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PneuMum: Impact from a randomised controlled trial of maternal 23-valent pneumococcal polysaccharide vaccination on middle ear disease amongst Indigenous infants, Northern Territory, Australia

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ABSTRACT

Background: We assessed maternal 23-valent pneumococcal polysaccharide (23vPPV) vaccine efficacy (VE) against middle ear disease and pneumococcal carriage amongst Australian Indigenous infants.
Methods: In an open label, allocation concealed, outcome-assessor blinded, community stratified, randomised controlled trial, healthy pregnant Indigenous women aged 17–39 years in the Northern Territory of Australia received the 23vPPV (1:1:1) at: 30–36 weeks gestation, birth, or were unvaccinated (ClinicalTrials.gov NCT00714064). Co-primary outcomes were the point prevalences of infant middle ear disease and 23vPPV-type carriage at age 7 months.
Results: The consent rate was 50% (313/632). Among 227 eligible participants randomised, retention rates were 86% (66/77) controls; 89% (67/75) pregnancy vaccinees; 88% (66/75) birth vaccinees. At infant age 7 months, ear disease prevalence was: 71% (47/66) controls, 63% (42/67) pregnancy vaccinees, 76% (50/66) birth vaccinees; and 23vPPV-type carriage was: 26% (17/66) controls, 18% (12/67) pregnancy vaccinees, 18% (12/66) birth vaccinees. For pregnancy vaccinees, VE was 12% (95% CI −12% to 31%) against infant ear disease and 30% (95% CI −34% to 64%) against 23vPPV-type carriage. In a post-hoc analysis, VE against infant ear disease concurrent with carriage of 23vPPV or related types was 51% (95% CI −2% to 76%). There were no serious adverse effects following receipt of the 23vPPV in pregnancy or at birth.
Conclusions: In a high risk population, our study was unable to demonstrate efficacy of 23vPPV in pregnancy against the co-primary outcomes of either all-cause infant ear disease or 23vPPV-type nasopharyngeal carriage at age 7 months. Efficacy against ear disease concurrent with carriage of vaccine-related serotypes (a more specific outcome) suggests 23vPPV in pregnancy may complement childhood pneumococcal vaccination programs.

1. Introduction

Indigenous Australian children experience the highest published rates of acute and chronic ear infections in the world [1]. This may result in permanent middle ear damage, hearing loss and educational disadvantage. These infections are mainly bacterial and the pneumococcus and non-typeable *Haemophilus influenzae* are the predominant pathogens. Pneumococcal colonisation begins within days of birth [2], months before direct immunological protection from infant pneumococcal conjugate vaccine (PCV) may be expected. Early recurrent pneumococcal infections cause inflammation and mucosal damage that precede and predispose to the

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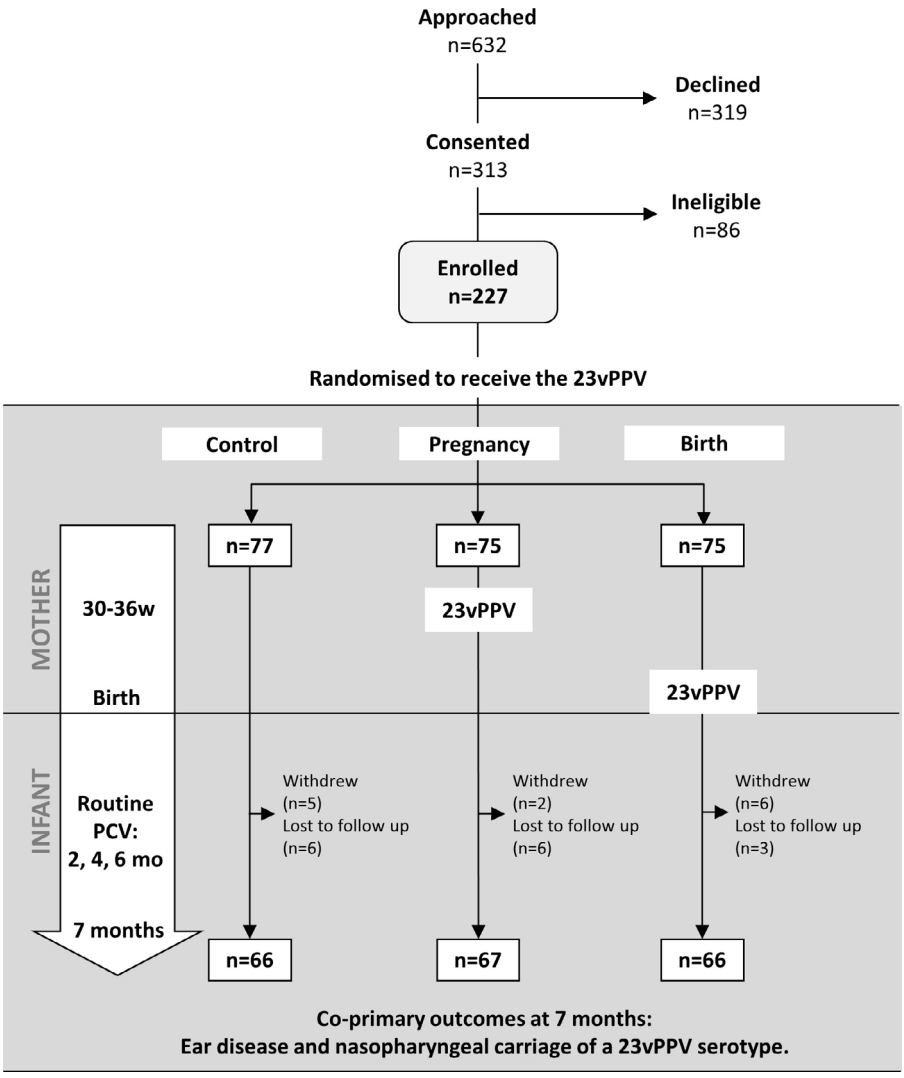

