PneuMum:
A randomised controlled trial of pneumococcal polysaccharide vaccination for Aboriginal and Torres Strait Islander mothers to protect their babies from ear disease

Protocol

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1. GENERAL INFORMATION

1.1 Protocol full title

PneuMum: A randomised controlled trial of pneumococcal polysaccharide vaccination for Aboriginal & Torres Strait Islander mothers to protect their babies from ear disease.

Short Title

PneuMum: will an injection for mothers help to protect their babies from ear disease?

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1.8 Confidentiality statement

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2. Executive Summary

PneuMum is a randomised controlled trial that aims to find out if pneumococcal immunisation for Aboriginal and Torres Strait Islander women, in the last few months of pregnancy or at delivery, can prevent ear disease in infants.

The vaccine, called Pneumovax, is a 23 valent pneumococcal polysaccharide vaccine (23vPPV) that is recommended for all Indigenous people in the Northern Territory from 15 years of age.

Background and rationale

Aboriginal and Torres Strait Islander children experience the highest rates of acute and chronic ear infections in the world, resulting in permanent ear damage, hearing loss and educational disadvantage. These infections are mainly bacterial. *Streptococcus pneumoniae* (pneumococcus) is the predominant pathogen. Pneumococcal colonisation and infection begins within days of birth, months before any potential immunological protection from infant pneumococcal conjugate vaccine may be expected. New strategies are needed to eliminate, or at least delay, this early-onset pneumococcal colonisation.

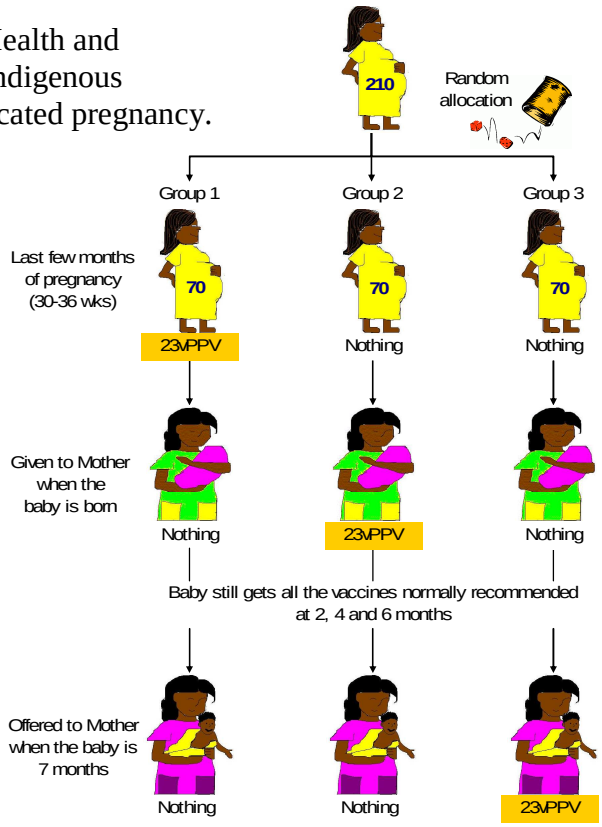
Maternal immunisation with polysaccharide pneumococcal vaccine is one strategy that may protect newborn infants through transplacental antibody transfer, increased secretory antibody in breast milk, and/or by reducing carriage (and transmission to the infant) of maternal pneumococci. Previous small studies using this strategy have been encouraging, but there have been no studies properly evaluating carriage or disease endpoints in infants.

Methodology

The study, which has been funded by National Health and Medical Research Council, aims to recruit 210 Indigenous women aged 17-39 years who have an uncomplicated pregnancy.

Potential subjects will be contacted to seek informed consent to participate. Written and pictorial information has been developed for use with local interpreters to explain the disease, reasons for the study, and potential harms / benefits of involvement. All participants will have documentation of appropriate antenatal investigations to ensure pregnancy is proceeding normally, as far as can be determined.

Eligible subjects will be randomly assigned to one of three groups (see Box), receiving Pneumovax (23vPPV) in the last few months of pregnancy, immediately postpartum, or seven months after childbirth (the control group).



Boostrix, recommended for new parents to prevent pertussis (whooping cough), will also be offered at delivery to all women who have not previously been vaccinated in accordance with NT government policy.

Although currently recommended for all Indigenous persons in the Northern Territory from 15 years of age and for others in high-risk groups, uptake of Pneumovax among women of child-bearing age has been low.

Outcomes

The primary outcome will be prevalence of ear infection at seven months of age, defined as middle ear effusion or tympanic membrane perforation or acute otitis media, identified by video-otoscopy and tympanometry. The primary analyses will be a direct comparison of the proportion of infants in the control group (Group 3) who have carriage of vaccine type pneumococci at seven months of age compared to infants in each of the other two groups and a similar comparison of the proportion with middle ear disease at that age.

There is a clear link between carriage of pneumococci in the first few months of life and the extraordinarily high rates of acute and chronic ear infections among Indigenous children, leading to hearing loss and educational disadvantage. Our study has the potential to demonstrate clinically significant benefits for infants in the first few months of life.

- If maternal immunisation can eliminate, or at least delay, early-onset pneumococcal colonisation it could have a substantial impact on ear disease.
- If the results of our study suggests antenatal vaccination reduces pneumococcal carriage or ear disease it would support moves to establish a much larger safety and efficacy studies of pneumococcal vaccination during pregnancy.
- If similar results are achieved from postnatal vaccination, it would provide evidence that such studies are not warranted and that the policy emphasis should be to ensure unvaccinated mothers are offered pneumococcal vaccination prior to discharge from hospital.

