

Role of Emerging Respiratory Viruses in Children With Severe Acute Wheezing

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Summary. Background Acute wheezing episodes are frequently associated with respiratory viral infections in children. However, the role of the recently described respiratory viruses is not yet fully understood. Objective The main objective of this study was to estimate the frequency of human metapneumovirus (HMPV), human bocavirus (HBoV), and 14 other respiratory viruses in hospitalized children with acute wheezing. Methods A prospective study was conducted on children <14 years old, admitted with an acute expiratory wheezing episode from September 2005 to June 2008. Viruses were detected in nasopharyngeal aspirates by polymerase chain reaction. Clinical data were prospectively recorded. Results A viral pathogen was identified in 444 (71%) out of 626 hospitalized acute wheezing episodes. Respiratory syncytial virus (RSV) was the most frequently detected (27%), followed by rhinovirus (24%), adenovirus (17.8%), HBoV (16%), and HMPV (4.7%). The rate of viral detection was significantly higher in infants (77.3%), than in older children (59.8%) ($P < 0.001$). RSV and HBoV were more prevalent in infants ($P < 0.001$) than in older children. Conclusion The most prevalent viruses found in severe acute wheezing episodes were RSV and rhinovirus not only in childhood, but also in infancy. However, other emerging viruses such as HBoV and metapneumovirus also play an important role in wheezing episodes. **Pediatr Pulmonol.** 2010; 45:585–591. © 2010 Wiley-Liss, Inc.

Key words: respiratory viruses; wheezing; human bocavirus; human metapneumovirus; rhinovirus; children; infants.

INTRODUCTION

Acute viral respiratory infection is one the leading cause of hospitalization of young children and may be related to a large number of wheezing episodes in all age groups, but especially in children.¹ A wide range of respiratory viruses, including rhinoviruses, respiratory syncytial virus (RSV), influenza viruses, coronaviruses, human parainfluenza viruses, human metapneumovirus (HMPV), and the recently discovered human bocavirus (HBoV) have been detected in pediatric patients with wheezing.^{2–8} Rhinovirus seems to be the most common respiratory virus associated with acute wheezing in school-age children, whereas RSV appears to be more frequent in younger children. However, the role of the novel respiratory viruses—HMPV and HBoV—has not been well studied yet and further studies are needed to better understand the relationship between these new respiratory viruses and acute wheezing in children.

We conducted this study to estimate the frequency of detection of HBoV, HMPV, and 14 other respiratory viruses in hospitalized children with an acute wheezing episode and to compare it with that observed in asymptomatic children. Clinical and epidemiological features associated with the newer and traditional respiratory viruses were also compared.

PATIENTS AND METHODS

This was a cross-sectional, prospective, and descriptive substudy of an ongoing investigation of respiratory tract infections in children, funded by FIS (Fondo de Investigaciones Sanitarias—Spanish Health Research Fund) Grants no.: 98/0310, PI06/0532 and approved by The Medical Ethics Committee. The study population was composed of all children <14 years of age, consecutively hospitalized at the secondary public hospital Severo Ochoa (Leganés, Madrid), for acute expiratory wheezing,

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from September 2005 to June 2008. Samples were collected after informed consent was obtained from the parents or legal guardians. In order to reduce as much as possible the variability of the patients included, and according to the criteria of McConnochie,⁹ acute expiratory wheezing was considered to be bronchiolitis when it occurred for the first time in children aged <2 years and these children were, therefore excluded.

All patients were evaluated by an attending physician. During the hospital stay, and as part of the study, a physician completed a standardized form with the following variables: age, sex, history of prematurity, and underlying chronic diseases, need for oxygen therapy assessed by transcutaneous oxygen saturation, axillary temperature $\geq 38^{\circ}\text{C}$, presence of infiltrate/atelectasis in radiographs, administration of antibiotic therapy, duration of hospital stay, total white blood cell (WBC) count, C-reactive protein (CRP) serum values, and result of blood culture when it was done. Oxygen therapy was provided in order to achieve oxygen saturation $\geq 94\%$.

Virus Sampling

On admission, a nasopharyngeal aspirate sample was obtained using a standardized procedure. Each specimen was sent to the Influenza and Respiratory Virus Laboratory at the National Microbiology Center (ISCIII, Madrid, Spain) for virological testing. Specimens were processed within 24 hr of collection. Upon receipt of NPA, 300 μl samples were aliquoted in the Sample Reception Unit, where a total of three aliquots were prepared and stored at -70°C . The reception area and the NPA sample aliquoting area were separate from the working areas.

