

## **Serial viral infections in infants with recurrent respiratory illnesses**

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**Short title:** Recurrent respiratory illnesses in infants

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**Abstract**

To better understand the viral etiology of recurrent and prolonged illnesses, we prospectively collected nasal secretions from 285 infants at increased risk of developing asthma. Of these, 27 infants had recurrent ( $\geq 5$ ) moderate-to-severe respiratory illnesses (MSIs), and the viral etiology of their 150 MSIs and 86 scheduled visits was analyzed by molecular diagnostics. Their demographic and clinical data were compared to those with 0-4 MSIs.

Frequently ill infants had higher exposure to other children and more wheezing illnesses than less symptomatic children ( $p < 0.0001$ ). Viruses were detected in 136/150 (91%) MSIs, 14/21 (67%) mild illnesses and 29/65 (45%) asymptomatic visits ( $p = 0.0001$ ). Rhinovirus (HRV) was the most common etiologic agent (61%, 43% and 35% respectively,  $p = 0.0020$ ). Mixed viral infections were generally associated with more severe illnesses (27%, 0% and 5%,  $p = 0.0001$ ). Among the 27 frequently ill infants, only 8/150 (5.3%) MSIs were prolonged ( $\geq 2$  weeks duration). Considering all samples, detection of the same virus strain  $\geq 2$  weeks apart was unusual (5.3% of all 244 positive findings).

HRV infections occur early, pervasively, and repetitively in these high risk infants. Infants with prolonged or recurrent respiratory illnesses most often have a series of infections rather than persistent infection with one virus strain.

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**Key words:**

Respiratory virus, rhinovirus, virus strain, virus persistence, infant, wheezing.

**Abbreviations:**

HRV	rhinovirus
COAST	Childhood Origins of ASThma
MSI	moderate-to-severe respiratory illness
PCR	polymerase chain reaction
RSV	respiratory syncytial virus
PIV	parainfluenza virus
Flu	influenza virus
hMPV	metapneumovirus
AdV	adenovirus
CoV	coronavirus
EV	enterovirus
cDNA	complementary deoxyribonucleic acid
Echo	echovirus
DNA	deoxyribonucleic acid

## Introduction

Acute viral infections are a major cause of respiratory morbidity in young children (1). Approximately 2-3% of all infants are admitted to hospital with bronchiolitis, usually during the seasonal epidemic (2). Emerging evidence from animal studies indicates that viral infections may be an important environmental stimulus for airway injury and remodelling, resulting in impaired lung function and potentially asthma (3).

Recent advances in viral diagnostics have increased the viral detection rate to as high as 95% in infants suffering from respiratory symptoms (4, 5). The availability of polymerase chain reaction (PCR) techniques, particularly for rhinoviruses (HRV), is one of the main reasons for the improved virus detection rate. Although HRV is principally known as the “common cold” virus, it is also found in up to 41-45% of young wheezing children, second only to respiratory syncytial virus (RSV) (4, 6-9). Interestingly, HRV-induced wheezing episodes in infancy are an important predictor of recurrent wheezing in four independent studies (10-13).

Limited data are available on the etiology and persistence of viruses in children with recurrent respiratory symptoms. HRV appears to be the most prevalent virus detected in this patient group and accounts for 48% of all upper respiratory tract illnesses (1). Although HRV infections can persist over a year in immunosuppressed patients (14), whether the same HRV strain can persist in children with frequent or prolonged illnesses is unknown (1, 15-17).

To better understand the nature and risk factors for recurrent illnesses and wheezing in infancy, we used two newly developed molecular techniques to analyze the viral etiology of illnesses in children who had family histories of allergic diseases or asthma and experienced

frequent moderate-to-severe respiratory illnesses in the first year of life (18, 19). We addressed three questions. First, how do children who suffer from frequent respiratory infections during infancy differ from other children with similar family histories? Second, what is the viral etiology of their respiratory illnesses during infancy? Third, a subset of infants have prolonged or seemingly continuous respiratory illnesses in the wintertime: are these prolonged illnesses due to persistent infections, or a series of different infections?

## Methods

### *Study subjects and study design*

Originally, 289 subjects were enrolled in the Childhood Origins of ASThma (COAST) Study at birth, and 285 were followed prospectively for 12 months. To be eligible, each of the COAST children were required to have one or both parents with allergic sensitization (one or more positive aeroallergen skin tests) and/or asthma (by history),  $\geq 37$  weeks gestation, and be otherwise healthy. Details of study population and design have been described previously (20). This study was approved by University of Wisconsin Human Subjects Committee. Enrollment occurred during the prenatal period, and commenced only after obtaining informed consent from the parents.

