Effect of the of 10-valent pneumococcal conjugate vaccine in Nepal 4 years after introduction: an observational cohort study

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Summary

Background In Nepal, *Streptococcus pneumoniae* (pneumococcus) is a common cause of bacterial pneumonia in children, and is a major health concern. There are few data on the effect of vaccination on the disease or colonisation with pneumococci in the nasopharynx of children in this setting. The 10-valent pneumococcal conjugate vaccine (PCV10) was introduced into the routine infant immunisation schedule in Nepal in 2015. We aimed to investigate the effect of the introduction of PCV10 on pneumococcal carriage and disease in children in Nepal.

Methods We did an observational cohort study in children in Nepal. The hospital surveillance study took place in Patan Hospital, Kathmandu, and community studies in healthy children took place in Kathmandu and Okhaldhunga district. For the surveillance study, all children admitted to Patan Hospital between March 20, 2014, and Dec 31, 2019, aged between 2 months and 14 years with clinician-suspected pneumonia, were eligible for enrolment. For the community study, healthy children aged 0–8 weeks, 6–23 months, and 24–59 months were recruited from Kathmandu, and healthy children aged 6–23 months were recruited from Okhaldhunga. We assessed the programmatic effect of PCV10 introduction using surveillance for nasopharyngeal colonisation, pneumonia, and invasive bacterial disease from 1·5 years before vaccine introduction and 4·5 years after vaccine introduction. For the surveillance study, nasopharyngeal swabs, blood cultures, and chest radiographs were obtained from children admitted to Patan Hospital with suspected pneumonia or invasive bacterial disease. For the community study, nasopharyngeal swabs were obtained from healthy children in the urban and rural settings. Pneumonia outcomes were analysed using log-binomial models and adjusted prevalence ratios (aPR) comparing each calendar year after the introduction of the vaccine into the national programme with the pre-vaccine period (2014–15), adjusted for calendar month, age, and sex.

Findings Between March 20, 2014, and Dec 31, 2019, we enrolled 2051 children with suspected pneumonia, and 11 354 healthy children (8483 children aged 6–23 months, 761 aged 24–59 months, and 2110 aged 0–8 weeks) to assess nasopharyngeal colonisation. Among clinical pneumonia cases younger than 2 years, vaccine serotype carriage declined 82% (aPR 0·18 [95% CI 0·07–0·50]) by 2019. There was no decrease in vaccine serotype carriage in cases among older unvaccinated age groups. Carriage of the additional serotypes in PCV13 was 2·2 times higher by 2019 (aPR 2·17 [95% CI 1·16–4·05]), due to increases in serotypes 19A and 3. Vaccine serotype carriage in healthy children declined by 75% in those aged 6–23 months (aPR 0·25 [95% CI 0·19–0·33]) but not in those aged 24–59 months (aPR 0·59 [0·29–1·19]). A decrease in overall vaccine serotype carriage of 61% by 2019 (aPR 0·39 [95% CI 0·18–0·85]) was also observed in children younger than 8 weeks who were not yet immunised. Carriage of the additional PCV13 serotypes in children aged 6–23 months increased after PCV10 introduction for serotype 3 and 19A, but not for serotype 6A. The proportion of clinical pneumonia cases with endpoint consolidation on chest radiographs declined from 41% in the pre-vaccine period to 25% by 2018, but rose again in 2019 to 36%.

Interpretation The introduction of the PCV10 vaccine into the routine immunisation programme in Nepal has reduced vaccine serotype carriage in both healthy children and children younger than 2 years with pneumonia. Increases in serotypes 19A and 3 highlight the importance of continued surveillance to monitor the effect of vaccine programmes. This analysis demonstrates a robust approach to assessing vaccine effect in situations in which pneumococcal disease endpoint effectiveness studies are not possible.

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Research in context

Evidence before this study

Nepal introduced the 10-valent pneumococcal conjugate vaccine (PCV10) into its routine infant immunisation schedule in 2015 using a unique 2 + 1 schedule, with priming doses at 6 weeks and 10 weeks of age plus a booster at 9 months of age, and with no catch-up campaign in older age groups. The effect on pneumococcal carriage and disease with this form of vaccine implementation had not previously been tested. A systematic database search was not done before the study commenced. However, a recent WHO systematic review found multiple studies assessing vaccine effect against invasive pneumococcal disease caused by pneumococcal serotypes covered by the PCV10 vaccine, but none in Asian or low-income or middle-income settings. Studies reporting the effect of a 2+1 schedule on pneumococcal pneumonia were limited to children younger than 5 years; however, a large proportion of pneumococcal pneumonia in Nepal is in children aged 5 years or older.

Added value of this study

In 2014, before the introduction of the vaccine, we commenced a vaccine impact study. The study assessed the

impact of the vaccine using the Nepali schedule with 2 initial doses at 6 weeks and 10 weeks of age followed by a booster at 9 months of age. This was not a schedule that had been used in other pneumococcal vaccine implementation programmes. In addition to measuring vaccine impact on carriage in healthy children, we also measured the impact on carriage of vaccine serotypes in children admitted to hospital with pneumonia. Blood cultures from admitted children were also serotyped for analysis.

Implications of all the available evidence

PCV10 introduction in Nepal resulted in a substantial decrease in carriage of vaccine serotypes in healthy children younger than 2 years and in clinical pneumonia cases in children of the same age. Increases in carriage of serotypes 19A and 3, which are not included in the PCV10 vaccine, were also observed. These data on PCV effect are useful for the planning of pneumococcal vaccine introduction programmes using a 2+1 regimen, especially in Asian settings.

Introduction

Streptococcus pneumoniae (pneumococcus) is a common cause of bacterial pneumonia, meningitis, and sepsis, and is a leading cause of morbidity and mortality from lower respiratory infections. Globally, it is estimated to cause more than a million deaths per year.[1,2]

Although the incidence of pneumonia has decreased in recent decades, about 138 million cases were estimated to have occurred in 2015 in children younger than 5 years.[3] In Nepal, pneumonia is a major health concern, with *S pneumoniae* being the most common cause of bacterial pneumonia in children.[4,5] Although many different serotypes of pneumococcus cause infection globally, in Kathmandu, Nepal, invasive pneumococcal disease among those aged 14 years and younger is predominantly caused by serotypes 1 and 5.[6,7] Additionally, in this setting, almost a third of all cases between age 2 months and 14 years occur in children aged 5–14 years, an age group that is not included in the WHO classification of childhood pneumonia and is therefore often excluded from many surveillance studies and global pneumonia statistics.[5,8]

Nepal introduced the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D-conjugate vaccine (PCV10) into its routine infant immunisation schedule in a phased manner in January, 2015, with support from Gavi, the Vaccine Alliance. By August, 2015, the vaccine was being used in all areas of the country. For operational reasons, Nepal implemented a 2 + 1 schedule, with priming doses at 6 weeks and 10 weeks of age and a booster at 9 months of age, and with no catch-up campaign in older age groups.

