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MAJOR ARTICLE

Nasopharyngeal Pneumococcal Density Is Associated With Viral Activity but Not With Use of Improved Stoves Among Young Andean Children

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Background. Indoor smoke exposure is common in developing countries and may influence nasopharyngeal (NP) pneumococcal colonization density and risk of acute respiratory illness. We compared colonization density among Andean children living in households previously enrolled in a randomized controlled trial of a home intervention package including improved stoves to reduce smoke, kitchen sinks, and water disinfection.

Methods. We enrolled 260 children aged <3 years and made weekly household visits to assess for acute respiratory illness (ARI) and collect nasal swabs for respiratory virus polymerase chain reaction (PCR) testing during ARI. At monthly intervals, NP swabs were collected to determine pneumococcal colonization density through quantitative *lytA* PCR. We used linear quantile mixed-effects models to compare median log-transformed colonization densities among children in households randomized to the control (n = 129) versus intervention (n = 131) in sequential time points, accounting for random effects of multiple samples from individual children. Other covariates included age, sex, month, antibiotic exposure, and timing of sample collection relative to ARI with and without viral detection.

Results. Age and sociodemographic characteristics were similar between groups. Although no differences were observed in densities between groups, colonization density varied significantly over time in both groups, with highest densities coinciding with spring months. Time during and after virus-associated ARI was also associated with higher pneumococcal colonization density than time remote from ARIs.

Conclusions. A home intervention package, including improved stoves, was not associated with changes in pneumococcal densities in young Andean children. However, increasing pneumococcal density was observed with spring season and viral-associated ARIs.

Keywords. indoor smoke exposure; pneumococcal density; pneumococcus; respiratory viruses.

Streptococcus pneumoniae is an important etiology of pneumonia in children. *Streptococcus pneumoniae* is also commonly detected in the nasopharynx of young children without respiratory symptoms. Nasopharyngeal (NP) pneumococcal colonization represents a critical initial step in the development of pneumococcal disease, and increased pneumococcal colonization density has been associated with increased risk of respiratory illness in children [1, 2]. Several factors have been associated with increased pneumococcal density, and thus they may also influence the risk of pneumococcal disease, acquisition of a new pneumococcal serotype, and the course of concomitant respiratory viral infections [3–5].

The use of indoor open fires or poorly functioning traditional stoves that rely on biomass fuels from wood, dung, or crop residues is common in rural areas of developing countries [6]. Evidence from some [6–8] but not all [9–12] studies suggests that indoor air pollution created by these stoves and other smoke exposures increases the risk of acute respiratory illness (ARI) in individuals living in these households, particularly women and young children, who are most heavily exposed. Limited data suggest that indoor air pollution from either cigarette exposure or open fires may be associated with increased risk of pneumococcal disease [13, 14]. However, the impact of indoor air pollution on NP pneumococcal colonization and density patterns in young children remains unclear.

From 2009 to 2011, we enrolled young children in the rural Peruvian Andes in a prospective cohort study of Respiratory Infections in Andean Peruvian children (RESPIRA-Peru) to evaluate the etiologies of ARI and to explore interactions of

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viruses with common respiratory bacteria [2, 15–19] among these children. A subset of children enrolled in the RESPIRA-Peru study lived in households that had been previously enrolled in a randomized controlled trial of a home intervention package that included improved stoves to reduce indoor smoke and enhanced water sanitation measures (the Integrated Home-based Intervention Package [IHIP] study) [10, 20]. We aimed to compare NP pneumococcal colonization density between children living in households in the IHIP control group and those in the intervention group and to evaluate the association between pneumococcal density and other factors, including age, sex, symptomatic status, antibiotic exposure, and viral detection.

METHODS

Study Setting

The IHIP and RESPIRA-Peru studies took place in the Province of San Marcos, Department of Cajamarca, located in the northern highlands of Peru. The population is primarily rural, with low educational level, low income, and limited access to healthcare services [16]. Altitude in San Marcos ranges from 1500 to 4000 meters. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the study communities starting in late 2009. The IHIP and RESPIRA-Peru studies were approved by the Institutional Review Boards of Vanderbilt University and the Instituto de Investigacion Nutricional in Peru. The IHIP study was also approved by the cantonal ethical review board of University of Basel, Switzerland (Ethikkommission Beider Basel).

