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Supplementary appendix

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Webappendix

Impact of the introduction of pneumococcal conjugate vaccination on pneumonia in The Gambia: population-based surveillance and case-control studies

1. Supplementary methods

1.1 Longitudinal study of vaccine impact

1.1.1 Study objective

The objective of this study was to determine the impact of the routine introduction of pneumococcal conjugate vaccine (PCV) on the incidence of radiologic pneumonia and invasive pneumococcal disease at the population-level.

1.1.2 Study setting

Clinical surveillance was conducted at all the health facilities which provide inpatient services in the area of the Basse Health and Demographic Surveillance System (BHDSS) in the Upper River Region, on the south bank of the River Gambia. The health needs of this population are served by the Basse Health Centre, which receives referrals from five smaller health facilities, Gambisara, Demba Kunda, Fatoto, Garawol, and Koina. There are no private health facilities in this area which provide inpatient services. Maternal-child-health teams provided immunization services at three base clinics and 37 outreach sites. In 2009, the estimated infant and under-5 year mortality rates in the BHDSS were 33 and 62 per 1,000 live births respectively. HIV prevalence among antenatal women in the area is around 1.0%.[1] *Plasmodium falciparum* is endemic with seasonal transmission during the wet season between August and December each year. The majority of the population engage in subsistence farming.

1.1.3 Surveillance population

The BHDSS, which covers an area of 1111 square kilometres, has been in operation since 2007. All residents of the BHDSS aged 2 months and greater were included in the study. Residence was defined as continual residence within the BHDSS for 4 or more months, or birth to a resident woman, as determined at 4-monthly visits to all households. The population of the BHDSS is enumerated every 4 months with recording of all births, deaths, and migrations. The estimated population in 2008 was 146 876, increasing to 179 108 in 2014. Although individuals who are not resident in the BHDSS do present to the health facilities in the area, this analysis includes only individuals who were confirmed resident at the time of illness. Residence was confirmed by the individual having been enumerated in the BHDSS population listing. Individuals enrolled in surveillance who are not found in the BHDSS population listing were visited at their household in order to ascertain residential status; if confirmed as residents they were enumerated through BHDSS procedures.

1.1.4 Case ascertainment

Patients were eligible for recruitment if they presented as an outpatient, or were admitted to one of the six health facilities in the BHDSS between May 12, 2008 and December 31, 2015. Patients who presented to any of these health facilities were screened for referral to a clinician by designated surveillance staff using standardised criteria (Table S1). Clinicians assessed patients referred to them using standardised diagnostic criteria (Table S2). A diagnosis of suspected pneumonia, septicemia, and/or meningitis led to standardized investigations (Table S3). Chest radiographs were taken on all cases of suspected pneumonia and if the attending clinician judged the investigation beneficial to patient management.

Clinicians recorded findings of clinical history and examination of every patient on standardized forms. For those aged 2-59 months, the surveillance diagnosis of suspected pneumonia was based on the clinician's clinical judgement or a presenting complaint of cough or difficulty breathing for less than 14 days and the presence of one or more of the following criteria: raised respiratory rate for age (>50 breaths/min for children aged 2-11 months, >40 breaths/min for children aged 12-59 months), lower chest wall indrawing, nasal flaring or grunting, peripheral oxygen saturation less than 92%, or focal chest signs (dull percussion note, coarse crackles, or bronchial breathing). During the course of the project it became apparent that there was a group of children who presented repeatedly with

an illness that met criteria for undertaking a chest X-ray and blood culture, but for whom, the clinicians' clinical diagnosis was one of reactive airways disease. After repeated presentations of a child for whom a diagnosis of reactive airways disease had been made, clinicians exercised their judgment regarding requests for further X-rays and blood cultures.

Weight was recorded using a digital scale (TANITA, Arlington Heights, USA) and height using a ShorrBoard® (Weigh and Measure, Olney, USA). Peripheral O_2 saturation (Nellcor N-65, Covidien, Colarado) was recorded routinely for all enrolled patients. Rapid malaria tests (ICT Diagnostics, Cape Town) were performed for all patients during the malaria transmission season from August to December each year.

Blood cultures were performed for all cases of suspected pneumonia, sepsis, or meningitis. Lumbar puncture was undertaken in cases of suspected meningitis. Lung aspiration was undertaken in selected cases of pneumonia when the following criteria were met: a) a large radiographic area of dense, peripheral, pneumonic consolidation, b) stable respiratory status, and c) written informed consent provided by the patient or parent or guardian of the participant child. Pleural fluid was aspirated for selected patients if a large pleural effusion was demonstrated on chest radiograph.

Radiographs were obtained using a portable system (HF-110A, DynaRad, Illinois, USA) with a consistent radiographic technique and digital processing (CR 120/140, Kodak, New York, USA). Procedures to produce digital images were in accordance with WHO recommendations.[2] Two readings of each radiograph (masked to identity and date) were undertaken by three independent readers and readings discordant for end-point consolidation were resolved by a paediatric radiologist. All readers were calibrated to the WHO standard for radiologic pneumonia with consolidation.[2] All readers were required to achieve very high levels of agreement on end-point consolidation with blinded samples of the WHO standard set of 222 radiographs (kappa statistic >0.8) before they read the study radiographs.

Blood was collected for culture using a sterile technique and inoculated into a culture bottle (Bactec Peds Plus, Bactec Anaerobic, Bactec Aerobic, Becton Dickinson; or conventional tryptone soy and brain heart infusion bottles for a minority of samples collected at night in outlying clinics). The weight of blood culture bottles was measured before and after sample collection. Lung aspirates were transported immediately to the MRC Basse laboratory and inoculated onto agar and examined using Gram stain. Cerebrospinal fluid, pleural fluid, and other microbiological samples were processed consistently using standard methods.[3]

All-cause mortality was defined as death of a resident recorded in the BHDSS database. Trained field workers administered standardised verbal autopsy questionnaires to respondents concerning all deaths in the BHDSS area. Possible pneumococcal deaths were defined as deaths of individuals with a verbal autopsy categorised as pneumonia, sepsis, or meningitis by the Inter-VA algorithm.[4] As complete data for possible pneumococcal mortality are not yet available this analysis is being deferred until data are complete.

1.1.5 Case definitions

All events included in the analysis of longitudinal data were restricted to residents of the BHDSS. Events were defined as radiological pneumonia if a patient had a suspected diagnosis of pneumonia and the radiological end-point consolidation as per the procedure for classification according to the WHO standard.[2] If multiple radiographs were taken during an episode of illness the worst radiographic appearance in the 3 days following the date of screening was accepted at final.

Clinical pneumonia, defined as cough or difficulty breathing for <14 days accompanied by: raised respiratory for age, lower chest wall indrawing, nasal flaring, grunting, O_2 saturation <92%, altered consciousness, inability to sit or feed, convulsions, dull chest percussion note, coarse crackles, or bronchial breathing, with isolation of *S. pneumoniae* from a sterile site.

Pneumococcal pneumonia was defined as a patient with clinical pneumonia (Table S2) accompanied by isolation of *S. pneumoniae* from a normally sterile site. Pneumococcal radiologic pneumonia was defined as WHO-defined end-point consolidation accompanied by isolation of *S. pneumoniae* from a normally sterile site. We classified pneumococcal pneumonia as being caused by a) PCV13 vaccine serotypes: 1, 3, 4, 5, 6A, 6B, 9V, 14, 18C, 19A, 19F, and 23F or b) non-PCV13 vaccine serotypes, being all other serotypes. Non-typeable isolates were excluded.

Episodes were considered as separate events if the first and subsequent consultations were at least 30 days apart, or if a pneumococcus of a different serotype was isolated during each episode. Cases of pneumococcal pneumonia in which two different serotypes were isolated were classified as two different episodes of serotype-specific IPD if the two different serotypes belonged to different serotype categories.

Hypoxic pneumonia was defined as clinical pneumonia with peripheral O_2 saturation <90%. Non-pneumococcal bacterial pneumonia was defined as a patient with clinical pneumonia accompanied by isolation from a normally sterile site of a bacterial pathogen other than *S. pneumoniae* or a contaminant*; Serratia marcescens, Serratia liquifaciens,* and *Neisseria meningitidis* were also excluded because of epidemics during the study period. Bronchiolitis was defined as clinical pneumonia with wheeze on chest auscultation without bronchial breathing or dullness to percussion combined with an absence of end-point consolidation on chest radiograph.

1.1.6 Laboratory methods

An automated system (Bactec 9050, Becton Dickinson, UK) was used for blood cultures. Bottles that signalled positive were sub-cultured onto blood agar, chocolate agar, and McConkey agar. Bottles which failed to signal within 5 days were considered negative. Isolates grown on these sub-cultures were identified using conventional microbiological techniques and biochemical tests (API, Biomerieux). Other sterile site samples were processed using consistent and standardized techniques.[3] *S. pneumoniae* was identified by colony morphology, susceptibility to ethylhydrocupreine and, if susceptibility was equivocal, by bile solubility, and reaction with polyvalent antisera (Statens Serum Institut, Copenhagen, Denmark). Isolates classified as contaminants included coagulase-negative staphylococcus, bacillus species, micrococcus species, and *Streptococcus viridans*.