In April–May, 2015, major earthquakes near Kathmandu led to more than 9000 deaths, internal displacement of populations, and damage to infrastructure. In winter 2015–2016 a shortage of fuel led to disrupted medical supplies and burning of low-quality fuel, worsening air pollution in the Kathmandu valley. Despite these difficulties, PCV10 was implemented into the Nepalese infant immunisation schedule with high coverage.

In March, 2014, before the introduction of the vaccine, we commenced an observational cohort study to assess the impact of the vaccine. This supplemented ongoing surveillance of invasive bacterial disease (including invasive pneumococcal disease) at Patan Hospital.[6] To supplement the invasive pneumococcal disease surveillance and understand the broader effect of PCV10 on pneumococcal carriage, we also undertook surveys of nasopharyngeal carriage of *S pneumoniae* among healthy children in urban and rural cohorts each year from Kathmandu Valley and Okhaldhunga district. We aimed to report the effect of the introduction of PCV10 on pneumococcal carriage and disease in children in Nepal, before the COVID-19 pandemic.

Methods

Study design and participants

We did an observational cohort study set in Patan Hospital, Kathmandu, Nepal. Patan Hospital is the teaching hospital for Patan Academy of Health Sciences and, with more than 600 beds, is one of the largest hospitals in the Kathmandu Valley. The hospital provides a wide range of public and private inpatient and

outpatient services for adults and children and serves as both the main hospital care facility for the local surrounding community and as a tertiary care facility for the Kathmandu Valley.

Vaccine impact on clinical and radiographic pneumonia in children younger than 14 years was measured prospectively at Patan Hospital, Kathmandu, in our hospital pneumonia surveillance part of the study. All children admitted to Patan Hospital between March, 2014, and December, 2019, aged between 2 months and 14 years with clinician-suspected pneumonia were eligible for enrolment. Study staff in the hospital identified eligible participants from daily admission logs. Written informed consent was obtained from parents or guardians at enrolment, followed by collection of basic demographic and clinical details.

In addition to the hospital surveillance study, community studies were undertaken to assess the effect of vaccine introduction on nasopharyngeal carriage of vaccine-serotype *S pneumoniae* in healthy children from one urban (Patan, Kathmandu) and one rural (Okhaldhunga) region. For the urban site in Patan, healthy children aged 0–8 weeks, 6–23 months, and 24–59 months were recruited at Patan Hospital following informed consent from parents or guardians. These were children attending outpatient vaccination clinics, routine check-ups, or visiting other relatives in hospital. Minor respiratory illness was not an exclusion criterion. In the rural Okhaldhunga district (eastern Nepal), healthy children aged 6–23 months were recruited through community clinics following informed consent from parents or guardians.

The study was approved by the Patan Academy of Health Sciences Ethical Review Committee, the Nepal Health Research Council (NHRC 04-2014 and 39-2014), and the Oxford Tropical Research Ethics Committee (OxTREC 05-14 and 28-14).

Procedures

For the hospital pneumonia surveillance, nasopharyngeal swabs from patients aged between 2 months and 14 years with suspected pneumonia admitted to hospital were cultured and serotyped to assess the impact of the vaccine on nasopharyngeal carriage of *S pneumoniae* in pneumonia cases. The presence of *S pneumoniae* in the nasopharynx during pneumonia illness does not prove the pneumonia to be pneumococcal in origin. However, the serotype carried can be indicative of the serotype responsible for the infection, because invasive serotypes are carried more commonly in pneumonia cases than in healthy controls.[9]

Nasopharyngeal swab samples were collected from participants at enrolment using sterile nylon flocked swabs. Blood samples for routine clinical care were taken for bacterial culture in Bactec Peds Plus culture bottles (Becton Dickinson, NJ, USA). Both sample types were processed in the microbiology laboratory at Patan Hospital with a randomly selected 10% of isolates also serotyped independently for quality control purposes in Oxford.

A digitalised chest radiograph taken as part of routine care at the time of enrolment was independently interpreted, using standardised WHO criteria,[10] as primary endpoint pneumonia (consolidation or effusion); other infiltrate; or no consolidation, effusion, or infiltrate by two readers (a paediatrician and a radiologist). A second senior radiologist arbitrated upon all discordant results and reviewed 10% of other radiographs. Readers were masked to clinical data.

In addition to surveillance of children with pneumonia, all febrile patients in hospital younger than 14 years (including those enrolled in the hospital pneumonia surveillance) were eligible for enrolment in the WHO enhanced invasive bacterial disease surveillance programme for invasive bacterial diseases, and results from blood, pleural fluid, and cerebrospinal fluid cultures or antigen tests were also available for inclusion in the analysis.

For the community carriage studies, nasopharyngeal samples were collected using sterile nylon flocked swabs for both the urban and rural study sites. Swabs were placed into tubes containing 1 mL skim milk tryptone glucose glycerol (STGG) transport medium. In the urban site, swabs were collected in the study room at Patan Hospital and transported to the microbiology laboratory within an hour of collection. In the rural site, swabs were transported to Okhaldhunga Community Hospital from the collection site and stored at –20°C. These swabs were transported to Patan Hospital in liquid nitrogen after up to a maximum of 4 days of storage in Okhaldhunga, before storage at Patan Hospital at –80°C.

For all swabs (from participants admitted to hospital and from both community carriage sites), aliquots of STGG media were made before freezing and transported to the UK on dry ice. The frozen nasopharyngeal samples (STGG) were thawed and cultured along with other recently collected swabs on Columbia agar containing 5% sheep blood and gentamicin and were incubated overnight at 37°C with 5% CO_2. Pneumococci were identified by standard phenotypic methods before serotyping by the Quellung reaction.[11,12]

Statistical analysis

For the community carriage study, surveys of 1151 healthy children for each yearly urban cohort were planned in children aged 6–23 months to have 80% power (two-sided alpha level of 0·05) to detect an overall reduction of 5 percentage points in carriage prevalence of at least one vaccine type (from 22% to 17%) or an increase of 5 percentage points (from 38% to 43%) in carriage prevalence of at least one non-vaccine type serotype. Thus, each year was a standalone study with full power in the main age group of 6–23 months for the urban cohorts. Additionally, the study was designed to have 80% power to detect decreases in serotype-specific analyses for