During the first year of life, participating families were asked to contact the study center each time the child had respiratory illnesses. If the illnesses were classified as moderate-to-severe based on predefined respiratory symptom score, 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 5 scheduled visits (2, 4, 6, 9 and 12 months) whether the child was sick or well. Twenty-seven children (9.3%) had  $\geq 5$  moderate-to-severe illnesses (MSI) during the first year of life, and were classified as children with “frequent illnesses”. Collectively, these children had a total of 150 MSIs and 86 additional scheduled visits (65 visits with no symptoms and 21 visits with mild symptoms) with nasal washes during the first year of life between March 1999 – April 2001.

Viruses illnesses in COAST participants were previously analyzed for respiratory viruses, and these data have been compared to blood cell cytokine responses (21, 22) and the risk of

recurrent wheeze (11). Furthermore, molecular typing of samples from COAST participants has led to the identification of new HRV strains (19). The goals of the current study are to identify the pattern and persistence of viral infections and HRV strains in 27 individual infants with frequent MSIs. We also investigated demographic and clinical differences between the infants with frequent MSIs and those infants with less respiratory symptoms.

#### *Definitions of illnesses and viral infections*

An illness was considered moderate-to-severe if a clinical symptom score was  $\geq 5$  and mild if the score was 1-4 (table E1). To be defined as a well child, the score had to be zero. The onset day of MSI was considered to be the first day with score  $\geq 5$ . Illness was considered as prolonged if moderate-to-severe symptoms persisted  $\geq 2$  weeks with the score remaining  $\geq 5$ . Consequently, a new patient evaluation and nasal wash sample was taken every 2 weeks in these prolonged illnesses. The last day of MSI was the last day with a score  $\geq 5$ . If such information was not available, the last day was estimated as occurring halfway between the last day of confirmed MSI and the next follow-up visit day (within 2 weeks) when the child no longer had MSI. Viral infections were considered persistent or recurrent if they reoccurred  $\geq 2$  weeks apart.

#### *Total IgE and allergy tests*

Total and allergen-specific IgE for *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, cat, dog, milk, egg, soy bean and peanut were analyzed in all 27 subjects by UniCAP 100<sup>E</sup> (Pharmacia and Upjohn Diagnostics, Kalamazoo, MI, USA) as described previously (23). The sensitivity for detection of total IgE was 2 kU/L. Allergen-specific IgE values of  $\geq 0.35$  kU/L were considered positive.

### *Clinical definitions*

Sensitization was defined by positive allergen-specific IgE test result at age 12 months. Atopic dermatitis at age 12 months was defined either as parental report of physician-diagnosed atopic dermatitis during the first year of life or by physician report of atopic dermatitis at physical examination performed at 12 months of age. Wheezing was determined for each illness or outpatient visit by report of the caretaker or by physical examination as recorded in the medical record (11).

### *Microbiology*

Diagnostic virology was performed for all nasal samples in the first year of life whether a child had symptoms or not. Results and methods for viral culture, immunofluorescent antibody staining for RSV, and nested polymerase chain reaction (PCR) for first year were previously described (11). All the samples of the 27 children with frequent respiratory illnesses were reanalyzed with a new multiplex PCR-based assay (Respiratory Multicode Assay, EraGen Biosciences, Madison, WI, USA) that detects the following viruses: HRV, EV, AdV (B, C, and E), Flu (A, B), PIV (1, 2, 3, 4a, 4b), CoV (SARS, OC43, 229E, and NL63), RSV (A, B) and hMPV (18). Molecular typing of HRV performed as described (19). More details are shown in online repository material. Bacterial etiology was not studied.

### *Statistics*

No statistical power calculations were performed. The predefined cut-off  $\geq 5$  MSIs was chosen because it represented approximately the top 10% of infants with recurrent illnesses. Normality of data distribution was tested with Kolmogorov-Smirnov test. Skewed data were analyzed with Kruskal-Wallis ANOVA. Otherwise, one-way ANOVA or linear regression



analysis was used. Dichotomous data were analyzed by Chi square test or Fisher's Exact Test (for counts less than 5). A nominal p-value of 0.05 was regarded as statistically significant.

## Results

### *Study subjects*

A total of 285 children completed the first year of follow-up. Of these 285 children, 27 had  $\geq 5$  MSIs, 204 had 1-4 MSIs and 54 did not have any MSIs during the first year of life (overall median, 2 MSIs [interquartile range 1-3], table 1). Exposure to other children was clearly associated with frequent MSIs. All the children with at least 5 MSIs either had older siblings or were in day care, compared to 82% in children with 1-4 MSIs ( $p = 0.018$ ) and 63% in the children without MSIs ( $p = 0.0003$ ). The infants with  $\geq 5$  MSIs also differed from the rest of the cohort by having more wheezing illnesses: 85% of them had wheezed at least once during infancy versus 32% in children with 1-4 MSIs and 2% in the children without MSIs ( $p < 0.0001$  for both comparisons). They also had more systemic corticosteroid courses ( $p < 0.0001$ ), antibiotic treatments ( $p < 0.0001$ ) and asymptomatic viral infections ( $p = 0.0058$ ). Children with  $\geq 5$  MSIs also tended to more often have a maternal history of asthma compared to other children ( $p = 0.083$ ). Other demographics and atopic characteristics were comparable.

