deceased, clinician advice, unable to locate / contact mother), 112 miscarriage / stillborn / unknown pregnancy outcome, 8 pending birth, 145 refused / unable to contact within window, 2 withdrawn post consent prior to randomisation], 425 randomised

Fig. 1. Number of infants seen and procedures completed by age and vaccine group.

2.3. Statistical analysis

The enrolment target of 425 infants had over 90% power to detect reductions in NTHi and 19A carriage at 7 months of age, from 80% to 50% (for NTHi) and 20% to 5% (for 19A), respectively, between the COMBO group and the PCV13 group (for NTHi), and the COMBO group and PHiD-CV10 group (for 19A) [13,23,24]. Binomial exact 95% confidence interval was calculated for all carriage outcomes and two-sided Fisher's exact test used in vaccine group comparisons. In secondary analyses, these comparisons were also applied to ear discharge samples and all samples at other timepoints. Data for serotypes 3, 6A, and 19A were aggregated for comparison due to the small number isolated. Cumulative carriage was calculated using all swabs, once an infant is observed to be carriage positive (by organism, Spn, NTHi and Mc) they are counted as positive at their age in days. New acquisition of a serotype is defined as a serotype different to the carriage type first isolated in the infant and includes multiple carriage serotypes counted individually. If multiple carriage was observed at the first colonisation the next serotype had to be different to both isolated serotypes to be counted as a new acquisition. If a specific serotype was observed in an infant at non-consecutive visits this was not counted as a new acquisition. Where there was no difference in serotype or antibiogram the data for the first isolate only was included in the analyses. For multiple isolates of the same serotype but different antibiograms, both were included in the analysis of the antimicrobial sensitivity data. Thus, per swab data may add to more than 100%. Analyses of ear discharge are presented per child. Where infants have bilateral ear discharge data were combined. Logistic regression was used to determine the impact of time on carriage of PCV13 serotypes, adjusted for clustering by child with robust SE to understand the herd impact of changing the infant pneumococcal vaccine schedule to PCV13 in the first year of the trial. Statistical significance was set at $\alpha \leq 0.05$. All statistical analyses were performed using Stata 15 (StataCorp, USA). Although we have multiple comparisons, no adjustments were made to significance thresholds.

2.4. Role of the funding source

This study was funded by the National Health and Medical Research Council (NHMRC) of Australia (GNT605810). The NHMRC has no input into the study design, collection, analysis, interpretation of findings or decision to publish these findings.

3. Results

3.1. Demographics and risk factors

Baseline characteristics and prevalence of risk factors were similar across the three groups (Table 1). Risk factors for OM remained

Table 1
Baseline and 7 month characteristics.

Characteristics	PCV13 PREVENAR13 (PPP) N = 143	PHiD-CV10 SYNFLORIX (SSS) N = 141	PCV13 and PHiD-CV10 (SSSP) N = 141
Characteristics at baseline (1 month of age)			
Sex			
Male	77 (53.8%)	69 (48.9%)	70 (49.6%)
Female	66 (46.2%)	72 (51.1%)	71 (50.4%)
Gestational age, weeks			
Mean (SD)	38.4 (1.4)	38.4 (1.4)	38.1 (1.6)
Birthweight, kg			
Mean (SD)	3.2 (0.5)	3.2 (0.5)	3.10(0.5)
Weight at randomisation, kg			
Mean (SD)	4.3 (0.5)	4.2 (0.6)	4.0 (0.7)
Age at randomisation, days			
Mean (SD)	33.1 (3.3)	32.5 (3.8)	32.5 (3.9)
Community – total randomisation			
Wurrumiyanga (Nguiu)	29 (20.3%)	33 (23.4%)	30 (21.3%)
Wadeye	53 (37.1%)	50 (35.5%)	51 (36.2%)
Kununurra	26 (18.2%)	25 (17.7%)	26 (18.4%)
Alice Springs (CAAC)	7 (4.9%)	6 (4.3%)	6 (4.3%)
Maningrida	28 (19.6%)	27 (19.1%)	28 (19.9%)
Risk factors for OM at visit 1			
Family history of TMP (mother)	22 (19.3%)	19 (15.7%)	17 (14.4%)
Family history of TMP (siblings)	19 (19%)	22 (23%)	19 (20%)
Siblings under 5 years			
Median (IQR)	2.0 (1.0–2.0)	2.0 (1.0–2.5)	2.0 (1.0–2.0)
Breast fed	120 (96.8%)	117 (94.4%)	116 (95.9%)
Breast fed only	98 (83.8%)	98 (85.2%)	93 (81.6%)
Bottle fed	24 (19.7%)	26 (20.8%)	25 (21.0%)
Bottle fed only	4 (3.3%)	6 (5.0%)	4 (3.4%)
Mother smoked during pregnancy	58 (48.7%)	60 (48.4%)	61 (50.8%)
Others smoked in household	27 (21.8%)	31 (24.8%)	22 (18.2%)
Use cooking fire	23 (18.7%)	22 (17.6%)	28 (23.1%)
Characteristics at 7 months of age			
Age in months,			
Mean (SD)	7.1 (0.72)	7 (0.66)	7 (0.93)
Risk factors for OM at visit 1			
Sibling in the study			
1	116 (84.7%)	109 (83.2%)	117 (85.4%)
2	21 (15.3%)	20 (15.3%)	20 (14.6%)
3	0 (0.0%)	2 (1.5%)	0 (0.0%)
Breast fed	110 (90.2%)	108 (90.8%)	115 (91.3%)
Mother smokes	72 (58.5%)	70 (58.8%)	83 (65.9%)
Others smoking at house	30 (24.6%)	26 (21.8%)	23 (18.3%)
Use cooking fire	33 (27.0%)	33 (27.7%)	36 (28.6%)

