ORIGINAL ARTICLE Asthma

The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity

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To cite this article: Turunen RM, Koistinen A, Vuorinen T, Arku B, Söderlund-Venermo M, Ruuskanen O, Jartti T. The first wheezing episode: respiratory virus etiology, atopic characteristics, and illness severity. *Pediatr Allergy Immunol* 2015: **25**: 796–803.

Keywords
atopy; bocavirus; bronchiolitis; child; cough; respiratory syncytial virus; rhinovirus; smoking; virus; wheezing

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Accepted for publication 30 November 2014

DOI:10.1111/pai.12318

Abstract

Background: Susceptibility to early rhinovirus-induced wheezing has been recognized as an important risk factor for childhood asthma, but data on the first wheezing episode are limited. The aim of this selected population study was to investigate virus etiology, atopic characteristics, and illness severity, as well as their interrelation, among first-time wheezing children.

Methods: We studied 111 first-time wheezing children aged between 3 and 23 months (88/23 in-/outpatients). The investigated factors included atopy, food, perennial and aeroallergen sensitization, eczema, atopic eczema, elevated blood eosinophil count, and parental allergic rhinitis, asthma, and smoking. Nasopharyngeal aspirates were analyzed for adenovirus, coronaviruses, enteroviruses, bocavirus-1 (also serologically confirmed), influenza viruses, metapneumovirus, parainfluenza viruses, rhinovirus, and respiratory syncytial virus using PCR methods.

Results: The mean age of the study patients was 12 months (standard deviation 6.0). Atopic characteristics could be found in 56%, atopic eczema in 16%, and sensitization in 23% of the cases. In all samples (100%), ≥1 viruses were detected as follows: rhinovirus (76%), respiratory syncytial virus (29%), bocavirus (18%, acute infections), and other viruses <10% each. Virus coinfections occurred in 38% of the children. Rhinovirus infection was positively associated with age, blood eosinophil count, eczema, and duration of cough, as well as parental allergic rhinitis and smoking but negatively associated with virus coinfection (all $p < 0.05$).

Conclusions: A respiratory virus infection can be detected in all first-time wheezing children. Rhinovirus dominated the findings and was linked to atopic characteristics, prolonged cough, and parental smoking.

Rhinovirus-induced early wheezing is an important risk factor for recurrent wheezing and childhood asthma (1–4). It has been linked to biased immunity (pronounced atopic characteristics and low interferon responses), lower airway inflammation (may respond to systemic corticosteroid), environmental factors (maternal smoking and allergen exposure), and genetics (family history of asthma and allergic rhinitis) (1, 2, 4–6). The detection of rhinovirus in wheezing children increases by age, and rhinovirus starts to dominate the virus etiology after the age of 9–12 months (Table S1) (7–9). These detection rates, however, have shown a 10-fold variability among young children (3–34%) (Table S1). The reasons for this may include insufficient characterization of wheezy episodes or atopy status, age of the subjects, and technical issues, such as the samples used, or the use of less sensitive multiplex PCR and its lower ability to detect different rhinovirus species (7, 10, 11). Data are also limited in how atopic characteristics, virus coinfection, and severity of illness are interrelated in children experiencing the first wheezing episode (12, 13).

Abbreviations
AdV, adenovirus; CV, coronavirus; EV, enteroviruses; Flu, influenza virus; HBoV, human bocavirus 1; Ig, immunoglobulin; MPV, metapneumovirus; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.

Of other major respiratory viruses, acute human bocavirus 1 (HBoV) infection has been serodiagnosed in up to 27% of the first-time wheezing children aged less than 2 yr (14). A prolonged HBoV DNA shedding occurs in the airways, and it may be the reason for the high HBoV coinfection rate (up to 90% of HBoV-positive cases) (15, 16). HBoV infection may modify the course of the rhinovirus-induced wheezing (17).

The aim of this study was to investigate virus etiology, atopic characteristics, illness severity, and their interrelation among 3- to 23-month-old first-time wheezing children. We hypothesized that rhinovirus etiology is linked to atopic characteristics and severity of illness already at the first wheezing episode.

Methods

Patients

Patients were consecutively recruited from the Department of Pediatrics, Turku University Hospital, Turku, Finland, from June 2007 to March 2009, and participated Vinku2 randomized controlled trial with oral prednisolone (4). Inclusion criteria included age between 3 and 23 months, delivery at ≥36 wk, first wheezing episode, and written informed consent from the guardian. Exclusion criteria were chronic illnesses excluding atopic illnesses, previous systemic or inhaled corticosteroid treatment, and/or need for intensive care unit treatment. The Ethics Committee of the Turku University Hospital approved the study protocol.