Nucleic Acid Extraction

Total nucleic acid was extracted from a 200 μl aliquot of each clinical specimen using the BioRobot M48 workstation and the MagAttract Virus Mini M48 Kit (Qiagen, Hilden, Germany) for PCR assays. A cloned amplified product of a DNA fragment supplied by the Access RT-PCR system kit (Promega, Madison WI) was added to the lysis buffer as an internal control for checking both the efficiency of nucleic acid extraction and the presence of amplification inhibitors. Internal control was quantified in such a way that a total of 100 molecules per tube were included.

Virus Detection Methods

Nucleic acids extracted from all NPAs were tested for HBoV using a sensitive and specific nested PCR method described previously.⁶ Influenza virus A, B, and C, RSV A and B and adenoviruses were detected by using a multiplex RT-nested PCR,¹⁰ and parainfluenza viruses 1–4, human coronaviruses 229E and OC43, enteroviruses

and rhinoviruses by using a second multiplex RT-nested PCR¹¹ as described previously by our group. HMPV was investigated in all samples using a RT-nested PCR to amplify the matrix gene.¹²

Prevention of PCR Contamination

Because of the high sensitivity of nested PCR, precautions were taken to prevent contamination of reaction tubes with previously amplified product or target RNA or DNA from other specimens and controls. The aliquoting of respiratory samples, processing of samples, preparation of reagents and nested PCR were performed in safety cabinets located in separate laboratories. Each cabinet was equipped with an independent batch of reagents, micropipette sets, sterile reagent tubes, and filtered pipette tips.

Statistical Analysis

Values were expressed as percentages for discrete variables, or as mean and standard deviation for continuous variables. Clinical characteristics and laboratory variables were compared using the Student *t*-test, the Mann–Whitney *U*-test, the one-way ANOVA test with the Games–Howell post hoc test for multiple comparisons, the χ^2 test, and Fisher's exact test. In order to avoid confusion, patients were stratified in two groups, younger and older than 2 years of age, to better analyse clinical and virological features. A two-sided value of $P < 0.05$ was considered statistically significant. All analyses were performed with the Statistical Package for the Social Sciences (SPSS), Version 15.0.

RESULTS

Viral Findings

During the study period 707 wheezing episodes were diagnosed. Eighty-one patients were excluded, either because of lack of NPA samples or because they refused to participate. Therefore, a total of 626 episodes corresponding to 539 patients were analyzed. A viral pathogen was identified in 444 episodes (71%); RSV was the most frequent virus detected (27%), followed by rhinovirus (24%), adenovirus (17.8%), HBoV (16%), and HMPV (4.7%). All coronaviruses were identified as OC43. Mixed viral infections were found in 34.5% of positive episodes. HBoV, adenovirus, coronavirus, and parainfluenza virus were identified more frequently as coinfection than as a single infection (see Table 1).

The rate of viral detection was significantly higher in infants <2 years than in older children: 77.3% versus 59.8% ($P < 0.001$; OR: 1.398; 95% CI: 1.192, 1.640); Coinfections were also more frequent in the younger group: 30.8% versus 19% ($P = 0.002$; OR: 1.225; 95% CI: 1.086, 1.382). Differences in the relative frequency of the

TABLE 1—Respiratory Viruses Identified in 626 Acute Wheezing Episodes During a 3-Year Study Period (Single Versus Dual or Multiple Infection)

Virus	Single infection, N	Dual or multiple infection, N
Respiratory syncytial virus	102	68
Rhinovirus	84	73
Adenovirus	47	63
Human bocavirus	33	67
Human metapneumovirus	15	15
Parainfluenza virus type 1–4	8	21
Influenza A, B, and C viruses	8	8
Coronavirus types OC43 and 229E	1	8
Enterovirus	4	6
Total	302	269

different viruses according to age were also detected: RSV and HBoV were more prevalent in infants than in older children ($P < 0.001$) (Fig. 1). Six out of nine coronaviruses were detected in infants <2 years.