Intergrated Home-based Intervention Package-Peru Community Trial

The IHIP trial was a community-cluster, randomized, controlled trial conducted from 2008 to 2010 that randomized 51 rural communities to either a control or intervention arm. Households that used open indoor fires for cooking and had at least 1 child < 3 years were eligible for enrollment. The intervention consisted of improved, vented stoves, kitchen sinks with running water, handwashing promotion, and installation of solar water disinfection units. The IHIP follow-up started after randomization and installation of new stoves and water disinfection units in the intervention group [10, 20]. A total of 499 children <3 years lived in households that participated in the IHIP study: 251 in control households and 248 in intervention households. Children living in households that participated in the IHIP trial were eligible for subsequent enrollment in the RESPIRA-Peru study if they met enrollment criteria as described below [16].

The Study of Respiratory Infections in Andean Peruvian Children Prospective Cohort Study

The RESPIRA-Peru study was a prospective cohort study conducted from May 2009 through September 2011. The study setting, enrollment, and follow-up have been previously described [2, 15, 16, 18, 19]. Field activities began with a local census to identify households with potentially eligible children (aged <3 years, including newborns). Identified households were visited and, after informed consent was obtained, young children were enrolled and followed prospectively. Newborns identified during the study period were added, with the goal of maintaining a dynamic cohort with approximately 500 children under observation at any one time. Trained field workers conducted weekly household visits and collected detailed clinical and sociodemographic data at enrollment and follow-up visits. Given that PCV7 was introduced into the study area beginning in late 2009, very few subjects selected for this study that were enrolled in 2009 had received any PCV7 doses at the time of RESPIRA-Peru enrollment.

Enrollment and follow-up in the RESPIRA-Peru study started approximately 3 months after the IHIP trial follow-up started. Thus, the present study included all children enrolled in the RESPIRA-Peru study who lived in a IHIP trial household. Follow-up for study children continued from the time of enrollment in the RESPIRA-Peru study through the date of death, attainment of 3 years of age, loss to follow-up, or November 2009, whichever came first.

Respiratory samples were systematically collected from each child under observation as previously described [2, 15, 16, 18, 19]. In brief, NP samples were collected monthly from all enrolled children regardless of clinical or symptomatic status, processed according to World Health Organization recommendations for detection of pneumococcus [21, 22], cultured and tested by quantitative PCR for the pan-pneumococcus *lytA* gene, and serotyped through multiplex PCR [2, 23]. In addition, nasal swabs (NS) were collected from each child during ARI, defined as the presence of either cough or fever [24]. For each ARI, the "ARI period" encompassed the period from the date of symptom onset to the last day of ARI symptoms that was followed by at least 7 days free of ARI symptoms. Nasal swab samples were shipped on dry ice in batches to Vanderbilt University for detection of the after respiratory viruses using real-time, monoplex, reverse-transcription PCR as previously described: influenza viruses A, B, and C; respiratory syncytial virus (RSV); human metapneumovirus (MPV); parainfluenza viruses (PIVs) 1–3; human rhinovirus (HRV); and adenovirus (AdV) [25–27]. If a child was experiencing an ARI at the time of the visit in which a monthly NP sample was collected, an NS sample was also collected at that visit.

Statistical Analysis

This study includes the subset of children who lived in households that participated in the IHIP study and were enrolled and followed in the RESPIRA-Peru study during 2009. We compared pneumococcal colonization densities among children in households randomized to the control versus the intervention group in the IHIP study at sequential time points. To retain samples with zero pneumococcal density for our analyses, we applied $\log_{10}(x+1)$-transformation to colonization density values, where

