UNIVERSITY OF OTAGO

Impact of whole population pneumococcal vaccination on pneumococcal carriage in infants in the first 8 weeks of life; a randomised controlled trial in The Gambia

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Abstract

Background: Pneumococcal diseases are responsible for over 1 million deaths every year and most of these are children in sub-Saharan Africa. The 7-valent pneumococcal conjugate vaccine (PCV7) has recently been introduced into child immunisation schedule in The Gambia. Early age at acquisition and high pneumococcal serotype diversity in The Gambia demand close monitoring after PCV7 introduction.

Objective: To determine the impact of community vaccination with PCV7 on nasopharyngeal carriage of pneumococci in newborn infants up to the age of 8 weeks.

Methods: Twenty one representative rural Gambian villages were randomly allocated to intervention and control groups. One dose of PCV7 was given to older children and adults, 2 doses for children aged 12 – 30 months and 3 doses for infants less than 12 months of age. In control villages, PCV7 was given to those aged 30 months and below and one dose of meningococcal group C conjugate vaccine (MnCC) was given to those >30 months of age. Babies born during the trial were recruited and nasopharyngeal swabs (NPS) collected as soon after delivery as possible and then weekly till end of 8 weeks. Transport and processing of NPS were by standard methods. Prevalence and rates of acquisition of any pneumococcus, vaccine serotypes (VT), vaccine associated serotypes (VAT), non-vaccine serotypes (NVT) and non-typeable pneumococci (NT) were studied. Kaplan-Meier failure functions were used to study time to first acquisition of pneumococci and acquisition rates were compared using Cox regression model adjusting for clustering at village level.

Results: One hundred eighty nine and 155 infants were recruited in intervention and control villages respectively. Overall prevalence of carriage by the 8th week of life was 75% and 80% in intervention and control arms respectively. Sixty seven serotypes were isolated in the study. Prevalence of VT carriage was significantly lower in intervention villages compared to control (11.1% vs. 27.4%, p<.001). NVT carriage was higher in intervention villages (68.3% vs. 60.6%, p=0.141) and there were statistically significant differences in the carriage rates of individual NVT; 7F (2.5% vs. 1.1%, p=.016), 10A (3.9% vs. 2.3%, p=.026) and 15B (4% vs. 1.6%, p=.001) in intervention compared to control villages. VAT and NT carriage were similar in both arms. The risk of acquisition of VT was significantly lower in intervention compared to control villages (hazard ratio 0.37; 95%CI 0.22 – 0.6, p<.001). For the NVT,

risk of acquisition was higher in intervention villages compared to controls but was not statistically significant (hazard ratio 1.26; 96CI 0.94 – 1.68, p=.12). There was a slight but non-significant increased risk of acquisition of NT in intervention compared to control villages (hazard ratio 1.13; 95%CI 0.61 – 2.09). VAT 6A carriage was significantly lower in intervention compared to control villages (1.8% vs. 4% respectively, p=.002) and VAT 19A carriage was higher in intervention villages compared to controls but this was not statistically significant (2.3% vs. 1.6% respectively, p=.205). The presence of children <5years of age was associated with an increased risk of acquisition of VAT (hazard ratio 1.07; 95%CI 1.03 – 1.12, P<.001).

Conclusion: High immune pressure through community vaccination with PCV7 in The Gambia resulted in a significant 'indirect' reduction in the carriage of pneumococci of vaccine serotypes in infants in their first 8 weeks of life in these communities. This was associated with increased acquisition of pneumococci of non-vaccine serotypes. These findings support both the introduction of PCV7 into The Gambia and the need for ongoing active surveillance.

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Contents

Definitions

Compound: A group of households occupying the same piece of land and usually under the control of the eldest man. In most cases, this would be the grandfather of the study subjects.

Household: A group of individuals living under one roof and eat from one cooking pot. The members are usually of the same nuclear family but could include members of the extended family.

‘Bantaba’: A central meeting place in the village where inhabitants could gather for meetings and other activities. This could be an open space shaded with a canopy or under a big tree. Sensitisation meetings with the village heads were mostly held in Bantabas.

‘Alkalo’: This is the official head of the village usually selected based on inheritance (ie a member of the family of a deceased Alkalo, usually the son or brother, would normally replace the Alkalo). There are also a handful of female Alkalos.

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List of Abbreviations

ALRI – Acute lower respiratory tract infection

AOM – Acute otitis media

CO2 – Carbon dioxide

Hib – *Haemophilus influenzae* type b

HIV – Human immunodeficiency virus

HR – Hazard ratio

IPD – Invasive pneumococcal disease

MRC – Medical research council

NPS – Nasopharyngeal swab

NT – non typeable pneumococci

NVT – Non vaccine serotypes

OR – Odds ratio

PCV – Pneumococcal conjugate vaccine

PPS – Pneumococcal polysaccharide vaccine

RR – Relative risk

SCC – Scientific co-ordinating committee

SD – Standard deviation

SP – *Streptococcus pneumoniae*

STGG - skim milk-tryptone-glucose-glycerol

SVT – Sibanor vaccine trial

UK – United Kingdom

USA – United states of America

VAT – Vaccine associated serotypes

VT – Vaccine serotypes

WHO – World health organisation

Chapter 1: Introduction

Streptococcus Pneumoniae (SP) is the leading bacterial cause of infection worldwide and results in a spectrum of clinical conditions ranging from otitis media to life threatening invasive infections such as sepsis, pneumonia and meningitis (1-2). In both industrialised and developing countries, SP ranks very high in its public health and economic impacts compared to other bacterial infections(1). Invasive pneumococcal disease is a major cause of morbidity in The Gambia with rates 2 – 8 times that in the USA(2). The most common form of pneumococcal disease is bacteraemic pneumonia, the highest proportion of which occurs at both extremes of age; children < 2 years of age and adults > 65 years of age(3). Of the estimated 2.6 million deaths due to pneumonia among children less than 5 years of age in the developing world, about 1 million is attributable to SP either alone, or in conjunction with viral respiratory infections, malnutrition or HIV infection. In addition, SP is the leading cause of non-epidemic childhood meningitis in the developing countries(4). In sub-Saharan Africa, SP has been shown to be responsible for 25% to >30% of all cases of meningitis in children aged less than 5 years and had a case fatality rate exceeding 50%(5). Two separate studies involving hospitalised south African children showed that SP was the predominant pathogen isolated during the study period, accounting for 23% and 33% respectively, of all isolated organisms (6-7). The prevention of pneumococcal disease in young children is therefore of major international public health importance.

Nasopharyngeal colonisation is very common in childhood and usually asymptomatic. Colonisation with a homologous strain of SP is necessary for the development of pneumococcal disease(8). In developing countries, nasopharyngeal colonisation with SP starts soon after birth and by the end of the first year, almost all children become carriers(9-10). Carriage rates stay high among older children and adults who then may become important sources of infection in children. Active case detection and antibiotic treatment of ALRI have been shown in developing countries to significantly reduce mortality due to pneumonia(11), but this modality is threatened by the rapid and increasing emergence of pneumococci that are resistant to the commonly used and easily available antibiotics such as

co-trimoxazole and penicillin. In addition, case detection and treatment of index cases present several challenges to health systems in the developing countries. The success achieved by the *Haemophilus influenzae* type b (Hib) conjugate vaccine in reducing the incidence of radiological pneumonia in both The Gambia and Chile(12-13) led to hopes that a conjugate pneumococcal vaccine would be even more effective against pneumococcal disease.

In 2000, the 7 valent pneumococcal conjugate vaccine (PCV7), which contains polysaccharide of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, was licensed for use against invasive pneumococcal disease (IPD) in children in the USA. This followed a successful efficacy trial of the vaccine among infants during which the vaccine reduced the incidence of IPD due to the serotypes included in the vaccine by 90%, the incidence of pneumonia by 33% and the incidence of otitis media by 7% (14). The vaccine has since been licensed in several other developed countries where invasive pneumococcal disease is predominantly caused by the serotypes included in the vaccine. In these populations, PCV7 provides coverage for 70 – >90% of pneumococcal serotypes causing invasive disease in the age group targeted for vaccination(15). The use of PCV7 has resulted in dramatic reductions in the incidence of IPD in the US not only in vaccinated children, but also in their unvaccinated contacts, older children, young adults and the elderly in whom the incidence of IPD has also markedly reduced (16). However in several developing countries of Africa and Asia, a significant proportion of invasive disease is caused by pneumococcal serotypes not included in the vaccine(17-18). Also, there have been reports of increases in the rates of IPD due to pneumococcal serotypes not included in the vaccine in populations where widespread use of this vaccine has been achieved (19-22).

The PCV7 has recently been introduced into the routine childhood immunisation schedule of two African countries – Rwanda and The Gambia. Previous pneumococcal carriage studies in The Gambia have shown that the pneumococcus is acquired very early in life, with about 93% of infants already colonised in the first week of life(23). In addition, as much as 65 different serotypes were isolated from samples collected from infants over a 2 year period, providing a ready pool of pneumococci for replacement of the vaccine types in the event of widespread pneumococcal vaccination. Another study in The Gambia has shown that although pneumococcal serotypes 1 and 5 were rarely isolated from the nasopharynx of children, they were nevertheless responsible for about one third of all cases of invasive

pneumococcal disease in The Gambia (24). Serotypes 1 and 5 are not covered in PCV7. A recent report from Portugal has shown an increasing prevalence of a pneumococcal serotype 1 lineage from the nasopharynx of children between 2001 and 2006 and this trend parallels the introduction and increased use of PCV7 among the study subjects (25). This finding is of strong relevance to The Gambia, an environment where serotype 1 is low in carriage but contributes significantly to the burden of invasive disease. It is therefore not certain what the impact of routine use of a pneumococcal conjugate vaccine of limited valence such as PCV7 would be in Gambian infants.

The overall aim of this study was to determine the impact of 7-valent pneumococcal conjugate vaccination on nasopharyngeal carriage of pneumococcus in infants in the first 8 weeks of life in vaccinated and unvaccinated communities in The Gambia. The specific objectives are:

1. To determine the effect of vaccination on the prevalence of carriage of any Pneumococci, vaccine type and non-vaccine type pneumococci in infants born in vaccinated and unvaccinated communities in the first 8 weeks of life
2. To determine the effects of vaccination on the acquisition rates of any pneumococci, vaccine type and non-vaccine type pneumococci in infants born in vaccinated and unvaccinated communities in the first 8 weeks of life

The findings from this study will form an important baseline data for the assessment of long term impact of the newly introduced PCV7 in The Gambia

My role in the study

I was the research clinician for the village randomised controlled study with full responsibility for the day to day running of the trial. My main duties were:

Community activities: I organised meetings with village heads during which the study objectives were explained, questions entertained and consent of the communities obtained. I took responsibility for the periodic organisation of ‘open days’ during which the trial progress was reviewed and presented to the participating communities in ordinary language. I supervised the mapping of villages for the study as well as periodic census updates which was done in the study villages at the end of every year.

Managerial duties: I supervised over 20 field workers and nurses who were involved in the trial and organised training for the consenting process and administration of questionnaires, NPS collection and vaccination. I was responsible for the co-ordination of a two weekly meeting of all investigators and support staff from the microbiology laboratory, data staff and field staff. During these meetings, I presented status report of the trial. In addition, I kept and maintained a comprehensive trial master file which was periodically reviewed with the trial support managers.

Sample collection and vaccination: I maintained a hands-on involvement with the clinical examination, NPS collection and vaccination of study subjects with the field nurses, including monitoring, reporting and management of vaccine adverse events. I supervised handling and transportation of NPS specimen to ensure full compliance with the established standard methods.

Data handling and analysis: I ensured at the end of each day that vetted data from the field was submitted to the data management team and questionnaires and forms for the following sampling period promptly produced. I worked with the trial data manager to ensure double entry and verification of data and took personal responsibility for the cleaning and analysis of the newborn longitudinal study which is presented in this thesis. Cleaning of this data involved detection of incomplete and duplicated data, verification of subject IDs, location of missing data, sorting out of data transcription errors and merging of the two datasets – risk factor questionnaire data and the NPS data. To ensure I played the major role in the analysis of data, I took the Biostatistics course – HASC 413- during the diploma year and learnt to use the stata statistical package which was used in the analysis of data for this thesis. I performed the analysis of the data and had expert help from a senior statistician for the time to acquisition and risk factor analysis in particular.

Chapter 2: Literature Review

Part one: Epidemiology of Pneumococcus, nasopharyngeal carriage and pneumococcal conjugate vaccine

2.1 The Pneumococcus

Streptococcus pneumoniae (SP) is an encapsulated, non-motile, non-sporulating Gram positive organism and appears typically as a 0.5 – 1.25μm diplococcus(26). The organism is oval or lancet shaped and is usually arranged in pairs or short chains. Being fastidious, it grows only in enriched bacteriological media where the colonies exhibit the typical alpha haemolysis when incubated under aerobic conditions. Virulent strains of SP are covered by capsular polysaccharides which form the basis for classification of the organism into serotypes(26). To date, 91 serotypes of SP have been described(27). The cell wall is made up of oligopeptide chains which are attached to alternating subunits of N-acetyl glucosamine and N-acetyl muramic acid. The cell wall also contains teichoic acid which exists in two forms; one which is exposed to the peptidoglycan layer and extending through the overlying capsule and a second which is covalently bound to the plasma membrane lipids.

2.1.1 Pathogenesis of Pneumococcal disease

The pathogenesis of SP is mediated by the host response to infection by the organism. The process begins with colonisation of the nasopharynx and the oropharynx to a lesser extent. Colonisation occurs when the organism gains access into the upper respiratory tract and binds to the host epithelial cells(28). This binding process is mediated by certain proteins called ‘surface protein adhesins’ released by the organism which aides the organism in attaching to host epithelial cells. Host defence against pneumococcal infection is mediated by both immunological and non-immunological mechanisms(29). Non-immunological factors relate to the integrity of the mucosal surfaces of the respiratory tract. Abnormalities of the respiratory mucosal surface occur in viral infections of the respiratory tract and in persons exposed to air-borne pollutants notably indoor fires for heating and cooking. It has been suggested, for instance, that prior influenza virus infection enhances adherence of pneumococci to tracheal epithelial cells(30).To counteract host mucociliary action required to inhibit colonisation, SP releases secretory immunoglobulin A (sIgA) protease and pneumolysin. Secretory immunoglobulin A protease counteracts host IgA while pneumolysin

binds to cholesterol on host cell membrane creating pores through which the organism gains access into normally sterile sites. In addition, ciliated epithelial cells are damaged and phagocytic cells are inhibited. Inflammation results from the release of peptidoglycan fragments, teichoic acid and pneumolysin as the bacteria gain entrance into the cells. The process stimulates both the classical and alternative complement pathways leading to the release of inflammatory cytokines which then cause tissue damage.

The colony morphology of pneumococci shows three interchangeable variants – opaque, semi-transparent and transparent – with different abilities to colonise the nasopharynx(31).The transparent variant tends to possess a selective advantage in nasopharyngeal colonisation leading to local infection by the more adhesive strains and invasive disease such as meningitis and bacteraemia by the less adhesive strains(32).

The processes by which the pneumococcus is translocated from the nasopharyngeal site to other sites remain incompletely understood and are probably mutifactorial(33). Pneumococcal pneumonia, for instance, is believed to result from aspiration of pneumococci from the upper respiratory tract though the possibility of blood-borne dissemination from the upper respiratory tract cannot be ruled out(33). Aspiration of pneumococci or penetration of the mucosa by the organism would result in bacteraemia whereas seeding in different body systems may lead to meningitis or other disease syndromes such as arthritis.

2.2 Pneumococcal Carriage

The human nasopharynx is an important ecological niche for many bacterial species, including the pneumococcus. For the pneumococcus however, only the human nasopharynx provides the environment necessary for survival and therefore the pneumococcus depends on successful transmission between humans to stay alive (34). The phenomenon collectively described as ‘carriage’ is actually divided into three distinct phases, namely, acquisition, carriage and termination of carriage. Acquisition refers to the point when SP gains access into the host nasopharynx and establishes itself leading to carriage, which refers to a period during which the organism is resident in the host nasopharynx. Loss of the organism from the human nasopharynx is referred to as termination of carriage(34). Nasopharyngeal colonisation is common and usually asymptomatic. The epidemiology of carriage and pattern of serotypes vary by geographical location(15, 17). High carriage rates have been reported from several

developing countries: 100% of children aged 3 months in Papua New Guinea (9), 93% of infants aged 1 month in The Gambia (23) and 50% of infants aged 8 weeks in Bangladesh (35). The rates are much lower in industrialised countries. In a population based prospective cohort study in the Netherlands, carriage rate was 44.5% in children aged 14 months(36). In Singapore, carriage rate of 14.1% was reported in children attending day care (37). It is believed that factors such as crowding, large number of siblings and frequent upper respiratory tract infections as seen in developing countries could contribute to the higher carriage rates observed in developing countries compared to the industrialised countries.

2.3 Risk factors for pneumococcal carriage

Certain environmental and host factors have been explored as possible risk factors for pneumococcal carriage in children.

2.3.1 Age

Series of longitudinal studies of carriage in children have consistently shown a significant age-specific increase in carriage over the first two years of life. Young children constitute the largest proportion of colonised individuals in the community while adults are far less colonised. In the early 1900s, investigators found that approximately 65% of adults carried Pneumococcus as against 35% of children(34). This is contrary to findings from recent studies. Prevalence of carriage has varied among studies, with a progressive rise during the first year from a prevalence of <10% in the weeks after birth to nearly 100% at 1 year of life in some studies(34). In Finland, pneumococcal nasopharyngeal carriage during health was found to increase gradually with age. Twelve percent (12%) of infants aged less than 3 months were found to be carriers and this rate increased to 43% at the age of 2 years(38). Similarly, carriage significantly increased in a cohort of Dutch children followed from birth and sampled on three occasions in the first year of life. Carriage was 8.3% at age 1.5 months, 31.3% at 6 months and increased to 44.5 at 14 months(36). In Papua New Guinea, 84% of babies were found to be carriers in the first month of life and by the 3rd month, 100% of babies had been colonised(9, 39). A longitudinal study of nasopharyngeal carriage in children in 21 rural Gambian villages showed a 65% prevalence of carriage by the age of 1 month and this increased to about 85% by the 4th month of life(23). A cross-sectional study carried out in the same population about the same time showed a carriage prevalence of 51% among adults aged 40 years and over (37). The reasons for the lower carriage in adults compared to

children are unclear. An association with the presence of antibodies to pneumococci in adults has been proposed as a possible explanation(40). The Gambian study additionally shows a much earlier age at first acquisition; mean and median times to first acquisition were 33days and 24 days respectively(23). In the US, time to first acquisition was shown to be approximately 6 months(8) while in Finland, 34% of infants had been colonised by 6 month of age(38).

Carriage therefore shows an age specific increase in the first year of life and remains higher among young children compared to adults.

2.3.2 Crowding

It is known that crowding as occurs in day care centres, military camps, hospitals and prisons, is associated with an increase in the transmission of pneumococci among individuals(41-42). Day care centres are the most common form of out-of-home care for children in the Western world(43). Day care centres present a unique environment for interaction of children with incompletely mature immune systems and susceptibility to both viral and bacterial upper respiratory tract infections. The hours which they spend each day in crowded conditions facilitate colonisation and spread of pneumococci(44) and this predisposes day-care attendees to pneumococcal infections, often with antibiotic-resistant strains(45). In a longitudinal study of nasopharyngeal carriage in children attending day care in Sweden, carriage was higher in children attending day-care centres with over 45 children in the class compared to those with less than 45 children in the class(46). This further emphasises the importance of crowding as a risk factor for pneumococcal carriage in children.

2.3.3 Respiratory infections

Pneumococcal carriage has been shown to increase in the presence of respiratory infection. Syrjanen et al(38) found a higher carriage rate (45% - 56%) during respiratory infection without acute otitis media (AOM) compared to rates during health (13% - 43%). During AOM, the pneumococcal carriage rates were high regardless of age, and during pneumococcal AOM, practically every child carried *S. pneumoniae*. Similarly, a longitudinal carriage study among adolescents identified the presence of respiratory tract infection at recruitment as an independent risk factor for pneumococcal carriage (47). Respiratory

infections are known to undermine the integrity of the mucosal epithelium thereby enhancing the ability of the pneumococcus to adhere to the epithelium(17). This could then lead to increased carriage rates.

2.3.4 Siblings

The presence of a colonised sibling has been documented as one of the strongest risk factors for pneumococcal carriage in infancy(34). This has been observed in both the developed and developing country settings. In separate studies conducted in Israel and Finland, infants have been shown to acquire pneumococci from their older siblings. A study in Israel study compared pneumococcal carriage in adults with that of children aged 6 years and below and identified young age and having young siblings as important risk factors for carriage in children(40). In a second Israeli study(48), pneumococcal strains acquired by younger siblings were compared with those present in day care centres in order to determine the association between carriage among infants cared for at home and carriage among their older siblings who attended day care centres. One or more strains identical by serotype and antibiotic susceptibility were isolated in the older sibling's day care centre in 76% of cases compared to 32% - 63% in all other day care centres. In the Finish study, nasopharyngeal swabs were collected on 10 occasions from 100 children and their family members (49). Simultaneous carriage of the same serogroup by another family member was found to be the strongest predictor of carriage in children older than 6 months. Having siblings and day-care attendance were also identified as significant risk factors for pneumococcal carriage in children in the Dutch cohort study (36).

2.3.5 Breastfeeding

Breastfeeding has been shown to be protective against otitis media and some respiratory tract infections(50). Protection against invasive pneumococcal disease has also been demonstrated in a case control study to identity risk factors for invasive pneumococcal disease in children aged 2 – 59 months (45). But studies investigating the role of breastfeeding in pneumococcal carriage have shown conflicting results. Rosen et al analysed antibodies to pneumococcal polysaccharides in human milk obtained from mothers and their effect on nasopharyngeal colonisation of their infants(51). This study showed that pneumococcal capsular antibodies in human milk did not protect against carriage. Similarly in a study of nasopharyngeal colonisation of respiratory pathogens among infants aged 1 – 2 months, Kaleida et al did not

demonstrate any difference in rates of colonisation between exclusively breastfed and exclusively bottle fed infants(52). On the other hand, Duffy et al observed lower rates of pneumococcal carriage in exclusively breastfed compared to formula fed infants in a longitudinal follow up of new born infants to assess the relationship of exclusive breastfeeding to episodes of acute otitis media(53). There is thus some uncertainty as to the true association between breastfeeding and pneumococcal carriage and the effect of breastfeeding on pneumococcal carriage may vary between populations.