Fig. 1. PneuMum participant flow diagram.

development of chronic suppurative otitis media, chronic bronchitis and bronchiectasis, all common among Indigenous populations [1,3,4]. New strategies are needed to prevent, or delay, early pneumococcal colonisation and middle ear disease.

Maternal immunisation with the 23-valent pneumococcal polysaccharide vaccine (23vPPV) is one strategy that may offer infant protection from birth. Randomised controlled trials of 23vPPV in pregnancy have been conducted in Asia [5–8], Africa [9,10] and the Americas [11,12]. These trials have consistently demonstrated higher vaccine-specific antibody levels in maternal, cord and infant blood, and in breast milk samples following 23vPPV in pregnancy compared to controls [5–7,9–11]. Two studies have assessed infant pneumococcal carriage at age 6–7 months following maternal 23vPPV. Whereas Munoz et al. [11] reported 34% (13/38) carriage in the control group compared with 11% (2/18) in the 23vPPV group, Lopes et al. [12] reported 15% (7/46) carriage in the control group compared with 17% (7/42) among pregnancy vaccinees and 18% (8/45) among birth vaccinees. Only one study, O'Dempsey et al. [10] in the Gambia, investigated infant otitis media. By one year of age, otitis media cases were reported in 4% (3/75) of controls compared to none (0/75) among 23vPPV vaccinees. It remains unclear whether 23vPPV in pregnancy can impact infant carriage and clinical outcomes. Recently, Daly et al. [13] reported use of an investigational 9-valent PCV in pregnancy. The vaccine was well tolerated but appeared to interfere with subsequent infant serotype specific immune responses to the 7-valent PCV (7vPCV) and increased the risk of otitis media before age 6 months, compared to controls.

Our randomised controlled trial, "PneuMum", aimed to determine whether maternal 23vPPV, in pregnancy or at birth, could reduce ear disease and 23vPPV-type nasopharyngeal colonisation among infants at 7 months of age in a setting where both are endemic.

2. Methods

2.1. Study design

We conducted an open-label, allocation concealed, outcome-assessor blinded, community stratified, three arm parallel group (1:1:1) randomised controlled trial (Fig. 1) of maternal 23vPPV (PNEUMOVAX® 23, Merck, USA).

2.2. Outcomes

Primary study outcomes at infant age 7 months were: prevalence of middle ear disease; and nasopharyngeal carriage of

23vPPV-type pneumococci. Secondary outcomes included the same assessments at infant age 1 and 2 months.

2.3. Participants, eligibility and randomisation

Pregnant Indigenous women in Darwin, Alice Springs and remote communities of the Northern Territory of Australia were recruited during public hospital antenatal screening visits (Royal Darwin or Alice Springs Hospitals) between August 2006 and January 2011. Eligibility, confirmed prior to randomisation but no earlier than 28 weeks gestation, was as follows: Indigenous Australian women aged 17–39 years with a singleton uncomplicated pregnancy (no existing or pre-existing condition judged by the clinical investigator to make pregnancy high-risk); resident of the catchment area; intending to give birth at a participating hospital; no HIV, history of severe allergy, uncontrolled asthma or splenectomy; and no history of 23vPPV within the previous three years.

At 30–36 weeks gestation (inclusive), eligible participants were stratified by community of residence and randomised in blocks of six by an independent statistician using a computerised random number generator. Allocation concealment was maintained using sealed, opaque envelopes. Randomisation was to one of three groups:

- Pregnancy vaccinees—maternal 23vPPV at the randomisation visit.
- Birth vaccinees—maternal 23vPPV within 72 h of infant birth.
- Control group—maternal 23vPPV offered at study exit (7 months post-partum).

Throughout the study, infants received their routine PCV's via primary health care providers, recommended at 2, 4 and 6 months of age [14,15]. Two PCV's in use over this period for infants were the 7vPCV from June 2001 and the 10-valent pneumococcal *H. influenzae* protein D conjugate vaccine (10vPHiD-CV) from October 2009.

2.4. Sample collection and testing

Maternal nasopharyngeal swabs were collected at birth and infant nasopharyngeal swabs at 1, 2 and 7 months. Pneumococcus was isolated according to World Health Organization (WHO) guidelines [16], identified by α-haemolysis, colony morphology and susceptibility to optochin. Where identified, four colonies per swab (including those of different morphologies) were serotyped by Quellung reaction using serotype-specific antisera (Statens Serum Institute, Copenhagen, Denmark). Non-typeable pneumococci and optochin non-susceptible isolates were not considered further. Nontypeable *H. influenzae* and *Moraxella catarrhalis* were cultured using standard microbiological methods [17].