x represents the measured density. Unadjusted group comparisons were performed using the χ^2 test, Wilcoxon rank-sum test, or Kruskal-Wallis test as appropriate. A multivariable linear quantile mixed-effects model was used to model the conditional median of log-transformed pneumococcal density against IHIP control/intervention group, with random effects of children to account for correlation between sequential measurements from the same child. Other covariates included age at the time of sample collection, sex, recent antibiotic exposure (aminopenicillins, cephalosporins, cotrimoxazole, chloramphenicol, or furazolidone within the past 7 days), calendar month, and timing of sample collection relative to ARI episodes with detection of any respiratory virus during or immediately (1–7 days) after ARI. From each child, samples were categorized as follows: no recent ARI (>14 days apart from ARI), 8–14 days before ARI, 1–7 days before ARI, ARI (during an individual ARI period), 1–7 days after ARI, and 8–14 days after ARI. A second similar model used the same outcome and covariates but assessed the association of specific viruses detected during ARI or in the 1- to 7-day post-ARI period. In this model, viral detection was categorized into 3 mutually exclusive groups (HRV only, codetection of 2 or more viruses, and other single virus detected) for each period, because the number of detections of non-HRV respiratory viruses was small. Predicted values were estimated from the final regression models, using 100 bootstrap replications, and violin plots [28] of predicted log-transformed pneumococcal densities were generated. Detailed model results of fixed effects are included in the Supplementary Materials. All analyses were completed with STATA version 14.2 (StataCorp, College Station, TX) except multivariable linear quantile mixed-effects models, which were conducted in R 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) using lqmm [29] package version 1.5.3.

RESULTS

Study Population

We identified 260 subjects enrolled and followed in the prospective RESPIRA-Peru study in 2009 who lived in 260 households that had previously enrolled in the IHIP study (n = 129 from control households and n = 131 from intervention households). These 260 subjects contributed 994 sequential (monthly) NP samples, with an average of 3.8 samples (range, 1–6) per child during study follow-up. Follow-up started 3 months after IHIP trial follow-up started, included 49% of children enrolled in the trial, and continued through November 2009.

The sociodemographic characteristics participants according to study group are displayed in Table 1. The median age of children at the time of enrollment was 23.8 months. The median household altitude was 2726 meters, and this was similar between groups. At RESPIRA-Peru enrollment, only 1 child had received a dose of PCV7 (1 of 129 [0.8%] in the IHIP control group). Household characteristics were similar between groups. As expected, the only significant difference between groups was type of cooking stove, with the majority of households in the intervention group using the IHIP-improved stove (89%), whereas most kitchens in control households used open stone fireplaces (59%; $P < .001$), followed by self-improved (22%) or traditional (13%) stoves. Almost all homes (255 of 258; 99%) in both groups used wood as cooking fuel. Only 13 of 260 children (5%) were never colonized with pneumococcus at any time point; 7 of these were in IHIP intervention households, and 6 were in IHIP control households.

Table 1. Sociodemographic Characteristics of 260 Participants <3 Years of Age According to Their IHIP Trial Assignment; Peru, 2009

Characteristic	Control (n = 129)	Intervention[a] (n = 131)	P[b]
Female, no. (%)	66 (51)	62 (47)	.54
Age (month) at enrollment, median (IQR)	24.8 (17.7–28.1)	23.3 (18.5–27.9)	.79
Children <5 years old in household, no. (%)			.17
1 (only the index child)	102 (79)	92 (70)	
2	23 (18)	37 (28)	
3 or more	4 (3)	2 (2)	
Child attends daycare-equivalent, no. (%)	18/125 (14)	16/128 (12)	.66
Received any dose(s) of PCV at the time of RESPIRA-Peru enrollment, no. (%)	1 (0.8)	0 (0)	1.00
Any smoker in home, no. (%)	6/75 (8)	6/73 (8)	.96
Bedrooms in home, no. (%)			.8
1	85 (68)	86 (67)	
2	30 (24)	34 (27)	
3	10 (8)	8 (6)	
Shares a bed, no. (%)	122/125 (98)	125/128 (98)	.98
Home dirt floor, no. (%)	124 (96)	127 (97)	.35
Home adobe or mud walls, no. (%)	124 (96)	128 (98)	.42
Home water supply, no. (%)			.26
Pipeline	102 (79)	99 (76)	
Lake, spring, or underground aqueduct	10 (8)	20 (15)	
Other (community spout, well, open canal)	17 (14)	12 (8)	
Sewage disposal, no. (%)			.68
Private latrine	97 (75)	98 (75)	
Septic tank	14 (11)	14 (11)	
Other (river, ditch, canal, or pond)	18 (14)	19 (14)	
Electric service, no. (%)			.39
Candle	67 (52)	67 (51)	
Electricity	36 (28)	29 (22)	
Kerosene or other	26 (20)	35 (27)	