Clinical Characteristics of Hospitalized Children

Three hundred ninety-seven (63.4%) children were <2 years of age (mean age: 12.0 months) and 229 (36.6%) were >2 years (mean age 4.6 years), with a clear predominance of males (63%). Over the 3-year study period most cases of wheezing admissions, in both groups, occurred in November and December (Fig. 2). However, some differences were found when monthly distribution was analyzed in relation to age and virus detection. In fact,

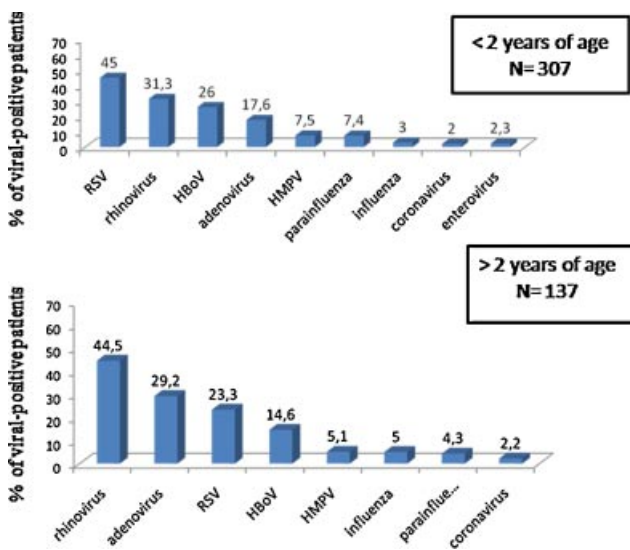


Fig. 1. Percentage of viral detection in virus-positive patients stratified by age. HBoV, human bocavirus; HMPV, human metapneumovirus.

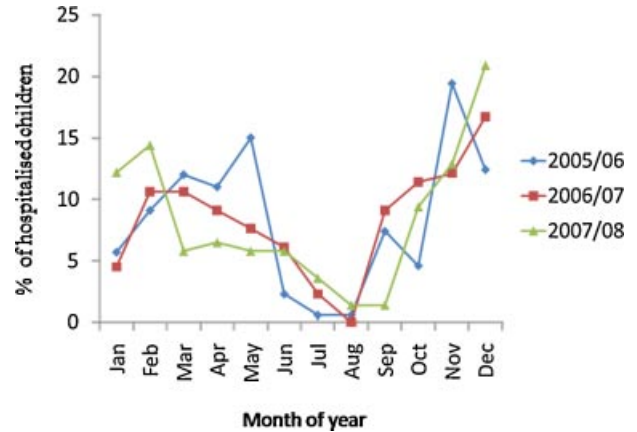


Fig. 2. Percentage of patients hospitalized monthly with acute wheezing episode during the 3-year study period.

whereas the peak of wheezing hospitalization in infants reached its maximum intensity in November and December, school-age children showed clearly two peaks; the first one in May and the second started in September and remained quite stable during fall and winter months (Fig. 3).

Admission to hospital for virus-associated wheezing episodes occurred mainly in November. The rate of viral detection rose in these months to 90% in infants <2 years of age and to 70% in older children. RSV and HBoV wheezing episodes occurred mainly in November and December, whilst most HMPV-wheezing was diagnosed in spring months. Rhinovirus infections occurred throughout the year although the highest overall activity was observed during fall (September to December) and spring months (March to May) (Fig. 4). Seven of nine coronavirus-associated wheezing patients were admitted in spring and summer. By contrast, the highest frequency of wheezing admissions without any viral detection occurred in May ($P < 0.001$), when the rate of viral detection was only 57.6% and 34.4% in younger and older children, respectively.

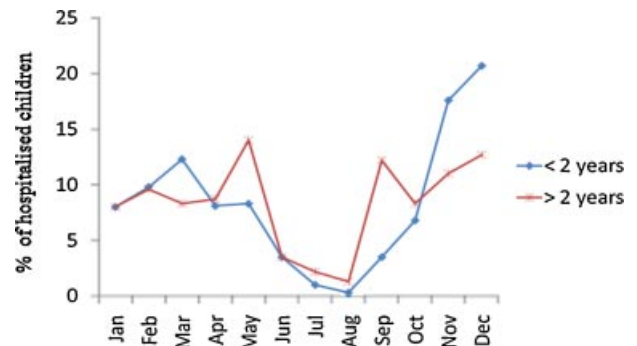


Fig. 3. Monthly distribution of hospitalized wheezing episodes by age.

