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Background: Human rhinoviruses (HRVs) cause common colds, and the recently discovered HRV-C is increasingly associated with lower respiratory illness among populations such as children and asthmatic patients.

Objective: To determine how HRV-C is associated with respiratory illness and to evaluate changes in prevalence and species over 2 decades.

Methods: A prospective study of children younger than 5 years was performed at the Vanderbilt Vaccine Clinic over a 21-year period. Nasal-wash specimens from children presenting with upper or lower respiratory illness at acute care visits were tested for HRV and HRV-positives genotyped. Demographic and clinical features were compared between children with or without HRV, and with different HRV species.

Results: HRV was detected in 190 of 527 (36%) specimens from a population of 2009 children from 1982 through 2003. Of these, 36% were HRV-C. Age (P = .039) and month of illness (P < .001) were associated with HRV infection and HRV species. HRV-C was significantly associated with lower respiratory illness, compared with HRV-A (P = .014). HRV-A and HRV-C prevalence fluctuated throughout the 21-year period; HRV-C was more prevalent during winter (P = .058).

Conclusions: HRV-C is not a new virus but has been significantly associated with childhood lower respiratory illness in this population for several decades. Temporal changes in virus prevalence occur, and season may predict virus species. Our findings have implications for diagnostic, preventive, and treatment strategies due to the variation in disease season and severity based on species of HRV infection. (J Allergy Clin Immunol 2013;131:69-77.)

Key words: Rhinovirus, HRV-C, children, season, lower respiratory illness

Human rhinovirus (HRV), a small nonenveloped RNA virus in the Picornaviridae family, is the predominant cause of the common cold. Since its discovery in 1956, approximately 150 serotypes have been identified.1-3 Although the common cold is often clinically benign, HRV infections can be severe in certain populations such as infants, children with wheezing or asthma, and older adults. HRV is associated with both upper respiratory illness (URI) and lower respiratory illness (LRI), including pneumonia,4 bronchiolitis,5 and asthma.6 HRV is associated with the hospitalization of 5 of 1000 US children younger than 5 years and 18 of 1000 US children younger than 6 months.7 HRV-associated wheezing during infancy predicts the development of subsequent childhood asthma.6,8 HRV infection also triggers many wheezing and asthma exacerbations.7,9 Especially in the spring and fall seasons, HRV-associated asthma exacerbations10 cause missed school, hospitalizations, economic loss, and suffering in those affected.

The recently discovered HRV-C species is genetically distinct from the classic species HRV-A and HRV-B.11-13 Multiple HRV strains and species may circulate concurrently even within small populations.14 Studies have suggested that HRV-C and HRV-A may exhibit different seasonality patterns15-17 and that HRV-C may be associated with increased disease severity and asthma risk.18-22 However, most of these studies were limited to 1 or 2 seasons, and thus did not account for random variation by year, which may influence a given seasonal trend. In the current study, we examined 21 years of data and specimens that were collected prospectively year-round from children with acute respiratory illness, allowing us to measure the impact and seasonal trends of HRV species over a prolonged period.

METHODS

Study design

Nasal-wash specimens were prospectively collected from 1982 through 2003 at the Vanderbilt Vaccine Clinic (VVC) in Nashville, Tennessee.21-27 The VVC was originally established to create a cohort of children for the evaluation of new vaccines, as well as determine the etiology of acute respiratory disease in early childhood in otherwise healthy young children. Thus, children with comorbid conditions other than mild asthma were excluded. Healthy,
full-term infants were enrolled at birth and followed up to age 5 years. The VVC was the primary care provider for these children, and children were evaluated in the VVC for all well and acute care visits. All visits were conducted within the General Clinical Research Center, with care provided by the pediatric infectious diseases faculty and research nurse practitioners. Comprehensive care was offered to these children with doctors on call 24 hours a day; this increased the likelihood of capturing all illnesses in these children. Questions involving whether the child had atopy and/or food allergies were not specifically asked by providers, and so children with those conditions were not explicitly excluded from the study. Thirteen children were older than 5 years at their last visit and were also included in this study. Conditions were not explicitly excluded from the study. Time between infections ranged between 2 months and 133 months old. During visits for illness, symptoms were recorded and nasal-wash samples were obtained and cultured for viruses. All studies were approved by the Vanderbilt University Medical Center Institutional Review Board. Parents provided written informed consent.