In addition to funding support from the National Health and Medical Research Council, the PneuMum study has been endorsed as an inkind project for the Cooperative Research Centre for Aboriginal Health and has received in-principle support from the World Health Organization and the Centre for Disease Control of the Northern Territory Department of Health and Community Services.

3. Aims

Primary aim:

- To determine if maternal immunisation with the 23-valent pneumococcal polysaccharide vaccine (23vPPV), given antepartum or immediately postpartum, can reduce pneumococcal carriage and the prevalence of middle ear disease among Indigenous children at seven months of age.

Secondary aims:

- To assess the acceptance of maternal immunisation in this population (as judged by the enrolment rate);
- To assess the impact against infant pneumococcal carriage and middle ear disease at one month and at two months of age;
- To assess maternal antibody response to antepartum or postpartum 23vPPV; and
- To assess the antibody response in infants of 23vPPV-immunised mothers following completion of the primary course of 7-valent pneumococcal conjugate vaccine (7vPCV).

4. Rationale

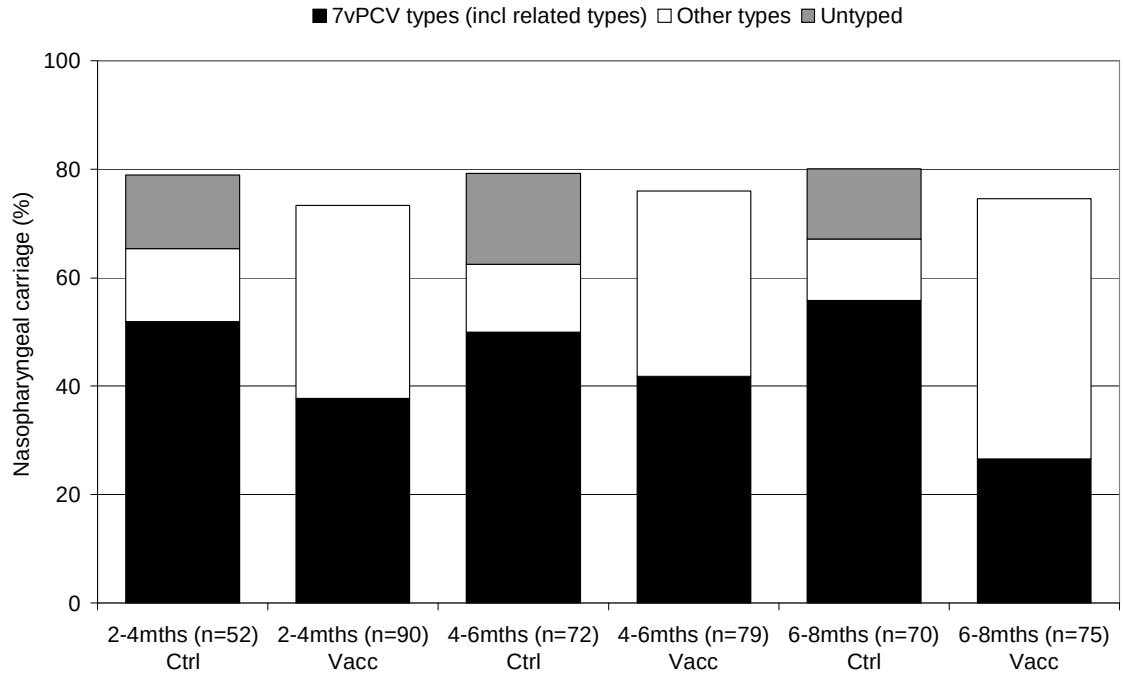
Aboriginal and Torres Strait Islander children experience the highest rates of acute and chronic ear infections in the world, with resultant permanent ear damage, hearing loss and educational disadvantage. These infections are mainly bacterial, and *Streptococcus pneumoniae* (pneumococcus) is the predominant pathogen. Pneumococcal colonisation and infection begins within days of birth, months before any potential immunological protection from infant pneumococcal conjugate vaccine may be expected. New strategies are needed to eliminate, or at least delay, this early-onset pneumococcal colonisation. Maternal immunisation with 23vPPV (known as Pneumovax) is one strategy that may protect newborn infants through transplacental antibody transfer, increased secretory antibody in breast milk, and/or by reducing carriage (and transmission to the infant) of maternal pneumococci. Previous small studies using this strategy have been encouraging, but there have been no studies properly evaluating carriage or disease endpoints in infants.

5. Ear disease among Aboriginal and Torres Strait Islander infants in the NT

By school entry, two thirds of Aboriginal and Torres Strait Islander children in the NT have hearing impairment or ear disease.[(1)] Once established, ear disease in these infants tends to persist and rarely, if ever, responds to treatment.[(2)] Pneumococcal colonisation commences shortly after birth and leads to recurrent acute otitis media and almost universal middle ear effusion (MEE) in the first year of life.[(3)] The carriage rates increase rapidly in the first few months (most of which is caused by serotypes contained in the 23vPPV). Carriage of vaccine types increases from 20% at one month to 39% at two months and 64% at three months, as does the prevalence of middle ear disease rising from 44% at one month to 90% at six months (AM, PM unpublished data).

