

# **An Open Label, Randomised Controlled Non-inferiority Trial, Comparing Two-Dose Priming with the 10-Valent Pneumococcal Conjugate Vaccine at 6 and 10 Weeks to 6 and 14 Weeks in Nepali Children.**

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## SUMMARY

**Background:** Nepalese infants receive PCV10 with a one-month interval between priming doses for programmatic reasons. We aimed to investigate whether immune responses to PCV10 serotypes were non-inferior if the second priming dose of PCV10 was delivered at a one-month as opposed to a two-month interval.

**Methods:** We conducted an open-label, randomised, parallel group trial in healthy Nepalese infants aged 40-60 days in Kathmandu, Nepal. Participants were randomised (1:1) using a computer-generated list with randomly varying permuted block sizes accessed through a validated web-based interface, to receive PCV10 at 6 and 10 weeks (6+10) or 6 and 14 weeks (6+14) of age, with both groups receiving a booster at 9 months of age. Laboratory staff, blinded to study intervention, analysed sera for antibodies against PCV10 serotypes by ELISA. The primary objective, using the intention-to-treat population, was to determine whether the 6+10 schedule was non-inferior to the 6+14 schedule at 9 months of age, based on the proportion of infants with serotype-specific IgG  $\geq 0.35$   $\mu\text{g/mL}$  and a 10% margin. Secondary objectives included, determining if there were any differences between the groups when comparing the proportion of infants with serotype-specific IgG  $\geq 0.35$   $\mu\text{g/mL}$ . This trial is registered at ClinicalTrials.gov, number NCT02385513.

**Findings:** Between August 21<sup>st</sup>, 2015 and April 4<sup>th</sup>, 2016, 304 Nepalese children were randomised to the 6+10 (n=152) and 6+14 groups (n=152). At 9 months of age, the 6+10 schedule was non-inferior for serotypes 5 (95% CI -9.6 to 13.25), 9V (95% CI -2.84 to 19.8), 14 (95% CI -7.27 to 12.54), and 19F (95% CI -9.86 to 0). At the same time-point, non-inferiority was not demonstrated for serotypes 1 (95% CI -13.56% to 6.77%), 4 (-21.64% to 1.18%), 6B (95% CI -20.65% to -0.18%), 7F (95% CI -15.26% to 5.42%), 18C (95% CI -25.78% to -4.83%), and 23F (95% CI -12.19% to 8.16%). After the booster dose, at 10 months of age, immunogenicity was similar for the two schedules.

**Interpretation:** Immunogenicity is lower in early infancy, for some serotypes, among children receiving PCV10 at 6 and 10 weeks, than in a 6 and 14 week schedule. The 6, 14 week and 9 month schedule should be implemented where possible. However, post-booster responses, which are thought to drive herd immunity, are similar with the two schedules. Therefore, the 6, 10 week and 9 month schedule is an alternative that can be used when logistically necessary, and will also provide herd protection.

**Funding:** Gavi - the vaccine alliance.

## **Research in context**

### **Evidence before this study**

We searched PubMed on May 1, 2018, for studies comparing the immunogenicity of different PCV schedules in children using the search terms "immunogenicity" AND "children" AND "PCV" AND "clinical trial" [publication type]. The search was unrestricted by language or publication date. Using this search strategy, we identified one systematic review which had meta-analysed studies reporting immunogenicity data up until 2011 and two further randomised controlled trials making a head-to-head comparison of PCV schedules, since the systematic review. The prior systematic review examining the effect timing of PCV7 priming schedules has on immunogenicity found that schedules with two month intervals between priming doses had improved immunogenicity for three of the PCV7 serotypes prior to boosting when compared with those with one month interval. Notably there were no RCTs which assessed the interval of PCV priming in a head-to-head design included in this review, with comparisons of intervals made across studies conducted in different settings. As such the role of co-variables, which have been shown to affect immunogenicity, should be considered. A study conducted since this review, compared four PCV13 infant schedules (2, 4, and 6 months; 2, 3, and 4 months; 2 and 4 months; or 3 and 5 months) in a head-to-head design among healthy Dutch children, and showed that PCV13 priming schedules with two month intervals had improved immunogenicity post-priming, when compared with one month intervals (2, 4, and 6 months superior for eleven serotypes compared with 2, 3, and 4 months). Although the differences between the schedules diminished with time, with few differences detected after boosting at 11.5 months of age (2, 4, and 6 months superior for two serotypes compared with 2, 3, and 4 months). Of note this study was designed in such a way that it could not completely differentiate the interplay between age of initial vaccination and interval of dosing. Another study compared PCV13 administered in three doses at one month intervals (2, 3, and 4 months) with two month intervals (2, 4, and 6 months) among premature infants in the United Kingdom, and showed significantly higher immunogenicity for seven serotypes after the priming series in the children who received PCV13 with two month intervals. Children in this study then received a booster at 12 months of age with those children who had PCV13 at one month intervals having better immunogenicity for three of the PCV13 serotypes. It should be noted that because the findings from this study of premature infants should be translated to healthy infants with caution. It is also difficult to generalise the findings of both of these head-to-head studies, conducted in the European children using 11.5-12 month of age boosters, to resource-limited settings where 9 month boosters are used.