TMP, tympanic membrane perforation.

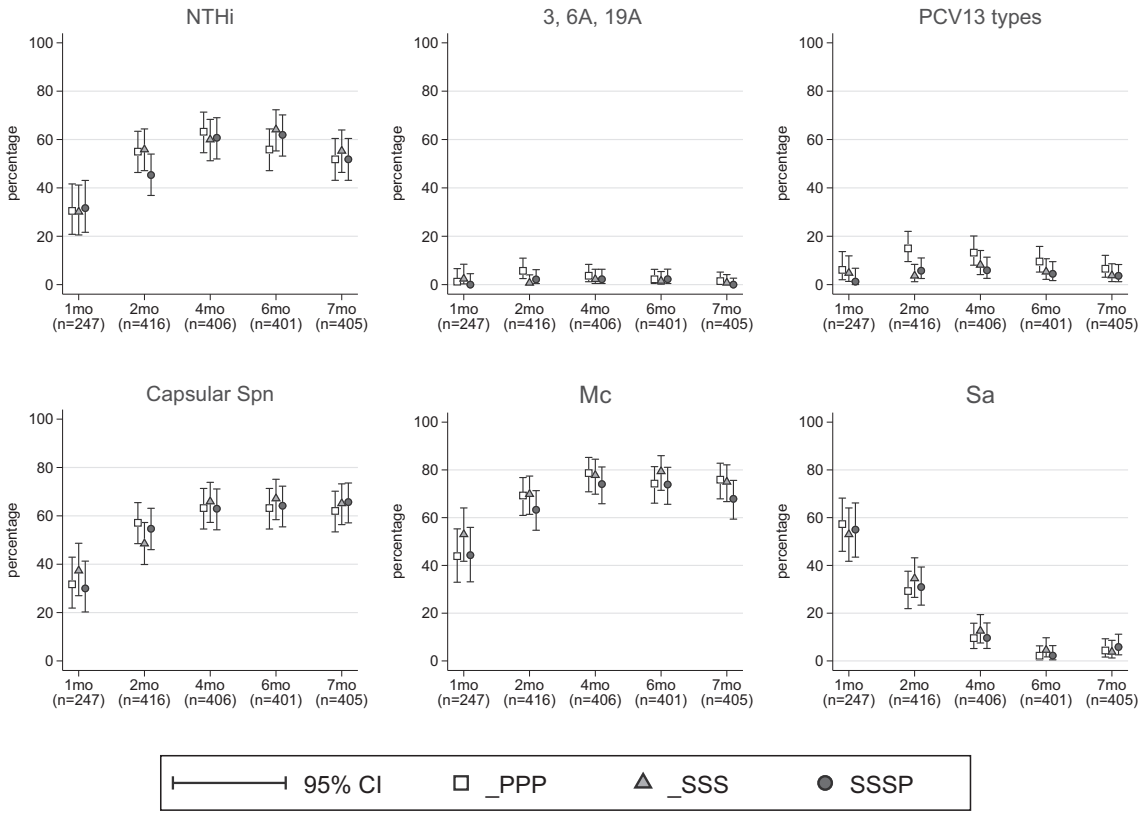


Fig. 2. Proportions of nasopharyngeal swabs positive for NTHi, combined serotypes 3, 6A and 19A, any PCV13-types, any capsular Spn, any Mc and Sa at ages 1-7mo by vaccine group with 95% CI.

similarly distributed between vaccine groups at 6 months of age. Overall breast feeding at 6 months was 91%, maternal smoking was 61%, smoke exposure in the home was 22%, and use of a fire for cooking or social gatherings was 28%. The number of Np and ear discharge swabs collected at each timepoint by vaccine group are shown in Fig. 1.

3.2. Primary microbiology outcomes:

3.2.1. Np carriage of 3, 6A, and 19A and NTHi by vaccine group at 7 months of age

Of the PCV13 exclusive serotypes only two isolates of serotype 3, four isolates of 6A, and 31 isolates of 19A were cultured from Np swabs over the life of the study. There were no statistically significant differences for the primary outcomes of combined Np carriage prevalence of serotypes 3, 6A, or 19A in the SSSP group compared to the SSS group (0% vs 0.8%; p = 0.491), or NTHi carriage in the SSSP group compared to the _PPP group (52% vs 52%, p = 1) at 7 months of age (Fig. 2, Table 2).

