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# Human rhinovirus C: Age, season, and lower respiratory illness over the past 3 decades

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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.<sup>1-3</sup> 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,<sup>4</sup> bronchiolitis,<sup>5</sup> and asthma.<sup>6</sup> 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.<sup>7</sup> HRV-associated wheezing during infancy predicts the development of subsequent childhood asthma.<sup>6,8</sup> HRV infection also triggers many wheezing and asthma exacerbations.<sup>7,9</sup> Especially in the spring and fall seasons, HRV-associated asthma exacerbations<sup>10</sup> 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.<sup>11-13</sup> Multiple HRV strains and species may circulate concurrently even within small populations.<sup>14</sup> Studies have suggested that HRV-C and HRV-A may exhibit different seasonality patterns<sup>15-17</sup> and that HRV-C may be associated with increased disease severity and asthma risk.<sup>18-22</sup> 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.<sup>23-27</sup> 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,

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**Abbreviations used**

HMPV: Human metapneumovirus  
 HRV: Human rhinovirus  
 LRI: Lower respiratory illness  
 PIV: Parainfluenza virus  
 RSV: Respiratory syncytial virus  
 URI: Upper respiratory illness  
 VVC: Vanderbilt Vaccine Clinic

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 analysis. Four children were between 60 and 70 months, 4 between 71 and 80 months, 3 between 81 and 100 months, and 2 between 100 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.

**Study groups**

Over the course of the study from 1982 through 2003, 2009 children were followed in the clinic, with approximately 200 children enrolled at any given time. Diagnoses were grouped into LRI (bronchiolitis, pneumonia, croup, and asthma) and URI (coryza, pharyngitis, and acute otitis media).<sup>28</sup> Nasal-wash samples collected from children with URI or LRI were cultured for virus.<sup>29</sup>

Samples that were culture negative for parainfluenza virus (PIV), respiratory syncytial virus (RSV), influenza, adenovirus, and samples that were culture positive for HRV were tested in our study. Samples that were culture positive for other viruses were not retested for HRV. All samples that were culture negative for PIV, RSV, adenovirus, influenza, as well as those associated with LRI were tested in our study. A random sample of available culture-negative URI specimens was drawn and tested alongside the LRI samples. Culture-negative URI specimens were randomly drawn from each quarter of each year on the basis of the number of samples available for a given quarter to yield approximately 25% of all specimens in each quarter. Of the 1735 URI culture-negative samples available, we tested 407. Thus, there were a total of 527 specimens with aliquots available for testing in our study (either culture-negative or HRV culture-positive), 190 from patients diagnosed with LRI and 407 from patients diagnosed with URI. On average, there were 24 (SE  $\pm$  2.33) samples each year available for testing. Figure E1 (in the Online Repository available at [www.jacionline.org](http://www.jacionline.org)) depicts the number of samples tested by year. Data from children who were infected with human metapneumovirus (HMPV), PIV, RSV, influenza, or adenovirus were previously published<sup>23-26,30</sup> and were analyzed in this study by using our models for comparison with HRV.

**Molecular analysis**

RNA was extracted from nasal-wash specimens, and real-time RT-PCR was used to detect HRV.<sup>31</sup> Conventional RT-PCR was performed on HRV-positive samples by using primers that amplified a 548 nt fragment encompassing the VP4/VP2 region and the hypervariable region in the 5' noncoding region.<sup>32</sup> Amplified fragments were gel-purified, cloned, and then sequenced at the Vanderbilt Sequencing Core. Sequences were aligned with each other and with published HRV sequences by using MacVector 11.0 (MacVector, Inc, Cary, NC), and phylogenetic analysis was conducted by using Mega 5.05.<sup>33</sup>

**TABLE I.** Frequency (and percentage) of specimens positive for HRV and type of HRV

Feature	n (%)				Total	+ Negative
	HRV-A	HRV-B	HRV-C	Untypeable		
No. of patients	91	5	69	25	190	336
Median age (mo)	17.7	15.2	15.4	14.76	11.47	14.45
Female	34 (37)	1 (20)	37 (54)	11 (44)	83 (44)	146 (43)
White	62 (67)	1 (20)	44 (64)	18 (72)	125 (66)	194 (58)
Black	19 (21)	4 (80)	24 (35)	7 (28)	54 (28)	125 (37)
Other ethnicity	10 (11)	0 (0)	1 (1)	0 (0)	11 (6)	17 (5)
Winter	21 (23)	0 (0)	26 (38)	8 (32)	55 (29)	121 (36)
Spring	28 (31)	1 (20)	18 (26)	8 (32)	55 (29)	75 (22)
Summer	17 (19)	1 (20)	9 (13)	1 (4)	28 (15)	62 (18)
Fall	25 (27)	3 (60)	16 (23)	8 (32)	52 (27)	78 (23)

