Seasonal Drivers of Pneumococcal Disease Incidence: Impact of Bacterial Carriage and Viral Activity

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(See the Editorial Commentary by van Hoek and Miller on pages 195–6.)

Background. Winter-seasonal epidemics of pneumococcal disease provide an opportunity to understand the drivers of incidence. We sought to determine whether seasonality of invasive pneumococcal disease is caused by increased nasopharyngeal transmission of the bacteria or increased susceptibility to invasive infections driven by co-circulating winter respiratory viruses.

Methods. We analyzed pneumococcal carriage and invasive disease data collected from children <7 years old in the Navajo/White Mountain Apache populations between 1996 and 2012. Regression models were used to quantify seasonal variations in carriage prevalence, carriage density, and disease incidence. We also fit a multivariate model to determine the contribution of carriage prevalence and RSV activity to pneumococcal disease incidence while controlling for shared seasonal factors.

Results. The seasonal patterns of invasive pneumococcal disease epidemics varied significantly by clinical presentation: bacteremic pneumococcal pneumonia incidence peaked in late winter, whereas invasive nonpneumonia pneumococcal incidence peaked in autumn. Pneumococcal carriage prevalence and density also varied seasonally, with peak prevalence occurring in late autumn. In a multivariate model, RSV activity was associated with significant increases in bacteremic pneumonia cases (attributable percentage, 15.5%; 95% confidence interval [CI], 1.8%–26.1%) but was not associated with invasive nonpneumonia infections (8.0%; 95% CI, −4.8% to 19.3%). In contrast, seasonal variations in carriage prevalence were associated with significant increases in invasive nonpneumonia infections (31.4%; 95% CI, 8.8%–51.4%) but not with bacteremic pneumonia.

Conclusions. The seasonality of invasive pneumococcal pneumonia could be due to increased susceptibility to invasive infection triggered by viral pathogens, whereas seasonality of other invasive pneumococcal infections might be primarily driven by increased nasopharyngeal transmission of the bacteria.

Keywords. pneumococcal; co-infections; RSV; seasonality; pneumonia.

The incidence of disease caused by pneumococcus, like many other bacterial pathogens, follows a distinct winter-seasonal pattern in temperate climates [1]. These seasonal epidemics probably result from a combination of increased carriage of the bacteria and increased susceptibility to developing disease [2, 3]. However, the relative importance of carriage and susceptibility is not known. Comparisons of seasonal variation in pneumococcal disease and carriage prevalence provide an opportunity to identify the drivers of pneumococcal disease incidence. This information, in turn, could help to clarify the health impacts of vaccination or other interventions that influence bacterial carriage prevalence or density.

Carriage is considered a prerequisite for disease, and the majority of young children carry pneumococcus in
the nasopharynx [4]. Carriage prevalence and acquisition rates vary seasonally, with peaks in wintertime [5–7]. Once a person is colonized with pneumococcus, the likelihood that invasive pneumococcal disease (IPD) will develop depends on the virulence of the strain and host susceptibility [4, 8–10]. Host susceptibility is influenced by seasonal factors, including recent infections with influenza, respiratory syncytial virus (RSV), or other respiratory viruses [11–14]. Likewise, environmental conditions, such as decreased sunlight and dryness, might affect immunity or the integrity of the respiratory mucosa [1].

In this study, we sought to determine how seasonal variations in bacterial carriage and respiratory virus activity influence the seasonal epidemics of IPD. We hypothesized that if seasonality of pneumococcal disease incidence is driven by increased bacterial transmission, then prevalence or density of nasopharyngeal carriage should increase around the same time and with a similar magnitude as disease incidence. In contrast, if incidence is primarily driven by increased host susceptibility, then disease incidence would increase independently of carriage and might be associated with respiratory virus activity. We used a unique collection of pneumococcal carriage and invasive disease data from the Navajo and White Mountain Apache (WMA) populations to test these hypotheses.

**MATERIALS AND METHODS**

**Data Sources**

We analyzed data from 4 sequential nasopharyngeal carriage studies conducted among the Navajo and WMA populations in the southwestern United States (Table 1). The details of the study objectives and designs have been described elsewhere and are summarized here briefly [6, 15, 16]. All 4 studies were community-based and focused on the evaluation of pneumococcal conjugate vaccine (PCV) effects among children. Study 1 was a randomized controlled trial designed to evaluate the direct and indirect effects of 7-valent PCV on nasopharyngeal colonization in vaccine recipients and their siblings (1998–2000) [15]. Study 2 examined the duration of the nasopharyngeal carriage effect in children and adults in those same communities shortly after the licensure of PCV (2001–2002) [16]. Study 3 examined the long-term effects of routine use of 7-valent PCV in children and adults in these communities with 6 consecutive monthly nasopharyngeal sample per person (2006–2008) [6]. Study 4 examined the effects of 13-valent PCV on pneumococcal carriage and invasive disease among children and adults (2010–2012; unpublished). All the analyses presented here focused on children <7 years old. We also conducted analyses stratified by pediatric age group, <2 and 2–6 years old, and found no significant differences in the seasonal patterns of carriage.