### **Added value of this study**

Our study is the first randomised trial to make a head-to-head comparison of a one month with two month interval PCV priming schedule followed by a 9 month booster. In this trial improved immunogenicity is conferred by a two month PCV priming interval, however the differences between the two schedules lessen over time, particularly after the booster dose.

### **Implications of all the available evidence**

A two month interval between priming doses is the preferred strategy for PCV delivery in infants. However, a recent WHO review, which includes the consideration of data from this trial, indicates that an accelerated priming schedule using a one month PCV priming interval may be used where programmatic reasons dictate, as there is little difference between groups post-boosting and there is still a significant impact expected to be had on invasive disease in resource limited settings.

## INTRODUCTION

*Streptococcus pneumoniae* is the leading cause of bacterial pneumonia, meningitis and septicaemia in children worldwide, and disproportionately affects children from developing countries.<sup>1</sup> Pneumococcal conjugate vaccines (PCVs) reduce pneumococcal disease burden by direct protection and by reducing nasopharyngeal carriage, thereby preventing transmission and inducing herd protection.<sup>2,3</sup>

In Nepal, invasive pneumococcal disease (IPD) is responsible for a substantial disease burden in children.<sup>4,5</sup> Surveillance conducted since 2005 at Patan Hospital, Kathmandu indicates the majority of IPD is due to serotypes 1, 5 and 14, and that the majority of IPD occurs in late infancy and toddlerhood.<sup>5-7</sup> The ten-valent pneumococcal conjugate vaccine (PCV10) was introduced into the routine infant immunization schedule of Nepal during 2015.<sup>8</sup> A randomised controlled trial, conducted at Patan Hospital, assessing the immunogenicity of PCV10, demonstrated that a two-dose prime (at 6 and 14 weeks) with a 9-month booster was non-inferior for IgG concentrations at 18 weeks and 10 months and superior at 2-4 years of age when compared with a conventional three-dose priming only schedule (6, 10, and 14 weeks).<sup>6</sup> This two-dose prime and boost schedule, with an 8-week interval between the priming doses, is endorsed by the World Health Organization (WHO) and recommended in late 2014 by the Nepal Ministry of Health.<sup>9</sup> The WHO however, has also recommended introduction of a single inactivated poliomyelitis virus vaccine (IPV) at 14-weeks of age to mitigate the risk of outbreaks from vaccine derived serotype 2 poliomyelitis virus, to protect against serotype 2 poliomyelitis virus once countries switch to bivalent OPV (containing only serotypes 1 and 3 polio-virus) from trivalent OPV, and to enhance immunogenicity of oral polio vaccine to serotypes 1 and 3 polio virus strains.<sup>10</sup> This has created a programmatic dilemma because it requires administering three injections at the 14-week visit (pentavalent vaccine, PCV and IPV). Based on concerns around public and provider acceptance, and feasibility, the Nepalese Ministry of Health, opted to move the second PCV10 priming dose from 14 weeks to 10 weeks of age, creating a 4-week rather than 8-week interval between the two priming PCV doses.<sup>11</sup> Given this decision it is important to evaluate the immunogenicity of this accelerated 2+1 schedule (i.e. with a one-month interval between priming doses), comparing it to the standard 2+1 schedule (i.e. with a two-month interval between priming doses) that has been shown to provide a level of immunogenicity that would predict substantial program impact on disease and colonisation. This is important because accelerated two-dose priming schedules have shown a reduction in immunogenicity in other settings.<sup>12,13</sup> Therefore, this study was undertaken to evaluate the immunogenicity of a schedule of immunisation with 10-valent PCV at 6 and 10 weeks which is currently being used in Nepal, compared with priming at 6 and 14 weeks of age in healthy Nepali infants. In both groups a PCV10 booster was given at 9 months of age.

## METHODS

### *Trial design and participants*

We conducted a single centre open-label, parallel group, randomised controlled trial, at Patan Hospital, Kathmandu, Nepal. Ethical approval was obtained from Oxford Tropical Research Ethics Committee (OXTREC 25-15) and the Nepal Health Research Ethics Committee (NHRC 90-2015). We recruited healthy Nepalese infants aged 40-60 days, who presented to the immunisation clinic at Patan Hospital and randomised them to receive PCV10 at either; 6 weeks, 10 weeks and 9 months of age (6+10) or 6 weeks, 14 weeks and 9 months of age (6+14). This trial is registered at ClinicalTrials.gov registration number NCT02385513. A copy of the study protocol can be obtained upon request from the corresponding author.

