Human Rhinovirus Species and Season of Infection Determine Illness Severity

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Rationale: **Human rhinoviruses (HRVs) consist of approximately 160 types that cause a wide range of clinical outcomes, including asymptomatic infections, common colds, and severe lower respiratory illnesses.**

Objectives: **To identify factors that influence the severity of HRV illnesses.**

Methods: **HRV species and types were determined in 1,445 nasal lavages that were prospectively collected from 209 infants participating in a birth cohort who had at least one HRV infection. Questionnaires were used during each illness to identify moderate to severe illnesses (MSI).**

Measurements and Main Results: **Altogether, 670 HRV infections were identified, and 519 of them were solitary infections (only one HRV type). These 519 viruses belonged to 93 different types of three species: 49 A, 9 B, and 35 C types. HRV-A (odds ratio, 8.2) and HRV-C (odds ratio, 7.6) were more likely to cause MSI compared with HRV-B. In addition, HRV infections were 5- to 10-fold more likely to cause MSI in the winter months ($P < 0.0001$) compared with summer, in contrast to peak seasonal prevalence in spring and fall. When significant differences in host susceptibility to MSI ($P = 0.004$) were considered, strain-specific rates of HRV MSI ranged from less than 1% to more than 20%.**

Conclusions: **Factors related to HRV species and type, season, and host susceptibility determine the risk of more severe HRV illness in infancy. These findings suggest that anti-HRV strategies should focus on HRV-A and -C species and identify the need for additional studies to determine mechanisms for seasonal increases of HRV severity, independent of viral prevalence, in cold weather months.**

Keywords: rhinovirus; severe illness; species; type; seasonality

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Why human rhinovirus (HRV) infections cause mild illness in some children and more severe illness in others is not understood.

What This Study Adds to the Field

We present definitive data to show HRV-A and HRV-C cause more severe respiratory illnesses in infants. Given the genetic diversity of HRVs, this provides an important advantage in the design of therapies that can now be more narrowly focused on HRV-A and HRV-C viruses. In addition, we report that season of infection has a major effect on HRV virulence. Although peak prevalence occurs in the spring and fall, virulence is greatest in the winter. These advances in understanding the pathogenesis and risk factors for more severe disease of HRV infections should lead to new strategies for prevention and treatment.

Human rhinoviruses (HRVs) are the most prevalent human respiratory viruses. Annually, they infect billions of people and are responsible for at least one-half of all acute upper respiratory illnesses (common colds), the most common illness of humans (1–3). In addition to common colds, infections with HRV result in a wide range of other clinical outcomes ranging from asymptomatic infection to severe lower respiratory illnesses, such as bronchiolitis, pneumonia, and exacerbations of asthma (1, 4–7). It is likely that host, viral, and environmental factors contribute to the severity of illness caused by HRV infection. Indeed, more severe HRV infections are associated with phenotypic characteristics such as extremes in age; chronic respiratory diseases such as asthma; reduced interferon responses in the blood and airway; and, in early life, male sex and reduced lung function (4, 8). Little is known about viral and environmental determinants of illness severity.

HRVs are a large group of genetically diverse RNA viruses and are classified phylogenetically into three species (A, B, and C). The 100 classical serotypes are found within species A and B, and approximately 50 newly identified types are HRV-Cs (5, 9–15). This tremendous genetic diversity represents a major obstacle toward developing both antivirals and vaccines for HRV, and identification of HRV species or types with greater virulence could help to focus these efforts.

The role of environmental and lifestyle determinants of HRV illness severity is also of great interest. Factors associated with more severe HRV respiratory symptoms include smoking, attendance at day care, and, in individuals with respiratory allergies, exposure to relevant allergens (4). There is some evidence that vitamin D status may also affect the frequency and severity of common colds (16), although data on HRV infections *per se* are lacking. The time-honored axiom that being chilled increases the susceptibility to or severity of colds is not supported by experimental data (17).

To test the hypothesis that HRV species and types differentially contribute to illness severity, we analyzed 1,445 samples collected from 209 infants during scheduled and sick visits in

(*Received in original form February 26, 2012; accepted in final form August 17, 2012*)

Funded by National Institutes of Health (National Heart, Lung, and Blood Institute) grant P01 HL070831 and (National Center for Advancing Translational Sciences) grant UL1TR000427.

