A Prospective Study of Agents Associated With Acute Respiratory Infection Among Young American Indian Children

Niranjan Bhat, MD, MHS, Rafal Tokarz, PhD,† Komal Jain, MS,† Saddef Haq, BS,† Robert Weatherholtz, BS,* Aruna Chandran, MD, MPH,* Ruth Karron, MD,‡ Raymond Reid, MD, MS,* Mathuram Santosham, MD, MPH,* Katherine L. O'Brien, MD, MPH,* and W. Ian Lipkin, MD†*

Background: Native American children have higher rates of morbidity associated with acute respiratory infection than children in the general US population, yet detailed information is lacking regarding their principal clinical presentations and infectious etiologies.

Methods: We pursued a comprehensive molecular survey of bacteria and viruses in nasal wash specimens from children with acute respiratory disease collected prospectively over 1 year (January 1 through December 31, 2009) from 915 Navajo and White Mountain Apache children in their second or third year of life who had been enrolled in an efficacy study of a respiratory syncytial virus monoclonal antibody in the first year of life.

Results: During the surveillance period, 1476 episodes of disease were detected in 669 children. Rates of outpatient and inpatient lower respiratory tract illness were 391 and 79 per 1000 child-years, respectively, and were most commonly diagnosed as pneumonia. Potential pathogens were detected in 88% of specimens. Viruses most commonly detected were respiratory syncytial virus and human rhinovirus; the 2009 pandemic influenza A (H1N1) illnesses primarily occurred in the fall. *Streptococcus pneumoniae* was detected in 60% of subjects; only human rhinovirus was significantly associated with *S. pneumoniae* carriage. The presence of influenza virus, human rhinovirus or *S. pneumoniae* was not associated with increased risk for lower respiratory tract involvement or hospitalization.

Conclusions: Acute lower respiratory illnesses occur at disproportionately high rates among young American Indian children and are associated with a range of common pathogens. This study provides critical evidence to support reducing the disproportionate burden of acute respiratory disease among young Native Americans.

Key Words: American Indian, respiratory infections, child, diagnosis, influenza

(*Pediatr Infect Dis J* 2013;32: e324–e333)

Accepted for publication March 01, 2013.

From the *Center for American Indian Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; †Center for Infection and Immunity, Columbia University Mailman School of Public Health, New York, NY; and ‡Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD. N.B. and R.T. contributed equally.

The findings and conclusions in this report are solely the responsibility of the authors and do not necessarily represent the official view of the Indian Health Service, National Center for Research Resources or National Institutes of Health.

This work was supported by the National Center for Research Resources at the National Institutes of Health (grant 1KL2RR025006-01 to N.B.); the National Institutes of Health (grant AI57158 to the Northeast Biodefense Center - W.I.L.); the Bill and Melinda Gates Foundation and the Defense Threats Reduction Agency (to the Center for Infection and Immunity, Columbia University). The parent clinical trial was funded by MedImmune, who played no role in the design, conduct, analysis or funding of the molecular diagnostic pathogen substudy. N.B is currently an employee of the United States Food and Drug Administration. K.L.O., A.C., R.W., R.R. and M.S. received funding for the parent trial from MedImmune. The authors have no other funding or conflicts of interest to disclose.

Address for correspondence: Katherine L. O'Brien, MD, MPH, Center for American Indian Health, 621 N. Washington Street, Baltimore, MD 21205. E-mail: klobrien@jhsph.edu.

ISSN: 0891-3668/13/3208-e324
DOI: 10.1097/INF.0b013e31828ff4bc

Native American (NA) children, particularly Alaska Natives and American Indians in the southwestern United States, have high rates of morbidity and mortality related to acute lower respiratory infections (ALRIs).[1,2] Prospective, population-based studies in these communities thus far have examined only specific clinical presentations associated with single pathogens, such as invasive disease due to *Streptococcus pneumoniae* or *Haemophilus influenzae* type b, or bronchiolitis due to respiratory syncytial virus (RSV).[3–10] Data regarding the impact of influenza in NA communities, even during the 2009 pandemic, have been limited to retrospective observational studies.[11–15] Although these investigations all demonstrate disproportionate rates of acute disease, the restricted range of their clinical and microbiologic evaluations prevents a more complete characterization of this elevated burden, which could direct where prevention efforts should be focused. Here, we describe the major clinical presentations and respiratory pathogens associated with ALRI events requiring medical attention in a cohort of young American Indian children followed prospectively through the 2009 calendar year.

METHODS

Study Population

Subjects for this analysis were drawn from a prospective clinical trial evaluating the effectiveness of motavizumab, an investigational monoclonal antibody directed against the RSV F protein, in preventing severe RSV-related disease in American Indian infants of the American Southwest (NCT00121108, www.clinicaltrials.gov).[16] Cohorts of children 6 months of age or younger belonging to the Navajo and White Mountain Apache or San Carlos Apache tribes were recruited each autumn between 2004 and 2007 and randomized in a 2:1 ratio to receive study drug or placebo in 5 monthly doses during their first RSV season. Subjects were monitored for all medically attended acute respiratory illnesses (MAARI) until their third birthday. Following reports of elevated morbidity related to 2009 pandemic influenza A/H1N1 (H1N1pdm) within NA communities,[11,12] we sought to investigate the impact of influenza in relation to other respiratory pathogens in this group of children during that time period. Therefore, all children from the third and fourth enrollment seasons (2006 and 2007) who were still participating in the trial follow-up as of January 1, 2009, were included in this report.

Clinical Surveillance

Active surveillance was conducted 6 days a week at Indian Health Service clinical facilities where the study subjects primarily sought care. A nasal wash specimen was collected in the clinic or at home by a study nurse within 72 hours of any visit with a respiratory illness diagnosis, or if presenting signs/symptoms suggested a respiratory illness. Because specimen collection occurred before formal assessment of the respiratory category of the illness, nursing staff were instructed to collect specimens in children with diagnoses suggesting a lower respiratory illness (pneumonia, bronchiolitis,

TABLE 1. Guidelines for Clinical Syndrome Categorization of Acute Respiratory Illness Episodes

Diagnostic Category	Categorization Guideline
Pneumonia	Takes precedence over any codiagnoses of a wheezing disorder or an etiology (eg, RSV, influenza)
Bronchiolitis	Includes bronchitis
Wheezing illnesses	Includes asthma, reactive airways disease, wheeze, small airways disease
ALRI not otherwise specified	Takes precedence over any codiagnosis of upper respiratory infection
Croup	Includes laryngotracheobronchitis
Allergic rhinitis	Includes seasonal allergies
Viral syndromes	Includes flu-like illness, viral syndrome, and stand-alone diagnoses of RSV, influenza, etc.
Pharyngitis, tonsillitis	Includes sore throat
Sinusitis, otitis media	Takes precedence over any codiagnosis of upper respiratory infection
Upper respiratory infection, cough	Takes precedence over any codiagnosis of an etiology

asthma, croup, etc.) or common presenting signs/symptoms from a prespecified list (cough, wheeze, crackles, shortness of breath, etc.). Therefore, samples were obtained from a subset of upper respiratory illnesses when the determination of lower versus upper respiratory involvement had not been made within the specimen collection window. Nasal washes were obtained by instilling 15–20 mL of lactated Ringers solution into the nasopharynx with a bulb syringe and passively collecting the fluid into a sterile specimen cup. Specimens were combined with viral transport medium, snap frozen in liquid nitrogen and stored at –70°C.