**Statistical analysis**

Poisson regression was used to model the prevalence of HRV over time as a function of age, race (white/black/other), sex, fever (yes/no), and season when the visit occurred. Generalized estimating equations methods were used to account for a small number of repeated measures. A similar model was used to compare the incidence of HRV species A versus C, adjusting for race, sex, and season. Both HRV-B and untypeable HRVs were excluded from analysis because of the small sample size. Month, year, and season were also factored into the analysis. Season was grouped into 4 categories: winter (December through February), spring (March through May), summer (June through August), and fall (September through November). Poisson regression was used to model the prevalence of diagnosis (bronchiolitis, pneumonia, croup, asthma, corzya, pharyngitis, and acute otitis media) separately as a function of HRV. Poisson regression with generalized estimating equations method was used to assess the association between HRV species (A, C) and classification of URI or LRI adjusting for race. A Poisson regression model was used to assess the seasonality of other respiratory viruses (HMPV, PIV, RSV, influenza, and adenovirus). Estimated relative risks with 95% CIs and/or P values are reported.

**RESULTS**

**All HRV infections**

From a population of 2009 children, 527 samples were available (either culture-negative or HRV culture-positive) and tested for HRV and 190 (36%) were positive. Of the HRV-positive samples, 91 (48%) were HRV-A, 5 (3%) HRV-B, 69 (36%) HRV-C, and 25 (13%) untypeable (Table I). Forty-eight of the 190 HRV-positive samples were collected from children diagnosed with LRI (25%) and 142 from those diagnosed with URI (75%). Detection of any HRV in the subpopulation was significantly correlated with the age of the child (P = .039), with younger children more likely to be infected with HRV (Fig 1, A). Any HRV detection marginally correlated with race (P = .054) and season (P = .08; Fig 1, A). Black children had a lower relative risk of being diagnosed with HRV than white (0.73; 95% CI, 0.58-0.93). Month was also a significant predictor of HRV detection (P < .001; Fig 1, B). Samples from children who were sick during the month of September had a relative risk for HRV diagnosis of 2.47 (95% CI, 1.40-4.36) compared with those samples from sick children during July. Sex of the child, any contact with a smoker, whether they attended daycare, and diagnosis of fever did not alter the likelihood of any HRV infection. Sample sizes for partial and total breast-feeding were too low to analyze. Only 11 of 527 subjects partially breast-fed their children (2.1%) and 12 of 527 (2.3%) exclusively breast-fed their children.

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29 months, with an average of 9.4 months (SE, 2.4 months). Of those 16, 9 (56%) were homologous infections (the same species of HRV, both HRV-A, eg). Four of these subjects were infected with HRV-A twice, 4 with HRV-C twice, and 1 had an unknown type of HRV both times. Of those 7 (44%) infections with different HRV species, 5 subjects were infected with HRV-A first, one with HRV-B first and one with HRV-C first. One of the 16 subjects was infected a total of 3 times, all HRV-As, and 1 was infected a total of 4 times, once with HRV-A and 3 times with HRV-C.

**HRV-A compared with HRV-C**

To examine more closely how the species of HRV may affect morbidity in young children, we compared HRV-A with HRV-C,
controlling for race and season. As with the detection of any HRV, we found that the age of the child was a significant predictor of HRV species \((P = 0.005)\), where older children were more likely to be diagnosed with HRV-C (Fig 2, A, shows the modeled negative binomial regression derived from the data). There was also a significant difference between males and females in the likelihood of having HRV-A versus HRV-C detected \((P = 0.02; \text{Fig} \ 2, \ B)\). Female children had a 1.4-fold relative risk of contracting HRV-C compared with male children (95% CI, 1.2-1.1). As expected, the trend was reversed with HRV-A infection, where females had a 0.69-fold (95% CI, 0.48-1.0) relative risk of contracting HRV-A compared with males. Again, season was marginally significant
where children were more likely to be diagnosed with HRV-C in the winter months (Fig 2, A). The relative risk of HRV-C in the winter months was 1.7 (95% CI, 1.0-2.9) compared with the fall months ($P = .058$; Table I).