Pregnancy vaccinees had maternal blood collected immediately prior to vaccination. All groups had maternal venous and cord blood collected at birth and infant blood at age 7 months. Breast milk was collected at birth, 1, 2 and 7 months post-partum. Serum immunoglobin (Ig) G specific for each 23vPPV-type were measured by trained laboratory staff blinded to the randomised allocation using the methodology of a validated 3rd generation WHO ELISA [18,19]. Breast milk IgA specific for 15 of the 23vPPV-types were measured using a novel ELISA method (Supplementary Method 1). A comprehensive analysis of vaccine immunogenicity will be the subject of a separate report.

2.5. Ear assessments

At the 1, 2 and 7 month visits, each child had bilateral clinical ear assessments performed by trained research nurses using tympanometry and otoscopy. Tympanocentesis was not performed. An independent assessor (CW), blinded to the randomisation allocation and the research nurse diagnosis, reviewed recordings of the tympanometry and pneumatic video-otoscopy. Where the independent assessor and research nurse diagnosis (ear disease present/absent) was concordant this was accepted as final. Where discordant, a second independent assessor (PSM) reviewed the tympanometry and pneumatic video-otoscopy blinded to all previous diagnoses and the randomisation allocation. The diagnosis from the second independent review was accepted as final. The presence or absence of ear disease was based on the infant's worst ear and determined according to recommended guidelines for clinical practice in this population (Supplementary Table 1) [20]. Outcomes for sub-categories of ear disease are not presented.

2.6. Analyses

Given an expected prevalence at age 7 months of 90% for middle ear disease and 55% for nasopharyngeal carriage of 23vPPV-types [1,21], the intended sample size of 210 participants (randomised 1:1:1), provided 80% power to demonstrate a 23% (90 to 69%) relative reduction in ear disease and a 45% (55 to 30%) relative reduction in 23vPPV-type carriage.

Mother-infant pairs were considered to have successfully completed the study follow-up if the infant had both a nasopharyngeal swab cultured and a valid independent ear assessment performed (tympanometry and/or video-otoscopy) at the 7 month visit. Data were analysed according to randomisation groups. Withdrawals, infants lost to follow up, or those without assessment data for both primary outcomes at age 7 months were excluded from analysis of study outcomes.

Primary analyses were independent comparisons between the point prevalence at age 7 months of ear disease and of 23vPPV-type carriage between infants of the control group compared with those of pregnancy vaccinees and birth vaccinees respectively. VE was calculated (1 minus the risk ratio) with 95% confidence intervals (95% CI). Confidence intervals were not adjusted for multiple comparisons, those excluding zero were considered statistically significant. Secondary analyses were comparisons of any pneumococcal carriage between controls and the respective vaccinees at age 7 months as well as comparisons of ear disease and carriage outcomes at ages 1 and 2 months.

A post hoc analysis at age 7 months, intended to maximise sensitivity for ascertainment of potentially vaccine-preventable ear disease, compared the prevalence of ear disease concurrent with nasopharyngeal carriage of a 23vPPV-type or the related serotype 6A (23v6A ear disease) between controls and the respective vaccinees. Serotype 6A was included based on demonstrated cross-reactivity with serotype 6B (a 23vPPV-type) [22,23].

2.7. Ethics, clinical trial registration and protocol

Approval was obtained from the Human Research Ethics Committee of the Northern Territory Department of Health and Community Services and Menzies School of Health Research (05/52). The trial was registered at clinicaltrials.gov (NCT00714064, formerly NCT00310349) and the protocol is available online at http://www.menzies.edu.au/icms_docs/213758_PneuMum_Protocol.pdf. The 23vPPV was administered according to Australian guidelines [14,15]. 23vPPV batch numbers were as follows: 2006—G1629, G1812; 2007—G1629, G3836, G3837, H5496; 2008—H5946, J3354; 2009—J3354, K1284, K2436, K2437, K3610, L0220, N1234 N1419; 2010—L0224, N0397, N1127, N1419, N1516, N1560, N1561, N3244, N3494, N3644; 2011—N3032, N3644, N3851.

Table 1
Participant characteristics.

Maternal characteristics at enrolment	Control group $n=77$	Pregnancy vaccinees $n=75$	Birth vaccinees $n=75$
Median maternal age (range), years	24 (17–38)	23 (17–39)	25 (17–37)
Household occupancy (range), people	5 (2–11)	4 (1–12)	4 (2–15)
	***n* (%)**	***n* (%)**	***n* (%)**
Remote community residence	24 (31%)	23 (31%)	24 (32%)
Primigravida	29 (38%)	29 (39%)	22 (29%)
Smoker	34 (44%)	36 (48%)	36 (48%)
Influenza vaccine in pregnancy	16 (21%)	9 (12%)	14 (19%)
Infant characteristics at birth	$n=76$	$n=75$	$n=74$
Median gestational age (range), weeks	39 (35–42)	39 (35–41)	39 (34–41)
Median birthweight (range), grams	3381 (2132–4620)	3293 (2060–4425)	3334 (2080–4330)
	***n* (%)**	***n* (%)**	***n* (%)**
Male	42 (55%)	36 (48%)	42 (57%)
Low birth weight (<2500 grams)	4 (5%)	7 (9%)	4 (5%)
Premature (<37 weeks)	2 (3%)	7 (9%)	4 (5%)
Special or intensive care admission	16 (21%)	14 (19%)	15 (20%)
Infant characteristics at 7 month visit	$n=66$	$n=67$	$n=66$
Median visit age (range), months	7.2 (6.7–8.1)	7.2 (6.6–9.2)	7.2 (6.6–8.3)
	***n* (%)**	***n* (%)**	***n* (%)**
Breast fed	38 (58%)	43 (64%)	45 (68%)
Breast fed exclusive	28 (42%)	30 (45%)	35 (53%)
Mother smoking	37 (56%)	49 (69%)	37 (56%)
≥2 doses of any PCV	57 (86%)	56 (84%)	59 (89%)