A study physician categorized each clinical visit; an MAARI episode was defined as an event in a study child with any lower or upper respiratory symptoms; the presence of an abnormality on chest examination (eg, wheeze, crackles, rhonchi); or where an acute respiratory illness diagnosis was made by the primary care provider based on clinical judgment. Study physicians then categorized the episode as an upper versus lower respiratory illness and assigned a final study diagnosis using a standardized protocol based on clinical examination findings, imaging reports, laboratory testing, clinical diagnoses and, where necessary, discussion with the provider. For study purposes, the protocol diagnosis took precedence when the assessments of the study and treating physicians were discordant. Study physicians communicated regularly with each other to discuss cases in which the final diagnosis was not straightforward, to maximize consistency and standardization across the study.

Diagnostic Testing

All specimens obtained between January 1 and December 31, 2009, were tested for this study. Total nucleic acid was extracted from 250 µL aliquots of the nasal wash samples using the easyMAG extraction platform (bioMérieux, Marcy l'Etoile, France) and eluted in 35 µL. Complementary DNA was generated from 10 µL aliquots of total nucleic acid using the Superscript II reverse transcription kit (Invitrogen, Carlsbad, CA) and analyzed by the MassTag polymerase chain reaction respiratory panel.[17–21] Each sample was screened using a panel of viral and bacterial respiratory pathogens, which included influenza A, influenza B, human rhinoviruses (HRVs), human enteroviruses, human metapneumovirus, human parainfluenza viruses 1–4, human coronaviruses 229E and OC43, RSV A and B, adenovirus, *Chlamydophila pneumoniae*, *H. influenzae*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Legionella pneumophila* and *S. pneumoniae*. Samples positive by MassTag polymerase chain reaction were reamplified by singleplex polymerase chain reaction and confirmed by sequencing.

Statistical Analysis

Data were analyzed using Stata 11.0 (StataCorp, College Station, TX). Healthcare provider records were reviewed by a study physician. MAARI episodes were categorized as outpatient or inpatient ALRI as appropriate, and diagnoses were classified by major clinical syndrome (Table 1). All clinical data were recorded on paper forms and submitted to the trial sponsor (MedImmune, Gaithersburg, MD), after which a cleaned, locked data set was

TABLE 2. Demographic, Clinical and Environmental Factors Among Study Subjects*

	2006 Enrollment Cohort	2007 Enrollment Cohort	Total
	n = 260	n = 655	N = 915
Gender (n, % male)	136 (52)	326 (50)	462 (50)
Assigned to study drug (motavizumab)	176 (68)	435 (66)	611 (67)
Age on January 1, 2009 (in mo; median, range)	26.3 (24.1–31.2)	15.4 (12.2–20.6)	16.9 (12.2–31.2)
Breastfed as an infant (n, %)	224 (86)	561 (86)	785 (86)
Hospitalized before enrollment in parent trial (n, %)	30 (13)	62 (11)	92 (12)
Attended day care at enrollment (n,%)	5 (2)	19 (3)	24 (3)
Siblings <6 years of age at home (n, %)	168 (65)	436 (67)	604 (66)
Smoker in the household	53 (20)	123 (19)	176 (19)
Mother smoked during pregnancy	11 (4)	27 (4)†	38 (4)
Wood/coal stove in home	155 (60)	409 (62)	564 (62)
Family member with asthma‡	92 (35)	180 (27)	272 (30)
Family member with wheeze	61 (23)	114 (17)†	175 (19)
Family member with hay fever‡	36 (14)	60 (9)†	196 (10)
Family member with eczema	31 (12)	73 (11)	104 (11)
Assigned to motavizumab treatment	176 (68)	435 (66)	611 (67)

*Baseline demographic and exposure data were collected by parent or guardian interview at the time of enrollment.
†Data not available for 1 subject.
‡$P < 0.05$ comparing 2006 cohort with 2007 cohort; $P \geq 0.05$ for all other variables.

TABLE 3. Episodes and Incidence Rates* of Medically Attended Acute Respiratory Infections, Outpatient Acute Lower Respiratory Illnesses and Inpatient Acute Lower Respiratory Illnesses, by Seasonal Cohort

	2006 Cohort	2007 Cohort	Total	*P*†
	n = 260	n = 655	N = 915	
MAARI				
No. of children (%)	163 (63)	506 (77)	669 (73)	<0.0001
No. of episodes	283	1193	1476	
Incidence	1428 (1267–1605)	1858 (1754–1966)	1756 (1668–1848)	<0.0001
Outpatient ALRI				
No. of children (%)	44 (17)	196 (30)	240 (26)	0.0001
No. of episodes	56	273	329	
Incidence	283 (214–367)	425 (376–479)	391 (350–436)	0.0020
Inpatient ALRI				
No. of children (%)	6 (2)	52 (8)	58 (6)	0.0016
No. of episodes	6	60	66	
Incidence	30 (11–66)	93 (71–120)	79 (61–100)	0.0014

*Episodes per 1000 child-years (95% confidence interval).
†Comparing 2006 cohort with 2007 cohort.

returned to the study investigators for further analysis. Clinical episodes from which multiple nasal washes were collected were reviewed and molecular testing results were consolidated if clinically compatible. Illness incidence rates were calculated based on the subjects under active surveillance, and were compared using rate ratios and exact binomial 95% confidence intervals. Person-time at risk for each participant was calculated from January 1, 2009, through the date of study withdrawal/completion or December 31, 2009, whichever occurred first. Pathogen-specific incidence rates were adjusted for the proportion of episodes sampled. Associations between diagnostic categories or clinical syndromes and individual pathogens or pathogen combinations were examined using the 2-sample test of proportions, χ^2 analysis or Fishers exact test, as appropriate. Baseline characteristics (Table 2) were evaluated as risk factors for lower respiratory tract disease or hospitalization using multiple logistic regression. The median ages between groups were compared using the Mann–Whitney test. A *P* value of <0.05 was considered statistically significant.

Human Subject Research Issues

The study was approved by the Navajo and White Mountain Apache tribes, as well as the institutional review boards of the Johns Hopkins Bloomberg School of Public Health, the Navajo Nation and the Phoenix Area Indian Health Service.