Prevention of early-infant acquisition of pneumococci through vaccination could substantially reduce the burden of ear disease in Aboriginal and Torres Strait Islander infants. However, the timing and nature of vaccination is critical because, in the absence of a major herd immunity effect, infant vaccination from two months of age will not prevent early onset carriage and ear disease. Preliminary data suggests the 7vPCV has reduced carriage of vaccine/vaccine related types in infants aged 2-4 months but has had no effect on overall carriage because of replacement by other pneumococcal serotypes not contained in the 7vPCV (Figure 1).

Figure 1 Nasopharyngeal carriage of pneumococci by serocategory and agegroup for control (Ctrl) and vaccine (Vacc) groups, Tiwi Islands

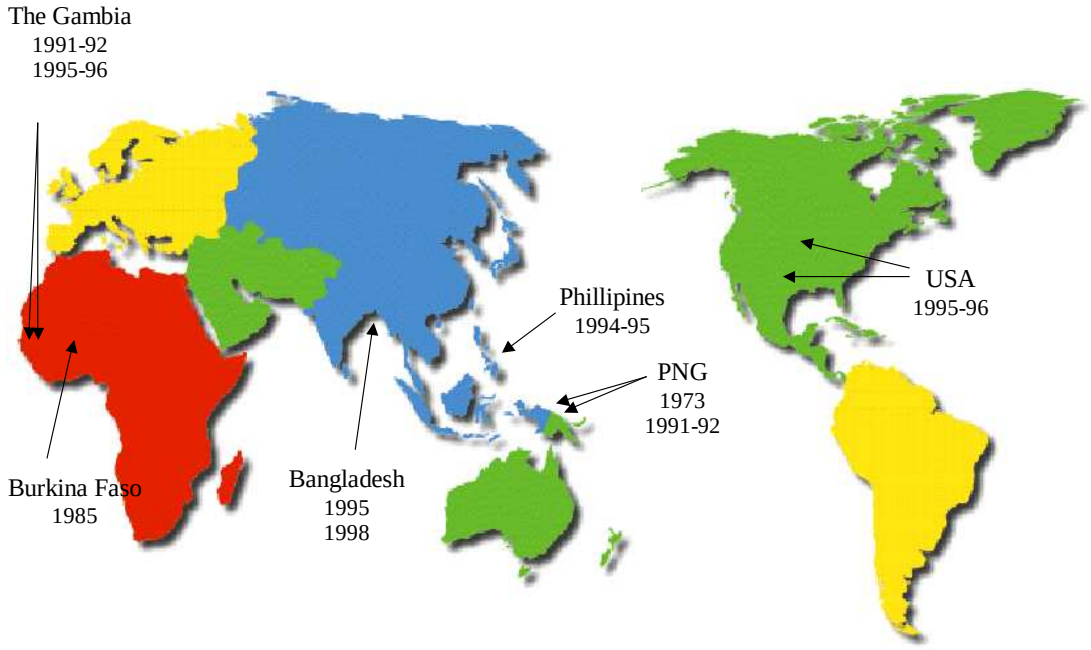


Source: G Mackenzie, unpublished data from a cohort of infants enrolled in a longitudinal study (PRIORiTI), Tiwi Islands, October 2001-April 2003 and historical controls

6. Previous studies of pneumococcal vaccination in pregnancy

The data relating to pneumococcal vaccination in pregnancy have been systematically reviewed by the World Health Organization.[4] The first trial involving pneumococcal vaccination during pregnancy was conducted in Papua New Guinea in 1973 when 187 women recruited for a larger vaccine trial were subsequently found to be pregnant after vaccination. Local custom had prevented enquiries about pregnancy at the time of recruitment so it is likely that most of the women were vaccinated early in pregnancy.[5,6] Trials have now been conducted in Asia, Africa and North America involving over 800 pregnant women (Table 1).[5-14]

Vaccination during pregnancy has been shown to increase maternal anti-pneumococcal IgG antibody and increase transplacental transfer to the infant through the cord blood as well as increase levels of specific antibody in breast milk.[8,10-13] The proportion of maternal anti-pneumococcal antibody transferred to the infant in these studies varied from 30% to 50% in the Gambia and Bangladesh to 89% in the USA.[8,11,13] There was also variation in the extent and duration of the antibody response for different serotypes and in different settings. Even so, the data suggest infants of vaccinated mothers have higher anti-pneumococcal antibody levels against most vaccine types during the first few months of life than infants of unvaccinated mothers with the added potential of mucosal protection through secretory IgA in the mother's milk.



Other places in the world where pregnant women have had pneumococcal vaccines

Note: The second arrow in the USA relates to a separate study that has not yet been completed (KM personal communication)

Table 1 Studies involving administration of pneumococcal polysaccharide vaccine to pregnant women

Author[ref]	Country	Study period	Age of mother	When vaccinated	Doses[#]
Riley[(5)], Lehmann[(6)]	Papua New Guinea	1973	Not specified	1st trimester?	187
Vincent-Ballereau[(7)]	Burkina Faso	1985	Mean 23 years	TBA*	37
O'Dempsey[(8)]	The Gambia	1991-92	Not specified	3rd trimester	75
Lehmann[(9)]	Papua New Guinea	1991-94	Mean 29 years	28-38 weeks	235
Quiambao[(10)]	Phillipines	1994-95	Not specified	25-33 weeks	106
Munoz[(11)]	USA	1995-96	19-39 years	30-36 weeks	20
Obaro[(12)]	The Gambia	1995-96	Not specified	24-32 weeks	56
Shahid[(13)]	Bangladesh	1995	15-40 years	30-34 weeks	36
Shahid[(14)]	Bangladesh	1998	15-40 years	30-34 weeks	82
				Total	834