2.3.6 Previous antibiotic use

Recent antibiotic use in the last month irrespective of antibiotic class, duration of use and the number of courses of antibiotics was strongly associated with the carriage of anti-biotic resistant pneumococci in a nationwide survey of pneumococcal carriage in Greek children in the pre-pneumococcal conjugate vaccination era (54). In addition, increasing number of courses of antibiotics in the last month increased the likelihood of isolating a resistant pneumococcal strain.

Therefore prudent use of antibiotics could be an important measure to check the increasing levels of carriage and infection with antibiotic-resistant pneumococcal strains.

Other risk factors that have been suggested but remain inconclusive include sex, maternal smoking and nutritional status. Sleeman et al investigated the factors associated with acquisition using data from a large longitudinal study of a birth cohort in the United Kingdom. Multivariate analysis was performed for the association between pneumococcal acquisition and several factors including breastfeeding, number of siblings, day care attendance, exposure to cigarette smoke, consultations to general practitioners for infections, antibiotic treatment and season. Of these factors, only number of siblings (hazard ratio 1.5; 95% CI 1.3 – 1.8; P <0.001) and visits to a general practitioner for mild upper respiratory infection (hazard ratio 1.8; 95% CI 1.1 – 2.9; P=0.02) were independently associated with acquisition of pneumococci(55). Among adolescents (aged 10 – 19 years) in Brazil, pneumococcal carriage was independently associated with younger age, male sex, exposure to passive smoke in the household and a history of acute asthma in the previous year(47).

2.4 Pneumococcal conjugate vaccines

The first preventive strategy against pneumococcal disease was introduced by Sir Almroth Wright in 1911 when he suggested that the inoculation of killed whole pneumococci might protect against pneumococcal infections(56). Unfortunately, this pioneering process could not be continued because of technical challenges. Later on in the 1920s, the isolation of the pneumococcal capsular polysaccharides led to the development of the first capsular polysaccharide vaccine. This vaccine successfully helped abort an outbreak of pneumonia in a state hospital in Worcester, Massachusetts in 1931(57). However, the development of successful antibiotic therapy which could effectively deal with pneumococcal disease in the 1950s led to declining interest in the development of anti-pneumococcal vaccines(58). With the emergence, spread and worldwide dissemination of penicillin resistant pneumococci mostly involving multi-drug resistant clones of serotypes 6B, 9V, 14, 19A, 19F and 23F, there was a renewed interest in vaccine strategies for the control of pneumococcal diseases (58). The 14 valent pneumococcal polysaccharide (PPS) vaccine was developed in 1977 and expanded in 1983 to a 23-valent vaccine which theoretically provided coverage for >80% of the pneumococci causing infections in adults. The currently licensed 23-valent PPS vaccine is effective in adults and children greater than 2 years but ineffective in children less than 2 years who suffer the greatest burden of pneumococcal disease and carriage(59). In addition, PPS vaccines do not induce T cell-dependent immune response which is required for induction of B cell immunological memory after vaccination. They have little or no impact on nasopharyngeal carriage(60).

To overcome these limitations, the pneumococcal conjugate vaccines are now being developed, employing the same principles used in the development of the haemophilus influenza type b (Hib) vaccine. This involves the conjugation of the capsular polysaccharide of the organism to a protein carrier such as tetanus toxoid, diphtheria toxoid and outer membrane protein from meningococcus group B(61). The 7-valent pneumococcal conjugate vaccine (PCV7) employing the diphtheria protein CRM197, was the first to be developed. It contains the polysaccharides of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F and induces a T cell-dependent response, memory B cells and an improved B cell response(62). This vaccine was introduced into the EPI schedule of US infants in 2000 and is now widely used for the prevention of pneumococcal diseases in children. Recently, the Food and Drug Administration approved an expanded valency pneumococcal conjugate vaccine

containing 13 pneumococcal serotypes, for use among US children aged 6 weeks to 71 months for prevention of invasive pneumococcal disease and Otitis media caused by pneumococcal serotypes contained in the vaccine(63).

2.5 Impact of vaccination with pneumococcal conjugate vaccine on pneumococcal carriage

2.5.1 Reduction in vaccine type carriage

Various studies have demonstrated a reduction in the nasopharyngeal carriage of vaccine-type (VT) pneumococci in infants and children after vaccination with PCV of varying valencies while the overall carriage rates remained essentially unchanged. Reduction in VT pneumococcal carriage was found among Israeli toddlers vaccinated with either one dose or two doses of PCV7 but not in those vaccinated with the 23-valent pneumococcal polysaccharide vaccine (64). A second study in Israel, a randomised controlled trial among day care attendees, also reported a reduction in VT pneumococcal carriage in these children following vaccination with the PCV9 as well as an associated reduction in the carriage of antibiotic resistant pneumococci among their siblings(65). In a longitudinal study of US infants from Texas, NPS was collected before each PCV7 dose in the primary series of vaccination , at 9 months of age and at 15 – 18 months of age, 2 - 3 months after the booster vaccination. Vaccine type pneumococcal carriage dropped from 18% to 9% (P=.001) between the 12- and 18 month follow up visits (66). In this cohort, however, there was a significant increase in the carriage of non-vaccine serotypes.

Two point prevalence studies conducted among day care centre attendees in Norway in 2006 (before PCV7 introduction) and 2008 (after widespread use of PCV7) revealed that overall carriage rate remained the same (77.7% in 2006, 80.2% in 2008)(67). On the other hand, overall VT carriage decreased by 48.4% (P<0.001). In children who had received just one dose of vaccine or no immunisation at all, a decrease of VT carriage by 37% was observed in 2008, reflecting herd effect on pneumococcal transmission and carriage. An increase in non-VT carriage caused by emergence of 37 sequence types and expansion of 17 existing ones was also observed.

PCV7 therefore reduces carriage of VT pneumococci both in vaccinated children (direct effect) and unvaccinated contacts (indirect effect). A concomitant increase in carriage of serotypes not included in the vaccine could occur in parallel.

2.5.2 Reduction in drug resistant pneumococcal carriage.

Vaccine serotype isolates of the pneumococci are almost exclusively associated with high level penicillin resistance presumably because selective pressures for resistance are strongest on isolates carried in the nasopharynx of children and these are predominantly the vaccine serotypes(68). A study investigating the impact of PCV9 on nasopharyngeal pneumococcal carriage in South African children showed a significantly reduced carriage of antibiotic resistant pneumococci among vaccinees compared to controls. Carriage of penicillin-resistant pneumococci was also significantly reduced in vaccinees compared to controls (21% vs. 41%, P <.001), and this was regardless of serotype carried(69). In southern Israel where a high prevalence of carriage of antibiotic resistant pneumococci has been demonstrated, Dagan et al showed a reduction in the carriage of antibiotic resistant pneumococci one year after vaccination among PCV9 vaccinees compared to controls who received the 23 valent polysaccharide vaccine(64). The carriage of antibiotic resistant pneumococci is therefore reduced following vaccination in parallel with the reduction of carriage of vaccine type pneumococci. However, this does not appear to be the only mechanism by which the PCV reduces carriage of antibiotic resistant pneumococci in children. Lower rates of otitis media resulting from reduction in vaccine type pneumococci was associated with lower rates of antibiotic use in Finish children(70). This reduced antibiotic use would potentially further reduce the development of antibiotic resistance. In communities where herd immunity is achieved, there is the potential for further reduction in the use of antibiotics and hence of carriage and transmission of antibiotic resistant pneumococci(71). Thus, the pneumococcal conjugate vaccine can potentially reduce transmission of antibiotic resistant pneumococci through pathways other than direct effect on carriage of resistant pneumococcal strains. While this is a reason to cheer, replacement of vaccine serotypes by antibiotic resistant non vaccine serotypes could erode this benefit. In a prospective follow up of 685 Portuguese day care centre attendees aged 6 months to 6 years , overall carriage and carriage of drug resistant pneumococci were determined over 6 sampling periods. Though a marked reduction in

the prevalence of carriage of antibiotic resistant vaccine serotypes was observed in vaccinees compared to controls, the total carriage of antibiotic resistant pneumococci did not change during the follow up period(72). This was because in parallel with the decline in prevalence of antibiotic resistant vaccine type pneumococci, there was a rise in the prevalence of antibiotic resistant non vaccine type which replaced vaccine serotypes in vaccinees. This phenomenon was thought to be due to the combined effect of vaccination and high rate of antibiotic use. It underlines the importance of close monitoring and surveillance after introduction of conjugate vaccines of limited valency(73).

2.5.3 Indirect effect of vaccination with PCV7

The PCV has been shown in randomised controlled trials and observational studies to reduce the nasopharyngeal carriage of pneumococcal serotypes included in the vaccine in recipients of the vaccine (65, 73-74). Reduction of vaccine type carriage was also observed in contacts of vaccine recipients who had not been vaccinated with PCV7 due to reduction in transmission of the vaccine serotypes in the community. Since colonisation is the first step in invasive disease, reductions in IPD due to vaccine serotypes and IPD due to any pneumococci have also been observed.

The Active Bacteria Core Surveillance network of the Centres for Disease Control and Prevention in the USA has routinely kept IPD data from a network of laboratories across several states. In an analysis of IPD rates between the periods 1998 /1999 (pre-PCV7 period) to 2003 (3 years after PCV7 inclusion into routine EPI), incidence rates of IPD among non PCV7- vaccinated persons in the target population reduced substantially over the review period(74). A reduction of 62% (95% CI 59 – 66) was observed among persons aged 5 years and above with the highest rate reduction observed among individuals aged 65 years and above. In this group, IPD rate dropped from 33.6/100,000 to 11.9/100,000 cases over the review period and in 2003, more than twice as many cases of VT IPD were found to have been prevented by indirect protection of unvaccinated individuals as direct protection. In a carriage study following group randomised trial of PCV7 among American-Indian children, the impact of the vaccine was assessed among trial participants and their unvaccinated household contacts(75). This study revealed that adults and unvaccinated children aged <5 years who live in the same household with a PCV7 vaccinated child were less likely to be colonised with PCV7 serotypes compared to

those in households without PCV7 vaccinees (OR for adults 0.57; 95%CI 0.33 – 0.99: OR for children 0.57; 95%CI 0.26 – 0.98). This finding was similar to that of a population based observational study among 8 Alaskan communities in which vaccine type pneumococcal carriage rates of adults dropped to 4.5% after introduction of routine PCV7 infant vaccination compared to carriage rate of 28.4% in the pre-vaccine era (76).

Unvaccinated individuals living in the same environment as vaccinated children experience indirect protection against pneumococcal carriage and invasive disease. It is expected that as vaccination coverage increases, indirect protection of unvaccinated individuals would also increase. This is potentially of huge public health importance in high risk populations such as American Indian children, Aboriginal children, sub-Saharan African children and children attending child-care centres.

2.5.4 Immunological hypo responsiveness

An interesting recent finding following vaccination of infants with the PCV7 was that there was immunological hypo responsiveness to a pneumococcal serotype if it was carried in the nasopharynx just prior to vaccinations. Dagan et al(77) obtained NPS from healthy children shortly before the first PCV dose in a randomised trial of 2 dosage regimens of the PCV7. In children who carried pneumococcus before the first dose of vaccine, IgG response to the carried serotype was significantly lower than in non-carriers. This phenomenon lasted for several months and was only partially reversed by the booster dose given at 12 months. A similar phenomenon has also recently been demonstrated in Filipino children who participated in an immunogenicity and carriage study nested in a trial of PCV11(78). Antibody concentration to the most commonly carried serotypes 6B, 19F and 23F were determined at 18 weeks and 9 months of age and compared between PCV11 groups stratified according to their carriage status at 6 weeks of age. IgG response was observed to be significantly lower at 18 weeks and 9 months among carriers of the specific serotypes at 6 weeks compared to non-carriers. The importance of this phenomenon is not well understood and requires further research especially in settings such as The Gambia where most children are likely to be carrying pneumococci at the time of vaccination.

Impact of vaccination on Invasive pneumococcal disease

Randomised controlled trials conducted among children in different populations have investigated the impact of pneumococcal conjugate vaccination on IPD. In the Northern California trial of PCV7 involving over 37,000 children, interim analysis showed that all 17 cases of IPD observed among fully vaccinated children and all 5 cases of IPD among partially vaccinated children were found only among controls, giving a vaccine efficacy of 100%. At the end of the trial, only 1 of the 40 IPD cases due to vaccine serotypes was observed among PCV7 vaccinees, giving an efficacy of 97.3% against IPD due to vaccine serotypes (19). Similarly, in a group randomised trial of PCV7 among Navajo and White Mountain Apache reservations children, a high risk population with very high burden of IPD, only 2 of the 13 cases of IPD observed during the trial was among PCV7 Vaccinees (79). The vaccine efficacy against IPD due to vaccine serotypes was 82.6% after controlling for group randomisation. The significance of this trial was that PCV7 was likely to be efficacious in high risk populations such as the children in developing countries where the burden of IPD is highest. A South African trial of PCV9 among children with and without HIV also demonstrated significant efficacy against IPD (80). Of the 20 cases of IPD observed among children without HIV infection during the study period, 17 were among controls while only 3 were among vaccinees, giving a vaccine efficacy of 83%. Among those with HIV infection, 26 cases of IPD were seen among controls while 9 cases were seen among vaccinees for a vaccine efficacy of 65%. In a Gambian study, though IPD was evaluated as a secondary end point and the cases were very few, a significant reduction in IPD due to PCV9 serotypes was observed among vaccinees compared to controls and a vaccine efficacy of 77% against IPD due to PCV9 serotypes was documented (81).

Following the introduction of PCV7 into routine infant vaccination schedule in the US in 2000, profound reductions in incidence of IPD due to serotypes included in the vaccine were observed within the first three years (14, 79). These reductions were also observed in infants too young to receive the vaccine as well as the very elderly who also did not receive the vaccine(82), a result of the indirect effect of the vaccine. A review of the impact of pneumococcal conjugate vaccination in the US in 2003 using population based data from a co-operative surveillance network revealed that PCV7 had prevented more than twice as many IPD cases through indirect effects on pneumococcal transmission than

through directly protecting vaccinated children(83). In addition, IPD due to high level penicillin resistant pneumococci was shown to have drastically reduced in a post-licensure surveillance for IPD after use of PCV7 in the Northern California Kaiser Permanente Trial. High level resistance of pneumococci to penicillin fell from a peak of 15% in 2000 to 5% in the first half of 2003(84).

Increases in the incidence of non vaccine type IPD following routine use of PCV7 (replacement disease) have now been reported from several studies. The first documentation of replacement in invasive disease was in a multicenter study of children in the USA hospitalised for IPD where researchers noted a concomitant increase in disease caused by non-vaccine serotypes alongside marked decline in vaccine type IPD(85). This involved mainly pneumococcal serogroups 15 and 33. Reporting on the serogroups of pneumococci isolated from children with IPD in Utah between 1997 and 2003, Byington et al found a significant decrease in disease due to vaccine serogroups after 2000 (when PCV7 was introduced in the US) but with a concomitant and significant increase in disease caused by non-vaccine groups(86). Similarly, investigators in Spain carried out a 10 year prospective study involving children with culture proven IPD in a large urban hospital and compared rates of vaccine and non-vaccine type IPD between pre-vaccine and vaccine periods(87). In the Spanish study, there was a significant increase in incidence of IPD between the pre-vaccine and vaccine periods. IPD rates increased by 58% between the two periods among children less than 2 years of age, and by as much as 135% among children aged 2 -4 years between the two periods. Of major concern was the marked increase in rates of non-vaccine serotype IPD in all the age groups and markedly increased rates of pneumonia with empyema caused by serotype 1 between the two periods especially in children aged 2 – 4 years of age. In addition, there was an emergence of previously established virulent clones of non-PCV7 serotypes 1 and 5. In surveillance studies, serotype 19A strains have been identified as major causes of replacement disease (88-89) and this probably reflects their greater prominence in carriage(90). Other significant non-vaccine serogroups that have become important causes of IPD following introduction of PCV7 include 11, 15, 33 and 35 (87).
The finding of replacement disease following introduction and routine use of PCV7 emphasises the importance of ongoing surveillance especially in high risk populations and the need for the development of conjugate vaccines of expanded valency.

2.6 Impact of vaccination on Pneumonia

Streptococcus Pneumoniae is a leading cause of pneumonia in children aged <5 years, the majority of whom live in the developing countries and childhood pneumonia is the leading single cause of child mortality worldwide(3, 91). The development of vaccines against measles, pertusis and Hib led to a remarkable fall in pneumonia morbidity and mortality worldwide but pneumonia deaths have remained high despite these interventions(3). The WHO recommends pneumococcal conjugate vaccination as a strategy to prevent childhood pneumonia.

Randomised controlled trials investigating effectiveness of pneumococcal conjugate vaccines against radiologically defined pneumonia in children have been conducted across different populations using PCVs of different valencies. A trial of PCV7 in Northern California showed a reduction in the incidence of radiologically defined pneumonia among the vaccinees of 20.5% (CI 4.4 – 34.0; P=0.02) compared to controls in the per protocol analysis (92). In the intention to treat analysis, a reduction of 17.7% (P = 0.01) was observed. This impact was much more among children in the first year of life (32.2% reduction in radiological pneumonia) and 23.4% in those in the second year of life. Similarly, incidence of clinically diagnosed pneumonia reduced by 4.3% (CI -3.5 – 11.5) among vaccinees compared to controls, but this did not reach statistical significance. A similar trial of PCV11 among Filipino children showed a 22.9% reduction in first episode of radiologically defined pneumonia among vaccinees compared to controls (93). Though this was not statistically significant, the magnitude of the effect was similar to the South African trial where PCV9 was shown to also reduce first episode of radiologically defined pneumonia among HIV negative children by 25% (CI 4 – 41) (80). Like the Northern California trial, the Filipino trial also showed a strong age dependent vaccine effect. Efficacy against radiologically defined pneumonia was 34% among children in the first year of life and only 2.7% among those aged 12 – 23 months. There was no significant vaccine efficacy against clinically diagnosed pneumonia. In The Gambia, the efficacy of PCV9 against first episode of radiologically defined pneumonia was 37% (CI 27 – 45) (81). In addition, PCV9 was also found to be 7% efficacious against clinically diagnosed pneumonia (CI 1 – 12).

With pneumonia deaths contributing to over 19% of all under-5 child deaths worldwide(91), demonstration of efficacy of PCV7 against pneumonia is an important public health milestone. Poor protection against clinical pneumonia could have been due to lack of standardisation of diagnostic criteria which would introduce misclassification. On the other hand, a more standardised definition employing radiological features showed an efficacy of 20 – 37 % between the trials.

Part Two: Systematic review of the evidence for Replacement carriage in children vaccinated with pneumococcal conjugate vaccine

Background

Infants and children younger than 2 years of age bear the greatest burden of pneumococcal diseases the world over. Pneumococcal polysaccharide vaccines which are effective against pneumococcal disease in older children and adults are ineffective in children younger than 2 years because these children respond poorly to T cell independent antigens such as pneumococcal polysaccharides. However, they are able to mount a brisk immune response to T-cell dependent antigens (94). The pneumococcal polysaccharide –protein conjugate vaccines which elicit a T-cell dependent immune response have been shown to reduce nasopharyngeal carriage of pneumococcal serotypes included in the vaccine in different populations.

Prior to the development of PCV7, a safety and immunogenicity trial of a 5 valent pneumococcal conjugate vaccine (PCV5) containing glycoprotein of pneumococcal serotypes 6B, 14, 18C, 19F and 23F was conducted in 1993 among infants in The Gambia(95). Infants in the vaccine group were vaccinated with PCV5 at ages 2, 3 and 4 months and revaccinated with the 23-valent pneumococcal polysaccharide vaccine at age 18 months. Six months after revaccination, these infants were compared with 160 matched controls who did not receive any pneumococcal vaccination. Though there was a significant reduction in the carriage of PCV5 serotypes in the vaccinees compared to the

controls, there was also a significant increase in the carriage of non-PCV5 serotypes among vaccinees compared to the controls, and the overall pneumococcal carriage rate among the vaccinees remained essentially the same. This was the first time this phenomenon, now termed 'Replacement' was demonstrated. Replacement carriage occurs when serotypes not included in a conjugate vaccine colonise the nasopharynx and 'replace' the vaccine serotypes whose colonisation has been prevented by the conjugate vaccine(96).

The introduction and widespread use of PCV7 in the US was followed by phenomenal success; not only did invasive disease almost disappear in the vaccinated population of children but the incidence of invasive pneumococcal disease also markedly reduced in their elderly and younger adult contacts due to reduction of transmission of pneumococci from the vaccinated children (16). However, reports of large increases in incidence of IPD due to NVT pneumococci have followed this success story not only in the US(19, 85) but also in Europe (72, 87) and among Aboriginal children in Australia(97). The changes in pattern of IPD following long term use of PCV7 mirror changes in pattern of carriage in the paediatric population. Replacement carriage with non-vaccine serotypes was observed in most randomised controlled trials of PCV7 among children (65, 98-101).

Reports of replacement with NVT pneumococci in carriage and invasive disease have more commonly come from vulnerable and high risk populations such as American Indian and Alaskan children, and Australian Aboriginal children. These populations share similar epidemiological characteristics of pneumococcal carriage and invasive disease with the developing countries of Africa such as The Gambia. The PCV7 was recently introduced in The Gambia amidst fears that replacement with NVT pneumococci in carriage and invasive disease may erode the benefits of vaccination. The need to establish the extent to which pneumococcal carriage replacement has resulted from PCV vaccination as baseline information for surveillance in The Gambia forms the main reason for this systematic review.

Objectives

To determine the effect of vaccination of children with the pneumococcal conjugate vaccine on nasopharyngeal pneumococcal carriage and to determine if replacement has occurred in the vaccine recipients compared to the non-recipients.