The use of a 50 μl inoculum (compared to 10 μl) for infants aged 1, 2 or 4 months slightly improved the Np carriage detection, particularly for NTHi (up to 5%). These additional positives are included in all reporting.

3.3. Secondary microbiology outcomes

3.3.1. Np carriage overall

A total of 1873 Np swabs were collected. Overall, 1159 Spn isolates were serotyped. One Np swab was not able to be assessed for NTHi and Mc due to swarming overgrowth.

Overall Np carriage of Mc was 70%, Spn 58%, NTHi 53%, and Sa 18%. Carriage of Hi type B was 1.3% and capsule types a, c-f 3.3%. Proportions by age and overall are reported in Table 2, with all vaccine group comparisons. The cumulative proportion of infants with Np carriage of Spn, NTHi and Mc is presented in Fig. 3; Mc was acquired first, followed by Spn and NTHi acquisition from a similar age. Cumulatively 92%, 91% and 96% were positive for Spn, NTHi and Mc respectively by age 7 months.

Of the ten serotypes common to both vaccines, no isolate of serotypes 1, 5 or 7F was cultured. The most common vaccine types were 19F (3%), 19A (3%), 23F (3%) and 9 V (1%). PCV13-VTs declined during the study period, from 15% of isolates (9% of Np swabs) collected in 2012 to 8% of isolates (4% of Np swabs collected) in 2017–18. Serotype 19A was 3% of isolates (2% of Np swabs) in 2012 and 0.5% of isolates (0.3% of Np swabs) in 2017–18. For vaccine groups combined, in a logistic regression, adjusted for clustering by child, there was a statistically significant temporal trend of declining Np carriage of PCV13-types from 9% to 4% between late 2011 to May 2018 (OR 0.840, 95%CI 0.617–0.900 p = 0.029). Carriage was dominated by non PCV13 vaccine serotypes (1032/1159, 89%), the most common types were 16F (15%), 11A (9%), 10A (6%), 7B (6%) and 6C (5%) (Fig. 4, Table 2).

Concurrent carriage of Spn, NTHi and Mc was 7%, 22%, 33%, 33% and 30% of swabs at 1, 2, 4, 6 and 7 months respectively. At one month of age the most prevalent colonisation pattern was Sa alone (23%), and co-colonisation with Spn, NTHi and Mc was the most common pattern at all other ages. At each age, more than 40% of infants had two or more co-colonising respiratory pathogens.

3.3.2. Np carriage of vaccine serotypes by age and vaccine group

At 1 month of age, pre-vaccination, combined Np carriage of serotypes 3, 6A, or 19A in the PPP, _SSS, and SSSP groups respectively was 1.2%, 2.4%, and 0%. At 2 months of age, carriage was 6%, 0.7%, and 2%, and the difference between the non-vaccinated groups was significant (p = 0.036). There were no other significant between group differences at any time point (Table 2).

Table 2
Number of nasopharyngeal swabs positive and percent (%) by vaccine group for NTHi positive swabs and swabs positive for serotypes, 3, 6A and 19A, any PHiD-CV10 types, any PCV13 types, any capsular Spn and any non-VT Spn.

Category	Age (mo)	N, number positive, percent (%) per group									
		N	_PPP	%	N	_SSS	%	N	SSSP	%	Total (%)
NTHi pos	1	82	25	(30.5)	83	25	(30.1)	80	25	(31.3)	(30.7)
	2	140	77	(55)	136	76	(55.9)	139	63	(45.3)	(52.1)
	4	136	86	(63.2)	135	81	(60)	135	82	(60.7)	(61.3)
	6	136	76	(55.9)	131	84	(64.1)	133	83	(62.4)	(60.6)
	7	137	71	(51.8)	132	73	(55.3)	136	71	(52.2)	(53)
Any 3, 6A, 19A	1	82	1	(1.2)	83	2	(2.4)	80	0	(0)	(1.2)
	2	140	**8**	**(5.7)**	136	**1**	**(0.7)**	139	3	(2.2)	(2.9)
	4	136	5	(3.7)	135	4	(3)	135	3	(2.2)	(2.7)
	6	136	3	(2.2)	131	2	(1.5)	133	3	(2.3)	(2.0)
	7	137	2	(1.5)	132	1	(0.8)	136	0	(0)	(0.7)
Any PHiD-CV10 VT Spn	1	82	4	(4.9)	83	2	(2.4)	80	1	(1.3)	(2.9)
	2	140	**14**	**(10)**	136	**4**	**(2.9)**	139	5	(3.6)	(5.5)
	4	136	13	(9.6)	135	8	(5.9)	135	5	(3.7)	(6.4)
	6	136	10	(7.4)	131	6	(4.6)	133	3	(2.3)	(4.5)
	7	137	8	(5.8)	132	5	(3.8)	136	5	(3.7)	(3.9)
Any PCV13 VT Spn	1	82	5	(6.1)	83	4	(4.8)	80	1	(1.3)	(4.1)
	2	140	**21**	**(15)**	136	**5**	**(3.7)**	139	**8**	**(5.8)**	(8.2)
	4	136	18	(13.2)	135	12	(8.9)	135	8	(5.9)	(9.1)
	6	136	13	(9.6)	131	8	(6.1)	133	6	(4.5)	(6.5)
	7	137	10	(7.3)	132	6	(4.5)	136	5	(3.7)	(4.7)
Capsular Spn	1	82	26	(31.7)	83	31	(37.3)	80	24	(30)	(33.1)
	2	140	80	(57.1)	136	66	(48.5)	139	76	(54.7)	(53.5)
	4	136	86	(63.2)	135	89	(65.9)	135	85	(63)	(64)
	6	136	86	(63.2)	131	88	(67.2)	133	85	(63.9)	(64.8)
	7	137	85	(62)	132	86	(65.2)	136	90	(66.2)	(64.3)
Any non-VT Spn	1	82	22	(26.8)	83	27	(32.5)	80	23	(28.8)	(30)
	2	140	62	(44.3)	136	61	(44.9)	139	68	(48.9)	(45.8)
	4	136	69	(50.7)	135	78	(57.8)	135	78	(57.8)	(55.7)
	6	136	74	(54.4)	131	81	(61.8)	133	81	(60.9)	(59.4)
	7	137	77	(56.2)	132	80	(60.6)	136	85	(62.5)	(60.1)