[#] Refers to the number of doses of 23 valent pneumococcal polysaccharide vaccine (23vPPV) except for the 1973 PNG study where a 14 valent pneumococcal polysaccharide vaccine (14vPPV) was used[(5)]

* TBA – to be advised. This study was published in French, translation pending

Two studies suggested that infant carriage of pneumococci may have been reduced through maternal immunisation but the sample size was insufficient to confirm a reduction. Munoz et al found none of the infants of 23vPPV recipients were colonised with pneumococci at two months of age compared to 8% of infants of mothers in the control group. By seven months of age the carriage rate was 6% in the 23vPPV group and 19% in the control group.[(11)] Similarly, O'Dempsey et al reported there were no cases of otitis media by one year of age among infants of vaccinated mothers compared to three cases among the control group.[(8)]

The aforementioned trials focussed on vaccination during pregnancy as a source of antibody transfer to the infant and one additional study assessed the antibody response of women and infants to 23vPPV given prior to pregnancy.[15] In each of these studies it was not possible to establish whether breast milk antibody alone could generate an adequate infant immune response in the absence of transplacental transfer. Vaccination of lactating women with 23vPPV has been shown to produce capsule-specific secretory IgA in breast milk with the potential for killing pneumococci at mucosal sites.[16] If adequate antibody protection can be provided through breast milk, postpartum vaccination may be a preferred option for some unimmunised mothers.

7. Safety during pregnancy

From the trials conducted to date (Table 1), there has been no evidence of adverse events from vaccination of pregnant women with 23vPPV.[4-14] While there are no biological reasons to suspect that vaccination toward the end of pregnancy would not be safe, these trials have been too small to exclude rare, serious adverse events. The World Health Organization has acknowledged the limited data on this issue and the need for large-scale safety and efficacy studies. WHO also makes the point that smaller studies should continue to be supported where they are able to test additional hypotheses relevant to maternal pneumococcal immunisation.[4] The protocol for the PneuMum trial has been forwarded to WHO, which has provided in-principle support for the study (see letter attached).

In Australia, national guidelines allow for 23vPPV to be given in pregnancy if the risk of pneumococcal disease is very high but otherwise recommend deferral of vaccination due to the limited data.[17] The likelihood that administration of 23vPPV in pregnancy is safe is supported by the experience of meningococcal polysaccharide vaccines, which are similar in formulation and have been used extensively with no documented adverse events either for pregnant women or their infants.[18]

8. Vaccination of Aboriginal and Torres Strait Islander women

Due to high rates of invasive pneumococcal disease, the 23vPPV is recommended for all Indigenous Australians aged 15-49 years who have any high-risk underlying conditions and for other high risk groups.[17] Within the Northern Territory, the 23vPPV is provided free for all Indigenous persons in this age group, regardless of other risk factors. However, coverage with the 23vPPV among Indigenous women of child-bearing age is estimated to be only 24% (personal communication, Christine Selvey, NT DHCS).

Our study will investigate two options for maternal immunisation: antenatal 23vPPV or immediate postnatal 23vPPV. The former is not routinely recommended at present, whilst the latter, although consistent with current recommendations, is not standard practice. At present uptake of the vaccine among Indigenous women of child-bearing age has been low. Vaccination of the mothers will protect them against invasive pneumococcal disease, and could potentially have an added benefit of protecting their infants against colonisation during the first few months of life. The extraordinarily high rates of infant pneumococcal carriage and of middle ear disease among Indigenous communities in the Northern Territory provides an opportunity to assess large effects on these outcomes from a relatively small study.

A larger study than we have proposed would be able to detect smaller differences in rates of otitis media at seven months of age (if they exist), however smaller differences may be less likely to lead to changes in practice. In addition, we will be monitoring other secondary endpoints such as anti-pneumococcal antibody production. These will be useful indicators of

whether a larger study is warranted to assess the effect on less common outcomes (eg. pneumonia, invasive pneumococcal disease), more long term outcomes related to the prevention of persistent disease, ear drum perforation and hearing loss, or to evaluate smaller reductions in carriage and ear disease at the younger age points (one month and two months).

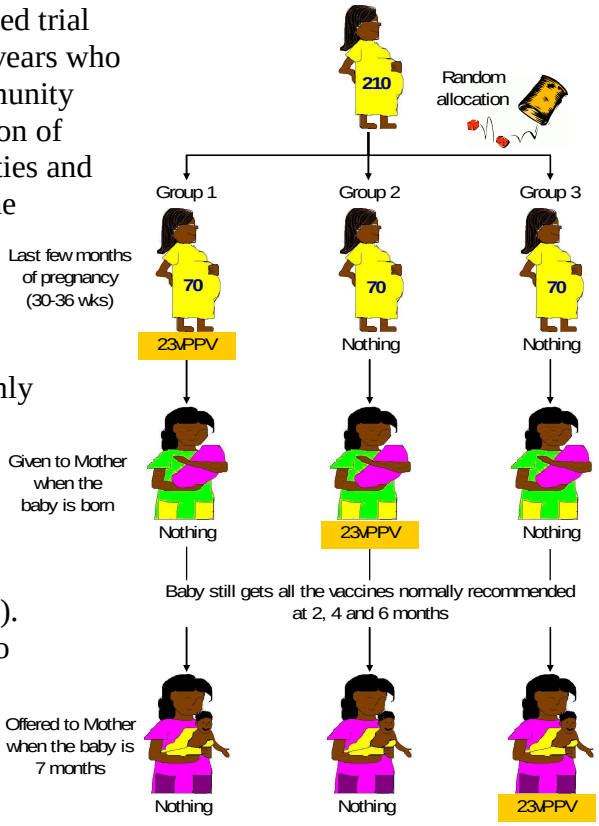
9. Research Plan

9.1 Setting

We propose to conduct the study in Darwin and, subject to community consent, other remote communities in the Top End of the Northern Territory. By limiting recruitment and follow-up to pregnant Indigenous women from larger communities, we will maximise use of available resources within this region where there are very high rates of pneumococcal carriage and middle ear disease among Aboriginal and Torres Strait Islander infants. Another important factor is the high rate of breast feeding (84% at three months and 79% at six months).[19]