All studies received ethical approval from the institutional review boards at Johns Hopkins School of Public Health, the Navajo Nation, and the Indian Health Service, Phoenix Area, and from the Navajo and WMA tribes. Parents or guardians of children enrolled in the carriage studies provided written informed consent.

Pneumococcal disease and viral activity data were obtained from 2 sources: the monthly number of IPD cases was derived from population-based active bacterial surveillance conducted at health facilities serving the Navajo and WMA reservations, as described elsewhere (1996–2012) [17]; IPD cases were divided into those with and those without a diagnosis of pneumonia. Those without a diagnosis of pneumonia had the following clinical syndromes (not mutually exclusive): bacteremia without focus (49%), bacteremia (10%), fever or febrile illness (29.3%), otitis media (25%), meningitis (19%), and other syndromes (eg, urinary tract infection, septicemia, cellulitis, dehydration, gastroenteritis, osteomyelitis, arthritis, viral syndrome; <5% each).

As a proxy for viral activity, we used weekly hospitalization data drawn from the State Inpatient Databases (SIDs) of the Healthcare Cost and Utilization Project, maintained by the Agency for Healthcare Research and Quality, through an active collaboration. This database contains a census of hospital discharge records from community hospitals in participating states [18]. For the primary analyses, we included only SID records from the 3 counties in northeastern Arizona that account for the majority of the Navajo reservation (Apache, Navajo, and Cococino counties). We determined the weekly incidence of hospitalizations among children <5 years old for bronchiolitis (*International Classification of Diseases, Ninth

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**Table 1. Carriage Study Characteristics in Children <7 Years Old**

<table>
<thead>
<tr>
<th>Carriage Study</th>
<th>Dates</th>
<th>Participants. No.</th>
<th>Total Swab Samples, No.</th>
<th>Positive Swab Samples, No.</th>
<th>Median Age, y</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>February 1998 through May 2000</td>
<td>844</td>
<td>2220</td>
<td>1429</td>
<td>1.5</td>
<td>O’Brien et al [15]</td>
</tr>
<tr>
<td>Study 3</td>
<td>March 2006 through April 2008</td>
<td>549</td>
<td>3329</td>
<td>1835</td>
<td>2.6</td>
<td>Scott et al [6]</td>
</tr>
<tr>
<td>Study 4</td>
<td>January 2010 through March 2012</td>
<td>3259</td>
<td>3260</td>
<td>1686</td>
<td>1.5</td>
<td>Unpublished</td>
</tr>
</tbody>
</table>

*From 1996/97 to 2012/13 there were 496 cases of invasive pneumococcal disease, including 210 bacteremic pneumonia and 286 nonpneumonia cases.*
Definitions of Pneumococcal Carriage Prevalence, and Carriage Density
Monthly pneumococcal carriage prevalence was defined as the number of swabs samples positive for pneumococcus divided by the total number of swab samples collected that month. Data from all 4 studies were used to estimate seasonal variations in carriage prevalence among children in the community.

Data on colonization density were available from study 1. Density of colonization was measured on a semiquantitative 4-point scale after inoculation of the plate with 10 µL of skim milk, tryptone, glucose, and glycerin (STGG) media, as described elsewhere [15]; 1 indicated <25 colonies on the plate, 2 indicated 25 to <100 colonies, 3 indicated ≥100 colonies but not confluent growth, and 4 indicated confluent growth. For our analyses, categories 1–3 were considered low density and category 4, high density.

Association Between Disease, Carriage, and Viral Activity
Monthly carriage prevalence, carriage density, and monthly disease incidence were estimated using regression models (details in Supplementary Appendix). To test for univariate associations between pneumococcal carriage and disease, we estimated the Pearson correlation coefficient between the monthly modeled carriage prevalence and monthly modeled number of IPD cases with or without pneumonia presentation.