Study protocol

At study entry, a study physician clinically examined the patients and verified wheeze and breathing difficulty and thereafter examined and scored the patients daily at ward. The respiratory symptom score was calculated as the sum of scores for the degree of dyspnea (0 none, 1 mild, 2 moderate, 3 severe), type of breathing (0 normal, 1 use of stomach muscles, 2 use of intercostal muscles, 3 nasal flaring), severity of auscultatory findings on wheezing (0 none, 1 expiratory, 2 inspiratory and expiratory, 3 audible without stethoscope), and assessment of expiratory:inspiratory time ratio (0 = 1:2; 1 = 1:1; 2 = 2:1; 3 = 3:1) (18). Wheezing was defined as a high-pitched whistling sound in expiration with breathing difficulty (1). The guardian was interviewed using a standardized health questionnaire. The first wheezing episode was defined by the interview and revision of medical records. Also at study entry, nasopharyngeal aspirate (NPA) was taken for virus diagnostics and blood was drawn (19). The patients were ready for discharge when difficulty of breathing had passed. After discharge, respiratory symptoms (rhinitis, cough, and wheezing) were recorded on a daily symptom diary for 2 wk. See more details in Supporting Information.

Laboratory data

Nasopharyngeal aspirates were stored at +4°C until analyzed within 3 days for the presence of rhinovirus, respiratory syncytial virus (RSV), and enteroviruses (EV) using *in-house* single PCR tests (19). Thereafter, the samples were stored at −70°C until analyzed for HBoV DNA using *in-house* single PCR (20) and for a panel of respiratory viruses [adenovirus (AdV), coronavirus (CV) 229E, NL63, OC43, and HKU1, human metapneumovirus (MPV), influenza (Flu) A or B virus, parainfluenza virus (PIV) types 1-3, rhinovirus, and RSV A and B] by multiplex PCR (Seeplex RV12 ACE Detection; Seegene, Seoul, Korea). The HBoV serology was carried out from paired serum samples taken 2–3 wk apart at the Haartman Institute, Helsinki, Finland (14, Data S1.).

Allergen-specific immunoglobulin E (IgE, cutoff 0.35 kU/l, CAP FEIA, Phadiatop Combi®, Phadia, Uppsala, Sweden), total IgE, and blood eosinophil count were analyzed according to the routine diagnostics of Central Laboratory, Turku University Hospital, Turku, Finland (7). The diagnosis of eczema was based on typical symptoms including pruritus, typical morphology, and chronicity of illness. Eczema was defined as atopic if sensitization was found. The term 'atopic characteristics' included specific sensitization, eczema, and/or blood eosinophil count $\geq 0.4 \times 10^9$/l.

Statistics

Statistical power calculations were carried out for the randomized trial (4), but not for the current analysis. In basic statistics, t-test, Mann–Whitney U-test, Pearson's chi-square test, or Fischer's exact test (when count <5) were used when appropriate. The associations between virology, atopic status, and illness severity variables were analyzed using univariable and multivariable logistic regression adjusted to age and sex when relevant. Calculations for illness severity included inpatient status, severity score, duration of hospitalization, and total duration of wheezing and cough. Patients receiving prednisolon (n = 38) were excluded from the illness severity analyses at the time point of study drug initiation because prednisolon is associated with short-term outcomes of acute wheezing (4). The associations between different viruses and virus coinfections were analyzed using logistic regression analysis. Logistic regression results are expressed as odds ratio (OR) and 95% confidence intervals (CI). Statistical significance was established at the level of $p < 0.05$. Data were analyzed using JMP software (Version 8.0.2, SAS Institute, Gary, NC, USA).

Results

Study population

Of the 125 consecutive children that were eligible for the study, 113 patients were enrolled (Fig. 1). After excluding two children with misdiagnoses, 111 children were finally analyzed. Seasonality of the subject recruitment is shown in Table 1.