The probability of HRV-A diagnosis varied significantly over the 21-year course of this study ($P = .03$). Although no particular year dominated, there was a trend toward more HRV-A diagnoses in 1990 as well as in the most recent samples from the years 2000 to 2003. However, inference regarding estimates from recent years is limited because of small sample sizes. Fig 3 represents the negative binomial regression fitted to the data, suggesting that more recently there has been a rise in HRV-A-associated acute respiratory illness in this study population.

### HRV compared with other viruses

Detection of the other study viruses (HMPV, PIV, RSV, influenza, and adenovirus) varied over time (see Figs E2-E6 in this article’s Online Repository available at www.jacionline.org). HMPV varied significantly over both month ($P < .001$) and year ($P = .012$), with highest rates in April and March and lowest in September and November (Fig E2). PIV varied over both year ($P = .02$) and month (highest rate in September, lowest in January and February; Fig E3). RSV significantly varied over month (high in December, low in July and August; $P < .001$; Fig E4), as did influenza (binned months, because of small sample size: high in January/February, low in May through September; $P < .001$; Fig E5). Adenovirus showed significant variability for both year ($P < .001$) and month (high in May, low in September; $P < .001$; Fig E6).

### Association of HRV species with URI or LRI

We next examined whether LRI or URI diagnosis was associated with species of HRV infection. There was a significant difference in the proportion of HRV-A and HRV-C in these grouped diagnoses. HRV-C was significantly more common among children with LRI (60%; relative risk C vs A = 2.152 [1.17-3.97]; $P = .014$; Fig 4). HRV-A was marginally more common among children with a diagnosis of URI (60%; relative risk of C vs A for URI = 0.81 [0.76-1.01]; $P = .069$).

We also examined how HRV and individual diagnosis were related. Because of the small sample size of HRV-positive specimens within each individual diagnosis category (bronchiolitis, croup, pneumonia, asthma, coryza, pharyngitis, or acute otitis media), corresponding tests likely suffered from low power and we were unable to detect significant differences among specific diagnoses, even when grouping all positive HRV species together. The original goals of the VCC were to conduct respiratory surveillance and investigational vaccine trials in otherwise healthy young children, and thus children with chronic conditions including moderate or severe asthma were excluded. Because of this, the sample size for patients with mild asthma was only 19, 10 of which were positive for any HRV. Even with this low number,
we found a 2.86-fold relative risk of asthma diagnosis when patients were infected with HRV, but the precision of that estimate is wide (95% CI, 0.23-35.47). Two of these patients were diagnosed with HRV-A and 6 with HRV-C, and 2 were untypeable.

The HRV sequences were aligned and a phylogenetic tree constructed to determine evolutionary relationships. As expected, 3 distinct groups emerged comprising species HRV-A, HRV-B, and HRV-C (Fig 5). Sequences in this article have been deposited into Genbank under accession numbers JX560565- JX560730. Labeling of individual virus sequences with the clinical diagnosis of URI or LRI illustrated the association of HRV-A with URI (red circles) and HRV-C with LRI (blue squares). Furthermore, we compared the similarity of the sequences to known HRV-A, HRV-B, and HRV-C sequences. HRV-A sequences had on average a similarity of 78.0% (±0.27). HRV-B sequences had an average similarity of 79.7% (±0.42), and HRV-C sequences were 73.9% (±0.16) similar on average. This confirms the discovery of more new strains of HRV and suggests that HRV-C strains exhibited greater genetic diversity within the species compared with either HRV-A or HRV-B.

