Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations

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Background: Detection of either viral or bacterial pathogens is associated with wheezing in children; however, the influence of both bacteria and viruses on illness symptoms has not been described.

Objective: We evaluated bacterial detection during the peak rhinovirus season in children with and without asthma to determine whether an association exists between bacterial infection and the severity of rhinovirus-induced illnesses.

Methods: Three hundred eight children (166 with asthma and 142 without asthma) aged 4 to 12 years provided 5 consecutive weekly nasal samples during September and scored cold and asthma symptoms daily. Viral diagnostics and quantitative PCR for *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* were performed on all nasal samples.

Results: Detection rates were 53%, 17%, and 11% for *H influenzae*, *S pneumoniae*, and *M catarrhalis*, respectively, with detection of rhinovirus increasing the risk of detecting bacteria within the same sample (odds ratio [OR], 2.0; 95% CI, 1.4-2.7; $P < .0001$) or the following week (OR, 1.6; 95% CI, 1.1-2.4; $P = .02$). In the absence of rhinovirus, *S pneumoniae* was associated with increased cold symptoms (mean, 2.7 [95% CI, 2.0-3.5] vs 1.8 [95% CI, 1.5-2.2]; $P = .006$) and moderate asthma exacerbations (18% [95% CI, 12% to 27%] vs 9.2% [95% CI, 6.7% to 12%]; $P = .006$). In the presence of rhinovirus, *S pneumoniae* was associated with increased moderate asthma exacerbations (22% [95% CI, 16% to 29%] vs 15% [95% CI, 11% to 20%]; $P = .01$). Furthermore, *M catarrhalis* detected alongside rhinovirus increased the likelihood of experiencing cold symptoms, asthma symptoms, or both compared with isolated detection of rhinovirus (OR, 2.0 [95% CI, 1.0-4.1]; $P = .04$). Regardless of rhinovirus status, *H influenzae* was not associated with respiratory symptoms.

Conclusion: Rhinovirus infection enhances detection of specific bacterial pathogens in children with and without asthma. Furthermore, these findings suggest that *M catarrhalis* and *S pneumoniae* contribute to the severity of respiratory tract illnesses, including asthma exacerbations. (J Allergy Clin Immunol 2014;133:1301-7.)

Key words: *Rhinovirus, bacteria, asthma*

There is mounting evidence that asthma is associated with changes in the airway microbiome. For example, detection of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* within the upper airway in early infancy is associated with an increased risk of recurrent wheezing and then asthma.[1,2] In addition, culture-independent methods of bacterial detection demonstrate that Proteobacteria, a phylum of bacteria containing a majority of gram-negative bacteria, appeared more frequently in both nasal and bronchial samples in patients with stable asthma and are linked to increased bronchial hyperresponsiveness.[3-5] However, prospective studies have not used molecular methods of detection to analyze these bacteria between children with and without asthma.

Recently, we reported that 90% of children with asthma are infected with rhinoviruses during the month of September and that the severity of clinical illness varies from no symptoms to severe wheezing illnesses.[6] These findings strongly suggest that there are cofactors that affect the relationship between rhinovirus infection and illness and suggest that airway bacteria might contribute in this regard. Furthermore, a recent study found an increase in invasive pneumococcal disease during peak rhinovirus activity.[7] Therefore we hypothesized that detection of specific bacterial pathogens would be increased in children with asthma

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Abbreviations used
FENO: Fraction of exhaled nitric oxide
OR: Odds ratio

compared with children without asthma and that codetection of these bacteria with rhinovirus would be associated with increased respiratory symptoms compared with rhinovirus detection alone. To test these hypotheses, we prospectively obtained weekly nasal secretion specimens from children with and without asthma during the fall of 2007-2009 and then compared detection of viruses and select bacteria with cold and asthma symptoms and moderate asthma exacerbations.

METHODS

Study subjects and design

Children included in this analysis were enrolled in a larger study (RhinoGen) to determine genetic correlates with more severe rhinovirus-induced illnesses. Of the 383 children participating in RhinoGen, 308 children aged 4 to 12 years submitted samples during a peak rhinovirus season. Starting the first Saturday of September 2007, 2008, or 2009, nasal samples were collected weekly for a total of 5 consecutive weeks. Children, with the help of their parents, were instructed to record upper respiratory tract illnesses and asthma symptoms, morning peak expiratory flow, and albuterol use on daily diary cards from 1 week before the first nasal sample submission through 1 week after the final (fifth) sample submission. See the Methods section in this article's Online Repository at www.jacionline.org for additional details about recruitment and inclusion criteria. This study was approved by the University of Wisconsin Human Subjects Committee, and written informed consent was obtained from the parents.