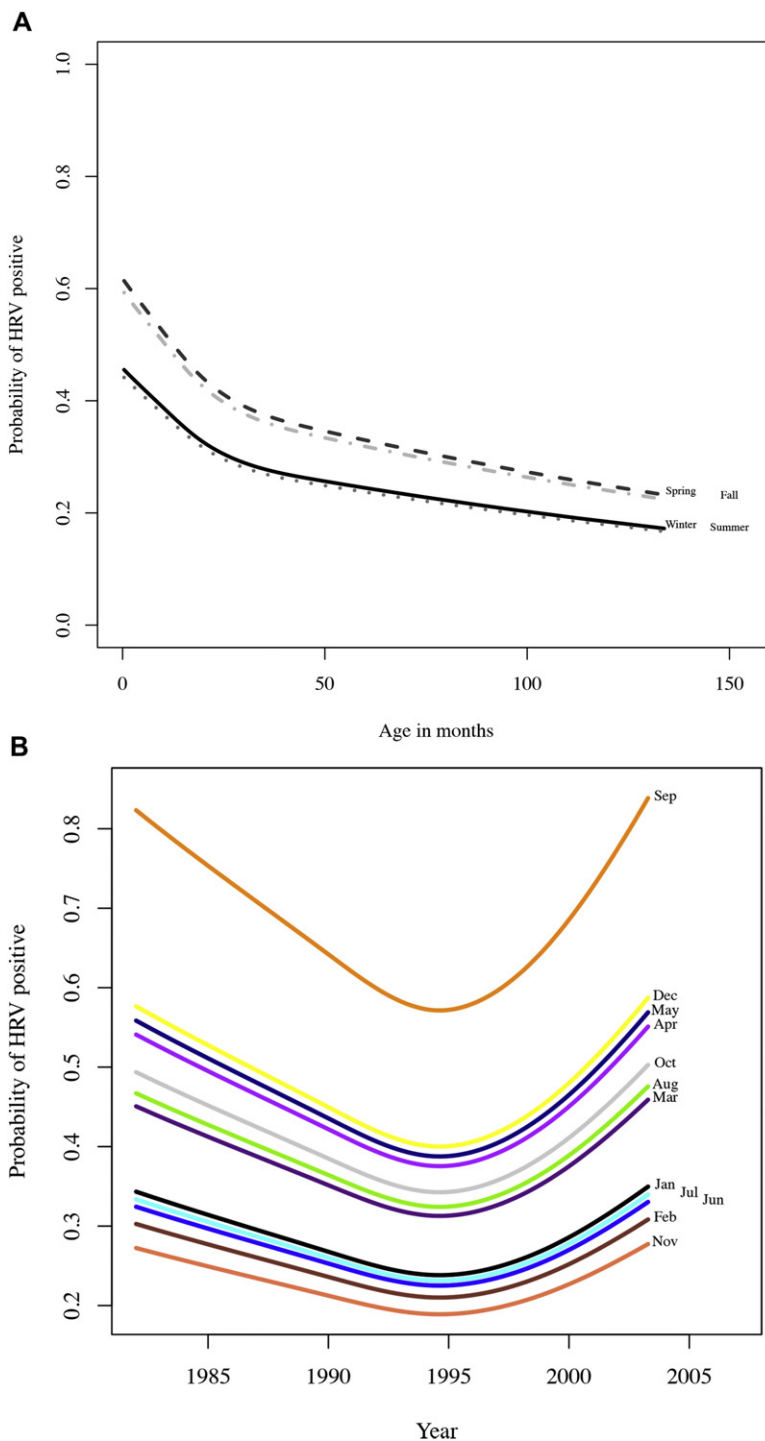
**Statistical analysis**

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<sup>34</sup> 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, coryza, 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.

Sixteen of 171 subjects (9.3%) were infected with HRV more than once. Time between infections ranged between 2 months and