Author Contributions: W.-M.L. directed and performed the virologic analyses and wrote the manuscript; R.F.L. is the principal investigator of the birth cohort study and contributed to study design and interpretation; M.D.E. and R.G. performed the statistical analysis; F.V. and T.P. performed the viral diagnostics; D.J.J. contributed to study design and interpretation; J.E.G. directed all aspects of the project. All authors contributed to editing the manuscript.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 186, Iss. 9, pp 886–891, Nov 1, 2012

Originally Published in Press as DOI: 10.1164/rccm.201202-0330OC on August 23, 2012
Internet address: www.atsjournals.org

the first year of life. These infants were enrolled in the COAST (Childhood Origins of ASThma) cohort study (18) and are at increased risk for developing allergies and asthma based on parental histories. Sensitive and specific molecular assays (14, 19) were used to identify the HRV infections that were caused by only one HRV type (solitary infection), and then HRV species and type were compared with illness severity. In addition, we tested whether the month of the year in which the illness occurred could additionally influence illness severity. Preliminary findings from this study were previously published in abstract form (20).

METHODS

Study Subjects and Study Design

The Human Subjects Committee of the School of Medicine and Public Health, University of Wisconsin-Madison approved this study. Written informed consent was obtained from the parents. Of the 289 subjects enrolled in the COAST Study at birth, 285 were followed prospectively for 12 months. Eligibility criteria included having one or both parents with allergic sensitization (one or more positive aeroallergen skin tests) and/or asthma, and birth at 37 weeks' gestation or more (18, 21–24). Enrollment occurred during the prenatal period and commenced only after obtaining informed consent from the parents. All specimens were collected between November 1998 and May 2001.

Families were asked to contact the study center each time the infant had respiratory symptoms. Arrangements were made with the family for study personnel to perform a nasal wash at home or in the clinic. In addition, a nasal wash was performed at each of five scheduled visits (2, 4, 6, 9, and 12 mo) whether the infant was sick or well. Nasal washes were tested for solitary HRV infection as described below. Altogether, 209 infants (72.3%) had at least one solitary HRV infection during the first year of life and are included in this analysis.

Definitions of Illnesses

Symptom score was assigned to each visit according to a predefined respiratory symptom scorecard (21). Illness was considered mild if the score was 1 to 4 and moderate to severe (MSI) if a clinical symptom score was 5 or greater. Viral detection in the absence of symptoms (score = 0) was considered asymptomatic infection.

HRV Molecular Detection and Typing

All nasal mucus samples were screened by respiratory multicode assay (19) for infection of 20 different common respiratory viruses: HRVs, enteroviruses, adenoviruses (B, C, and E), influenza (A, B), parainfluenza (1–3, 4a, 4b), coronaviruses (SARS, OC43, 229E, and NL63), RSV (A, B), metapneumovirus, and bocavirus. Typing of HRV-positive samples was performed as described (14) or by direct sequencing of polymerase chain reaction fragments of 5′ noncoding region. An isolate is assigned as a classical serotype (prefixed with R) or a new type (W) by phylogenetic tree analysis. Briefly, if the new sequence clusters with the sequence of one of the 101 classical serotypes, it was assigned as that serotype, and if the new sequence has 9% pairwise nucleotide divergence from the nearest serotype or type, it was designated as a new type (*see* online supplement for additional data). To confirm the species assignment of the new types, representative samples of each type were sequenced at the VP4-VP2 coding region (420-nt) (25). All new sequences described in this report are deposited in the GenBank (accession numbers JX041186-JX041253).

Statistical Analysis

Logistic regression models were used to estimate the proportion of HRV-induced MSIs detected at scheduled visits as a function of month and age. To account for differential sampling of asymptomatic/mild infections and MSIs, observed asymptomatic/mild infections were weighted by the inverse probability of detection of MSI at scheduled visits (8.5) for analysis. Because a total of 214 HRV MSIs were detected and 25 of them occurred at scheduled visits, the probability of detecting MSI at scheduled visits is calculated to be 11.7%. Assuming that the detection probability of asymptomatic/mild infections is similar to that of MSI at scheduled visits, each asymptomatic/mild infection observed at a scheduled visit is representative of a total of 8.5 (= 100/11.7) asymptomatic/mild infections throughout the year. Mixed effects logistic regression models were constructed in terms of month and HRV group (A/B/C) as fixed effects, subject and type as random effects, and the log-transformed probability of detection as an offset term. Best linear unbiased estimators of the type effects, subject effects, and their corresponding standard errors were used to construct 95% empirical Bayes credible intervals for the type (virulence) and subject (susceptibility) effects.