Maternal characteristics are described at time of enrolment (prior to withdrawals/loss to follow-up). Infant characteristics at birth exclude 2 pre-birth withdrawals. Infant characteristics at 7 month visit exclude 13 withdrawals and 15 lost to follow-up. PCV coverage calculated at 14 days prior to 7 month visit, included ≥2 doses of the 7-valent pneumococcal conjugate vaccine (7vPCV; 59% overall) or the 10-valent pneumococcal *Haemophilus influenzae* protein D-conjugate vaccine (10vPHID-CV; 28% overall) which were in similar proportion in each group (data not shown). No infant received only two doses of a mixed schedule.

3. Results

3.1. Participants

The consent rate was 50% (313/632). Among 227 eligible participants subsequently randomised (Fig. 1), the retention rate was: 86% (66/77) controls; 89% (67/75) pregnancy vaccinees; 88% (66/75) birth vaccinees. Neither the withdrawals ($n=13$) nor those lost to follow-up at 7 months ($n=15$) were differentially clustered by randomisation group. Reasons for withdrawal were: moved outside study catchment area ($n=3$); personal ($n=6$); not specified ($n=4$). Reasons for loss to follow-up were: moved outside study catchment area ($n=6$); unable to be located ($n=8$); refusal of 7 month ear exam ($n=1$). Median time between receipt of the 23vPPV in pregnancy and birth was 6 weeks (IQR 4–8 weeks), range 1–10 weeks. All participants that completed the 7 month follow-up received the 23vPPV as allocated.

Participant characteristics were similar among the allocated groups (Table 1). Stratification by location of residence resulted in equal proportions of remote dwelling participants in each randomisation group. Median maternal age at enrolment was 23–25 years with self-reported smoking rates high, both during pregnancy (44–48%) and at infant age 7 months (56–69%). Few women (12–21%) received seasonal influenza vaccine during pregnancy, whilst most infants (84–89%) had received ≥2 PCV (7vPCV or 10vPHiD-CV) doses at least 14 days prior to the 7 month visit.

3.2. Safety

Four pregnancy vaccinees (5%) reported local pain, swelling or mild nausea post 23vPPV. Two pregnancy vaccinees had preterm births (35 weeks, 37 weeks) of otherwise healthy babies. Prevalence of preterm birth, low birth weight, or neonatal intensive and special care admission were similar for pregnancy vaccinees compared with those not in the pregnancy vaccine group (Table 1). Two study participants (non-pregnancy vaccinees) withdrew prior to birth.

3.3. Co-primary outcomes

At age 7 months (Table 2), middle ear disease prevalence among infants was 71% (47/66) for the control group compared with 63% (42/67) for infants of pregnancy vaccinees (VE 12%, 95% CI −12% to 31%) and 76% (50/66) for infants of birth vaccinees (VE −6%, 95% CI −31% to 13%). At the same age, 26% (17/66) of infants in the control group had nasopharyngeal carriage of a 23vPPV-type compared with 18% (12/67) for infants of pregnancy vaccinees (VE 30%, 95% CI −34% to 64%) and 18% (12/66) for infants of birth vaccinees (VE 29%, 95% CI −36% to 63%).

Table 2
Co-primary ear disease and carriage outcomes at infant age 7 months.

Outcomes at infant age 7 months	Control group $n=66$	Pregnancy vaccinees $n=67$		Birth vaccinees $n=66$	
	n (%)	*n* (%)	VE[1]% (95% CI)	*n* (%)	VE[2]% (95% CI)
Ear disease	47 (71)	42 (63)	12 (−12,31)	50 (76)	−6 (−31,13)
NP carriage of a 23vPPV-type	17 (26)	12 (18)	30 (−34,64)	12 (18)	29 (−36,63)

NP: nasopharyngeal; VE[1]: vaccine efficacy of pregnancy vaccinees versus control group; VE[2]: vaccine efficacy of birth vaccinees versus control group; VE = 1 minus the risk ratio.

Table 3
Ear disease and nasopharyngeal pneumococcal carriage outcomes at 1, 2 and 7 month visits among infants of controls, pregnancy and birth vaccinees.