Patient characteristics

Twenty-one percent of the subjects were aged 3–5 months, 30% 6–11 months, and 50% 12–23 months (Table 1). Fifty-six

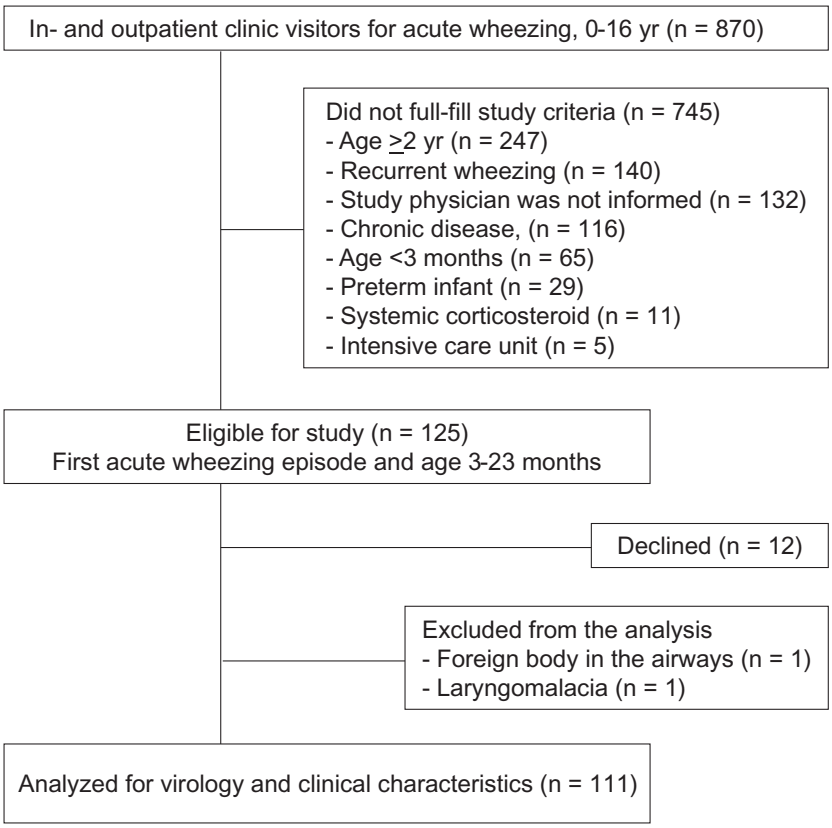

Figure 1 Study flow chart.

percent of the subjects had at least one atopic characteristic. The median duration of respiratory infection symptoms before recruitment was 2 days. Seventy-nine percent of the subjects were hospitalized. For more on patient characteristics see Data S2.

Virus etiology

One or more viruses were detected in the nasopharynx of all the study children (111/111; 100%). A total of 167 PCR-positive virus detections were made in 111 nasopharyngeal aspirates, with one or more viruses detected in each of the 111 (100%) children (Table 2). The most common agent was rhinovirus (76%), followed by RSV (28%), HBoV (PCR or serodiagnosis, 18%), and other viruses <10% each. Overall, 69/111 (62%) single virus infections were found, of which rhinovirus was the most common causative agent (n = 50, 72%), followed by RSV (n = 11, 16%). Viruses other than these two were detected in ≤10% of the cases with single virus infection.

A serodiagnosed acute HBoV infection occurred in 18/20 patients with HBoV DNA (90%). In 2/20 patients (10%), the presence of viral DNA in nasopharynx was found without serologic evidence of an acute infection; a high HBoV IgG antibody level was already detected in the first serum sample without IgM antibody response indicating previous infection.

Virus coinfections were found in 42/111 (38%) of children (Tables 2 and 3). In these 42 patients, rhinovirus was the most commonly detected virus (n = 34, 81%), followed by RSV (n = 20, 49%), HBoV (n = 16, 38%), PIV (n = 9, 22%), CV (n = 5, 12%), EV and AdV (each n = 4, 9.8%), MPV (n = 3, 7.3%), and Flu A or B (n = 2, 4.9%). Two viruses occurred in 71% of the coinfections. The most common dual infection was rhinovirus+HBoV (33% of dual infections). Of the coinfections, 3 viruses were found in 24% of the cases and 4 viruses in 4.8% of the cases. Parainfluenza virus (OR 19 [95% CI 3.3, 350], p = 0.0003), HBoV (OR 8.1 [95% CI 2.7, 31], p = 0.0001), and RSV (OR 4.8 [95% CI 2.0, 12], p = 0.0003) were associated with virus coinfection. Metapneumovirus and rhinovirus were not associated with virus coinfection ($p \geq 0.2$). The total amount of AdV, CV, EV, and Flu A or B detections were too small for this analysis.

Most of the viruses were detected during the winter season (n = 85, 51%) (Table 1). The peak epidemics of rhinovirus, RSV, HBoV, and coinfections can be seen on Fig. S1.

Atopic characteristics and virus etiology

The etiology of rhinovirus was positively associated with age, blood eosinophil count $\geq 0.4 \times 10^9$/l, eczema, atopic eczema, parental allergic rhinitis, and parental smoking (all $p < 0.05$, adjusted to age except eczema and atopic eczema, Table 4, Table S2).