Of the 150 MSIs in the 27 frequently ill infants, symptoms included cough (99%; 9% mild, 29% moderate, 61% severe), rhinorrhea (97%; 28% mild, 69% moderate-to-severe), duration of illness  $>4$  days (59%), wheezing (45%), hoarseness (32%), dyspnea (10%), retractions (8%), fever (5%), and apnea (1%). Of the 21 mild illnesses in the same children, symptoms included rhinorrhea (84%; 53% mild, 32% moderate-to-severe), cough (47%; 26% mild, 16% moderate, 5% severe), duration of illness  $>4$  days (26%), and fever (11%).

### *Viral etiology of MSIs and scheduled visits*

A total of 150 MSIs, 21 mild illnesses and 65 well visits with nasal wash were recorded during the first year of life in the 27 frequently ill infants (fig. 1). Viral detection rate increased with the severity of illness: 45% (29/65) in asymptomatic infants, 67% (14/21) in infants with mild illness and 91% (136/150) in infants with MSI ( $p = 0.0001$  overall, fig. 2). HRV detection rate (including mixed infections) increased with the severity of illness, respectively: 35% (23/65), 43% (9/21) and 61% (91/150) ( $p = 0.0020$  overall). Multiple viruses were most often found in infants with MSI (27%, 39/150) and rarely in other infants (asymptomatic, 5%, 3/65; mildly symptomatic, 0% 0/21,  $p = 0.0001$ ). Of the 39 cases with MSI and mixed viral infection, 86 viruses were detected: most often HRV ( $n = 30$ ), followed by RSV ( $n = 13$ ), AdV ( $n = 13$ ), PIV ( $n = 8$ ), Flu ( $n = 6$ ), hMPV ( $n = 6$ ), coronavirus ( $n = 5$ ) and enteroviruses ( $n = 5$ ). Only two double HRV infections (2 different strains) were found. When viruses were detected in asymptomatic children, an MSI associated with the same viral agent occurred within 2 weeks in 4/29 (14%) of these cases.

### *Viral etiology of wheezing in frequently ill infants*

Of the 27 children with  $\geq 5$  MSIs during infancy, 23 (85%) wheezed with viral infection (table E2). Seventy-eight percent (18/23) of them wheezed at least once with HRV, followed by RSV (70%, 16/23) and other viruses. A total of 73 wheezing episodes were recorded in these 27 infants (mean 3.2 [sd 1.9] wheezing episodes per child). Of the 68 wheezing episodes with a corresponding nasal wash, virus was detected in 60 (88%) specimens. HRV was the most prevalent agent whether including (47%, 32/68) or excluding multiple viral infections (25%, 17/68), followed by RSV (total 24% [16/68] and as sole agent 10% [7/68]).

### *Viral findings in prolonged illnesses*

The duration of a single MSI was usually less than 2 weeks (142/150, 95%). Eight (5.3%) MSIs lasted  $\geq 2$  weeks and were considered prolonged (fig. 3). Only one prolonged MSI was linked to same viral finding in the consecutive samples taken  $\geq 2$  weeks apart (HRV-12 at both visits 14 days apart, and AdV C only at the second visit).

### *Recurrent infections*

We then analyzed the frequency of recurrent infections, defined as the same viral strain occurring in separate clinical illnesses or well visits  $\geq 2$  weeks apart in same infants.

Considering all 244 positive viral findings in the 27 frequently ill infants, 13 (5%) viral findings met these criteria and can be considered as recurrent infections with the same or similar viral agents (AdV, n = 6; HRV, n = 4; RSV, n = 1; hMPV, n = 1). Twelve viruses reoccurred once, and one virus reoccurred twice. Consecutive recurrent viral findings are shown in fig. 4. Non-consecutive similar viral findings reoccurred within 83-116 days.

Combinations of viruses were common: multiple viruses were detected in over half of the samples associated with persistent or recurrent viral findings.

### *Comparisons between HRV and other viral infections*

HRV infections tended to occur earliest (mean age at first HRV infection was 4 months compared to  $\geq 6$  months for other viruses), were most frequent (mean 3.6 episodes compared to  $< 1.0$  episodes for other viruses) and had similar severity compared to other infections. The first HRV infections occurred before first RSV infections in 73% of cases (19/26, excluding one case with simultaneous HRV and RSV infection). HRV and RSV infections had comparable severity (mean [SEM] symptom score, HRV 8.0 [0.3] vs RSV 9.1 [1.0], mean

difference -1.0, 95% CI -2.9 to 0.7,  $p = 0.23$ ,  $n = 102$ ; after adjusting for or excluding mixed viral infections, the difference remained non-significant [ $p = 0.30$  or  $p=0.71$  respectively]).