Procedures and definitions

At the first study visit, subjects were taught to collect samples of their own nasal mucus using a nose-blowing technique, as previously described.[6,8] These samples were analyzed for common respiratory tract viruses by using the Respiratory Multicode Assay (EraGen Biosciences, Madison, Wis).[9]

Nasal samples were also analyzed for *S pneumoniae*, *H influenzae*, and *M catarrhalis* by using quantitative real-time PCR. DNA was extracted with the BiOstic Bacteremia DNA Isolation Kit (Mo Bio laboratories, Carlsbad, Calif). The quantitative Spn9802 PCR for the detection of *S pneumoniae*[10] was combined with the P6 PCR for the detection of *H influenzae*[11] and the copB PCR for the detection of *M catarrhalis*,[12] as previously described. All primers and probes were obtained from Applied Biosystems (Foster City, Calif), and the real-time PCR assay was performed in a 7300 Applied Biosystems instrument. We confirmed double- and triple-positive results obtained by using a Multicode assay by repeating quantitative PCR with single-target assays. Standard curves consisted of bacterial DNA extracted from known quantities of clinical isolates of each bacterium obtained from the University of Wisconsin Hospital Clinical Microbiology Laboratory, and PCR results are expressed as colony-forming unit equivalents.

Children scored cold and asthma symptom severity based on a 4-point scoring system (see Table E1 in this article's Online Repository at www.jacionline.org).[6,8] Moderate asthma exacerbations were defined as at least moderate asthma symptoms (score ≥2) and either a decrease in peak expiratory flow of at least 20% or increased use of albuterol for 2 days or more in accordance with National Heart, Lung, and Blood Institute and American Thoracic Society definitions.[13,14] Current asthma was diagnosed at study completion based on previously reported criteria.[15] Skin prick testing and measurement of total and allergen-specific IgE levels in plasma were performed on enrollment (UniCAP 100; Phadia, Uppsala, Sweden).

Children who collected at least 4 of 5 weekly nasal specimens and were missing less than 20% of diary card data were included in the analysis. A week was defined as the 7-day period surrounding (±3 days) the day of nasal specimen collection. Each week was designated as positive or negative for virus, bacteria, or both based on viral detection, bacterial detection, or both. Cold and asthma symptom burdens for each week were calculated by summing the daily symptom scores across the week. An illness was defined as a period of 2 or more consecutive days of cold or asthma symptoms that did not contain any periods of consecutive asymptomatic days. All nasal samples obtained between 3 days before the first day of the illness and 3 days after the last day of the illness were considered to be associated with that illness. Cold and asthma symptom burdens for each illness were calculated by summing daily symptom scores across the illness.

Statistical analysis

Atopic status (positive determined based on either specific IgE level or skin prick test response), race, and sex were compared by asthma status with the χ^2 test for association. Age and fraction of exhaled nitric oxide (FENO) were compared by asthma status with the Wilcoxon rank sum test. FEV_1, forced vital capacity, and FEV_1/forced vital capacity ratio were compared by asthma status with linear models that also included covariates for age, sex, race, and height. Viral infection and bacterial detection rates were compared by asthma status with logistic regression models, both univariate and adjusted for sex, race, and sensitization. Generalized linear mixed-effects models with a random effect for subject to account for repeated measures within subjects were used to compare weekly symptom burdens and occurrences of moderate asthma exacerbations by virus and bacterial detection and to assess whether infection with a given virus or bacterium increased or decreased susceptibility to another bacterium or virus during the same week or 1 week later. A matched-pairs case-control design was used in which each illness (case) was matched with an asymptomatic period (control) of the same length in another subject to assess the association between viral detection, bacterial detection, or both and the occurrence of an illness. Conditional logistic regression was then used to estimate odds ratios (ORs) of illness occurrence with respect to detection of the virus, bacterium, or both. A 2-sided P value of less than .05 was regarded as statistically significant.

RESULTS

Subjects' characteristics

Of the 383 children enrolled in RhinoGen, 54 had insufficient quantity of nasal samples to allow analysis for both viruses and bacteria, whereas an additional 19 subjects did not submit at least 4 (80%) of the 5 scheduled nasal samples, and 2 additional subjects did not complete at least 40 days (80%) of symptom diaries. Therefore a total of 308 (80%) subjects were included in the final analysis (see Fig E1 in this article's Online Repository at www.jacionline.org).