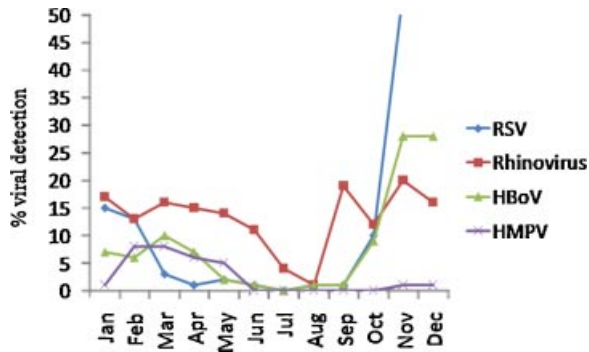


Fig. 4. Percentage of monthly virus-associated wheezing episodes.

Regarding clinical characteristics, virus-positive and virus-negative wheezing episodes were compared after stratifying both groups by age—younger and older than 2 years (Tables 2 and 3). Fever was more frequent with a higher temperature in documented viral infections in both

age groups. Antibiotics were prescribed more frequently in infants with viral infection, who also needed longer oxygen therapy. However, no significant differences could be found regarding the presence of infiltrate/atelectasis or length of hospital stay between viral positive and negative wheezing episodes. On the other hand, and irrespectively of viral detection, children <2 years of age needed a longer hospital stay ($P = 0.001$), longer oxygen therapy ($P = 0.04$), and had fever ($P < 0.006$) more frequently than older children.

Clinical characteristics of wheezing episodes associated with single viral infections were compared (Table 4). As RSV infections were the most common, all other respiratory infections were compared with them. Firstly, some significant differences could be recorded with respect to rhinovirus-positive episodes: rhinovirus-positive children were older ($P = 0.003$), presented less frequently with fever ($P < 0.001$) and hypoxia ($P = 0.06$), and had shorter hospital stays ($P < 0.001$)

TABLE 2—Clinical Characteristics of Virus-Positive Versus Virus-Negative Asthma Exacerbations in Children <2 Years of Age (N = 397)

	Patients with ≥ 1 virus, N (%)	Patients with no virus detected, N (%)	P
Males	200 (65.1)	56 (62.2)	0.610
Prematurity	53 (17.3)	16 (18)	0.886
T > 38°C	210 (68.4)	49 (54.4)	0.014
T (°) max	38.5 ± 0.8	38.2 ± 0.9	0.038
Duration of fever (days, mean)	3.0 ± 2.0	3.2 ± 2.3	0.577
SatO ₂ <95%	198 (64.5)	55 (61.8)	0.641
Duration of hypoxia (days, mean)	2.6 ± 2.1	1.9 ± 1.6	0.009
Length hospital stay (days, mean)	4.6 ± 2.7	4.3 ± 2.4	0.238
Infiltrate/atelectasis	86 (28.1)	33 (36.7)	0.264
Antibiotic treatment	54 (17.8)	8 (8.9%)	0.042
WBC count × 10 ⁹ cells/L	12.9 ± 5.3	12.0 ± 4.4	0.278
CRP level mg/L	29.0	32.0	0.664
Total	307	90	

Boldface values indicate $P < 0.05$.

TABLE 3—Clinical Characteristics of Virus-Positive Versus Virus-Negative Acute Wheezing Episodes in Children >2 Years of Age (N = 229)

	Patients with ≥ 1 virus, N (%)	Patients with no virus detected, N (%)	P
Males	84 (61.3)	55 (59.8)	0.816
Prematurity	18 (13.1)	6 (6.5)	0.109
T > 38°C	75 (54.7)	35 (38)	0.013
T (°) max (mean)	38.6 ± 0.7	37.9 ± 1	<0.001
Duration of fever (days, mean)	2.8 ± 2.1	2.6 ± 2.0	0.728
SatO ₂ <95%	100 (73)	61 (66.3)	0.277
Duration of hypoxia (days, mean)	2.1 ± 2.1	1.7 ± 1.9	0.250
Length hospital stay (days, mean)	3.5 ± 2.4	3.2 ± 2.0	0.256
Infiltrate/atelectasis	42 (30.7)	19 (21)	0.218
Antibiotic treatment	25 (18.4)	10 (11)	0.123
WBC count × 10 ⁹ cells/L	14.6 ± 10.4	14.2 ± 6.0	0.827
CRP level mg/L	34.0 ± 39	55.9 ± 95	0.123
Total	137	92	

Boldface values indicate $P < 0.05$.