	Infant age 1 month			Infant age 2 months			Infant age 7 months		
	Control group $n=66$	Pregnancy vaccinees $n=67$	Birth vaccinees $n=66$	Control group $n=66$	Pregnancy vaccinees $n=67$	Birth vaccinees $n=66$	Control group $n=66$	Pregnancy vaccinees $n=67$	Birth vaccinees $n=66$
Blinded assessor diagnosed infant ear disease, *n* (%)	13 (25%)	14 (25%) $p^1=1.000$	12 (28%) $p^2=0.817$	17 (33%)	21 (41%) $p^1=0.418$	23 (45%) $p^2=0.228$	47 (71%)	42 (63%) $p^1=0.358$	50 (76%) $p^2=0.694$
23vPPV carriage types, (*n*)									
Serotype 3	1	0	0	0	1	0	1 (m**1**)	2	0
Serotype 6B (PCV)	0	0	0	1	2	0	0	2	1
Serotype 8	0	1	0	0	0	0	0	1	0
Serotype 9N	0	0	1	0	0	1	0	1	1
Serotype 9V (PCV)	0	0	0	1	0	0	0	0	0
Serotype 10A	0	1	0	1	1	2	1	2 (m**5**)	6 (m**6**)
Serotype 11A	1	0	2	1	0	1	1	1	0
Serotype 15B	0	0	0	0	1	0	4 (m**1**) (m**3**)	0	0
Serotype 19A	0	0	0	0	1	1	6 (m**2**) (m**4**)	2	3 (m**7**)
Serotype 19F (PCV)	0	1	1	1	2	0	1 (m**2**)	0	1
Serotype 22F	0	0	0	1	0	0	1	1	0
Serotype 23F (PCV)	0	1	0	0	0	1	0	0	0
Serotype 33F	0	0	0	0	0	0	4	0	0
Overall 23vPPV carriage, *n* (%)	2 (3%)	4 (7%) $p^1=0.680$	4 (8%) $p^2=0.423$	6 (10%)	8 (14%) $p^1=0.777$	6 (10%) $P^2=1.000$	17 (26%)	12 (18%) $p^1=0.300$	12 (18%) $p^2=0.401$
Non-23vPPV carriage types, (*n*)									
Serotype 6A	0	0	0	0	0	1	4	0	1
Other serotypes	6	5	5	12	11	11	22 (m**3**) (m**4**)	21 (m**5**)	28 (m**6**) (m**7**)
Any pneumococcal carriage, *n* (%)	8 (14%)	9 (15%) $p^1=1.000$	9 (17%) $p^2=0.793$	18 (31%)	19 (32%) $p^1=1.000$	18 (31%) $P^2=1.000$	41(62%)	32 (48%) $p^1=0.118$	39 (59%) $p^2=0.859$
No nasopharyngeal swab, (*n*)	8	7	13	7	8	7	0	0	0
No blinded tympanometry, (*n*)	14	12	23	14	16	15	6	6	4

The primary infant ear disease outcome at age 7 months was determined by expert independent blinded assessors using tympanometry and video-otoscopy recorded by research nurses in the field. Infants were only included in the table if they completed the 7 month study follow-up: both a nasopharyngeal swab cultured and a valid independent ear assessment performed (tympanometry and/or video-otoscopy). Difficulty obtaining high quality video-otoscopy images at one or two month visits precluded their use for blinded diagnosis at these visits. Ear disease outcomes at age 1 and 2 months were determined solely by blinded assessment of the tympanometry. **Multiple serotype carriage**: (**m1**) 3 and 15B; (**m2**) 19F and 19A; (**m3**) 15C and 15B; (**m4**) 19A and 35F; (**m5**) 10A and 22A; (**m6**) 6C and 10A; (**m7**) 6C and 19A. **PCV serotypes**(**PCV**): pneumococcal serotypes contained in the 7vPCV or 10vPHID-CV. **23vPPV carriage**: nasopharyngeal carriage of pneumococcal serotypes contained in the 23vPPV. **p**^{1}: 23vPPV in pregnancy versus controls and **p**^{2}: 23vPPV at birth versus controls; significance determined using Fisher's exact test. Missing data are described at the bottom of the table. Nasopharyngeal swabs were not available for 28 infants at 1 month and 22 infants at 2 months. No blinded assessment of the tympanometry was available for 49 infants at 1 month, 45 infants at 2 months and 16 infants at 7 months.