Bold type indicates p-value < 0.05 for group comparisons (Fisher's exact test); Culture of 50 μl added 10 positives for Spn, 1(0.4%), 7 (2%) and 2(0.5%); 41 for NTHi, 6(2%), 13 (3%) and 22(5%); 22 for Sa, 6(2%), 11(3%), and 4(1%) at 1, 2 or 4 months respectively.

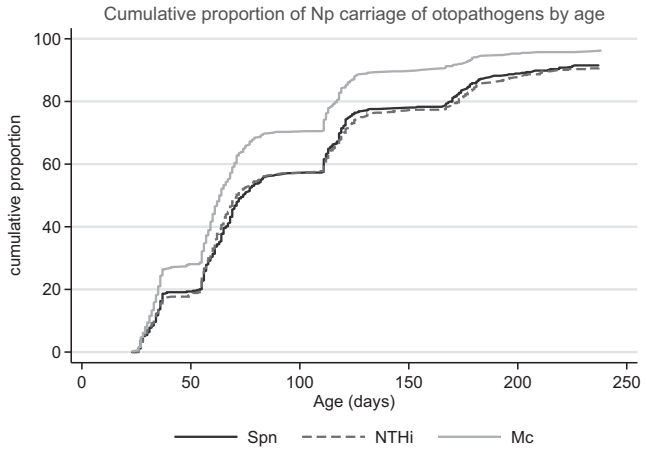


Fig. 3. Cumulative proportion of children positive for the otopathogens Spn, NTHi and Mc by age.

At 2 months of age, one month after first dose of PHiD-CV10 in the SSSP group, Np carriage of ten common serotypes was not significantly lower in the SSSP group (3.6%) compared to either of the non-vaccinated groups (10% and 2.9%). However, as with 3, 6A and 19A, the difference in Np carriage of ten common serotypes between non-vaccinated groups was statistically significant (10% vs 2.9%; p = 0.026). This difference was not sustained at subsequent timepoints.

At 4 months, following two doses of PHiD-CV10 in the SSSP group compared to single doses in the _PPP and _SSS groups, Np carriage of PCV13 serotypes remained highest in the _PPP group at 13.2% compared to 8.1%, and 5.9% in _SSS and SSSP groups but differences were non-significant (p = 0.062). Differences at other timepoints were also non-significant.

3.3.3. Np carriage of non-PCV13 types

Over all ages, Np carriage of non-PVC13 types was 48%, 54% and 54% in the _PPP, _SSS and SSSP groups, respectively. There were no statistically significant differences at any age (Fig. 2, Table 2). There was a transient significant difference in proportion positive for serotype 6C at 6 months of age, between the SSSP group (0.7%) and the _SSS group (5.3%), p = 0.035.

3.4. Antimicrobial sensitivity for Spn and NTHi

Antimicrobial testing (Fig. 4) showed that overall 52% of the capsular isolates were sensitive, 47% were non-susceptible and 1% were resistant to penicillin; while 71% were sensitive and 29% were resistant to azithromycin. At age 7 months, the proportion of infants with an azithromycin resistant isolate was lower in the _PPP group compared to the SSSP group (15.3% vs 27.7%; p = 0.018) but not the _SSS group (15.3% vs 24.2%; p = 0.091). There were no other between group differences in antimicrobial sensitivity at any age (data not shown).

At a serotype level, over 90% of 7B and 15A isolates were macrolide resistant (Fig. 4). The resistance pattern was largely found among infants living in two specific regions during 2012/13 for 15A and 2013/14 for 7B.

Approximately 9% of NTHi positive swabs had a β-lactamase producing isolate.