The etiology of RSV was positively associated with inpatient status, but negatively associated with age, male sex, eczema, blood eosinophil count $\geq 0.4 \times 10^9$/l, and parental smoking (all $p < 0.05$, adjusted age and sex, Table 4, Table S2).

Table 1 Patient characteristics

Factor	N = 111
Age, months	12.0 (6.0)
Male sex	74 (67%)
Inpatients	88 (79%)
Atopic characteristics	62 (56%)
Eczema	32 (29%)
Atopic eczema	17/108 (16%)
Sensitization	25/108 (23%)
Food	24/108 (22%)
Aeroallergen	12/108 (11%)
Perennial	11/107 (10%)
Total immunoglobulin E, kU/l*	16 (6, 41)
Blood eosinophil count, 10^9/l*	0.34 (0.09, 0.57)
Season of recruitment	
Spring (March–May)	17 (15%)
Summer (June–August)	12 (11%)
Fall (September–November)	37 (33%)
Winter (December–February)	45 (41%)
Duration of symptoms before recruitment, days	
Rhinitis	3 (2, 6)
Cough	3 (2, 5)
Wheezing	1 (1, 2)
Fever	1 (0, 2)
At study entry	
Heart rate, per minute*	140 (19)
O_2-saturation, %*	97 (95, 98)
Respiratory frequency, per minute*	49 (13)
Blood leukocyte level, $\times 10^9$/l*	11 (3.5)
C-reactive protein, mg/l*	9 (2, 21)
Temperature, °C*	37 (37, 38)
Severity score, scale 0–12†	5.5 (2.4)
Parental self-reported allergic rhinitis	66 (59%)
Maternal self-reported allergic rhinitis	42 (38%)
Parental physician-diagnosed asthma	22 (20%)
Maternal physician-diagnosed asthma	21 (19%)
Parental smoking	45 (41%)
Maternal smoking	21 (19%)
Indoor pets	33 (30%)

Values are presented as number of cases (%), mean (standard deviation), or median (interquartile range). * Data available: total immunoglobulin E, n = 102; blood eosinophil count, n = 107; heart rate, n = 109; O_2-saturation, n = 104; respiratory frequency, n = 110; blood leukocyte level, n = 108; C-reactive protein, n = 101; temperature, n = 105. †Severity scores were assessed on a scale from 0 (none) to 12 (severe) (18).

Table 2 Virus etiology

Virus	RT-PCR (n = 111) (%)	Multiplex PCR (n = 111) (%)	Sero-diagnosis (n = 101) (%)	Total (n = 111) (%)
Rhinovirus	81 (73)	57 (51)		84 (76)
RSV	23 (21)	32 (29)		31 (29)
RSV A		15 (14)		
RSV B		17 (15)		
Human bocavirus 1	19 (17)		18 (20)	20 (18)
Parainfluenza virus		10 (9.0)		10 (9.0)
Parainfluenza virus 1		1 (0.9)		
Parainfluenza virus 2		3 (2.7)		
Parainfluenza virus 3		6 (5.4)		
Metapneumovirus		7 (6.3)		7 (6.3)
Adenovirus		4 (3.6)		4 (3.6)
Coronavirus		5 (4.5)		5 (4.5)
OC43/HKU1		3 (2.7)		
229E/NL43		2 (1.8)		
Enterovirus	4 (3.6)			4 (3.6)
Influenzavirus		2 (1.8)		2 (1.8)
Influenzavirus A		2 (1.8)		
Influenzavirus B		0 (0.0)		
1 virus				69 (62)
2 viruses				30 (27)
3 viruses				10 (9.0)
4 viruses				2 (1.8)
≥1 viruses/sample				111 (100)
≥2 viruses/sample				42 (38)
≥3 viruses/sample				12 (11)
≥4 viruses/sample				2 (1.8)

RT-PCR, reverse transcriptase PCR; RSV, respiratory syncytial virus.

The etiology of acute HBoV was associated with older age (p = 0.015, Table 4, Table S2). Virus coinfection was associated with maternal allergic rhinitis (p = 0.039, adjusted to age).

Illness severity

Rhinovirus etiology was associated with prolonged cough (p = 0.0.12, adjusted to age, Table 5), whereas RSV was associated positively with inpatient status (p = 0.0013, adjusted to age and sex) and duration of hospitalization (p = 0.047, adjusted to age and sex). Virus coinfection, irrespective of the virus etiology, was associated with prolonged wheezing (p = 0.013, adjusted to age).