RESULTS

Infections and Illnesses

Overall, 209 infants had 1,445 nasal wash specimens in their first year of life (Table 1). Of these specimens, 958 were from scheduled study visits and 487 were obtained during unscheduled sick visits. Of the scheduled visits, 707 involved healthy infants (well visit, symptom score = 0), 223 involved mild respiratory illnesses (symptom score 1–4), and 28 involved MSIs. All of the unscheduled samples (n = 487) were obtained during MSI. Virus was identified in 962 of the 1,445 samples (66.6%), and the frequency of viral detection increased with illness severity (Figure 1; no illness, 44.6% [315 of 707]; mild illness, 78.5% [175 of 223]; MSI, 91.7% [472 of 515]). All common respiratory viruses were detected (HRV, RSV A and B; coronaviruses NL63 and OC43; parainfluenza 1, 2, 3, 4a, and 4b; human metapneumovirus; influenza A and B; adenoviruses B, C, and E; enterovirus; and bocavirus), and HRV was most common (696 [72.3%] of virus-positive samples). More than one nasal mucus specimen was collected during some illnesses: 223 specimens obtained during mild illnesses were linked to 205 discrete illnesses, and 515 specimens were linked to 488 MSIs (Table 1).

HRV was detected in 696 of 1,445 samples (48.2%), and typing was successful in 98.7% of these samples. After accounting for repeat samples during the same infection (n = 26), coinfection with viruses other than HRV (n = 123), coinfections with two different HRV types (n = 21), and negative typing results (n = 7), there were 519 infections with single HRV types.

Seasonality of HRV Infection and Illness

After statistically weighting to account for missed asymptomatic/mild infections not seen at scheduled visits, there were 12.2 HRV infections per infant-year (95% confidence interval [CI]

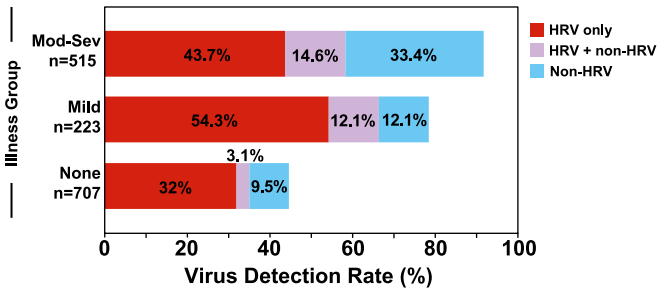

Figure 1. Prevalence of human rhinovirus (HRV) infection according to severity of illness. A total of 1,445 samples of nasal mucus collected from 209 infants in their first year of life between March 1999 and May 2001 were tested for common respiratory viruses. Viruses in the non-HRV category included respiratory syncytial virus A and B, coronaviruses NL63 and OC43, parainfluenza viruses 1 to 4, human metapneumovirus, influenza A and B, adenovirus B and C, enteroviruses, and bocavirus.

TABLE 1. NASAL MUCUS SPECIMENS AND TOTAL ILLNESSES

Illness Category	Specimens According to Visit Type			Total Illnesses*
	Scheduled	Unscheduled	Total	
Well	707	0	707	—
Mild	223	0	223	205
MSI	28	487	515	488
Total	958	487	1445	693

MSI = moderate to severe illness.
* Some illnesses (n = 45) had more than one associated nasal mucus specimen.

8.7–18.9), with 49% asymptomatic, 46% mild illnesses, and 5% MSI. HRV infections occurred year-round and were greatest in the spring and fall (Figure 2A). Rates of HRV infection were approximately threefold higher during peak prevalence months in March, September, and October (4.6, 4.3, 4.3 infections per 100 child-days, respectively), compared with January and July (1.3, 2.0 infections per 100 child-days).

We tested whether the month of infection influenced the probability that an HRV infection would produce MSI among the 519 infections caused by a single virus. The severity of HRV illnesses had a clear seasonal pattern ($P < 0.0001$), and infections were most likely to cause MSI during December to February (12–23%) and least likely to cause MSI during April to August (2–3%, Figure 2B).

Virulence of HRV Species and Types

The HRV isolates in the 519 solitary infections included 257 HRV-A viruses, 37 HRV-B, and 225 HRV-C. Both HRV-A (odds ratio [OR], 8.2; 95% confidence interval [CI], 2.7–25) and HRV-C (OR, 7.6; 95% CI, 2.6–23) were significantly more likely than HRV-B to induce MSI (Figure 3).