9.2 Study design

The study is an open label randomised controlled trial involving 210 Indigenous women aged 17-39 years who have an uncomplicated pregnancy. Once community consent is obtained, we will seek the cooperation of general practitioners in participating communities and visiting District Medical Officers to assist in the identification of pregnant women so that we can then contact them about possible involvement in the trial.



Following recruitment, subjects will be randomly assigned to one of three groups (see Box).

- Women in Group 1 will receive 23vPPV (Pneumovax) during the third trimester of pregnancy. We will aim to give the vaccine at 32 weeks gestation (range 30-36 weeks). As discussed later, we have opted not to use an alternative vaccine at this time point for the other groups.
- Women in Group 2 will receive 23vPPV (Pneumovax) at delivery.
- Women in Group 3 will be offered 23vPPV when their infant is aged at 7 months (the end of the observation period).

All women will be offered adult diphtheria, tetanus and acellular pertussis vaccine (Boostrix) at delivery in accordance with NT government policy. Those women who had not received Boostrix by the time of the 7 month visit will again be offered the vaccine at that time.The provision of *Boostrix* for mothers soon after delivery is consistent with the recommendations of the Australian Immunisation Handbook[17] and is now an NT government funded program to minimise the risk of transmission of pertussis (whooping cough) from the parent to the newborn infant.

9.3 Primary and secondary analyses

The primary outcome will be prevalence of ear infection at seven months of age defined as middle ear effusion or tympanic membrane perforation or acute otitis media identified by video-otoscopy and tympanometry. Seven months was chosen as the age to assess the primary outcome because both carriage and middle ear disease are well established by this age and the child's language development may be at risk from the associated hearing loss.[3] Further, all children should have received their primary course of 7vPCV by this age.[17] Thus assessment at seven months will provide the best opportunity to identify an additional impact of each maternal immunisation strategy on pneumococcal carriage and ear disease in children.

Analysis will be performed on an intention-to-treat basis, with a secondary per-protocol analysis performed as well. The primary analyses will be a direct comparison of the proportion of infants in the control group who have carriage of vaccine type pneumococci at seven months of age compared to infants in each of the other two groups and a similar comparison of the proportion with middle ear disease at that age. We used recent unpublished data from a longitudinal study in the NT to calculate baseline rates (Table 2) since these rates were lower than those of previously published data.[3] We aim to recruit 70 evaluable subjects per group, which will be sufficient to demonstrate a 45% reduction in pneumococcal carriage or a 23% reduction in middle ear disease at seven months of age. In the event that maternal immunisation in the third trimester is effective, a difference of this size would mean that five pregnant women would need to be immunised to prevent one child from having otitis media at seven months of age. Given the uncertainty surrounding the use of vaccines during pregnancy, we believe differences smaller than this may be unlikely to change practice.

Table 2 Sample size estimates per group for primary and secondary endpoints

Endpoint	Infant Age	Control group	Vaccine group (reduction)	Sample size
Nasopharygeal carriage of pneumococci serotypes contained in the 23vPPV	7 months	55%	30% (-45%)	68
	2 months	39%	16% (-59%)	67
	1 month	20%	3% (-85%)	66
Middle ear effusion or acute otitis media or tympanic membrane perforation	7 months	90%	69% (-23%)	66
	2 months	70%	45% (-36%)	68
	1 month	44%	20% (-55%)	67

Baseline data from a cohort of Aboriginal infants enrolled in a longitudinal study (PRIORiTI), October 2001-November 2003 (AJM, PM unpublished data)
Sample size required for 80% power and two-tailed alpha of 0.05 for the absolute difference described

Secondary analyses will assess the same endpoints at one and two months of age but we may have power to detect only larger reductions given the sample size of 70 subjects per group (Table 2). We will also examine the relationship of maternal pneumococcal carriage, maternal anti-pneumococcal antibody levels, cord blood antibody levels and breast milk antibody levels to infant carriage and middle ear disease at one, two and seven months of age. In addition, we will assess the impact of each maternal immunisation strategy on breast milk antibody levels to all 23 serotypes contained in the vaccine, breast milk antibody avidity (to four selected serotypes), maternal antibody response to antepartum or postpartum 23vPPV, and infant anti-pneumococcal antibody levels at seven months of age (following the 3rd recommended dose of 7vPCV).

We will monitor adverse vaccination advents and assess effects of each vaccine on gestational age, birth weight, common pregnancy outcomes, premature rupture of membranes, pre-eclampsia, labour (spontaneous/induced/augmented), proportion delivering by caesarean section, post-partum haemorrhage, Apgar scores, maternal pyrexia during labour, perinatal mortality and stillbirths.