TABLE 4. Number of Times a Child Was Seen, According to Visit Type

Number of Visits of this Type	Number of Children			
	All MAARI	All ALRI	Outpt ALRI	Inpt ALRI
None	246	642	675	857
Once	267	197	179	53
Twice	198	49	43	3
Three times	101	16	11	1
Four times	51	5	5	1
Five times	23	4	1	0
Six times	19	2	1	0
Seven times	8			
Ten times	1			
Eleven times	1			

RESULTS

Incidence of MAARI

At the start of the analysis period, 915 children (median age at start on January 1, 2009: 16.9 months) were under active follow-up; 260 (28%) enrolled in 2006, and 655 (72%) enrolled in 2007 (Table 2). The 2 cohorts were well balanced at enrollment in the parent trial, with the exception of having a family history of asthma or hay fever. A high proportion of subjects was breastfed in infancy (86%), had young siblings at home (66%), was exposed to second-hand smoke (19%) or wood-burning or coal-burning stoves (62%) in the home or had been hospitalized before enrollment in the parent trial (12%). Although two thirds of subjects lived with other young children, formal day-care attendance (3%) was low.

Of the 915 subjects under surveillance, 669 (73%) experienced a total of 1476 MAARI episodes, resulting in an incidence rate of 1756 episodes per 1000 child-years (Table 3). Of these episodes, 329 were ALRI managed on an outpatient basis, whereas 66 were ALRI requiring hospitalization, resulting in incidence rates of 391 and 79 episodes per 1000 child-years, respectively. Rates of respiratory illness were higher in the younger cohort compared with the older cohort for all categories ($P < 0.05$ for all). Subjects experienced 2.2 MAARI episodes on average (range 0–11), whereas 246 (27%) experienced none. Repeat visits accounted for 27% and 12% of inpatient and outpatient ALRI episodes, respectively (Table 4). Of the 240 children who experienced an outpatient ALRI, 25 (10%) also experienced an inpatient ALRI episode. Episodes occurred in 2 waves during 2009 (Fig. 1). The first wave, peaking in mid to late winter, represented the second or third winter respiratory season experienced by the 2 cohorts, whereas the second wave occurred throughout the autumn, after a proportion of the older cohort had completed follow-up.

Of the 1476 episodes, 989 (67%) were diagnosed as upper respiratory infection or cough, whereas pneumonia, bronchiolitis and other wheezing illnesses accounted for 23% (Table 5). Among outpatient ALRI, bronchiolitis was seen in one fifth (21%), whereas pneumonia accounted for more than one quarter (28%) and other wheezing illnesses accounted for one third (32%). Pneumonia was diagnosed in a majority (59%) of ALRI hospitalizations, followed by bronchiolitis and other wheezing illnesses.

Detection of Viruses and Bacteria

A total of 699 respiratory specimens were collected from 639 (43%) of the 1476 MAARI events, including 295 (30%)

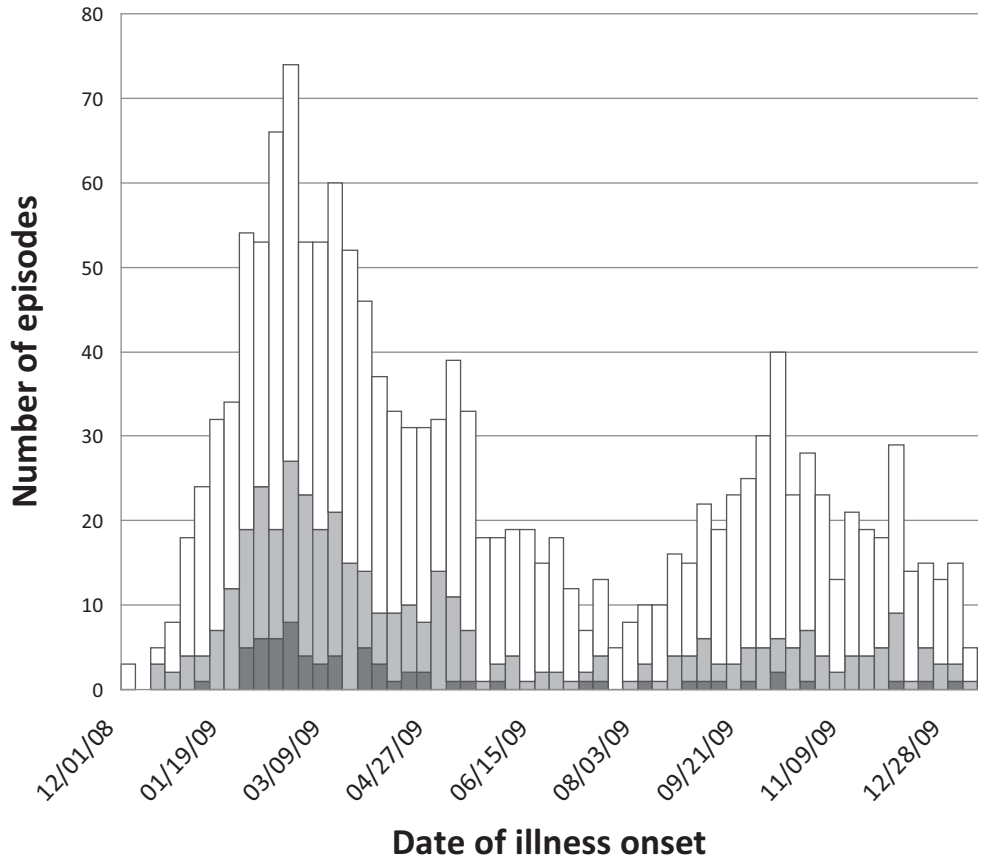

FIGURE 1. MAARIs by date of onset among participants (N = 915), January 1 to December 31, 2009, including inpatient acute lower respiratory illnesses (black), outpatient acute lower respiratory illnesses (gray) and all other MAARIs (white).

of the 984 upper respiratory infection episodes, 236 (72%) of the 329 outpatient ALRI episodes and 59 (89%) of the 66 ALRI hospitalizations. Samples were collected a median of 4 days (interquartile range 2–6 days) after illness onset. Molecular diagnostic testing detected ≥1 potential pathogens in 88% of episodes overall, including 91% of outpatient ALRI and 93% of inpatient ALRI (Table 6). Virus detection decreased in frequency as the number of days between illness onset and sample collection increased ($P = 0.001$). Among the respiratory viruses, HRV and RSV were most commonly detected across all categories, with RSV predominant among the more severe episodes (eg, 44% of ALRI hospitalizations). RSV episodes were seasonal, peaking in early February, and ending by early May (Fig. 2). HRV was detected in roughly one quarter of episodes, with winter and fall waves similar to those observed for MAARI overall. HRV was detected among outpatient and inpatient ALRI cases in similar