Pneumococcal isolates were transported to the MRC Fajara laboratory for serotyping using a latex agglutination assay which employs factor and group-specific antisera (Statens Serum Institut, Copenhagen, Denmark). Serotypes 6A and 6B were differentiated from 6C by polymerase chain reaction using a 6C-specific DNA primer (CDC, Atlanta, USA). External quality control serotyping was performed at the National Institute for Infectious Diseases in South Africa for 10% of isolates and for all isolates of serogroups 6 or 9. The laboratories in Basse and Fajara submitted to external quality assurance programmes throughout the study (UK National External Quality Assessment Service, WHO reference laboratory Denmark, Royal Australasian College of Pathologists).

1.1.7 Vaccination

All vaccines were delivered by the Gambia Government EPI. PCV7 was introduced on August 19, 2009 without a catch-up programme and PCV13 was introduced in May 2011. The schedule included three doses at ages 2, 3, and 4 months. Children aged <6 months were eligible to receive three doses, while older children were eligible for one dose. Surveillance staff attended all vaccination clinics and recorded the administration of all vaccine doses in the BHDSS using a real-time, electronic system.

In order to illustrate the rate of uptake of PCV in different age groups included in the analysis, we plotted the coverage over time of two or more doses of PCV7 and PCV13 in the 2-11 month, 12-23 month, and 2-4 year age groups. To demonstrate vaccine coverage during the 2014/15 period which was used for comparison with the baseline period, we calculated the coverage of two or more doses of PCV before 12 months of age in a cohort of BHDSS residents born in the second half of 2014.

1.1.8 Population denominator

Annual incidence was calculated using BHDSS estimates of the age-specific mid-point population in each year. Comparisons of before and after incidence used weighted averages of the mid-point populations in the respective age-strata and annual time periods, May 12, 2008 – May 11, 2010 and 2014/2015.

1.1.9 Extrapolation of case counts in 2008 and 2010

We extrapolated the number of unobserved cases between January 1 and May 11, 2008 and also during the flood period between October 5 and November 3, 2010; radiologic pneumonia surveillance was interrupted for 8 days between October 5–12. These extrapolated cases were used for plots of annual incidence over time but not used in calculations of incidence rate ratios.

The expected number of cases from January 1 to May 11, 2008 was the product of the number of cases between May 12 and December 31, 2008 and the average of the ratios of annual cases occurring during January 1 to May 11 compared to May 12 to December 31 in 2009 and 2011-2015. The age and serotype distribution of the extrapolated cases was the same as the age and serotype distribution of observed cases in 2008 and 2009.

Unobserved cases of radiologic pneumonia in 2010 were extrapolated for the eight days of interrupted pneumonia surveillance during the flood period. Pneumococcal pneumonia cases were extrapolated for the 30 days of interrupted IPD surveillance. The expected number of unobserved radiologic and pneumococcal pneumonia cases respectively was the product of the number of observed cases in 2010 and the average of the annual ratios of cases which occurred between October 5–12, and October 5 and November 3, in 2009 and 2011-2014. The age and serotype distribution of the extrapolated cases was the same as for the observed cases in 2010.

1.1.10 Adjustment of annual case counts

The number of children presenting to health facilities and screened for referral to surveillance clinicians per unit population increased over time. We corrected the annual count of events using the same procedure as we used in an earlier analysis,[5] an approach which has been used by a number of other investigators.[6;7] We corrected for these changes in the sensitivity of case ascertainment adjusting the annual crude counts of pneumonia cases, by age group, and assuming the serotype distribution was the same as that of the observed cases each year. Annual, age-specific case counts were adjusted using the difference between the mean rate of referral of patients to surveillance clinicians during the study period and linear regression curves fitted to plots of annual rates over time. The peak rate of referral of children occurred in 2014 and 2015 and our adjustment reduced the number of cases in the latter years of surveillance and increased the number of cases in the first years of surveillance. Thus, a result of our adjustment of case counts was that the number of cases in children that are included in the incidence rate ratio calculations, comparing the first and final two years of surveillance, are slightly greater in the adjusted analysis than in the crude analysis. The proportion of children with clinical pneumonia who had a chest radiograph in the baseline and PCV13 period was not significantly different.

1.1.11 Surveillance time periods for the before and after analysis

The baseline period was from May 12, 2008 until May 11, 2010. The vaccine period was from January 1, 2014 until December 31, 2015.

Incidence was defined as the number of cases divided by the mid-point population at risk. Vaccine impact was calculated as the difference in age-specific incidence subtracting the incidence after vaccination from the incidence before the introduction of PCV,

$$IRD = Incidence\ before\ PCV - Incidence\ after\ 13PCV$$

The incidence rate ratio (IRR) was calculated as the ratio of incidence after to that before PCV, introduction indicating vaccine effectiveness at the population level. Vaccine effectiveness was calculated as (1-IRR) x 100.

$$IRR = Incidence\ after\ 13PCV / Incidence\ before\ PCV$$

The IRR analysis was based on a Poisson distribution. We assessed the validity of this distributional assumption by modelling the age-specific incidence of radiologic pneumonia using pre-PCV data from 2008–2009. Our assessment of distributional validity indicated the Poisson distribution was acceptable for the 2-11 and 12-23 month age groups but that there was significant over dispersion in the 2-4 year age group. We therefore used a variance inflation factor of 1.22 to account for the overdispersion in the 2–4 year age group.

1.1.12 Bias and confounding

To investigate potential bias due to temporal changes in health care seeking behaviour we conducted an *a priori* stratified analysis excluding outpatients. The IRR estimates using the crude data and data excluding outpatients were compared and if they differed by greater than 20% we concluded that clinically significant confounding was present. We conducted these stratified analyses of the IRR using crude data and data with adjusted case counts accounting for changes over time in the sensitivity of case ascertainment (section 3.5). We determined the adjusted, stratified,

and age-specific annual case counts by multiplying the adjusted count by the proportion of radiological pneumonia cases treated as an outpatient in the baseline and PCV13 periods and by age strata.

In order to account for potential bias due to temporal changes in patient investigation and confounding due to secular trends in serotypes we conducted *a priori* stratified analyses excluding cases of pneumococcal pneumonia detected by lung aspiration alone and cases caused by serotype 1 or 5. We determined the adjusted, stratified, and age-specific annual case counts by multiplying the adjusted count by the proportion of cases detected by lung aspiration alone and the proportion due to serotype 1 or 5 in the baseline and PCV13 periods within age strata.

To assess the effect of temporal trends related to bacterial pneumonia, we evaluated the crude and adjusted incidence of clinical pneumonia due to bacteria other than pneumococcus obtained by the surveillance system, as a control condition. This is an ideal control condition because it is diagnosed in the same manner as the target condition and has the same risk factors but is not influenced by PCV. Non-pneumococcal bacterial pneumonia was defined as a positive blood culture in which the isolate was not a contaminant, *S. pneumoniae*, *Serratia liquefaciens*, or *N. meningitidis*. *S. liquefaciens* was not included as we experienced a nosocomial epidemic of this bacterium in the latter half of 2010. Meningococcal isolates were not included in non-pneumococcal bacteraemia because of the epidemic nature of meningococcal disease; in fact, the surveillance area experienced an epidemic of meningococcal disease in 2012.[6] Non-pneumococcal bacterial pneumonia was detected using the same procedures as for detection of pneumococcal pneumonia and it is unlikely to be influenced by PCV. Therefore, the incidence of non-pneumococcal bacterial pneumonia is a robust control condition indicating potential changes in the sensitivity of surveillance and/or changes in population risk factors for bacterial pneumonia and pneumococcal pneumonia which are unrelated to PCV. Annual counts of non-pneumococcal bacterial pneumonia were extrapolated in 2008 and 2010 in same manner as for radiologic pneumonia. We also adjusted the annual and comparison period counts of non-pneumococcal bacterial pneumonia in order to plot incidence over time and to evaluate the IRR.

In our setting, significant risk factors for radiologic pneumonia are malnutrition and malaria. Therefore, we also evaluated whether there were any changes over time in the prevalence of malnutrition or malaria in patients who were enrolled in surveillance with suspected pneumonia.

1.2 Case-control study of PCV13 effectiveness against radiologic pneumonia

1.2.1 Study objective

The objective of this study was to determine the individual-level effectiveness of PCV13 against radiological pneumonia with consolidation in a population-based case-control study.

1.2.2 Study design

The study was conducted within the BHDSS (estimated population in 2015 – 179,550), located in the Upper River Region of The Gambia and in the Fuladu West Health and Demographic Surveillance System (FWHDSS, estimated population in 2014 - 92 464) in Central River Region (Fig. S1). The Fuladu West population is served by Bansang Hospital and two outlying clinics (Jakhaly and Brikamaba). The BHDSS setting is described in sections 2.1.2 and 2.1.3.