	March–December, 2014 (n=210)	2015 (n=267)	2016 (n=461)	2017 (n=380)	2018 (n=411)	2019 (n=322)
Clinical details						
Clinical discharge diagnosis of pneumonia	184 (88%)	239 (90%)	440 (95%)	353 (93%)	385 (94%)	298 (93%)
Age, years						
Median (IQR)	1·3 (0·6–2·6)	1·3 (0·6–2·7)	1·2 (0·6–2·5)	1·6 (0·8–3·1)	1·0 (0·6–2·3)	1·6 (0·7–3·3)
Younger than age 2	118/184 (64%)	155/239 (65%)	299/440 (68·0%)	206/353 (58·4%)	268/385 (69·6%)	164/298 (55·0%)
Between 2 and younger than 5	45/184 (24%)	56/239 (23%)	100/440 (23%)	102/353 (28·9%)	98/385 (25·5%)	87/298 (29·2%)
5–13	21/184 (11%)	28/239 (12%)	40/440 (9%)	45/353 (12·7%)	19/385 (4·9%)	47/298 (15·8%)
Missing	0	0	1/440 (<1%)	0	0	0
Sex						
Male	112/184 (60·9%)	150/239 (62·8%)	261/440 (59·3%)	200/353 (56·7%)	238/385 (61·8%)	178/298 (59·7%)
Female	72/184 (39·1%)	89/239 (37·2%)	179/440 (40·7%)	153/353 (43·3%)	147/385 (38·1%)	120/298 (40·3%)
Wheezing						
Wheeze on admission	70/184 (38·0%)	78/239 (32·6%)	230/440 (52·3%)	132/353 (37·4%)	93/385 (24·2%)	55/298 (18·5%)
Missing	9/184 (4·9%)	0	0	0	0	0
Temperature						
Temperature >38°	70/184 (38·0%)	82/239 (34·3%)	135/440 (30·7%)	135/353 (38·2%)	156/385 (40·5%)	119/298 (39·9%)
Missing	1/184 (0·5%)	1/239 (0·4%)	0	0	0	0
Antibiotic use						
Recent antibiotic use	72/184 (39·1%)	123/239 (51·5%)	214/440 (48·6%)	163/353 (46·2%)	152/385 (39·5%)	117/298 (39·3%)
Missing	2/184 (1·1%)	1/239 (0·4%)	3/440 (0·7%)	4/353 (1·1%)	11/385 (2·9%)	9/298 (3·0%)
Blood culture positive for S pneumoniae	5/184 (2·7%)	2/239 (0·8%)	6/440 (1·4%)	2/353 (0·6%)	2/385 (0·5%)	7/298 (2·3%)
Chest radiograph						
Chest radiograph obtained in those with clinical discharge diagnosis of pneumonia	167/184 (90·8%)	224/239 (93·7%)	434/440 (98·6%)	338/353 (95·8%)	379/385 (98·4%)	291/298 (97·7%)
Chest radiographs missing/uninterpretable	17/184 (9·2%)	15/239 (6·3%)	6/440 (1·4%)	15/353 (4·2%)	6/385 (1·6%)	7/298 (2·3%)
Chest radiograph classification						
Primary endpoint pneumonia (consolidation or effusion)	76/167 (45·5%)	83/224 (37·1%)	149/434 (34·3%)	106/338 (31·4%)	96/379 (25·3%)	106/291 (36·4%)
Other infiltrates	33/167 (19·8%)	36/224 (16·1%)	47/434 (10·8%)	44/338 (13·0%)	46/379 (12·1%)	44/291 (15·1%)
Normal	58/167 (34·7%)	105/224 (46·9%)	238/434 (54·8%)	188/338 (55·6%)	237/379 (62·5%)	141/291 (48·5%)
Nasopharyngeal samples						
Carriage of S pneumoniae	70/184 (38·0%)	81/241 (33·6%)	143/448 (31·9%)	135/360 (37·5%)	169/393 (43·0%)	113/308 (36·7%)
Carriage of PCV10 serotypes of S pneumoniae*	26/184 (14·1%)	32/241 (13·3%)	49/448 (10·9%)	40/360 (11·1%)	26/393 (6·6%)	20/308 (6·5%)

Data are n (%) or n/N (%). N values in column headings are number enrolled with suspected pneumonia. PCV10=10-valent pneumococcal conjugate vaccine. *Participants could be carrying more than one serotype of pneumococcus in their nasopharynx, so percentages represent percent of total carriage rates.

Table: Demographic and clinical characteristics of patients admitted to hospital with pneumonia

five serotypes in the vaccine that had been detectable in at least 1% of swabs taken in previous studies.

For the hospital pneumonia surveillance study, a sample size of 414 children would have 80% power to detect a 75% reduction in carriage of serotypes 1 and 5, the major serotypes isolated for invasive disease. There was no target for enrolment, as all eligible children within the age range were approached for recruitment and numbers varied from year to year depending on the number of pneumonia cases being admitted to the hospital. We expected that there would be enough participants for most years for the study to be fully powered to detect the difference specified. Further details on sample size assumptions are provided in the appendix (pp 1–2).

We assessed nasopharyngeal carriage prevalence of pneumococci for each study population, cohort, and year, and classified these as any PCV10 serotypes (PCV10: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F), additional PCV13 serotype (PCV13+: 3, 6A, and 19A), additional PCV20 serotype (PCV20+: 8, 10A, 11A, 12F, 15B, 22F, and 33F), or other non-vaccine types.

We calculated prevalence ratios by comparing each post-introduction year with the 2014–15 pre-vaccine years. Log-binomial regression models incorporating variables for sex, age, month of enrolment, and recent antibiotic use were fitted to estimate adjusted prevalence ratios. Models of carriage in healthy community children were adjusted for sex, age, and month of enrolment.

For chest radiographs of pneumonia cases, we calculated the prevalence of primary endpoint pneumonia in those with clinically diagnosed pneumonia and calculated adjusted prevalence ratios. We present point estimates with 95% CIs without p values and with no adjustment for multiplicity.

Data were entered into Microsoft Access or OpenClinica databases by staff at Patan Hospital. Data were queried and cleaned regularly with a further review at the end of each year. All analyses were done with SAS (version 9.4).

All participants with available data from nasopharyngeal swabs were included in the analysis. The analysis of primary endpoint pneumonia included only those with interpretable radiographs and missing outcome data were not imputed.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

There were 2051 cases of suspected pneumonia in children aged between 2 months and 14 years enrolled between March 20, 2014, and Dec 31, 2019, at Patan Hospital: 477 during the pre-vaccination period (2014–15) and 1574 in the 4 years post vaccine introduction. Of these, 1899 (92·6%) had a clinical diagnosis of pneumonia on discharge (table).