Children were eligible for inclusion if they were healthy, born at > 37 weeks gestation, residing in Kathmandu, and had not had any prior vaccinations other than BCG, and oral polio vaccine (OPV). Children with any significant condition that may have affected the outcome of the study (for example a significant congenital syndrome), had been previously admitted to hospital (except where it was judged not to compromise the study), were born prematurely, had previous immunisation, or any investigational or non-registered product within 30 days of vaccination, were excluded from the trial. Any children with a systemic illness or fever over 38 degrees Celsius at the time of a scheduled study visit, had immunisation deferred until they had recovered. Written parental consent was obtained for all participants. Enrolled children received all other vaccines recommended in the Nepal immunisation program according to the routine schedule (appendix table S1).

### *Randomisation and masking*

After enrolment, participants were randomised by study staff (1:1) to receive PCV10 as either the 6+10 or 6+14 group. Participants and clinical staff were not blind to group allocation after the randomisation process was complete; however, all laboratory staff were blinded. Randomisation was computer-generated, with randomly varying permuted block sizes using a fully validated web-based interface system, Sortition.<sup>14</sup>

### *Procedures*

Participants received PCV10 according to their allocated treatment arm in addition to their other routine vaccines according to the Nepal vaccination program schedule. Three blood samples were collected for analysis of serum antibody responses to the PCV10 serotypes, diphtheria, pertussis, *Haemophilus influenzae* type b (Hib), and tetanus antigens. We collected a nasopharyngeal swab at two time points (6 weeks and 10 months of age) to examine carriage of pneumococcus over time and to assess differences between the two groups. At 12 months of age all participants received a dose of Varicella-Zoster virus vaccine.

Blood was centrifuged for ten minutes at 3000 rpm and serum stored at -20°C or below. Serum samples, masked by study code, were analysed for the 10 vaccine serotypes by ELISA at the WHO reference laboratory (University College London), and for other vaccine antigens (pertussis, diphtheria, tetanus, and Hib) by multiplex immunoassay (Bio-Rad Laboratories, Hercules, CA).<sup>15,16</sup> Nasopharyngeal swabs were collected and processed according to the WHO guidelines.<sup>17</sup> Swabs were placed in a tube of skim-milk-tryptone-glucose-glycerine transport medium and subsequently plated on sheep blood agar. Following overnight incubation at 35-37°C in 5% carbon dioxide, morphologically distinct colonies were selected and sub-cultured overnight before undergoing serotyping by Quellung reaction.

### *Outcomes*

The primary outcome measure was the percentage of infants with PCV10 serotype specific IgG  $\geq 0.35$   $\mu\text{g/mL}$  at 9 months of age. Secondary outcomes were: the percentage of infants with PCV10 serotype-specific IgG  $\geq 0.35$   $\mu\text{g/mL}$  one month after the second priming dose of PCV10 and one month following the 9 month booster; the geometric mean concentrations of PCV10 serotype specific IgG one month following the second priming dose of PCV10, at 9 months of age, and one month following the 9 month booster for each of the two study groups; the percentage of infants who experienced an adverse event following the 14 week visit in each of the study groups; and serotype specific pneumococcal carriage at age 6 weeks, and at age 10 months.

### *Statistical analysis*

The pre-specified primary analysis was by intention-to-treat. Percentages of participants with IgG  $\geq 0.35$   $\mu\text{g/mL}$  were calculated for each group with 95% confidence intervals (CIs) calculated using the Binomial Exact method. For the 9-month visit, the assessment of non-inferiority for each serotype, was carried out using Farrington-Manning method with the non-inferiority margin set at 10%.<sup>18</sup> At other time-points, Fisher's Exact test was used to assess differences between the two groups. Serum antibody concentrations were log-transformed and geometric mean concentrations (GMCs) were compared by calculating the geometric mean ratio. Comparisons between groups were carried out using t-test. Fisher's Exact test was used to compare the proportion of vaccine type

carriage between the two groups. A p-value < 0.025 (1-sided) was considered to be statistically significant for the non-inferiority analysis of the primary objective and < 0.05 (2-sided) was considered to be statistically significant for other comparisons of superiority. P-values were not adjusted for multiple comparisons. All analyses were carried out using SAS version 9.4 and R version 3.3.1.<sup>19</sup>

#### ***Sample size calculation***

Sample sizes were based on a prior study in which, at 9 months of age, there were 5 out of 10 serotypes with serotype-specific IgG  $\geq$  0.2  $\mu\text{g/mL}$  (measured by the GSK ELISA) in at least 93% of participants.<sup>6</sup> We used a WHO reference laboratory ELISA in this study; measures of 0.2  $\mu\text{g/mL}$  in the GSK ELISA correlates with 0.35  $\mu\text{g/mL}$  in the WHO ELISA.