Selection Criteria for inclusion of studies in the review

All cluster or individual randomised controlled studies comparing any of 7-valent pneumococcal conjugate vaccine (PCV7) and 9-valent pneumococcal conjugate vaccine (PCV9) with placebo, or with no treatment, given to children up to the age of 5 years, with

1. Pneumococcal carriage as primary outcome measure
2. Invasive pneumococcal disease and pneumococcal carriage outcomes
3. Pneumococcal carriage determined by isolation of SP from the nasopharynx according to WHO standard criteria(102)
4. At least six months of follow-up after completion of the primary series of vaccination

Excluded from the review were

1. Non-randomised trials
2. Trials with less than six months of follow-up after completion of primary series of vaccination
3. Trials with pneumonia, otitis and other outcomes but without pneumococcal carriage outcomes
4. Trials recruiting children more than 5 years of age
5. Safety and immunogenicity trials without carriage outcome measure

Search strategy

The following databases were searched to identify reports of randomised trials of the pneumococcal conjugate vaccines through the Medline search engine: Cochrane Central Register of Controlled Trials 2009, Database of Abstracts of Reviews of effects, EMBASE database 1947 – present and Ovid MEDLINE (R) 1950 – present. The

following medical subject headings, terms and text words were used in varied combinations to achieve maximum sensitivity: “pneumococcal conjugate vaccine”, “pneumococcal conjugate vaccination”, “nasopharyngeal carriage”, “nasopharyngeal colonisation”, “efficacy”, “effectiveness”, “randomised controlled trial”, “invasive pneumococcal disease”, “children” and “infants”. In addition, a manual search of the reference section of relevant trials and review articles was done. Details of the search strategy are shown separately.

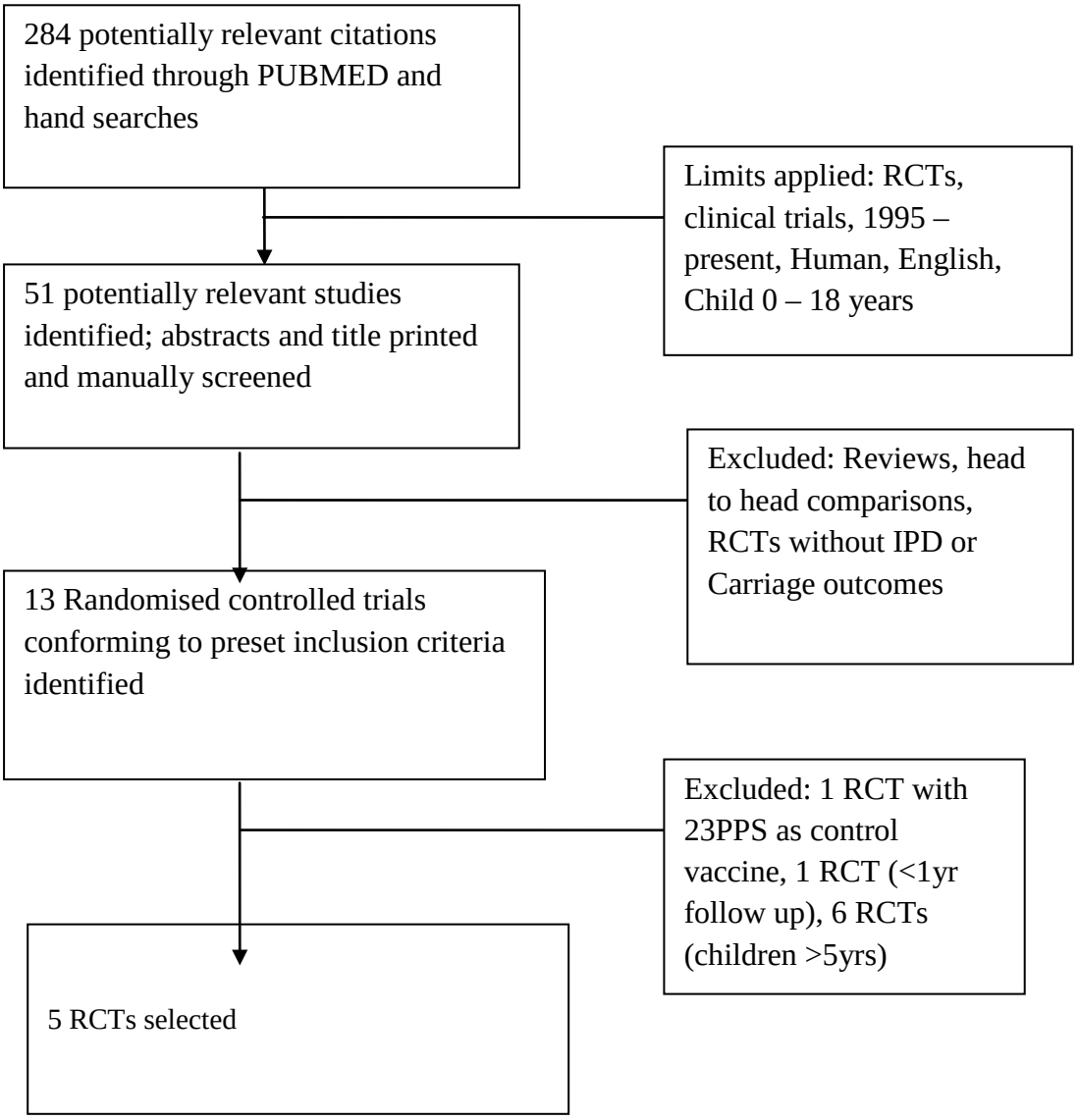


Figure 1: Flow diagram of selection of studies included in the review

Note: RCT – randomised controlled trial, IPD – invasive pneumococcal disease

Methods

The identified trials were assessed for eligibility using the above pre-determined criteria. Since the authors' names, institution and the source of publication were known, the trials were not assessed blind.

Data collection and Analysis: Data were extracted from the results and other relevant sections of the individual papers. The following data were extracted: total number of children involved in the trial, vaccination schedules, length of follow-up period, carriage rates of vaccine- and non-vaccine type pneumococci in treatment and control groups and the commonest non-vaccine serotypes isolated from recipients of PCV.

Description of Studies

Five reports from four studies met the pre-set inclusion criteria (table 1). Altogether, these studies involved 3, 082 subjects (65, 69, 98-100). The studies were conducted between late 1990s and mid 2000s and reported from 1999 to 2009. Three of these studies used PCV7 as study vaccine while 2 studies used PCV9. The Meningococcal C conjugate vaccine (MnCC) was used as control vaccine in 3 studies; an identical appearing placebo (actual ingredient not stated) was used in one study while in one study no vaccine was given to the controls. Children were recruited into the studies from birth to approximately three years of age. Two of the studies were conducted in the USA while one study was conducted in each of Israel, South Africa and the Netherlands. The primary vaccination series ranged from two doses in one study to three doses in four of the studies. The PCV7 was given in two of the studies as booster vaccination after the primary series of vaccination while no boosters were given in the other three studies. The period of follow up after primary vaccination series ranged from 6 months to 2 years. One study (van Gils et al) had two treatment groups; one group which had 2 doses of PCV7 and another given a third dose of PCV7 7 months after the second dose.

Table 1: Characteristics of studies included in the review

Author	Country	Enrolment age	Study vaccine	Vaccination schedule	Follow up
Mbelle et al	South Africa	6 wk	PCV9	6,10,14 weeks; no booster	6 Mo
Millar et al	U S A	0-6yrs	PCV7	2,4,6Mo,booster 12-15Mo	24 – 48 Mo
Van Gils et al	Netherlands	<12wk	PCV7	2,4Mo OR 2,4,11Mo;no booster	2yrs
O'Brien et al	USA	2yrs	PCV7	2,4,6Mo,booster 12-15Mo	3-14Mo
Dagan et al	Israel	12-35Mo	PCV9	1dose or 2doses 1Mo apart	2 yrs

Main results

1. **Overall pneumococcal carriage**: Overall pneumococcal carriage remained essentially unchanged in vaccinees compared to controls in all the reviewed studies. Mbelle et al(69) found carriage rates of 54% and 61% among vaccinees and controls respectively at 6 months after primary series of PCV9 vaccination (at 9 months of age). A carriage rate of 63.9% and 60.5% respectively were found among vaccinees and controls 27 months after primary series of vaccination with PCV7 in the study by Millar et al (98). In the study by van Gils et al (100), overall carriage rates at age of 12 months were 62% and 67% (RR 0.93[95% CI 0.83 – 1.04]) among 2-dose PCV vaccinees and controls respectively (8 months after primary series of vaccination). O'Brien et al (99) reported no difference in the rate of overall pneumococcal carriage among vaccinees and controls after primary series of vaccination but did not show data while Dagan et al (65) showed only prevaccination data (80% vs. 81% in vaccine recipients compared to controls).

2. **Vaccine serotype carriage**: Nasopharyngeal carriage of pneumococcal serotypes included in the trial vaccine was significantly reduced among vaccinees compared to controls in all the studies.
    a. Mbelle et al: Carriage of vaccine type Pneumococcus at 6 months after primary series of vaccination was 18 % (43 of 242) of vaccinees compared to 36 % (866 of 239) of controls (P<.001).

b. Millar et al: VT carriage at mean time of 29 months after primary series of vaccination was 10% among vaccinees compared to 17.1% among controls (P=.01; OR 0.55[95%CI 0.36 – 0.85]).
c. Van Gils et al: VT carriage was determined at two time points; at age 12 months and 24 months. At 12 months of age, carriage rates were 25% among 2-dose vaccinees (group 1), 20% among 3-dose vaccines (group 2) and 38% among controls. Reduction in VT carriage was significant when both groups were individually compared to controls (RR 0.64 [95%CI 0.51 – 0.81] for group 1 versus controls, and RR 0.52 [0.41 – 0.68] for group 2 versus controls). At age 24 months, the difference was even more significant; carriage rates were 15% among group 1 vaccinees, 14% among group 2 vaccinees and 36% among controls. The RR of VT carriage were 0.42 (95%CI 0.31 – 0.56) for group 1 compared to controls and 0.40 (95%CI 0.29 – 0.54) for group 2 compared to controls.
d. O'Brien et al: VT carriage was significantly lower among vaccinees compared to controls 1 month after primary series of vaccination (OR 0.40 [95%CI 0.23 – 0.67]) and just prior to booster vaccination (6 – 9 months) after primary series of vaccination (OR 0.51 [95%CI0.34 – 0.78]). However, by 6 months after booster vaccination (12 – 15 months after primary series), no significant difference was found in the rate of carriage among vaccinees and controls (data not documented).
e. Dagan et al did not document carriage rates among study subjects after vaccination but documents that VT acquisition was much less among vaccinees compared to controls.

The commonest vaccine serotypes isolated in the studies include 6B, 19F and 23F (Mbelle et al, Millar et al and van Gils et al). In the study by Dagan et al, the most commonly isolated serotypes were 14, 19F and 23F.

In general, VT carriage was significantly lower among PCV recipients compared to their controls and this difference was already present in the first month after primary series of vaccination in the study by O'Brien et al.

3. **Non-vaccine serotype carriage**: All the selected studies also demonstrated significantly higher carriage rates of NVT among vaccinees compared to controls. By

the age of 9 months (6 months after primary series), NVT carriage was significantly higher among vaccinees compared to controls in the study by Mbelle et al (36% [87 of 239] vs. 25% [59 of 239]; P =.007) respectively. Overall NVT carriage was also significantly higher in the studies by Millar et al among vaccinees (39.3% [95%CI 34.8 – 43.9] compared to controls (29.9% [24.6 – 35.6]); van Gils et al (RR 1.32 [1.06 – 1.63] for group 1 vaccinees vs. controls and RR 1.43 [95%CI 1.16 – 1.76] for group 2 vaccinees compared to controls at age 24 months and in O'Brien et al (OR 1.67 [95%CI 1.02 – 2.78] at 6 months after booster vaccination. Dagan et al measured NVT acquisition rates among study participants and documented 479 acquisitions among 132 vaccinees and 403 acquisitions among 130 controls (P=0.013). NVT did not include serotype 6A in the study by Dagan et al because serotype 6A was found to decrease in vaccinees in response to vaccination with serotype 6B antigen.

Overall, NVT carriage showed a significant increase across the studies and this difference was seen as early as 6 months and up to 18 months after primary series of vaccination.

4. **Commonest non-vaccine serotypes**: Pneumococcal serotypes 15, 6A and 19A were the three most commonly isolated NVT among vaccinees in the study by Mbelle et al. Serotypes 6A, 35B and 22F were the commonest NVT among vaccinees in the study by Millar et al while the overall commonest isolated NVT were 6A, 19A, 11A and 23B in van Gils et al study.

Summary of the main findings

These randomised controlled trials of the pneumococcal conjugate vaccines have shown statistically significant reduction in the prevalence of vaccine serotype carriage in recipients of PCV as well as nasopharyngeal replacement carriage with pneumococcal serotypes not contained in the study vaccine. These effects were observed after vaccination with two or more doses of PCV. In the studies where risks of NVT carriage were compared, vaccinated children were at least 1.3 times more likely than the controls to carry NVT pneumococci after PCV vaccination. Replacement carriage was observed as early as 6 months after primary series of vaccination as well as 18 months afterwards. Replacement carriage was therefore a consistent finding in children of diverse backgrounds and from different epidemiological settings following PCV vaccination.

Reduction in vaccine type carriage seemed to have resulted from reductions in the acquisition of new pneumococci and not elimination of the already carried pneumococci as Dagan et al showed(65). The duration of this reduction remains to be clearly understood. In the study by O'Brien et al(99), reduced carriage of vaccine serotypes was no longer evident one year after primary series of vaccination while van Gils et al found persisting lower carriage of vaccine serotypes among vaccinees 29 months after primary series vaccination.

Findings from some important excluded studies: Two studies with otitis media as primary outcome which also investigated nasopharyngeal carriage were excluded on account of the age of the participants (>5 years). Both studies had findings consistent with the reviewed studies which further confirm the development of replacement carriage in recipients of pneumococcal conjugate vaccine. Van Kempen et al investigated the impact of PCV vaccination on recurrent episodes of otitis media and VT and NVT carriage in a randomised controlled trial. Subjects were recruited at age 12 – 24 months and randomised to receive either two doses of PCV7 1 month apart and a booster vaccination with the 23-valent pneumococcal polysaccharide vaccine 6 months later, or hepatitis A control vaccine. Though no difference in NVT carriage was seen 7 months after primary series of vaccination, a statistically significant difference was observed at 14 months post-vaccination between vaccinees and controls (37% vs. 3%; P=.002). In a similar study investigating pneumococcal carriage in children with a history of recurrent otitis media, Veenhoven at al also demonstrated significantly higher carriage of NVT among PCV recipients compared to controls (73% vs. 54%; P=.001). This difference was observed after booster vaccination which was given 6 months after primary series of vaccination. These studies therefore strongly corroborate the findings in the selected studies.

Discussion of the evidence

1. Role of Chance, Bias and Confounding

The finding of statistically significant evidence for replacement carriage in separate randomised controlled trials with the same outcome measure strongly speaks against chance as possible explanation for the findings. All the confidence intervals of the relative risks and odds ratios measured in the studies were narrow and on the same side of the null

value, ruling out chance. Where proportions were compared, the P values were all well below the stated alpha level (0.05).

Prior heavy use of antibiotics could confound the finding of reduced carriage in vaccinees compared to controls. One study(100) specifically compared antibiotic use one month before vaccination among controls and vaccinees and found no difference. Furthermore, there was no difference in various risk factors for carriage measured in the study between vaccinees and control such as number of siblings, day care attendance and prevaccination pneumococcal carriage rates. Therefore known confounding cannot account for the findings. The randomised controlled nature of the studies would also reasonably distribute any possible unknown confounders between the study arms thereby making unknown confounding an unlikely explanation of the findings.

2. Causality

Though the consistent finding of replacement in different populations with differing pneumococcal epidemiology strongly suggests that replacement carriage in these studies was a result of PCV vaccination, a recent report from Korea(103) indicating that serotype 19A was already on the increase among children before introduction of PCV into the population makes it important to establish causality in the relationship between PCV vaccination and replacement carriage. In addition, Dagan et al recently implicated antibiotic usage pattern as an important possible causal factor in the development of replacement carriage in a particular population(104). However, these reports have centred mainly on serotype 19A, a NVT pneumococci commonly carried in the nasopharynx even before advent of the PCV(105) but not on other NVT pneumococci which have become important carriage and invasive disease isolates only after the introduction of PCV7 such as 15 and 33 (87, 101).There was a strong temporal relationship in these reviewed studies between replacement carriage and PCV vaccination. Finally, replacement of certain commonly carried microorganisms by the uncommonly carried ones in response to pressures as from vaccines or antibiotics is a biologically plausible phenomenon which has been demonstrated for pneumococci and speculated for other upper respiratory tract organisms such as Haemophilus influenza type b and staphylococcus aureus(106).

Summary

In summary, well conducted randomised controlled trials with primary endpoint of pneumococcal carriage have demonstrated that following vaccination of children with PCV: VT carriage is reduced among PCV recipients and this could begin as early as after the first dose; overall pneumococcal carriage remains essentially unchanged and NVT pneumococcal carriage is significantly increased among PCV recipients compared to controls. Serotypes 6A and 19A are by far the commonest replacing serotypes.

Mean duration of carriage after acquisition of SP did not differ between PCV7 recipients and controls in two studies where it was investigated (65, 99). This was true for both VT and NVT pneumococci, suggesting that the reduction in VT carriage observed in these studies occurred due mainly to prevention of new acquisitions.

Reports of invasive disease due to these replacement serotypes add further urgency to the need for increased vigilance and surveillance as PCV of limited valency is introduced across various high-risk populations across the world including The Gambia.

Chapter3: Methods

3.1 Study Design – This study was one component of a large single blind cluster randomised controlled trial of the 7 valent pneumococcal conjugate vaccine. The control vaccine was the meningococcal C conjugate vaccine which protects against meningococcal meningitis and has no effect on nasopharyngeal carriage of Pneumococcus.

3.2 Brief Description of The Gambia – The Gambia is a small country on the West African coast and derives its name from the river Gambia along which it is situated. The country is entirely surrounded by Senegal except for a 60 km border along the Atlantic Ocean. The total population in 2010 is estimated to be about 1.7 million people. The population is made up of several ethnic groups including Mandinka, Jola, Fula, Wolof, Sarahule and Serere. The Gambia is divided into five administrative regions, namely the Central River, Upper River, Lower River, North bank and Western.

3.3 Study Population – An area ~30 x 20 km in dimension and containing 55 villages in the Western division of The Gambia was selected for the study. This area is in the rural area of the country and is representative of other rural areas of The Gambia while being accessible to the coastal town of Fajara where the main site of the MRC is situated (Figure 2). This area is also located away from the area where a trial of the 9 valent pneumococcal conjugate vaccine was conducted. Twenty one villages were selected from the 55 in the area, because they had a population of 100 – 500 inhabitants, and were not on the main highway that transects the area from east to west. In addition, these villages were separated from each other by a distance of at least 3 km and this helped to limit the effect of population movements between them. The population comprised mainly subsistence farmers who grow millet and maize for home consumption and groundnuts as a cash crop. The majority of the people belonged to the Jola and Mandinka ethnic groups.

Village reporters were contracted in each village to report any pregnancies and births to the field workers. The parents of the newborn child were then approached for collection of informed consent. Newborn babies of consenting parents were then enrolled into the study.

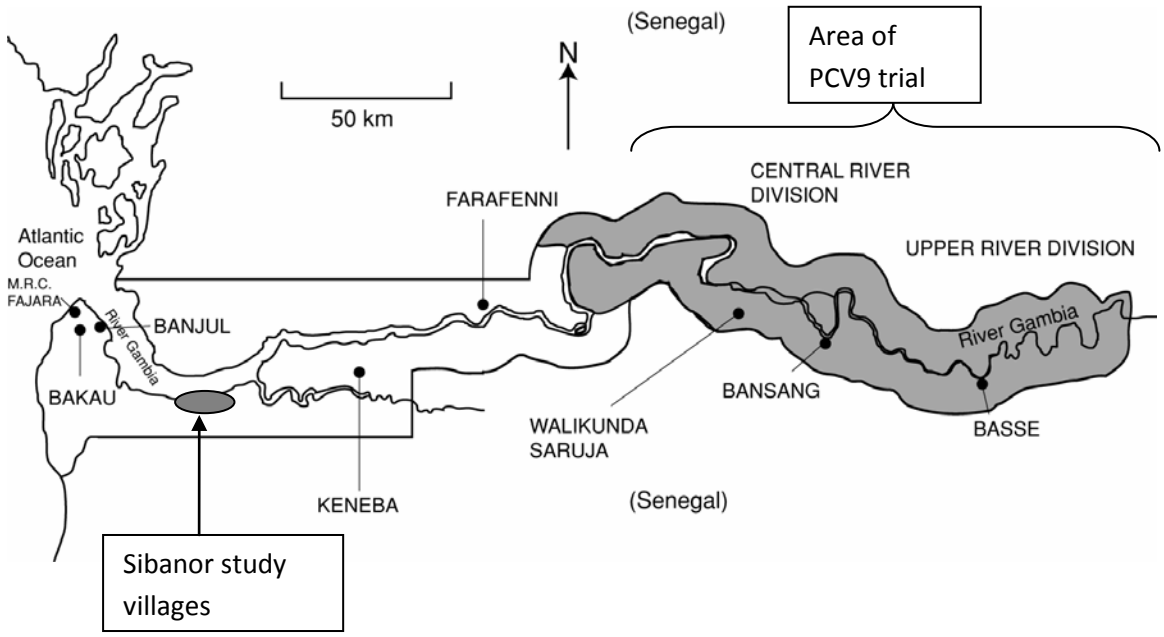


Figure 2: Map of The Gambia showing the locations of the study area, the MRC laboratory and the area where a previous trial of the 9-valent pneumococcal conjugate vaccine was conducted

3.5 Approval for the study

The study protocol was approved by the Scientific Co-ordinating Committee (SCC) of the MRC, Gambia and the joint Gambian government / MRC Ethics Committee. To obtain the approval of the study communities, trained field workers and the study investigators visited the 21 selected villages to set up introductory meetings with the village heads, called 'Alkalos' in the local languages. On agreed dates, follow up meetings were held from village to village with the Alkalos and elders to explain the study objectives in their local languages. During these meetings, the purpose of the study, the nature of the sampling process and the importance of the study were explained in detail in the local languages. Ample time was give for questions and for clarification of any concerns raised at these meetings. General approval was then obtained from the Alkalos and the elders for the study and for a meeting with the entire village to be convened. Village meetings were then organised in each of the villages where the study objectives were once again explained to the entire village population and questions and concerns raised by the people addressed. A general approval was then obtained in the form of an oral affirmation. For each compound and household, approval was sought from the heads prior to enrolment of the members of the compound or household. Approval for the study granted by these heads then paved the way for the recruitment of individual members of the community, with written informed consent including from primary caregivers on behalf of their children.