The median age of pneumonia cases was 1·3 years (IQR 0·6–2·6) before vaccine introduction and the median ranged from 1·0 year (0·6–2·3) to 1·6 years (0·7–3·3) during the 5 subsequent years of follow-up. S pneumoniae was isolated from blood cultures in only 24 cases with a discharge diagnosis of pneumonia, 19 (79%) of which were due to serotype 1 (table).

The proportion of clinical pneumonia cases with primary endpoint pneumonia on chest radiographs declined after the introduction of the vaccine from 159 (40·6%) of 391 in the pre-vaccine period (2014 and 2015) to 96 (25·3%) of 379 by 2018, but increased again in 2019 to 106 (36·4%) of 291 (table). The overall adjusted prevalence of primary endpoint pneumonia in those with clinical pneumonia was 40% lower in 2018 (adjusted prevalence ratio [aPR] 0·60 [95% CI 0·48–0·74]) compared with the 2014–15 pre-vaccine period (figure 1A; appendix p 10). However, in 2019 the overall aPR was not significantly lower than in the pre-vaccine period (aPR 0·87 [95% CI 0·72–1·06]). The increase in the proportion of children with primary
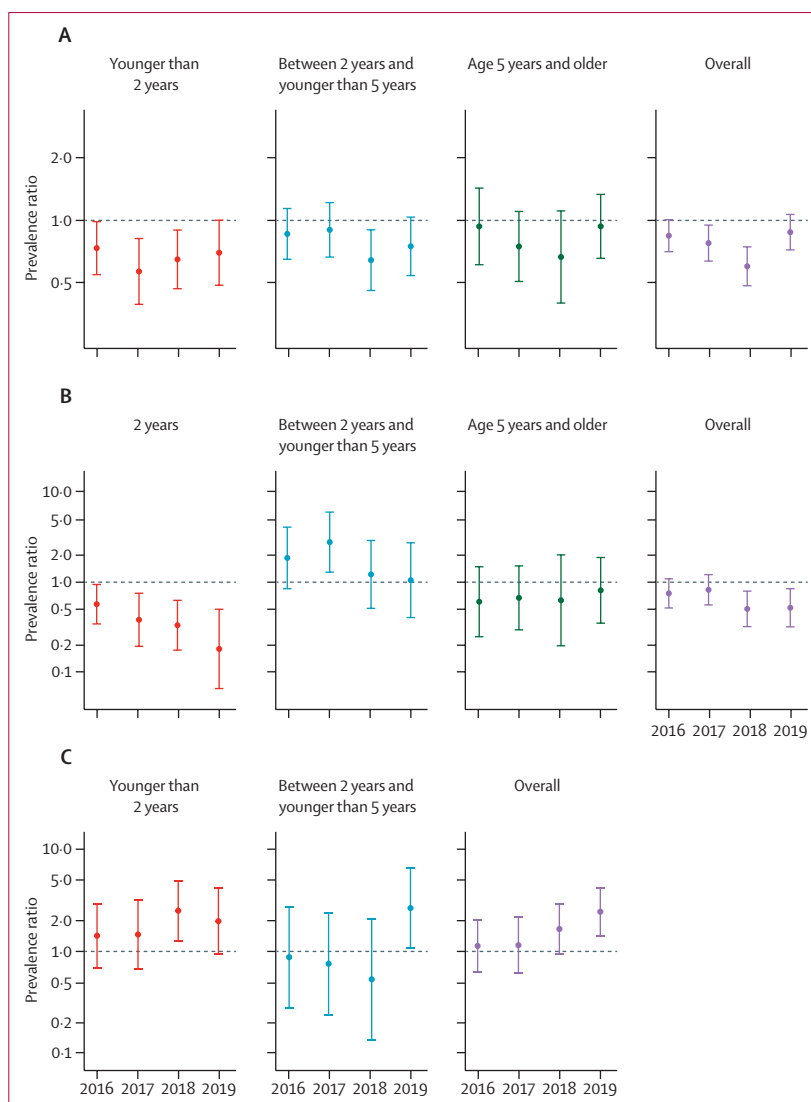

Figure 1: Primary endpoint pneumonia on chest radiographs (A), carriage of a PCV10 serotype (B), and carriage of a PCV13+ serotype (C) in those with clinically diagnosed pneumonia in the post-vaccine years compared with pre-vaccine years
Prevalence ratios compare the prevalence of the endpoint in the post-vaccine years with the pre-vaccine years (2014–15) combined from a log-binomial model. In part (A) estimates are adjusted for age, sex, recent antibiotic use, and month of admission; in parts (B) and (C) estimates are adjusted for age, sex, and month of admission. PCV10 serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F. PCV13+ serotypes: 3, 6A, and 19A. There were too few cases in those aged 5–14 years to enable calculation of prevalence ratios in this age group. 2014–15: pre-vaccine roll-out of PCV10; 2016–19: post-vaccine introduction years. PCV=pneumococcal conjugate vaccine.

endpoint pneumonia between 2018 and 2019 was most pronounced in children older than 5 years, too old to have been eligible for routine infant immunisation with PCV10. In children younger than 2 years primary endpoint pneumonia remained low in 2019.