The case control study was embedded within a larger programme of surveillance in the BHDSS to assess the impact of the introduction of PCV into routine immunisation.

During the period of surveillance (BHDSS, January 2011 – September 2014; FWHDSS, September 2011-September 2014), all paediatric patients who presented at one of the clinics were screened by a surveillance nurse using a list of criteria suggestive of pneumonia, meningitis or sepsis. Patients who met screening criteria were referred to a surveillance clinician in Basse or Bansang (patients in Jakhaly and Brikamaba were only referred if the surveillance nurse suspected meningitis). Standardised criteria were used to categorise patients within the clinical syndromes of suspected pneumonia, sepsis, and/or meningitis. Patients with suspected pneumonia had a chest X-ray and blood culture. All children who were admitted with a medical problem had a blood culture and patients with suspected meningitis had a lumbar puncture if there were no contraindications. Patients in Brikamaba and Jakhaly with suspected pneumonia had chest X-rays performed locally. Initially, cases were restricted to children who were admitted overnight. In March 2012 the protocol was modified to screen and investigate children being treated as outpatients for suspected pneumonia.

Radiographic procedures in the BHDSS were described in section 2.1.4. In the FWHDSS radiographs were performed using the same procedures as in the BHDSS but films were processed using automated wet development (Agfa Curix 60, Agfa-Gevaert, Mortsel, Belgium) and digitization of the hard radiographs (Sierra Advantage, Vidar, Herndon, VA, USA). All radiographs were interpreted by two independent, trained and calibrated readers using the WHO standard for radiologic pneumonia with consolidation in children.[2] Discordant readings were resolved by a third trained and calibrated reader. All readers were recalibrated to the WHO standard every 6 months achieving kappa scores ≥0·7 before reading radiographs.

1.2.3 Case definition

Radiologic pneumonia with consolidation was diagnosed when two readers agreed that dense fluffy, or alveolar, consolidation was present in a segment, lobe, or whole of a lung, or when a pleural effusion was present – as described in the WHO standard for radiologic pneumonia.

1.2.4 Exposure definition

Vaccine exposure was determined by the number of PCV13 doses received 14 days before the date of clinical evaluation for the index case. The primary comparison was between vaccinated and unvaccinated individuals:

1. An unvaccinated individual was defined as someone who has not received any doses of PCV13 or PCV7 (i.e., no doses recorded in the BHDSS or FWHDSS vaccination records, on the health card, and when the health card was unavailable, no vaccination according to clinic records or parental recall).
2. An individual was considered vaccinated if they had received three doses of PCV13 (by BHDSS or FWHDSS vaccination records, health card and/or were definitively identified in a vaccination register).

Cases or controls without an immunisation history were excluded from the analysis (i.e., they were excluded if they were without immunisation data from the BHDSS and FWHDSS vaccination records, the health card, the clinic vaccination register, or parental recall).

1.2.5 Inclusion criteria

To be eligible for the study, cases of radiologic pneumonia had to be:

- Aged 90 days or greater.
- Resident in the study area since age 2 months.
- Eligible to have received at least one dose of PCV13, 14 days or more before the date of illness.

1.2.6 Exclusion criteria

Children were excluded from the study if:

- Younger than 90 days.
- They had received three doses of PCV7.
- Resident outside the study area.
- They had a major congenital malformation.
- They or their mother were enrolled at an HIV clinic.
- They had clinical findings consistent with immune deficiency.
- They had been previously enrolled as a case.

1.2.7 Enrolment of controls

Initially, two controls were enrolled for each case of radiologic pneumonia. The protocol was modified in March 2012 to enrol three controls for each case of radiologic pneumonia. Controls were enrolled using up-to-date lists of 10 resident children matched to the index case on date of birth +/- 15 days. The lists were created using the population registers of the BDHSS and FWHDSS, and children were ordered in terms of their proximity in age to the index case. Individual children could be enrolled more than once as controls but were excluded if previously enrolled as a case. When the chosen control could not be recruited at an initial visit, up to two additional attempts, a week apart, were made to recruit the control before the next control from the list was approached. Potential controls were approached in order of their proximity in age to the index case. A control was usually recruited within 3 months of the case being identified, although occasionally controls were recruited outside this time window. A proportion of the potential controls could not be matched to the index case because their true date of birth was

different to that recorded in the population register (true dates of birth were defined as dates recorded on antenatal cards, child health cards, and which agreed with the date of first immunisation). In such cases the next child was selected from the list of potential controls - i.e., children with incorrect dates of birth were not eligible for enrolment.

Field workers visited the child's mother at home (both cases and controls) to collect socio-demographic and vaccination data using a structured questionnaire. The mothers of cases were interviewed at home within 1 month of visiting the health facility. Data on routine vaccinations, including PCV, were collected from the child's health card; when this was not available, the clinic register was used, or the mother was asked to recall if the child had been vaccinated.

1.2.8 Sample size calculation

To determine the sample size, we assumed a vaccine coverage of 90% in controls (10% received 0 doses and 90% received 3 doses). Based on this assumption, we calculated that 881 cases of radiological pneumonia would give 80% power to detect a vaccine effectiveness of 35% against radiological pneumonia.

1.2.9 Statistical methods

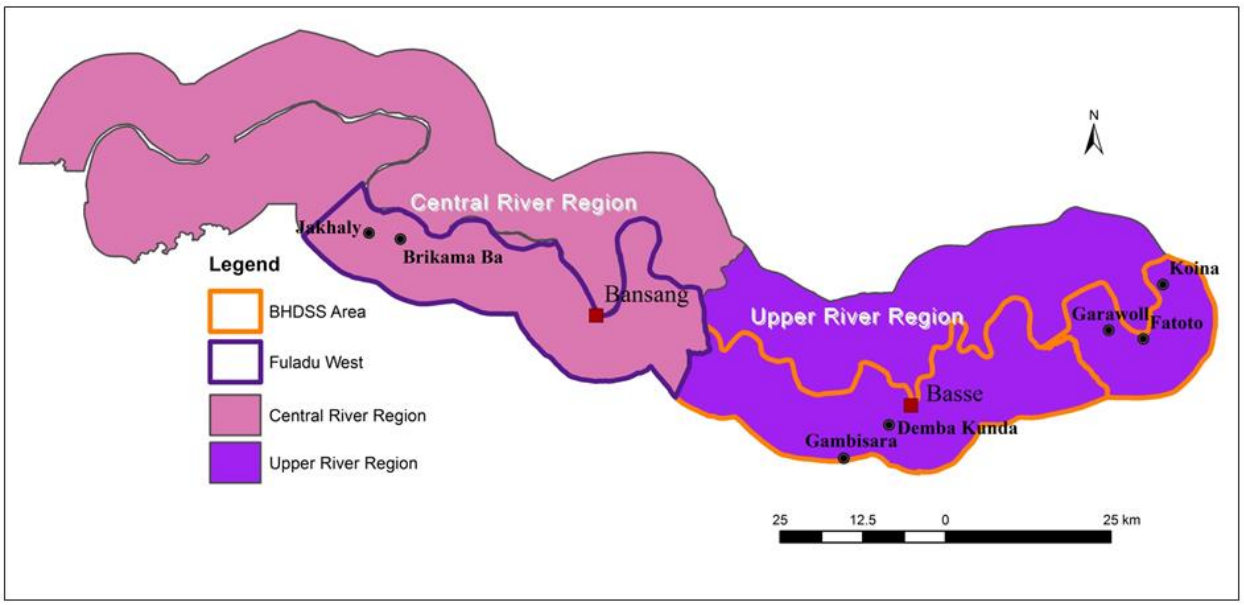
The primary study question was the effectiveness of PCV13 against radiologic pneumonia with consolidation. Thus, children who had received one or more doses of PCV7 were excluded from the analysis. Vaccine effectiveness was defined as 1-OR, where OR is the odds ratio comparing 0 doses of PCV (i.e., no PCV7 or PCV13) with three doses of PCV13 (and no doses of PCV7). We also included analyses of vaccine effectiveness for one, two, and two or more doses of PCV13. The odds ratio was estimated using a conditional logistic regression to account for the age-matching.

By design, estimates of vaccine effectiveness were adjusted for age, since cases and controls were matched on age. We tested for and failed to detect collinearity between the potential confounding variables that were associated with cases compared to controls. Estimates of effectiveness were also adjusted for potential confounding due to sex, mother's age, mother's educational level, number of children in the household aged <5 years, number of children sleeping in the room where the child sleeps, socioeconomic status based on asset score, illness within the last 3 months, previous hospitalisation, distance to health care facility, and nutritional status (*Z*-scores were computed with the zanthro command in Stata, using the 2000 US growth standards as the reference distribution). All potential confounding variables were included in the model as no selection method is uniformly superior to adjusting for all well-measured confounders and numbers of cases were sufficient to fit the model.[8] Statistical significance was set at an alpha level <0·05.