The 519 solitary HRV isolates belonged to 93 types: 49 HRV-A, 9 HRV-B, and 35 HRV-C types. Eighteen types were found only once. The remaining 75 types were identified two or more times, and 19 types were detected ten or more times (R19, R28, R52, R56, R78, R88, R89, W01, W05, W06, W11, W12, W23, W24, W26, W28, W29, W32, and W38; *see* online supplement for details in type assignment). The 93 types segregated into two groups according to their probability of inducing MSI ("virulence"). The nine HRV-B types clustered together in the low-virulence group, and HRV-B-R52 had the lowest virulence (0.5%; 95% CI, 0.3–0.8%). The 84 HRV-C and HRV-A types were more virulent; in this cluster, the probability of MSI ranged from 3.1% (HRV-C-W36; 95% CI, 2.1–4.4%) to 11.7% (HRV-C-W12; 95% CI, 8.7–15.5%).

Of the 214 HRV-induced MSIs, 41 (19%) were associated with wheezing. Although the number of wheezing illnesses was too low to be modeled, there were numerically fewer wheezing illnesses caused by HRV-B (n = 0) compared with either HRV-A (n = 27) or HRV-C (n = 14). There were 186 acute care visits for HRV illnesses, including 111 HRV-A, 4 HRV-B, and 71 HRV-C. Only two HRV illnesses required hospitalization, and both of these were due to HRV-A infections.

Individual Susceptibility

Host susceptibility to HRV-induced MSI also varied significantly ($P = 0.004$). When significant differences in host susceptibility to MSI ($P = 0.004$) were considered, strain-specific rates of HRV MSI ranged from less than 1% to more than 20% (Figure 4). The mean risk of MSI per infection was approximately 2% for children in the least susceptible tertile, 5% for average children, and about 10% for more susceptible children. Given the estimated frequency of 12.2 infections per child per year, the corresponding risks of at least one HRV MSI per year were 22%, 47%, and 72%, respectively. The effect of virus type on MSI risk was most pronounced for more susceptible infants; however, HRV-B types were unlikely to cause MSI regardless of individual susceptibility (Figure 4).

DISCUSSION

The advent of sensitive molecular techniques for viral diagnosis has led to an increased appreciation of the strikingly broad range of clinical illness caused by HRVs. Epidemiologic studies routinely report high rates of HRV detection in children with common colds, wheezing illnesses, and pneumonia, and even in the absence of symptoms. In this study of infants participating in a birth cohort study who were prospectively monitored for evidence of infection and illness, the results provide definitive evidence that HRV species and type impact the severity of respiratory illness. HRV species A and C were each about seven times more likely than HRV-B to be associated with MSI. In fact, the nine individual HRV-B types clustered together into a low-virulence group and were unlikely to cause MSI even in more susceptible children. Collectively, these findings strongly suggest that antiviral strategies aimed at reducing HRV-related morbidity in high-risk infants should focus on HRV-A and HRV-C species viruses. Another novel finding was that the peak prevalence and severity of HRV infections did not correspond; HRV infections were most frequent in the spring and fall but were more likely to cause severe illnesses in winter (December to February). Identifying factors related to season and host that promote