In children younger than 2 years with clinical pneumonia, carriage of any PCV10 vaccine serotype was lower in all years after the introduction of the vaccine, such that the aPR of carriage of a PCV10 serotype was 0·18 (95% CI 0·07–0·50) in 2019 (figure 1B; appendix p 10). There was no decrease in
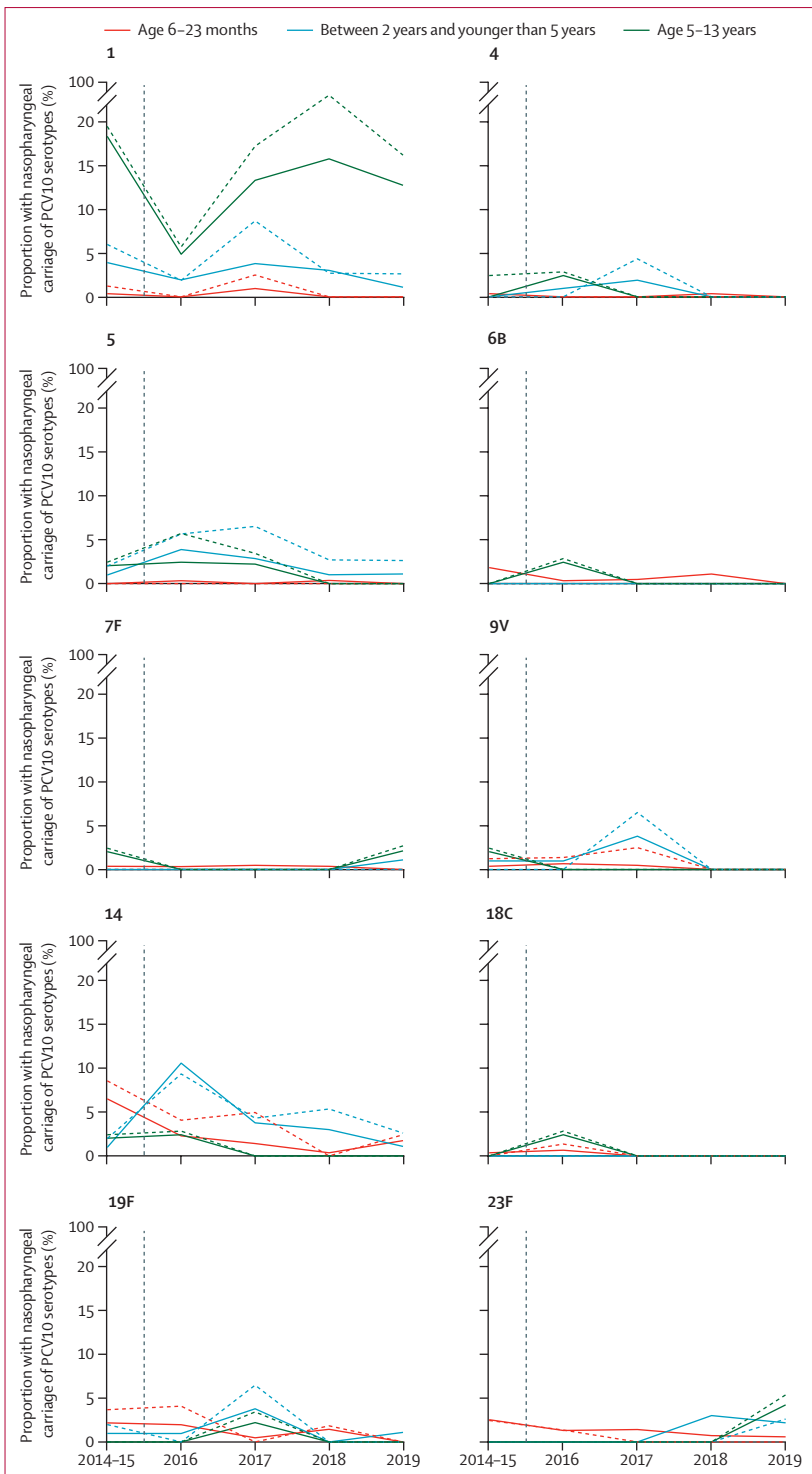
Figure 2: Nasopharyngeal carriage of PCV10 serotypes of *S pneumoniae*
Proportions shown are clinically diagnosed pneumonia cases (solid lines) or those with primary endpoint pneumonia (dotted lines), by age group. 2014–15: pre-vaccine roll-out of PCV10; 2016–19: post-vaccine introduction years. Dotted vertical line separates pre-vaccine and post-vaccine periods. PCV=pneumococcal conjugate vaccine.

vaccine-type carriage in older age groups (figure 1B; appendix p 10).

Serotype 14 was the most carried vaccine serotype in those with clinical pneumonia before the introduction of PCV10 (20 [5%] of 423), followed by serotype 1 (14 [3%] of 423; figure 2; appendix p 6). Serotype 14 was more commonly carried in those younger than 2 years with clinical pneumonia and its prevalence declined after vaccine introduction. Before introduction of the vaccine, 18 (90%) of the 20 cases of serotype 14 carriage were in children younger than 2 years. By 2019, there were four (1%) of 298 children carrying this serotype, three of whom were younger than 2 years. By contrast, serotype 1 was carried predominately in those aged 5 years or older. Of the 14 (3%) of 423 children carrying serotype 1 before vaccine introduction, nine were aged 5 years or older and prevalence of this serotype remained high. In 2019, there were seven (2%) of 298 clinical pneumonia cases with carriage of serotype 1, six of whom were aged 5 years or older (appendix pp 6–9).

Carriage of the three additional PCV13+ serotypes in clinical pneumonia cases increased after PCV10 introduction (figure 1C; appendix p 10). In 2019, the prevalence of additional PCV13+ serotypes was 2·2-fold higher than in the pre-PCV10 period (aPR 2·17 [95% CI 1·16–4·05]). Carriage of additional PCV20 serotypes was lower in 2019 than in the pre-PCV10 period (aPR 0·34 [95% CI 0·13–0·90]).

Increases in carriage among clinical pneumonia cases occurred for all additional PCV13+ serotypes. By 2019, serotype 6A had increased from 2·4% to 3·4%, serotype 3 had increased from 0·2% to 1·3%, serotype 19A had increased from 1·2% to 3·7%, and serotype 6C had increased from 0·7% to 2·7% (appendix p 4).

11 354 healthy children (8483 children aged 6–23 months, 761 aged 24–59 months, and 2110 aged 0–8 weeks) were recruited for the community studies. In the pre-vaccine period, carriage of any PCV10 vaccine serotype in healthy children in the local communities of Patan and Okhaldhunga was higher for rural (Okhaldhunga) children than urban (Patan) children (27·7% in rural children in 2015, and 20·2% in urban children in 2014 and 17·1% in urban children in 2015; appendix p 19). Overall carriage of any *S pneumoniae* was also higher in the rural community sampled (55·4% in urban children *vs* 81·3% in rural children in 2015).