## Discussion

Understanding the epidemiology and natural history of prolonged and recurrent respiratory viral infections has been hampered by shortcomings associated with traditional viral diagnostics. This prospective study used molecular viral diagnostic to analyze the etiology of frequent respiratory infections in a group of infants at increased risk of developing allergies and asthma. The findings demonstrated that HRV were most often associated with MSIs and wheezing during infancy, lending further support to the close association between HRV infections and respiratory morbidity in this age group. In addition, prolonged MSIs occurred infrequently, even in this selected population, and were rarely due to persistent viral infection with the same pathogen. Instead, infants with frequent MSIs typically had a series of infections with different viruses or virus strains.

HRV infections have increasingly been associated with wheezing in infants during the last decade (4, 6-7, 10, 11, 13). The HRV detection rates have reached as high as 41-45% of young wheezing children (6, 9). In our study of infants with frequent illnesses, 78% of the wheezing infants wheezed at least once with HRV, and this is comparable to the 70% wheezing rate associated with RSV. In addition, HRV was associated with illnesses of comparable severity to other viruses such as RSV. Finally, molecular analysis revealed that simultaneous infections with more than one HRV strain were rare. When considered collectively with other recent reports (10-13), our data suggest that the clinical impact of early HRV infections can be similar to that of early RSV infections and forms an important basis for further research.

HRV belong to the *Picornaviridae* family, and to date, more than 100 HRV serotypes have been identified by traditional viral culture and serology techniques (24). In our study, HRVs were typed by a sensitive molecular assay based on phylogenetic comparisons of their 260-bp variable sequences in the 5' noncoding regions with homologous sequences of the 101 known serotypes. This molecular typing assay directly identified HRV from the original clinical specimens, and we previously reported data from this population in which several new strains that evaded traditional serotyping assays were identified (19).

Previous studies have suggested that HRV might cause persistent infections, although definitive conclusions were impossible because HRV strains were not identified (15-17). In our study, despite the high prevalence of HRV positive samples, only one of the prolonged MSIs (fig. 3) and 4 additional cases of persistent or recurrent infection (fig. 4) were associated with the same HRV; infections lasting longer than 2 weeks were unusual. Whether prolonged shedding is an indication of immunologic anomaly and linked to poorer long-term prognosis remains to be determined. In a previous study of asthmatic and normal adult volunteers, viral shedding was still detectable in 54% of adult subjects 14 days after experimental inoculation with HRV-16 (25). However, the quantity of virus was quite low at this point, and cold symptoms were generally either gone or resolving. Although our study design is not optimal for determining the exact duration of illness, sampling during regularly scheduled visits even when symptoms were absent reduced the possibility of missing persistent infections. The virus most likely to be persistent in our study was AdV which was detected for longer than 2 weeks in 6 of 8 cases (75%). This DNA virus, however, can be latent and intermittent detection by PCR does not necessarily mean reinfection (26). With the possible exception of AdV, our study conclusively demonstrates that recurrent or prolonged illnesses in infants tend to be caused by serial infections rather than prolonged infections with one organism.

Viruses were also detected more often in frequently ill infants when they were asymptomatic (table 1). Nearly half (45%) of the scheduled visits without any respiratory symptoms were associated with positive viral finding. At these well-visits, HRV was again the most frequently detected virus (35%, vs  $\leq 5\%$  for other viruses), which is in agreement with previous reports of 10-41% HRV detection rates in asymptomatic children, with higher rates generally associated with younger age (6, 15, 27). Interestingly, the frequently ill infants in our study were all exposed to other children at home or day care.

Considering the tremendous sensitivity of PCR-based assays, what does it mean to detect a respiratory virus in an asymptomatic child? Viruses detected in asymptomatic children may represent a low level infection without associated symptoms, although it is difficult to prove that a young child is totally asymptomatic. In addition, we found evidence that this may also represent the first sign of a developing clinical illness (15), since 14% of these were linked to MSI associated with the same viral agent within the following 2 weeks. The low percentage of persistent or recurrent viral infections, however, argues against the suggestion that viruses detected by PCR are likely to be residual nucleic acids left over from distant infections. Instead, our findings suggest that PCR is likely to detect true infections with or without symptoms. Furthermore, infants with high exposure to other children appear to have more asymptomatic viral infections.

In agreement with a previous study of infants (7), mixed viral infections were linked to more severe respiratory symptoms. Persistent or recurrent infections were also associated with a high prevalence of mixed viral infections. HRV was the virus most often associated with mixed viral infections as previously reported (5, 7, 8). Whether mixed infections or higher



frequency of asymptomatic infections are primarily due to increased exposure, or host factors such as weak interferon responses deserves further study. More data is available on HRV infections, and recent studies in humans have linked susceptibility to HRV infections to suboptimal immunologic responses of blood or airway cells (10-13, 21, 22, 28-30). Interestingly, HRV associated risk for recurrent wheezing remained unchanged after adjusting to exposure to other children and other relevant factors in the COAST and Australian cohorts (11, 13).