TABLE 5. Number and Incidence Rates* of Clinical Syndrome Categories by Visit Type

Diagnostic Category	All MAARI	All ALRI		Outpatient ALRI		Inpatient ALRI	
	N (%)	N (%)	Incidence	N (%)	Incidence	N (%)	Incidence
Total	1476	395		329		66	
Pneumonia	132 (9)	132 (33)	157 (131–186)	93 (28)	111 (89–136)	39 (59)	46 (33–63)
Bronchiolitis	83 (6)	83 (21)	99 (79–122)	68 (21)	81 (63–103)	15 (23)	18 (10–29)
Wheezing illnesses†	114 (8)	114 (29)	136 (112–163)	104 (32)	124 (101–150)	10 (15)	12 (6–22)
ALRI not otherwise specified	47 (3)	47 (12)		46 (14)		1 (2)	
Croup	65 (4)	11 (3)		10 (3)		1 (2)	
Allergic rhinitis	11 (1)	0 (0)		0 (0)		0 (0)	
Viral syndromes	23 (2)	2 (1)		2 (1)		0 (0)	
Pharyngitis, tonsillitis	2 (<1)	0 (0)		0 (0)		0 (0)	
Sinusitis, otitis media	10 (1)	1 (<1)		1 (<1)		0 (0)	
Upper respiratory infection or cough	989 (67)	5 (2)		5 (2)		0 (0)	

*Episodes per 1000 child-years (95% confidence interval).
†Asthma, reactive airways disease, wheeze, small airway disease (other than bronchiolitis).

TABLE 6. Number (%) and Incidence Rates* of Pathogens Detected, by Visit Type

	All MAARI Episodes		Outpatient LRI		Inpatient LRI	
	N (%)	Incidence	N (%)	Incidence	N (%)	Incidence
Total episodes tested	639 (100)		236 (100)		59 (100)	
Influenza						
All type A	59 (9.2)		19 (8.1)		3 (5.1)	
H1N1pdm	33 (5.2)	91 (63–128)	11 (4.7)	18 (9–33)	3 (5.1)	4 (1–12)
Type B	4 (1)		1 (0.4)		0 (0)	
Total	63 (9.9)	174 (134–223)	20 (8.5)	33 (20–51)	3 (5.1)	4 (1–12)
Enteroviruses	29 (4.5)	80 (54–115)	9 (3.8)	15 (7–28)	6 (10.2)	8 (3–17)
Rhinoviruses	171 (26.8)	473 (405–550)	61 (25.9)	101 (77–129)	14 (23.7)	19 (10–31)
Coronaviruses 229E and OC43	11 (1.7)	30 (15–54)	4 (1.7)	7 (2–17)	0 (0)	0 (0–5)
Human metapneumovirus	51 (8.0)	141 (105–186)	18 (7.6)	30 (18–47)	5 (8.5)	7 (2–16)
Parainfluenza						
Type 1	18 (2.8)		5 (2.1)		0 (0)	
Type 2	7 (1.1)		1 (0.4)		0 (0)	
Type 3	57 (8.9)		20 (8.5)		6 (10.2)	
Type 4	5 (1)		3 (1.3)		1 (1.7)	
Total	87 (13.6)	241 (193–297)	29 (12.3)	48 (32–69)	7 (11.9)	9 (4–19)
RSV						
RSV A	32 (5.0)		12 (5.1)		10 (17.0)	
RSV B	82 (12.8)		37 (15.7)		16 (27.1)	
Total	114 (17.8)	315 (260–379)	49 (20.1)	81 (60–107)	26 (44.1)	35 (23–51)
Adenovirus	14 (2.2)	39 (21–65)	3 (1.3)	5 (1–14)	5 (8.5)	7 (2–16)
S. pneumoniae	381 (59.6)		148 (62.7)		34 (57.6)	
H. influenzae	120 (18.8)		47 (19.9)		13 (22.0)	
C. pneumoniae	20 (3.1)	55 (34–85)	9 (3.8)	15 (7–28)	1 (1.7)	1 (0–7)

*Episodes per 1000 child-years (95% confidence interval), adjusted by the proportion sampled.

proportions; of these, less than one fifth were associated with HRV alone. Influenza, parainfluenza (PIV; particularly type 3), and human metapneumovirus (hMPV) were detected at comparable rates (8–13%). *S. pneumoniae* was detected in 60% of episodes, with a smaller proportion positive for *H. influenzae* (all types; 19%) and *C. pneumoniae* (3%).

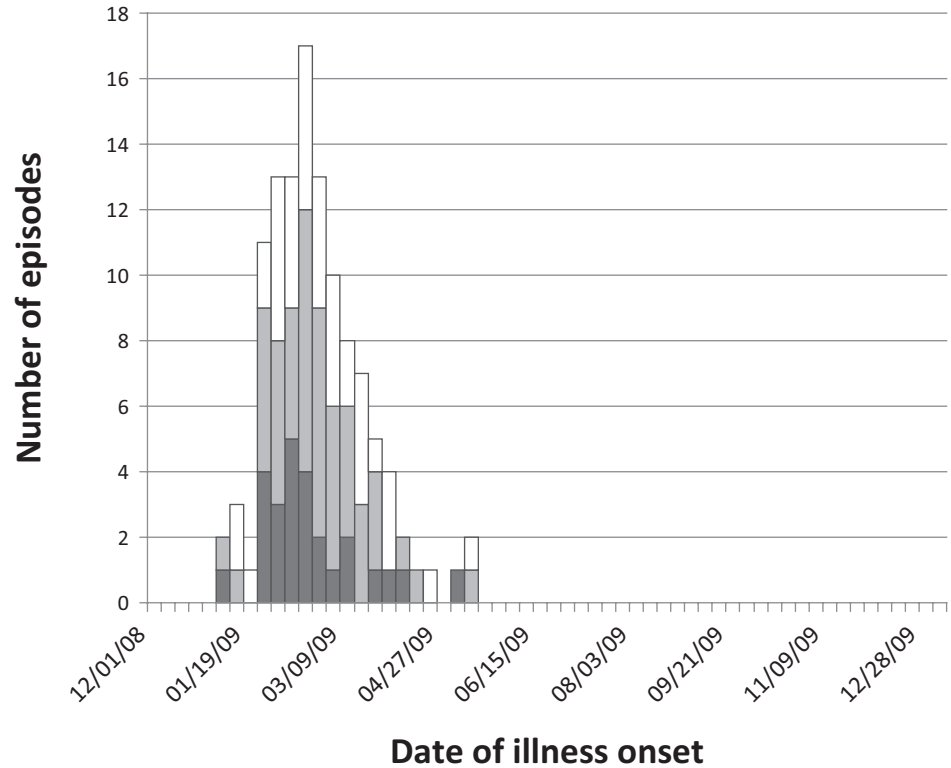

FIGURE 2. MAARIs by date of onset and visit type associated with RSV including inpatient acute lower respiratory illnesses (black), outpatient acute lower respiratory illnesses (gray) and all other MAARIs (white).