Of the 308 subjects enrolled, 166 (54%) had current asthma. In comparing the groups, there was no significant difference in age or baseline lung function; however, the asthma group had a higher rate of allergic sensitization and higher baseline FENO values and contained more male and fewer white subjects (Table I).

Identification of pathogenic bacteria

The 308 subjects who completed the study submitted 1394 nasal samples. One hundred seventy-three samples positive for other viruses were excluded from analysis, resulting in 867 (71%) samples positive for bacteria and 481 (39%) samples positive for rhinovirus. Of the bacteria detected, *H influenzae* was detected most frequently (53% of samples), followed by *S pneumoniae* (17%) and *M catarrhalis* (11%). Detection rates were similar between children, regardless of asthma (Table II) and allergic sensitization status (data not shown).

TABLE I. Study subjects

	Asthma (n = 166)	No asthma (n = 142)	*P* value
Age (y)	8.3 ± 2.1	8.6 ± 1.6	.27
Sex (male)	67%	56%	.03
Race (white)	81%	92%	.007
Atopic status	73%	53%	.0003
FENO	23 ± 22	13 ± 13	<.0001
FEV_1	1.71 ± 0.44	1.76 ± 0.45	.31
FVC	2.12 ± 0.59	2.14 ± 0.59	.99
FEV_1/FVC ratio	0.81 ± 0.07	0.83 ± 0.07	.07

FVC, Forced vital capacity.

TABLE II. Detection of bacteria and viruses in relation to asthma status (n = 1221)

	No asthma (n = 576)	Asthma (n = 645)	*P* value
No bacteria	167 (29%)	187 (29%)	.99
M catarrhalis	56 (10%)	68 (11%)	.64
S pneumoniae	90 (16%)	108 (17%)	.60
H influenzae	308 (53%)	350 (54%)	.78
Rhinovirus	230 (40%)	251 (39%)	.72

Examination of immunization records revealed that 95% of subjects were vaccinated with the full series of *H influenzae* type B (HIB) vaccine, whereas 61% of subjects were vaccinated with the pneumococcal (PCV-7) vaccine. There were no differences in bacterial detection rates or symptom reporting based on pneumococcal vaccine status (data not shown).

Bacterial detection associated with viral infection

We next analyzed whether viral detection increased the likelihood for bacterial detection or *vice versa*. Rhinovirus detection increased the risk for detection of a bacterial pathogen in the same specimen (OR, 2.0 [95% CI, 1.4-2.7]; $P < .0001$) and for detection of bacteria the following week (OR, 1.6 [95% CI, 1.1-2.4]; $P = .02$; Table III). In contrast, detection of bacteria did not increase the chance of viral detection the following week (OR, 0.9 [95% CI, 0.6-1.3]; $P = .42$). Similarly, when examining each bacteria individually, rhinovirus was more likely to precede *S pneumoniae* detection (OR, 3.4 [95% CI, 2.0-5.5]; $P < .0001$) and *M catarrhalis* detection (OR, 3.7 [95% CI, 1.5-9.0]; $P = .004$). Rhinovirus infection was also associated with an increased likelihood of concurrent detection of *S pneumoniae* (OR, 3.0 [95% CI, 2.1-4.3]; $P < .0001$), with a similar trend for *M catarrhalis* (OR, 1.5 [95% CI, 1.0-2.3]; $P = .08$). Rhinovirus infection was also associated with greater quantities of *S pneumoniae* and *H influenzae*, with a similar trend observed for *M catarrhalis* (Table IV).

Time-based analysis of bacterial effects on illness severity

We used 2 approaches to determine whether detection of bacteria was related to illness severity. First, we conducted a time-based analysis to determine whether detection of rhinovirus, bacteria, or both in a given week was related to the severity of cold symptoms during that same week. Compared with weeks with no pathogen detected (mean, 1.8 [95% CI, 1.5-2.2]), cold symptom burden was significantly greater with either *S pneumoniae* detection (mean, 2.7 [95% CI, 2.0-3.5]; $P = .006$) or rhinovirus infection (mean, 3.3 [95% CI, 2.8-3.9]; $P < .0001$; Fig 1, *B*). In addition, concurrent detection of *S pneumoniae* and rhinovirus was associated with increased cold symptoms (mean, 3.8 [95% CI, 3.0-4.7]; $P < .0001$), even when compared with detection of *S pneumoniae* alone ($P = .02$). Neither *M catarrhalis* nor *H influenzae* was associated with increased cold symptoms (Fig 1, *A* and *C*).