Geometric mean anti-capsular pneumococcal antibodies at delivery in the mother and at age seven months in the infant will be compared using a two-tailed t-test, after adjusting for the mother's pre-vaccine titres. Chi-squared analysis will be conducted to determine the effects of the vaccine on the proportion of infants who have middle ear disease and for those carrying vaccine type pneumococci at each of the various time points.

The true immune correlate for protection from pneumococcal disease is not known. Based on experience with the comparable Hib vaccines, data from animal models and the limited efficacy data available, regulatory agencies now believe that anti-capsular antibody levels represent the best guide to immunity following vaccination. While the actual protective level probably varies with serotype and disease manifestation, antibody levels greater than 0.35 mcg/ml are expected to indicate protection against invasive disease.[(20)] We will conduct a chi-squared analysis to determine the effects of the vaccine on the proportion of mothers with anti-capsular pneumococcal antibodies greater than 0.35 mcg/ml at delivery and the proportion of infants with anti-capsular pneumococcal antibodies greater than 0.35 mcg/ml at seven months of age.

9.4 Eligibility for recruitment

Subject to community consent, all women of Aboriginal and/or Torres Strait Islander descent aged 17 to 39 years who have a singleton uncomplicated pregnancy and reside in Darwin or participating communities will be eligible for recruitment in the trial provided that:

- the subject intends to deliver her child at the Royal Darwin Hospital or Darwin Private Hospital and
- has given informed consent to participate.

A complicated pregnancy is defined as an existing pregnancy complication (eg significant pre-eclampsia with hypertension, proteinuria and oedema) or any pre-existing condition judged by the clinical investigator to make pregnancy high-risk. Eligibility will be confirmed after the routine antenatal at 28 weeks to ensure that the pregnancy is proceeding normally. Subjects will be excluded if they:

- have had 23vPPV (Pneumovax) within the previous three years;
- intend to leave the study area during the follow-up period;
- are HIV positive, have a history of severe allergy, uncontrolled asthma or splenectomy.

A record will be kept of the reasons for exclusion.

9.5 Enrolment and consent

Consistent with our previous clinical trials involving Indigenous communities, we will first obtain community consent before approaching any individuals. Community meetings will be conducted to explain the trial objectives, the potential risks and benefits associated with involvement. These meetings will be conducted with community groups such as women's centres, Councils and health clinic staff, providing written and pictorial information, translated as appropriate.

Once community consent is obtained, we will seek the cooperation of general practitioners in participating communities and visiting District Medical Officers to identify pregnant women of Aboriginal and/or Torres Strait Islander descent so that they and their families can then be contacted by members of the project team about possible involvement in the trial. All participants will have documentation of appropriate antenatal investigations to ensure pregnancy is proceeding normally, as far as can be determined. We will provide a participant information package that explains the disease, reasons for the study, and potential harms/benefits of the study protocol. This will be in line with a previous informed consent package that we developed and piloted for a proposed effectiveness trial in NT Aboriginal communities of an unlicensed pneumococcal conjugate vaccine.[21] Written informed consent will be obtained from all participants.

9.6 Randomisation and vaccination

Subjects will be randomised to one of three groups:

(1) antepartum 23vPPV (given during the 3rd trimester)
(2) postpartum 23vPPV (given immediately following delivery)
(3) control group (23vPPV given seven months after delivery).

The allocation of subjects to each group will occur following enrolment and be conducted by a person independent of the study. Allocation will be stratified by the urban or remote nature of the participant's community of residence and concealed using sequentially numbered opaque sealed envelopes. The random allocation sequence will be computer generated by the Menzies School of Health Research statistician who will be independent of the study.

The conjugate vaccine (7PCV) was not considered a viable alternative to 23vPPV because of limited serotype coverage when used alone and the likelihood of increased reactogenicity if combined with a polysaccharide booster in the third trimester. We considered including a saline placebo during the third trimester for groups 2 and 3 but did not proceed with this design due to concerns about the acceptability of a placebo during pregnancy.

All laboratory assessments will be undertaken without knowledge of the subject's vaccination status and all the results of all video-otoscopy recorded during the field visits will be reviewed by an independent investigator blinded to the subject's vaccination status.

The vaccines will be given by an approved immunisation provider as an intramuscular injection to the upper outer arm using sterile technique. Subjects will be observed for up to one hour after vaccination with resuscitation equipment available and a physician in attendance. An adverse events form (including any abnormal pregnancy events) will be placed in the medical records of participants for completion by health staff. For those women vaccinated in pregnancy, a project team member will reassess the study participant at 1-3 days post-vaccine and will review hospital admissions for the period 7 days post-vaccination in order to confirm no potentially serious adverse events (SAEs) have occurred. An independent Data Safety Monitoring Board (DSMB) will be established to assess safety of the trial and to safeguard the interests of trial participants (see Section 8). The DSMB will be informed within 24 hours of receipt of a SAE.