Our study has some limitations. The study cohort included only infants at high risk for allergies and asthma. Since these children may have increased susceptibility for lower respiratory HRV infections (6), additional studies are needed to determine whether there are any differences in outcomes of a generalized population. We systematically studied only MSIs and not mild colds. Finally, although our diagnostic virology had a high yield during times of illness, several new viruses have recently been discovered (e.g. human bocavirus, coronavirus HKU1, and respiratory polyomaviruses) and may account for some of the unexplained illnesses.

In conclusion, this study demonstrates that frequently ill infants at increased risk for chronic allergic diseases and asthma most often have a series of infections with different virus strains rather than persistent infection with one virus. Interestingly, HRV infections were clearly most often associated with MSIs and wheezing. Thus, our study provides additional evidence that HRV can produce more than the “common cold” in high risk infants and these infections occur early, pervasively, and repetitively with different strains.

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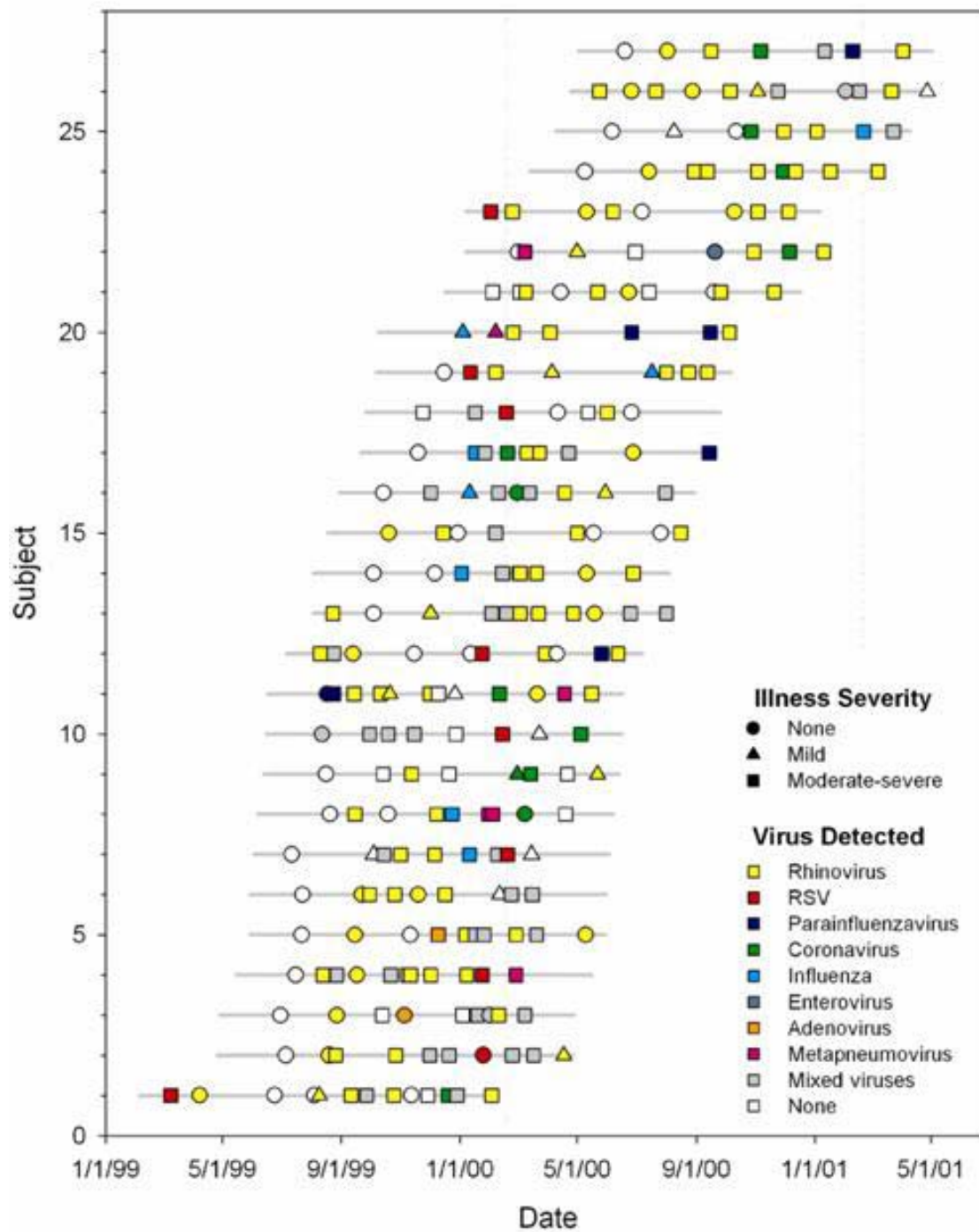
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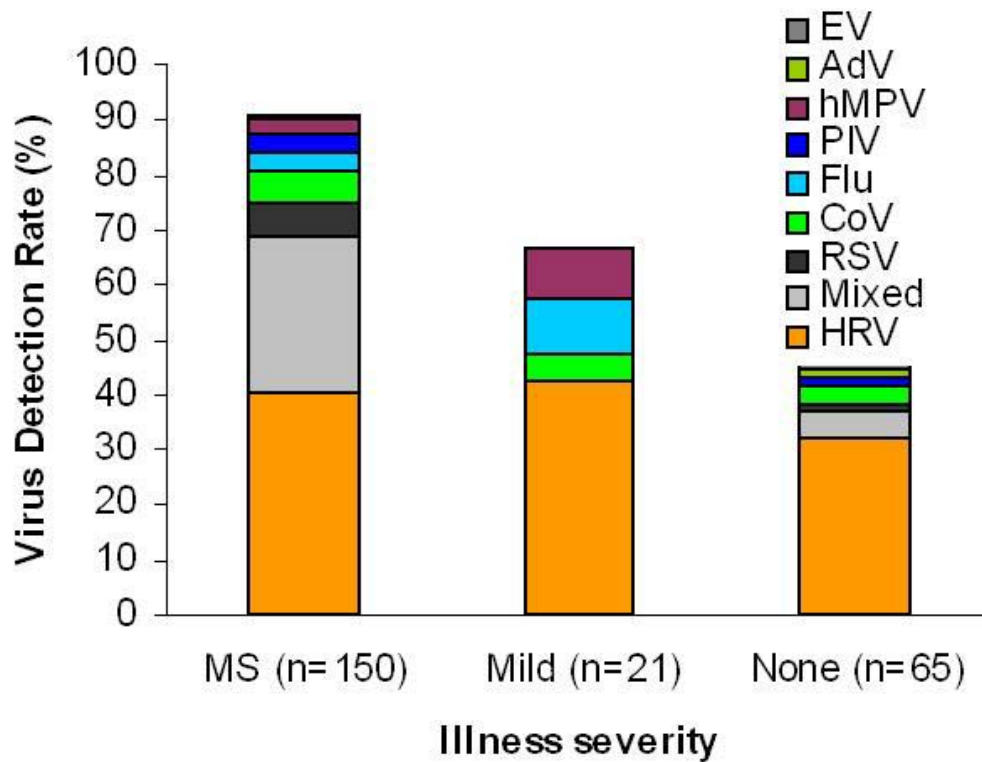
### **Legends to the figures**