3.6 Mapping and census of the study villages

After the consenting process, mapping of the compounds and households was performed. The study villages were arranged in alphabetical order and coded from A to U. Compounds and Households in each village were assigned identification numbers in an orderly fashion starting from the centre of the village outwards. The centre of the village was taken as the focal point where the villagers would normally gather for meetings called 'Bantaba' in all local Gambian languages. Compounds were first grouped into Blocks, where a block represents all compounds within a distinct area which are separated from the other compound by a road or a clear boundary. Blocks were numbered starting from the Bantaba northwards

until all households have been covered. The first block is numbered '01' and the second '02' in that order. Within each block, compounds were also given numbers beginning from '01' until all compounds within the block had been numbered. Within each compound, households were also assigned numbers beginning from '01'. Compound number was then printed with indelible ink on a conspicuous part of the front wall of each house in the compound as allowed by the head of the household. Finally, individual members of the household were enumerated and assigned numbers starting from the household head, usually the father. The census list was stored in the study database and updated yearly throughout the duration of the study. A combination of the village code and the block, compound, household and individuals' numbers then made up the identification number of each participant. For instance, a participant with the number A 0101 0101 would be the household head in the first household within the first compound of the first block of village A.

3.7 Randomisation

Computer generated allocation schemes which took into consideration the population of the villages were used to randomise the study villages into 11 villages for vaccination with the 7 valent pneumococcal conjugate vaccine and 10 villages for vaccination with the control vaccine (Meningococcal C conjugate vaccine). The allocation scheme was such that the difference in total population size between the two sets of villages did not exceed 10 percent.

3.8 Sample size determination

A previous pre-vaccination baseline survey showed that the prevalence of carriage of pneumococci of the 7 valent pneumococcal conjugate vaccine was about 40% in infants up to the age of 2.5 months and about 30% in those aged 30 months or more(23). The coefficient of variation between villages was 0.3. Based on these and assuming that the study would recruit at least 200 newborns , the trial would have about 90% power with a type1 error of 5% to detect a 50% reduction in the colonisation of infants with pneumococci of vaccine type before the age of two months.

3.8 Community Vaccination

The vaccination was carried out village by village on days when the villagers would normally not go to farms or the local market. All participants above 30 months of age in the vaccine villages were vaccinated with the one dose of 7PCV while those in the control villages were vaccinated with one dose of the meningococcal C conjugate vaccine (MnCC). All children between two months of age up to the age of 30 months residing in the study villages were given 7PCV regardless of the vaccine given in their individual villages. This was because the pneumococcal conjugate vaccine has already been shown to be efficacious against invasive pneumococcal disease among infants in The Gambia (81) and elsewhere (14). Withholding the vaccine from the infants was therefore considered unethical. Children aged between 2 and 11 months of age received 3 doses of the vaccine given approximately one month apart. Those aged between 12 and 30 months received two doses also given one month apart. All the children born during the study were given three doses of the vaccine at the ages of 2, 3 and 4 months. In both intervention and control villages, mop-up vaccinations were conducted from time to time to ensure high rate of vaccination in the villages.

3.9 Adverse events monitoring

Infants were vaccinated with the 7-valent pneumococcal conjugate vaccine after the final nasopharyngeal sample was collected (see section 3.11) in the 8^{th} week of life. Field workers went back to the villages the first two days after vaccination to ascertain any adverse events according to approved standard operating procedures. Mothers and care givers of the children were also encouraged to report any adverse events to village reporters who were contracted for the study or to the village health centre. Any reported event was followed up with clinical examination, treatment or referral as the case may be. Adverse events forms were completed and kept in the trial master file for subsequent reporting to the appropriate authorities and follow up of the infant.

3.10 Consenting for the newborn study

Approval of the study by the village heads was followed by formally reading out and explaining the study information in the local languages and allowing for further questions and clarifications. The village heads would then either append a signature or make a thumb print on the signature section of the consent form in duplicates to signify consent on behalf of the community. A copy of the consent form was left with the village head while a copy was kept

by the study team. The consenting process was then extended to the various compound heads and the household heads in the same manner. Where a household head failed to give consent, the household was excluded from the study. We contracted village reporters throughout the period of trial who reported any births in the villages to the field workers as soon as possible. Upon the report of the birth of any child, field workers visited the household to obtain consent from the caregiver. This was usually the mother or the grandmother, and in few occasions the fathers and other adult members of the household. The study information was read out and interpreted in the local language by trained field workers. The information included the nature and frequency of nasopharyngeal sampling. Where the care-giver consented, she appended a signature or a thumb print on the consent form to allow administration of the risk factor questionnaire and subsequent collection of nasopharyngeal sample from the child. On subsequent sampling days, the consent of the care-giver was sought but there was no requirement for the consent form to be signed.

3.11 Nasopharyngeal swabbing and transport

Nasopharyngeal swabs (NPS) were collected from the participating children as soon after birth as possible and then weekly until the age of 8 weeks. The baby was first clinically examined by the field nurse or the research clinician. The weight and length were obtained and documented. After placing on the laps or any soft surface, the head was slightly tilted backwards and the calcium alginate tipped swab gently advanced into one nostril, parallel to the floor of the nasopharynx until about half of the length had gone in or a gentle resistance was felt. The swab was left in position for about five seconds to saturate the tip and then gently removed. It was then immediately inserted into a vial containing 1ml of skim milk-tryptone-glucose-glycerol (STGG) transport medium .A pair of scissors wiped with 70% alcohol was used to cut off the excess wire handle, leaving the tip in the transport medium. The cap was then tightened and the vial labelled and placed in an ice box. The vials were labelled with unique identifiers which showed no information regarding the vaccine received or the name of the child. In accordance with the WHO protocol for the evaluation of pneumococcal carriage (102), the swabs were transported within 8 hours of collection to the main MRC laboratory in Fajara which is about 90 kilometres away from the field. Prior to departing the field sites, the presence of a laboratory technician waiting to receive the samples in Fajara was confirmed with a phone call. On public holidays, a designated laboratory technician was assigned by the study team to await, collect and process the NPS

samples according to a standard operating procedure. NPS was collected weekly from each infant till the age of 2 months. A risk factor questionnaire was administered to the mother or care-giver during each sampling episode. The risk factors covered included ethnic group of the child, breastfeeding, use of antibiotics, movements out of the study village and whether there was a history of chest infections or ear discharge in the week preceding sampling. Chest infections were taken as reports of cough, difficulty with breathing, fast breathing or any combination of these for which the parent or caregiver sought medical treatment for the baby.

3.12 Laboratory processing of NPS

Immediately upon reception of the NPS specimens in the laboratory, they were vortexed for 10 – 20 seconds to disperse organisms from the swab tip and then stored at -70^{o}C until they were tested in batches. Inocula of thawed STGG media were plated onto gentamicin blood agar for the identification of *Streptococcus Pneumoniae* and incubated overnight at 37^{o}C in 5% carbon dioxide (CO2). Growth of alpha-haemolytic colonies was then documented semi-quantitatively as follows; 4+ if >10 colonies were in quadrant 4, 3+ if <10 colonies were in quadrant 4 but > 10 colonies in quadrant 3, 2+ if < 10 colonies were in quadrant 3 and > 10 colonies in quadrant 2 and 1+ if there were < 10 colonies in quadrant 1. From this primary agar plate, 2 presumptive pneumococcal colonies were picked and streaked out onto another blood agar plate. An optochin disc was placed in the centre of each streak and the agar plate incubated overnight at 37^{o}C in 5% CO2. A zone of inhibition greater than 14 mm was regarded as susceptibility, 7 – 13 as indeterminate and less than 7mm as resistant to optochin. Isolates with indeterminate susceptibility to optochin were tested for bile solubility. All optochin susceptible and bile soluble isolates were regarded as pneumococci while the optochin resistant isolates were regarded as species other than pneumococci. Serotyping of the pneumococcal isolates was done using the latex agglutination technique. Equivocal results were confirmed by the Quellung reaction. The antibiotic sensitivity patterns of selected isolates were determined using the disk-diffusion method.

3.13 Data management and Statistical analysis - All data were double entered into a Microsoft Access database specifically designed for the project. Datasets from the study were grouped into two: one for the risk factor data and the other for the laboratory results (pneumococcal isolates and the antibiotic sensitivity pattern).The datasets were checked for errors using a computer programme. Incongruence and data duplication were corrected.

Where required, references were made to the hard copies of the risk factor questionnaire and laboratory forms to sort out inconsistencies in the entered data. The individual cleaned risk factor and NPS results datasets were then merged using commands from the Stata statistical package version 11 (Stata Corporation, TX, USA) which was also used for the analysis of the data. A separate census data which contains demographic information of all subjects in the 21 villages was kept and updated yearly. Household size data and data on number of individuals in the under 5 years age group were extracted from the census data and added on to the Newborn data using a "merge m:1" stata command. Data for fifteen infants who were not included in the Census data because they were born after the last update period, were manually searched out using the subject id identifier which contains unique identifiers of each child's village, compound and household.

Kaplan-Meier failure functions were used for the study of time to first acquisition of pneumococci. The mean survival times were calculated by extending the tail of the survival curves to reach the x-axis assuming an exponential distribution if the longest follow-up time was a censored observation. Acquisition rates were compared using Cox regression model with robust standard errors adjusting for clustering at village level and controlling for various risk factors for carriage including sex, household size, ethnic group and number of children <5 years in the household. Comparison of acquisition rates was by hazard ratios with 95% confidence intervals. For the regression model, the four level variable 'ethnic group' was reduced to three levels comprising 'Jola' 'Mandinka' and 'others' because of the small number of infants from these ethnic groups. Differences in carriage prevalence of vaccine groups and individual pneumococcal serotypes were tested by chi square and Fischer exact tests. A p-value of <0.05 was considered statistically significant. First acquisition of carriage was defined as the isolation of any pneumococcus for the first time in the individual infant. Prevalence of carriage was defined as the number of infants carrying pneumococcus at any point in time divided by the total number of infants studied and this ratio is expressed as a percentage.

Vaccine serotypes were defined as the pneumococcal serotypes contained in the 7 valent pneumococcal conjugate vaccine (PCV7). These are serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Vaccine-associated serotypes were defined as isolates of the serogroups 6, 9, 19 and 23 which are not contained in PCV7. These include serotypes 6A, 9A, 9F, 9L, 9N, 19A, 19B,

23A and 23B. Serotypes 23 and 19 were also included in this group because they are not vaccine serotypes but are serogroups associated with PCV7. Non vaccine serotypes were defined as all other serotypes except the non-typeable pneumococci. The non-typeable pneumococci are a mixed group of pneumococci which lack the characteristic pneumococcal polysaccharide capsule and are therefore unable to be typed using standard methods employed in this study. They are reported as a group in this study.

Chapter 4: Results

4.1 Characteristics of study villages

The intervention and control study villages were similar in number of inhabitants at the start of trial, age and sex distribution, number of compounds and number of households per compound and distance from the main road dividing the study area into east and west (Table 2). However, the ethnic distribution of the villages was different ($p<0.001$), with the Mandinka ethnic group over-represented in intervention villages. The majority of the inhabitants of the study villages were of Jola ethnic group (92.4% vs. 58.7% in control and intervention villages respectively) and the predominant occupation was subsistence farming (30.2% vs. 32.9% of adults in control and intervention villages respectively). Older children and adults in the age group 15 - <50 years were the largest group in both control (45.7%) and intervention (41.7%) villages and the median ages were 18 and 16 years respectively.

Table 2: Characteristics of the Study villages and their inhabitants

Characteristics	Control villages	Intervention villages
Village populations at start of trial		
Total	2765	2676
Median (Range)	198 (140 – 528)	251 (69 – 634)
Age distribution (at start of trial)		
<30 mo (%)	6.2	6.8
30mo – 59mo (%)	7.4	8.3
5y - <15yrs (%)	28.2	29.9
15yrs - <50 (%)	45.7	41.7
>=50yrs (%)	12.4	13.3
Median	18y	16y
Sex distribution		
Male (%)	49.0	47.8
Female (%)	51.0	52.2
Median number of compounds (Range)	13 (4 – 27)	11 (6 – 37)
Median number of households per compound		
(Range)	1 (1 – 9)	1 (1 – 17)
Ethnic distribution		
Mandinka	0.4	36.2
Jola	92.4	58.7
Fula	4.1	2.3
Other	3.1	2.8
Occupation		
Farmer	30.2	32.9
Unskilled worker / maid	1.3	0.8
Student	41.6	40.5
Other	26.9	25.8
Distance from Sibanor main road		
Median (IQR)	3 (2 – 4)	4 (2 – 6)
Distance from closest health centre		
Median (IQR)	9.5 (5 – 12)	6 (3 – 13)
Number of rooms per household		
Median (IQR)	7 (5 – 9)	6 (4 – 9)

4.2 Characteristics of the infant study population

Three hundred and sixty two (362) new births were reported in the 21 study villages during the study period (July 2006 -June 2008) and parents' consents were obtained before enrolment of infants for participation in the study. Five infants were withdrawn from the study by the parents after consenting to participate and just before first week samples could be taken. Two infants died within the first week of life and 3 infants moved out of the study villages before commencement of NPS collection. Samples were not collected from 6 other infants who were not available through the 8 week sampling period despite being resident in the study villages. Three hundred and forty six infants therefore participated in the study (Table 3).Two infants, one infant who had just one nasopharyngeal swab collected during the study period and one missing from the risk factor database, were also excluded from analysis. This leaves 344 infants for the analysis. Males accounted for 51.6% and 52.4% in control and intervention villages respectively. The majority of the infants were of Jola ethnic group (89.7% vs. 56.1%) in control and intervention villages respectively. The infants were of similar birth weight and were predominantly exclusively breastfed in the first 8 weeks of life (87.6%% vs. 89.9% in control and intervention villages respectively). Antibiotics were given in the first week of life to 30 of the 344 infants, 15 (9.7%) in control villages and 15 (7.9%) in intervention villages. Similar numbers of infants were reported to have travelled out of the study villages in the week preceding NPS sampling in both study arms but reports of chest infection were more common in intervention villages (2.6% vs. 10.6%; $p<0.001$).

Table 3: Characteristics of Infants in the Newborn study

Characteristics	Control Villages	Intervention Villages
Total number of infants recruited	155	189
Males	80 (51.6)	99 (52.4)
Birth weight, Mean (SD) kg	3.5 (.52)	3.4 (.53)
Exclusive breastfeeding (%)	135 (87.1)	170 (89.9)
Ethnic group		
Jola	139 (89.7)	106 (56.1)
Fula	11 (7.1)	9 (4.8)
Mandinka	1 (0.6)	68 (35.9)
Others*	4 (2.6)	6 (3.2)
Antibiotics	15 (9.7)	15 (7.9)
Ear discharge	9 (5.8)	8 (4.2)
Chest Infection	4 (2.6)	20 (10.6)
Travelled at anytime during study	35 (22.6)	39 (20.6)

Note: * includes Serere, Manjago and Sarahule groups present in very small numbers in the villages where the study took place. Numbers in parenthesis are percentages except as specified for birth weight.

4.3 Nasopharyngeal samples (NPS)

Altogether, 2328 NPS were collected representing a mean of 6.8 samples per infant (median n of samples per infant, 7; range 6 – 8 per infant). One thousand and thirteen (43.5%) of these were from control villages while 1315 (56.5%) were from intervention villages. In control villages, 61.3% of the infants were sampled in the first week of life while in intervention villages, 67.2% of infants were sampled in the first week of life.

Streptococcus pneumoniae (SP) was isolated in 1308 (56.2%) of all samples in the study villages. Of the 1013 samples from control villages, 587 (57.8%) were positive for SP while 721 (54.8%) of 1315 samples from intervention villages were positive for SP. There was no

significant difference in the rate of pneumococcal isolation from samples in control and intervention villages (p=0.122).

4.4 Overall nasopharyngeal pneumococcal carriage

Figure 3 shows the prevalence of pneumococcal carriage by study arm and age. Carriage was about 20% in the first week, doubling by the 4th week to about 50% and increasing further to 70% in control and intervention villages by the 5th and 6th weeks respectively. Overall pneumococcal carriage was 70% in both study arms in the 7th week but reached 80% and 75%in the 8th week in control and intervention villages respectively. Pneumococcal carriage was similar in each of the 8 weeks of follow up in both arms of the study

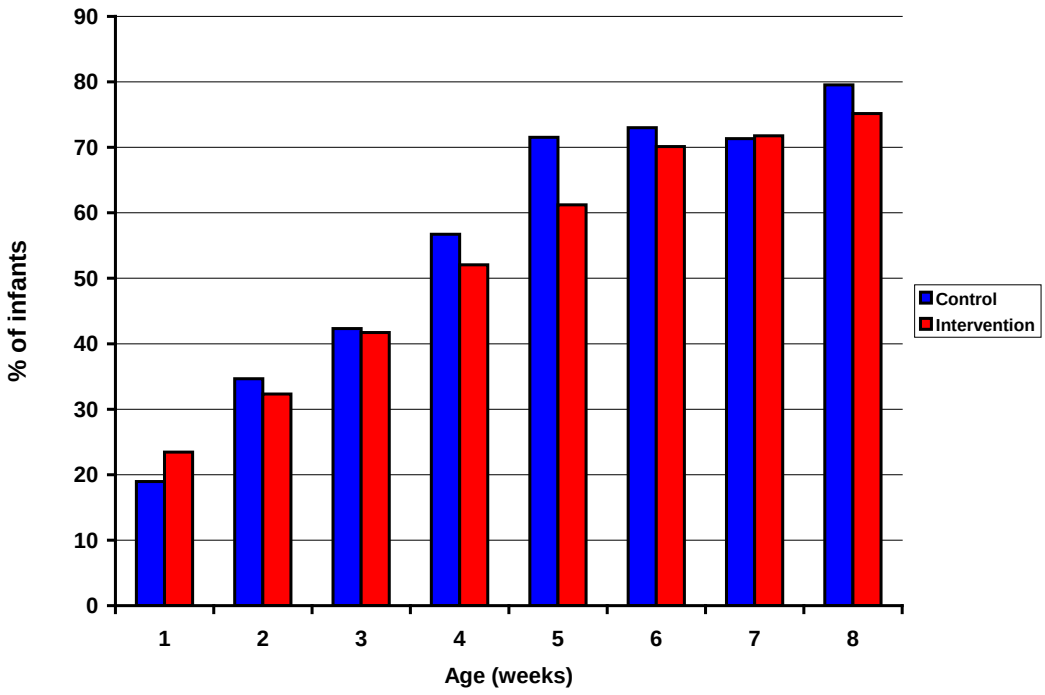
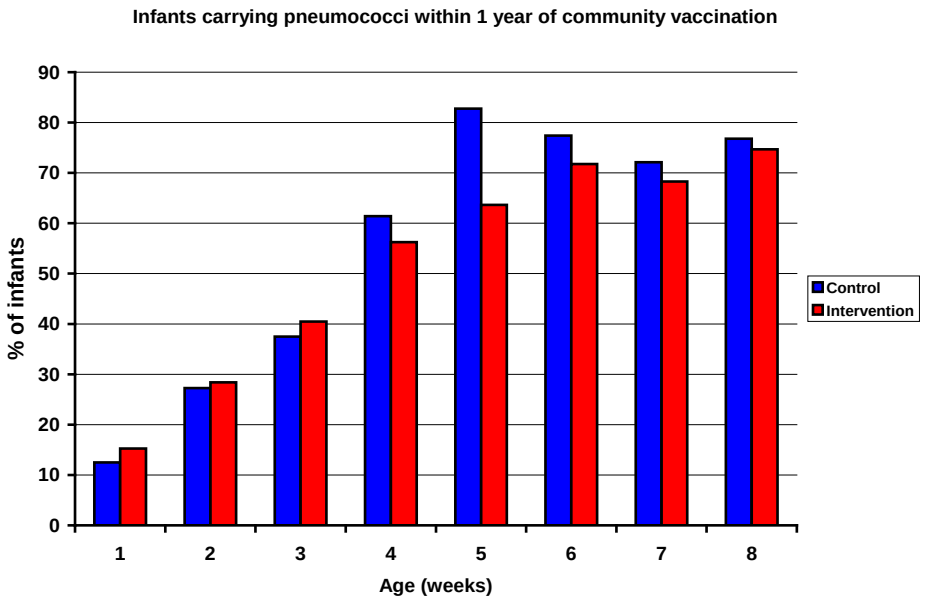


Figure 3: Prevalence of pneumococcal nasopharyngeal carriage in infants by age and study arm

The pattern of carriage remained similar in both study arms when the infants were stratified by the time of birth during the trial (Figure 4 A & B). However, carriage in the first week was lower in the first year of trial compared to second year in both study arms (12.5% vs. 23.6% in control villages and 15.3% vs. 28.9% in intervention villages) but this was not statistically

significant in both study arms. A similar but less pronounced trend was also observed in carriage rates in the second week but overall carriage remained essentially the same in both study arms in the two different birth periods.

A



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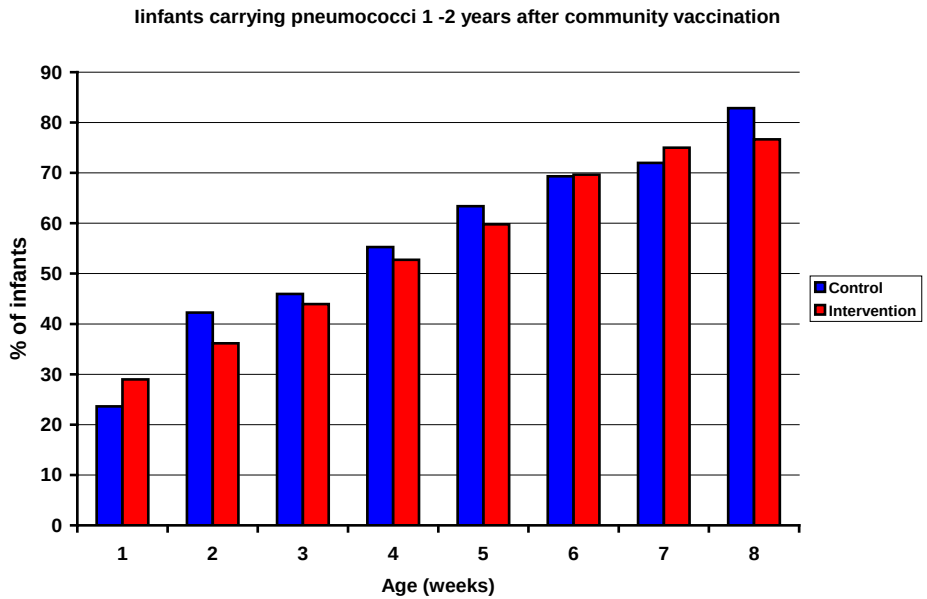


Figure 4: Prevalence of pneumococcal nasopharyngeal carriage in infants by study arm and age stratified by time of birth during trial (A: Infants born within first year of community vaccination; B: Infants born 1 – 2 years after community vaccination).