Discussion

This study shows that acute wheezing in young children is exclusively caused by virus infection. Previous studies on bronchiolitis and acute wheezing have reached an 89–95% overall virus infection rate among children <1 yr of age and a 66–94% overall virus infection rate among children aged <2 yr (Table S1) (9, 12, 21). Before our study, only one seasonal subanalysis, from April to August, resulted in a 100% virus infection rate among under 36-month-old children hospitalized for acute wheezing (22). The 5–11% increase in virus detection rate in our study can probably be explained by good clinical samples (i.e., nasopharyngeal aspirates) and comprehensive virus diagnostics (i.e., sensitive *in-house* PCR for the most important viruses and multiplex PCR for other respiratory viruses) (10, 16, 19).

The rhinovirus detection rate of 76% was clearly higher than in previous studies focusing on the first wheezing episode (7, 9). Previously, this level of rhinovirus detection rate has only been found in recurrently ill infants with a high risk for asthma (23).

Table 3 Virus coinfections, n = 42

Coinfections with 2 viruses	30 (71%)
Rhinovirus+HBoV	10 (24%)
Rhinovirus+RSV	6 (14%)
Rhinovirus+MPV	3 (7.1%)
Rhinovirus+PIV	2 (4.8%)
Rhinovirus+CV	2 (4.8%)
RSV+CV	2 (4.8%)
RSV+PIV	2 (4.8%)
AdV+PIV	1 (2.4%)
Rhinovirus+EV	1 (2.4%)
RSV+HBoV	1 (2.4%)
Coinfections with 3 viruses	10 (24%)
Rhinovirus+RSV+EV	2 (4.8%)
Rhinovirus+RSV+HBoV	2 (4.8%)
Rhinovirus+RSV+Flu	1 (2.4%)
Rhinovirus+RSV+PIV	1 (2.4%)
Rhinovirus+MPV+PIV	1 (2.4%)
Rhinovirus+HBoV + CV	1 (2.4%)
RSV+AdV+PIV	1 (2.4%)
RSV+Flu+HBoV	1 (2.4%)
Coinfections with 4 viruses	2 (4.8%)
Rhinovirus+AdV+HBoV+PIV	1 (2.4%)
Rhinovirus+AdV+EV+RSV	1 (2.4%)

RSV, respiratory syncytial virus A/B; HBoV, human bocavirus 1; EV, enteroviruses; PIV, parainfluenza virus types 1-3; AdV, adenovirus; Flu, influenza A/B virus; MPV, metapneumovirus; CV, coronavirus OC43/HKU1/229E/NL43.

The high prevalence of rhinovirus is most likely explained by a more sensitive real-time PCR than that of the conventional PCR (11). As rhinovirus infection is closely linked to wheezing status, atopy, and increasing of age (3, 7), the dominance of rhinovirus in our findings may also be explained by our solely wheezing cohort, high prevalence of atopic characteristics (56%), and the relatively high mean age (12 months) of the study patients. Our in-house rhinovirus PCR is known to detect also rhinovirus C genotypes. The sequencing of the amplicons is currently under investigation.

Our study shows that already the first rhinovirus-induced wheezing episode was closely associated with atopic characteristics, especially with typical first manifestations of atopic march, eczema, and blood eosinophil count. These findings are in agreement with previous studies (2, 7, 9, 24); however, the association of the first rhinovirus-induced wheezing episode with prolonged cough has not been reported earlier (1, 3). This finding supports the hypothesis that children susceptible to rhinovirus-induced wheezing may already have a chronic, partly atopy-related inflammation in the lower respiratory tract manifesting as cough, and this condition is exacerbated by rhinovirus infection (4). Importantly, already the first rhinovirus-induced wheezing episode has been associated with an increased risk of recurrent wheezing as a long-term sequel and this risk may be even greater if the patient has atopic characteristics or a family history of asthma (2, 7). In the cohort of slightly older children with recurrent wheezing, many studies have linked rhinovirus-induced wheezing to atopic characteristics, that is, specific IgE sensitization, increased eosinophil count, atopic eczema, and maternal atopic asthma (1, 3, 13, 25, 26).

Earlier research has shown that maternal smoking during pregnancy is an independent risk factor for rhinovirus-induced wheezing (27). Our study adds that there is a link between parental smoking and rhinovirus etiology of the first wheezing episode. The exact mechanism behind this association is yet unclear. One potential mechanism could be a bronchial inflammation caused by tobacco smoke (6), which could increase the risk for rhinovirus-induced lower airway illness.