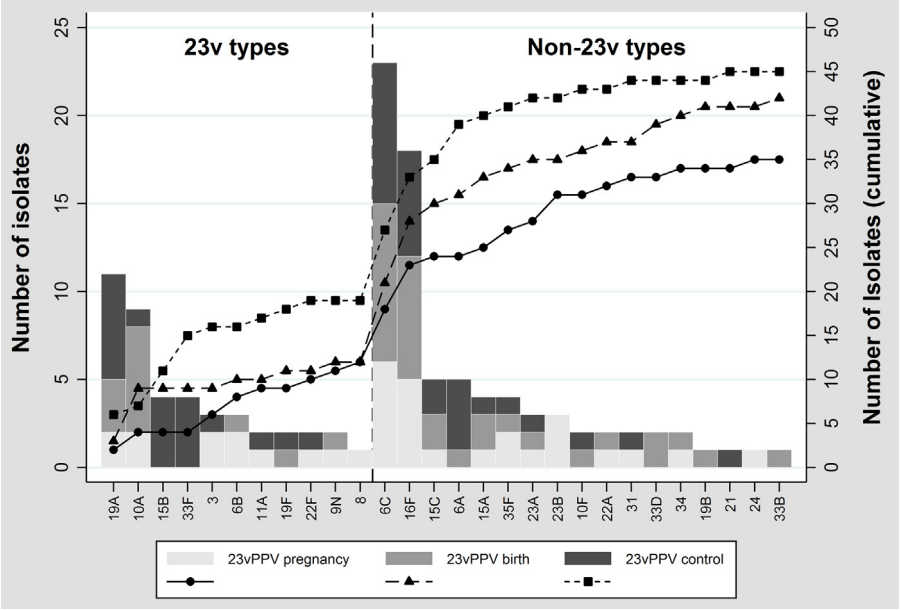

Fig. 2. Pneumococcal nasopharyngeal carriage isolates detected at infant age 7 months by serotype for controls, pregnancy and birth vaccinees. Individual (bars; left axis) and cumulative (lines; right axis) serotypes detected in the nasopharynx of infants at 7 months of age are shown for each randomisation group. Multiple carriage of 23vPPV-types occurred in two infants (infant 1: serotypes 15B and 3; infant 2: serotypes 19A and 19F), both of whom were in the control group. 23v types: pneumococcal serotypes contained in the 23vPPV.

3.4. Secondary outcomes

At age 7 months, carriage of any pneumococcal serotype was 62% (41/66) for control infants compared with 48% (32/67) for infants of pregnancy vaccinees ($p = 0.118$) and 59% (39/66) for infants of birth vaccinees ($p = 0.859$) (Table 3). The most common serotypes at age 7 months (Fig. 2), were non-23vPPV-types 6C and 16F ($n = 23$ and $n = 18$ infants respectively). Of the 23vPPV-types ($n = 41$ infants in total), 19A, 10A, 15B and 33F predominated ($n = 28$ infants in total). Serotypes 19A, 15B and 33F were each less common among infants of vaccinees compared to controls while serotype 10A was most common among birth vaccinees (Fig. 2, Table 3). Five infants had serotype 6A carriage, four of which were among the control group. Collectively, 7vPCV-types were infrequent at age 1 ($n = 3$), 2 ($n = 8$) and 7 months ($n = 5$).

Maternal 23vPPV-type carriage rates at birth were 2% (3/132) among non-pregnancy vaccinees compared to 6% (4/67) among pregnancy vaccinees ($p = 0.228$). Difficulty obtaining high quality video-otoscopy images at one or two month visits meant the blinded assessor diagnosis at these visits relied solely on the tympanometry recording. At the one and two month visits, neither the prevalence of infant middle ear disease nor nasopharyngeal carriage of 23vPPV-types was significantly different between controls and respective maternal vaccination groups (Table 3).

Immune responses in maternal sera, cord blood and breast milk were consistently higher amongst pregnancy vaccinees than controls for the commonly isolated serotypes 19A, 15B, 33F and 10A, though the responses varied by serotype (Fig. 3). In terms of magnitude, antibody concentrations for serotypes 19A, 15B and 33F were higher than serotype 10A. For all other 23vPPV-types (data not shown): maternal serum IgG concentrations were significantly higher post-23vPPV compared to controls, either at birth or 1 month post-partum (including for the 23vPPV-related serotype 6A); IgG concentrations in cord blood were significantly higher amongst pregnancy vaccinees than controls (but not for serotype 6A); and infant serum IgG concentrations at age 7 months were similar across all three groups, including to the PCV serotypes with no significant evidence of interference from maternal vaccination. In breast milk, when compared to controls, pregnancy vaccinees had higher IgA for 14/15 serotypes tested at 1 month, 15/15 at 2 months and 13/15 serotypes at 7 months while birth vaccinees had higher IgA for 12/15 serotypes at 1 month, 13/15 serotypes at 2 months and 6/15 serotypes at 7 months. Overall serotype-specific breast milk IgA was generally higher among pregnancy compared to birth vaccinees at 1 month (0.9 to 1.61 fold), 2 month (1.2 to 1.5 fold) and 7 months (1.1 to 1.7 fold). IgA concentrations were all <0.4 μg/ml.

3.5. Post hoc analysis

At age 7 months, infant ear disease prevalence associated with concurrent carriage of a 23v6A serotype (Supplementary Table 2, Fig. 4) was 27% (18/66) in the control group compared with 13% (9/67) among pregnancy vaccinees (VE 51%, 95% CI −2% to 76%) and 17% (11/66) among birth vaccinees (VE 39%, 95% CI −19% to 69%). In contrast, the prevalence at age 7 months of ear disease associated with non-23v6A serotypes, nontypeable *H. influenzae* and/or *M. catarrhalis* in the absence of pneumococcus, or where no detectable pathogen was found, were similar between vaccinees and controls (Fig. 4).

At age 7 months, 12 serotypes were associated with the 23v6A ear disease in 38 children (Supplementary Table 2). Of these, serotypes 19A, 10A, 15B, 33F and 6A predominated (76%; 29/38). Serotypes 19A, 15B, 33F and 6A were proportionally more common among controls than the vaccinees whereas serotype 10A was more common among the vaccinees than the controls. The other seven children with 23v6A ear disease had serotypes 3, 6B, 8, 9N, 11A, 19F, 22F (each serotype had ≤1 occurrence per group). There were no children with ear disease and concurrent nasopharyngeal carriage of the other 23vPPV-types (1, 2, 4, 5, 7F, 9V, 12F, 14, 17F, 18C, 20 & 23F). Only three infants (one in each group) had ear disease associated with a PCV-type (7vPCV or 10vPHiD-CV).