4.5 Prevalence of Carriage by vaccine group

4.5.1 Overall prevalence

The overall prevalence of carriage of any pneumococcus was similar in both arms of study (table 4). Overall pneumococcal carriage prevalence was 87.7% in control villages and 85.2% in intervention villages. However, the prevalence of vaccine serotype carriage was significantly lower in intervention compared to control villages. Twenty one (11.1%) of the 189 infants in intervention villages and 43 (27.4%) of 155 infants in control villages carried vaccine serotypes at least once during the study ($p<0.001$). The prevalence of carriage of vaccine associated serotypes and non-typeable pneumococci were similar in both arms of study. Prevalence of non-vaccine serotype carriage was higher in intervention compared to control villages but this did not reach statistical significance (68.3% vs. 60.6%, $p=0.141$).

Table 4: Prevalence of pneumococcal carriage by vaccine group and study arm

Vaccine group	Control N (%)	Intervention N (%)	p-value
Vaccine serotypes	43 (27.4)	21 (11.1)	<.001
Vaccine associated serotypes	31 (20.0)	38 (20.1)	0.981
Non-vaccine serotypes	94 (60.6)	129 (68.3)	0.141
Non typeable pneumococci	19 (12.3)	26 (13.8)	0.682
Any pneumococcus	136 (87.7)	161 (85.2)	0.492

Note: N refers to number of infants who carried pneumococci at least once during the study period. Percentages are with reference to total number of infants in the study arm.

4.5.2 Weekly point prevalences

Vaccine serotypes

The proportion of children carrying pneumococcal vaccine serotypes (VT) was consistently lower in the intervention villages compared to the control villages throughout the follow up period (Figures 5, 9 - 13). Prevalence of VT was 7.3% in control villages in the first week, increasing rather sharply to 15.4% in the 3rd week and thereafter maintained a steady increase

to about 19% by the 8th week of life. In intervention villages, prevalence of VT carriage was 2.3% in the 1st week of life and remained under 5% all through the rest of the follow-up period except in the 8th week of life when it rose to 6.5%. Though there was no statistically significant difference in VT prevalence between the study arms in the 1st week of life (P=.073), the difference in VT prevalence achieved statistical significance the rest of the follow-up period (P=.027 week 2, P=.001 in week 3, P<.001 in weeks 4 – 7, P=.002 in week8).

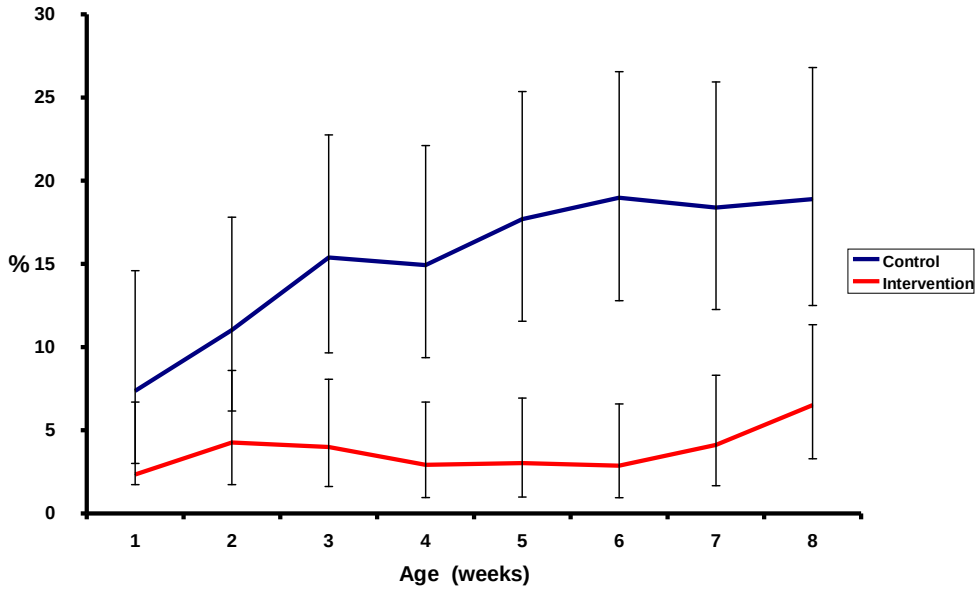


Figure 5: Line graph showing prevalence of vaccine serotypes by study arm and age
Note: Bars indicate 95% confidence intervals

Vaccine-associated serotypes (VAT)

In the first week of life, VAT prevalence was 4.2% in control villages and rose steadily to 9.7% in the 4th week (Figures 6, 9 - 13). By the 8th week of life, VAT carriage prevalence was 16.5%. In intervention villages, VAT prevalence was 3.1% in 1st week of life and also maintained a steady but less pronounced rise to 4.1% in the 4th week of life and finally to 10.1% in the 8th week. Though the proportion of children colonised with VAT in control villages in the 4th week was twice the proportion in intervention villages (9.7% vs. 4.1%), this difference did not reach statistical significance (P=.06).

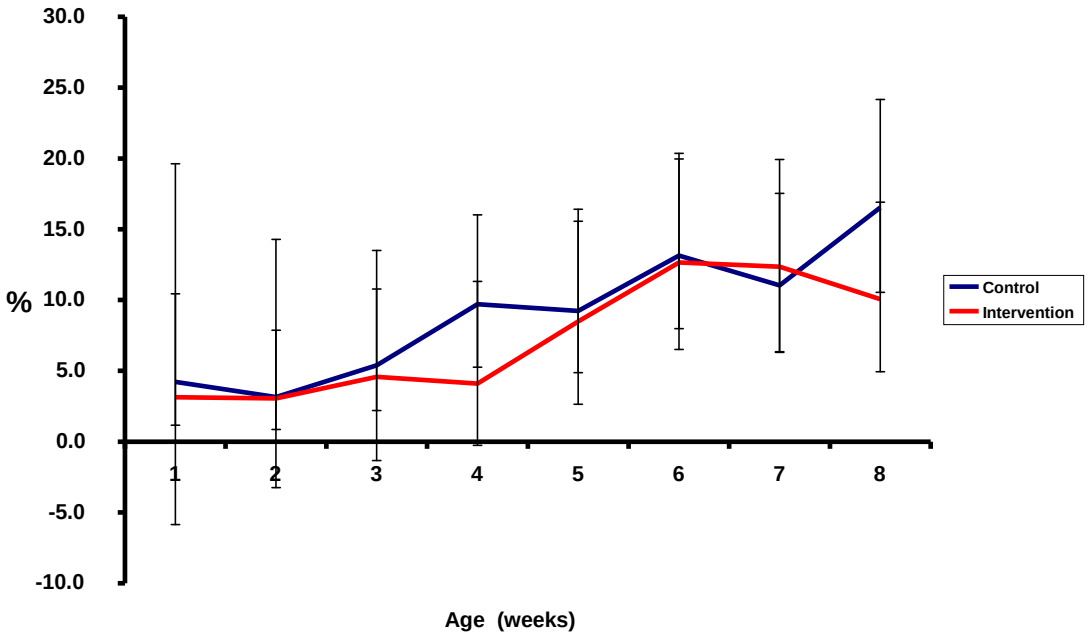
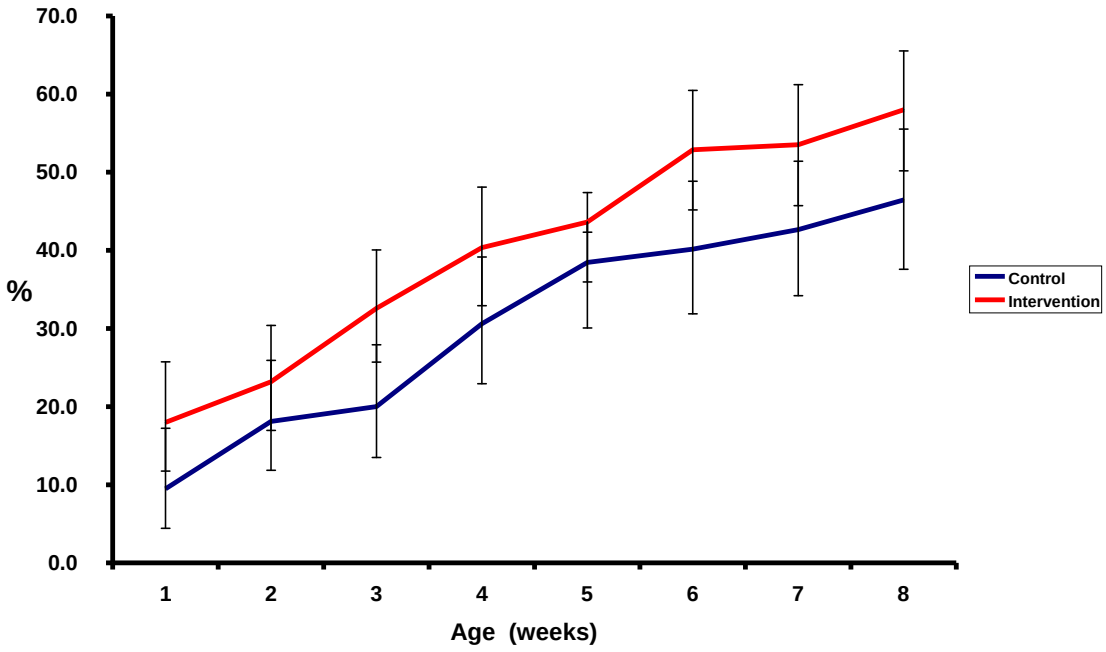


Figure 6: Line graph showing prevalence of vaccine-associated serotypes by study arm and age

Note: Bars indicate 95% confidence intervals

Non-vaccine serotypes (NVT)

A comparison of prevalence of carriage of NVT between the study arms is shown in figures 7, 9 - 13. NVT carriage prevalence was consistently higher in intervention villages through the first 8 weeks of life. NVT carriage prevalence was 18% in the first week. By the 4th week, the prevalence was 40% and by the 8th week, 58% of all infants in intervention villages carried NVT. In control villages, there was also a consistent increase in the prevalence of NVT carriage but the rates were lower compared to those of intervention villages. NVT prevalence was 9.5% in the first week and increased to 30.6% by the 4th week of life. By the 8th week of life, 46.5% of infants carried NVT. The differences in weekly point prevalences between both arms did not reach statistical significance.

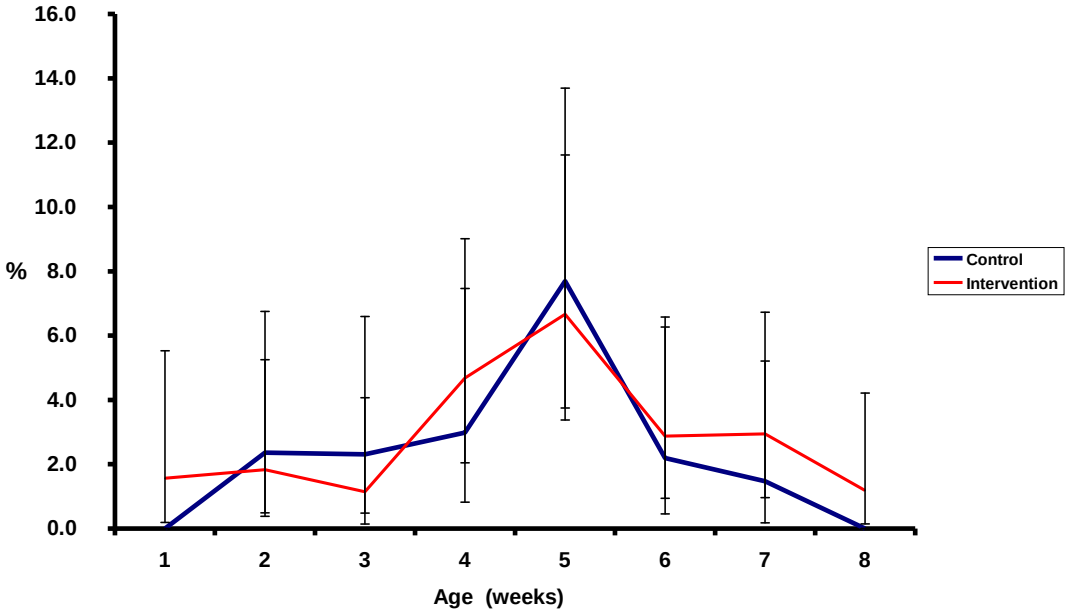


Figures 7: Line graphs showing prevalence of carriage of non-vaccine serotypes by study arm and age

Note: Bars indicate 95% confidence intervals

Non-typeable pneumococci (NT)

An initial increase in the proportion of NT carriers in both arms which attained a peak in the 6th week was followed by a consistent fall in these proportions until the end of the follow-up period (Figure 8). In control villages, no infant carried NT in the first week of life. However, the carriage prevalence increased steadily from 2.4 % (3/127) in 2nd week to a maximum of 7.7% (10/130) in the 5th week of life. Thereafter there was a decrease in proportion of infants carrying NT until the 8th week when there was no carrier, as in the 1st week of life. In intervention villages, NT carriage prevalence was 1.6% (2 / 128) in the first week of life. There were 2/175 carriers (1.1%) in the 3rd week and by the 5th week of life, NT carriage prevalence was 6.8% (11/165) in intervention villages.



Figures 8: Line graphs showing prevalence of carriage non-typeable pneumococci by study arm and age

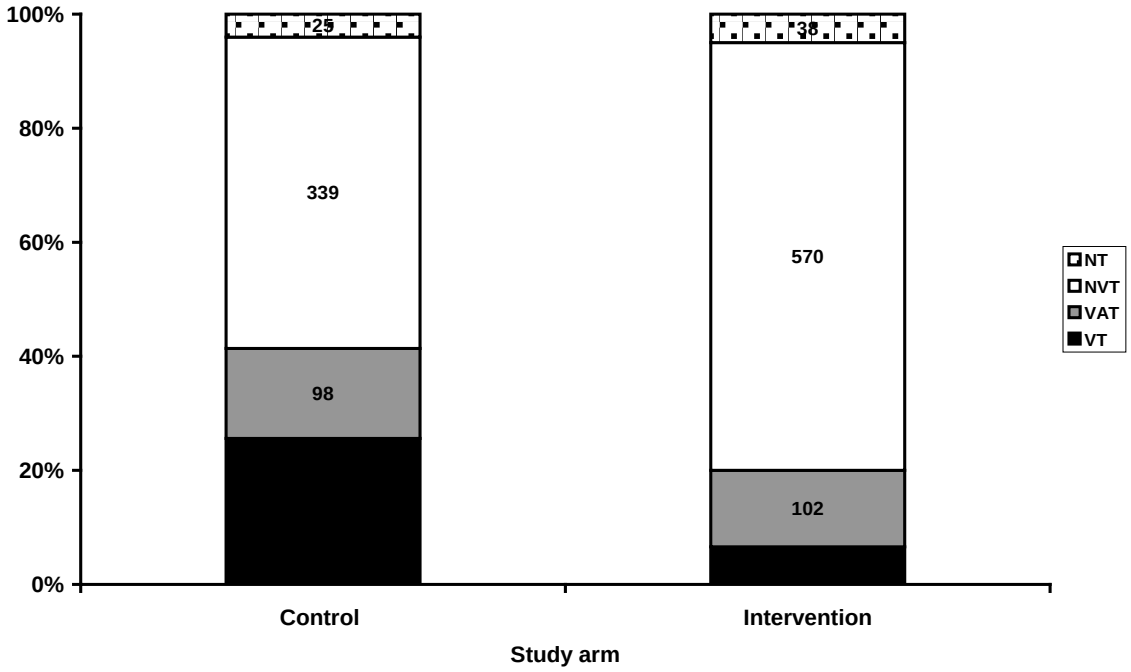


Figure 9: Overall distribution of isolates showing number and proportion of isolates by vaccine groups and non-typeable pneumococci

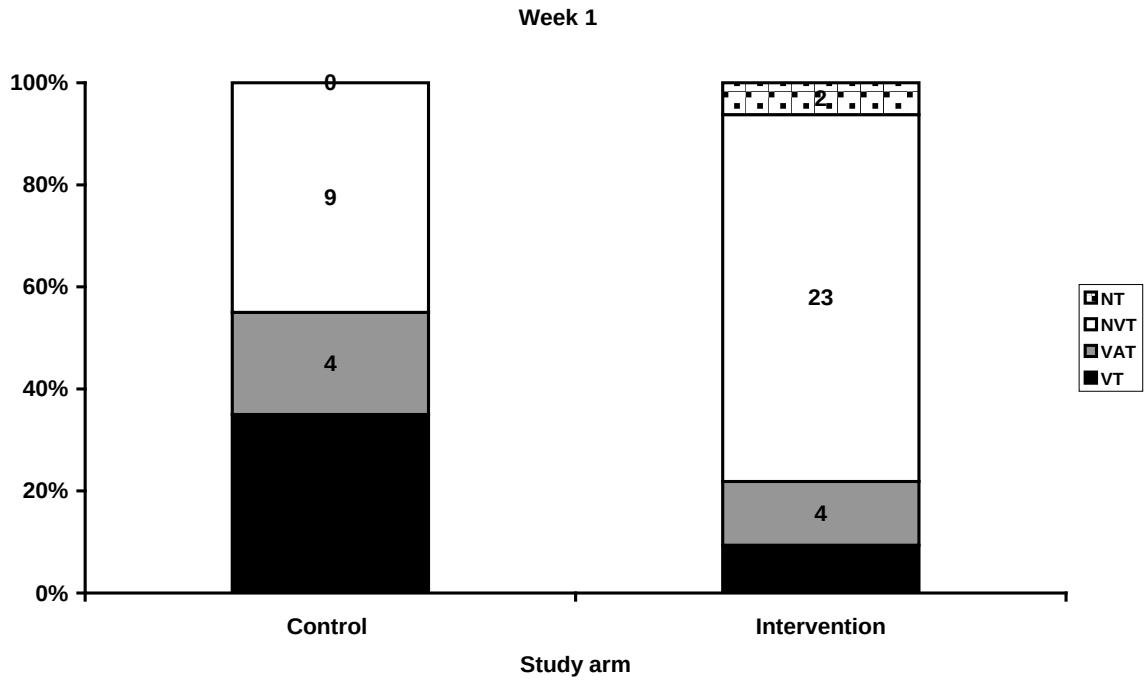


Figure 10: Number of infants and proportions of vaccine groups and non-typeable pneumococci carried during 1st week of life

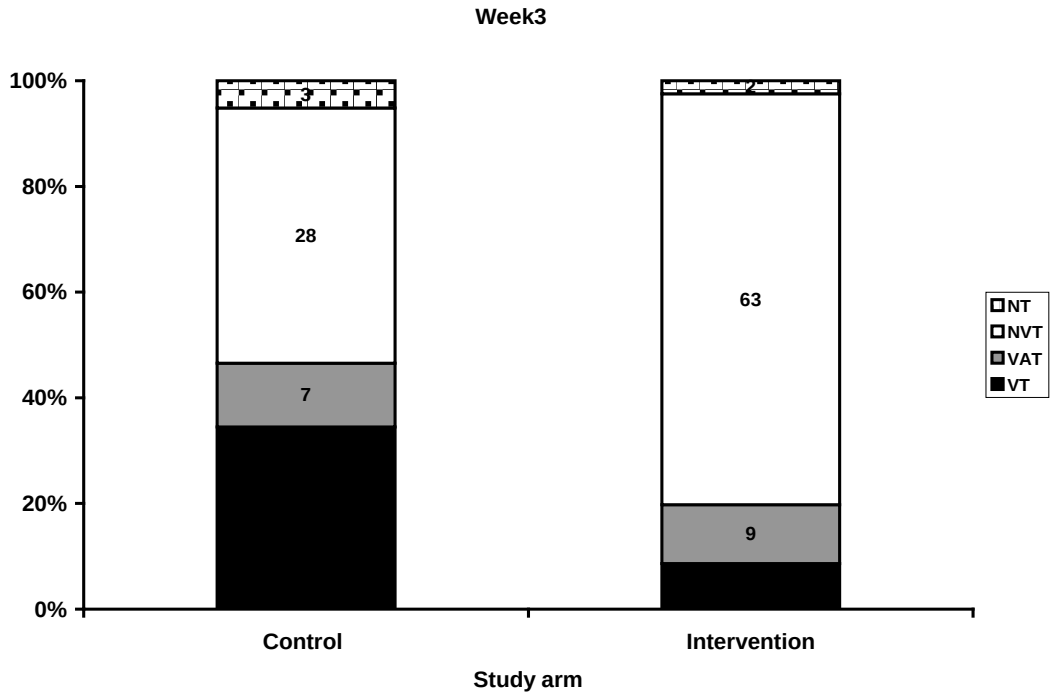


Figure 11: Number of infants and proportions of vaccine groups and non-typeable pneumococci carried during 3rd week of life