As a high proportion of Gambian children are fully vaccinated before 12 months of age, estimates of PCV13 effectiveness were stratified by age 3-11 months and ≥12 months.

The inference from the calculated vaccine effectiveness in this population-based case-control study is that exposure of the population to vaccination reduces the incidence of radiologic pneumonia by number of percentage points of vaccine effectiveness.[9]

2. Supplementary figures



Basse Health and Demographic Surveillance System, in Upper River Region outlined in orange; Fuladu West Health and Demographic Surveillance System, in Central River Region outlined in purple.

Figure S1: Map showing the location of the Basse and Fuladu West Health and Demographic Surveillance Systems in the rural east of The Gambia, West Africa, with the position of study health facilities shown

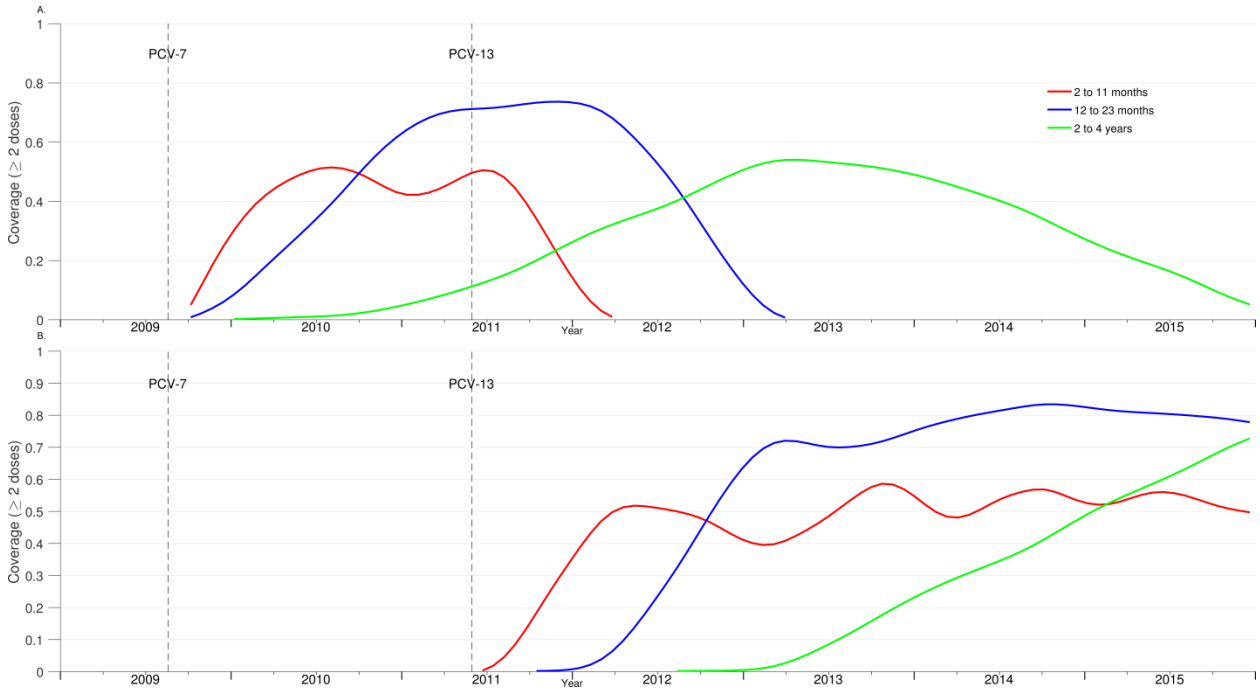


Figure S2: Coverage of two or more doses of A) PCV7 and B) PCV13, by age group over time

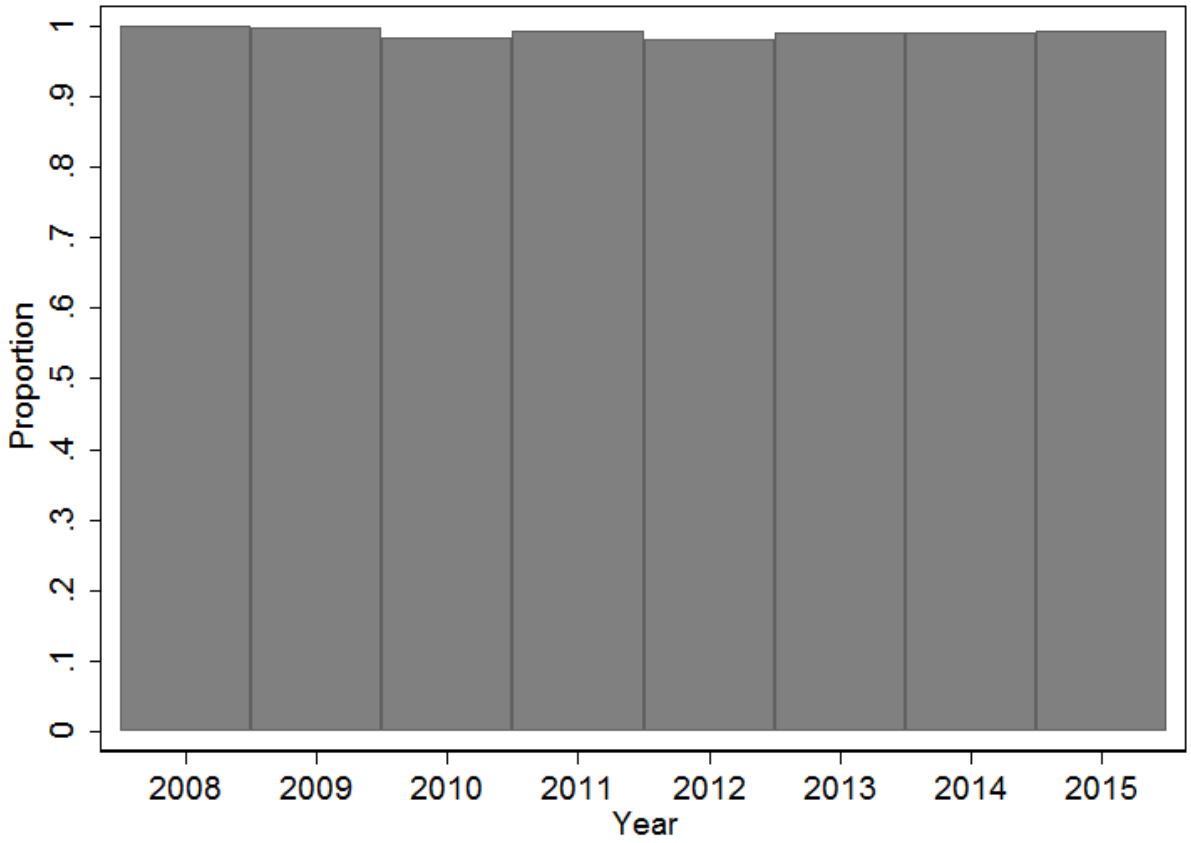


Figure S3: Proportion of children aged 2-59 months meeting referral criteria who were assessed by a clinician, by year of the study