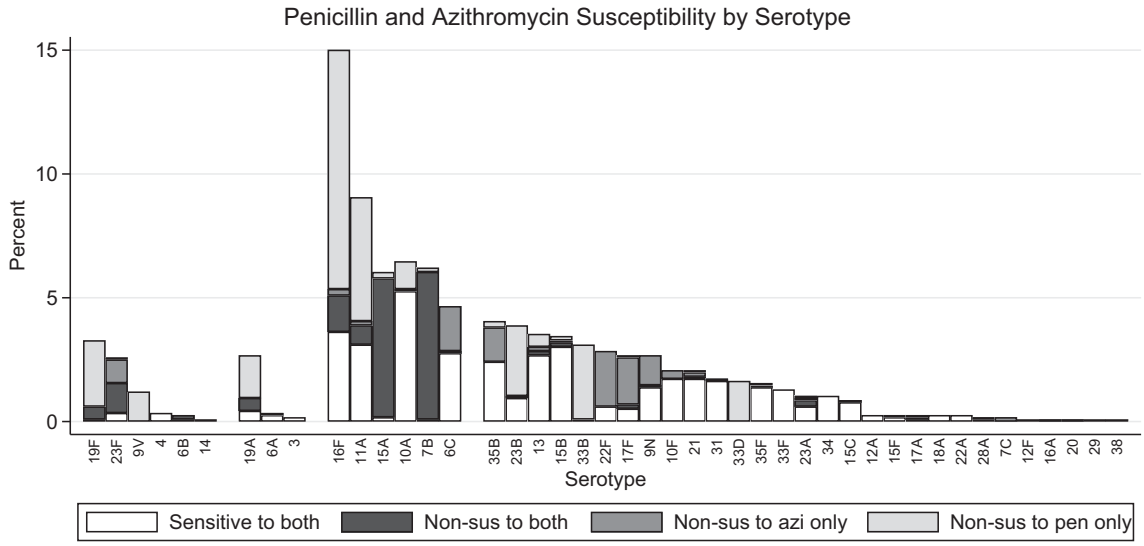


Fig. 4. Percent of isolates with sensitivity to penicillin and azithromycin, in order of PCV7, PHiD-CV10 and PCV13 types, followed by hierarchy of remaining serotypes.

Table 3
Serotype breakdown of consecutive visit carriage.

	Same serotype at consecutive visits		
Serotypes (n)	5 visits (N = 2) 10A (1) 23B (1)	4 visits (N = 12) 15A (3) 10F (2) 1 each of 10A, 11A, 16F, 19F, 33B, 35B, 35F	3 visits (N = 43) 7B (9) 16F (8) 11A (4) 15B (3) 2 of each 15A, 31, 19F, 6C, 9 V 1 each of 13, 21, 10A, 10F, 22F, 23B, 33B, 33F, 35B, 6C, 9 N

3.5. Serotype carriage patterns, new acquisitions, and multiple carriage

Of the 425 infants randomised, at least one Np swab was collected from 422 infants, 33 of whom were negative for capsular Spn or did not contribute swabs at all timepoints. There were 60 (3%) swabs with two different serotypes isolated, one had three serotypes. Of the 389 infants with serotype data, 20 infants had a serotype at all five study visits. Consecutive carriage of the same serotype at 2 or more visits was seen in 166 infants for 2 visits, 43 for 3 visits, 12 for 4 visits and 2 for 5 visits. Consecutive serotypes at 3 or more visits are shown in Table 3. Serotype 19F was the only vaccine serotype to be detected at 3 or more consecutive visits. The remaining infants had different serotypes or were Spn-negative visit to visit.

3.6. NP carriage and antimicrobial sensitivity of other otopathogens

Np carriage of NTHi by age and vaccine group (Fig. 2), as reported in Table 2 shows no statistically significant differences.

There were no statistically significant differences at any time point between the vaccine groups for carriage of either Sa or Mc (Fig. 2). Almost all (97%) of the Mc positive swabs (97% of infants) had a β-lactamase producing Mc isolate. Overall, 22% of the Sa positive swabs (4% infants) had a cefoxitin resistant isolate.

3.7. Ear discharge swabs

Overall, 108 swabs were collected from 124 discharging ears in 57 infants (13%) visited between 2 and 7 months of age; 74 swabs were from AOMwiP and 34 were from CSOM. At least one pathogen was isolated in 40 of the 57 infants. One ear discharge swab was not able to be assessed for NTHi and Mc due to swarming overgrowth.

3.7.1. Ear discharge culture at the primary 7 month endpoint, by vaccine group

There were 17, 12 and 10 infants with ear discharge in the _PPP, _SSS and SSSP groups 7 months of age (9%). None were culture positive for serotypes 3, 6A, or 19A at this age. There were no significant vaccine group differences (Table 4), other than an unexpected difference in NTHi-positive ear discharge at 7 months between the _SSS and SSSP groups (3/11, 27.3% vs 8/10, 80%; p = 0.030) but the low numbers limit robust interpretation.

