ORIGINAL ARTICLE

First Human Challenge Testing of a Pneumococcal Vaccine
Double-Blind Randomized Controlled Trial

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Abstract

Rationale: New vaccines are urgently needed to protect the vulnerable from bacterial pneumonia. Clinical trials of pneumonia vaccines are slow and costly, requiring tens of thousands of patients. Studies of pneumococcal vaccine efficacy against colonization have been proposed as a novel method to down-select between vaccine candidates.

Objectives: Using our safe and reproducible experimental human pneumococcal colonization model, we aimed to determine the effect of 13-valent pneumococcal conjugate vaccine (PCV) on colonization.

Methods: A total of 100 healthy participants aged 18–50 years were recruited into this double-blind randomized placebo-controlled trial. They were randomly assigned to PCV (n = 49) or hepatitis A (control, n = 50) vaccination and inoculated with 80,000 CFU/100 μl of *Streptococcus pneumoniae* (6B) per naris.

Measurements and Main Results: Participants were followed up for 21 days to determine pneumococcal colonization by culture of nasal wash. The PCV group had a significantly reduced rate of 6B colonization (10% [5 of 48]) compared with control subjects (48% [23 of 48]) (risk ratio, 0.22; confidence interval, 0.09–0.52; $P < 0.001$). Density of colonization was reduced in the PCV group compared with the control group following inoculation. The area under the curve (density vs. day) was significantly reduced in the PCV compared with control group (geometric mean, 259 vs. 11,183; $P = 0.017$).

Conclusions: PCV reduced pneumococcal colonization rate, density, and duration in healthy adults. The experimental human pneumococcal colonization model is a safe, cost-effective, and efficient method to determine the protective efficacy of new vaccines on pneumococcal colonization; PCV provides a gold standard against which to test these novel vaccines.

Clinical trial registered with ISRCTN: 45340436.

Keywords: pneumonia; vaccination; challenge; pneumococcus; colonization

Pneumococcal disease is the most common cause of preventable death in children and a major cause of death among adults worldwide (1). Major impact on disease prevention requires interruption of colonization (2). Pneumococcal conjugate vaccine (PCV) has been effective in the prevention of pneumococcal colonization (3, 4) and disease (5) in young children, with indirect herd protection in unvaccinated adults because of reduced community colonization rates. However,

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At a Glance Commentary

Scientific Knowledge on the Subject: New vaccines are urgently needed to protect the vulnerable from bacterial pneumonia. Clinical trials of pneumonia vaccines are slow and costly, requiring tens of thousands of patients. Studies of pneumococcal vaccine efficacy against colonization have been proposed as a novel method to down-select between vaccine candidates.

What This Study Adds to the Field: The experimental human pneumococcal colonization model is a safe, cost-effective, and efficient method to determine the protective efficacy of new vaccines on pneumococcal colonization. Pneumococcal conjugate vaccine (Prevenar-13) provides a gold standard against which to test these novel vaccines.

the lack of a serotype-independent vaccine and the level of protection afforded against mucosal diseases, such as pneumonia and otitis media, remain problematic in the current pneumococcal vaccination strategy. As such there is a need for new vaccines with several in development, some of which are at the stage of phase 1 trials (6). However there is a bottleneck in non–PCV-related vaccine development because clinical trials with tens of thousands of participants are required to compare a new vaccine with the current gold standard vaccine (PCV) using an outcome of disease reduction. A pathway for licensure of PCV-related products now exists based on noninferiority of immunogenicity bypassing the need for phase III trials (7).

A reduction in experimental colonization acquisition rates after vaccination would provide proof-of-concept for both individual protection and an indication of potential reduction in transmission, essential for herd protection. These results would generate confidence for pursuing large and expensive clinical trials with pneumonia, otitis media, or invasive pneumococcal disease as endpoints (8). Studies of pneumococcal vaccine efficacy against pneumococcal colonization have been proposed as an effective method to down-select between vaccine candidates and lend support to phase III trial choice (8). We have developed a safe and reproducible experimental human pneumococcal colonization (EHPC) model. In this model, carefully screened healthy adult participants are inoculated with doses of pneumococci with a 50% colonization rate at a density typical of natural colonization and duration of 1–3 weeks (9, 10).