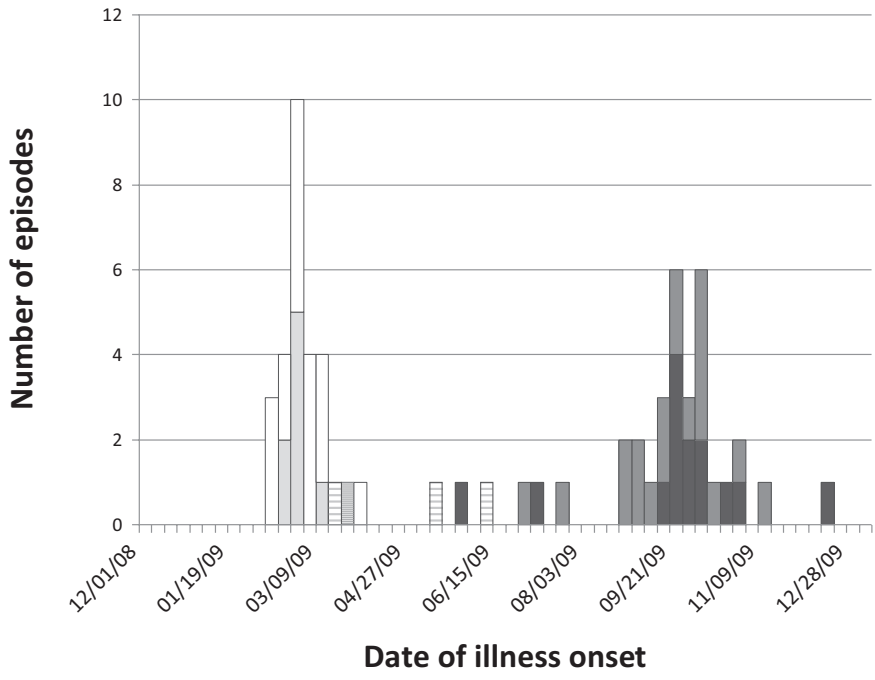

FIGURE 3. MAARIs associated with influenza by subtype and illness category. Seasonal influenza A acute lower respiratory illnesses (light gray) and other MAARIs (white), influenza B acute lower respiratory illnesses (heavy stripe) and other MAARIs (light stripe), and H1N1pdm acute lower respiratory illnesses (dark gray) and other MAARIs (medium gray).

Among influenza detections, episodes during the first 3 months of the year were associated with seasonal influenza A(H1N1) and influenza B viruses, whereas pandemic influenza A(H1N1) (H1N1pdm) was first identified in late May (Fig. 3). Although the first spring wave was largely absent in this population, H1N1pdm ultimately accounted for more than half of all influenza detections, including 11 (64%) of the 22 ALRI cases and all 3 illnesses requiring hospitalization.

Lower respiratory illnesses were more likely to be associated with RSV compared with upper respiratory illness (detected in 25% versus 11%, $P < 0.001$) whereas inpatient status was associated with the detection of RSV ($P = 0.029$), enterovirus ($P < 0.001$) and adenovirus ($P < 0.001$) when compared with all outpatient MAARI. By contrast, the detection of influenza virus (including H1N1pdm), rhinovirus or pneumococcus did not increase the risk of either lower respiratory tract involvement or hospitalization. It is notable that all 5 inpatient episodes associated with adenovirus involved the detection of an additional virus (RSV in 4, HRV in 1), and only 1 of the 6 enterovirus-associated hospitalizations involved its detection as a single pathogen.

TABLE 7. Multiple Pathogen Detections, by Visit Type

Number of Pathogens Detected in Specimen	Number of Specimens in Category (%)			
	All MAARI	All ALRI	Outpatient ALRI	Inpatient ALRI
0*	75 (12)	26 (9)	22 (9)	4 (7)
1	192 (30)	96 (33)	78 (33)	18 (31)
2	261 (41)	114 (39)	94 (40)	20 (34)
3	98 (15)	50 (17)	38 (16)	12 (20)
4	12 (2)	8 (3)	3 (1)	5 (8)
5	1 (<1)	1 (<1)	1 (<1)	0 (0)
Virus + *S. pneumoniae*	307 (48)	148 (50)	114 (48)	34 (58)
Total	639	295	236	59

*Specimen negative.

Approximately one third of episodes were associated with only 1 pathogen across all visit types; the largest proportion demonstrated 2 pathogens (Table 7). Detection of ≥3 pathogens was more frequently seen among hospitalized episodes (29%) than among MAARI episodes overall (17%; $P = 0.030$). *S. pneumoniae* was identified in combination with a virus in 48–58% of episodes, yet HRV was the only virus specifically associated with its presence ($P = 0.028$ for all MAARI; $P = 0.034$ for all ALRI; $P = 0.145$ for outpatient ALRI; $P = 0.069$ for inpatient ALRI). Influenza virus, RSV and rhinovirus were negatively associated with each other ($P < 0.05$ for all by pairwise combination).

The frequencies of pathogens according to diagnostic category among ALRI were unevenly distributed (Table 8). As expected, RSV was prominently seen among pneumonia and bronchiolitis cases, whereas rhinovirus was more frequent in other wheezing illnesses and pneumonia. Influenza and parainfluenza cases were notable for pneumonia and nonspecific ALRI, but were seen in a considerable proportion of wheezing illnesses as well. In contrast with previous reports,[22,23] and similar to more recent data,[24] hMPV was most frequently detected with pneumonia and nonspecific ALRI as opposed to bronchiolitis and other wheezing illnesses.

Risk Factor Analyses

Subjects who experienced ≥1 MAARI (n = 669) were more likely to have been younger at the start of the study period compared with those who had not (n = 246; median age 16.5 versus 18.4 months, $P = 0.0002$). These 2 groups were otherwise similar in terms of baseline characteristics. Among subjects who had an MAARI, those who experienced lower respiratory involvement (n = 273) were younger ($P = 0.0001$), were more likely to be male ($P = 0.017$), to have had an ALRI as an infant before enrollment in the trial ($P = 0.034$) and to have a family history of an atopic

TABLE 8. Pathogen Detection (Number, % Positive Among Those tested) by Diagnostic Category Among Outpatient and Inpatient ALRI Episodes