4. Discussion

In our outcome-assessor blinded trial, we were unable to demonstrate significant efficacy of 23vPPV in pregnancy against either infant ear disease (VE 12%, 95% CI −12% to 31%) or

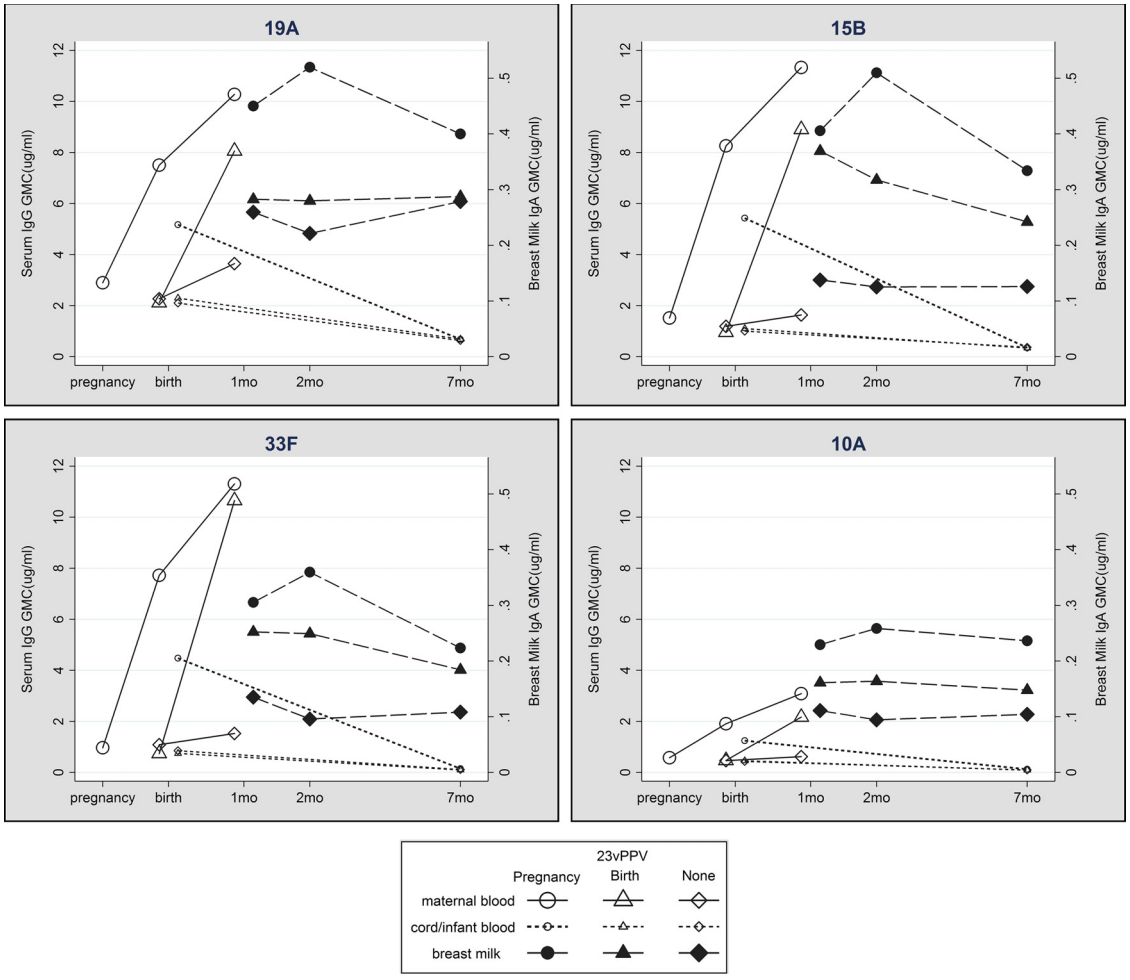

Fig. 3. Immunogenicity of 23vPPV in venous blood, cord blood and breast milk among controls, pregnancy and birth vaccinees for serotypes 19A, 15B, 33F and 10A. Geometric mean concentrations (GMC) of antibodies (immunoglobin, Ig) to four commonly carried serotypes (19A, 15B, 33F and 10A) as measured in mothers (venous blood, cord blood and breast milk) and their infants (blood) at scheduled study visits for each randomisation group. For the other 23vPPV-types, relative trends in antibody concentrations were similar by visit and vaccine group, despite variations in absolute magnitude.