Assuming the same response, 304 participants would provide 90% power ( $\alpha=0.025$ ) to determine if the 6+10 schedule was non-inferior to the 6+14 schedule in 5 out of 10 serotypes, and 80% power to determine if the 6+10 schedule was non-inferior to the 6+14 schedule in 7 out of 10 serotypes. These calculations allowed for a 10% sample loss (due to laboratory error or loss to follow up). There was no accounting for multiple comparisons in sample size calculations.

#### ***Role of the funding source***

This study was funded by Gavi - the vaccine alliance. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors had complete access to all study data, did not receive any funding to write this article, and had final responsibility for the decision to submit this publication.

## RESULTS

Between the 21st of August 2015 and 4th of April 2016, 850 children were screened and 304 enrolled (figure 1 and appendix table S2). 152 children each were randomised to receive PCV10 at either 6 and 10 weeks (6+10), or 6 and 14 weeks (6+14) of age. Both groups received PCV10 at 9 months of age. The baseline characteristics of the study participants are shown in table 1.

At 9 months of age, the proportion of children achieving serotype-specific IgG  $\geq 0.35$   $\mu\text{g/mL}$  was non-inferior following the 6+10 PCV10 schedule compared with the 6+14 schedule for serotypes 5 (95% CI -9.61% to 13.25%), 9V (95% CI -2.84% to 19.8%), 14 (95% CI -7.27% to 12.54%), and 19F (95% CI -9.86% to 0%) (all  $p < 0.025$ ), while for serotypes 1 (95% CI -13.56% to 6.77%), 4 (95% CI -21.64% to 1.18%), 6B (95% CI -20.65% to -0.18%), 7F (95% CI -15.26% to 5.42%), 18C (95% CI -25.78% to -4.83%), and 23F (95% CI -12.19% to 8.16%), non-inferiority was not demonstrated (table 2). Sensitivity analysis was done on the per protocol population by imputing missing data in either a best (IgG assumed to be  $\geq 0.35$   $\mu\text{g/mL}$ ) or worst (IgG assumed to be  $< 0.35$   $\mu\text{g/mL}$ ) case scenario. In the best case scenario, non-inferiority was shown for serotypes 5, 9V, 14, and 19F, whilst in the worst case scenario non-inferiority was shown for serotype 9V (appendix tables S3 and S4).

One month following the second priming dose of PCV10 the proportion of participants with IgG  $\geq 0.35$   $\mu\text{g/mL}$  in the 6+10 group compared with the 6+14 group was significantly lower for serotypes 1, 6B, 18C, and 23F (figure 2 and appendix table S5). At 10-months (one month following the 9-month booster dose) there were no measurable differences between the groups for serotypes 4, 18C, and 19F as 100% of participants in both groups had IgG  $\geq 0.35$   $\mu\text{g/mL}$ . For the remaining serotypes (serotypes 1, 5, 6B, 7F, 9V, 14, and 23F) there were no statistically significant differences found (figure 2, and appendix table S6).

Geometric mean concentrations of serum IgG one month following the second priming dose were significantly lower in the 6+10 compared with the 6+14 group for serotypes 6B, 7F, 18C, 19F, and 23F (figure 3 and appendix table S7). At the same time point the GMC of serum IgG to serotype 5 was significantly higher in the 6+10 compared with the 6+14 group. At 9 months and 10 months of age GMCs were significantly lower in the 6+10 compared with the 6+14 group for serotypes 18C and 19F (figure 3, and appendix tables S8 and S9).

Adverse reactions for seven days following the 14-week visit were solicited using a diary card. No significant differences across the seven-day period were seen between the two trial groups for fever, pain, vomiting, diarrhoea, or sleepiness. A significantly higher proportion of children in the 6+14 compared with the 6+10 group on day 1 had a change in feeding, and crying and irritability reported (appendix tables 10-16).

Nasopharyngeal swabs were collected from participants at 6 weeks and 10 months of age to examine whether any effect on PCV10 carriage may be detected between the trial groups. 60/304 (19.7%) and 170/287 (59.2%) participants had pneumococcus detected at 6 weeks and 10 months of age respectively. 5/152 (3.3%) and 2/152 (1.3%) children were carrying PCV10 serotypes at 6 weeks of age in the 6+10 and 6+14 groups respectively. At 10 months of age 13/143 (9.1%) and 12/144 (8.3%) of children were carrying a PCV10 serotype in the 6+10 and 6+14 groups respectively (6+10 vs 6+14 at 10 months,  $p = 0.8375$ ).