In terms of global impact, PCV is currently considered to be an outstanding vaccine success; it sets the standard for future pneumococcal vaccines and is therefore the ideal gold standard for EHPC testing. Here we use this EHPC model to assess whether 13-valent PCV (PCV-13, Prevenar-13) has a direct impact on experimental pneumococcal colonization rates, density, and duration. Some of the results of this study have been previously reported in the form of an abstract (11).

Methods

Trial Design and Participants

A total of 100 nonsmoking, healthy participants aged 18–50 years old were recruited between September 2013 and April 2014 (Figure 1). Participants were screened as discussed and then randomized to receive either PCV-13 (Prevenar-13 containing serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; Pfizer, New York, NY) or hepatitis A (Avaxim; Sanofi Pasteur MSD, Lyon, France) vaccination (control group). Natural nasopharyngeal pneumococcal colonization at the time of recruitment/screening was not an exclusion criteria. Serotypes included in a vaccine are termed vaccine types (VTs); those not included are termed nonvaccine types (NVTs). The National Health Service Research and Ethics Committee approved the study (12/NW/0873 Liverpool).

Randomization, Blinding, Vaccination, and Unblinding

A double-blind randomized controlled trial (RCT) was performed at a city-center university teaching hospital in Liverpool, United Kingdom. Randomization was computer-generated and occurred in blocks of 10. An independent statistician from the tropical Clinical Trials Unit at the Liverpool School of Tropical Medicine produced the randomization schedule and the sealed envelopes containing the study group allocations. Research (clinical and laboratory) staff and participants were blinded to the vaccination allocation. An unblinded vaccination team was used to vaccinate study participants. Vaccines were prepared out of sight of the study participants. At the end of the study, the participants were unblinded to the vaccine that they received.

Hepatitis A vaccine (Avaxim) was chosen as a suitable control because of its safety profile, adjuvant content (aluminum-containing vaccine), (assumed) lack of effect on nasal colonization/immunity, and health benefit for those involved in the study.

Participant Monitoring and Safety

A Data Monitoring and Safety Committee monitored the study throughout. At the prevaccination visit a clinical examination, history, and pregnancy test (where appropriate) were performed. Participants remained at the clinic for 20–30 minutes after vaccination to monitor for any immediate side effects. Full resuscitation equipment and an anaphylaxis trolley were immediately available. Four weeks after vaccination, participants were inoculated with pneumococcus.

Study screening to minimize the risk of pneumococcal infection to participants or contacts included the following:

- Study team selection, experienced in human challenge studies
- Study design
- Serotype selection (6B) and dosing
- Participant selection and exclusion criteria (*see* supplemental material E1 in the online supplement; exclusion criteria)
- Participant education and rigorous safety procedures including (*1*) a 24-hour emergency telephone contact with researchers (including close individual daily monitoring for 7 d postinoculation via text contact) and access to hospital facilities with prompt treatment if required, (*2*) a postinoculation advice sheet, (*3*) a thermometer, and (*4*) a course of amoxicillin tablets in case of emergency

Data on adverse events were collected and categorized as follows: headache, sore throat, nasal congestion/running/sneezing, myalgia, lethargy, earache/muffling/popping, pyrexia, neck stiffness, hospital admission, and other (including shivering, wheezy, cough, abdominal cramps, photophobia, sinus pain, and generally unwell). As per sponsor guidance, any serious adverse events were recorded and