**Fig. 1.** Temporal occurrence of viral infections in 27 infants with recurrent moderate-to-severe respiratory illnesses during infancy according to the severity of symptoms. Cases 1, 5, 7, 9, 10, 15, and 17 had also one MSI each without nasal wash, which are not shown. HRV, rhinovirus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; CoV, coronavirus; hMPV, metapneumovirus; Flu, influenza virus; AdV, adenovirus; EV, enteroviruses.



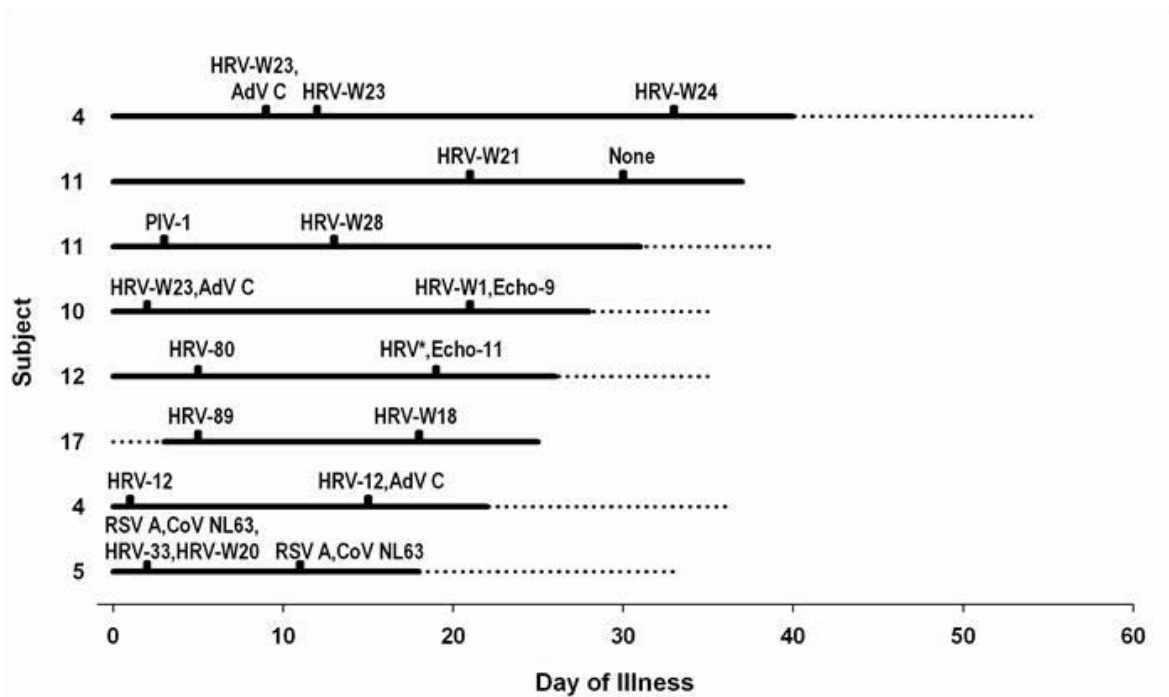
**Fig. 2.** Viral findings in 27 infants with recurrent moderate-to-severe respiratory illnesses (MSIs) during infancy according to the severity of symptoms at each visit. EV, enteroviruses;