Antibody responses to diphtheria, pertussis, *Haemophilus influenzae* type b, and tetanus antigens (diphtheria toxin, pertactin, filamentous haemagglutinin (FHA), pertussis toxin, *Haemophilus influenzae* type b, protein D, and tetanus toxin) were assessed at each blood collection time point and showed that the 6+10 group had lower GMCs of IgG than the 6+14 group to all antigens except FHA at one month post the second dose of PCV10 (14 weeks in the 6+10 group and 18 weeks in the 6+14 group). At the later time points GMCs were similar between the two groups (appendix table S17).

Serious adverse events (SAEs) occurred in 32 participants, 11/152 in the 6+10 group and 21/152 in the 6+14 group (table 3). Fourteen SAEs were deemed to be related to PCV10 administration with five and nine events reported for the 6+10 and 6+14 groups respectively. No deaths were reported for any of the participants. Medical histories were collected for each of the SAEs and are shown in appendix table S18.

At each study visit, illnesses experienced by the participant since the prior visit were recorded. There was no evidence of difference between the groups in the number of overall illness episodes across the entire study period. At an illness-specific level there were significantly more cases of pneumonia between the second dose and the booster dose at 9 months of age in the 6+10 group, (15/144 versus 5/147 in the 6+10 and 6+14 groups respectively,  $p = 0.021$ ) (appendix table S19). However, when all pneumonia cases prior to the booster were analysed there was no significant difference between groups (18/147 versus 9/149 in the 6+10 and 6+14 groups respectively,

p=0.071). Across comparable time periods, no differences were seen between the groups for reported cases of tonsillitis, upper respiratory tract infections, bronchiolitis, and gastroenteritis (appendix tables 20-23).



## DISCUSSION

This is the first trial to compare antibody responses in children who received two doses of PCV10 in early infancy with either a one-month or two-month interval between doses, followed by a booster at 9 months of age. Our results show that at 9 months of age, prior to the booster, the immunogenicity of the 6+10 schedule was non-inferior to the 6+14 group for four of the ten vaccine serotypes (serotypes 5, 9V, 14, and 19F). Non-inferiority was not shown for the other six serotypes (1, 4, 6B, 7F, 18C, and 23F). For these serotypes, the 6+10 group had between 2% and 15% fewer participants with antibody levels achieving the 0.35 µg/mL threshold. At one-month following the second priming dose of PCV10, the 6+10 group had significantly fewer children with IgG ≥ 0.35 µg/mL for four of the ten serotypes (serotypes 1, 6B, 18C, and 23F). These results indicate that children in the 6+10 group had a period of lower antibodies to six vaccine serotypes in early infancy, prior to their 9 month booster dose. However, one-month after receipt of the booster dose, antibody responses were high for all serotypes and there were no significant differences between groups in the proportions of children with IgG ≥ 0.35 µg/mL.

Whether differences in immunogenicity between the study groups in early infancy, prior to the booster, translate to an increased risk of pneumococcal disease in this community is not known. A re-analysis of a trial of nine-valent PCV in the Gambia classified infants according to the spacing of doses received (three doses with one-month or two month intervals), and found no evidence for an increased risk of pneumonia, hospitalization, or mortality, in those with the shorter gap between doses.<sup>20</sup> In our study high antibody responses after the booster dose were achieved for both groups, with at least 92% of children achieving the 0.35 µg/mL threshold for all serotypes, implying no difference in disease risk between groups following the booster during early childhood. In many settings sustained population-wide PCV use in infancy has induced an indirect effect, protecting the unvaccinated population by interrupting transmission of vaccine serotypes and removing them from circulation.<sup>21</sup> In this region, as in others, carriage of pneumococcus is more prevalent in late infancy and early childhood, compared with early infancy.<sup>22</sup> This means that higher post-booster antibody levels in late infancy and their persistence through early childhood are thought to be of more importance to the interruption of transmission, than antibody levels in early infancy. Indeed, data from this study supported the WHO advice on pneumococcal vaccine schedules indicating that, while the 6, 14 week and 9 month schedule is preferred, the 6, 10 week and 9 month schedule could be used where programmatic issues demand it.<sup>23</sup> The differences in immunogenicity seen for some serotypes between the 6+10 and 6+14 groups after the second priming dose of PCV10, may translate to only a limited effect on vaccine preventable IPD risk during the early phases of nation-wide vaccine introduction (prior to establishment of herd protection), after which the timing of infant doses is likely to become of lesser importance. This is exemplified by the fact that some settings with established herd protection are considering the use of one-dose prime and boost (1+1) PCV schedules.<sup>21,24</sup>

The findings from this study are consistent with prior observations showing that administering a PCV at two-month as opposed to one-month intervals results in improved immunogenicity.<sup>25,26</sup> This is exemplified by a recent trial that showed a three dose PCV13 schedule with longer intervals (doses at 2, 4, and 6 months of age) had superior immunogenicity when compared with a three dose schedule with shorter intervals (doses at 2, 3, and 4 months of age) for 9 of the 13 vaccine serotypes one month after the priming series.<sup>25</sup> Following boosting these differences diminished, with the 2, 4, and 6-month schedule superior to the 2, 3, and 4-month schedule for GMCs of IgG to serotypes 18C and 23F only.