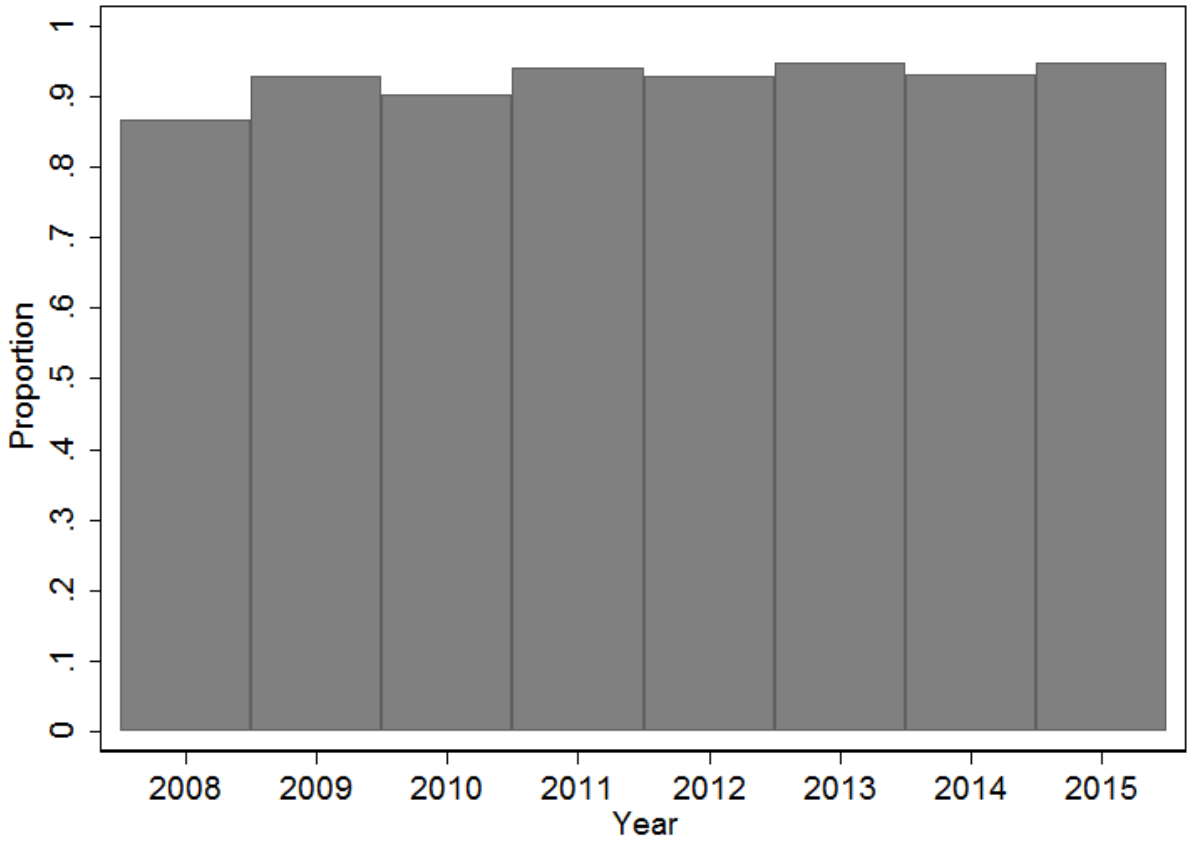


Figure S4: Proportion of children aged 2-59 months meeting referral criteria who had suspected pneumonia, by year of the study

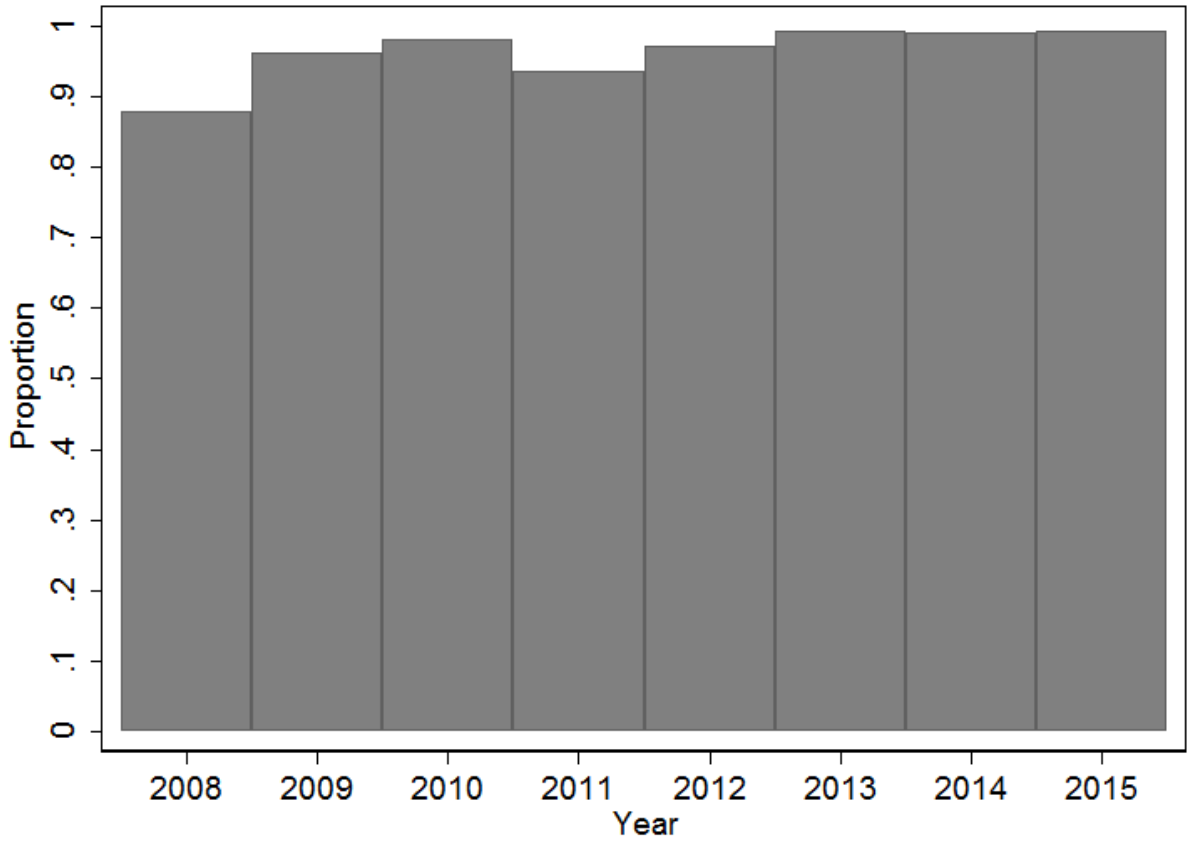


Figure S5: Proportion of children aged 2-59 months with suspected pneumonia who had a chest radiograph, by year of the study

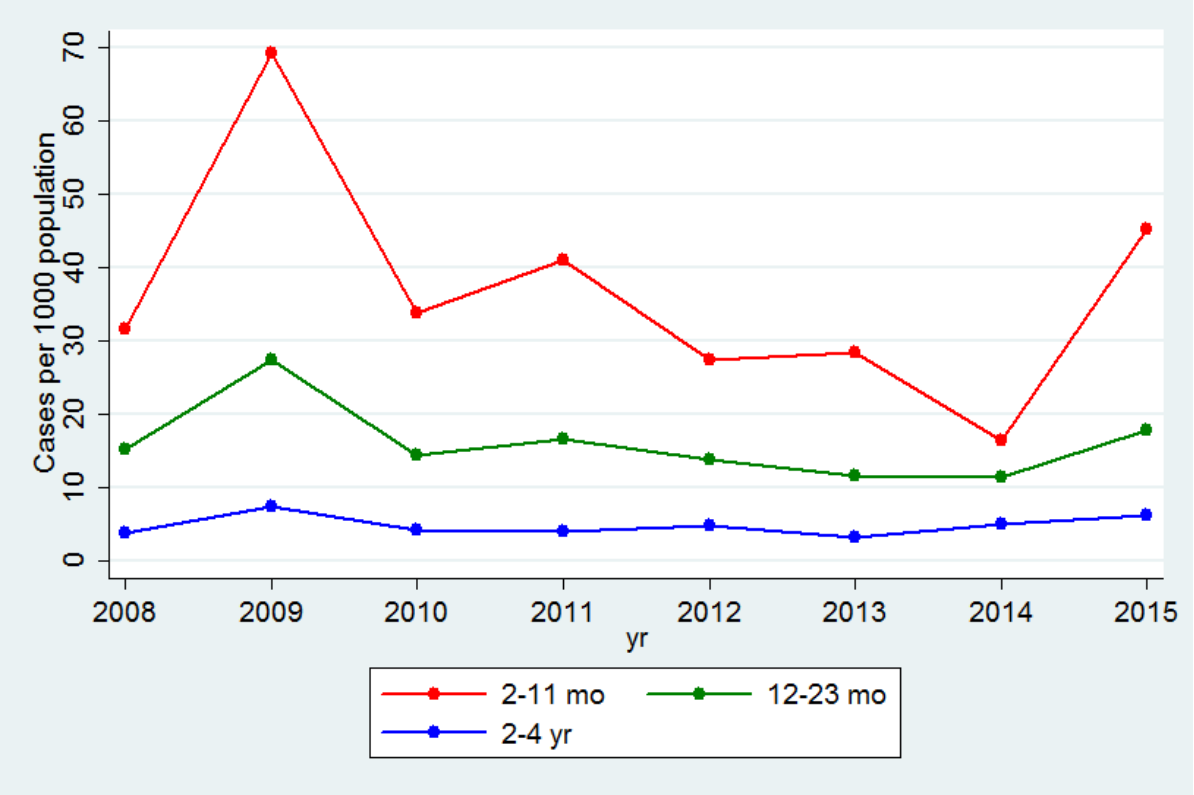


Figure S6: Adjusted incidence of bronchiolitis between 2008 and 2015, by age group