Despite a major RSV epidemic, its detection rate within this study reached only 28%, whereas earlier RSV has been detected in up to 80% of wheezing children aged <24 months (21). Traditional bronchiolitis with non-specific noisy breathing is typically an RSV-induced illness in infants, whereas wheezing typically occurs in older children and links more closely to rhinovirus (2, 7, 9). Thus wheezing, being our inclusion criterion, biases the prevalence numbers toward rhinovirus. The terms 'rhinovirus-induced wheezing' and 'RSV-induced bronchiolitis' well describe the typical clinical picture of these illnesses. In agreement with previous studies, RSV etiology has been linked to hospitalization (12, 28) and not to atopic characteristics (25, 29).

Although HBoV is the third most common pathogen of lower airway illnesses among young children, its causative role is still being questioned (7, 9, 24, 30). Earlier HBoV DNA has been found in up to 20% of respiratory samples from wheezing children aged <24 months, but with serology HBoV infections, detection rate has increased to 27%, indicating that serological diagnosis is not only more accurate, but also more sensitive for HBoV diagnostics (14, 16). These numbers together suggest that HBoV commonly causes lower airway symptoms. Of our study patients, only two older children (10% of our HBoV cases) appeared to have prolonged HBoV shedding. The number is too low to allow for a comparison with previous studies, which have indicated HBoV shedding in up to 38% of the cases (16, 30). In agreement with previous studies, HBoV did become more prevalent with age in childhood and was not associated with illness severity (14, 16). HBoV-induced bronchiolitis/wheezing has not been associated with atopic characteristics as was also observed in our study (3, 25, 29).

Virus coinfections occurred in 38% of the cases, which is close to earlier detection rates among children with the first wheezing episode (7, 9). However, the coinfection rate can be as high as 51% among wheezing children aged <24 months (15). In our study, PIV, HBoV, and RSV were significantly linked to virus coinfections. The high coinfection rates of PIV and HBoV are in concordance with previous studies (15, 16), whereas RSV has not shown close associations with the presence of other viruses (15). Even though in our study rhinovirus was the most often detected virus (81%) in coinfections, it was not—due its overall high prevalence—statistically associated with them. This emphasizes its role as a single causative agent of wheezing. Importantly, we found coinfections with up to four different viruses, which highlights

Table 4 The associations between patient characteristics and virology

Factor	Rhinovirus n = 84	RSV n = 31	HBoV n = 18	Virus coinfection n = 42
Age, months	***1.1 (1.0, 1.2)***	***0.90 (0.83, 0.97)***	***1.1 (1.0, 1.2)***	0.98 (0.92, 1.0)
Male sex	2.3 (0.95, 5.7)	***0.33 (0.14, 0.79)***	0.56 (0.20, 1.6)	0.94 (0.45, 2.3)
Atopic characteristics				
Univariable	***5.4 (2.1, 12)***	***0.26 (0.10, 0.61)***	0.99 (0.36, 2.8)	0.72 (0.33, 1.6)
Multivariable	***4.0 (1.5, 12)***	***0.37 (0.14, 0.97)***	0.52 (0.16, 1.7)	0.73 (0.32, 1.7)
Sensitization				
Univariable	2.8 (0.87, 13)	0.55 (0.17, 1.5)	1.0 (0.27, 3.3)	0.90 (0.34, 2.2)
Multivariable	1.6 (0.42, 7.5)	0.99 (0.27, 3.3)	0.53 (0.12, 1.9)	0.98 (0.35, 2.6)
Food sensitization				
Univariable	2.6 (0.81, 12)	0.59 (0.17, 1.6)	1.1 (0.28, 3.5)	1.2 (0.47, 3.1)
Multivariable	1.6 (0.43, 7.6)	0.98 (0.27, 3.2)	0.65 (0.16, 2.3)	1.4 (0.50, 3.6)
Aeroallergen sensitization				
Univariable	p = 0.066*	**p = *0.018*†**	0.45 (0.024, 2.5)	0.29 (0.043, 1.2)
Multivariable			0.16 (0.0081, 1.1)	0.29 (0.040, 1.3)
Total IgE ≥45 kU/l				
Univariable	1.5 (0.18, 2.0)	0.29 (1.1, 16)	1.7 (0.49, 5.4)	0.83 (0.30, 2.1)
Multivariable	0.86 (0.24, 3.5)	0.46 (0.090, 1.8)	0.99 (0.25, 3.4)	0.98 (0.34, 2.7)
B-Eos ≥ 0.4 × 10^9/l				
Univariable	***15 (4.0, 94)***	***0.17 (0.080, 0.78)***	0.96 (0.32, 2.7)	0.53 (0.23, 1.2)
Multivariable	***11 (2.9, 72)***	***0.27 (0.068, 0.63)***	0.51 (0.15, 1.6)	0.51 (0.21, 1.2)
Eczema				
Univariable	***4.2 (1.3, 19)***	***0.19 (0.042, 0.59)***	0.94 (0.28, 2.8)	0.44 (0.17, 1.1)
Multivariable	3.4 (1.0, 15)	***0.23 (0.050, 0.73)***	0.63 (0.17, 2.0)	0.45 (0.17, 1.1)
Atopic eczema				
Univariable	***6.1 (1.1, 110)***	0.29 (0.04, 1.1)	1.2 (0.25, 4.2)	0.45 (0.12, 1.4)
Multivariable	3.2 (0.54, 62)	0.56 (0.077, 2.6)	0.57 (0.11, 2.3)	0.45 (0.11, 1.5)
Parental allergic rhinitis				
Univariable	***2.8 (1.1, 6.9)***	0.53 (0.23, 1.2)	2.0 (0.68, 6.5)	1.6 (0.74, 3.7)
Multivariable	***2.5 (1.0, 6.5)***	0.69 (0.28, 1.7)	1.6 (0.18, 1.8)	1.7 (0.77, 3.9)
Parental asthma				
Univariable	2.3 (0.71, 11)	0.51 (0.14, 1.5)	0.78 (0.17, 2.7)	1.2 (0.44, 3.0)
Multivariable	2.4 (0.66, 12)	0.51 (0.13, 1.7)	0.77 (0.16, 2.7)	1.2 (0.44, 3.1)
Parental smoking				
Univariable	***3.0 (1.2, 9.0)***	0.50 (0.20, 1.2)	0.69 (0.22, 1.9)	0.61 (0.27, 1.3)
Multivariable	***3.4 (1.2, 10)***	***0.38 (0.14, 1.0)***	0.67 (0.21, 2.0)	0.61 (0.27, 1.3)