Examination of immunogenicity using GMCs showed similar observations to those made when comparing the groups using the 0.35 µg/mL threshold. Interestingly, GMCs for serotypes 18C and 19F were higher in the 6+14 compared with 6+10 group at 9 and 10 months. A finding which may be related to the difference in interval between priming doses, or age maturation of the immune system differing between the two groups when the second PCV10 dose is given. Notably, the pneumococcal polysaccharides for serotypes 18C and 19F contained within PCV10 are conjugated to tetanus toxoid and diphtheria toxoid respectively. In this trial the penta-valent vaccine which contains tetanus and diphtheria toxoids was administered at 6, 10 and 14 weeks of age. As such it may also be that the extra dose of penta-valent vaccine that the 6+14 group receive before the second dose of PCV10 has a role in augmenting the immunogenicity to serotypes 18C and 19F.

Between the second dose and the 9 month booster, children in the 6+10 group had significantly more cases of pneumonia compared with the 6+14 group. This may be a chance finding, or potentially due to the four-week longer observation period in the 6+10 group for this analysis. In addition, this study was not designed to measure the differences in pneumonia cases in a standardized way. It is unknown whether the cases of pneumonia in this study were due to PCV10 covered serotypes, non-PCV10 serotypes, other bacteria, or viruses. Larger epidemiological studies, specifically designed to measure pneumonia incidence among children receiving different PCV schedules would be better suited to identifying any difference in the risk of developing pneumonia as a result of vaccine timing.

The strengths of this trial are its randomised design, careful timing of sample collection allowing valid comparisons across groups, sample size powered for the primary outcome, low rate of loss to follow-up, and use of a standardized assay to assess the primary outcome. In this trial, timing of vaccine administration was strictly adhered to however, it is important to consider the effect of vaccine adherence in the general population, and how transferable findings in the trial are to those receiving the vaccine in the community. In Nepal the trial findings are likely to correlate well with observations in the community with the WHO estimating coverage of the third pentavalent vaccine (at 14 weeks of age) to be 87% in 2016, whilst one study reports adherence to the EPI for delivery of the first pentavalent vaccine dose (at 6 weeks of age) to be almost 80%.<sup>27,28</sup> How well the community may adhere to the 6+14 or the 6+10 PCV10 schedule is difficult to predict from this trial alone, and further study into community and health care provider preferences are needed to address this question. Limitations of this trial include the open-label design, the timeframe of blood sampling, multiple comparisons, and single-centre design. As participants and staff were aware of study arm allocation, there is the possibility that this influenced the reporting of any safety signals. Blood sampling following the second PCV priming dose was performed at 14 and 18 weeks of age in the 6+10 and 6+14 groups respectively, and as such there is a one month age difference between the groups when comparing this time point, which is not the case with the 9 and 10 month time points. There were multiple time points and multiple serotypes included in the analysis. The study was powered only on the primary outcome and not powered to account for multiple comparisons and therefore only unadjusted p-values are presented. It is therefore possible that some findings are due to chance alone and should be interpreted with caution. The single-centre design means that careful consideration should be taken when translating the findings to other settings. Should comparable studies be performed in the future, meta-analysis of the unadjusted data reported in this study may provide more robust results.

Overall this trial demonstrates that the safety profile of the 6+10 and 6+14 PCV10 schedules were similar however, improved immunogenicity in early infancy was seen for the 6+14 group. Direct protection may be reduced in early infancy among children receiving PCV10 at 6 and 10 weeks during the course of vaccine introduction. However, the interruption of transmission and creation of herd protection through high-coverage, nation-wide implementation of PCV10 is likely to progressively ameliorate any difference in disease risk due to lower immunogenicity in early infancy for the 6+10 schedule. As such the data reported in this trial should not impair flexibility in programmatic implementation of either schedule, as advised in recent WHO recommendations.<sup>23,29</sup>

## **Contributors**

RK designed the study, coordinated study implementation, analysed the data, and led the writing of the report. MG, ST, and BW designed the study and coordinated local study implementation. L-MY and UG conducted the statistical analysis. IA, KP, DRM, KLO'B, DFK, and SS designed the study. SK designed the study and coordinated study implementation. GB led the analysis of serum for antibodies to penta-valent vaccine antigens. KF conducted analysis of serum for anti-pneumococcal antibodies. DG led the analysis of serum for anti-pneumococcal antibodies. AJP designed the study and coordinated study implementation. All authors contributed to the interpretation of data and subsequent writing, reviewing, and revision of the manuscript.