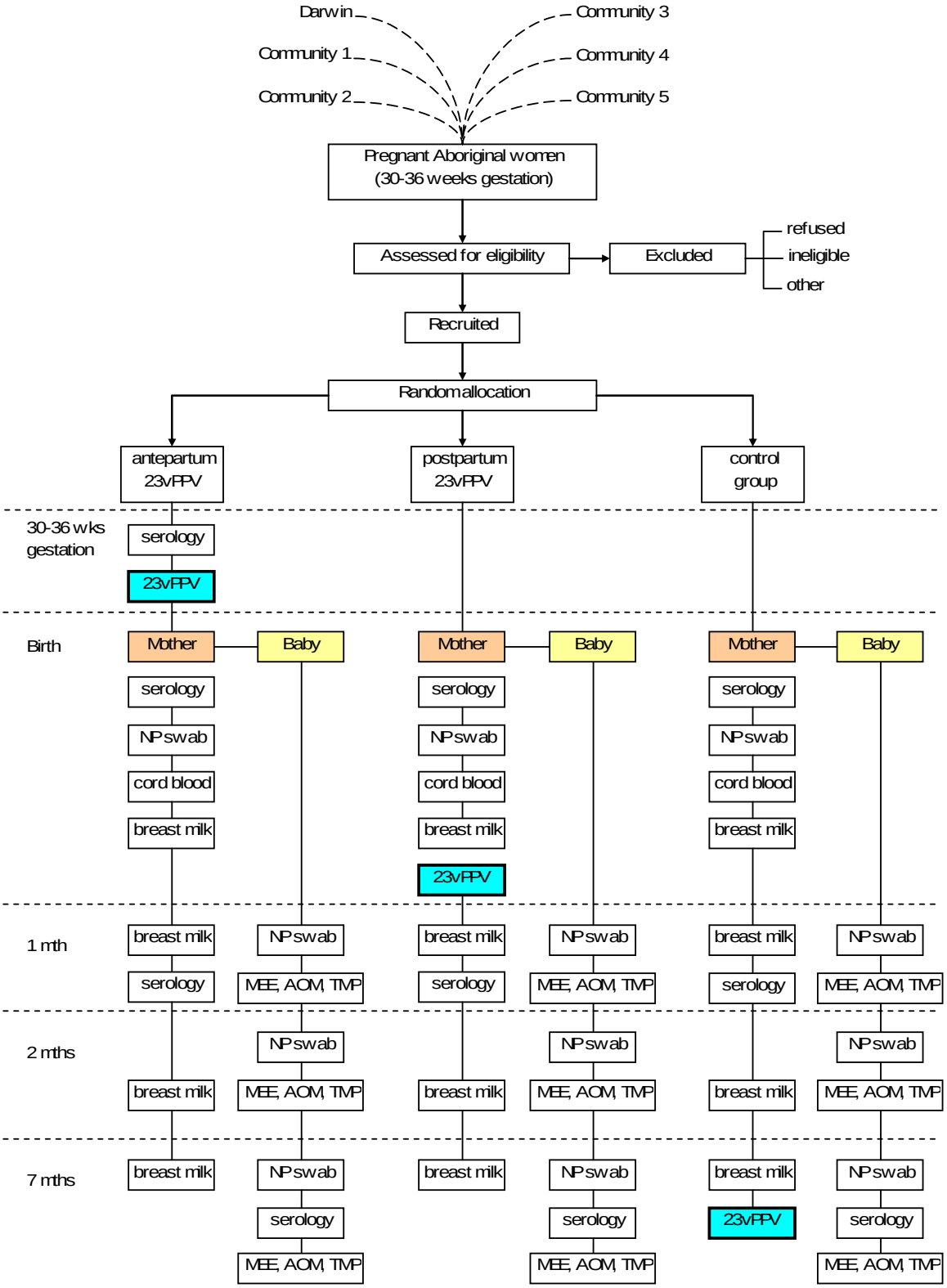
Subjects with severe acute illness at the time of presentation will not be vaccinated. Following the conclusion of the observation period, seven months after childbirth, those subjects who have not received 23vPPV or dTpa (Boostrix) will be offered the vaccine. All subjects will be advised about other vaccines that they are recommended to receive in future.

9.7 Follow-up and specimen collection

Each participant and newborn child will followed-up by a member of the study team at birth, one, two and seven months after birth (Figure 2). Infant MEE, AOM and TMP will be assessed using video-otoscopy and typanometry at age one, two and seven months. At their first assessment, infants will also receive a full '6 week' postnatal clinical examination. Infants will also have their nutritional, respiratory, and skin status assessed and treated in accordance with current standard treatment guidelines at every assessment. All assessments will be conducted by a clinician trained in the effective management of common medical problems of infants in remote communities. These examinations and interventions are likely to mean that all children participating in the study will benefit from additional medical care. Parent interview and medical record review will be used to record all potential factors that could affect acquisition of the pneumococcus in mother and baby (including number of siblings, antibiotic usage, housing, nutritional status, and smoking exposure status).

Maternal specimens will be collected immediately prior to vaccination (to determine baseline anti-capsular pneumococcal antibodies); at delivery (cord blood, serology, nasopharyngeal swab, breast milk); and at one month (breast milk), two months (repeat serology and breast milk) and seven months after childbirth (breast milk). Infant nasopharyngeal swabs will be collected at one, two and seven months with a follow-up serology at seven months (one month following completion of the primary course of 7vPCV).

Figure 2 Flowchart of the proposed study design



9.8 Laboratory testing

Nasopharyngeal swabs will be immediately placed in STGG transport medium, vortexed and frozen (initially at -20°C for transport, and then at -70°C) until plated, according to methods we have described and which are now standard WHO recommendations.[22] A scraping of the transport medium will be inoculated onto both a gentamicin free horse blood-agar plate and a gentamicin 2.5 mg/L blood-agar plate. Plates will be incubated at 37°C in 5% CO_2 for 18 – 24 hours. Pneumococci will be isolated and identification made by colony morphology and susceptibility to optochin. Susceptibility to penicillin will be determined using the E test. Serotyping will be performed by Quellung reaction using specific antisera.