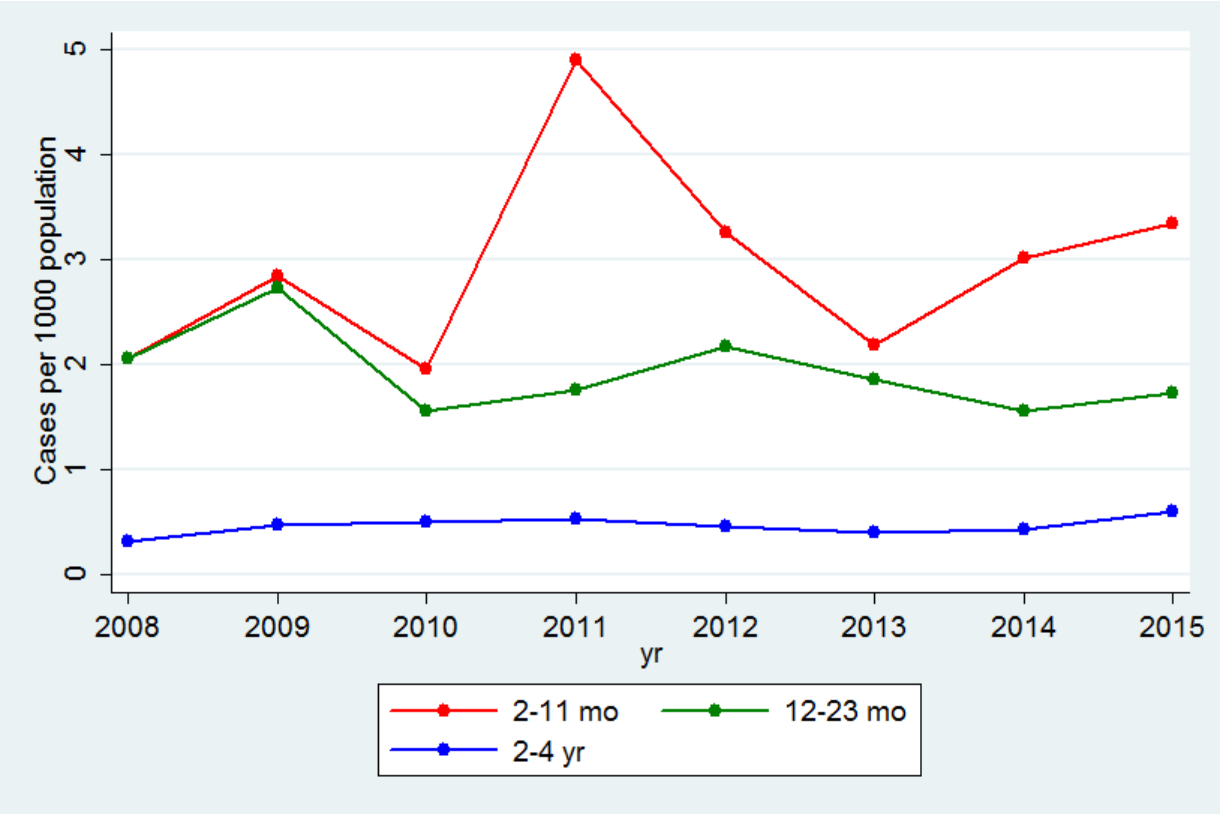


Figure S7: Adjusted incidence of non-pneumococcal bacterial pneumonia between 2008 and 2015, by age group

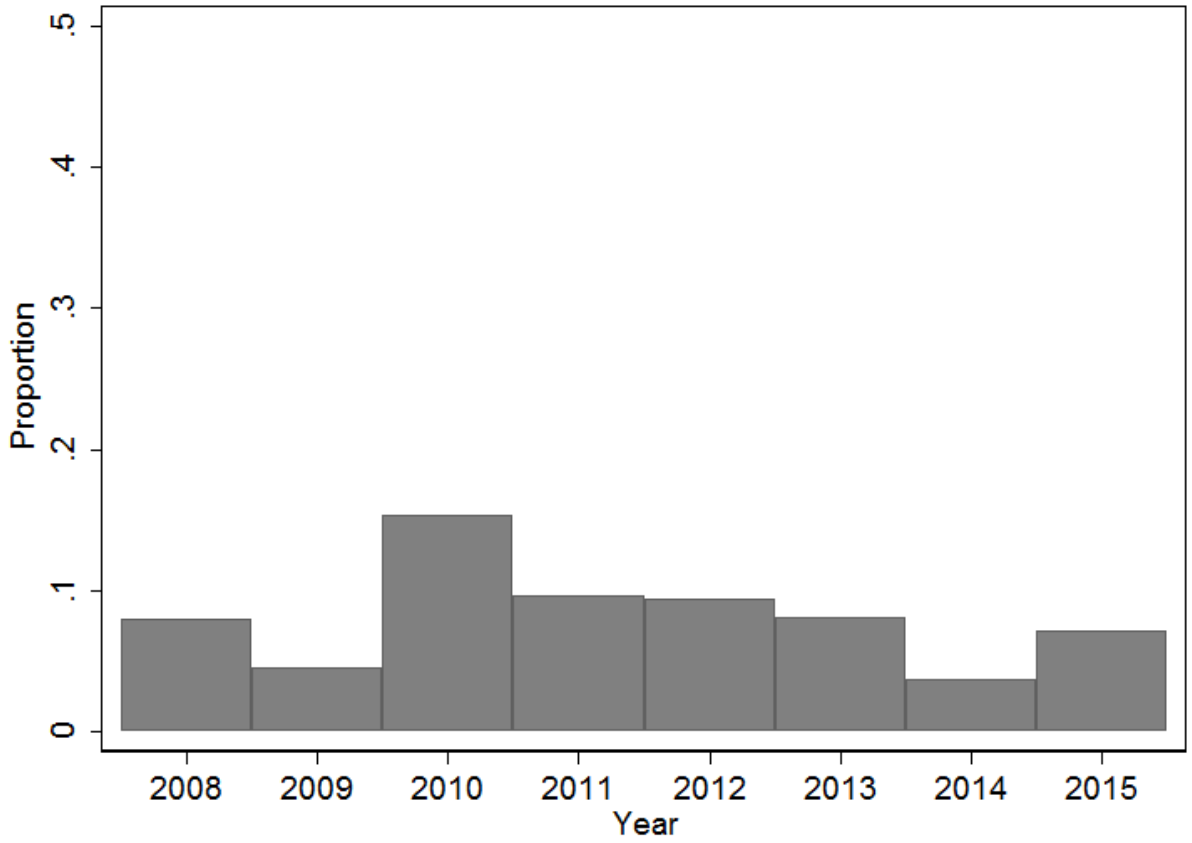


Figure S8: Proportion of children aged 2-59 months with suspected pneumonia in the wet season who had a positive malaria test, by year of the study

Note: the prevalence of malaria in study participants aged 2-59 months in the 'baseline' period and 2014/15 period was not significantly different.

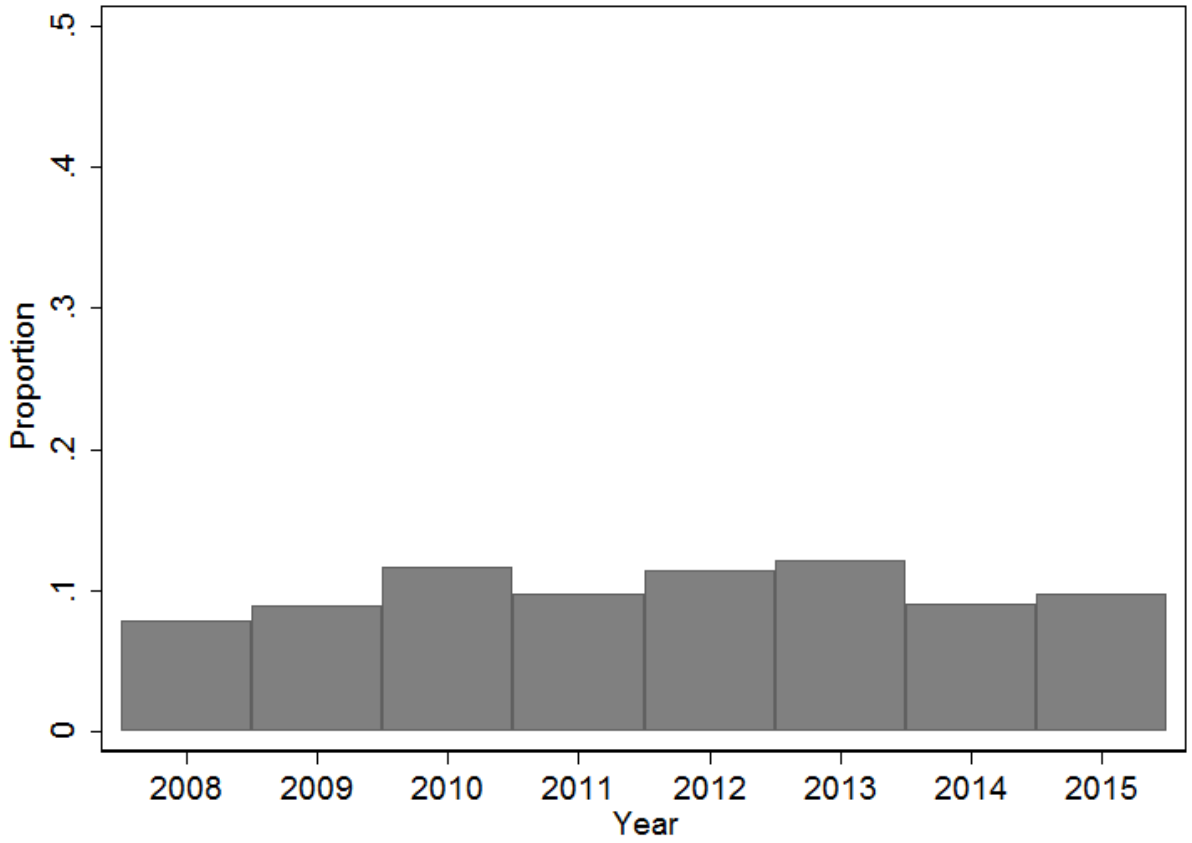


Figure S9: Proportion of children aged 2-59 months with suspected pneumonia who had weight-for-height z-score < -3 by year of the study