TABLE III. Influence of virus on bacterial detection

	Rhinovirus preceding bacteria	Bacteria preceding rhinovirus	Concurrent bacteria and rhinovirus detection	OR	95% CI	*P* value
Any bacteria	No	—	—	1	—	—
	Yes	—	—	1.6	1.1-2.4	.02
	—	No	—	1	—	—
	—	Yes	—	0.9	0.6-1.3	.42
	—	—	No	1		
	—	—	Yes	2.0	1.4-2.7	<.0001
M catarrhalis	No	—	—	1	—	—
	Yes	—	—	3.7	1.5-9.0	.004
	—	No	—	1	—	—
	—	Yes	—	0.9	0.5-1.6	.67
	—	—	No	1		
	—	—	Yes	1.5	1.0-2.3	.08
S pneumoniae	No	—	—	1	—	—
	Yes	—	—	3.4	2.0-5.5	<.0001
	—	No	—	1	—	—
	—	Yes	—	0.9	0.6-1.4	.54
	—	—	No	1		
	—	—	Yes	3.0	2.1-4.3	<.0001
H influenzae	No	—	—	1	—	—
	Yes	—	—	0.9	0.7-1.3	.69
	—	No	—	1	—	—
	—	Yes	—	1.1	0.8-1.5	.75
	—	—	No	1		
	—	—	Yes	1.2	0.9-1.6	.14

Next, we compared pathogen detection with asthma symptoms. Compared with weeks with no pathogens (mean, 1.6 [95% CI, 1.3-2.1]), asthma symptoms were increased when rhinovirus was detected together with either *S pneumoniae* (mean, 2.9 [95% CI, 2.1-4.0]; $P = .0001$) or *M catarrhalis* (mean, 2.7 [95% CI, 1.8-4.0]; $P = .01$), with a trend for increased symptoms with both rhinovirus and *S pneumoniae* compared with rhinovirus alone ($P = .08$; Fig 1, *E*). *H influenzae* detection was not associated with increased asthma symptoms (Fig 1, *F*).

We next compared detection of bacteria, either individually or in combination with a viral illness, with the prevalence of moderate asthma exacerbations. Compared with when no pathogen was detected (mean, 9.2% [95% CI, 6.7% to 12%]), exacerbations were more frequent with detection of either rhinovirus (mean, 15% [95% CI, 11%-20%]; $P = .0005$) or *S pneumoniae* (mean, 18% [95% CI, 12% to 27%]; $P = .006$; Fig 1, *H*). Weeks in which rhinovirus and *S pneumoniae* were detected together had the highest frequencies of exacerbations (mean, 22% [95% CI, 16% to 29%]; $P < .0001$), and this combination was more likely to be associated with asthma exacerbation when compared with rhinovirus infection alone ($P = .01$). The presence of *M catarrhalis* or *H influenzae* did not increase the risk of asthma exacerbation (Fig 1, *G* and *I*).

TABLE IV. Association of rhinovirus detection with bacterial quantity*

	Bacterial quantity detected (CFUe)	Rhinovirus detection	*P* value: vs no bacteria	vs lower half/lowest quartile	vs second quartile	vs third quartile
M catarrhalis	0	38%	—	—	—	—
	1-202	45%	.42	—	—	—
	203-161,000	53%	.07	.45	—	—
S pneumoniae	0	35%	—	—	—	—
	1-322	48%	.03	—	—	—
	323-8,770,000	73%	<.0001	.0002	—	—
H influenzae	0	37%	—	—	—	—
	1-3	34%	.42	—	—	—
	4-12	42%	.23	.11	—	—
	13-79	34%	.42	.98	.10	—
	80-102,000	57%	<.0001	<.0001	.005	<.0001

CFUe, Colony-forming unit equivalents.
*A value of 0 indicates a value of less than the detection limit. Detectable bacteria were divided into greater than and less than median values. Because we detected *H influenzae* in a large quantity of samples, we divided this group into quartiles.

Finally, the associations between pathogens and study outcomes (cold symptoms, asthma symptoms, and asthma exacerbations) did not differ by atopic status (interaction $P > .2$ for all).