	Outpatient ALRI								Inpatient ALRI					
Diagnostic Category	All Diagnoses	Pneumonia	Bronchiolitis	Wheezing Illnesses*	LRI NOS	Croup	Viral Syndromes	URI or Cough	All Diagnoses	Pneumonia	Bronchiolitis	Wheezing Illnesses*	LRI NOS	Croup
Total episodes	329	93	68	104	46	10	2	5	66	39	15	10	1	1
Episodes tested (N, %)	236 (72)	72 (77)	47 (69)	72 (69)	32 (70)	9 (90)	1 (50)	3 (60)	59 (89)	35 (90)	15 (100)	7 (70)	1 (100)	1 (100)
Influenza														
All type A	19 (8)	5 (7)	3 (6)	8 (11)	3 (9)	0 (0)	0 (0)	0 (0)	3 (5)	2 (6)	0 (0)	0 (0)	1 (100)	0 (0)
H1N1pdm	11 (5)	2 (3)	2 (4)	5 (7)	2 (6)	0 (0)	0 (0)	0 (0)	3 (5)	2 (6)	0 (0)	0 (0)	1 (100)	0 (0)
Type B	1 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	20 (8)	6 (8)	3 (6)	8 (11)	3 (9)	0 (0)	0 (0)	0 (0)	3 (5)	2 (6)	0 (0)	0 (0)	1 (100)	0 (0)
Enteroviruses	9 (4)	1 (1)	2 (4)	5 (7)	1 (3)	0 (0)	0 (0)	0 (0)	6 (10)	3 (9)	1 (7)	1 (14)	1 (100)	0 (0)
Rhinoviruses	61 (26)	16 (22)	7 (15)	25 (35)	9 (28)	3 (33)	0 (0)	1 (33)	14 (24)	5 (14)	4 (27)	4 (57)	1 (100)	0 (0)
Coronaviruses (229E and OC43)	4 (2)	2 (3)	0 (0)	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Human metapneumovirus	18 (8)	4 (6)	3 (6)	5 (7)	6 (19)	0 (0)	0 (0)	0 (0)	5 (8)	3 (9)	2 (13)	0 (0)	0 (0)	0 (0)
Parainfluenza														0 (0)
Type 1	5 (2)	2 (3)	0 (0)	1 (1)	2 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Type 2	1 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Type 3	20 (8)	6 (8)	3 (6)	8 (11)	2 (6)	0 (0)	0 (0)	1 (33)	6 (10)	4 (11)	2 (13)	0 (0)	0 (0)	0 (0)
Type 4	3 (1)	0 (0)	1 (2)	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Total	29 (12)	8 (11)	4 (9)	12 (17)	4 (13)	0 (0)	0 (0)	1 (33)	7 (12)	5 (14)	2 (13)	0 (0)	0 (0)	0 (0)
RSV														
RSV A	12 (5)	2 (3)	3 (6)	1 (1)	6 (19)	0 (0)	0 (0)	0 (0)	10 (17)	4 (11)	3 (20)	2 (29)	0 (0)	1 (100)
RSV B	37 (16)	11 (15)	12 (26)	7 (10)	4 (13)	2 (22)	1 (100)	0 (0)	16 (27)	12 (34)	3 (20)	1 (14)	0 (0)	0 (0)
Total	49 (21)	13 (18)	15 (32)	8 (11)	10 (31)	2 (22)	1 (100)	0 (0)	26 (44)	16 (46)	6 (40)	3 (43)	0 (0)	1 (100)
Adenovirus	3 (1)	0 (0)	2 (4)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	5 (8)	2 (6)	3 (20)	0 (0)	0 (0)	0 (0)
S. pneumoniae	148 (63)	37 (51)	33 (70)	41 (57)	28 (88)	7 (78)	0 (0)	2 (67)	34 (58)	19 (54)	10 (67)	4 (57)	1 (100)	0 (0)
H. influenzae	47 (20)	13 (18)	10 (21)	16 (22)	4 (13)	3 (33)	1 (100)	0 (0)	13 (22)	5 (14)	5 (33)	3 (43)	0 (0)	0 (0)
C. pneumoniae	9 (4)	5 (7)	0 (0)	3 (4)	0 (0)	0 (0)	0 (0)	1 (33)	4 (7)	3 (9)	1 (7)	0 (0)	0 (0)	0 (0)
None detected	22 (9)	9 (13)	3 (6)	7 (10)	1 (3)	2 (22)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Asthma, reactive airways disease, wheeze, small airway disease (other than bronchiolitis).

LRI NOS indicates lower respiratory illness, not otherwise specified; URI, upper respiratory infection.

condition ($P = 0.001$) compared with those who had not had ALRI (n = 396), by multiple logistic regression. Among the children with ALRI, those who required hospitalization (n = 58) were more likely to have been younger ($P = 0.017$), or to have a history of eczema ($P = 0.014$), compared with subjects who were managed as outpatients (n = 215), by multiple logistic regression. The effects of motavizumab on bronchiolitis and RSV will be reported separately.

DISCUSSION

Here we provide, for the first time, prospective data regarding the major presentations and pathogens associated with acute respiratory illness from a cohort of young American Indian children. Collected through a full calendar year, these findings document a wide spectrum of respiratory pathogens, including the emergence of pandemic influenza, in this population known to be at high risk for respiratory illness. These data indicate high rates of medical attendance beyond infancy for respiratory disease, particularly involving the lower respiratory tract. Our rates of ALRI hospitalizations exceed estimates of 17–20 per 1000/year derived from retrospective database analyses.[2,25] This difference may be due to our use of active surveillance in a well-defined prospective cohort. Similarly, the incidence of pneumonia observed in this study exceeds published rates of community-acquired pneumonia for children in the general US population (16.9–22.4 outpatient visits per 1000 children aged 1–5 years[26] and 8.1–9.1 and 3.9–4.8 hospitalizations per 1000 children aged <2 years and 2–4 years, respectively).[27] Although limited by differences in age ranges, case definitions and laboratory methods, such comparisons provide strong indication of the higher burden of acute respiratory disease among young American Indian children relative to the general US pediatric population. These elevated rates of pneumonia, despite the presence of high vaccine coverage among NAs against important serotypes of *S. pneumoniae* and *H. influenzae* type b,[28] demonstrate the relevance of other respiratory pathogens. Our data also confirm the continuing high burden of bronchiolitis among young American Indian children, even into their second and third respiratory seasons, underscoring the need for additional strategies in the prevention and control of this condition.

We demonstrate the continued dominance of RSV in the years following infancy, responsible for a large proportion of pneumonia, bronchiolitis and other acute wheezing illnesses. We also confirm the high prevalence of HRV among these children, although it is unclear if HRV was the causative pathogen in all cases, as multiple detections were common. An additional virus was detected in approximately one third of HRV ALRI episodes, compared with 17% of ALRI cases positive for hMPV, 18% of those with influenza and 24% of those with RSV. Nevertheless, HRV may play at least a contributing role, as codetection of HRV with bacteria was common, and among ALRI cases and hospitalizations, was statistically associated with *S. pneumoniae*.