3.7.2. Ear discharge culture-positive swabs by pathogen and age, vaccine groups combined

By age, ear discharge swabs were positive for Spn in up to 23% and NTHi in up to 61% of infants with perforated tympanic membranes. Sa declined from 100% at 2 months of age to 26% at 7 months of age. NTHi was the most common ear discharge pathogen detected in either AOMwiP or CSOM (48% and 51%, respectively) (Table 4, Fig. 5). Among 22 Spn isolates cultured from 19 ear discharge swabs taken from 15 infants the serotype hierarchy was 16F(4) and 7B(4, all from one infant), 11A(2), 13(2), 15A(2), 19A(2), 19F(2), 33F(2), 10A(1) and 33B(1). Mixed serotype infections were detected on several occasions but are not described here.

4. Discussion

4.1. Nasopharyngeal carriage

In this 3-arm trial of a head to head comparison of PCV13 and PHiD-CV10 standard 3-dose schedules, and comparison with a novel 4-dose early combination of PHiD-CV10 at 1, 2 and 4 months plus PCV13 at 6 months we found no difference in primary microbiological outcomes between the SSSP and _PPP groups (for NTHi),

Table 4
Vaccine group comparisons of ED culture-positive for NTHi, serotypes 3, 6A, and 19A, any PCV13 types, any capsular Spn, any Sa, or Spn and NTHi co-colonisation.

Category	Age (mo)	N, number positive, percent (%) per group									Total (%)
		N	_PPP	%	N	_SSS	%	N	SSSP	%	
NTHi	2	0	0	(0)	1	0	(0)	2	0	(0)	(0)
	4	5	3	(60)	1	1	(100)	4	2	(50)	(60)
	6	16	6	(37.5)	6	3	(50)	9	5	(55.6)	(45.2)
	7	17	12	(70.6)	11	**3**	**(27.3)**	10	**8**	**(80)**	(60.5)
Any 3, 6A, 19A	2	0	0	(0)	1	0	(0)	2	0	(0)	(0)
	4	5	0	(0)	1	0	(0)	4	0	(0)	(0)
	6	16	0	(0)	6	1	(16.7)	9	0	(0)	(3.2)
	7	17	0	(0)	11	0	(0)	10	0	(0)	(0)
Any PCV13 VT Spn	2	0	0	(0)	1	0	(0)	2	0	(0)	(0)
	4	5	1	(20)	1	0	(0)	4	0	(0)	(10)
	6	16	0	(0)	6	1	(16.7)	9	0	(0)	(3.2)
	7	17	1	(5.9)	11	0	(0)	10	0	(0)	(2.6)
Capsular Spn	2	0	0	(0)	1	0	(0)	2	0	(0)	(0)
	4	5	1	(20)	1	0	(0)	4	0	(0)	(10)
	6	16	2	(12.5)	6	2	(33.3)	9	3	(33.3)	(22.6)
	7	17	3	(17.6)	11	1	(9.1)	10	4	(40)	(21.1)
Sa	2	0	0	(0)	1	1	(100)	2	2	(100)	(100)
	4	5	2	(40)	1	0	(0)	4	2	(50)	(40)
	6	16	3	(18.8)	6	1	(16.7)	9	4	(44.4)	(25.8)
	7	17	6	(35.3)	11	**0**	**(0)**	10	**5**	**(50)**	(29)
Capsular Spn & NTHi	2	0	0	(0)	1	0	(0)	2	0	(0)	(0)
	4	5	1	(20)	1	0	(0)	4	0	(0)	(10)
	6	16	1	(6.3)	6	2	(33.3)	9	2	(22.2)	(16.1)
	7	17	2	(11.8)	11	**0**	**(0)**	10	**4**	**(40)**	(15.8)

Bold type indicates p-value < 0.05 for group comparisons (Fisher's exact test); Culture of 50 μl added 1(10%) additional Sa positive. At 2, 4, 6 and 7 months respectively there were 0, 3 (30%), 14(45%) and 14(37%) of ED with no otopathogen growth.

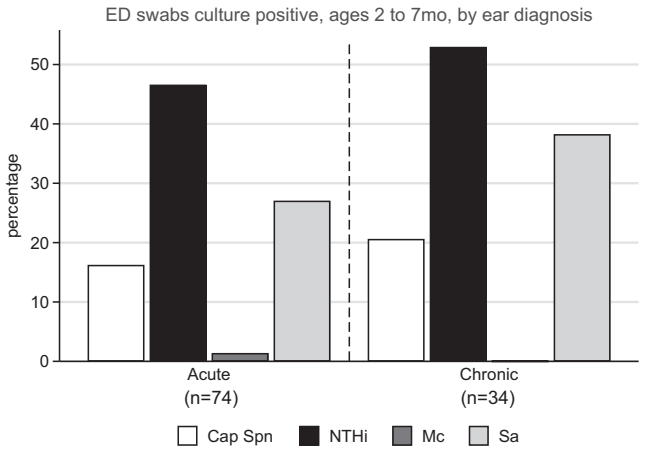


Fig. 5. Proportion of ear discharge swabs positive for Spn, NTHi, Mc and Sa, by ear diagnosis, vaccine groups combined.

or SSSP and _SSS groups (for serotypes 3, 6A and 19A). There were no significant vaccine group differences in Np carriage of other pathogens investigated at the primary 7 month endpoint.