Serum will be separated from blood and stored at -70°C until transported to the Immunology Department, Royal Children's Hospital, Melbourne, on dry ice. Anticapsular pneumococcal antibody levels will be determined using standardized ELISA methodology. Techniques to assay serotypes have been developed in conjunction with Dr H Kayhty's laboratory in Finland, for our current randomised trial of pneumococcal conjugate vaccine in Fijian infants (NHMRC ID Number 251648). Enzyme immunoassay (EIA) will measure IgG to 23vPPV serotypes. Microtitre wells will be coated with 2.5-10 μg/mL pneumococcal polysaccharide depending on the serotype. This will be diluted in phosphate buffered saline by incubating at 22°C overnight. Methylated human serum albumin (5 μg/mL) will be used to improve coating of serotype 3. To neutralise unspecified cell wall antibodies, 1-100 diluted serum samples will be incubated for 30 minutes with 10 μg/ml of cell wall polysaccharide before further dilutions. Alkaline phosphatase conjugated anti-human IgG and its substrate will be used for detection. A reference serum will be used. Two additional control sera will be used on each plate to assess interassay variation.

Techniques to assess avidity have also been developed in conjunction with Dr H Kayhty's laboratory in Finland. Avidity of IgG antibodies to capsular polysaccharide of pneumococci will be determined by EIA methods. Serum samples will be diluted 1:50 or 1:100 in PBS-10% fetal calf serum (F-PBS) containing 20 or 10 μg/ml of pneumococcal cell wall polysaccharide. After 30 minutes incubation with the absorbent at room temperature, 3-fold dilutions will be made in F-PBS and the sera will be incubated on the plates for 2 hours at 37°C. After washing, 0.5 M sodium thiocyanate (NaSCN) in F-PBS will be added to dissociate antibody-antigen complexes. NaSCN or F-PBS will be incubated for 15 minutes at room temperature, after which the plates will be washed and antibody binding will be detected by the addition of alkaline phosphatase-conjugated anti-human IgG. The colour will be developed by the substrate *p*-nitrophenyl phosphate. Absorbance at 405 nm will be read on an EIA reader. Results will be expressed as an avidity index, assigned as the percentage of antibodies that remain bound to the antigens after thiocyanate treatment. The avidity index will be calculated by dividing the end point titre of the serum sample with NaSCN treatment by the end-point titre of the sample without NaSCN treatment and multiplying by 100. Control serum will be added to each plate to assess reproducibility. Capsule-specific secretory IgA in breast milk will be determined using the methods described by Finn et al.[16]

9.9 Public health significance

As we have outlined, there is a clear link between carriage of pneumococci in the first few months of life and the extraordinarily high rates of acute and chronic ear infections among Aboriginal children that lead to permanent ear damage, hearing loss and educational disadvantage. Whereas treatment strategies have been largely unsuccessful in controlling ear disease, our study has the potential to demonstrate clinically significant benefits for infants in the first few months of life. If maternal immunisation can eliminate, or at least delay, early-onset pneumococcal colonisation it could have a substantial impact on ear disease.

If the results of our study suggest antenatal vaccination reduces pneumococcal carriage or ear disease it would support moves to establish much larger safety and efficacy studies of pneumococcal vaccination during pregnancy. If similar results are achieved from postnatal vaccination, it would provide evidence that such studies are not warranted and that the policy emphasis should be to ensure unvaccinated mothers are offered 23vPPV prior to discharge from hospital. Given the limited sample size in our study, it is possible that a smaller but still clinically significant reduction in carriage or ear disease may go undetected at one and two months. However, we will also be establishing anti-pneumococcal antibody levels from cord blood, maternal serum and breast milk at various time points that can be used as other indicators to establish if there has been a differential response between either of the vaccine groups and the control group. A larger study would then be indicated to confirm the long-term outcomes in terms of prevention of persistent disease, ear drum perforation and hearing loss.

10. Management structure

A community forum was held at Menzies School of Health Research on 11 and 12 July 2005 involving health service providers and community representatives. Agreement was reached on a management structure for the trial including an Indigenous Reference Group (IRG) with a representative to be nominated on the independent Data Safety Monitoring Board and nominated health professionals acting as advisors to the IRG. We have subsequently written to leaders in each community and key health service providers seeking written expressions of interest/support for the proposed study and nominations for representatives on the IRG where appropriate.

Members of the DSMB are Prof Don Roberton (Chair), Prof Lyn Gilbert (Deputy Chair), Dr Graham Byrnes (independent biostatistician), Dr Ros Webby (designated safety monitor), Prof David Isaacs, A/Prof Deborah Lehmann, Dr Christine Selvey, Ms Sandra Nelson (IRG representative).

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Appendix 1 Brief Summary
A brief summary of the rationale and methodology of PneuMum study was prepared for distribution to community groups, health service providers and other key stakeholders. Copy lodged with HREC

Appendix 2 Pictorial Flip Chart
A copy of the current Pictorial Flip Chart has been lodged with the HREC

Appendix 3 Plain Language Statement
A copy of the current Plain Language Statement has been lodged with the HREC

Appendix 4 Consent Form
A copy of the current Consent Form has been lodged with the HREC

Appendix 5 Indigenous Reference Group – Terms of Reference
A copy of the current Terms of Reference for the IRG has been lodged with the HREC

Appendix 6 Data Safety Monitoring Board – Terms of Reference
A copy of the current Terms of Reference for the DSMB has been lodged with the HREC

Appendix 7 Serious Adverse Event – Standard Operating Procedure
A copy of the current Standard Operating Procedures for Serious Adverse Events has been lodged with the HREC