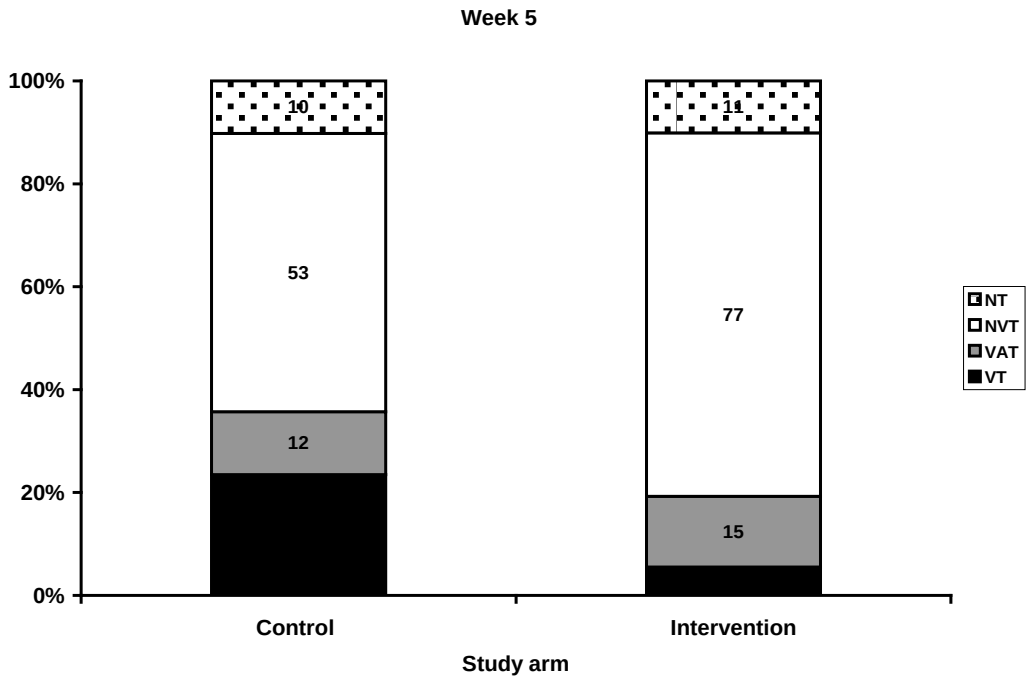


Figure 12: Number of infants and proportions of vaccine groups and non-typeable pneumococci carried during 5th week of life

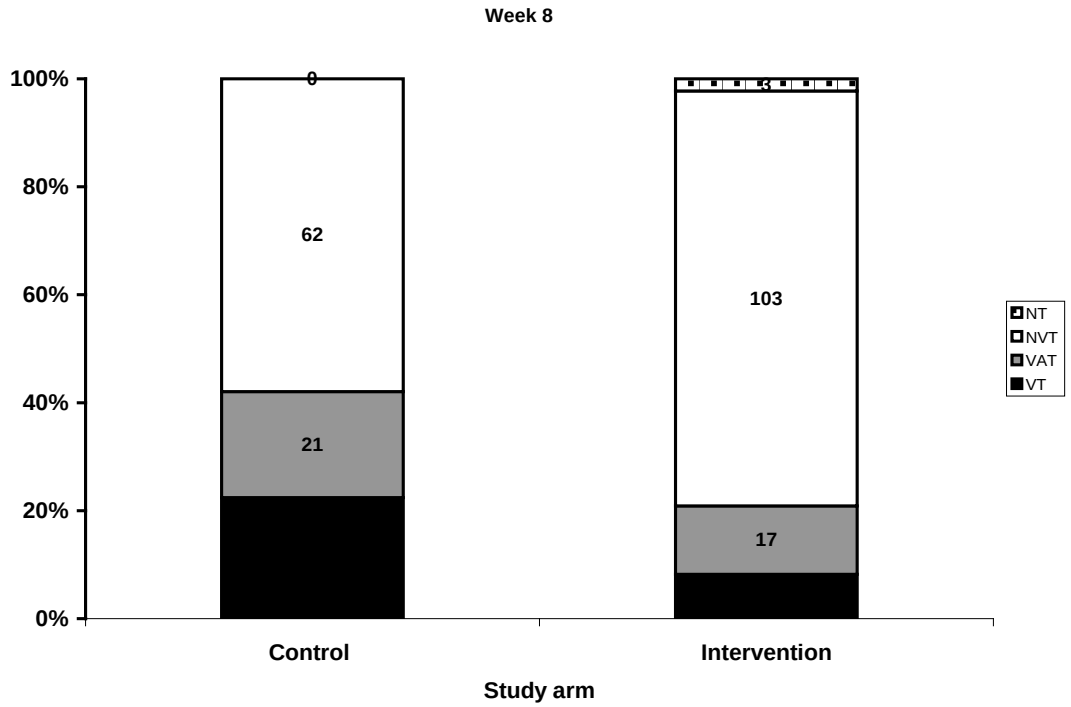


Figure 13: Number of infants and proportions of vaccine groups and non-typeable pneumococci carried during 8th week of life

4.6 Distribution of pneumococcal serotypes

4.6.1 9 commonest serotypes

Overall, 67 individual serotypes were represented among the isolates (n=1308). There was a statistically significant difference in the distribution of serotypes between the study arms (p<.001). The 9 most common serotypes accounted for 55.6% (n=728) of the isolates. These are serotypes 13 (6.3%), 11 (6.3%), 19F (6.2%), 16F (5.85), 10A (5.6%), 15B (5.2%), 6A (4.9%), 23F (4.5%) and 19A (3.6%). Five of these (serotypes 13, 11, 16F, 10A and 15B) were non-vaccine serotypes, 2 (serotypes 6A and 19A) were vaccine-associated serotypes and 2 (serotypes 19F and 23F) were vaccine serotypes (table 5).

All 5 non-vaccine serotypes and serotype 19A were present in higher proportions in intervention villages compared to controls (table 3) but this difference reached statistical significance only in serotype 10A (6.9% vs. 3.8%; p=.013) and serotype 15B (7.1% vs. 2.7%; p<.001). On the other hand, vaccine serotypes 19F and 23F and vaccine-associated serotype 6A, were significantly lower in intervention compared to control villages (3.0% vs. 9.9%, p<.001 for serotype 19F, 1.1% vs. 8.7%, p<.001 for serotype 23F and 3.3% vs. 6.7%, p=.003 for serotype 6A).

The other 58 serotypes accounted for 47.2% of all isolates in the study and accounted for similar proportions of all isolates in both arms of the study.

4.6.2 Serotypes 1 and 5

: These serotypes are of particular concern in The Gambia. They are responsible for a significant proportion of invasive diseases but tend to be carried transiently in the nasopharynx. There were only 6 isolates of serotype 1; one from control villages and 5 from intervention villages. Carriage of this serotype involved 4 infants, one from control and 3 from intervention villages (prevalence of carriage 0.6% vs. 1.6% respectively, p=0.232). One infant in intervention arm carried this serotype for two consecutive weeks. Serotype 5 on the other hand, had 5 isolates all of which were from control villages and were carried by 3 infants (prevalence of carriage 1.9%). Two of these infants carried this serotype for two consecutive weeks.

Table 5: Distribution of pneumococcal serotypes at any time during the first 8 weeks of life stratified by study arm

		Number of isolates (%)		
Rank	Serotype	Control	Intervention	Total
1	13	34 (5.48)	53 (6.99)	87 (6.31)
2	11	32 (5.15)	55 (7.26)	87 (6.31)
3	19F	62 (9.98)	23 (3.03)	85 (6.16)
4	16F	30 (4.38)	50 (6.60)	80 (5.80)
5	10A	24 (3.86)	53 (6.99)	77 (5.58)
6	15B	17 (2.74)	54 (7.12)	71 (5.15)
7	6A	42 (6.76)	25 (3.30)	67 (4.86)
8	23F	54 (8.70)	8 (1.06)	62 (4.50)
9	19A	17 (2.74)	32 (4.22)	49 (3.55)
	Other	284 (45.73)	367 (48.42)	651 (47.21)
	VT	159 (25.6)	50 (6.59)	209 (15.15)
	VAT	98 (15.78)	102 (13.45)	200 (14.5)
	NVT	339 (54.58)	570 (75.19)	909 (65.92)
	NT	25 (4.03)	38 (5.01)	63 (4.57)

Note: The 9 commonest pneumococcal isolates are shown and ranked by frequency of isolation. Numbers of isolates include counts from multiple carriages. Difference in distribution of serotypes, vaccine, vaccine-associated and non vaccine serotypes between study arms was P<0.001 (chi2 test)

4.7 Distribution of individual vaccine, vaccine-associated and non-vaccine pneumococcal serotypes

Figures 14 - 16 show a comparison of the relative distribution of pneumococcal serotypes among all samples taken during the study period in control and intervention villages.

Vaccine serotypes

The proportions of all vaccine serotypes were higher in control villages compared to intervention villages (Figure 14). The statistically significant difference in the distribution of the vaccine serotypes (P=.018) appeared to be accounted for mainly by serotypes 14, 19F and 23F. The relative proportions of serotypes 19F and 23F were 5.9% vs. 1.7% (p<.001) and 5.1% vs. 0.6% (p<.001) in control and intervention villages respectively. There were 24 isolates of serotype 14 isolated during the study, 18 from Control villages and 6 from Intervention villages constituting 1.7% and 0.4% of samples from the villages respectively (p=.003). Only small numbers of serotypes 4, 9V and 18C were isolated. All pneumococci of serotypes 4 (n=6) and 18C (n=3) were from the control villages. Of the 9 isolates of serotype 9V, 6 were from control villages and 3 were from intervention villages. The proportions of serotype 6B were similar in Control and Intervention villages (0.9% vs. 0.7% respectively, p=.658).

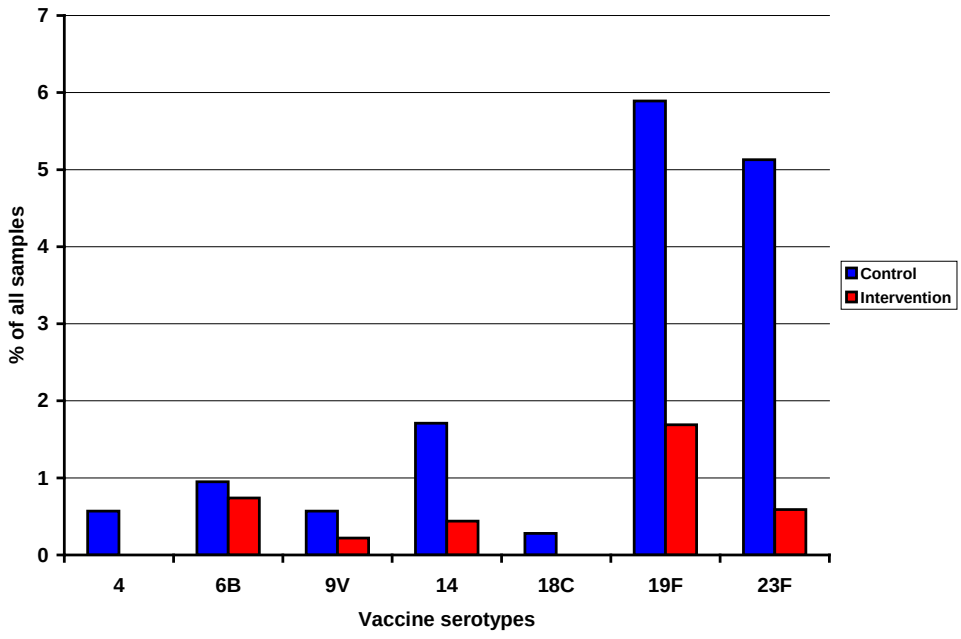
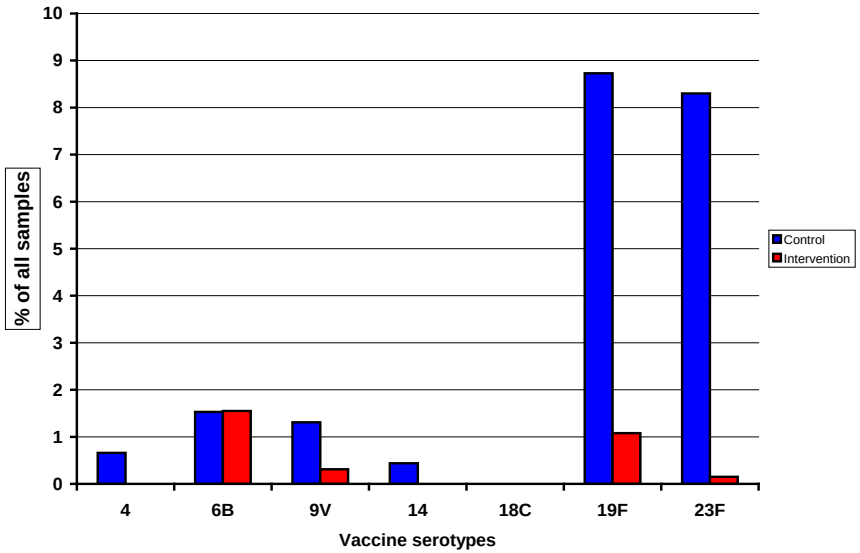


Figure 14: Comparison of individual vaccine serotypes between Control and Intervention villages. Note: Values on y-axis are percentage of serotypes as proportions of all samples taken during the study

The relative proportions of serotypes were also compared by time of birth of the infants in relation to vaccination of the communities (Figure 15; A & B). Overall, the proportions of vaccine serotypes remained much lower in intervention villages compared to control in both periods except for serotype 6B which was the same in both arms in the first year. In the first year of vaccination, serotypes 19F and 23F predominated (8.7% & 8.3% of isolates respectively) in control villages. By the second year, these serotypes maintained predominance in control villages but dropped in their relative proportions to 3.7% & 2.6% respectively. Serotype 14 increased in control villages (0.4%, n=2 in first year vs. 2.8%, n=16 in second year) while being isolated in intervention villages for the first time in 2nd year. On the other hand, serotypes 6B and 19F predominated in intervention villages in first year but by the second year, no serotype 6B pneumococcus was isolated and proportion of serotype 19F had doubled, relative to the first year.

A



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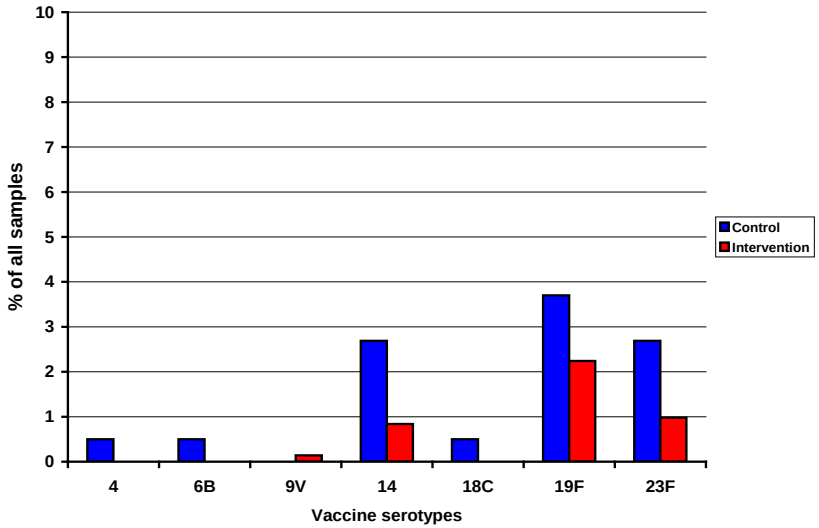


Figure 15: Comparison of individual isolates of vaccine serotypes by study arm and time since completion of community vaccination with the 7-valent pneumococcal conjugate vaccine.

Note: Values on y-axis are percentage of serotypes as proportions of all samples taken during the study. A: from infants born in 1st year of vaccination; B: from infants born in second year of vaccination

Vaccine-associated serotypes

Serotype 6A was the predominant VAT in both arms of the study (Figure 16). The proportion was twice as much in control villages as in intervention villages (4% vs. 1.8% respectively) and this difference was statistically significant (P=.002). Serotype 23B showed similar picture (1.8% in control vs. 0.9% in intervention), though the numbers were much smaller and the difference not statistically significant. On the other hand, serotypes 19A and 19B proportions were higher in intervention compared to control villages (2.3% vs. 1.6%, p=.205) and (1.9% vs. 1%, p=0.087) respectively but these were not statistically significant.

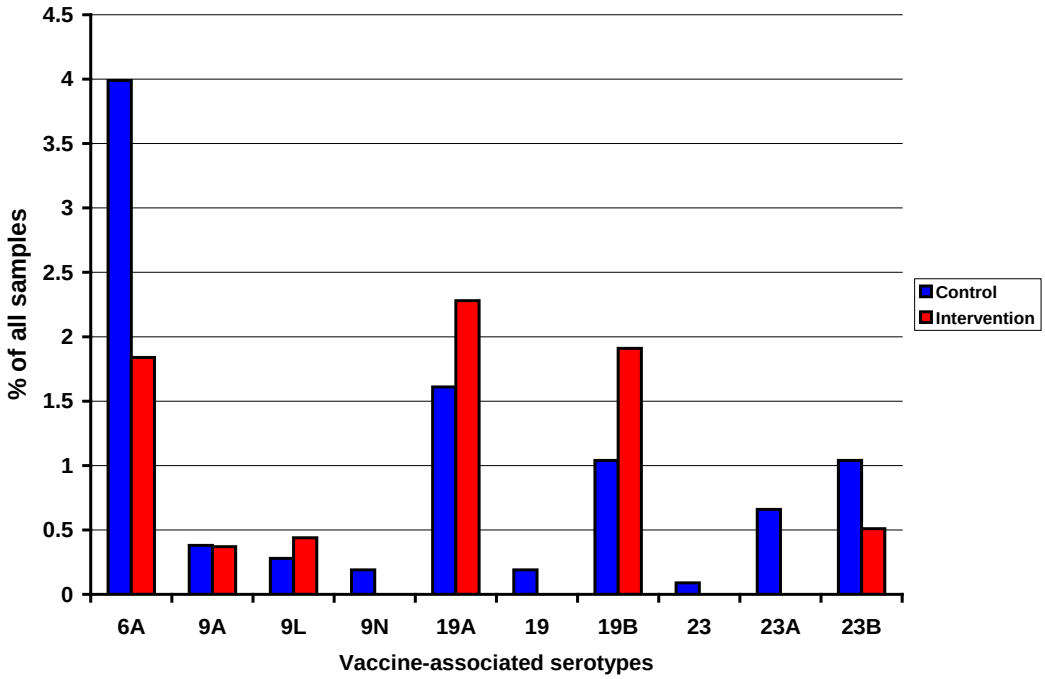


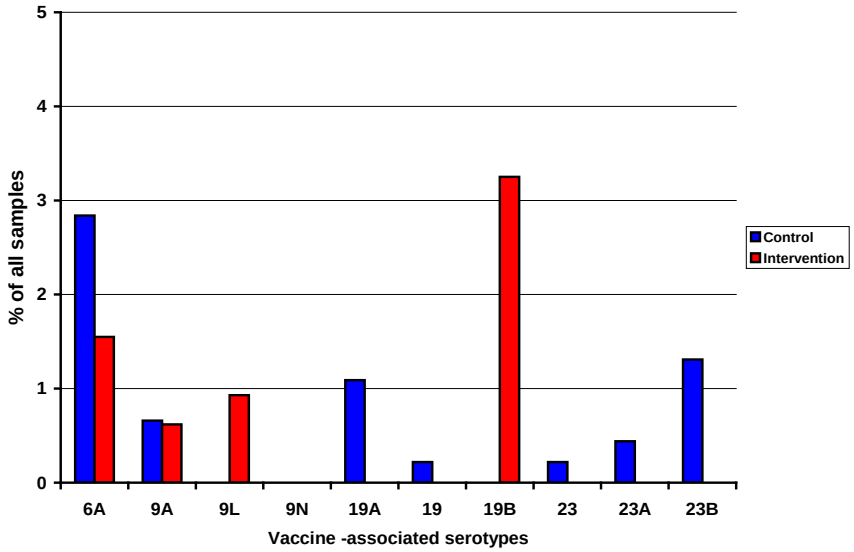
Figure 16: Comparison of individual vaccine-associated serotypes between Control and Intervention villages.

All 7 isolates of serotype 23A were from control villages. The other VAT isolates were present in small numbers. Serotype 9A was present in equal proportions in both arms of the study whereas serotype 9L was isolated in 0.3% and 0.4% of samples in control and intervention villages respectively.

In the first year of community vaccination, serotype 6A was the most predominant VAT isolate in control villages but had markedly increased in relative importance the second year

(from 2.8% to 4.8% of all samples in control villages). An increase in proportion of serotype 6A was also observed in intervention villages but the rate was much less (from 1.5% to 2.0%) as shown in Figure 16 A&B. Serotype 19A was not isolated in intervention villages in the first year of vaccination whilst being isolated in 1.09% of all samples in control villages in the first year. However, serotype 19A was the predominant isolate in intervention villages in second year and was isolated in 4.3% of samples. Similarly, serotype 29B was not isolated in control villages in first year but was isolated in about 2% of all samples in the second year. Other VAT isolates were as shown in Figure 17 A & B.

A



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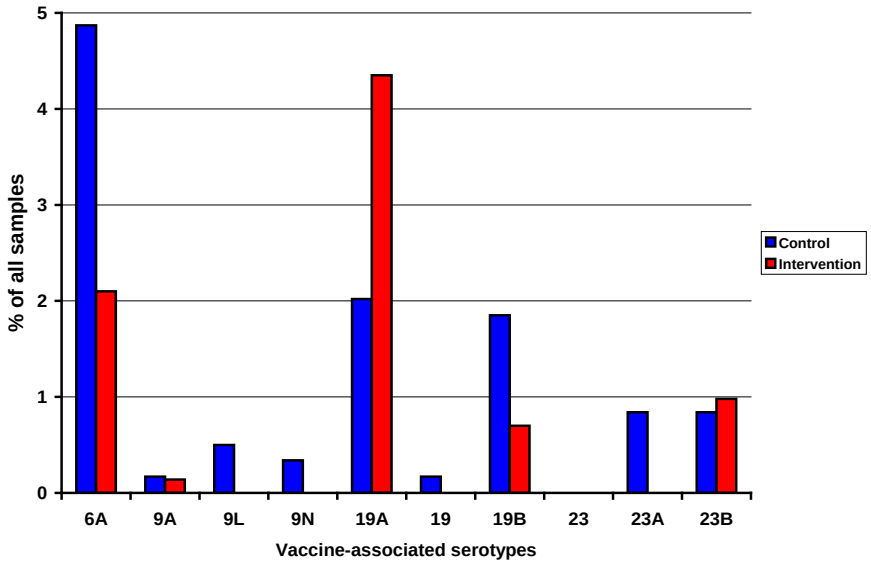


Figure 17: Comparison of individual isolates of vaccine-associated serotypes by study arm and time since completion of community vaccination with the 7-valent pneumococcal conjugate vaccine.