3. Supplementary Tables

Table S1: Screening criteria for referral of out- and in-patients for clinician assessment (if one or more criteria are present for 14 days or less)

Patients aged 2 to 59 months

- History of cough or difficulty breathing, plus raised respiratory rate for age
- Lower chest wall indrawing, nasal flaring, or grunting
- Oxygen saturation less than 92%
- History of convulsion
- Impaired consciousness[*]
- Bulging fontanelle
- Stiff neck
- Axillary temperature at least 38°C, or less than 36°C, in a patient admitted or being admitted
- Prostration[†]
- Weight below -3 z score for age
- Local musculoskeletal swelling or tenderness
- **Irrespective of age or residential location, any child with possible meningitis**

[*]Impaired consciousness is defined as V, P, or U on the AVPU score, where A is if the patient is alert, V if responsive to verbal stimulus, P if responsive to pain stimulus, and U if unresponsive. [†]Prostration is defined as an inability to drink or breast feed, or to remain in a seated position in a child otherwise able to do so.

Table S2: Clinical definitions for suspected pneumonia, septicaemia, and meningitis

	Age ≥2 months and <5 years
Suspected pneumonia	Suspected pneumonia is defined if there is a history of cough or difficulty breathing of less than 14 days' duration, accompanied by one or more of: 1. Raised respiratory rate for age* 2. Lower chest wall indrawing, nasal flaring or grunting 3. Oxygen saturation less than 92% 4. Focal chest signs (dull percussion note, coarse crackles, bronchial breathing)
Suspected meningitis	Suspected meningitis will be defined according to clinical judgement and is to be considered if any of the following are present: 1. Neck stiffness 2. Impaired consciousness† 3. Prostration‡ 4. History of convulsion 5. Bulging fontanelle
Suspected septicaemia	Suspected septicaemia will be defined as one or more of: 1. Clinician diagnosis of focal sepsis (including but not limited to: septic arthritis, osteomyelitis, endocarditis, peritonitis, liver abscess, soft tissue abscess, cellulitis) 2. For a patient admitted, or being admitted, axillary temperature is <36°C or ≥38°C and no obvious cause of fever 3. For a patient admitted, or being admitted, the clinical impression is of severe malnutrition§

*Raised respiratory rate for age is defined as greater than 50 breaths per minute for children at least 2 months but less than 12 months, and as greater than 40 breaths per minute for children at least 12 months but less than 60 months.
†Impaired consciousness is defined as V, P, or U on the AVPU score, where A is if the patient is alert, V if responsive to verbal stimulus, P if responsive to pain stimulus, and U if unresponsive.
‡Prostration is defined as inability to drink or breast feed, or to remain in a seated position in a child otherwise able to do so.
§Severe malnutrition is defined according to the WHO definition.[10]

Table S3: Guideline for investigation of patients referred to clinicians and diagnosed with suspected pneumococcal disease according to clinical definitions

a.	Patients with suspected pneumococcal disease are to have blood culture.
b.	Patients with suspected meningitis are to have lumbar puncture.
c.	Patients with suspected pneumonia are to have chest X-ray.
d.	Chest X-ray should also be considered in patients with meningitis or septicaemia if the clinician's impression is of co-existing pneumonia or if it is judged that a chest X-ray will assist in management.
e.	Lung aspirate should be considered for a patient if peripheral consolidation has been demonstrated, preferably by X-ray.
f.	Other investigations including pleural tap and joint aspirate may be considered according to the clinical indication.
g.	Patients with suspected pneumococcal disease are to have,
	i. a rapid diagnostic test for malaria (January – July; only if surveillance number ends in '0').
	ii. serum collection for antibiotic activity detection if surveillance number ends in '0' or '5' and the patient is enrolled in Basse.

Table S4: Crude and adjusted number of cases and incidence of bronchiolitis in the baseline period of 12 May 2008 to 11 May 2010 and the 2014/15 period post-vaccine introduction, by age group

Age group	5/2008–4/2010 adjusted (crude) cases	5/2008–4/2010 adjusted (crude) incidence per 1000 person-years	2014/15 adjusted (crude) cases	2014/15 adjusted (crude) incidence per 1000 person-years	Crude incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)	Adjusted incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)
2–11 months	524 (441)	51·7 (43·5)	413 (495)	31·4 (37·6)	0·86 (0·76–0·98)	0·61 (0·53–0·69)
12–23 months	258 (208)	21·4 (17·3)	233 (286)	15·2 (18·7)	1·08 (0·90–1·29)	0·71 (0·59–0·85)
2–4 years	191 (169)	5·9 (5·2)	234 (273)	5·8 (6·7)	1·29 (1·06–1·56)	0·98 (0·81–1·18)

Adjusted case counts take account of changes over time in the rate of patients meeting criteria for referral, and are rounded to the nearest integer. Bronchiolitis was defined as clinical pneumonia with auscultatory wheeze without radiologic pneumonia, dullness to percussion or bronchial breathing.

Table S5: Crude and adjusted number of cases and incidence of pneumococcal pneumonia endpoints, excluding cases identified by lung aspiration alone, in the baseline period of 12 May 2008 to 11 May 2010 and in the 2014/15 period post-vaccine introduction, by age group

	5/2008–4/2010 adjusted (crude) cases	5/2008–4/2010 adjusted (crude) incidence per 1000 person-years	2014/15 adjusted (crude) cases	2014/15 adjusted (crude) incidence per 1000 person-years	Crude incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)	Adjusted incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)
Age 2-11 months						
Radiologic pneumonia						
Pneumococcal	13 (11)	1·3 (1·1)	6 (7)	0·5 (0·5)	0·49 (0·19–1·26)	0·36 (0·14–0·93)
PCV13 vaccine-type	7 (6)	0·7 (0·6)	1 (1)	0·08 (0·08)	0·13 (0·02–1·07)	0·11 (0·01–0·89)
Non-vaccine-type	5 (5)	0·5 (0·5)	5 (6)	0·4 (0·5)	0·92 (0·28–3·03)	0·77 (0·22–2·65)
Clinical pneumonia						
Pneumococcal	27 (23)	2·7 (2·3)	16 (19)	1·2 (1·4)	0·64 (0·35–1·17)	0·46 (0·25–0·85)
PCV13 vaccine-type	16 (14)	1·6 (1·4)	3 (3)	0·2 (0·2)	0·16 (0·05–0·57)	0·14 (0·04–0·50)
Non-vaccine-type	11 (9)	1·1 (0·9)	13 (16)	1·0 (1·2)	1·37 (0·60–3·10)	0·91 (0·41–2·03)
Age 12-23 months						
Radiologic pneumonia						
Pneumococcal	25 (20)	2·1 (1·7)	3 (3)	0·2 (0·2)	0·12 (0·03–0·40)	0·09 (0·03–0·31)
PCV13 vaccine-type	23 (19)	1·9 (1·6)	0 (0)	(0·0)	ns	ns
Non-vaccine-type	1 (1)	0·08 (0·08)	3 (3)	0·2 (0·2)	2·36 (0·25–22·7)	2·36 (0·25–22·7)
Clinical pneumonia						
Pneumococcal	28 (22)	2·3 (1·8)	7 (9)	0·5 (0·6)	0·32 (0·14–0·70)	0·20 (0·09–0·45)
PCV13 vaccine-type	26 (21)	2·2 (1·7)	2 (2)	0·1 (0·1)	0·07 (0·02–0·32)	0·06 (0·01–0·25)
Non-vaccine-type	1 (1)	0·08 (0·08)	6 (7)	0·4 (0·5)	5·50 (0·68–44·7)	4·72 (0·57–39·2)
Age 2-4 years						
Radiologic pneumonia						
Pneumococcal	24 (21)	0·7 (0·7)	10 (12)	0·2 (0·3)	0·46 (0·21–0·99)	0·33 (0·15–0·75)
PCV13 vaccine-type	20 (18)	0·6 (0·6)	4 (4)	0·1 (0·1)	0·18 (0·05–0·59)	0·16 (0·05–0·52)
Non-vaccine-type	3 (3)	0·09 (0·09)	7 (8)	0.2 (0·2)	2·12 (0·49–9·19)	1·86 (0·42–8·27)
Clinical pneumonia						
Pneumococcal	34 (30)	1·1 (0·9)	18 (20)	0·4 (0·5)	0·53 (0·28–0·99)	0·42 (0·22–0·79)
PCV13 vaccine-type	30 (26)	0·9 (0·8)	5 (6)	0·5 (0·1)	0·18 (0·07–0·49)	0·13 (0·05–0·38)
Non-vaccine-type	5 (4)	0·2 (0·1)	12 (14)	0·3 (0·3)	2·79 (0·82–9·51)	1·91 (0·60–6·05)

PCV13=serotypes covered by PCV13. Non-vaccine type=serotypes not covered by PCV13. Exclusion of cases from adjusted counts used the age- and year-specific distributions of cases identified by lung aspiration alone. Adjusted case counts are rounded to the nearest integer. Confidence intervals calculated taking into account overdispersed Poisson distributions in the 2–4 years age group.