B-Eos, blood eosinophil count; IgE, immunoglobulin E; RSV, respiratory syncytial virus; acute HBoV, acute human bocavirus 1. Data expressed as OR (95% confidence interval). Bold and italic face indicates a significant result, $p < 0.05$. B-Eos and total IgE were log-transformed. In regression analysis, factor positive values were compared to factor negative values. Multivariable analyses were adjusted to age (rhinovirus, HBoV, and virus coinfection), or to age and sex (RSV).

*Odds ratio for rhinovirus etiology and aeroallergen sensitization was not calculable, because there was no aeroallergen-sensitized patients in rhinovirus-negative group: Odds Ratio = (rhinovirus-positive patients with aeroallergen sensitization/rhinovirus-negative patients with aeroallergen sensitization)/(rhinovirus-positive patients with no aeroallergen sensitization/rhinovirus-negative patients with no aeroallergen sensitization) = (12/0)/(70/26) = not calculable.

†Odds ratio for RSV etiology and aeroallergen sensitization was not calculable because there was no aeroallergen-sensitized patients in RSV-positive group: Odds Ratio = (RSV-positive patients with aeroallergen sensitization/RSV-negative patients with aeroallergen sensitization)/(RSV-positive patients with no aeroallergen sensitization/RSV-negative patients with no aeroallergen sensitization) = (0/12)/(31/65) = 0.

the need of quantitative PCR with standardized sampling procedures and serology. These methods in turn would give more accurate information on which virus is actively replicating at the very moment, and which viral infections are incipient or waning as reviewed in Jartti et al. (10). Virus–virus cross talk may also modulate the immune responses of viruses in coinfections, as we have recently shown between HBoV and rhinovirus (17). The association between coinfections and longer duration of wheezing may, however, indicate a more severe illness, due to either simultaneous, or more often, consecutive viral infections (23, 24).