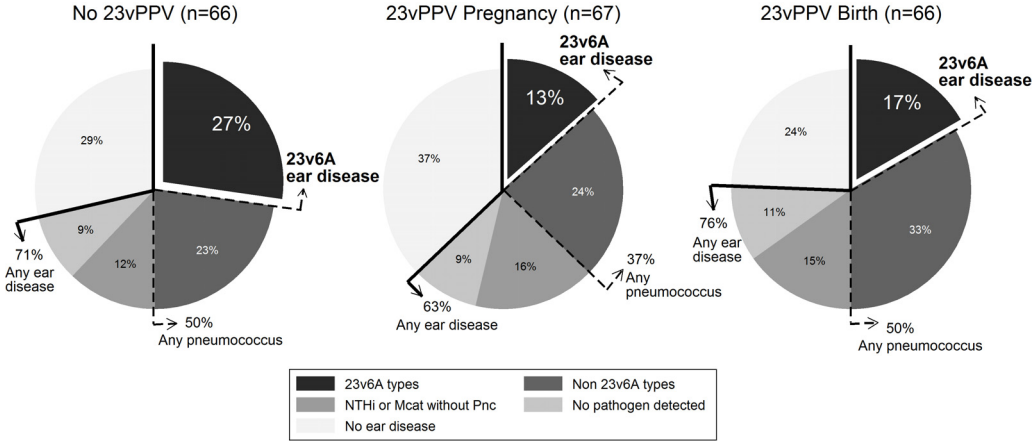

Fig. 4. Nasopharyngeal carriage concurrent with ear disease at infant age 7 months among controls, pregnancy and birth vaccinees.
The proportion of infants with **Any ear disease** (bold lines) are sub-grouped (dotted lines) as follows: **Any pneumococcus**: concurrent nasopharyngeal carriage of any pneumococcal serotype; **23v6A ear disease**: ear disease with concurrent nasopharyngeal carriage of a 23v6A serotype (black wedge, exploded). Note: Two infants, both in the control group, had ear disease with concurrent multiple 23v6A serotypes (serotypes 15B and 3; 19A and 19F). Infants with multiple serotype carriage of a 23v6A and a non23v6A serotype are shown exclusively within the 23v6A wedge: one infant of pregnancy vaccinees (serotypes 10A and 22A), two infants of birth vaccinees (serotypes 6C and 10A; 6C and 19A) and two control infants (serotypes 15B and 15C; 19A and 35F). **NTHi**: Nontypeable *Haemophilus influenzae.* **Mcat**: *Moraxella catarrhalis.* **Pnc**: Pneumococcus.

23vPPV-type nasopharyngeal carriage (VE 30%, 95% CI −34% to 64%) at age 7 months nor for the same outcomes following 23vPPV at birth (Table 2). We achieved our intended sample size but had reduced study power due to lower than predicted prevalence amongst the controls of the a priori outcomes of ear disease (actual 71%; predicted 90%) and nasopharyngeal carriage of 23vPPV-types (actual 26%; predicted 55%) at infant age 7 months.

In post hoc analysis, the risk of ear disease concurrent with nasopharyngeal carriage of a 23vPPV-type or the vaccine-related serotype 6A (23v6A ear disease) was reduced by 51% (95% CI −2% to 76%) among infants of pregnancy vaccinees compared with controls. The lack of vaccine impact against other (non-23v6A) ear disease suggests the reduction was vaccine-specific. High maternal post-vaccination antibody concentrations to serotypes 19A, 15B and 33F but not to serotype 10A are also consistent with the observed impact against ear disease associated with these serotypes. We advocate the use of this more specific outcome strategy in future pneumococcal vaccine trials, particularly where tympanocentesis is not possible.

Previous maternal 23vPPV trials have also failed to demonstrate an impact against infant acute respiratory infections [12], otitis media [10] or pneumococcal carriage [11,12]. In contrast to the PneuMum study, these trials were smaller and conducted in lower risk settings without a routine childhood PCV program in place. Most infants (84–89%) in our study had received ≥2 doses of a PCV (7vPCV or 10vPHiD-CV) at least 2 weeks prior to the seven month visit and few ($n = 5$) had carriage of a PCV serotype. Any effect against ear disease or pneumococcal carriage in our setting was largely confined to 23vPPV serotypes not contained in PCV's.

Undertaking a maternal vaccination trial in this high risk setting was challenging; however, the consent rate (50%) and achievement of the intended sample size, demonstrate that such studies are possible. Engagement with participants, local communities [24], an Indigenous Reference Group [25] and the Independent Data Safety Monitoring Board that included community representation were integral to the success of this trial.

In rural and remote regions of Australia's Northern Territory the risk of ear disease remains high. In our control group at 7 months of age, seven out of ten children had middle ear disease. For every three control children with ear disease at age 7 months, one was potentially preventable by 23vPPV in pregnancy, one was associated with a non-vaccine type whilst the other had no associated pneumococcal carriage. Our data suggests the 23vPPV in pregnancy halved the preventable fraction of infant ear disease at 7 months but lacked the ability to impact all-cause ear disease, perhaps similar to the lack of effect against all-cause pneumonia in adults [26]. Future use of maternal 23vPPV in this region is likely to be confined to preventing the small fraction of infant ear disease associated with common non-PCV serotypes (examples 15B and 33F).

Contributions and acknowledgements

MJB and SAM co-authored the manuscript in consultation with RMA and the other authors. RMA, JRC, AJL, PSM, EKM, PT, MLKT designed the trial. SAM, AB, KMH, JN assisted with protocol development and study operating procedures. CW was the primary independent blinded assessor of ear disease outcomes. PSM was the study medical advisor, specialist ENT paediatrician and secondary independent blinded assessor of ear disease outcomes. SN engaged with Aboriginal Medical Services and advised on cultural matters. KMH was responsible for the microbiological analysis. AB performed vaccine antigen serum and breast milk ELISA assays. MJB performed statistical/scientific appraisal and data analysis. MDC provided statistical support.

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Notes

Conflict of interest statement

MJB, AJL, KMH have received research and conference support from GlaxoSmithKline (GSK) and Pfizer. RMA, PSM and EKM have received research funding from GSK. EKM has served on Advisory Boards for GSK and Pfizer. SAM, AB, SN, CW, JN, MDC, MLKT, PT and JRC declare no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.10.101.

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