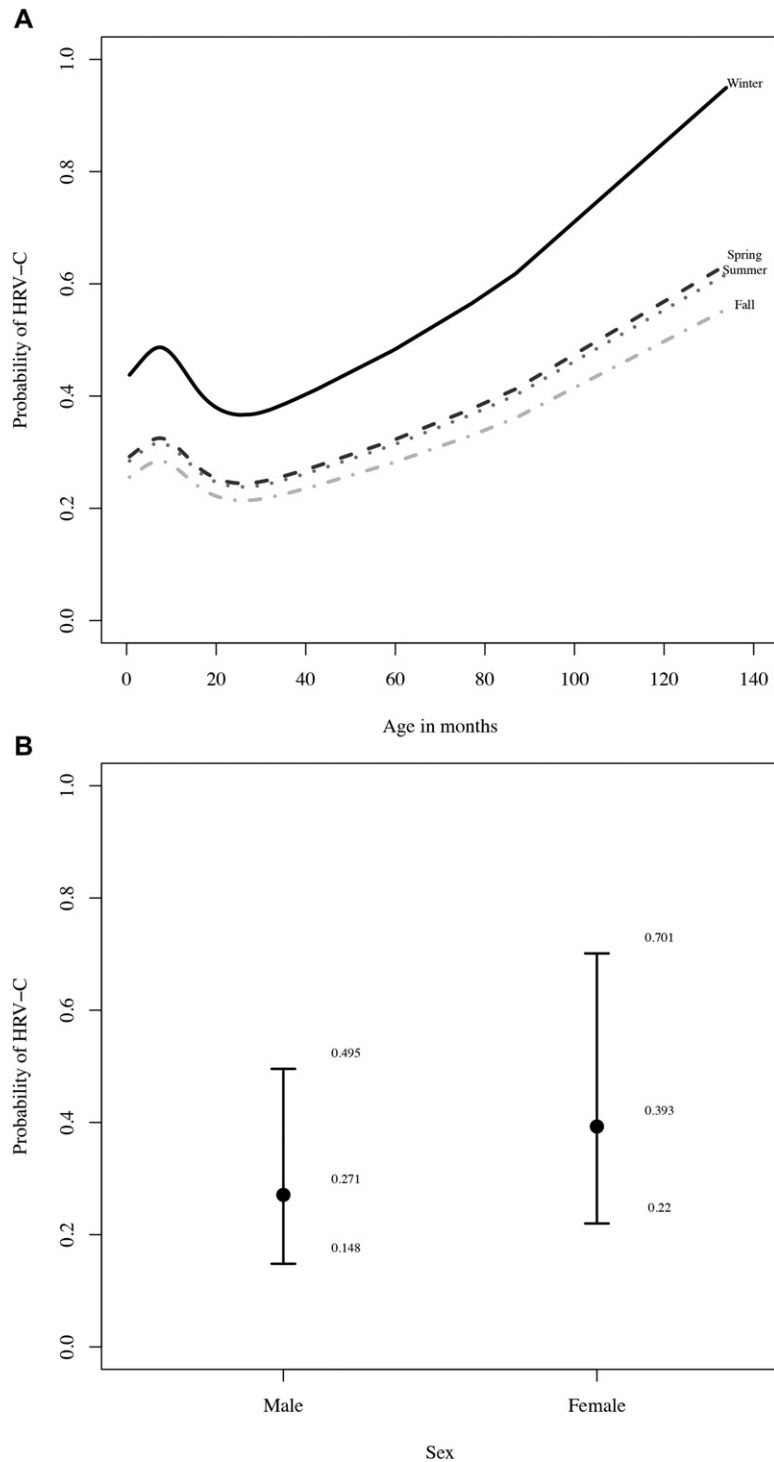
**FIG 1.** Age and month are significantly correlated with HRV infection. **A**, HRV is more common in younger children in fall and spring months. Winter, solid line; spring, dashed line; summer, gray dot; fall, gray dash/dot. Graph shown for white males. **B**, In September, children had a 2.47-fold increased risk of positive HRV diagnosis than in July. Lines represent modeled negative binomial regression.