Illness-based analysis of bacterial effects on illness severity

We also performed an illness-based analysis to determine whether rhinovirus detection, bacterial detection, or both influenced the probability of symptomatic illness, including moderate asthma exacerbations. For this analysis, illnesses were clinically defined by at least 2 consecutive days of respiratory symptoms (median, 6 days; range, 2-42 days), and then pathogens detected during the illness were analyzed. Thus an illness could be associated with no pathogens or rhinovirus, bacteria, or both (including sequential detection of >1 pathogen). Over the 5 weeks, 291 illnesses occurred; 11% of illnesses were associated with rhinovirus alone, 30% were associated with bacterial detection, 49% were associated with detection of both rhinovirus and bacteria, and 10% were not associated with either pathogen. Each illness was then matched with an asymptomatic period of the same length, and the probability of pathogen detection was compared. When detected as single pathogens, rhinovirus alone increased the risk of illness (OR, 1.5 [95% CI, 1.0-2.3]; $P = .03$), but this was not the case for any of the bacteria.

We next examined combinations of rhinovirus and specific bacteria and their relationships to illness (Fig 2, *A-C*). Among all study subjects, illnesses were significantly associated with detection of rhinovirus together with either *M catarrhalis* (OR, 3.1 [95% CI, 1.6-6.4]; $P = .001$) or *S pneumoniae* (OR, 2.2 [95% CI, 1.3-3.7]; $P = .004$). Furthermore, detection of the combination of rhinovirus and *M catarrhalis* (OR, 2.0 [95% CI, 1.0-4.1]; $P = .04$) increased the likelihood of illness compared with solitary detection of rhinovirus. In contrast, the presence of *H influenzae* in combination with rhinovirus was not associated with increased risk of illness.

Finally, we tested whether the combination of rhinovirus and bacteria was associated with increased illnesses with moderate asthma exacerbations. Exacerbations that were associated with rhinovirus together with *M catarrhalis* (OR, 7.1 [95% CI, 1.3-39]; $P = .02$), *S pneumoniae* (OR, 4.2 [95% CI, 1.4-13]; $P = .01$), or *H influenzae* (OR, 4.2 [95% CI, 1.1-16]; $P = .04$) had the greatest risk of exacerbations (Fig 2, *D-F*).

DISCUSSION

In this study we tested 2 hypotheses to determine the role of bacterial pathogens in rhinovirus-induced respiratory symptoms and exacerbations of asthma. First, we predicted that detection of bacterial pathogens would be increased in children with asthma compared with their nonasthmatic counterparts; however, this was not the case. We also hypothesized that common bacterial pathogens contribute to rhinovirus-induced illness severity, and thus these bacteria would be more prevalent during rhinovirus-induced illnesses than during asymptomatic rhinovirus infections. By prospectively monitoring viruses and bacteria at weekly intervals, we found several significant relationships between rhinovirus infections and detection of common bacterial pathogens. Rhinovirus infection was associated with a greater probability of detecting bacteria within concurrent and subsequent nasal samples and also an increase in the quantity of these bacteria. We then used 2 analytic strategies (time-based and illness-based strategies) to compare pathogen detection to respiratory symptoms, and the results provide evidence that specific bacteria and rhinovirus are both associated with respiratory and asthma symptoms. In the time-based analysis *S pneumoniae* was associated with moderate asthma exacerbations when detected either alone or together with rhinovirus, although our illness-based analysis demonstrates that illnesses associated with rhinovirus and *S pneumoniae*, *M catarrhalis*, or *H influenzae* are associated with an increased risk of moderate exacerbations compared with illnesses without pathogen detection. These findings suggest that during rhinovirus infection, these specific bacterial pathogens are cofactors that contribute to the severity of respiratory symptoms, including rhinovirus-induced exacerbations of asthma.

Regardless of the analytic approach, the detection of *H influenzae* alone was generally not associated with greater symptoms. A previous study of 33 subjects using culture-based methods reported an association between *H influenzae* in bronchoalveolar lavage samples and persistent wheezing in preschool children.[16] The differences in study results might be