This study highlights the ongoing early life Np colonisation by multiple otopathogens. Prevalence of Np carriage at 7 months of age was 64% for Spn, 53% for NTHi, 73% for Mc and 5% for Sa. Interestingly, Np carriage of Mc was higher than NTHi or Spn at 1 month of age indicating earlier colonisation or detection; however, almost no Mc was found in ear discharge. Although comparisons with historical data are less reliable, current prevalence may be lower than previously reported from this population. An RCT in 1994–2000 reported higher carriage prevalence for Spn (80%) and NTHi (77%) [23], and 2012–13 cross-sectional data from Indigenous children (mean age 16 months) detected 80%, 70% and 44% carriage of Spn, NTHi and Mc respectively and 19% Sa [12]. While current data indicate a decrease in Np carriage of Spn and NTHi, these rates are still high confirming ongoing early exposure to multiple bacterial respiratory pathogens. A major driver is likely to be household crowding, which continues to be a priority issue in remote communities.

We report low Np carriage rates of serotypes 3 (0.2%) and 6A (0.4%) and 19A (3%) overall. While this is consistent with our previous studies for serotype 3, [4,12] we expected higher carriage for 6A and 19A. Our power and sample size estimates predicted that the study was powered to detect a 15% absolute difference in 19A carriage, from 20% to 5%. During the course of this RCT with the introduction of PCV13 in the NT, surveillance indicated reductions in 19A carriage, limiting power to capture vaccine group differences.

There was an overall low percentage of all PCV13 serotypes isolated during this trial. PCV13-type carriage decreased slowly, but significantly over the course of the study. There was unexpectedly significantly higher PCV13-VTs in the _PPP group compared to both the _SSS and SSSP groups at 2 months, and the _PPP group continued to have higher (non-significant) carriage of PCV13 vaccine serotypes than _SSS and SSSP at 4, 6, and 7 months. This is an interesting observation as immunogenicity data from this study showed significantly lower IgG GMCs in the _PPP group to most PCV serotypes at 4 months [19].

A diversity of non-vaccine serotypes play an important role in otitis media in this population. Serotype 16F has been uniquely dominant in the NT but has not been the target of any expanded valency vaccine development.

We found reduced carriage and middle ear infection caused by vaccine type pneumococci, as had been reported previously [12,24], replaced in carriage and middle ear infection by numerous non-PCV Spn serotypes.

4.2. Ear discharge microbiology

NTHi was the most commonly cultured otopathogen from the ear discharge of infants with either AOMwiP or CSOM. No ear discharge was positive for serotypes 3, 6A, or 19A. Unexpectedly, the proportion of NTHi, Sa, and NTHi + Spn positive ear discharge sam-

ples were all lower in the _SSS compared to the SSSP group at 7 months of age. As both groups had 3 doses of the PHiD-CV10 vaccine, differences in NTHi-related middle ear infection are difficult to explain, also because there was no parallel difference in NTHi-Np carriage. It is plausible that maternal antibody against NTHi interfered with the infant responses to the earlier 1–2–4 month schedule compared to the 2–4-6 month schedule. Whilst these mothers were not vaccinated with a protein D vaccine, they may have natural antibodies from prior exposure to NTHi, especially those who live with other young children. We did not test maternal antibodies in this trial, however, in unvaccinated infants at 2 months of age protein D antibody levels (*trans*-placental) were very low [19].

We observed high density Sa infection in ear discharge of infants at 2 months of age. Whilst ear canal contamination could be the source of Sa in the ear discharge, our finding that peak Np carriage of Sa (55%) is also at 2 months of age implies that the Np may be a source at this early age.

For NTHi-OM, our study to 7 months of age was not able to confirm findings from observational studies in older post-booster dose children in this population that reported a potential compartmental effect of PHiD-CV10 on reducing NTHi middle ear infection, in the absence of an effect on Np carriage [12].

4.3. Mixed primary schedules

Prior to this study there were no trials describing mixed primary PCV schedules. Studies published during the time of this RCT with mixed primary and booster series trials report immunogenicity outcomes (safety, reactogenicity, IgG, OPA and memory B cells) [25,26], however, there are no other known published randomised trials of mixed primary course PCV schedules.