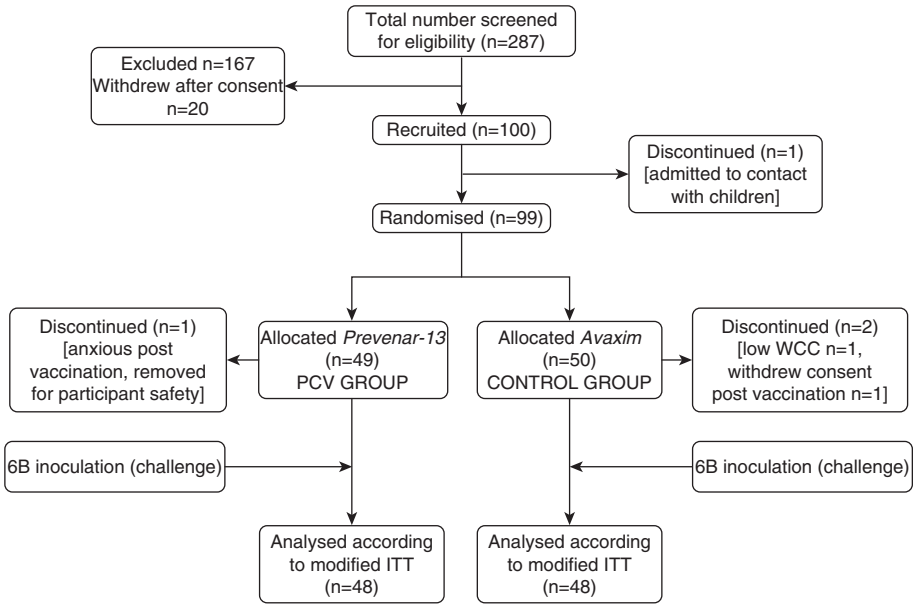

Figure 1. Consolidated Standards of Reporting Trials flow diagram showing numbers of participants screened, recruited, and randomized, and reasons for discontinuation in the study and timing of inoculation/challenge. Reasons for exclusion include the following: close contact with at-risk individuals (children, n = 16; patients, n = 41), did not attend appointment (n = 18), cannot commit time to the study (n = 13), current smokers or >10 pack-years (n = 11), lived in a hepatitis A endemic area (n = 11), previously involved in an experimental pneumococcal colonization study (n = 9), asthma (n = 8), allergic to penicillin/amoxicillin (n = 7), and other (n = 33). ITT = intention-to-treat; PCV = pneumococcal conjugate vaccine; WCC = white cell count.

reported to the Data Monitoring and Safety Committee and sponsor within 24 hours.

Experimental Human Pneumococcal Colonization

We used our published protocol with full safety cover (10). Briefly, a well-characterized penicillin-sensitive 6B serotype pneumococcus (BHN 418, sequence type 138) was grown to mid-log phase in Vegitone broth (Oxoid; Thermo Scientific, Hampshire, UK) and stored in 1-ml aliquots containing 20% glycerol at −80°C. Confirmation of serotype was performed using latex agglutination (Statens Serum Institute, Copenhagen, Denmark) and bacterial purity was confirmed by an independent reference laboratory (Public Health England, Colindale, UK).

On the day of inoculation, an aliquot was thawed, centrifuged, and the bacterial pellet was washed before being resuspended and diluted in 0.9% sterile saline to reach the desired concentration of bacteria. The prepared inoculum was taken to the clinical area where the participant was seated in a semirecumbent position. A total of 100 μl of inoculum containing the desired dose (80,000 CFU/100 μl) was instilled into each nostril in a circular motion (10). Following inoculation the participant remained in this position for 10–15 minutes. Serial dilutions of the inocula were plated onto blood agar for dose confirmation. The 6B serotype has been used previously by our group and others (9, 12). The strain was chosen because its genome is fully sequenced and there are negligible rates of natural colonization with 6B in Liverpool. We performed studies (using serotype 23F and 6B) to establish dose, safety, and achieve stable colonization rates (manuscript in preparation).

Nasal Washing and Detection of Pneumococcal Colonization

Nasal wash (NW) samples were collected prevaccine, post-vaccine/preinoculation, and postinoculation (Days 2, 7, 14, and 21). Samples were collected and processed for pneumococcal detection as previously described (10, 13). Briefly, 5 ml of 0.9% saline was instilled into each naris; this was repeated twice (10 ml total per nares). If less than 10 ml was returned, up to 40 ml normal saline was used as necessary. All CFU density data were calculated as CFU per milliliter of NW returned.

NW samples were transferred immediately to the laboratory and processed as previously described (9, 10). Briefly, NW samples were centrifuged at 3,345 × *g* for 10 minutes. Following centrifugation the supernatant was discarded and the NW bacterial pellet was resuspended in 100 μl of skim milk tryptone glucose glycerol medium. Serial dilutions of the pellet were plated on Columbia Horse Blood Agar containing 4 μg/ml gentamicin (Sigma, Dorset, UK) to quantify colonization density. Plates were incubated for 24 hours at 37°C with 5% CO_2 and inspected to identify pneumococcal phenotype. Serotype was confirmed by latex agglutination. Participants in whom experimental pneumococci were detected in NW samples at any visit postinoculation were defined as experimentally colonized. Isolates were frozen at −80°C for storage. All experimentally colonized participants who did not have two consecutive culture-negative NWs received amoxicillin, 500 mg three times per day, for 3 days at the end of the study to ensure 6B colonization clearance.