Prevalence of vaccine serotypes in community children declined substantially in both urban and rural communities in the post-vaccine period. For children aged 6–23 months, the aPRs were 0·25 (95% CI 0·19–0·33) in urban children in 2019, and 0·28 (0·19–0·37) in rural children in 2018 (not measured in 2019; figure 3). In the smaller sample of older children aged between 2 years and younger than 5 years, the 2019 aPR was 0·59 (95% CI 0·29–1·19). A decrease in carriage of PCV10 serotypes was also
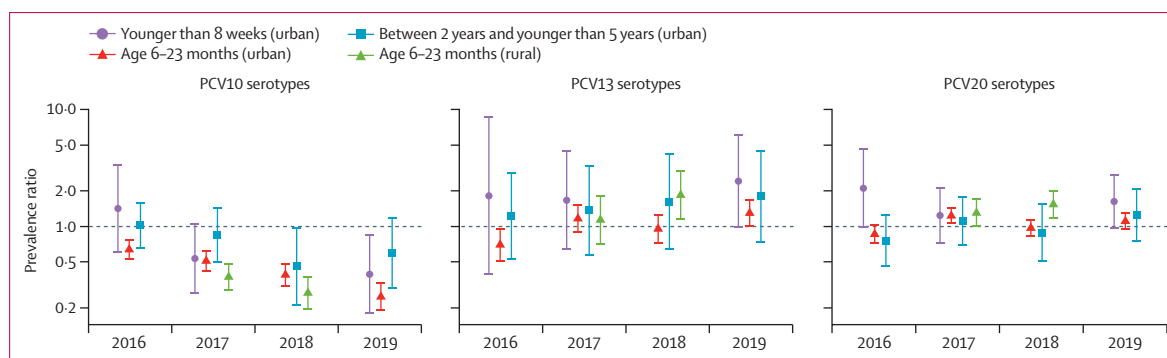
Figure 3: Adjusted prevalence ratios for nasopharyngeal carriage of vaccine serotypes in healthy children for each post-vaccine year compared with 2014–15
Rural cohorts were not enrolled in 2016 or 2019. The younger than 8 weeks cohort were not enrolled in 2018. Prevalence ratios are adjusted for age, sex, and calendar month. Dotted horizontal line shows the prevalence ratio of 1·0 representing no change from the pre-vaccine period. PCV10 serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F. Additional PCV13+ serotypes: 3, 6A, and 19A. Additional PCV20+ serotypes: 8, 10A, 11A, 12F, 15B, 22F, and 33F. 2014–15: pre-vaccine roll-out of PCV10; 2016–19: post-vaccine introduction years. PCV=pneumococcal conjugate vaccine.

observed in children younger than 8 weeks of age who had not yet been immunised, with a reduction of 61% by 2019 (aPR 0·39 [95% CI 0·18–0·85]; figure 3; appendix p 21).

Carriage of *S pneumoniae* in healthy community children aged 6–23 months was highest for serotypes 6B (4·2%), 19F (4·9%), and 23F (3·9%) in the pre-vaccination period in urban cohorts, and declined substantially for these serotypes to 0·5% for 6B, 0·9% for 19F, and 1·3% for 23F by 2019 (appendix pp 12–14). In rural children, serotypes 6B (6·5%), 14 (4·7%), and 19F (6·5%) were also the most prevalent in the pre-vaccination period, and were reduced to 1·9% for 6B, 2·3% for 19F, and 1·6% for 23F by 2018 (figure 4; appendix pp 17–18).

Carriage of the additional PCV13+ serotypes increased after PCV10 introduction for serotypes 3 and 19A, but not for serotype 6A, resulting in no overall change in urban cohorts (appendix p 5). However, an increase in carriage of additional PCV13+ serotypes was observed in rural children by 2018 (aPR 1·85 [95% CI 1·15–2·99]; appendix pp 5, 21). The prevalence of serotype 3 carriage increased from 0·5% in 2014 to 1·7% in 2019, in urban children aged 6–23 months and was 1·6% in rural children in 2018 but was not detected before PCV10 roll-out. Similarly, prevalence of 19A carriage rose from pre-vaccine levels of 2·0% in urban children and 2·2% in rural children to 4·0% in urban children and 7·0% in rural children (appendix pp 12, 17). Carriage of the additional serotypes in PCV20+ was also higher in rural children in 2018 than in the pre-vaccine period (aPR 1·55 [95% CI 1·17–2·05]) but was not higher in urban children (appendix pp 5, 21).

There were 72 (0·7%) of 9974 children who had a febrile illness and were enrolled in the surveillance for invasive disease who had either blood, cerebrospinal fluid (CSF), or pleural fluid samples positive for *S pneumoniae* during the study period, by culture or CSF Binax testing (figure 5). In 2019, there were 17 cases of invasive pneumococcal disease recorded. Information on serotype was available for 15 cases, which included

11 (73%) cases of serotype 1, and one each of serotypes 3, 5, 6B, and 19A. None of the 2019 invasive pneumococcal disease cases in children younger than 12 months were due to PCV10-covered serotypes. All serotype 1 and 5 cases in 2019 were in children aged 2 years and older and two cases of unknown serotype were in children older than 5 years. The single case of serotype 6B disease in 2019 was a vaccinated 19-month-old child.

Discussion

The introduction of the PCV10 in Nepal resulted in a substantial decrease in carriage of vaccine serotypes in healthy children younger than 2 years, and in carriage of vaccine serotypes in children of the same age with clinical pneumonia. Year-on-year declines in vaccine-type carriage in children younger than 2 years were observed both in urban and rural communities. Carriage of PCV10 serotypes was almost eliminated among clinical pneumonia cases, with only four clinically diagnosed pneumonia cases younger than 2 years having nasopharyngeal swabs positive for a vaccine serotype in 2019. Additionally, some evidence of indirect (herd) effects was seen in the reduction in carriage in healthy children who had not yet been vaccinated by at least 8 weeks of age.

These data on PCV effect are useful for planning of pneumococcal vaccine introduction programmes in Asia, where disease burden is high and where several countries have not yet introduced the vaccine.[13,14]