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

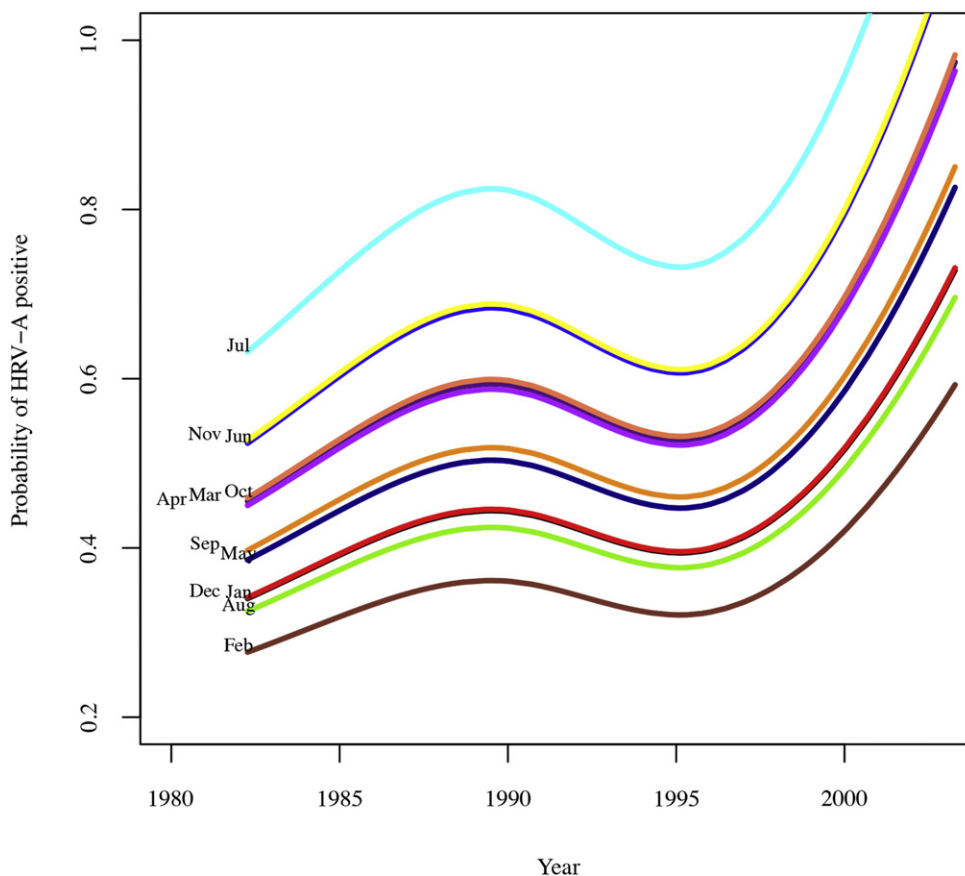
To examine more closely how the species of HRV may affect morbidity in young children, we compared HRV-A with HRV-C,



**FIG 2.** Older females in the winter months were more likely to present with HRV-C. **A**, Age and season predicted HRV species (shown for white males). The negative binomial regression modeled here predicts that HRV-C is more common with increasing age. **B**, Sex was also a significant factor in the likelihood of HRV-C (shown for 11.2-month-old white child in the fall).

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 ( $P = .005$ ), 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 ( $P = .02$ ; 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). 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



**FIG 3.** HRV-A rate varies from year to year. HRV-A fluctuated significantly over the years; recently, there was more HRV-A present in children with respiratory illness. Lines represent the negative binomial regression model based on the data.

( $P = .056$ ), 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 1).

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

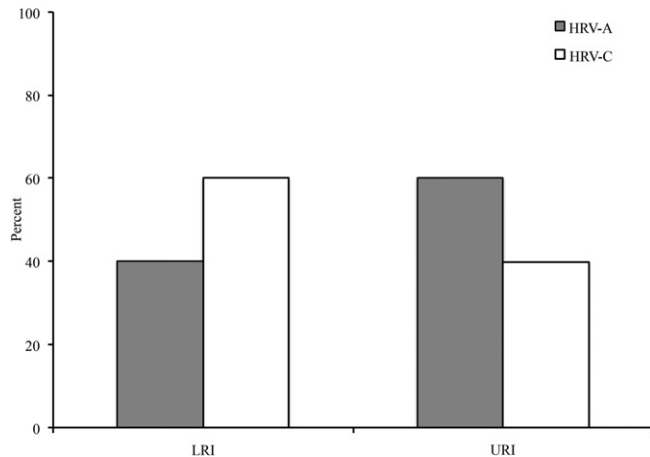
Detection of the other study viruses (HMPV, PIV, RSV, influenza, and adenovirus) varied over time (see Figs E2-E6 in this article's Online Repository available at [www.jacionline.org](http://www.jacionline.org)). HMPV varied significantly over both month ( $P < .001$ ) and year ( $P = .012$ ), with highest 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,



**FIG 4.** LRI diagnosis is more often associated with HRV-C and URI with HRV-A. Percentage of patients diagnosed with infection type: LRI (bronchitis, croup, pneumonia, and asthma) and URI (coryza, pharyngitis, and acute otitis media). White bars represent HRV-C, and gray bars represent HRV-A.

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% ( $\pm 0.27$ ), HRV-B sequences had an average similarity of 79.7% ( $\pm 0.42$ ), and HRV-C sequences were 73.9% ( $\pm 0.16$ ) 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.