AdV, adenovirus; hMPV, metapneumovirus; PIV, parainfluenza virus; Flu, influenza virus; CoV, coronavirus; RSV, respiratory syncytial virus; HRV, rhinovirus.

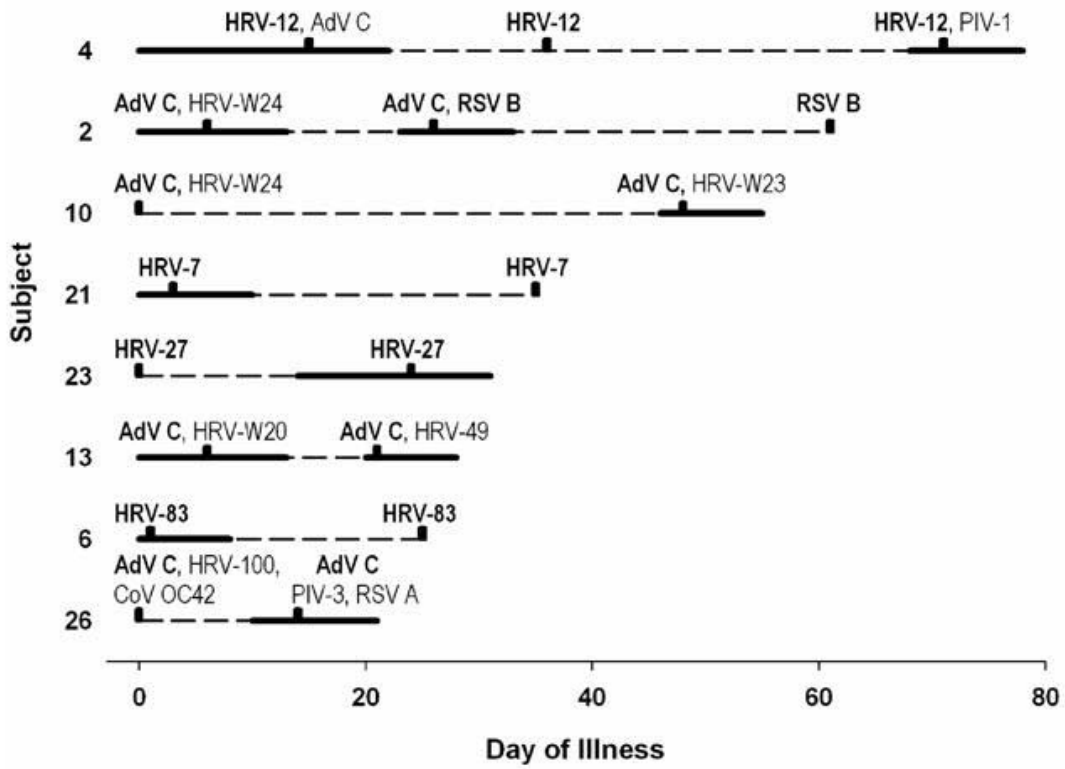


**Fig. 3.** Viral findings in moderate-to-severe illnesses lasting  $\geq 2$  weeks. Bolded line illustrates moderate-to-severe symptoms and dotted line illustrates the total duration of symptoms. \*No sample available for molecular typing. RSV, respiratory syncytial virus; CoV, coronavirus; HRV, rhinovirus; Echo, echovirus; AdV, adenovirus.





**Fig. 4.** Identical viral findings in consecutive nasal wash samples  $\geq 2$  weeks apart relative to illness (bolded line illustrates symptomatic and broken line asymptomatic periods). HRV, rhinovirus; AdV, adenovirus; PIV, parainfluenza virus; CoV, coronavirus; RSV, respiratory syncytial virus.