Abbreviations: IHIP, Intergrated Home-based Intervention Package; IQR, interquartile range; PCV, pneumococcal conjugate vaccine; RESPIRA-Peru, The Study of Respiratory Infections in Andean Peruvian Children.

[a]The intervention consisted of improved, vented stoves, kitchen sinks with running water, handwashing promotion, and installation of solar water disinfection units.

[b]$P < .05$.

Comparison of Pneumococcal Nasopharyngeal Density

In unadjusted analysis of the first samples collected from each child after enrollment (n = 260), there was no difference in

mean $\log_{10}$-transformed colonization density between groups (control 3.1 colony-forming unit [CFU]/mL [standard deviation {SD} = 1.8] versus intervention 3.0 CFU/mL [SD = 1.9]; P = .58). The majority (55%) of first samples were collected during the month of June (n = 143), followed by September (n = 48), July (n = 28), August (n = 19), October (n = 10), May (n = 7), and November (n = 5); this distribution was similar between groups.

When all 994 samples collected from the 260 children were considered, in unadjusted analysis, there were no differences in mean $\log_{10}$-transformed density between the control (3.3; SD = 2.3) and intervention (3.5; SD = 2.3) groups overall (P = .24) or at any time point by calendar month. However, pneumococcal densities varied significantly by calendar month in both groups, with densities peaking in October and November (P < .001) (Figure 1), coinciding with the local spring months and the beginning of the rainy season.

Among these 994 samples, 134 were collected during an ARI period, with at least 1 virus detected in 79 of 134 (59.0%) corresponding ARI samples. Viral detections were similar between groups. In 64 of these ARI samples, a single virus was detected (34 HRV, 9 AdV, 6 RSV, 7 PIV, 5 influenza, and 3 MPV), whereas 2 viruses were detected in 15. Human rhinovirus was present in 12 of 15 (80.0%) codetections, most commonly with AdV (7 of 15; 46.7%) or PIV (4 of 15; 26.7%). Of 994 samples, 88 were collected during the 1- to 7-day post-ARI period, and at least 1 virus was detected in 36 (40.1%) of these 1- to 7-day post-ARI samples. A single virus was detected in 30 of 36 (15 HRV, 8 AdV, 4 PIV, 2 influenza, 1 RSV), whereas 2 viruses were detected in 6 of 36 (HRV-AdV in each case).

In the multivariable mixed-effects quantile regression model, $\log_{10}$-transformed pneumococcal densities were not significantly associated with the IHIP intervention, age, sex, or recent antibiotic usage (Supplemental Tables). However, pneumococcal densities were significantly associated with calendar month and with ARI periods. In particular, pneumococcal densities were significantly higher during both the ARI period and the 1- to 7-day post-ARI period when at least 1 respiratory virus was detected compared with the reference (asymptomatic period more than 14 days apart from ARI) (Figure 2; Supplemental Table 1). When specific viruses were examined, detection of either HRV or a non-HRV virus was also significantly associated with pneumococcal density during the ARI period (Supplemental Table 2; Figure 3). In the 1- to 7-day post-ARI period, non-HRV detections were associated with significantly higher densities compared with the reference, although detections of HRV were not. During the ARI period, coinfection with 2 respiratory viruses was not associated with increased density. However, this association was significant in the 1- to 7-day post-ARI period when 2 viruses were detected (HRV-AdV in all cases).