In our study, HRV was more frequently detected in younger children and infants than in older children, in agreement with other studies. However, we found that when older children were infected with HRV, it was more often HRV-C. Few studies have analyzed differences in HRV species in relationship to the age of the child; however, a study in Thailand detected HRV-A most often in children younger than 1 year and in adults and HRV-C more frequently in children aged 1 to 4 years. If HRV-C is more strongly associated with LRI, it is possible that parents of older children seek medical attention only for these more severe illnesses. Similarly, parents of younger children may take children to the doctor more often, even for less severe symptoms, than do parents of older children. Further studies are necessary to determine why age is associated with differential HRV species infections.

We also found that when examining both age and season HRV-C demonstrated different trends than did HRV overall. One possible explanation is that viral interference alters seasonal peaks and prevalence between HRV-C and other HRV species. It has been suggested that HRV may interfere with several other respiratory viruses, such as adenovirus, influenza virus A, PIV, HMPV, and coronavirus, based on the fact these viruses are significantly less likely to occur when coinfected with HRV. Wisdom et al found that though 10.7% of singly infected patients had RSV, only 2.4% had RSV when coinfected with HRV-C. In one of our previous studies of young hospitalized children, HRV-C codetection was 10% compared with 23% for HRV-A (P = .037). Another explanation may be that HRV-C is more likely to be communicable in winter months. A recent study on guinea pigs suggested that influenza B was more transmissible (both with and without contact) at colder temperatures. Although it is unknown why HRV-C peaks in the winter, recent work from Japan also supports this trend. During the 2008-2009 season, researchers found that HRV-C was most common in December. In our study population because we observed a marginally significant trend over a 20-year period, it is likely that in Nashville the HRV-C burden is higher in the winter months. This suggests that rapid viral detection, particularly in winter months when HRV-C, RSV, and influenza all peak, could modify treatment.

In our study, HRV was associated with a 2.86-fold increased relative risk (95% CI, 0.23-35.47) of asthma diagnosis, and the majority of these patients with asthma were infected with HRV-C. Although this result did not reach statistical significance and has a large data spread, it may be biologically relevant. Because of the experimental design, children with moderate or severe asthma were excluded from the original study, limiting the power to analyze the relationship between asthma and HRV. We had only 10 samples from patients with mild asthma that were positive for HRV; more data are needed to obtain a precise estimate of the true relative risk of asthma among those with different HRV species.

One limitation of the study was that we did not retest specimens that were positive for other viruses for HRV, thus likely underestimating HRV prevalence. In addition, we did not test specimens for bacterial coinfections, which may complicate acute respiratory viral infections. Another limitation was that study viruses other than coronaviruses and HMPV were identified by viral culture or rapid antigen tests, which are less sensitive than RT-PCR used to detect HRV. Thus, we could not directly compare HRV results with those previously obtained for some other viruses. Furthermore, rates of medical visits may have differed over time and among families in Nashville during the 21-year period. Breast-feeding rates were low in the study population, which limited power to detect...
any effect on infection; some studies show that breast-feeding may protect children from respiratory tract infections.44,45 We also did not test healthy children for HRV, which has been shown to be present in asymptomatic individuals.46,47 Strengths of our analysis include the prospective nature of the data and specimen collection, the use of molecular viral diagnostics with cloning and sequencing for HRV typing, and more than 2 decades of year-round recruitment. The demographics of children enrolled in this study were similar to those in the United States,48 and so our results should be largely applicable to the rest of the country. We did have slightly more black children in this study compared with the general makeup of the United States (34% vs 13% black in the United States48). However, race may not be a consistent risk factor for HRV infection. In a previous study from Nashville, Tennessee, HRV was associated more often with black children,17 the opposite of the trend we found.