We also tested for associations between carriage and disease while estimating the importance of viral activity and controlling for other shared seasonal factors. We fit 2 Poisson regression models, with or without a predictor for respiratory virus activity (details in Supplementary Appendix). The outcome variable was either observed monthly cases of bactenemic pneumonia or cases of nonpneumonia IPD in children <7 years old since 1996–1997, and the predictors were observed monthly carriage prevalence, dummy variables for PCV periods, and 12- and 6-month sine and cosine terms to represent background seasonal factors. The alternative model added a term for monthly bronchiolitis incidence (a proxy for RSV activity) and included interpolated carriage data for years that had bronchiolitis and pneumococcal disease data but no carriage survey (Supplementary Appendix). The 95% confidence intervals (CIs) were obtained by using a seasonal block bootstrap [20] with 1000 replicates.

RESULTS
Seasonal Fluctuations in IPD
The incidence of IPD exhibited significant seasonal variation in children <7 years old, with peak IPD incidence occurring in late autumn (Figure 1A). Interestingly, the seasonal incidence patterns differed between bacteremic pneumonia and nonpneumonia IPD cases, with pneumonia diagnoses peaking in midwinter and nonpneumonia cases peaking in the autumn (Figure 1B). This difference was statistically significant (interaction term between disease presentation and month of the year, P < .001).

Given the small total number of IPD cases, we sought to confirm these patterns by using data from a larger database. Therefore, we evaluated the seasonal variations in hospitalizations owing to pneumococcal pneumonia or pneumococcal septicemia in 8 states in the United States. The incidence of these clinical diagnoses followed a pattern similar to those for invasive disease incidence among the Navajo—earlier increases in pneumococcal septicemia and later increases for pneumococcal pneumonia (Supplementary Figure 2).
Seasonal Fluctuations in Pneumococcal Carriage Prevalence, Acquisition Rate, and Carriage Density

Like IPD, carriage prevalence fluctuated seasonally. Prevalence began to increase in August and September, remained high through the autumn, and peaked in December. Carriage prevalence then declined in January and February (Figure 2A). Even during the lowest months, average carriage prevalence remained >50%. Interestingly, the seasonal increase in carriage prevalence was driven entirely by increases in the prevalence of high-density colonization episodes (Figure 2B). This was particularly pronounced in November, when 48.5% (95% CI, 41.3%–55.6%) of those tested were colonized at a high density, compared with 29.7% (95% CI, 23.0%–36.3%) in July. In contrast, the prevalence of low-density colonization was relatively constant through the year (Figure 2B). Seasonal variations in the carriage acquisition rate were comparable to the variations in carriage prevalence (Supplementary Figure 4).

Univariate Associations Between IPD Seasonality and Carriage

Model predictions of monthly carriage prevalence were well correlated with model predictions of monthly disease incidence ($\rho = 0.79$; 95% CI, .36–93). Because the seasonal disease curves differed by presentation, we then compared monthly carriage prevalence with monthly incidence of bacteremic pneumonia and nonpneumonia IPD (Figure 3). The association between carriage prevalence and IPD without a pneumonia diagnosis was strong ($\rho = 0.87$; 95% CI, .56–96), whereas the relationship between carriage prevalence and bacteremic pneumonia was weak ($\rho = 0.34$; 95% CI, −.31–76).

Contribution of Carriage and Viral Activity to Seasonal Variations in IPD Incidence

Carriage, IPD, and viral activity all vary seasonally and will therefore probably be highly correlated. To obtain accurate estimates of the relationships between them, it is necessary to use regression models that control for these shared seasonal variations [11, 12]. We first evaluated the association between observed monthly carriage prevalence and observed monthly cases of bacteremic pneumonia or nonpneumonia IPD. When carriage data from all 4 surveys were included, there was a borderline association between seasonal increases in carriage prevalence and the incidence of nonpneumonia IPD cases (attributable percentage, 25.6; 95% CI, −4.4% to 46.7%). In contrast, there was no association between carriage prevalence and bacteremic pneumonia cases (attributable percentage, −42.4%; 95% CI, −199% to 50.2%). A similar pattern is observed with data from just the period before 13-valent PCV (studies 1–3; nonpneumonia IPD attributable percentage 27.3% [95% CI, −11.1% to 51.6%; bacteremic pneumonia attributable percentage, −45.4% [−255 to 71.2%]).

Next, we built on this model by adding a predictor for bronchiolitis incidence (a marker of RSV activity, available only for the years before 13-valent PCV) and interpolating carriage prevalence values for seasons that lacked data. Increases in bacteremic pneumonia were associated with bronchiolitis incidence (attributable percentage, 15.5%; 95% CI, 1.8%–26.1%) but not with variations in carriage prevalence (attributable

Figure 2. A, Estimated pneumococcal carriage prevalence in each month among <7 year olds. B, Estimated prevalence of high- and low-density pneumococcal carriers among all individuals with swab samples in study 1. The markers indicate the average monthly prevalence, controlling for age and vaccination period. Estimates were calculated using generalized estimating equations (controlling for repeated sampling of individuals; A) and generalized logit regression (±95% confidence intervals; B).