Cross-sectional studies of PCV effect on pneumococcal carriage in the community have been conducted in multiple low-income and middle-income country settings.[15–17] However, serotype distribution in disease can be markedly different from serotypes circulating in healthy populations, and substantial geographic variation exists. Our study is the first to extend beyond community surveillance in a low-income Asian setting, to quantify the effects of PCV on vaccine-serotype-specific disease, as assessed by carriage of vaccine serotypes in those who were admitted to hospital with pneumonia. In our
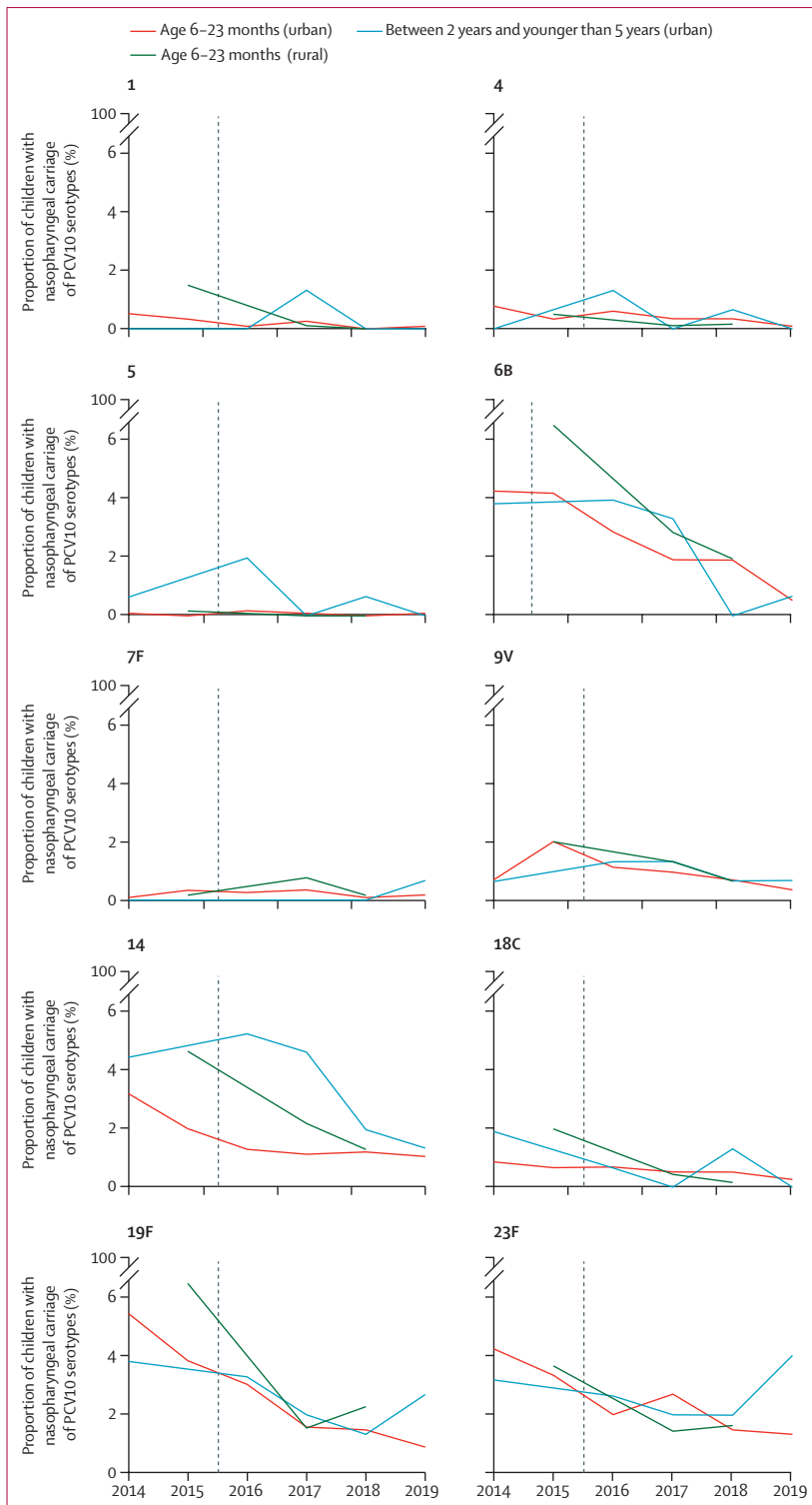
Figure 4: Percentage of children with positive nasopharyngeal swabs for PCV10 serotypes of *S pneumoniae* in healthy urban and rural communities in Nepal
Dotted vertical line separates pre-vaccine and post-vaccine years. 2014–15: pre-vaccine roll-out of PCV10; 2016–19: post-vaccine introduction years. PCV=pneumococcal conjugate vaccine.

comprehensive 6-year programme of surveillance, we investigated the effect of PCV10 introduction in urban and rural settings, enrolling both patients with pneumonia who had been admitted to hospital, as well as healthy community-based children, in one of the world's lowest-income countries.

There is a paucity of data on the effect of pneumococcal conjugate vaccines on vaccine serotype pneumococcal pneumonia, due to difficulties in measuring this outcome. Determining the true cause of pneumonia is challenging given the limitations of diagnostic tools.[18]

A recent WHO systematic review found multiple studies assessing vaccine effect against vaccine type invasive pneumococcal disease, but none in Asian or low-income or middle-income settings.[19] Only four studies reported the effect of the 2+1 schedule on pneumococcal pneumonia using pre-vaccine and post-vaccine introduction study designs. These studies were limited to children younger than 5 years. All four studies reported reductions of between 70% and 75% for overall pneumonia caused by all pneumococcal serotypes combined. However, serotype-specific estimates or vaccine-serotype estimates were unavailable due to the lack of available serotyping data, and studies were not done in low-income or middle-income countries.[19]

To overcome this gap in serotype-specific estimates for pneumococcal pneumonia from low-income or middle-income settings, we assessed vaccine-serotype pneumococcal nasopharyngeal carriage from patients with pneumonia. Carriage of *S pneumoniae* in children diagnosed with pneumonia is not proof that the pneumonia is pneumococcal in origin. However, carriage of serotypes that are rarely detected in healthy controls, but are common in pneumonia cases, is more likely to be indicative of a pneumococcal infection.[9] In 2019, serotype 1 was most often detected in invasive pneumo-coccal disease, and was found in nasopharyngeal swabs in 12·8% of patients with pneumonia aged 5 years or older, but was not detected at all in healthy children older than 2 years. Notably, the distribution of carried serotypes in those with pneumonia in our study differed from the distribution of serotypes carried in healthy child cohorts, but was similar to the distribution of serotypes found in invasive pneumococcal disease. These findings confirm the benefits of this approach in which nasopharyngeal samples are taken in pneumonia patients to measure the effect of pneumococcal conjugate vaccine against pneumococcal pneumonia. These data also highlight the unusual age distribution of disease caused by serotype 1 in this setting.

Indirect vaccine effects were not observed in older children in our urban community cohort, nor in those admitted to hospital with pneumonia, possibly due to the smaller sample size. However, in infants younger than 8 weeks of age, a decrease in vaccine type carriage was seen in 2019. There is variable evidence of indirect vaccine effects when PCV10 is introduced without a catch-up

campaign. In Mozambique, PCV10 was introduced using a 3+0 schedule with no catch-up campaign and no effect was seen in children aged 2–5 years.[20] By contrast, a significant effect in those aged 2–6 years was observed in Fiji as early as 1 year after vaccine introduction using a 3+0 schedule without a catch-up campaign, but was no longer significant after 3 years.[21] In Kilifi, Kenya, PCV10 was introduced using a 3+0 schedule with catch-up to 5 years and a large decrease in vaccine type carriage in those 5 years and older was observed.[22] In Ghana, the introduction of PCV13 with a 3+0 schedule and no catch-up campaign resulted in decreased incidence of vaccine type pneumococcal meningitis in children younger than 5 years of age. However, 4 years after vaccine introduction, serotype 1 remained the major cause of pneumococcal meningitis in older children and adults, showing an absence of established herd effects.[13]

In our community cohorts, detection of indirect vaccine effects was limited by the small sample size targeted for children aged 2–5 years: 150 children per year. In pneumonia surveillance, the number of children admitted with pneumonia was also low in the older age groups; between 58% and 70% of children with pneumonia each year were younger than 2 years, and the median age each year was between 12 months and 19 months. A catch-up campaign in older children might still be required to see effect on disease caused by this serotype which disproportionately affects older children.