## DISCUSSION

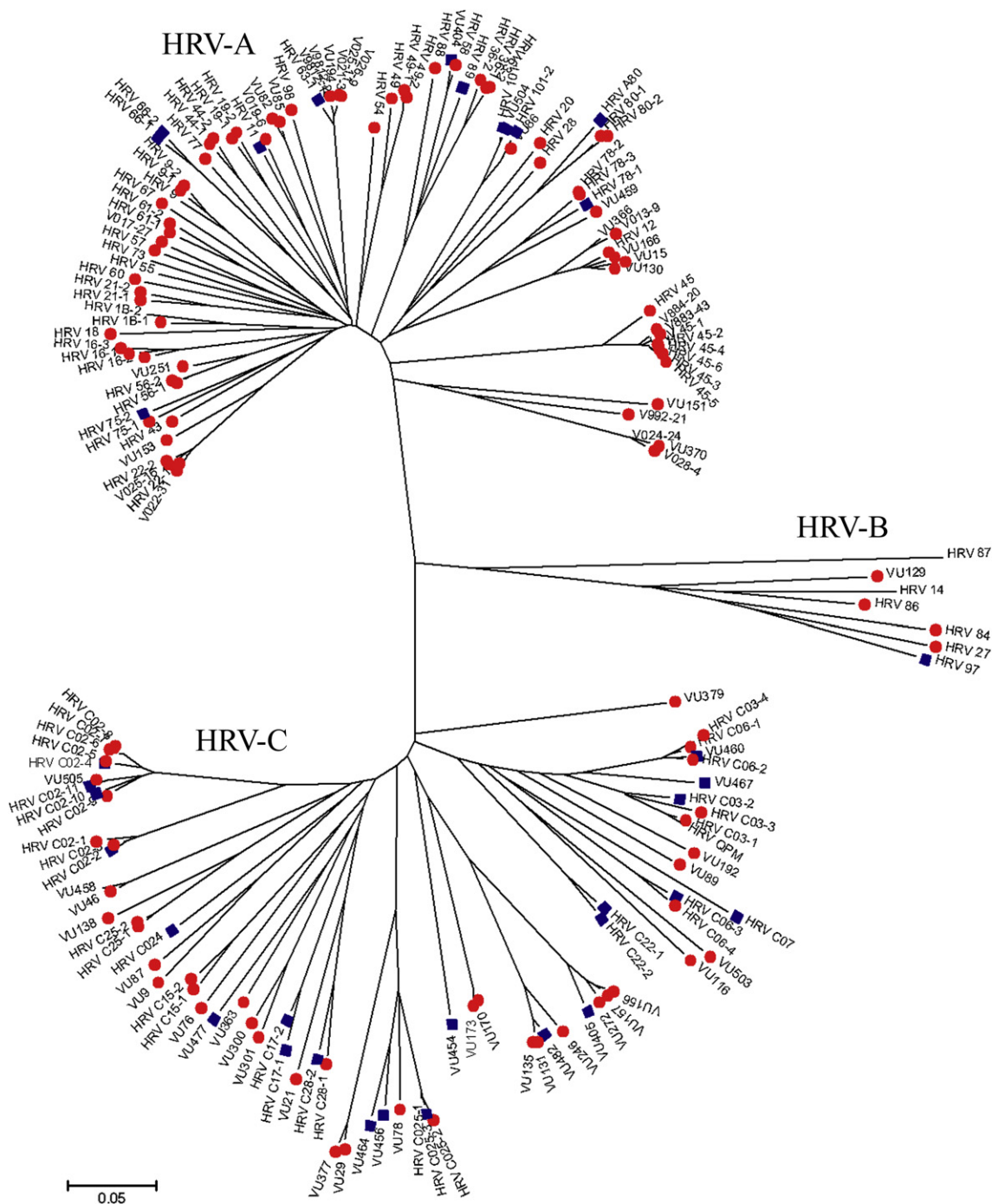
We retrospectively analyzed data and samples from a prospective 21-year cohort study. Our findings show that the recently described HRV-C is not a new virus but has played an important role in childhood respiratory disease for at least 20 years. The data also confirm the growing evidence that HRV-C is associated with more severe lower respiratory disease, compared with HRV-A.<sup>19-21</sup> Although other studies have examined HRV seasonality,<sup>15,16</sup> this is the first study to describe more than 20 years of data. This longitudinal cohort provides a unique ability to determine trends in HRV circulation over time without the risk of seasonal patterns attributable to random fluctuations. Interestingly, while HRV was prevalent in the fall and spring as expected,<sup>35,36</sup> HRV-C was found most commonly during winter months. Because these data encompass 21 years of prospective data, it is unlikely that the explanation is due to an atypical increase in HRV-C in a single winter.