Figure 3. Comparison of the relative monthly variations in carriage prevalence (dotted line), bacteremic pneumonia (light gray solid line), and nonpneumonia invasive disease (dark gray solid line) among children <7 years old. Disease curves represent incidence rate ratios relative to July, and carriage curves represent risk ratios for carriage relative to July. Numbers represent changes at the peaks (ratios) relative to July.
percentage, −23.8%; 95% CI, −111% to 38.2%). In contrast, increases in nonpneumonia IPD cases were associated with seasonal variations in carriage prevalence (attributable percentage, 31.4%; 95% CI, 8.8%–51.4%) but not with bronchiolitis incidence (attributable percentage, 8.0%, 95% CI, −4.8% to 19.3%). We also tested whether variations in influenza activity were associated with increases in bacteremic pneumonia or nonpneumonia IPD. However, there was no significant effect, and influenza was dropped from the final model.

As a sensitivity analysis, we ran the model while excluding IPD cases with a syndrome of meningitis or otitis media from the model. The estimate for the attributable percentage of carriage did not change appreciably after exclusion of meningitis (28.0%; 95% CI, −1.1% to 49.8%) and was modestly lower after exclusion of invasive disease cases that were also associated with otitis media (18.6%; 95% CI, −12.5% to 51.6%).

**DISCUSSION**

We have shown that both pneumococcal carriage prevalence and invasive disease incidence vary seasonally among children in the Navajo and WMA populations. Unexpectedly, the seasonal epidemics of bacteremic pneumonia and nonpneumonia IPD cases occurred at different times. Seasonal variations in the occurrence of nonpneumonia IPD cases were associated with changes in carriage prevalence, whereas seasonal variations in bacteremic pneumonia were associated with changes in RSV activity (bronchiolitis). These results suggest that although carriage might be necessary to cause pneumococcal pneumonia, it is not sufficient—a viral infection (or some other seasonal risk factor) is also required (Figure 4).

Few studies have explicitly examined the link between seasonal variations in carriage and disease for pneumococcus. Hodges and MacLeod [2] identified coincident curves of carriage and pneumococcal disease among Army recruits during World War II, and our findings largely support this link for nonpneumonia IPD in children. Our finding of shared seasonality of bronchiolitis (an RSV surrogate) and invasive pneumonia is consistent with the finding of Stensballe et al that RSV increased the risk for IPD [21].

The seasonal increase in carriage prevalence is consistent with findings in several previous studies [7, 22]. In contrast, other studies have not detected any seasonal variations in carriage prevalence [23, 24]. The reason for these discrepancies between studies could be related to year-to-year differences in the magnitude of seasonal variations. This is evident in our own data, which showed substantially more seasonal variation in 2006–2007 than in 1998–1999 (Supplementary Figure 5). Further studies in other populations and other climatic zones will be required to support our hypotheses and to determine whether the relationships between carriage, viral infections, and different pneumococcal syndromes can be generalized to other settings.

An important issue with public health implications is how vaccine-induced changes in pneumococcal carriage will affect disease incidence. Our results suggest that changes in carriage prevalence are sufficient to drive changes in the incidence of nonpneumonia invasive infections. Therefore, we might expect to observe a direct relationship between vaccine-associated changes in carriage and changes in nonpneumonia IPD, with the change dependent on the invasiveness (case-carrier ratio) of the serotypes [25]. In contrast, changes in carriage are not sufficient to influence invasive pneumonia incidence—a secondary infection or other seasonal risk factor might also be necessary. Therefore, there might not be a direct relationship between postvaccine increases in nonvaccine serotypes in carriage and disease. In particular, we might expect that the invasiveness of the serotype being carried might be less important for invasive pneumococcal pneumonia than for nonpneumonia IPD. Such an effect could explain why increases in disease caused by nonvaccine serotypes (serotype replacement) have been more pronounced for bacteremic pneumonia than for other invasive presentations [26].

Studies in animals demonstrate that infection with influenza increases transmission and shedding of pneumococcus in addition to increasing susceptibility to pneumococcal disease. If influenza, RSV, or other viruses play an important role in increasing transmission, we might expect to see higher carriage prevalence during periods of intense viral activity. We did not detect any such changes in carriage related to influenza or RSV. The seasonal peak in carriage prevalence and carriage density occurs in the late autumn, before influenza and RSV peak in this population. Because we did not detect any effect of viral...
infections on carriage, we expect that viral infections primarily increase susceptibility to secondary bacterial infections, rather than enhance bacterial transmission, at least in children.