Note: Values on y-axis are percentage of serotypes as proportions of all samples taken during the study. A: from infants born in 1st year of vaccination; B: from infants born in second year of vaccination

Non-vaccine serotypes

The most frequently isolated 9 non-vaccine serotypes were more predominant in intervention compared to control villages. The difference was more marked and statistically significant for serotypes 7F (2.5% vs. 1.1%, p=.016), 10A (3.9% vs. 2.3%, p=.026) and 15B (4% vs. 1.6%,p=.001) in intervention and control villages respectively (figure 18).

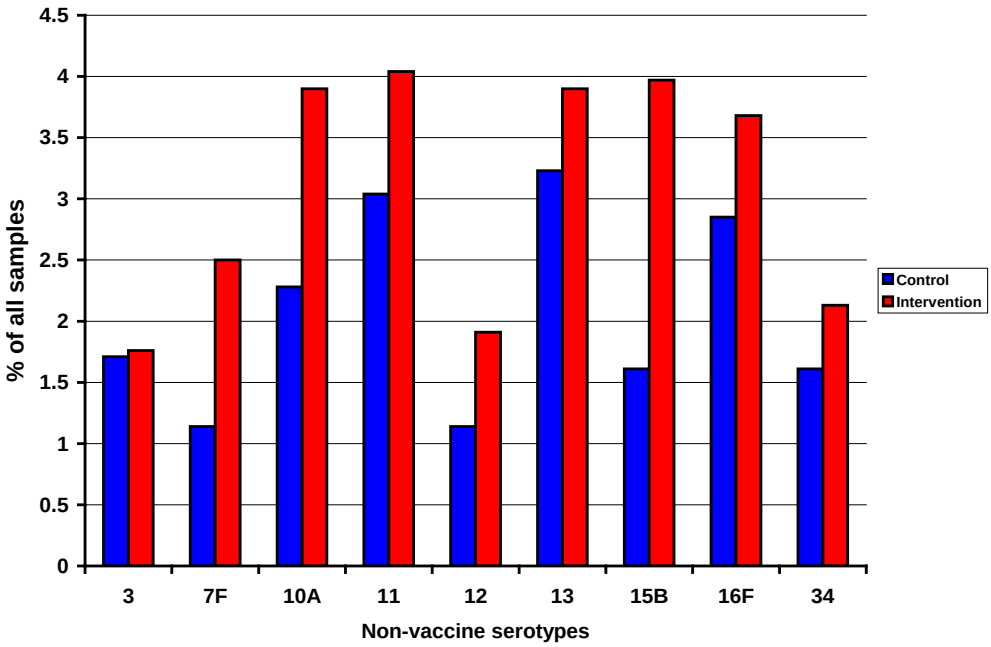


Figure 18: Comparison of the 9 most-frequently isolated non-vaccine serotypes between Control and Intervention villages

When stratified by time since community vaccination, the following features were apparent (figure 19 A & B);

Serotype 7F positive samples increased in control and intervention villages from 0.2% vs. 0.7% respectively in the first year, to 1.5% vs. 4.7% respectively in the second year;

Serotype 15B positive samples increased in intervention villages but dropped in control villages between the two periods (1.7% vs.3% respectively, in the 1st year and 5% vs. 0.5% respectively, in 2nd year).

Serotypes 3 and 12 positive samples were higher in control compared to intervention villages in the second year (2.8% vs. 1.9% for serotype 3, p=0.203 and 2.0% vs. 1.1% for serotype 12, p=0.137, respectively) but these were not statistically significant.

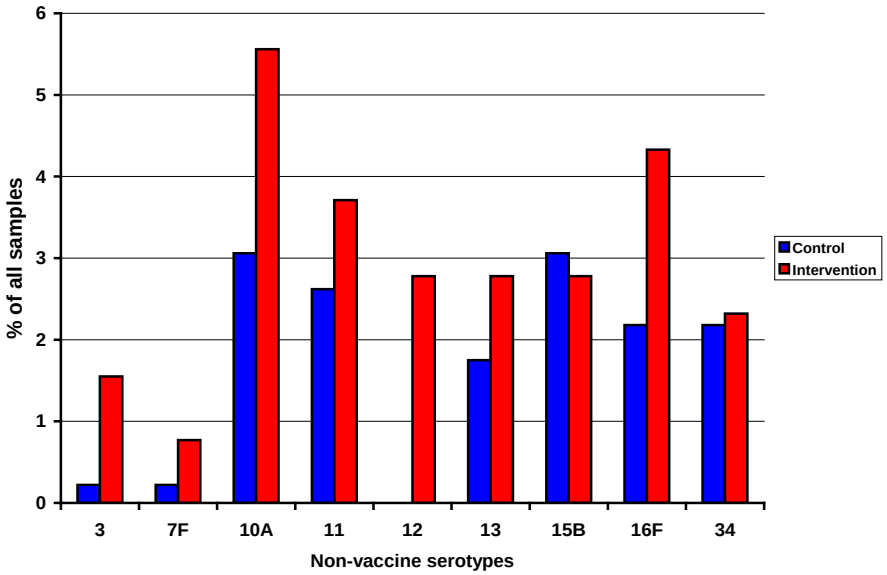
Serotypes 10A, 11 and 13 positive samples were higher in intervention compared to control villages in the 1st year (5.5% vs. 3.0%, p=0.025; 3.7% vs. 2.6%, p=0.212 and 2.8 vs. 1.7%, p=0.182 respectively) but by 2nd year, these differences became smaller but not statistically significant (2.4% vs. 1.7, p=0.491, 4.4% vs. 3.4%, p=0.526 and 4.9% vs. 4.3%, p=0.880) for serotypes 10A, 11 and 13 respectively.

Serotype 16F positive samples were significantly higher in intervention compared to control villages in the 1st year (4.2% vs. 2.2% respectively, p=0.028) but by 2nd year, there was no difference between the study arms (3.1% vs. 3.4% respectively, p= 0.818).

Non-typeable pneumococci

Non-typeable pneumococci were not significantly different between control and intervention villages overall (Figure 20) and accounted for less than 3% of all samples in both arms. While NT positive samples were much less in second year compared to the first (1.7%in control villages vs. 2.5% in intervention villages), this difference was not statistically significant (p=.607).

A



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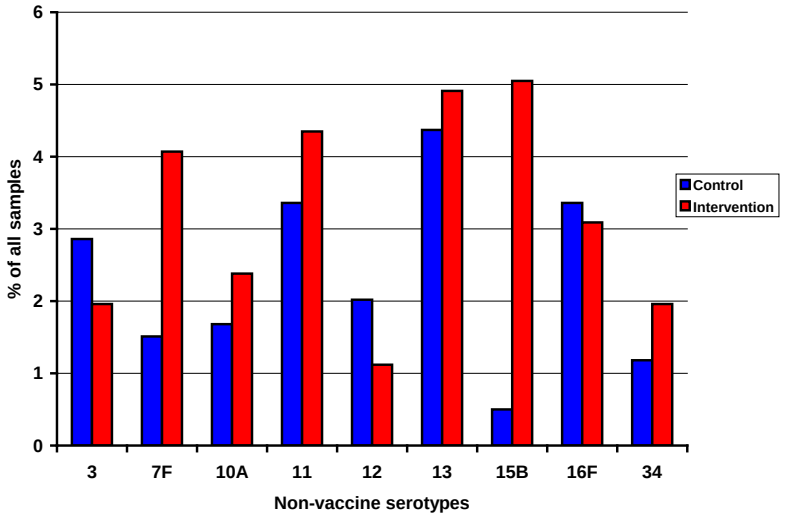

Figure 19: Comparison of individual isolates of non-vaccine serotypes by study arm and time since completion of community vaccination with the 7-valent pneumococcal conjugate vaccine.

Note: Values on y-axis are percentage of serotypes as proportions of all samples taken during the study. A: from infants born in 1st year of vaccination; B: from infants born in second year of vaccination

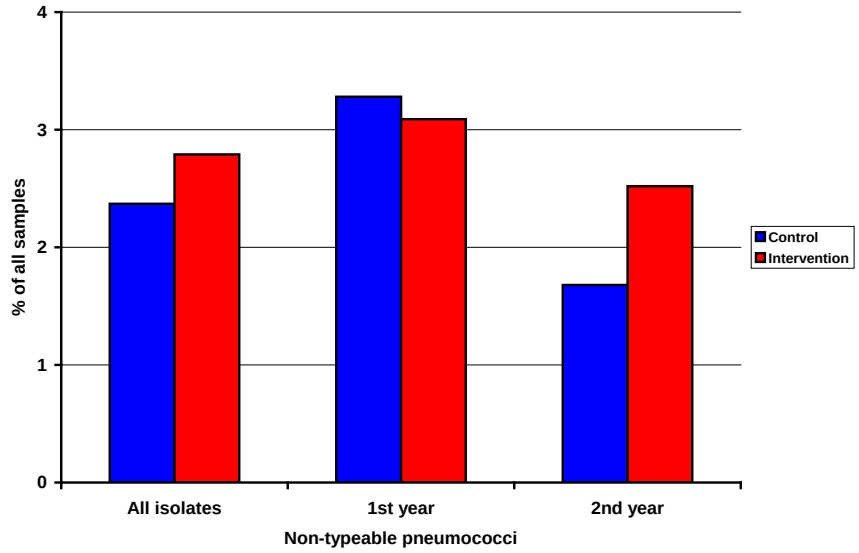

Figure 20: Comparison of non-typeable pneumococci by study arm and time since completion of community vaccination

4.8 Analysis of pneumococcal acquisition

Time to first acquisition: First acquisition of any Pneumococci occurred very early in control and intervention arms (mean times to acquisition 4.6 days vs. 4.8 days respectively). There was a constantly increasing rate of acquisition such that over 75% of all infants had acquired Pneumococcus by the end of the first 8 weeks of life.

Figures 21 and 22 show the survival curves for the first acquisition of carriage for the 6 commonest pneumococcal serotypes (13, 11, 19F, 10A, 16F and 15B), serotype 23F, vaccine groups, non-typeable pneumococci and any Pneumococcus. Pneumococcal serotypes 13, 11, 16F, 10A and 15B were acquired at the same rate in both arms of the study. However, serotype 19F was acquired faster and at a constant rate in control compared to intervention villages where only a negligible proportion of infants acquired 19F and the rate remained the same through the study period.

Acquisition of vaccine serotypes in control villages was earlier and increased at a constant rate with age. Mean time to acquisition for all vaccine serotypes was 24.5 days in control

villages and 67.9 days in intervention villages. Rates of first acquisition were much lower in intervention villages and did not increase for most of the first 8 weeks of life (Figure 22). First acquisitions of vaccine associated serotypes and the non-typeable pneumococci were the lowest and occurred at the same rate in both arms. For both groups, rates of first acquisition increased slightly about the 5th week of life compared to the first 4 weeks.

Non vaccine serotypes were acquired by the 1st week in both arms of the study and the rate consistently increased through the first 8 weeks of life. However the rates were higher in intervention villages. Mean times to first acquisition were 9 days vs. 7 days in control and intervention villages respectively. By the 5th week, 50% of infants had acquired a non-vaccine serotype at least once in intervention villages compared to about 35% in control villages

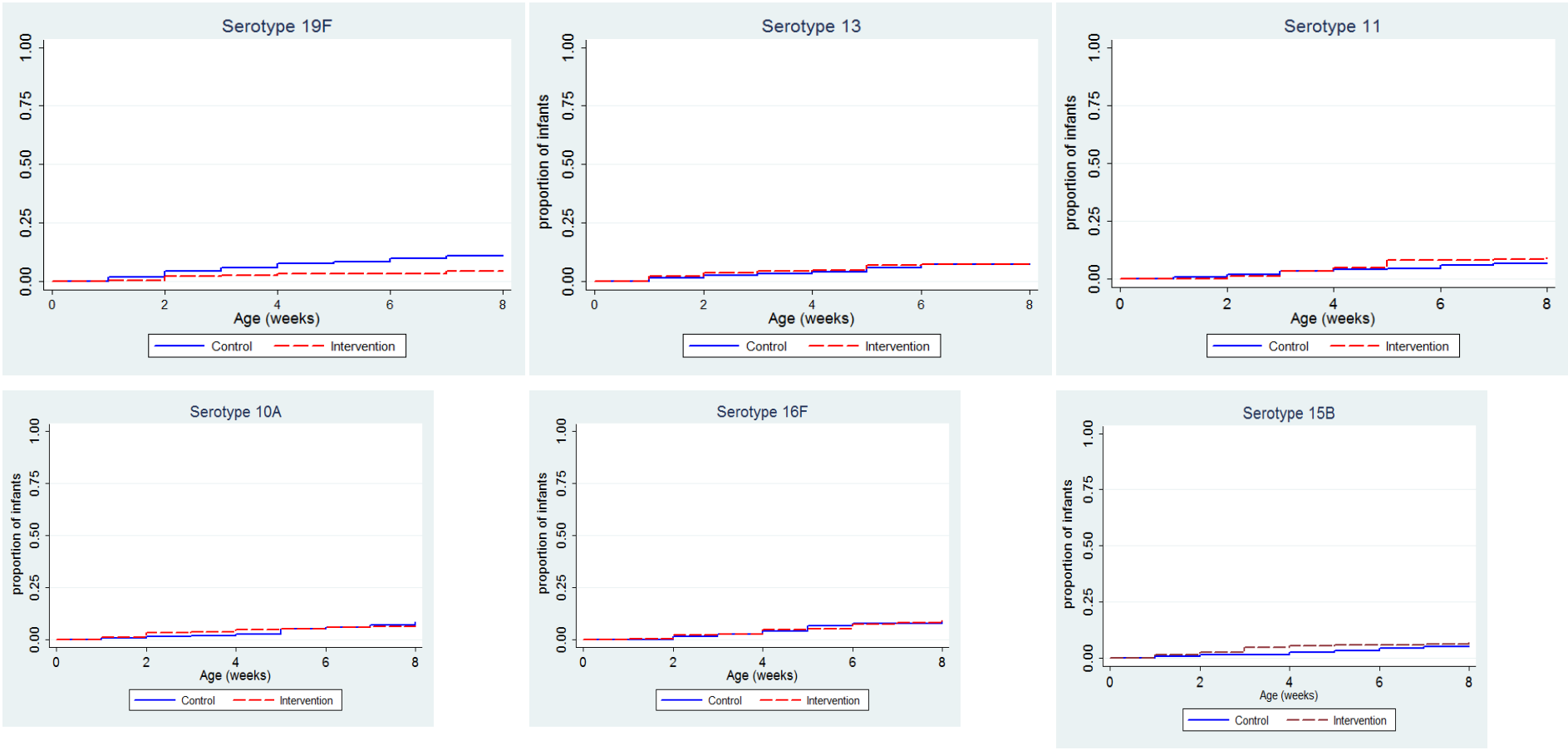

Figure 21: Kaplan-Meier curves showing a comparison of time to first acquisition of the 6 commonest serotypes by study arm and age

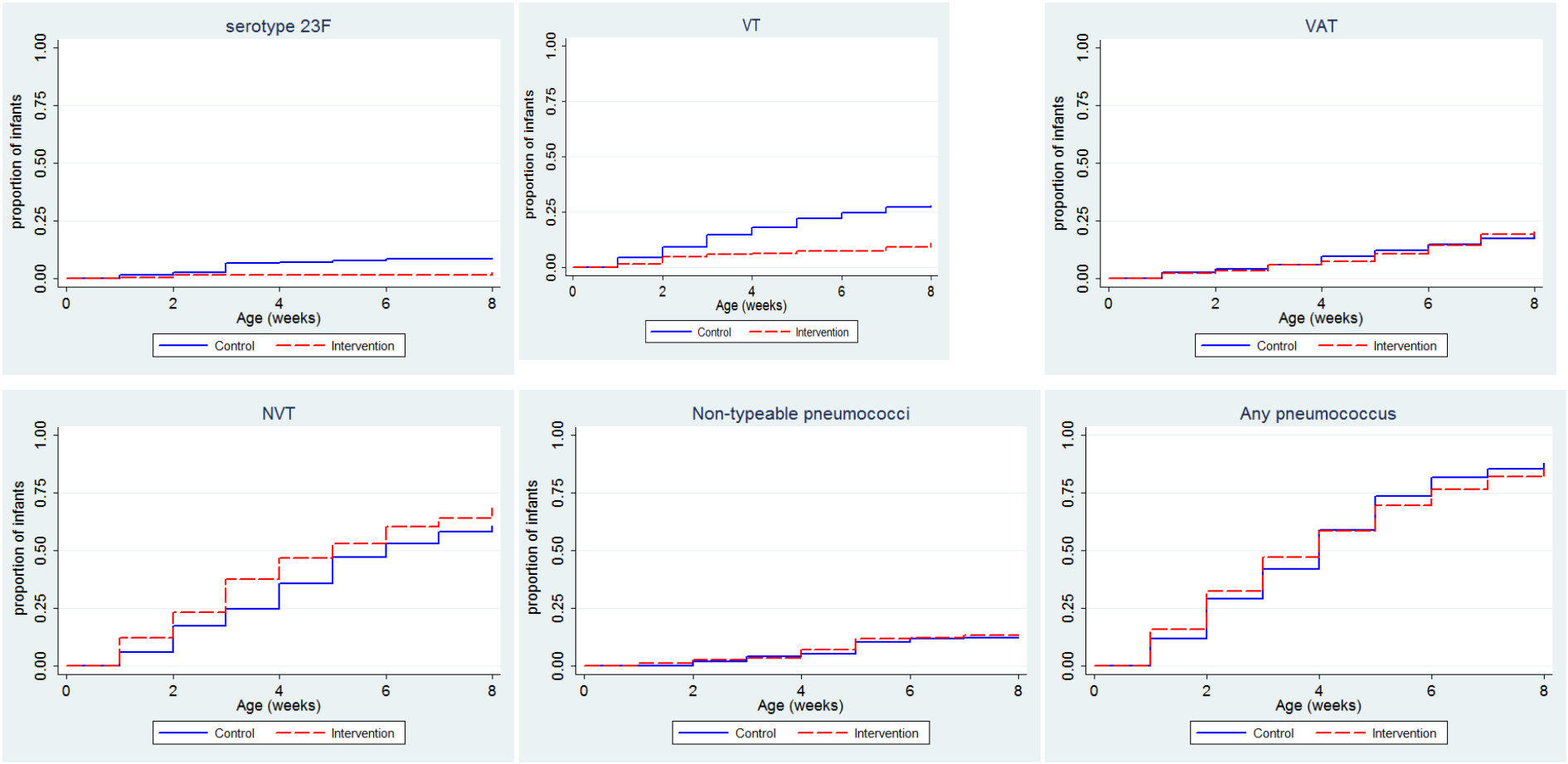

Figure 22: Kaplan-Meier curves showing comparison of times to first acquisition of serotype 23F, vaccine groups, non-typeable pneumococci and any pneumococcus in infants by study arm and age.

Note: VT – vaccine serotypes, VAT – vaccine associated serotypes, NVT – non vaccine serotypes, NT- Non-typeable pneumococci

Rates of first pneumococcal acquisition: The rates of acquiring Pneumococcus for the first time were compared using Cox regression model. Table 6 shows the hazard ratios for first acquisition of pneumococcal carriage in infants. For all 7 serotypes except 19F and 23F, infants from intervention villages acquired the serotypes earlier than in control villages and this was statistically significant for serotypes 13 and 10A. The hazard ratio was highest for serotype 15B (infants in intervention arm 67% more likely to acquire the serotype compared to controls) but this did not reach statistical significance.

Table 6: Risk of first acquisition of pneumococcal carriage among infants in Intervention villages compared to those in control villages

Serotype	n of isolates	Hazard ratio (95%CI)	p-value
19F	85	0.42 (0.21 -0.85)	0.02
13	87	1.1 (1.01 – 1.9)	0.02
11	87	1.4 (0.69 – 2.85)	0.34
10A	77	1.09 (1.01 – 1.18)	0.03
16F	80	1.07 (0.44 – 2.62)	0.87
15B	71	1.67 (0.84 – 3.32)	0.15
23F	58	0.31 (0.07 – 1.32)	0.113
Vaccine serotypes	209	0.37 (0.22 – 0.6)	<0.001
Vaccine-associated serotypes	200	1.03 (0.67 – 1.61)	0.87
Non-vaccine serotypes	909	1.26 (0.94 – 1.68)	0.12
Non-typeable pneumococci	63	1.13 (0.61 – 2.09)	0.7
Any Pneumococcus	1308	0.97 (0.71 – 1.32)	0.842

Note: Cox regression model was used to estimate hazard ratios and controlled for sex, ethnicity, breastfeeding, household size and number of children <5years of age in the household. First 6 serotypes shown were the commonest isolated in the study villages. Serotype 23F was the 2nd commonest vaccine serotype.

Infants in intervention villages had a 63% lower hazard for acquiring vaccine serotypes compared to those in control villages. This finding was statistically significant (P<.001). The vaccine serotype 19F also had a 58% lower hazard for acquisition in intervention villages (P=.02).

There was a generally increased hazard for acquisition of non-vaccine serotypes (26%) and non-typeable pneumococci (13%) in intervention infants compared to those in control villages. However, none of this was statistically significant. For the vaccine-associated serotypes, there was no difference in hazard for acquisition between the two arms.

Effect of risk factors on risk of acquisition: The impact of breastfeeding, ethnicity, gender and household size on first acquisition of pneumococcal carriage was evaluated. Belonging to ethnic group 3 (combination of small ethnic groups) was associated with statistically significant lower rate of acquisition of any pneumococci (HR 0.42; 95%CI 0.25 – 0.77, p=.005), vaccine serotypes (HR 0.32; 95%CI 0.17 – 0.59, p<.001) and non-vaccine serotypes (HR 0.56; 95%CI 0.32 – 0.96, p=.04). The presence of children under 5 years in the household was associated with an increased rate of acquisition of vaccine-associated serotypes (HR 1.07; 95%CI 1.03 – 1.12, P<.001) and of the non-vaccine serotype 13 (HR 1.1; 95%CI 1.01 – 1.9, p=0.02). The other risk factors were not associated with the rate of first acquisition of pneumococci in the infants and this was consistent between the two study arms.

Chapter 5: Discussion

The 7-valent pneumococcal conjugate vaccine has just been introduced in The Gambia for prevention of pneumococcal disease. Previous studies in The Gambia have documented some of the highest pneumococcal carriage rates among adults and children ever reported worldwide (13, 28, 37). Important features of pneumococcal carriage in The Gambia include very early age at first acquisition, a very high carriage rate in the newborn population and a large diversity of pneumococcal serotypes. These characteristics are major reasons for concern in the event of introduction of a pneumococcal vaccine of limited valency such as PCV7.