Our 6-year follow-up programme enabled the detection of delayed vaccine effects. The long length of this study is of particular value in the Nepalese setting because the vaccine was introduced using a unique 2+1 schedule, without a catch-up campaign, in a setting with an older age distribution of pneumococcal pneumonia cases. A reduction in vaccine type carriage in children with pneumonia aged 2–5 years can be seen by mid-2019, when most of this age group would have been vaccinated, showing direct protection provided by the vaccine, 2–3 years after the booster dose.

Widespread use of any serotype-specific polysaccharide-conjugate vaccines is often followed by increases in other serotypes not contained in the vaccine.[23,24] In patients admitted to hospital with pneumonia, we observed a rise in carriage of serotypes 3 and 19A, yet no rise in the carriage of 6A. A similar pattern was seen in community children younger than 2 years, for whom a decrease in carriage of 6A was observed, suggesting cross protection from serotype 6B in the vaccine.

Serotypes 19A and 6A are contained in the PCV13 vaccine and in the newer 10-valent pneumococcal conjugate vaccine (Pneumosil, Serum Institute of India);[25] thus, a change of vaccine product might prevent further increases in these serotypes. However, to gain control of the current high proportion of serotype 1 disease in older children, a catch-up campaign might be considered by policy makers. Modelling of different catch-up campaigns in Viet Nam before vaccine introduction concluded that
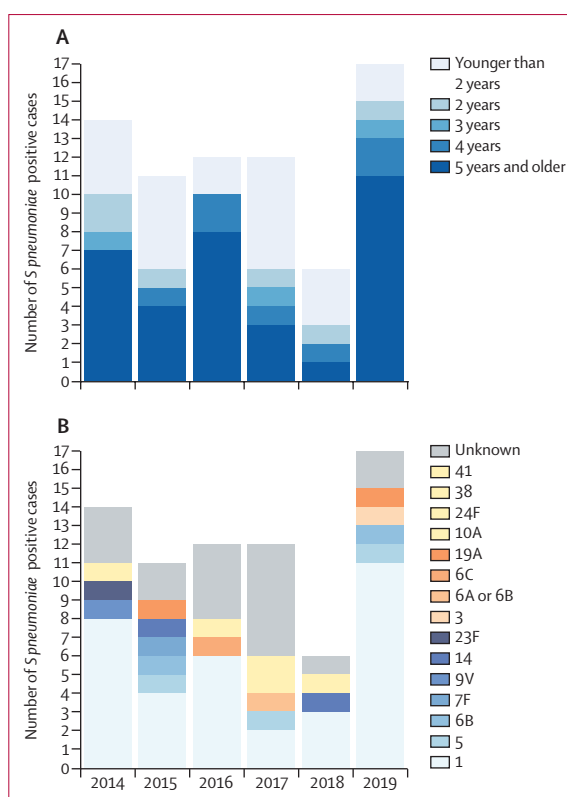

Figure 5: **Cases of invasive pneumococcal disease in children younger than 14 years at Patan Hospital by age (A) and by serotype (B)**
Serotypes are unknown in cases with positive CSF Binax test and negative CSF culture. Number of samples tested each year: 2014, 1306; 2015, 1285; 2016, 1954; 2017, 1728; 2018, 1892; 2019, 1809. The Bactec machine was recalibrated at the start of 2019, leading to an increase in detection of cases. CSF=cerebrospinal fluid.

catch-up campaigns can reduce the time to elimination of vaccine serotypes in the Vietnamese context.[26] However, similar modelling is not available for Nepal and the effect of commencing a catch-up campaign after routine vaccination programmes is unknown.

Consolidation of effusion on chest radiographs might indicate a pneumonia that is bacterial in origin. We observed a decline in the proportion of radiographs that showed primary endpoint pneumonia until 2018. However, the proportion of radiographs showing primary endpoint pneumonia in 2019 was higher than in previous years and, when broken down by age groups, age-specific trends varied and were not more pronounced in the youngest, most vaccinated age group. The proportion of children with endpoint consolidation can be affected by other circulating pathogens with prevalence varying from year to year. Detecting vaccine effects using this method is harder as it is a less specific outcome. Furthermore, several external factors such as air pollution and internal population migration might have affected the observations in this study.[27]

There are limitations to our study. We had 1·5 years of surveillance before the vaccine introduction and thus

the amount of year-to-year variation in case numbers that might exist in the absence of vaccination remains unclear. The planned 5-year follow-up after vaccine introduction was curtailed by 1 year due to the COVID-19 pandemic. Data for 2020 are not presented due to the influence of the pandemic on case numbers and social mixing. Because the study was powered to detect differences in each yearly cohort, the loss of 1 year of data does not affect power, and the original funding was for 3 years after vaccine introduction. Decreases in vaccine type carriage observed in the study were in keeping with the target effect sizes allowed for in the sample size calculations. Observational cohort studies can be influenced by temporal changes in disease due to non-vaccine causes and we cannot rule out some form of bias or confounding due to unknown or unmeasured factors on study outcomes, and our population might not be representative of the general population or children in other geographic regions. Vaccinations were not given as part of the study and vaccine history could only be elicited by caregiver recall and might not be correct, because vaccination rates were high by caregiver recall, including in older aged children who were ineligible for vaccination.

The inclusion of PCV10 given at 6 weeks, 10 weeks, and 9 months of age in the Nepalese infant immunisation schedule resulted in reductions in carriage of vaccine serotypes, for both healthy children in urban and rural communities, and in admitted children with pneumonia younger than 2 years. Emerging increases in carriage of serotypes 19A and 3 in healthy children highlight the importance of surveillance to provide data to inform optimal vaccine policy and product choice.

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Contributors
AJP, DFK, ShS, KLO'B, ST, and DRM conceptualised the study and AJP, DFK, DRM, ShS, KLO'B, MDK, ST, and MV supervised the study. MG, PA, SB, ASB, MJC, SG, JH, RK, SK, BK, PM, YFM, BP, RP, GPS, and BW curated the data. MCG and SoS did the laboratory work; MV did the statistical analysis; and AJP, DFK, ShS, KLO'B, ST, and DRM acquired funding. MV wrote the original draft of the manuscript; and ShS, AJP, MJC, DFK, JH, DRM, BW, RK, SB, and ST helped with writing, reviewing, and editing the manuscript. MV and AJP verified the underlying data. All authors critically reviewed and approved the final version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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