Consistent with prior reports,[14,15] we found substantially higher rates of influenza-related respiratory illness in our population compared with US children in general.[29,30] On a national level, the 2008-2009 influenza season was mild in terms of severity and extent, whereas pandemic influenza affected children disproportionately worldwide.[31–33] Therefore, it is not surprising that H1N1pdm accounted for all the influenza-related hospitalizations in this cohort. Nevertheless, seasonal H1N1 and B viruses contributed almost as much to the total influenza burden in terms of outpatient ALRI episodes (42%) and overall MAARI visits (44%), and within a shorter span of time. These unexpectedly comparable proportions of seasonal and pandemic influenza cases may be because young age is an independent risk factor for influenza-related complications, and the study cohort was younger at the start of 2009, when seasonal viruses were in circulation. Moreover, the spring wave of H1N1pdm was comparatively mild in this population, and our surveillance did not capture the fall wave experiences of the full 2006 cohort, as up to 30% of this group (representing 8% of the total cohort) had completed follow-up by September 1, 2009.

Similar to prior studies conducted in NA communities, pneumococcal colonization within this cohort exceeded rates found among similar age groups in the general US population.[34–40] Although these high rates have been implicated in the increased burden of invasive pneumococcal disease observed among NAs, the mechanisms underlying this relationship remain unclear. The potential for a synergistic effect when viruses and bacteria coinfect the respiratory tract has received increased attention in recent years, particularly between influenza and *S. pneumoniae*.[41,42] One study that used a similar diagnostic approach to ours demonstrated that codetection of *S. pneumoniae* in nasopharyngeal samples was associated with more severe H1N1pdm illness.[20] We did not observe such correlations, other than a potential interaction with rhinovirus, which has been reported previously.[43–46] The significance of this combination is unclear, as both pathogens can be detected in respiratory specimens in the absence of symptoms. The high overall burden of disease and elevated background rates of bacterial colonization present in this population may have obscured such statistical relationships in our study. In addition, we only established the presence or absence of *S. pneumoniae* and did not measure colonization density, which may be more closely associated with disease.[47,48] More generally, multiple pathogen detection appeared to be related to hospitalization in our study, supporting previous reports suggesting that coinfection increases severity.[49,50] This statistical association does not prove a causal relationship, however, and several studies dispute this effect.[51–53]

Our study was subject to limitations. First, our study did not include microbial testing in asymptomatic subjects, thus limiting our ability to draw causal inferences between the pathogens detected in the nasal wash and the clinical illness observed in the child. Second, clinical syndromes were assigned according to diagnoses made by the treating provider, and could only be standardized and confirmed at the level of medical record review by our study physicians. Third, we report data only from a single year, focusing on children in a restricted age range. Finally, the detection of bacteria or other pathogens in upper respiratory samples may not always be correlated with lower tract disease, thus limiting our ability to identify potential associations with coinfections or clinical outcomes.

In summary, we present the first comprehensive assessment of the clinical diagnoses and pathogens associated with acute respiratory illness among young American Indian children. These findings demonstrate the prevalence of numerous respiratory pathogens, including RSV, rhinovirus and pneumococcus in this community, and indicate that seasonal and pandemic influenza imposed a considerably higher burden compared with the general US population. Our observations offer valuable insights into the complex picture of respiratory infections and provide critical evidence to support prevention and control efforts that can be applied to epidemiologically similar communities throughout the world.

ACKNOWLEDGMENTS

The authors express their appreciation to the children and adults from the Navajo, White Mountain Apache and San Carlos Apache communities who participated actively in this study and the Indian Health Service physicians who cared for them. The authors gratefully acknowledge the dedicated efforts of the Center

for American Indian Health field staff who collected these data over many years. The authors also gratefully acknowledge the work by MedImmune staff on the parent clinical trial.