This randomised controlled study has demonstrated a significantly reduced carriage of vaccine serotypes in the first 8 weeks of life in infants born into PCV7 vaccinated communities compared to those born in control villages. Significantly reduced carriage of individual vaccine serotypes 19F, 23F and 14 in intervention villages compared to controls was demonstrated. Though vaccine serotypes carriage showed a decline in control infants in the second year of vaccination relative to the first, vaccine serotypes carriage remained lower in intervention infants through the study period. In parallel with the reduced vaccine type carriage was an overall increase in carriage of non-vaccine serotypes in intervention infants compared to controls, with statistically significant increased carriage of individual non-vaccine serotypes 7F, 10A and 15B. Furthermore, there was a 63% reduction in risk of acquisition of vaccine serotypes in intervention infants compared to controls which was statistically significant and a 26% increased risk of acquisition of non-vaccine serotypes in intervention infants compared to controls which did not reach statistical significance. However, a statistically significant increase in acquisition of non-vaccine serotypes 10A and 13 in intervention infants compared to controls was demonstrated. Vaccine associated serotype 6A carriage was significantly less in intervention infants compared to controls while serotype 19A carriage was higher in intervention infants but this was not statistically significant.

The data from this randomised controlled trial of PCV7 show some important key observations. First, vaccination of the community offered indirect protection of newborn babies against acquisition and carriage of vaccine serotypes while increasing their risk of

acquisition and carriage of non-vaccine serotypes compared to babies born into partially vaccinated communities. O'Brien et al demonstrated protection against vaccine serotypes carriage and increased risk of carriage of non-vaccine serotypes in American Indian infants living in PCV7-vaccinated communities compared to their counterparts living in control communities(99). In addition, proportion of isolates that were non-vaccine serotypes was significantly higher in infants in vaccinated communities compared to controls. This is similar to the findings from our study. The American Indian population in the US are known to exhibit pneumococcal epidemiological characteristics similar to The Gambia, with high carriage rates and increased risk of invasive disease (107). Other randomised controlled trials of pneumococcal conjugate vaccine among infants and children have similarly demonstrated reduced vaccine serotypes carriage in parallel with increased risk of carriage of non-vaccine serotypes. A trial of 9 valent pneumococcal conjugate vaccine among South African children showed that 18% of vaccinees versus 36% of controls carried pneumococci by 6 months after primary series vaccination. Carriage of non-vaccine serotypes was 36% vs. 25% in intervention arm compared to control. These were all statistically significant(69). Similarly, Millar et al showed a vaccine type carriage of 10% among vaccinated US children compared to 17.1% of controls as well as a significantly increased non-vaccine serotype carriage among vaccinated children compared to controls (98). However, these were results of direct effect of the vaccine. The uniqueness of the present study is the demonstration of an impact of PCV7 vaccination in newborn children as an indirect effect and at much younger age that the other studies.

Non-vaccine serotypes were the predominant serotypes isolated in infants in this study. This represents a significant shift to non-vaccine serotypes in the communities. In control villages which represented a setting of early use of PCV7 in a population, non-vaccine serotypes accounted for 54.5% of all isolates compared to 33.7% found in the same communities before start of the trial(23). In intervention villages, only non-vaccine serotypes were represented among the 6 commonest isolates. In addition, the increased carriage of individual non-vaccine serotypes 7F, 10A and 15B reached statistical significance in intervention compared to control infants, though the study was not particularly powered to detect this. This suggests replacement carriage with non-vaccine serotypes, a phenomenon that has been demonstrated in randomised controlled trials (65, 69-70, 98) and observational studies (66, 72, 108) involving the pneumococcal conjugate vaccine.

The demonstration of replacement carriage in this study is not surprising. A combination of high carriage rates, early acquisition, large pool of non-vaccine serotypes and transmission driven by young children as demonstrated in our previous studies (23, 109-110), favour the rapid development of replacement carriage in the presence of a pneumococcal vaccine of limited valency. Increased frequency and severity of non-vaccine serotype invasive disease has been observed in several populations where PCV7 has been in long term use (87, 90). The shift of predominant serotypes in the community to non-vaccine serotypes in this study is therefore worrisome, more so as this is being demonstrated in newborn babies among whom pneumococcal disease is being increasingly recognised(82).

In terms of individual serotypes, statistically significant reductions in carriage of individual vaccine serotypes (19F, 23F, 14) were demonstrated in vaccinated communities compared to controls. This shows a strong impact on carriage in newborn babies of community vaccination with PCV7. The American Indian study, though among older infants, demonstrated statistically significant reduction only in the case of serotype 19F, and this was in children who had received at least three doses of PCV7.

Acquisition of pneumococci occurred very early in this study and the overall pneumococcal carriage was high in keeping with findings from the prevaccination study(23). The previous study showed a mean time to first acquisition of 33days compared to 4.6 days in intervention and 4.8 days in control villages in the present study. Though it has been shown that most children are colonised with pneumococci at some point in their first two years of life(8), this study has shown the earliest time to first acquisition of pneumococci ever reported in a randomised controlled trial. Early acquisition of pneumococcus has been reported from Papua New guinea where all children had acquired pneumococci by the age of 3 months(39) and in Bangladesh where 50% of infants acquired pneumococcus by the 8^{th} week of life(35). Acquisition of pneumococci occurs even later in children from industrialised countries: in the USA, a mean time to first acquisition of 6 months was documented(8) while in Finland, 34% of infants were found have acquired pneumococci by age of 6 months(38). Very early acquisition in The Gambia compared to industrialised countries might reflect differences in carriage rates in the communities, number of children living in the same household as the child and the circulating pool of serotypes in the community. In this study, the presence of

children less than 5 years of age in the household was associated with an increased risk of acquisition of vaccine associated serotypes (HR 1.07; 95%CI 1.03 – 1.12). Using longitudinal carriage data from a birth cohort in the UK, Sleeman et al also demonstrated a similar association between number of siblings and pneumococcal acquisition(55). The importance of intra-family transmission of pneumococcus was highlighted in a previous study in The Gambia where children were found to introduce and transmit the pneumococci in the household(110). Transmission of pneumococcus to the infants in this study could therefore have been predominantly by the young children in their families and households.

Vaccine serotypes were acquired later among children in intervention villages compared to controls (mean times to acquisition 67.9 days vs. 24.5days) and intervention infants were 63% less likely to acquire a vaccine serotype compared to controls. Time to acquisition of non-vaccine serotypes was also shorter in intervention compared to control infants. Reduced risk of acquisition of vaccine serotypes and increased risk of acquisition of non-vaccine serotypes were also demonstrated in the American Indian study(99), and among Israeli toddlers attending day-care(65) in infants who had received pneumococcal conjugate vaccine compared to controls. Reduction in risk of acquisition is believed to be the mechanism by which PCV reduces carriage of vaccine serotypes.

An overall reduction in rate of carriage of vaccine serotypes in control villages compared to baseline carriage in the communities was also demonstrated in this study. A previous pre-vaccination longitudinal carriage study conducted in these same villages showed an overall carriage rate of vaccine serotypes of about 40% in the first 2 months of life(23). PCV7 was given to all residents of intervention villages but also to all children in control villages aged 30 months and below by the time of commencement of the trial. In addition, the vaccination of all newborn infants at the end of the 8^{th} week of life increased the pool of vaccinated children in the control villages over time, further helping to provide protection against pneumococcal colonisation in the community and leading to a reduction in the pool of vaccine serotypes available for transmission in the community. This observation of reduced vaccine serotype carriage in control villages is another evidence for a strong indirect effect of PCV7 among infants in our setting.

No difference in the carriage rate of vaccine-associated serotypes (P=0.11) and non-typeable pneumococci (P=0.53) was demonstrated in this study. However, the vaccine-associated serotype 6A was significantly less carried in intervention villages compared to control. It was the 3rd commonest serotype isolated from infants in these communities before the trial but had dropped in its relative importance (to 6th position) as a colonising serotype in both control and intervention villages in the present study. Significantly reduced carriage of serotype 6A was also demonstrated among toddlers in Israel who received PCV9 compared to controls(65) and this was thought to be due to cross-protection against this serotype by serotype 6B antibodies. Cross-protection of 6B against 6A has also been reported in other settings and the finding of this in The Gambia is good news especially given the increasing importance of serotype 6A in the post-conjugate vaccine era.

On the other hand, vaccine associated serotype 19A behaved like a non-vaccine serotype in this study. It appeared relatively later in the infants. Of 49 isolates overall, only five of these were isolated in the first year of community vaccination and these were all in control villages. But by the second year, 44 isolates were recovered, majority (32/44) of this in intervention villages where this serotype was being isolated for the first time. This is a significant finding, giving the increasing importance of serotype 19A in invasive disease and antibiotic resistance worldwide. It could also suggest that the replacement timelines may vary by serotype. Following the introduction of PCV7 in the US, serotype 19A has not only become the most frequently carried serotype in the nasopharynx(111), but has also become the leading pneumococcal serotype causing invasive disease and respiratory infections(112). Among children vaccinated with reduced doses of PCV7 in a randomised controlled study in Netherlands, an increase in serotype 19A nasopharyngeal acquisition was observed, compared to the controls (113). Though several observational studies and controlled trials have linked the use of PCV7 to increases in serotype 19A carriage and disease, a case has been made for the role of high antibiotic use in the countries where this trend has been demonstrated. Dagan et al, for instance, have demonstrated the potential promoting role of antibiotic use, especially Azythromycin, in the increases observed in multi-drug resistant serotype 19A otitis media which was independent of PCV7 vaccination(114). In addition, increases in serotype 19A carriage have been observed in some populations before the introduction of PCV7 vaccination(103). Other factors thought to be contributory include the baseline prevalence of this serotype and fluctuations of carriage serotypes with time (19, 108). In our study, only 7.9% of the infants in intervention villages had any antibiotics during the study period (9.7% in control villages) and antibiotic use is generally

low in rural Gambia. Antibiotic use is therefore unlikely to have played a significant role in the observed increased serotype 19A carriage in intervention villages. In the prevaccination study, serotype 19A was the 6th commonest serotype isolated in children in these study villages. Though this serotype has dropped in rank to 9th in the present study, the presence of higher proportion of the isolates in intervention villages may suggest a role for the serotype in replacement in our setting.

Only few serotypes 1 and 5 were isolated in this study (6 isolates of serotype 1 and 5 isolates of serotype 5). All 5 serotype 5 isolated were from 3 children in control villages. One serotype 1 isolate was carried by a child in control villages while 5 isolates were carried by 3 children in intervention villages. It has been shown that certain serotypes causing invasive disease are not commonly isolated from the nasopharynx(115). The finding of only very few isolates of serotypes 1 and 5 in this study is in keeping with this. These serotypes are responsible for over 30% of all invasive pneumococcal disease episodes in The Gambia (24) but were rarely isolated in previous carriage studies in The Gambia. In Spain, serotype 1 has emerged in the PCV era as an important cause of pneumonia with empyema in very young children(87). Previously established virulent strains of serotypes 1 and 5 have also emerged. The numbers of these isolates in this present study are too few to draw any meaningful conclusions. However, it is interesting that all 5 serotype 5 isolates were from control villages where the vaccine pressure was much less compared to intervention villages. This could be a chance finding but could also reflect carriage in the household of the individual infants rather than an effect of PCV7 vaccination. Carriage of this serotype involved two children who carried it on two consecutive weeks and a third child who carried once. A previous pre-vaccination study of transmission of pneumococcus in these communities revealed similar findings; all episodes of carriage of serotype 5 were limited to the same village and these serotypes were carried for a short period of time(110).

The high proportion of newborn infants who carried pneumococci at least once during the study period is consistent with high carriage rates previously documented among adults and children in these villages (23, 109-110). Sixty seven serotypes were isolated from infants in this study compared to 35 different serotypes in Bangladeshi children(35), 25 different serotypes in the USA(8) and 30 different serotypes in Finland(38). This large diversity of serotypes provides a large pool of non-vaccine types ready to occupy the nasopharyngeal niche in the event of use of a conjugate vaccine of limited valency such as PCV7. The PCV7 has already been introduced in The Gambia and there are plans to shift to the 13-valent vaccine which provides additional coverage to six other serotypes. The

six additional serotypes include serotypes 1 and 5 which cause a significant proportion of invasive pneumococcal disease in The Gambia and serotypes 3, 6A, 7F, 19A which were all important serotypes in this study. In spite of this, danger of replacement with serotypes not included in the vaccine remains a reality. The need for surveillance in the communities has been underlined by the findings in this study.

In summary, this randomised controlled trial of PCV7 among rural Gambian newborn babies has demonstrated a strong indirect protection of newborn babies living in vaccinated communities against nasopharyngeal acquisition and carriage of pneumococcal serotypes included in PCV7 in the first 8 weeks of life. An overall reduction in carriage of these serotypes was also demonstrated in control villages compared to prevaccination levels also reflecting strong indirect effect of PCV7 from the infant population who were vaccinated. However, significant replacement carriage with non-vaccine serotypes has occurred in the infants living in vaccinated villages and calls for caution as pneumococcal vaccines of limited valency are used in The Gambia.

Strengths of the Study

To the best of our knowledge this is the first randomised controlled trial of the PCV7 where newborns are recruited from birth and sampled weekly in the first 2 months of life in a developing country setting. An observational study of nasopharyngeal carriage among Bangladeshi infants recruited newborn babies but commenced sampling at two weeks of age, and at two weekly intervals(35).

The randomised controlled trial design and the use of a well established platform for the study of epidemiology of pneumococcal carriage are strengths of the study. The MRC (UK), The Gambia, has conducted several pneumococcal carriage studies in the Western division of The Gambia and has maintained a regular tracking of changes in the community including a regular census of the participating communities. The work was conducted by well trained and highly experienced field workers and nurses who were efficient in the processes involved with the organisation of community studies, NPS collection in newborn children, specimen handling and transport, and good clinical and laboratory practice methods.

A further strength of this study is that NPS were collected from newborn babies as soon after birth as possible, providing an opportunity to document carriage the earliest possible time it could have occurred. Over 60% of all newborns in the both arms of the study were sampled in the first week of life, with sampling commencing latest in most others by the second week of life. Furthermore, the weekly sampling frequency is about the most frequent sampling frequency possible in most settings, and has allowed for an enhanced understanding of the first pneumococcal acquisitions, the colonising pneumococcal serotypes and their relationship with one another.

Findings from this study will most likely apply to other developing countries in sub-Saharan African and could be used to guide pneumococcal vaccination policy in these countries.

Limitations of the Study

A follow up period of just 8 weeks weakened the ability of this study to demonstrate whether the observed increase in the NVT among infants in intervention villages would persist, disappear or even increase. Secondly, the follow up period of 8 weeks could not allow for a study of duration of carriage as time of loss of carriage was censored in most cases. Second and subsequent acquisitions were also very limited and could not be studied within the time of follow-up. A previous study of duration of carriage among infants in these study villages(23), showed a mean and median duration of carriage of 87.6 days (95%CI, 72.2 – 97.0days) and 70 days (95%CI, 59.5 – 77.0days) respectively, both of which were longer than the entire follow-up period of the present study.

The risk factor data obtained through the risk factor questionnaire in this study was based on reports of the primary caregivers which could not always be objectively ascertained. While there were no reasons to doubt them, use of antibiotics for instance could have been under- or over reported and antibiotic use could confound the association between PCV7 vaccination and carriage. To minimise this, the health cards of the infants were examined whenever this was possible, to confirm use of antibiotics. However, reports of antibiotic use were very few and similar in both study arms. There were no differences in the rates of report of any of the

other risk factors between the two arms, except for chest infection (2.6% [n=4] vs. 10.6% [n=20] p=, in control and intervention arms respectively).

Invasive disease end point would have afforded us an opportunity to determine the importance of the carriage findings in clinical invasive disease. Though the study was not designed for this, a correlation of the carriage findings with invasive disease within the setting of our study could have added more to the public health importance of the study.

Chapter 6: Conclusions and Recommendations

High immune pressure through community vaccination with PCV7 in The Gambia has resulted in a significant 'indirect' reduction in the carriage rate of pneumococci of vaccine serotypes in infants in their first 8 weeks of life in these villages. There was associated increased risk of acquisition and carriage of non-vaccine pneumococci in these infants compared to their counterparts in the control communities. These findings support both the introduction of PCV7 into The Gambia and the need for a robust surveillance mechanism to monitor changes over time.

Since replacement carriage is likely to develop in response to use of any pneumococcal vaccine of limited valency, pneumococcal conjugate vaccines should not be regarded as the final modality for the prevention of pneumococcal diseases in The Gambia. Efforts should be continued to strengthen and improve the existing health systems to enable early diagnosis and treatment of pneumococcal diseases as important measures in control of pneumococcal diseases in The Gambia.

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Appendix 1

MEDICAL RESEARCH COUNCIL (MRC)/GAMBIAN COMMUNITY PNEUMOCOCCAL CONJUGATE VACCINE TRIAL

INFORMATION SHEET TO BE EXPLAINED TO PARENTS AND ADULT PARTICIPANTS

Pneumonia, an infection of the lungs and meningitis an infection of the brain, are both serious diseases in The Gambia causing many deaths and much serious illness. These conditions are most frequent in young children but can also affect adults.

The germs that cause pneumonia and meningitis are often found in the throat of people who are quite healthy but the germs can spread from these healthy people to other people who may then become seriously sick. The germs have many different types. MRC is working with the Government of The Gambia on the evaluation of vaccines to prevent some kinds of pneumonia and meningitis. A successful trial of a pneumonia vaccine was concluded in URD and CRD last year.

MRC would want to know whether or not there would be changes in the types of this germ in the throat when the vaccine is used in a large scale in the future. You and members of your family are invited to take part in the study.

All children up to the age of 30 months will receive 3 doses, at one monthly interval, of a pneumococcal vaccine that has been licensed and in use in the USA since year 2000 without any serious adverse effects. A group of older children and adults in 10 villages will also receive pneumococcal conjugate vaccine. A second group of older children and adults in 11 villages will receive a single dose of meningococcal polysaccharide vaccine.

We will place a thin cotton-tipped soft plastic swab into one of the nostrils to collect some secretions from the throat. This procedure is not painful and is not associated with any risk of injury. It will only cause mild and temporary discomfort to the person. For most of the villagers it will be done only once. For a number of persons it will be done a number of times and we will explain to the parents or participants before it is done.

You and some members of your household will be asked to contribute a small volume of blood, about one spoonful (5 ml) and saliva when the swab is taken. These samples will be tested for the presence of substances that can neutralize the activities of the germs.

You and your family can withdraw from this study at any time. Participation in the study is entirely voluntary and will not interfere with standard healthcare that you and your family would normally receive or with their routine vaccination.

Do you have any questions about the study?

Do you agree to join the study? If you agree the consent form will be read to you before you sign the form.

The field assistant will countersign the consent form to indicate the compound heads understood the explanation and freely gave their consent

Contact information: If you require further information about the study, please contact the following people at the addresses shown below:

1. Dr Richard Adegbola
MRC Laboratories, Fajara

P O Box 273 Banjul. The Gambia

Phone: 495442 (work)

Phone: 496580 (home)

2. Dr Uzochukwu Egere
MRC Laboratories

Phone: 7820343.

Appendix 2

Gambian Community Pneumococcal Studies, Sibanor Vaccine Trial
Form 007: NASOPHARYNGEAL SWAB CULTURE REQUEST FORM

701 Subject ID

703 Health card number |__|__| |__|__|__|__|__| |__|__|

704 First name:

Last Name:

708. Laboratory number of specimen: **NPS**|__|__|__|__| |__|

709 Date specimen was received in laboratory |__|__|/|__|__|__|/|__|__|__|__|

710 Time specimen was received in laboratory |__|__| |__|__| *(Please24 Hour clock System)*

711 *Streptococcus pneumoniae* isolated |__| (0=No, 1=Yes)

If yes, antibiotics sensitivity (zone size in mm):

Oxacillin (1ug) zone size…….mm |__| (1=S, 2=R, 3=M)

Penicillin (10 units) zone size…….mm |__| (1=S, 2=R, 3=M)

Ampicillin (10 ug) zone size…….mm |__| (1=S, 2=R, 3=M)

Chloramphenicol (30ug)zone size…….mm |__| (1=S, 2=R, 3=M)

Appendix 3

Gambia Community Pneumococcal Studies, Sibanor Vaccine Trial

Form 011: Risk factor Data Questionnaire (New Births

1101 Child's Study ID **Sample serial ID:** |_ _|

(eg A for First sample, B for Second sample, C for Third sample etc..)

1102 Mother's First Name: **Last Name:**

1104 Child's First Name |__|__|__|__|__|__|__|__|	Last Name |__|__|__|__|__|__|__|__|
1105 Date of Birth:	
1106 Gender	(M=Male, F=Female)

1110 Relation of interviewee to this child |__|

(1=Mother, 2=Father, 3=Grandmother, 4=other blood relatives, 5= other adult)

1111 Is this child currently breastfed? |__|
(0=No, 1=Mixed, 2=Breast milk with water, 3=Exclusive breast feeding)

1112 Is this child currently receiving any other feed apart from breast milk? |__| (0=No, 1=Yes)

1113 Has the child had any antibiotics in the last two weeks? ***(Check health card)***|__|
(0=No, 1=Yes , 8=Not applicable, 9=Not known)

1114 If yes to question 1113, what were the antibiotics from most recent prescription within the last two weeks?

First antibiotic	Second antibiotic
|__|__|	|__|__|
(**1115**)	(**1116**)

(1=Co-trimoxazole (Septrin), 2=Amoxycillin, 3=Penicillin, 4=Ampicillin, 5=Chloramphenicol, 6=Erythromycin, 7=Gentamicin, 8=Cloxacillin, 10=Tetracycline, 11=Nitrofurantoin, 12=other, specify……., 88=not

1018 Has the child had any ear discharge visible in the last two weeks? (observe) |__|
(1=Right, 2=Left, 3=Both, 4=None, 5=child was not at home, 9=Not known)

1019 Has the child had any chest infection in the last two weeks? |__| (0=No, 1=Yes)

1020 Was nasopharyngeal swab taken? |__| (0=No, 1=Yes)

1021 If no to question 1119, why? | | **(Attach consent form)**