Endpoints

The primary endpoint was pneumococcal colonization at any time point (Days 2, 7, 14, or 21). The secondary endpoints were (*1*) pneumococcal colonization at individual time points (Days 2, 7, 14, or 21); (*2*) pneumococcal density at individual time points and the area under the density curve (AUC); and (*3*) pneumococcal colonization duration, defined as the duration from inoculation to the last confirmed positive NW sample. All endpoints were defined by culture of NW.

Statistical Methods and Analysis

The primary endpoint was analyzed using a generalized linear model with treatment as a single predictor, generating risk ratios and odds ratios together with their 95% confidence intervals (CIs) of being colonized with pneumococcus between the PCV and control groups. The presence of pneumococcus at individual time points was analyzed using a generalized estimating equation (GEE) model with treatment, time, interaction between treatment, and time as fixed effects and participant as cluster effect. The odds ratio together with their 95% CIs at each time point were derived from the GEE model.

Density of pneumococcal colonization at different time points was available for those with measured density. Log-transformed density was analyzed using

Table 1. Baseline Demographics of Participants

Characteristics	PCV (*n* = 49)	Control (*n* = 50)
Age, yr, mean ± SD	24.1 ± 6.1	23.2 ± 6.9
Male, number (%)	20 (40.8)	19 (38.0)
Time from vaccination to inoculation, d, mean ± SD	35.0 ± 3.9*	34.1 ± 2.2*
Dose inoculated, CFU/100 μl, mean ± SD	83,203 ± 8,026*	82,602 ± 8,098*

Definition of abbreviation: PCV = pneumococcal conjugate vaccine.
Data as per intention-to-treat analysis, n = 99.
*n = 48 in each group.

a GEE model with treatment, time, interaction between treatment and time as fixed effects, and participant as cluster effect. The ratio between PCV and control in geometric mean together with their 95% CIs at each time point was calculated. Also, the AUC of pneumococcal colonization at Days 2, 7, 14, and 21 was calculated using the trapezoidal rule and the log-transformed AUC was analyzed using a generalized linear model with a single factor of treatment. The ratio between PCV and control in geometric mean concentration together with their 95% CIs was derived.

Analysis was based on the intention-to-treat (ITT) principle. Participants not completing the inoculation limb of the study were removed leading to a modified ITT population. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC) and Stata 13 (StataCorp, College Station, TX).

Results

A total of 429 volunteers were screened and 100 participants were recruited. Reasons for nonrecruitment included close contact with children aged younger than 5 years old or at-risk individuals, such as the elderly or those on immunosuppressive medications; asthma (on regular medication); current smoker; greater than 10 pack-year smoking history; and penicillin/amoxicillin allergy (*see* supplemental material E1). A total of 99 participants were vaccinated (n = 49 PCV, n = 50 control subjects, ITT population); three participants were removed preinoculation (n = 96 modified ITT population) (Figure 1).

There were no significant differences between the groups with regards to age, sex, time from vaccination to inoculation, or dose of inoculum received (Table 1). Pneumococcal colonization with the inoculated 6B serotype at any time was found in 5 of 48 (10.4%) participants in the PCV group compared with 23 of 48 (47.9%) in the control group. The risk ratio of pneumococcal colonization following PCV compared with control vaccine was 0.22 (95% CI, 0.09–0.52; $P = 0.0007$). The corresponding odds ratio was 0.13 (95% CI, 0.04–0.3; $P = 0.0002$). The percentage of colonized participants tell from 8.3% to 4.3% in the PCV group and from 43.8% to 33.3% in the control group between Days 2 and 21, respectively (Table 2). Among the five participants colonized in the PCV group, two participants were still colonized at Day 21.

PCV reduced colonization density (Figure 2). The density of 6B pneumococci recovered from the nasopharynx of participants in the PCV group was on average three logs lower than the density recovered from the control group, with statistically significant differences seen up to 7 days postinoculation (Table 3, Figure 2). There was an overall significant reduction in colonization intensity as measured by the AUC (ratio, 0.02; 95% CI, 0.00–0.51; $P = 0.017$). We did not observe a statistical difference in duration of established colonization between the groups ($P = 0.1328$).

In total six participants were naturally colonized, four in the PCV and two in the control group. In the PCV group, two participants were colonized with VT (both serotype 3) and two with NVT. In the control group both were NVT (serotype 33 and 8). One participant in the control group was co-colonized with both the inoculated serotype 6B and a naturally acquired serotype 8 (*see* Table E1). In less than 3% (n = 15) of NW more than 20 ml of normal saline was required to obtain the required greater than or equal to 10 ml of NW return.