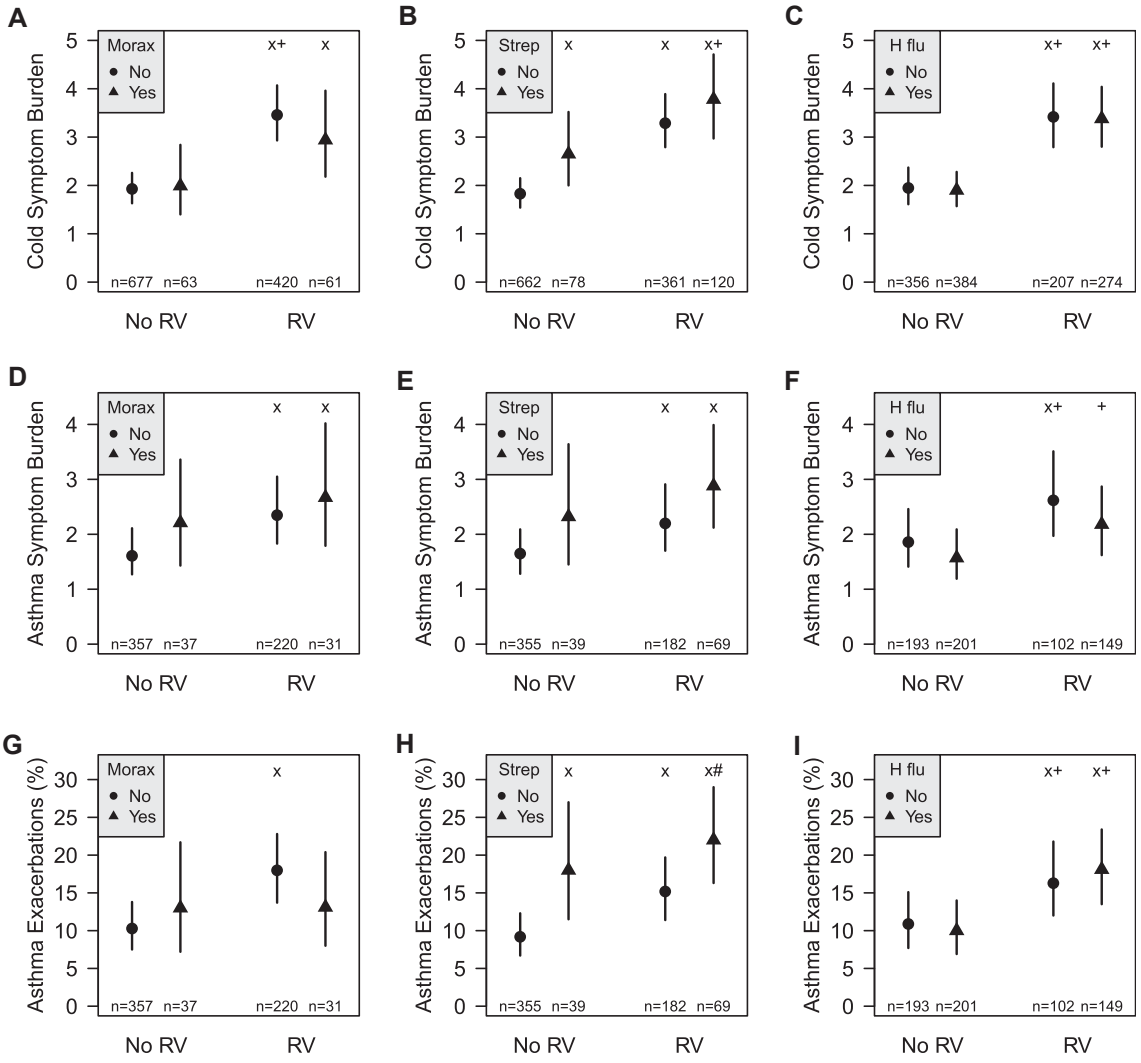

FIG 1. Weekly pathogen detection compared with concurrent weekly respiratory symptoms. Weekly specimens of nasal secretions were analyzed for rhinovirus *(RV)* and bacteria, and pathogen detection was compared with weekly measures of respiratory symptoms, including cold symptoms **(A-C)**, asthma symptoms **(D-F)**, and moderate asthma exacerbations **(G-I)**. x$P < .05$ versus no pathogen, +$P < .05$ versus bacteria only, and #$P < .05$ versus rhinovirus only.

due to the differences in ages of the study populations, or it could be that *H influenzae* can contribute to respiratory symptoms in young children but that this process is not related to rhinovirus infection. Another possibility is that only some *H influenzae* strains cause symptoms and that our PCR-based methods could have detected a broader range of nonculturable strains of *H influenzae* that are not associated with disease.

To our knowledge, this study was the first to prospectively follow both viral and bacterial infections in school-age children with and without asthma. Bacterial pathogens were detected at higher rates than in previous studies, possibly because of our use of molecular techniques rather than culture-based methods.[1,2] Alternatively, because season can affect the detection of *S pneumoniae*[17,18] and *M catarrhalis*,[19] the high rates of detection in our study might be related to sampling in the fall.