REFERENCES

1. Holman RC, Curns AT, Cheek JE, et al. Infectious disease hospitalizations among American Indian and Alaska native infants. *Pediatrics*. 2003;111:E176–E182.
2. Peck AJ, Holman RC, Curns AT, et al. Lower respiratory tract infections among american Indian and Alaska Native children and the general population of U.S. Children. *Pediatr Infect Dis J*. 2005;24:342–351.
3. O'Brien KL, Shaw J, Weatherholtz R, et al. Epidemiology of invasive Streptococcus pneumoniae among Navajo children in the era before use of conjugate pneumococcal vaccines, 1989-1996. *Am J Epidemiol*. 2004;160:270–278.
4. Bockova J, O'Brien KL, Oski J, et al. Respiratory syncytial virus infection in Navajo and White Mountain Apache children. *Pediatrics*. 2002;110(2 Pt 1):e20.
5. Millar EV, O'Brien KL, Levine OS, et al. Toward elimination of Haemophilus influenzae type B carriage and disease among high-risk American Indian children. *Am J Public Health*. 2000;90:1550–1554.
6. Weatherholtz R, Millar EV, Moulton LH, et al. Invasive pneumococcal disease a decade after pneumococcal conjugate vaccine use in an American Indian population at high risk for disease. *Clin Infect Dis*. 2010;50: 1238–1246.
7. Davidson M, Parkinson AJ, Bulkow LR, et al. The epidemiology of invasive pneumococcal disease in Alaska, 1986-1990–ethnic differences and opportunities for prevention. *J Infect Dis*. 1994;170:368–376.
8. Singleton R, Hammitt L, Hennessy T, et al. The Alaska Haemophilus influenzae type b experience: lessons in controlling a vaccine-preventable disease. *Pediatrics*. 2006;118:e421–e429.
9. Singleton RJ, Bruden D, Bulkow LR. Respiratory syncytial virus season and hospitalizations in the Alaskan Yukon-Kuskokwim Delta. *Pediatr Infect Dis J*. 2007;26(suppl 11):S46–S50.
10. Singleton RJ, Butler JC, Bulkow LR, et al. Invasive pneumococcal disease epidemiology and effectiveness of 23-valent pneumococcal polysaccharide vaccine in Alaska native adults. *Vaccine*. 2007;25:2288–2295.
11. Centers for Disease Control and Prevention. Deaths related to 2009 pandemic influenza A (H1N1) among American Indian/Alaska Natives - 12 states, 2009. *MMWR Morb Mortal Wkly Rep*. 2009;58:1341–1344.
12. La Ruche G, Tarantola A, Barboza P, et al.; epidemic intelligence team at InVS. The 2009 pandemic H1N1 influenza and indigenous populations of the Americas and the Pacific. *Euro Surveill*. 2009;14:19366.
13. Groom AV, Jim C, Laroque M, et al. Pandemic influenza preparedness and vulnerable populations in tribal communities. *Am J Public Health*. 2009;99(suppl 2):S271–S278.
14. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet*. 2011;378:1917–1930.
15. Dee DL, Bensyl DM, Gindler J, et al. Racial and ethnic disparities in hospitalizations and deaths associated with 2009 pandemic Influenza A (H1N1) virus infections in the United States. *Ann Epidemiol*. 2011;21:623–630.
16. Chandran A, Millar E, Weatherholtz R, et al. Safety and efficacy of motavizumab in the prevention of RSV disease in healthy infants. *Pediatr Academic Societies Annual Meeting Abstracts*. 2008:4460–4465.
17. Briese T, Palacios G, Kokoris M, et al. Diagnostic system for rapid and sensitive differential detection of pathogens. *Emerging Infect Dis*. 2005;11: 310–313.
18. Lamson D, Renwick N, Kapoor V, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004-2005. *J Infect Dis*. 2006;194:1398–1402.
19. Dominguez SR, Briese T, Palacios G, et al. Multiplex MassTag-PCR for respiratory pathogens in pediatric nasopharyngeal washes negative by conventional diagnostic testing shows a high prevalence of viruses belonging to a newly recognized rhinovirus clade. *J Clin Virol*. 2008;43:219–222.
20. Palacios G, Hornig M, Cisterna D, et al. Streptococcus pneumoniae coinfection is correlated with the severity of H1N1 pandemic influenza. *PLoS ONE*. 2009;4:e8540.
21. Tokarz R, Kapoor V, Wu W, et al. Longitudinal molecular microbial analysis of influenza-like illness in New York City, May 2009 through May 2010. *Virol J*. 2011;8:288.
22. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004;350:443–450.
23. Schildgen V, van den Hoogen B, Fouchier R, et al. Human Metapneumovirus: lessons learned over the first decade. *Clin Microbiol Rev*. 2011;24:734–754.
24. Edwards KM, Zhu Y, Griffin MR, et al.; New Vaccine Surveillance Network. Burden of human metapneumovirus infection in young children. *N Engl J Med*. 2013;368:633–643.
25. Singleton RJ, Holman RC, Folkema AM, et al. Trends in lower respiratory tract infection hospitalizations among American Indian/Alaska Native children and the general US child population. *J Pediatr*. 2012;161:296–302.e2.
26. Kronman MP, Hersh AL, Feng R, et al. Ambulatory visit rates and antibiotic prescribing for children with pneumonia, 1994-2007. *Pediatrics*. 2011;127:411–418.
27. Centers for Disease Control and Prevention. Pneumonia hospitalizations among young children before and after introduction of pneumococcal conjugate vaccine--United States, 1997–2006. *MMWR Morb Mortal Wkly Rep* 2009;58:1–4.
28. Groom AV, Santibanez TA, Bryan RT. Vaccination coverage among American Indian and Alaska native children, 2006-2010. *Pediatrics*. 2012;130:e1592–e1599.
29. Dawood FS, Fiore A, Kamimoto L, et al.; Emerging Infections Program Network. Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008. *J Pediatr*. 2010;157:808–814.
30. Poehling KA, Edwards KM, Griffin MR, et al. The burden of influenza in young children, 2004-2009. *Pediatrics*. 2013;131:207–216.
31. Louie JK, Acosta M, Winter K, et al.; California Pandemic (H1N1) Working Group. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA*. 2009;302: 1896–1902.
32. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic, Bautista E, Chotpitayasunondh T, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med*. 2010;362:1708–1719.
33. Dawood FS, Iuliano AD, Reed C, et al. Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infect Dis*. 2012;12:687–695.
34. Hennessy TW, Singleton RJ, Bulkow LR, et al. Impact of heptavalent pneumococcal conjugate vaccine on invasive disease, antimicrobial resistance and colonization in Alaska Natives: progress towards elimination of a health disparity. *Vaccine*. 2005;23:5464–5473.
35. Millar EV, O'Brien KL, Watt JP, et al. Effect of community-wide conjugate pneumococcal vaccine use in infancy on nasopharyngeal carriage through 3 years of age: a cross-sectional study in a high-risk population. *Clin Infect Dis*. 2006;43:8–15.
36. O'Brien KL, Millar EV, Zell ER, et al. Effect of pneumococcal conjugate vaccine on nasopharyngeal colonization among immunized and unimmunized children in a community-randomized trial. *J Infect Dis*. 2007;196:1211–1220.
37. Millar EV, O'Brien KL, Zell ER, et al. Nasopharyngeal carriage of Streptococcus pneumoniae in Navajo and White Mountain Apache children before the introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J*. 2009;28:711–716.
38. Millar EV, Pimenta FC, Roundtree A, et al. Pre- and post-conjugate vaccine epidemiology of pneumococcal serotype 6C invasive disease and carriage within Navajo and White Mountain Apache communities. *Clin Infect Dis*. 2010;51:1258–1265.
39. Scott JR, Hinds J, Gould KA, et al. Nontypeable pneumococcal isolates among navajo and white mountain apache communities: are these really a cause of invasive disease? *J Infect Dis*. 2012;206:73–80.
40. Wroe PC, Lee GM, Finkelstein JA, et al. Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatr Infect Dis J*. 2012;31:249–254.
41. Madhi SA, Klugman KP; Vaccine Trialist Group. A role for Streptococcus pneumoniae in virus-associated pneumonia. *Nat Med*. 2004;10:811–813.
42. McCullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clin Microbiol Rev*. 2006;19:571–582.
43. Templeton KE, Scheltinga SA, van den Eeden WC, et al. Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. *Clin Infect Dis*. 2005;41:345–351.

44. Jennings LC, Anderson TP, Beynon KA, et al. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax*. 2008;63:42–48.
45. Peltola V, Heikkinen T, Ruuskanen O, et al. Temporal association between rhinovirus circulation in the community and invasive pneumococcal disease in children. *Pediatr Infect Dis J*. 2011;30:456–461.
46. Launes C, de-Sevilla MF, Selva L, et al. Viral coinfection in children less than five years old with invasive pneumococcal disease. *Pediatr Infect Dis J*. 2012;31:650–653.
47. Vu HT, Yoshida LM, Suzuki M, et al. Association between nasopharyngeal load of Streptococcus pneumoniae, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. *Pediatr Infect Dis J*. 2011;30:11–18.
48. Albrich WC, Madhi SA, Adrian PV, et al. Use of a rapid test of pneumococcal colonization density to diagnose pneumococcal pneumonia. *Clin Infect Dis*. 2012;54:601–609.
49. Drews AL, Atmar RL, Glezen WP, et al. Dual respiratory virus infections. *Clin Infect Dis*. 1997;25:1421–1429.
50. Cilla G, Oñate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. *J Med Virol*. 2008;80:1843–1849.
51. Canducci F, Debiaggi M, Sampaolo M, et al. Two-year prospective study of single infections and co-infections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol*. 2008;80:716–723.
52. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol*. 2010;82:1282–1290.
53. Martin ET, Kuypers J, Wald A, et al. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respi Viruses*. 2012;6:71–77.