<b>Table 1.</b> Patient characteristics				
Factor	Infants with $\geq 5$ MSIs n = 27	Infants with 1 - 4 MSIs n = 204	Infants with 0 MSIs n = 54	p overall
MSIs during infancy, No. <sup>1</sup>	5 (5, 6)	2 (1, 3)	0 (0, 0)	–
Month of birth, range	Feb/99 – Apr/00	Nov/98 – Apr/00	Nov/98 – Mar/00	–
Birth weight, kg	3.5 (0.6)	3.5 (0.5)	3.6 (0.6)	0.23
Male gender, No.	18 (67%)	116 (57%)	27 (50%)	0.35
Wheezed during during infancy, No.	23 (85%)	65 (32%)	1 (2%)	< 0.0001 <sup>2</sup>
Wheezing episodes, No	3 (1, 4)	0 (0, 1)	0 (0, 0)	< 0.0001 <sup>2</sup>
$\geq 1$ hospitalization for wheezing, No.	2/27 (7%)	5/204 (2%)	0/54 (0%)	0.15
$\geq 1$ systemic corticosteroid course, No.	10/27 (37%)	18/202 (9%)	0/53 (0%)	< 0.0001 <sup>3</sup>
$\geq 1$ antibiotic treatment, No.	26/27 (96%)	138/204 (68%)	17/54 (31%)	< 0.0001 <sup>4</sup>
Atopic dermatitis during infancy, No.	11 (41%)	89 (44%)	22 (41%)	0.91
Sensitization at year 1, No.	9 (33%)	56/198 (28%)	13/53 (25%)	0.70
Food sensitization, No.	9 (33%)	48/198 (24%)	10/53 (19%)	0.36
Aeroallergen sensitization, No.	5 (19%)	24/198 (12%)	6/53 (11%)	0.61
Total IgE at year 1, kU/L	17.6 (7.0, 31.8)	13.6 (5.2, 32.4)	15.5 (5.6, 26.8)	0.82
B-eos at year 1, $\times 10^9/L^5$	2.0 (1.0, 3.5)	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	0.95
Breast feeding, No.	9 (33%)	64 (31%)	18 (33%)	0.95
Older sibling or day care, No.	27 (100%)	168 (82%)	34 (63%)	0.0002 <sup>6</sup>
Asymptomatic infection per visit, No.	28/60 (47%)	20/90 (22%)	12/43 (28%)	0.0058 <sup>7</sup>
Mixed viral infection per illness or asymptomatic visit, No.	36/150 (24%)	45/414 (11%)	3/187 (2%)	< 0.0001 <sup>8</sup>
Smoke exposure, No.	7 (26%)	54 (26%)	10 (19%)	0.48
Cat at home, No.	9 (33%)	62 (30%)	13 (24%)	0.60

Dog at home, No.	9 (33%)	69 (34%)	23 (43%)	0.47
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Abbreviations: MSI, moderate-to-severe illness; B-eos, blood eosinophil count.

Data presented as median (interquartile range), range, mean (sd), or No. (%).

Data analysed using one-way ANOVA, Kruskal-Wallis ANOVA or Chi square test.

<sup>1</sup>MSI defined as respiratory symptom score  $\geq 5$ .

<sup>2</sup> $\geq 5$  vs 1-4 MSIs and  $\geq 5$  vs 0 MSIs,  $p < 0.0001$  for both.

<sup>3</sup> $\geq 5$  vs 1-4 MSIs,  $p = 0.0003$ ;  $\geq 5$  vs 0 MSIs,  $p < 0.0001$ ;  $\underline{0}$  vs 0-4 MSIs,  $p = 0.029$ .

<sup>4</sup> $\geq 5$  vs 1-4 MSIs,  $p = 0.0012$ ;  $\geq 5$  vs 0 MSIs,  $p < 0.0001$ ;  $\underline{0}$  vs 0-4 MSIs,  $p < 0.0001$ .

<sup>5</sup>Infants with  $\geq 5$  MSIs,  $n = 16$ ; infants with 1-4 MSIs,  $n = 155$ ; infants without MSIs,  $n = 32$ .

<sup>6</sup> $\geq 5$  vs 1-4 MSIs,  $p = 0.018$ ;  $\geq 5$  vs 0 MSIs,  $p = 0.0003$ .

<sup>7</sup> $\geq 5$  vs 1-4 MSIs,  $p = 0.0030$ ;  $\geq 5$  vs 0-4 MSIs,  $p = 0.0030$ . There were 193 asymptomatic scheduled visits where multiplex PCR-based assay had been done.

<sup>8</sup> $\geq 5$  vs 1-4 MSIs,  $p = 0.0001$ ;  $\geq 5$  vs 0 MSIs,  $p < 0.0001$ ;  $\underline{0}$  vs 1-4 MSIs,  $p = 0.0002$ .