Table S6: Crude and adjusted number of cases and incidence of pneumococcal pneumonia endpoints, excluding cases of serotype 1 or 5, in the baseline period of 12 May 2008 to 11 May 2010 and in the 2014/15 period post-vaccine introduction, by age group and serotype

	5/2008–4/2010 adjusted (crude) cases	5/2008–4/2010 adjusted (crude) incidence per 1000 person-years	2014/15 adjusted (crude) cases	2014/15 adjusted (crude) incidence per 1000 person-years	Crude incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)	Adjusted incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)
Age 2-11 months						
Radiologic pneumonia						
Pneumococcal	12 (10)	1·2 (1·0)	6 (7)	0·6 (0·5)	0·54 (0·21–1·42)	0·38 (0·14–1·03)
PCV13 vaccine-type	5 (4)	0·5 (0·4)	1 (1)	0·08 (0·08)	0·19 (0·02–1·72)	0·15 (0·02–1·32)
Non-vaccine-type	7 (6)	0·7 (0·6)	5 (6)	0·4 (0·5)	0·77 (0·25–2·39)	0·55 (0·17–1·73)
Clinical pneumonia						
Pneumococcal	25 (21)	2·5 (2·1)	15 (18)	1·1 (1·4)	0·66 (0·35–1·24)	0·46 (0·24–0·88)
PCV13 vaccine-type	13 (11)	1·3 (1·1)	2 (2)	0·2 (0·2)	0·14 (0·03–0·63)	0·12 (0·03–0·52)
Non-vaccine-type	12 (10)	1·2 (1·0)	13 (16)	1·0 (1·2)	1·23 (0·56–2·71)	0·83 (0·38–1·83)
Age 12-23 months						
Radiologic pneumonia						
Pneumococcal	19 (15)	1·6 (1·2)	5 (6)	0·3 (0·4)	0·31 (0·12–0·81)	0·21 (0·08–0·55)
PCV13 vaccine-type	16 (13)	1·3 (1·1)	2 (2)	0·1 (0·1)	0·12 (0·03–0·54)	0·10 (0·02–0·43)
Non-vaccine-type	2 (2)	0·2 (0·2)	4 (4)	0·3 (0·3)	1·57 (0·29–8·58)	1·57 (0·29–8·58)
Clinical pneumonia						
Pneumococcal	19 (15)	1·6 (1·2)	10 (12)	0·7 (0·8)	0·63 (0·29–1·34)	0·41 (0·19–0·89)
PCV13 vaccine-type	16 (13)	1·3 (1·1)	4 (4)	0·3 (0·3)	0·24 (0·08–0·74)	0·20 (0·07–0·59)
Non-vaccine-type	2 (2)	0·2 (0·2)	7 (8)	0·5 (0·5)	3·14 (0·67–14·8)	2·75 (0·57–13·2)
Age 2-4 years						
Radiologic pneumonia						
Pneumococcal	16 (14)	0·5 (0·4)	10 (11)	0·2 (0·3)	0·63 (0·26–1·50)	0·50 (0·21–1·19)
PCV13 vaccine-type	12 (11)	0·4 (0·3)	2 (2)	0·05 (0·05)	0·14 (0·03–0·76)	0·13 (0·03–0·69)
Non-vaccine-type	3 (3)	0·09 (0·09)	8 (9)	0·2 (0·2)	2·39 (0·56–10·1)	2·12 (0·49–9·19)
Clinical pneumonia						
Pneumococcal	22 (20)	0·7 (0·6)	15 (17)	0·4 (0·4)	0·68 (0·33–1·38)	0·54 (0·26–1·12)
PCV13 vaccine-type	18 (16)	0·6 (0·5)	2 (2)	0·2 (0·05)	0·10 (0·02–0·50)	0·09 (0·02–0·44)
Non-vaccine-type	5 (4)	0·2 (0·1)	13 (15)	0·3 (0·4)	2·99 (0·88–10·1)	2·07 (0·66–6·47)

PCV13=serotypes covered by PCV13. Non-vaccine type=serotypes not covered by PCV13. Exclusion of cases from adjusted counts used the age-, year-, and serotype category-specific distributions of case due to serotypes 1 or 5. Adjusted case counts are rounded to the nearest integer. Confidence intervals calculated taking into account overdispersed Poisson distributions in the 2–4 years age group.

Table S7: Crude and adjusted number of cases and incidence of clinical pneumonia caused by non-pneumococcal bacteria in the baseline period of 12 May 2008 to 11 May 2010 and the 2014/15 period post-vaccine introduction, by age group

Age group	5/2008–4/2010 adjusted (crude) cases	5/2008–4/2010 adjusted (crude) incidence per 1000 person-years	2014/15 adjusted (crude) cases	2014/15 adjusted (crude) incidence per 1000 person-years	Crude incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)	Adjusted incidence rate ratio 2014/15 relative to 5/2008–4/2010 (95% CI)
2–11 months	25 (21)	2·5 (2·1)	42 (50)	3·2 (3·8)	1·83 (1·10–3·05)	1·29 (0·79–2·12)
12-23 months	27 (22)	2·2 (1·8)	26 (31)	1·7 (2·0)	1·11 (0·64–1·91)	0·76 (0·44–1·30)
2–4 years	12 (10)	0·4 (0·3)	22 (25)	0·5 (0·6)	1·99 (0·89–4·47)	1·46 (0·67–3·17)

Adjusted case counts take account of changes over time in the rate of patients meeting criteria for referral, and are rounded to the nearest integer.

Table S8: Characteristics of cases and controls in the Gambian case-control study of the effectiveness of 13-valent pneumococcal conjugate vaccine against radiologic pneumonia

Characteristic		Cases (N=733) n (%)	Controls (N=2105) n (%)	*p*-value
Demographics				
Age in months, median (IQR)		10·6 (6·4-17·1)	10·6 (6·4-17·1)	0·913
Male		399 (54·4)	1073 (51·0)	0·112
Year of enrolment	2011	51 (7·0)	114 (5·4)	
	2012	168 (22·9)	483 (22·9)	
	2013	288 (39·3)	839 (39·9)	
	2014	226 (30·8)	669 (31·8)	0·488
Maternal age in years, median (IQR)		28·0 (23·0-32·0)	28 (23·0-32·0)	0·78
Mother's education	None	128 (17·5)	408 (19·4)	
	Lower basic	71 (9·7)	194 (9·2)	
	Upper basic	32 (4·4)	147 (7·0)	
	Secondary	22 (3·0)	55 (2·6)	
	Koranic	480 (65·5)	1301 (61·8)	0·064
No. people living in household		25·0 (14·0-38·0)	24·0 (14·0-40·0)	0·724
No. children <5 years in household*		5·0 (3·0-8·0)	5·0 (3·0-7·)	0·028
No. people in same room as child		4·0 (3·0-5·0)	4·0 (3·0-5·0)	0·847
Risk factors				
Weight-for-height z-score <-2		186 (25·8)	397 (18·9)	<0·001
Birth weight <2·5kg		15 (9·5)	52 (11·3)	<0·001
Breast feeding		733 (100·0)	2105 (100·0)	1·00
Illness in previous 3 months		189 (25·8)	255 (12·1)	<0·001
Previous hospitalization		132 (18·0)	150 (7·1)	<0·001
Distance from hospital in km, median (IQR)		5·0 (1·2-10·0)	5·5 (1·0-12·0)	0·021
Age in months at first dose PCV13, median (IQR)		2·6 (2·3-3·2)	2·6 (2·2-3·1)	0·227
Age in months at second dose PCV13, median (IQR)		4·1 (3·4-4·9)	3·9 (3·4-4·8)	0·07
Age in months at third dose PCV13, median (IQR)		5·5 (4·7-6·8)	5·5 (4·6-6·6)	0·376

*Defined as individuals who eat from the one cooking pot.

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