Of those receiving Prevenar-13, 12 (24%) had local symptoms (sore arm, n = 12; injection site swelling, n = 2). Of those receiving Avaxim, nine (19%) had local symptoms (sore arm, n = 5; injection site pain/redness/swelling, n = 4; localized numbness, n = 1; neck stiffness, n = 1). For both vaccines no systemic symptoms were reported and there were no episodes of anaphylaxis.

Fourteen (48%) colonized and 25 (37%) noncolonized participants reported symptoms. Thirteen colonized (three PCV, 10 control subjects) and 23 noncolonized (14 PCV, nine control subjects) reported minor symptoms at least once up to Day 21 postinoculation. Minor symptoms included headache, sore throat, nasal congestion/running/sneezing, myalgia, lethargy, earache/muffling/popping, pyrexia, wheeze, mild photophobia, cough, abdominal pain, and sinus pain. One participant (PCV group and 6B colonized) was admitted to hospital overnight at 48 hours postinoculation complaining of pyrexia,

Table 2. 6B Pneumococcal Colonization Status Assessed According to Vaccination Group at Each and Any Time Point

Day	No. Colonized/Total No. (%)		Odds Ratio (95% CI)	*P* Value
	PCV Group (*n* = 48)	Control Group (*n* = 48)		
Day 2	4/48 (8.3)	21/48 (43.8)	0.12 (0.04–0.38)	0.0003
Day 7	4/48 (8.3)	21/48 (43.8)	0.12 (0.04–0.38)	0.0003
Day 14	1/48 (2.1)	19/48 (39.6)	0.05 (0.01–0.27)*	0.0004*
Day 21	2/46 (4.3)	15/45 (33.3)		
Any day	5/48 (10.4)	23/48 (47.9)	0.13 (0.04–0.37)	0.0002

Definition of abbreviations: CI = confidence interval; PCV = pneumococcal conjugate vaccine.
Note modified intention-to-treat analysis (n = 96) was used because participants excluded post-vaccination but preinoculation cannot develop experimental 6B colonization.
*Days 14 and 21 were combined to generate a stable estimate.

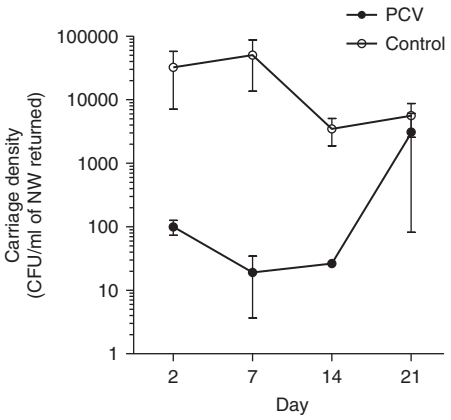

Figure 2. 6B pneumococcal colonization intensity (density [CFU/ml of NW returned] in relation to duration of colonization [days]) postinoculation at each time point according to vaccination group. Values shown are the mean CFU/ml ± SEM. Full data plotted in Figure E1. NW = nasal wash; PVC = pneumococcal conjugate vaccine.

lethargy, and sore throat. She was diagnosed with tonsillitis and a nontoxigenic *Corynebacterium diptheriae* was cultured from throat swabs. She received amoxicillin, 500 mg three times per day, for 10 days and made a full and uneventful recovery. The participant was included in the ITT analysis although, as expected, the antibiotic therapy terminated 6B colonization.

Discussion

We have demonstrated that 6B pneumococcal colonization acquisition was reduced by 78% in Prevenar-13 (PCV) vaccinated adults compared with control subjects. When 6B colonization did occur post-PCV vaccination, this was at a significantly lower density than in control participants. Furthermore, we demonstrated for the first time that EHPC can be used as an innovative approach to test vaccine efficacy in healthy adults using both pneumococcal colonization acquisition rates and density as important and relevant endpoints.