Intriguingly, our study identified a strong relationship between bronchiolitis (a marker for RSV activity) and pneumococcal pneumonia but not between influenza and pneumococcal pneumonia. This could result from the pediatric age distribution used in our analyses. Based on the ICD-9 codes, there were 10 times more RSV hospitalizations than influenza hospitalizations among children <5 years old in the Navajo region of Arizona. Influenza might also have some capacity to increase the risk for pneumococcal disease in this age group, but previous studies have estimated that the influenza attributable percentage in the United States is low (<10%) [11, 12], limiting our ability to detect an association.

The increase in pediatric carriage prevalence (and total disease incidence) that occurs in August and September suggest that school terms might play a role. The link between school terms and disease dynamics has been established for other infectious diseases, including pandemic influenza [27] and measles [28].

Our study has several limitations. Its design was not optimal to detect an association between carriage and disease. Carriage data were available for fewer years than disease data, and the number of disease cases in a given year was relatively small. The interpolation of carriage data for years without available observations was a compromise that allowed us to fit a full model that controlled for viral activity. This interpolation, though, might lead to underestimation of the true impact of carriage. This method is also relatively insensitive to fluctuations in carriage that occur over short periods (weeks) or in small geographic areas, such as within a family or daycare center. Such small-scale variations might nonetheless be important for increasing disease risk. We also assumed that disease is always preceded by carriage, a well-supported assumption [29]. It is possible, however, that the correlation between carriage and IPD simply reflects shared seasonal risk factors (eg, decreased immune function). Furthermore, there was not sufficient power to compare seasonal patterns across different IPD serotypes, an issue that has not been addressed, to our knowledge. Finally, because there is a lack of viral surveillance data in this population, we used bronchiolitis hospitalizations as a surrogate for RSV activity, an approach that has been validated elsewhere [19, 30, 31]. In our hospitalization data set, there was a strong correlation between hospitalizations coded as bronchiolitis and as RSV ($r^2 = 0.86$). However, the diagnosis of bronchiolitis probably includes disease caused by other respiratory viruses.

In summary, based on unique multiyear carriage and disease data from the Navajo and WMA pediatric populations, we identified a relationship between autumn seasonality in carriage prevalence and nonpneumonia IPD, and between the winter increase in RSV activity and bacteremic pneumonia. Furthermore, seasonal variations in carriage prevalence were driven by increases in high-density colonization episodes. Going back to our initial hypothesis, we conclude that increases in bacterial transmission and respiratory virus activity are probably 2 independent drivers of IPD seasonality, but, contrary to our expectations, they might affect different disease presentations. This work sheds new light on the drivers of seasonal variations for an important bacterial pathogen and provides information that may be help in understanding and predicting vaccine impacts.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. Thanks to Aruna Chandran and Dan VanDeRiet from the Center for American Indian Health for providing bronchiolitis data, which were used to validate the SID data, to Eugene Millar for data from the longitudinal colonization study, and to Ginny Pitzer for helpful discussion about the analyses.

Financial support. This work is supported by the Multinational Influenza Seasonal Mortality Study, with funding from the Office of Global Health Affairs’ International Influenza Unit in the Office of the Secretary of the Department of Health and Human Services.

Potential conflicts of interest. D. M. W. has received research support through a Pfizer grant to Yale University for other projects, L. R. G. and K. L. O. have research grant support related to pneumococcal vaccines from GlaxoSmithKline and Pfizer, and K. L. O. has received honoraria for participation in external expert advisory committees on pneumococcal vaccines convened by Merck, Sanofi-Pasteur, and GlaxoSmithKline. All other authors report no potential conflicts.

Support for the Navajo/WMA studies came from the Bill and Melinda Gates Foundation (grant 37875), The Native American Research Centers for Health (grants U26HS300013/03 and U269400012-01), the Centers for Disease Control, Prevention National Vaccine Program Office, the Thrasher Research Fund (grant 02820-9), Wyeth Vaccines (Pfizer Inc.), the US Agency for International Development, and the World Health Organization, National Institute on Minority Health and Health Disparities of the National Institutes of Health (Award Number R01MD004011) and Pfizer, Inc. (Protocol Number: 606A1-4013). 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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Pneumococcal Disease, Carriage, and RSV • CID 2014:58 (15 January) • 193