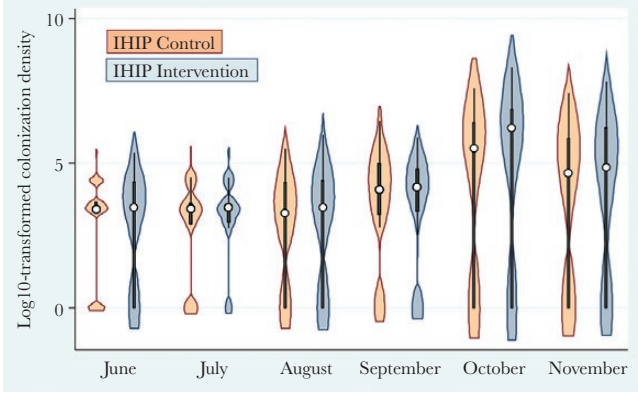

Figure 1. Distribution of pneumococcal densities among children <3 years of age by Integrated Home-based Intervention Package (IHIP) trial assignment and calendar month; Peru, 2009. Circles indicate median densities, bars represent interquartile range, lines represent the 95% confidence interval, and the density plot width indicates the frequency of observations.

DISCUSSION

Indoor smoke exposure from the use of biomass fuels in traditional open stoves is considered an important risk factor for the development of respiratory illnesses in children in developing countries, and strategies to effectively mitigate this risk are needed [6]. We assessed whether the use of improved stoves reduced the density of NP pneumococcal colonization, a crucial step in the development of pneumococcal disease. In our study, NP pneumococcal densities did not differ significantly between samples from children living in households with improved

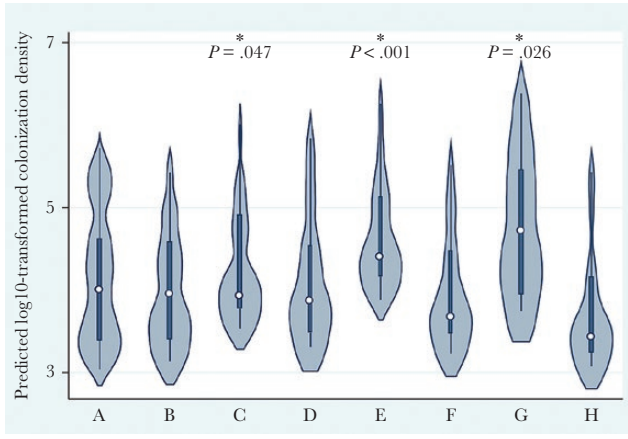

Figure 2. Predicted $\log_{10}$-transformed colonization densities relative to acute respiratory illness (ARI) and detection of viruses among children <3 years of age by Integrated Home-based Intervention Package trial assignment and calendar month; Peru, 2009. (A: no recent ARI; B: 8–14 days before ARI; C: 1–7 days before ARI; D: current ARI, no virus; E: current ARI, any virus; F: 1–7 days after ARI, no virus; G: 1–7 days after ARI, any virus; H: 8–14 days after ARI.) Predictive densities were estimated from the final multivariable linear quantile mixed-effects model. Circles indicate median densities, bars represent interquartile range, lines represent the 95% confidence interval, and the density plot width indicates the frequency of observations. Asterisks (*) indicate significantly increased predicted densities relative to the reference group, with P < .05 considered statistically significant.