FIG 5. Phylogenetic tree of HRV species depicting HRV species and URI (red circle) or LRI (blue square) diagnosis. Grouping URI and LRI diagnoses suggests that HRV species is associated with certain clinical phenotypes (or respiratory disease severity). Novel sequences are designated by “VU” followed by the sample number, and sequences matching published HRV strains are marked as HRV followed by the strain number.
In conclusion, HRV-C has circulated for many years, is prevalent in the winter, and is more strongly associated with LRI than is HRV-A. The temporal variation in virus detection we observed over 2 decades confirms that season is strongly linked with the likelihood of HRV infection and that HRV-C peaks during a different season than do other HRV species. These data suggest that HRV species can contribute to the severity of disease and asthma exacerbations in children and that certain HRV species are more common at different times of year. Further studies are required to better understand the pathogenesis of HRV species and their role in LRI and asthma to optimally target future diagnostic, preventive, and treatment strategies for specific HRV species and strains.

We thank Dr Kathryn Edwards and the families who participated in the Vanderbilt Vaccine Clinic. Statistical analysis was conducted by Z.L. and B.R.S.

Clinical implications: Since 1982, HRV-C has been associated with lower respiratory illness, particularly during the winter. Rapid viral detection during winter when HRV-C, RSV, and influenza peak is necessary to modify treatment.

REFERENCES

1. Pelon W, Mogabgab W, Phillips I, Pierce W. A cytopathogenic agent isolated from nasal recruits with mild respiratory illnesses. Proc Soc Exp Biol Med 1975;94:262-7.
2. H ampampian YY. A collaborative report—rhinoviruses, extensions of the numbering system from 89 to 100. Virology 1987;159:191-2.
3. Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T. Proposals for the classification of human rhinoviruses C into genotypically assigned types. J Gen Virol 2010;91:2409-19.
4. Kouider A, Kubo H, Takakura K-I, Togawa M, Shiomi M, Kohdera U, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. J Clin Virol 2009;46:85-9.
5. Z.L. Statistical analysis was conducted by Z.L. and B.R.S.
43. Bezerra PGM, Britto MCA, Correia JB, Duarte MdCMB, Fonseca AM, Rose K, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. Plos One 2011;6:e18928.

44. Miller EK, Bugna J, Libster R, Shepherd BE, Scalzo PL, Acosta PL, et al. Human rhinoviruses in severe respiratory disease in very low birth weight infants. Pediatrics 2012;129:E60-7.

45. Klein MI, Bergel E, Gibbons L, Coviello S, Bauer G, Benitez A, et al. Differential gender response to respiratory infections and to the protective effect of breast milk in preterm infants. Pediatrics 2008;121:E1510-6.

46. Wright PF, Deatly AM, Karron RA, Belshe RB, Shi JR, Gruber WC, et al. Comparison of results of detection of rhinovirus by PCR and viral culture in human nasal wash specimens from subjects with and without clinical symptoms of respiratory illness. J Clin Microbiol 2007;45:2126-9.

47. Sato M, Li H, Bziker MR, Werkhaven JA, Williams JV, Chappell JD, et al. Detection of viruses in human adenoid tissues by use of multiplex PCR. J Clin Microbiol 2009;47:771-3.

48. United States Census Bureau. Summary File 1. United States Census, 2010. Washington, DC. http://2010.census.gov/2010census/data.
FIG E1. Number of samples analyzed for HRV by year. Black bars indicate the number of positive samples.
FIG E2. The probability of HMPV varies significantly over month and year. Lines represent negative binomial regression model based on data.
FIG E3. The probability of parainfluenza virus varies significantly over month and year. January and February were least likely to be associated with positive PIV. Lines represent negative binomial regression model based on data.
The probability of RSV infection varies by month. December, January, and February were more likely to be associated with virus detection. Lines represent negative binomial regression model based on data.
FIG E5. The probability of influenza varies by month. Because of low sample size, data were binned into 2-month intervals. January/February was most likely to be associated with positive influenza diagnosis. *Lines* represent negative binomial regression model based on data.
FIG E6. The probability of adenovirus varies by both month and year. September was least likely to be associated with adenovirus, and May was most often associated with adenovirus. Lines represent negative binomial regression model based on data.