Bacteria were most likely to be detected when rhinovirus was present or in the week after rhinovirus infection. This relationship is in accordance with viral infections preceding bacterial infections in the middle ears,[20] sinuses,[21] and lungs.[22] Viral infections promote bacterial infections through several mechanisms, including induction of receptors used by bacteria to invade cells[23] and disruption of epithelial barrier function.[24,25] It is likely that rhinovirus uses some or all of these mechanisms to promote susceptibility to bacterial invasion.[26] In addition, microbial components can activate Toll-like receptors, prompting epithelial cells and leukocytes to release inflammatory cytokines and mediators. Therefore it is possible that children infected with both rhinovirus and bacteria experience greater airway inflammation that in turn contributes to symptoms and asthma exacerbations.

This study has a number of unique advantages and some limitations that should be considered in interpreting these data. The prospective study design allowed us to monitor children for rates of rhinovirus infection, bacterial detection, and associated cold and asthma symptoms. For detection of rhinovirus and bacteria, we used sensitive PCR-based diagnostics.[9] Also, we used a simple sample collection and scoring system to encourage adherence. The scoring system has proved informative in

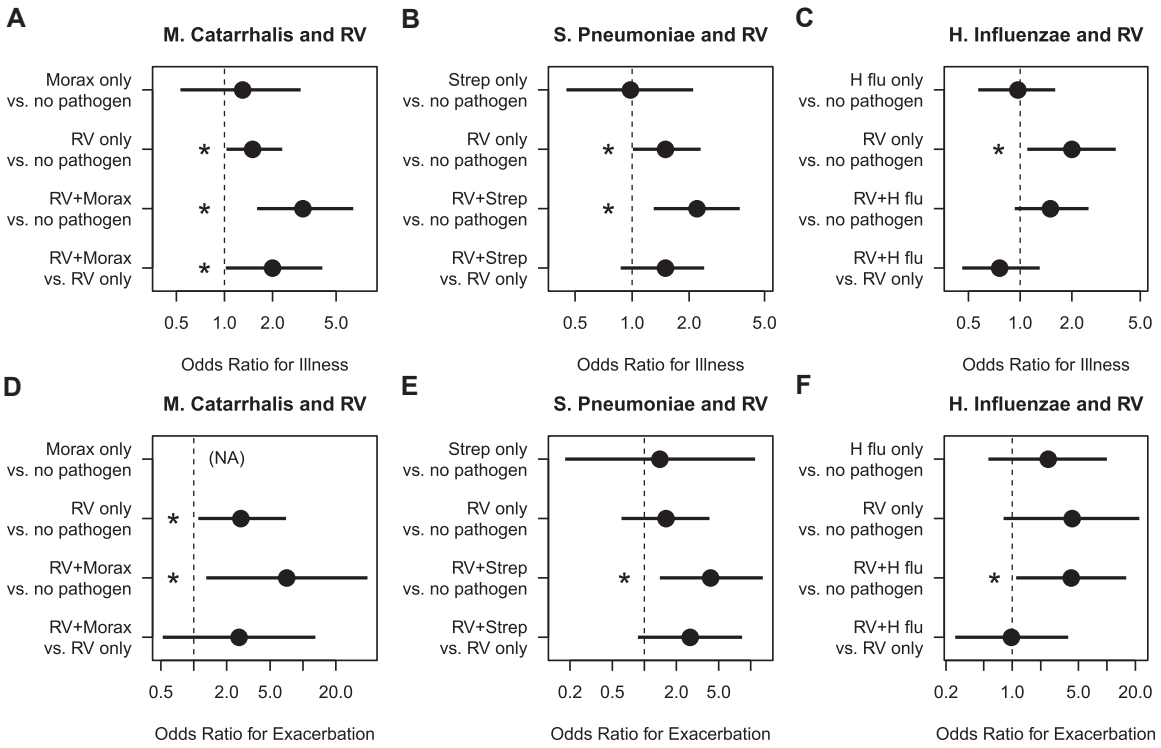

FIG 2. A-C, Probability of experiencing illness symptoms according to patterns of pathogen detection. Each illness was matched with an asymptomatic period of the same length, and the probability of pathogen detection was compared. **D-F,** Within the asthma group, the probability of experiencing an asthma exacerbation with pathogen detection was also compared. *$P < .05$.

previous studies[6,8] but has not been validated. Other common cold instruments are available (eg, Jackson score or Wisconsin Upper Respiratory Symptom Survey 21), but none have been formally validated in children. Asthma is a disease of the lower airways, and our conclusions are based on samples obtained from the upper airway because of the age of the subjects and the need for serial sampling. Nevertheless, previous studies have shown correlations between upper and lower airway microbiology[27] and virology.[28] The nasal blow technique that was used could have underestimated rates of bacterial detection during periods of health; however, in preliminary studies nasal blow samples and midturbinate swabs yielded similar rates of bacterial detection (data not shown). Finally, these studies were conducted in suburban children during the month of September (2007, 2008, or 2009), and additional studies are needed to confirm our findings in other populations and at other times of the year.