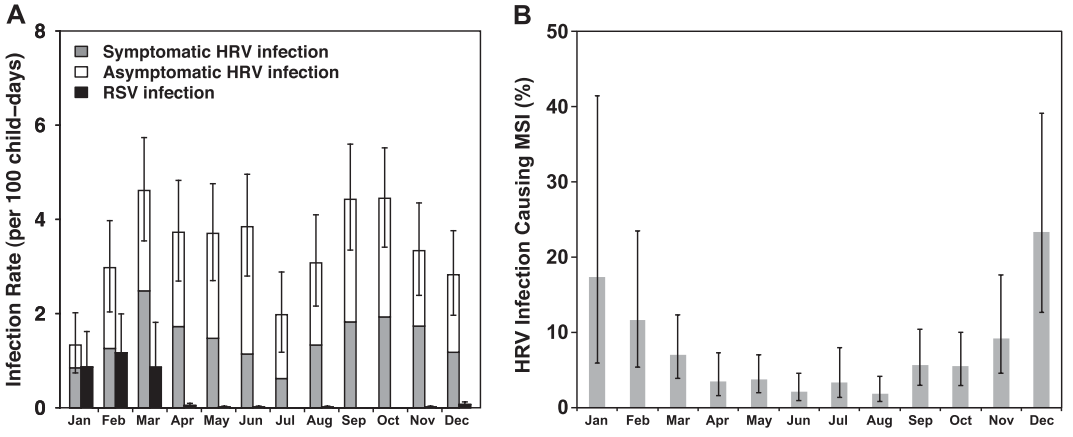

Figure 2. Epidemiology of human rhinovirus (HRV) infections and illnesses. HRV infections were identified in 670 of 1,445 samples, and infection rates (per 100 child-days) were plotted according to the month of sampling (*A*). The seasonality of respiratory syncytial virus infections in the study is included for contrast. To examine the relationship between season and severity of illness, the percent of solitary HRV infections (n = 519) that caused moderate to severe illness (MSI) was estimated using mixed effects logistic regression models (*B*). Month of infection was strongly associated with the risk of MSI ($P < 0.0001$). Error bars represent 95% confidence intervals.

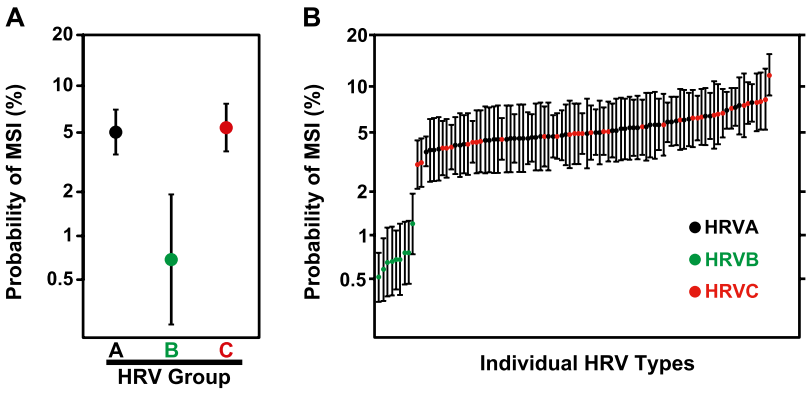

Figure 3. Differential virulence of human rhinovirus (HRV) species and types. The species and types of the 519 solitary HRV infections were determined by partial genome sequencing. The probability of inducing moderate to severe illness (MSI) by each species (*A*) and type (*B*) was estimated using mixed effects logistic regression models. Error bars represent 95% confidence intervals.

more severe HRV illnesses is an important goal, especially in the absence of specific therapies.

The recent discovery of the HRV-C species (12–15) prompted renewed interest in studying the etiology and epidemiology of HRV infections. Since then, multiple studies have compared the virulence of HRV-C relative to the classical HRV-A and -B species (13, 26–36). In several of these studies, HRV-C viruses appear to be overrepresented compared with HRV-A and -B in infants and children with lower respiratory infections and in exacerbations of childhood asthma. These studies focused on analysis of samples from ill (usually hospitalized) children and were limited by the lack or paucity of concurrent sampling of asymptomatic or mild illnesses. In this study, we prospectively obtained samples from infants with clinically significant respiratory symptoms and also from those with mild or no symptoms. Our model indicates that approximately one-half of the HRV infections were asymptomatic (Figure 2A), which underscores the importance of including routine sampling in determining patterns of HRV infection and illness. In addition, both HRV-A and HRV-C viruses were more virulent than HRV-B viruses in infants. This finding is in agreement with a recent case-control study of hospitalized children (37).

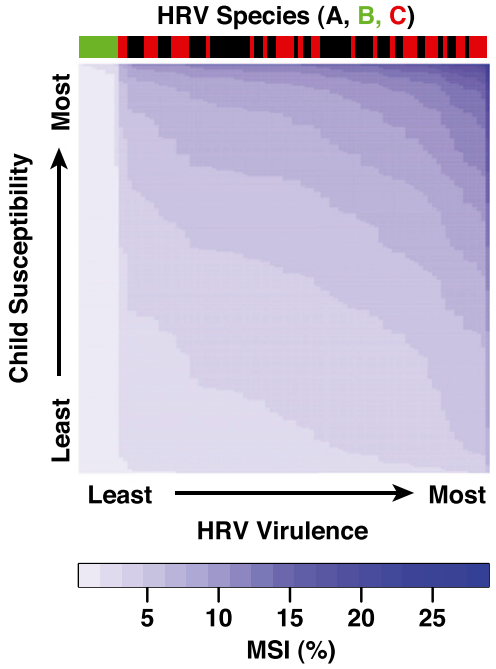

Figure 4. Risk of human rhinovirus (HRV) moderate to severe illness (MSI) depends on both susceptibility and virus type. Using mixed effects logistic regression models, we estimated the virulence (propensity to cause MSI) of the 93 different HRV types (*x axis*, ordered low to high), and the susceptibility to MSI for 219 study participants (*y axis*, ordered low to high). The graph illustrates the risk of MSI for all combinations of subject susceptibility and HRV type. The species of each type is indicated on the upper color bar (A = black; B = green; C = red).