4.4. Comparison with global experience, RCTs using PHiD-CV10 in a primary series

Few PHiD-CV10 studies report nasopharyngeal carriage during or at one month post primary infant series, and none are comparisons with PCV13. Infants in the Finnish RCT [27] received PHiD-CV10 in a 3 dose primary series compared to a 2 dose series and unvaccinated controls. They observed no significant difference in Np carriage of any Spn, NTHi, Mc, or Sa. Post primary series, at approximately 6 months of age, PHiD-CV10 serotype carriage was 183/1803 (10.1%), 159/1289 (12.3%) and 237/1897 (12.5%) and NTHi carriage was 51/1803 (2.8%), 33/1289 (2.6%) and 43/1897 (2.3%) in the 3-dose, 2-dose and control groups respectively. An OM and carriage RCT in Panama [15] comparing a 3 dose PHiD-CV10 primary series (2, 4, 6 months) with unvaccinated controls also reported no difference in NTHi (5.7% (n = 788) vs 5.6% (n = 784)), Sa, or Mc, with a significant difference in overall PHiD-CV10 types, which was lower in the PHiD-CV10 group (12% vs 16%) aged approximately 12 months. Both these studies report much lower Np carriage rates than our cohort. Comparisons of unvaccinated and a 2- or 3-dose primary series of PHiD-CV10 in Nepal also showed no difference in pneumococcal Np carriage (69/124, 55.6% vs 58/108, 53.7% vs 57/108. 25.8%) at 9 months of age (post primary series) [28]. NTHi was not reported. A RCT in the Netherlands [29] comparing PCV7 and PHiD-CV10 (both 2, 3, 4 month primary series) reported no significant difference at age 11–13 months in NTHi (138/255, 54% vs 296/512, 58%), overall Spn (119/258, 46% vs 249/514, 48%), or serotype 19A (19/257, 7% vs 33/514, 6%). The failure of primary series PHiD-CV10 to reduce NTHi carriage seems to have been established in these trials, supported by cross-sectional data in diverse settings [4,30–32]. However, as none compare PHiD-CV10 with PCV13, impact on Np carriage of serotypes 3, 6A, and 19A remain unclear. Our study had the potential to address this question, but concurrent introduction of PCV13 to the national immunisation schedule at commencement of our trial induced herd effects and thus diminished carriage of these serotypes during the trial.

4.5. Neonatal vaccination

We report NP carriage rates of approximately 30% for NTHi and Spn, and 47% for Mc at one month of age, indicating a possible role for vaccination earlier than the 1 month of age first dose given in this trial. There are two published trials of neonatal PCV7 vaccination, both in populations with high rates of Spn Np carriage and IPD. Both studies showed that safety and reactogenicity outcomes were not different between controls and vaccinees. The Kenyan trial found no statistically significant difference in Np Spn carriage at 18 or 36 weeks [33]. The Papua New Guinean study also found no difference in overall Spn Np carriage or PCV7 VTs and non-VTs at any age [34]. They report high rates of dense carriage, with 22% of infants positive for pneumococci at 1 week of age, and a broad diversity of serotypes. There have been no neonatal trials of PHiD-CV10 or PCV13. If the Australian Indigenous infant schedule was to add a neonatal PCV, efficacy and safety studies would be needed to confirm it could be co-administered with the neonatal Hepatitis B vaccine.

4.6. Maternal vaccination

Maternal vaccination can provide antibodies to protect the infant in the first weeks of life. A published study of maternal pneumococcal polysaccharide vaccine in Indigenous mothers in the NT, however the impact on infant Np Spn carriage was limited, and there was evidence that maternal antibodies interfered with infant response to PVC primary schedules [35]. A placebo controlled maternal immunisation study of a 9 valent conjugate vaccine in the US also showed evidence of antibody interference, with decreased responses to the infant primary series, which was associated with increased rates of OM in the vaccinated group [36].

4.7. Limitations

Our few significant findings from exploratory analyses could be significant due to chance alone, as we did not adjust for multiple comparisons. However, the observations are of interest and contribute to understanding early carriage.

The observed prevalence of serotype 19A carriage was lower than predicted as a result of PCV13 introduction across Australia in late 2011, this limited power to detect a significant difference. The use of PCR and culture in combination may have increased the detection sensitivity of ear discharge pathogens [7,37].

There were differences in the modifiable risk factors of smoking and breastfeeding over time and between communities, however the randomisation stratification at the community level was effective, and the factors were not dissimilar by vaccine group at both 1 and 6 months of age, and over time.

5. Conclusions

This RCT reports that PHiD-CV10 does not reduce early NTHi carriage in a high risk population compared to non-protein D PCV13. We found no differences in VT- or non-VT Spn carriage between 3-dose PHiD-CV10 or PCV13 schedules nor any difference between these and a mixed 4-dose primary schedule. NTHi and non-PCV serotypes are still major concerns for ear disease in this population. NTHi was the main pathogen cultured from ear discharge. These data suggest that multiple pathogens are driving

early OM episodes in this population. These infants continue to be at high risk of early and persistent Np carriage and progression to chronic OM with long term sequelae. Vaccines with efficacy and/or herd effects against both NTHi and Spn nasopharyngeal carriage in the first weeks of life will significantly improve the quality of life for children who are currently at great disadvantage in terms of language development, education, and later employment. In addition to, or in the absence of improved vaccines, other strategies to prevent early exposure to infection will require cross-sector investment to address vast gaps in social determinants of Aboriginal and Torres Strait Islander health.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Authors JB, NW, BA, MJB, VMO, KL, ASB, HCSV, ACC, have nothing to declare. EKM has received support for OPA assays for a completed PCV trial in Vietnam and has an ongoing collaboration with Pfizer on pneumonia in Mongolian adults. AJL received a donation of assay reagents for immunogenicity assays from GlaxoSmithKline.

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