Our findings suggest the possibility that prevention or treatment of *S pneumoniae* and *M catarrhalis* could reduce the frequency and severity of respiratory illnesses and, importantly, exacerbations of asthma. Antibiotic treatment is available for these pathogens, but the benefits of this approach must be weighed against potential risks, such as antimicrobial resistance, killing of beneficial microbes, side effects, and cost. Uncomplicated colds are relatively minor and self-limited illnesses, and even if bacteria contribute to symptoms, antibiotic treatment is not warranted. Exacerbations entail greater morbidity; however, our data suggest that 8 courses of antibiotics during colds would be required to prevent 1 asthma exacerbation. It is possible that a subset of asthmatic patients would benefit from antibiotics, and further studies are needed to investigate this possibility. Other methods to prevent *S pneumoniae* and *M catarrhalis* infections, such as vaccination and probiotics, are in use or development and could have efficacy for colds and exacerbations of asthma.

In summary, our findings demonstrate that during the peak fall season, rhinovirus infection increases the likelihood of detecting bacteria within the upper airway, and when detected along with *S pneumoniae* or *M catarrhalis*, children are at increased risk of experiencing illness symptoms and moderate asthma exacerbations. This association might help to explain why rhinovirus infections are associated with such a broad range of clinical illnesses. The implications of these findings are that solitary rhinovirus infections produce mild or asymptomatic infections, whereas rhinovirus infections that are complicated by secondary infection with (or expansion of) these airway bacteria cause more severe illnesses. If these findings are confirmed, understanding the mechanisms of rhinovirus/bacterial interactions could lead to new therapeutic approaches to respiratory illnesses and asthma.

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Clinical implications: In children codetection of rhinovirus with *S pneumoniae* or *M catarrhalis* leads to an increased risk of experiencing illness symptoms and moderate asthma exacerbations.

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METHODS

Recruitment and inclusion/exclusion criteria

The study population was recruited from the general population in Madison, Wisconsin, and surrounding areas through primary care physicians, allergy and asthma specialists, and advertisements in the community. The study was designed to be as inclusive as possible to reflect the general population. Any child with or without asthma aged 4 to 12 years was considered eligible for the study provided they did not have a history of prematurity, complications at birth, respiratory problems at birth, or any other significant medical illness. In addition, they could not be enrolled in another respiratory study.

The subject's reported asthma status was reported by the parent on enrollment. Then, in the main RhinoGen study we followed the subject's asthma symptoms and treatment over 1 year to confirm asthma status. Current asthma was diagnosed at the end of the study period based on the documented presence of 1 or more of the following characteristics in the previous year: (1) use of albuterol for coughing or wheezing episodes (prescribed by physician); (2) use of a daily controller medication; (3) step-up plan, including use of albuterol or short-term use of inhaled corticosteroids during illness; (4) use of prednisone for asthma exacerbation; and (5) reversibility of pulmonary function tests after administration of a short-acting β-agonist. Two separate investigators who were blind to any antecedent histories concerning viral illnesses or patterns of aeroallergen sensitization independently evaluated each subject for the presence or absence of asthma based on the above criteria.

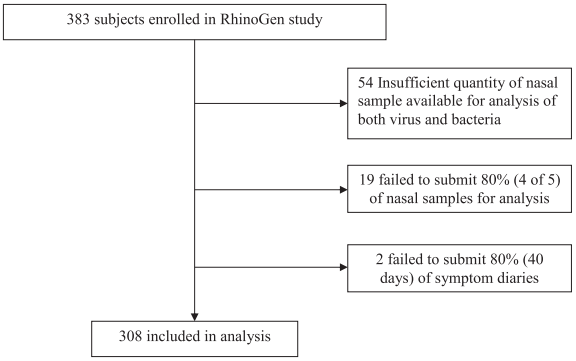

FIG E1. Subjects included in analysis.

TABLE E1. Definition of cold and asthma scores

		Cold symptoms	Asthma symptoms
0	Absent	None	None
1	Mild	Mild stuffy or runny nose but does not affect daily activity	Occasional cough or wheeze but does not affect daily activity
2	Moderate	Moderate stuffy or runny nose and reduced activity but does not affect sleep	Frequent cough or wheeze with some shortness of breath and reduced activity but not affecting sleep
3	Severe	Cannot breathe through nose and not able to sleep well because of symptoms	Unable to sleep well because of symptoms