In our study, HRV was more frequently detected in younger children and infants than in older children, in agreement with other studies.<sup>7,37</sup> 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.<sup>38</sup> 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.<sup>39</sup> 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 coinfecting with HRV.<sup>40</sup> Wisdom et al<sup>39</sup> found that though 10.7% of singly infected patients had RSV, only 2.4% had RSV when coinfecting with HRV-C. In one of our previous studies of young hospitalized children, HRV-C coinfection was 10% compared with 23% for HRV-A ( $P = .037$ ).<sup>41</sup> 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.<sup>42</sup> 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.<sup>15</sup> 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.<sup>43</sup> Another limitation was that study viruses other than coronaviruses<sup>30</sup> and HMPV<sup>25</sup> 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



**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 (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.

any effect on infection; some studies show that breast-feeding may protect children from respiratory tract infections.<sup>44,45</sup> We also did not test healthy children for HRV, which has been shown to be present in asymptomatic individuals.<sup>46,47</sup> 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,<sup>48</sup> 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 States<sup>48</sup>). 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,<sup>17</sup> the opposite of the trend we found.



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.**

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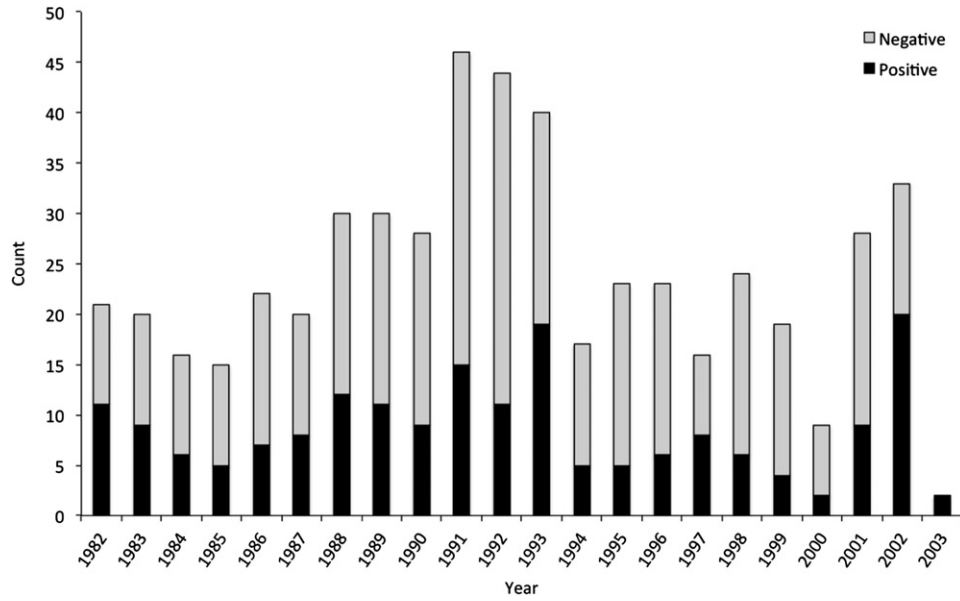
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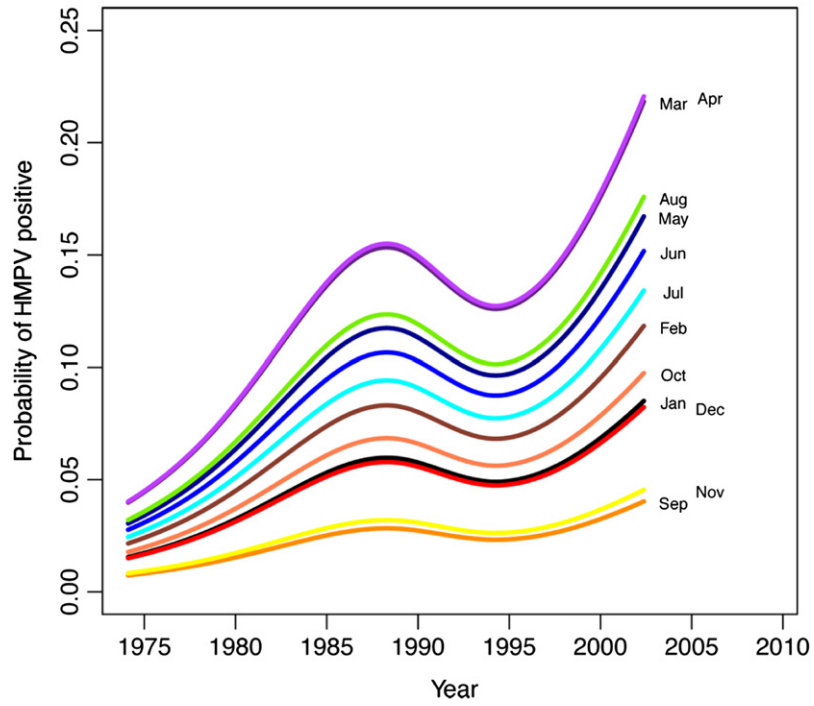
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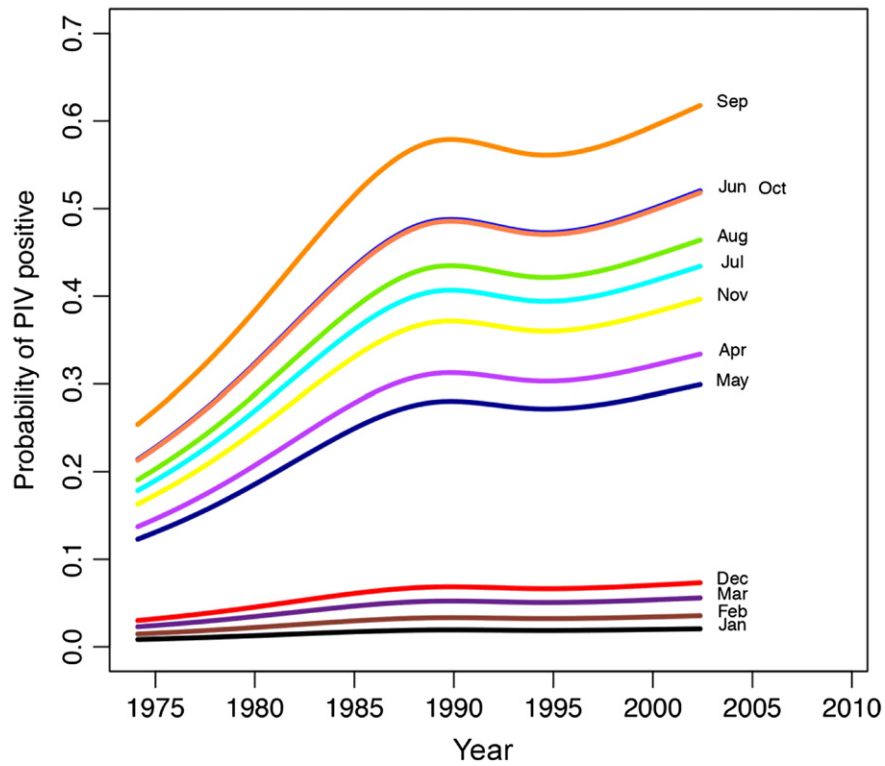
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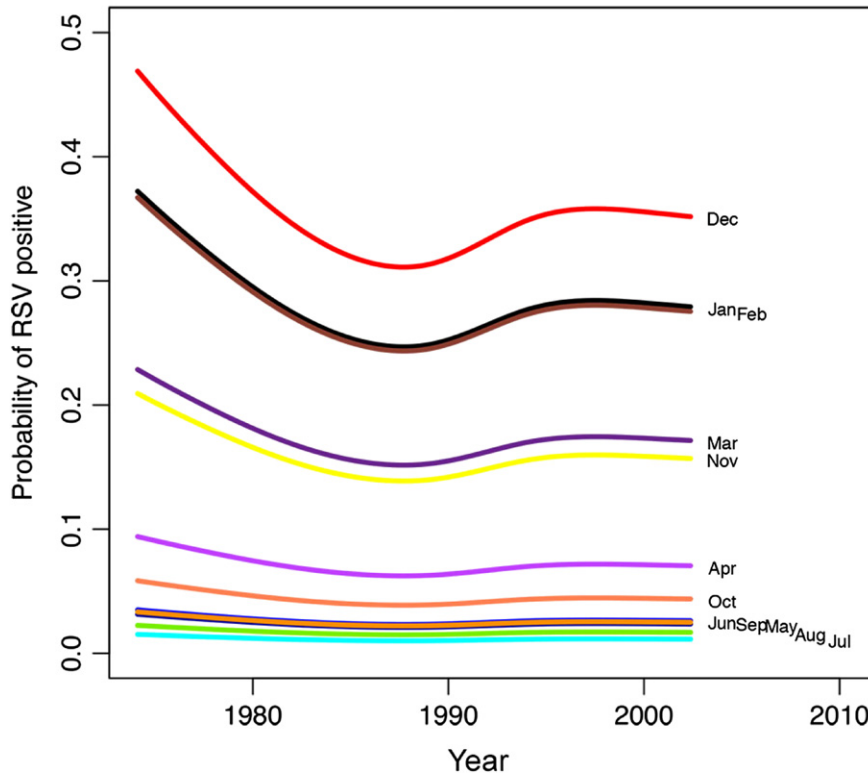
**FIG E1.** Number of samples analyzed for HRV by year. *Black bars* indicate the number of positive samples.