In conclusion, our study demonstrates that a respiratory virus can be detected in all children experiencing a first-time wheezing episode. Rhinovirus is an important etiologic agent

Table 5 The associations between virus etiology and the severity of illness

	Inpatients vs. outpatients n = 111	Severity score at study entry ≥6 vs. <6 n = 110	Duration of hospitalization ≥24 h vs. <24 h n = 81	Total duration of wheezing ≥3 days vs. <3 days n = 75	Total duration of cough ≥14 days vs. <14 days n = 74
Rhinovirus					
+ vs. −					
Univariable	0.59 (0.16, 1.8)	1.5 (0.63, 3.8)	0.57 (0.21, 1.5)	0.35 (0.050, 1.5)	***2.7 (1.0, 7.7)***
Multivariable	0.56 (0.15, 1.8)	1.5 (0.58, 3.8)	0.79 (0.27, 2.3)	0.44 (0.061, 2.0)	***3.5 (1.2, 11)***
RSV					
+ vs. −					
Univariable	***5.2 (1.4, 34)***	0.59 (0.25, 1.4)	***2.8 (1.1, 7.3)***	2.9 (0.67, 20)	1.3 (0.48, 3.3)
Multivariable	***9.5 (2.2, 68)***	0.69 (0.27, 1.7)	2.4 (0.83, 7.2)	2.2 (0.47, 16)	1.2 (0.44, 3.3)
HBoV					
+ vs. −					
Univariable	1.4 (0.40, 6.3)	0.57 (0.18, 1.6)	1.1 (0.35, 3.5)	2.3 (0.38, 44)	0.74 (0.20, 2.5)
Multivariable	1.4 (0.38, 6.5)	0.52 (0.16, 1.5)	1.5 (0.46, 5.1)	3.1 (0.49, 61)	0.81 (0.22, 2.8)
Virus coinfection vs. single virus infection					
Univariable	2.0 (0.74, 5.9)	1.0 (0.48, 2.3)	1.8 (0.74, 4.5)	***8.3 (1.5, 150)***	1.6 (0.64, 4.2)
Multivariable	2.0 (0.74, 5.9)	1.1 (0.49, 2.3)	1.7 (0.66, 4.3)	***8.7 (1.5, 160)***	1.6 (0.64, 4.2)

RSV, respiratory syncytial virus; HBoV, acute human bocavirus 1; coinfections, ≥1 virus detected. Data expressed as odds ratio (95% confidence interval). Bold and italic face indicates a significant result, $p < 0.05$. In regression analysis, factor positive values were compared to factor negative values. Multivariable analyses were adjusted to age (rhinovirus, HBoV and virus coinfection), or to age and sex (RSV). Severity score was calculated as a sum of score for degree of dyspnea, type of breathing, auscultatory findings, and expiratory:inspiratory time ratio (18).

already at the first wheezing episode and it closely associates with age, atopic characteristics, and parental smoking. In the age-group of this study, rhinovirus was more closely linked to wheezing than RSV. While RSV was associated with hospitalization, rhinovirus was linked to prolonged cough suggesting a more chronic type of illness in children susceptible to rhinovirus-induced wheezing. HBoV was the third most prevalent lower airway pathogen. It was not linked to atopic characteristics, and prolonged HBoV shedding was rare in this cohort. Our results call attention to the differences in clinical characteristics between these three major viral causes of wheezing in clinical practice and research (4).

Acknowledgments

We thank Helena Kantola, Johanna Vänni, and Heini Niskala for the virus analyses. This work was supported by grants from the Academy of Finland, Helsinki; the Finnish Medical Foundation, Helsinki; the Sigrid Jusélius Foundation, Helsinki; the Foundation for Pediatric Research, Helsinki; Allergy Research Foundation in Southwest Finland, Turku; and the Helsinki University Research Funds, all in Finland.

Conflict of interest

The authors have no conflict of interest in connection with this study.

Author contributions

Drs Turunen and Jartti have participated sufficiently in the work of this manuscript to take public responsibility for the whole content. Study concept and design: Turunen, Vuorinen, Ruuskanen, Jartti. Acquisition of data: Vuorinen, Jartti. Analysis and interpretation of data: Turunen, Koistinen, Vuorinen, Arku, Söderlund-Venermo, Jartti. Drafting of the manuscript: Turunen, Jartti. Critical revision of the manuscript for important intellectual content: Turunen, Koistinen, Vuorinen, Arku, Söderlund-Venermo, Ruuskanen, Jartti. Administrative, technical, or material support: Vuorinen, Söderlund-Venermo, Ruuskanen, Jartti. Study supervision: Jartti.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Data S2. Results.

Table S1. Molecular virus etiology of the first wheezing episode in children aged less than 3 yr.

Table S2. The associations between patient characteristics and virus etiology.

Figure S1. Virus etiology of rhinovirus (RV), respiratory syncytial virus (RSV), human bocavirus 1 (HBoV, DNA in NPA and/or serodiagnosed acute infection) and other sole viruses (metapneumovirus, influenza virus, parainfluenza virus, enterovirus, coronavirus) together with coinfection of each.