The finding that HRV-B types seldom cause more severe illness has implications for antiviral drug development. For example, the A and B species designations roughly correspond to patterns of sensitivity of HRV to capsid-binding drugs, such as pleconaril or WIN compounds (38). These data suggest that, at least for infants, capsid-binding agents could be designed to target HRV-A and HRV-C, which is phylogenetically closer to HRV-A than to HRV-B (11, 39). The same principles could also apply to other antiviral agents and to efforts to develop a vaccine for HRV. Once considered extremely unlikely due to the large number of serotypes, there have been reports of antibody responses to shared HRV epitopes with some neutralizing capability (40).

Multiple studies have shown that HRV illnesses have a predominant peak in the fall and a smaller but still distinct peak in the spring and are present at low rates (20–30% of the fall peak) throughout the rest of the year (2, 3, 41). The times of peak prevalence were similar in our study of infants, but in contrast to other studies the infection rate remained relatively high (63–85% of the fall peak) in 7 of the remaining 9 months and substantial (30 and 45%) even in the 2 lowest months. The higher-than-expected prevalence rate outside of fall and spring could be due to the inclusion of asymptomatic HRV infections in our analysis, in contrast to sampling only symptomatic infections in most other studies. However, a similar pattern of seasonality was obtained when we limited the analysis to symptomatic infections only (Figure 2A). Another potential explanation for the higher off-season HRV prevalence is that infants are more susceptible to HRV infection than older children and adults, which implies that young children could serve as an important reservoir for HRV during periods when prevalence is low in other age groups.

Despite the relatively low prevalence of HRV infections during the winter, infections at this time of year were more likely to cause MSIs. This finding raises interesting questions about underlying mechanisms for the observed seasonality of illness severity. The summer nadir and winter peak suggest the possibility that vitamin D reduces the severity of HRV illness, and there are epidemiologic studies that support this theory (16). Alternatively, the prevalence of many other respiratory pathogens peaks in the winter, and it is possible that recent infection with other respiratory viruses or interactions with colonizing bacteria enhance the severity of subsequent HRV illness. Additional studies are warranted to investigate these and other possibilities.

This study has a number of strengths and some limitations to consider in interpreting these data. Up to 20 HRV types circulate simultaneously in a community (2, 42), and the spectrum of types changes from season to season (3, 42, 43). To define the virulence of individual types, it is therefore advantageous to analyze a large number of samples collected over relatively few seasons. In this study, 1,445 samples were collected in 2.3 years, yielding 519 solitary HRV infections. State of the art viral diagnostics were used; typing of the HRVs detected in this study was successful in a very high percentage (98.7%) of samples, which provided an unbiased data set to test for relationships between species, type, and illness outcomes. The study included young infants with little previous exposure to other HRVs, which minimized the effects of adaptive immunity to prior infections. It should be considered that clinical responses to HRV infections could be age dependent, and whether these findings hold true for other age groups remains to be determined. Finally, our findings do not distinguish whether species-specific differences in illness severity are related to distinct patterns of viral replication or inflammatory responses.

In conclusion, the development of antiviral drugs for the treatment of HRV infections is an important and unmet medical need that is especially important for high-risk patients, including infants. The great genetic diversity of HRV has presented a major challenge to these efforts, and our findings suggest that narrowing the focus of anti-HRV medications or vaccines to target A and C species viruses is a viable strategy. Finally, understanding the individual and seasonal factors that contribute to more severe illnesses could lead to the development of new therapeutic approaches to reduce the overall burden of respiratory illness in infancy.

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

Acknowledgments: The authors thank the clinical coordinators for their efforts in patient recruitment, retention, and the procurement of all of the biologic specimens used in these analyses. They also thank the Wisconsin State Lab of Hygiene for support, the many health care professionals within the surrounding communities for their cooperation, and the COAST families for their enthusiastic participation. They also thank Mr. Robert Gordon (Department of Pediatrics, University of Wisconsin-Madison) for graphic illustrations.

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