## **Declarations of interest**

AJP reports grants from Okairos, and Pfizer, which finished within the past 36 months outside the submitted work. AJP is Chair of UK Dept. Health's Joint Committee on Vaccination & Immunisation & the EMA scientific advisory group, on vaccines and is a member of the WHO's SAGE. AJP is chair of the Department of Health's Joint Committee on Vaccination and Immunisation but the views expressed herein do not represent necessarily those of DH or JCVI. He is also a member of WHO's SAGE. DFK receives salary support from the NIHR Oxford Biomedical Research Centre. RK received the Robert Austrian Award in Pneumococcal Vaccinology, which was supported by Pfizer, at the 10<sup>th</sup> International Symposium on Pneumococci and Pneumococcal Diseases 2016. KOB has had grant funding in the preceding three years from GSK, Pfizer, the Bill & Melinda Gates Foundation and Gavi for pneumococcal vaccine related research. DG reports grants from vaccine manufacturers GSK, Sanofi Pasteur, and Merck, outside the submitted work.

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## REFERENCES

- 1 Wahl B, O'Brien K, Greenbaum A, *et al.* Global, regional, and national burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: updated estimates from 2000-2015. *Lancet Glob Heal* 2018.
- 2 Centers for Disease Control and Prevention (CDC). Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease-United States, 1998-2003. *MMWR Morb Mortal Wkly Rep* 2005; **54**: 893-7.
- 3 Flasche S, Van Hoek AJ, Sheasby E, *et al.* Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. *PLoS Med* 2011; **8**: e1001017.
- 4 Kelly DF, Thorson S, Maskey M, *et al.* The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal. *Int J Infect Dis* 2011; **15**: e17-23.
- 5 Shah AS, Deloria Knoll M, Sharma PR, *et al.* Invasive Pneumococcal Disease in Kanti Children's Hospital, Nepal, as Observed by the South Asian Pneumococcal Alliance Network. *Clin Infect Dis* 2009; **48**: S123-8.
- 6 Hamaluba M, Kandasamy R, Upreti SR, *et al.* Comparison of two-dose priming plus 9-month booster with a standard three-dose priming schedule for a ten-valent pneumococcal conjugate vaccine in Nepalese infants: a randomised, controlled, open-label, non-inferiority trial. *Lancet Infect Dis* 2015. DOI:10.1016/S1473-3099(15)70007-1.
- 7 Rijal B, Tandukar S, Adhikari R, *et al.* Antimicrobial susceptibility pattern and serotyping of Streptococcus pneumoniae isolated from Kanti Children Hospital in Nepal. *Kathmandu Univ Med J (KUMJ)*; **8**: 164-8.
- 8 Kumar R, Arora N, Santosham M. South Asia symposium on pneumococcal disease and the promise of vaccines - Meeting report. *Vaccine* 2016; **34**: 2622-6.
- 9 WHO recommendations for routine immunization - summary tables. [http://www.who.int/immunization/policy/immunization\\_tables/en/](http://www.who.int/immunization/policy/immunization_tables/en/) (accessed Jan 9, 2017).
- 10 Organization WH. Weekly epidemiological record. World Health Organization, 2014 <http://www.who.int/wer/2014/wer8921.pdf?ua=1>.
- 11 Ministry of Health: National Immunisation Programme. <http://www.mohe.gov.np/content/national-immunisation-programme> (accessed Dec 3, 2017).
- 12 Conklin L, Loo JD, Kirk J, *et al.* Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type invasive pneumococcal disease among young children. *Pediatr Infect Dis J* 2014; **33 Suppl 2**: S109-18.
- 13 Goldblatt D, Southern J, Ashton L, *et al.* Immunogenicity of a Reduced Schedule of Pneumococcal Conjugate Vaccine in Healthy Infants and Correlates of Protection for Serotype 6B in the United Kingdom. *Pediatr Infect Dis J* 2010; **29**: 401-5.
- 14 Sortition®: Clinical trial randomisation software. <https://www.phc.ox.ac.uk/research/technology-outputs/sortition-clinical-trial-randomisation-software>.
- 15 van Gageldonk PGM, van Schaijk FG, van der Klis FR, Berbers GAM. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to Bordetella pertussis, diphtheria and tetanus. *J Immunol Methods* 2008; **335**: 79-89.
- 16 de Voer RM, Schepp RM, Versteegh FGA, van der Klis FRM, Berbers GAM. Simultaneous detection of Haemophilus influenzae type b polysaccharide-specific antibodies and Neisseria meningitidis serogroup A, C, Y, and W-135 polysaccharide-specific antibodies in a fluorescent-bead-based multiplex immunoassay. *Clin Vaccine Immunol* 2009; **16**: 433-6.
- 17 Satzke C, Turner P, Virolainen-Julkunen A, *et al.* Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine* 2013; **32**: 165-79.
- 18 Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. *Stat Med* 1990; **9**: 1447-54.
- 19 R Core Team (2016). R: A language and environment for statistical computing. <https://www.r-project.org/>.
- 20 Mackenzie GA, Bottomley C, van Hoek AJ, *et al.* Efficacy of different pneumococcal conjugate vaccine schedules against pneumonia, hospitalisation, and mortality: Re-analysis of a randomised trial in The Gambia. *Vaccine* 2014; **32**: 2493-500.
- 21 Ladhani SN, Collins S, Djennad A, *et al.* Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000-17: a prospective national observational cohort study. *Lancet Infect Dis* 2018; **18**: 441-51.
- 22 Kandasamy R, Gurung M, Thapa A, *et al.* Multi-serotype pneumococcal nasopharyngeal carriage prevalence in vaccine naïve Nepalese children, assessed using molecular serotyping. *PLoS One* 2015; **10**: e0114286.