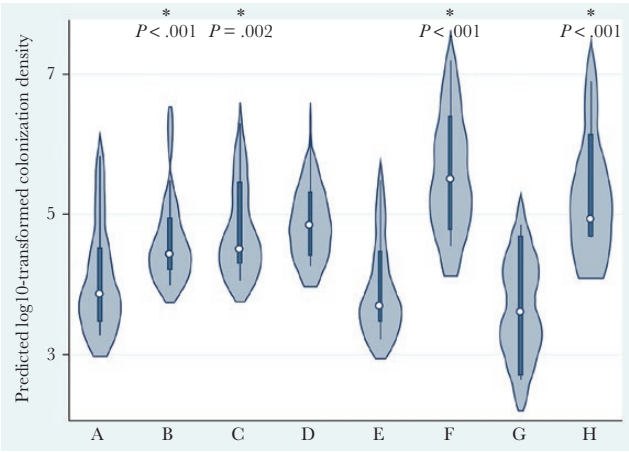

Figure 3. Predicted $\log_{10}$-transformed colonization densities relative to acute respiratory illness (ARI) (1–7 days post-ARI) and detection of specific viruses among children <3 years of age by Integrated Home-based Intervention Package trial assignment and calendar month; Peru, 2009. (A: current ARI, no virus; B: current ARI, any virus except human rhinovirus (HRV); C: current ARI, HRV only; D: current ARI, coinfection; E: 1–7 days after ARI, no virus; F: 1–7 days after ARI, any virus except HRV; G: 1–7 days after ARI, HRV only; H: 1–7 days after ARI, coinfection.) Predictive densities were estimated from the final multivariable linear quantile mixed-effects model. Circles indicate median densities, bars represent interquartile range, lines represent the 95% confidence interval, and the density plot width indicates the frequency of observations. Asterisks (*) indicate significantly increased predicted densities relative to the reference group, with $P < .05$ considered statistically significant.

stoves compared with traditional open fire stoves. This finding may not be unexpected, because improvements in indoor air quality, as measured by particulate matter and carbon monoxide measurements, were very modest in the IHIP intervention households and did not reach statistical significance [9]. However, in both groups, densities were significantly higher during viral-associated ARIs and immediately after these ARI periods. Densities were highest during October and November, the early spring months, coinciding with the beginning of the rainy season and with peak HRV activity [15].

Few studies have assessed the impact of indoor smoke exposure from cooking stoves on pneumococcal carriage and disease in children. Improved stoves designed to enhance air quality by reducing air particulate matter have demonstrated promise in reducing ARI risk [30–32], with greatest effects on women and young children, who have the most direct and prolonged exposure. However, other studies have demonstrated conflicting results on improved health outcomes [9, 11]. A recent community-level cluster-randomized controlled trial among 8626 Malawian households found no reduction in pneumonia episodes among children in households that received an improved, biomass-fueled cookstove [12]. The lack of effect in these studies has been partially attributed to alternative exposures to air pollution (ie, garbage burning, tobacco smoke) that may have overwhelmed any cookstove effect, insufficient reductions in smoke from the improved stoves, or poor adherence to use of improved stoves due to inconvenience associated with longer cooking times or increased wood consumption [9, 30].

For the IHIP study, there was community input on stove design and relatively persistent use of improved stoves several months after implementation. However, improvements in indoor air quality were very modest, and no reduction in ARI or pneumonia incidence was observed in the IHIP intervention group, although the assessment may have been limited by the small number of cases that occurred during follow-up [10]. In contrast, in a subset of children participating in the IHIP and the RESPIRA-Peru studies, we previously reported significantly lower influenza ARI rates in subjects in the intervention arm compared with the control arm of the IHIP study [33]. However, risk of influenza ARI was significantly lower in children in either arm of the IHIP trial compared with children from households that did not participate in IHIP. It is unclear whether this association was due to 1 component of the intervention, such as improved indoor air quality, or combined effects of multiple components, including improved water sanitation practices and hand hygiene, which has previously been associated with reduced ARI risk [33].

Although synergistic relationships between infection with certain respiratory viruses and pneumococcal colonization density have been reported from mouse models [34–37], data are limited on the specific impact of respiratory viruses on pneumococcal density in the pathogenesis of ARI in children. Although several studies have evaluated these associations using ecologic designs, few have used longitudinal individual-level data. From a much larger cross-sectional analysis of the RESPIRA-Peru cohort that was not restricted to IHIP participants, we previously reported that pneumococcal densities peaked during ARI, relative to asymptomatic periods before and after ARI periods, and were highest when respiratory viruses, specifically HRV, were present [2]. A 2011 case-control study from Vietnam found that pneumococcal densities were 15-fold higher in children hospitalized with radiologically confirmed pneumonia with concomitant respiratory viruses detected than in healthy controls [5]. Influenza A, RSV, and HRV detections in that study were all significantly associated with higher pneumococcal densities. Age, sex, and prior antibiotic use were not associated with pneumococcal density [5], consistent with our findings. In another study from Tanzania, pneumococcus was more prevalent in children with febrile ARI compared with children with febrile non-ARI, and density was increased in severe pneumonia compared with mild illness, although the presence of viral pathogens was not assessed [38]. It is interesting to note that a recent case-control study conducted in South Africa reported no significant differences in pneumococcal colonization densities between children with pneumonia and healthy controls [39]. Another separate, cross-sectional study of children and adults hospitalized with severe ARI in South Africa reported that detection of respiratory viruses was associated