Human challenge studies in vaccination are not unusual; they have been critical to the development of a wide number of candidate approaches to malaria vaccination (14) and are in development for other infectious diseases (15). We conducted this study as a registered double-blind RCT to demonstrate proof of principle for EHPC in pneumococcal vaccine testing. The study has a number of strengths in that it is small, quick, safe, economical, and precise. The study took 9 months to complete, required only 100 participants, had no serious adverse events related to pneumococcal inoculation, and had a budget considerably less than £1 million, with the precision of the culture-determined colonization endpoint giving the study a definitive answer. Our observations of a reduction in colonization acquisition rate and density, but not duration, are the same as shown in RCTs conducted in children (16, 17). Using disease as an endpoint, the CAPiTA (Community-Acquired Pneumonia Immunization Trial in Adults) study involved more than 85,000 participants from 58 hospitals in the Netherlands over 5 years from 2008, to evaluate the efficacy of Prevenar-13 in adults 65 years and older (18–20). EHPC studies could be used to down-select vaccine candidates. Using colonization as a surrogate endpoint, the cost and time taken from vaccine discovery to product registration and market (often over a 10-year process with current cost estimates of US$200–500 million per vaccine [21]) could be substantially reduced.

Table 3. 6B Colonization Density According to Vaccination Group at Each Time Point

Day	Density (*CFU/ml*) Geometric Mean ± SD		Ratio (95% CI)	*P* Value
	PCV (No. Colonized at Each Time Point)	Control (No. Colonized at Each Time Point)		
Day 2	99 ± 53 (4)	33,694 ± 120,812 (21)	0.17 (0.04–0.65)	0.0099
Day 7	19 ± 31 (4)	50,517 ± 169,172 (21)	0.02 (0.00–0.14)	<0.0001
Day 14	26* (1)	3,476 ± 6,962 (19)	1.94 (0.09–43.90)†	0.6767†
Day 21	3,085 ± 4,247 (2)	5,623 ± 11,855 (15)		

Definition of abbreviations: CI = confidence interval; PCV = pneumococcal conjugate vaccine.
Density is only reported in participants who are colonized with 6B.
*Only one observation, and SD is unavailable.
†Days 14 and 21 were combined to generate a stable estimate.

The main weakness of the study is that colonization in healthy adults is not a clinical disease endpoint; nevertheless, it is a critical determinant of transmission not simply a surrogate of protection. Other limitations are that this model is not suitable for use in children, because of ethical issues, and only a single serotype (6B) was used; our planned future work will also use other serotypes. Prevention of colonization in children and adults results in herd protection through reduced exposure, therefore this effect is directly relevant to predicting herd protection and hence informing vaccine strategy. The small number of participants with natural colonization (n = 6; 6%) in our study is typical of that expected for U.K. adults (4), but does not allow conclusions about the effect of PCV-13 vaccination on natural colonization or its interaction with 6B. We note that although it is theoretically possible to have both natural and 6B experimental colonization, this was only seen in one participant. A short interval between vaccination and pneumococcal challenge was chosen to assess the effect on colonization during the period of optimal immune response to vaccination. Future studies with longer intervals are planned to investigate whether protection wanes with time and will investigate the role of viral colonization. We have previously found no correlation between baseline anticapsular or antiprotein antibody levels and protection from colonization (9).

Epidemiologic data have shown a reduction in nasopharyngeal colonization after PCV in children and adults (4, 22) by direct protection of vaccinated individuals and by reduction in exposure of unvaccinated individuals through herd protection. The protective effectiveness of PCV-13 against VT colonization in children was approximately 74% (22). We have replicated these findings in adults demonstrating a 78% reduction against VT (6B experimental) colonization in adults. We have also shown a 10% PCV-13 failure rate. The large effect demonstrated here may either be caused by our protocol design (in which we chose to inoculate participants at optimal immunity post PCV-13 vaccination at 4 wk), or it may be that PCV-13 is particularly effective against type 6B (as has been shown in colonization studies in children when compared with other VTs, post PCV-7 and -13, such as serotype 19F and 3 [22, 23]). 6B colonization rates in children declined from 20% (24) to 0–2%

after the introduction of PCV-7 (3, 23, 25, 26) in Europe. Our findings replicate the known impact of PCV on colonization (impact on density and acquisition but no impact on duration) from both RCT and epidemiologic studies (16, 17).

This study provides data to confirm that PCV-13 not only leads to a significant reduction in pneumococcal colonization acquisition rates, but also reduces the density thereby offering further protection by reduced transmission. We suggest that this novel EHPC model can be used as a platform for future pneumococcal vaccine testing, using small sample sizes and shorter time scales than community studies to reduce time and cost to market. We recommend that colonization acquisition rate, density, and duration are all measured in these studies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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