- 23 Weekly epidemiological record. 2017  
<http://apps.who.int/iris/bitstream/handle/10665/259533/WER9248.pdf?sequence=1> (accessed April 17, 2018).
- 24 Goldblatt D, Southern J, Andrews NJ, *et al.* Pneumococcal conjugate vaccine 13 delivered as one primary and one booster dose (1 + 1) compared with two primary doses and a booster (2 + 1) in UK infants: a multicentre, parallel group randomised controlled trial. *Lancet Infect Dis* 2018; **18**: 171–9.
- 25 Spijkerman J, Veenhoven RH, Wijmenga-Monsuur AJ, *et al.* Immunogenicity of 13-valent pneumococcal conjugate vaccine administered according to 4 different primary immunization schedules in infants: a randomized clinical trial. *JAMA* 2013; **310**: 930–7.
- 26 Deloria Knoll M, Park DE, Johnson TS, *et al.* Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on immunogenicity. *Pediatr Infect Dis J* 2014; **33 Suppl 2**: S119–29.
- 27 Nepal: WHO and UNICEF estimates of immunization coverage: 2016 revision. 2016  
[https://data.unicef.org/wp-content/uploads/country\\_profiles/Nepal/immunization\\_country\\_profiles/immunization\\_npl.pdf](https://data.unicef.org/wp-content/uploads/country_profiles/Nepal/immunization_country_profiles/immunization_npl.pdf).
- 28 Hoest C, Seidman JC, Lee G, *et al.* Vaccine coverage and adherence to EPI schedules in eight resource poor settings in the MAL-ED cohort study. *Vaccine* 2017; **35**: 443–51.
- 29 Cohen O, Knoll M, O’Brien K, *et al.* Pneumococcal Conjugate Vaccine (PCV) Review of Impact Evidence (PRIME).  
[http://www.who.int/immunization/sage/meetings/2017/october/3\\_FULL\\_PRIME\\_REPORT\\_2017Sep26.pdf](http://www.who.int/immunization/sage/meetings/2017/october/3_FULL_PRIME_REPORT_2017Sep26.pdf).

## FIGURE LEGENDS

**Figure 1. Trial profile.** Nepalese infants were randomly assigned to receive PCV10 at 6 and 10 weeks of age followed by a 9-month booster (6+10 group) or 6 and 14 weeks of age followed by a 9 month booster (6+14 group). PCV10 = ten-valent pneumococcal conjugate vaccine. DTwP-HBV-Hib = diphtheria-tetanus-whole cell pertussis-hepatitis B virus-*Haemophilus influenzae* type b vaccine. OPV = oral poliomyelitis vaccine. IPV = inactivated poliomyelitis vaccine.

**Figure 2. Proportion of Nepalese children with serum pneumococcal serotype-specific IgG  $\geq$  0.35  $\mu$ g/mL.** Nepalese children were randomised to receive PCV10 at 6 and 10 weeks of age (6+10) or 6 and 14 weeks of age (6+14). Sera were collected and analysed at one-month following the second priming dose (A), 9 months of age (B), and 10 months of age (C). \*p < 0.05 for 6+14 versus 6+10 at one-month following the second priming dose and 10 months of age.

**Figure 3. Geometric mean concentrations (GMCs) of serum pneumococcal serotype-specific IgG amongst Nepalese children.** Participants were randomised to receive PCV10 at 6 and 10 weeks of age (6+10) or 6 and 14 weeks of age (6+14). Sera were collected and analysed at one-month following the second priming dose (A), 9 months of age (B), and 10 months of age (C). \*p < 0.05 for 6+14 versus 6+10. †p < 0.05 for 6+10 versus 6+14.