with higher likelihood of nasopharyngeal pneumococcal colonization and increased pneumococcal density, although the study was not powered to assess the association with specific viruses [4]. Children up to 4 years of age comprised only approximately half of the study participants, and the setting and high human immunodeficiency virus (HIV) prevalence make those results difficult to extrapolate to HIV-uninfected children in other settings.

Our study has several limitations. First, our analysis was based on the 49% of IHIP study participants who were age-eligible for enrollment into RESPIRA-Peru. Thus, our power to detect differences is reduced compared with assessments of the complete IHIP or RESPIRA-Peru cohort, which may account for some differences in these findings compared to our other studies [2]. The median age of children in this study was 2 years; patterns of pneumococcal density and viral detection may differ substantially in infants. We considered control/intervention group status as a proxy for indoor air pollution, but measurements of particulate matter and/or carbon monoxide were not consistently available and when they were, they were not very different. Seven months after use, the difference in air quality between groups was modest, and the level of indoor pollutants was high, even in households with improved stoves [9]. Adherence to the assigned intervention was measured by self-report [10], and modifications may have been made to the improved study stoves, or intervention households may have intermittently used indoor open fires, which could have diluted the intervention impact in real-world conditions. Our group previously reported that ARI incidence was higher in late fall, winter, and spring (May to December; range, 5.4–7.3 ARI/child-year), compared with the summer and early fall (January to April; range, 3.5–4.3 ARI/child-year) in the RESPIRA-Peru study [15]. Thus, months with higher ARI incidence are overrepresented in this study. We restricted our analysis to 2009 because this period represented the period in closest temporal proximity to implementation of the intervention packing in the IHIP trial; however, had a full calendar year of data been included after the intervention, including periods of lower ARI incidence, additional insight may have been gained regarding the relationship of ARI occurrence, pneumococcal density, and season. Although HRV was frequently detected during ARI [15], other viruses were less frequently detected, limiting assessments of individual viruses to pneumococcal colonization density. Furthermore, asymptomatic detection of respiratory viruses was common in this cohort [19]. Additional studies are needed to determine whether the observed association between viral detection and pneumococcal density is present during asymptomatic periods, which may shed light on the role of these interactions in ARI pathogenesis. Finally, although our study did not assess for codetection of multiple pneumococcal serotypes, recent studies have highlighted differences in patterns of colonization density in cocolonization with multiple pneumococcal serotypes, which may have important impacts on transmission dynamics [40–42].

CONCLUSIONS

In conclusion, we found that a home-based intervention targeting indoor air pollution and home hygiene was not associated with reduced pneumococcal density in young Peruvian children. Spring season and codetection of pneumococci with respiratory viruses during ARI and post-ARI periods were associated with increased pneumococcal density, an important factor in the development of pneumococcal disease. Future longitudinal assessments are needed to understand the interplay between nasopharyngeal pneumococcal colonization, cocolonization with multiple serotypes, respiratory viral coinfection, pneumococcal conjugate vaccination, and the impact of these factors on pneumococcal virulence and transmission dynamics and the development of respiratory illness in young children.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Potential conflicts of interest. C. G. G. has served as a consultant to Pfizer in unrelated work. M. R. G. receives grant funding from MedImmune. K. M. E. receives grant funding from Novartis in unrelated work. J. V. W. serves on a Scientific Advisory Board for Quidel and an Independent Data Monitoring Committee for GlaxoSmithKline, neither related to the present work. C. F. L. serves as a Scientific Advisor to Takeda and GlaxoSmithKline in subjects not related to the present work. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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