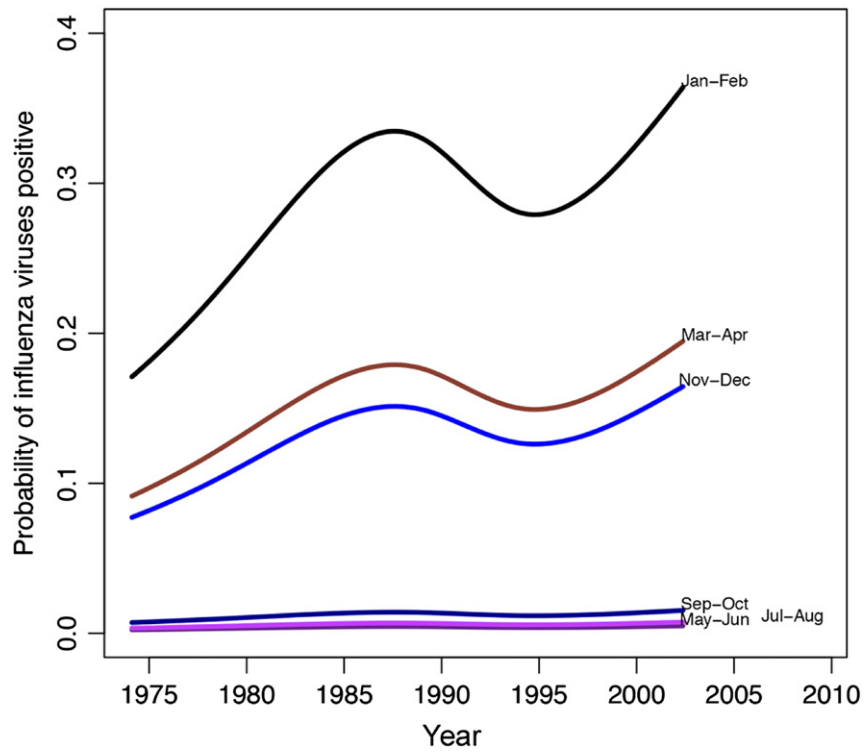
**FIG E2.** The probability of HMPV varies significantly over month and year. Lines represent negative binomial regression model based on data.



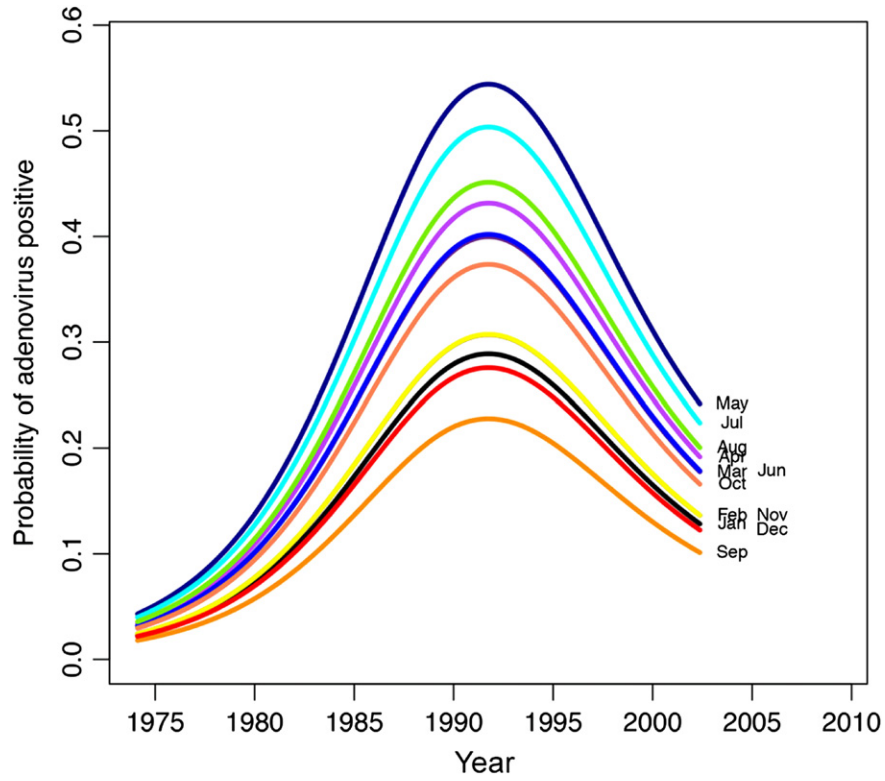
**FIG E3.** The probability of parainfluenza virus varies significantly over month and year. January and February were least likely to be associated with positive PIV. Lines represent negative binomial regression model based on data.



**FIG E4.** 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.