TABLE 4—Comparison of Clinical Presentation and Outcome of Patients With Single Infection Due To Different Viruses

	RSV	Rhinovirus	Adenovirus	HBoV	hMPV	<i>P</i>
Male	56 (56%)	57 (71.3%)	24 (57%)	19 (68%)	10 (62.5%)	0.261 ¹
T > 38°C	80 (80%)	34 (42.5%)	27 (64.3%)	18 (64.3%)	10 (62.5%)	<0.001 ¹
SatO ₂ <94%	75 (75%)	47 (58.8%)	28 (66.7%)	16 (57%)	13 (81.3%)	0.088 ¹
Antibiotic treatment	16 (16%)	8 (10%)	14 (33.3%)	3 (10.7%)	3 (18.8%)	0.019 ¹
Infiltrate/atelectasis	34 (41.5%)	17 (33%)	16 (61.5%)	8 (44.4%)	6 (40%)	0.221 ¹
Age <2 years	79 (79%)	45 (56.3%)	22 (52.4%)	20 (71.4%)	11 (68.8%)	0.005 ¹
Age (months, mean)	18	32.5	28	18.8	23.2	0.001 ²
Length hospital stay	5.12	3.5	4.3	3.8	3.8	0.001 ²
Duration of fever (days, mean)	3.2	2.2	3.3	2.8	4.3	0.07 ²
Duration of hypoxia (days, mean)	3.2	1.6	3.0	2.0	2.0	0.004 ²
WBC count × 10 ⁹ cells/L	11,452	17,268	17,495	11,341	11,222	0.01 ²
CRP level mg/L	28.8	33.8	31.3	16.1	15.3	0.611 ²

HBoV, human bocavirus; hMPV, human metapneumovirus; WBC, white blood cells; CRP, C-reactive protein.

¹Chi-square test.

²One-way ANOVA.

than RSV-positive patients. Secondly, adenovirus-positive patients were also older ($P=0.03$), showed infiltrate in chest radiographs ($P=0.05$), were prescribed antibiotic therapy ($P=0.002$), and had leukocytosis ($P=0.002$) more frequently than RSV-positive patients. Lastly, HBoV-wheezing episodes shared many similarities with those associated with RSV; notably the proportion of patients with hypoxia was smaller in the HBoV group ($P=0.06$). No significant differences could be found between RSV and HMPV wheezing episodes.

Finally, clinical characteristics of single and dual/multiple infections were compared. Children with dual/multiple infections were significantly younger (20.4 ± 18 months, $P=0.045$). No other noticeable differences could be shown between the two groups.

DISCUSSION

The frequency of respiratory virus detection in 626 wheezing episodes in hospitalized children was, in this prospective study, 71%. This figure was even higher in children <2 years of age, where the rate of viral detection reached 75.5%. Our results agree with most recent studies published in children with acute wheezing. Khetsuriani et al.¹³ identified at least one respiratory virus in 63% of 65 children older than 2 years with acute wheezing. Kusel et al.¹⁴ in a community-based cohort of 198 children at high atopic risk, found 68.9% of specimens from children with wheezing, tested positive for any respiratory virus. Gendrel et al.¹⁵ identified one respiratory virus in 34% of 50 children aged 2–15 years hospitalized with severe acute wheezing. A study recently published by Allander et al.,² which included 259 children hospitalized for acute wheezing, found a potential viral pathogen in 95%. This high rate of viral detection is probably related to the use of very sensitive diagnostic methods, but that these authors also included infants with a first episode of acute bronchiolitis. A viral etiology for bronchiolitis is well

known^{16,17} and in recent reports up to 90% of infants with a first episode of wheezing have evidence of a viral infection.¹⁸ A major strength of our study is, besides the use of a panel of very sensitive PCR assays for a full range of respiratory viruses—described by our group elsewhere,^{6,10–12} the exclusion of those patients with a first episode of wheezing and the enrollment of a high number of patients—almost 630—over three full calendar years. This long inclusion period reduces potential biases towards seasonal differences in respiratory virus circulation. Therefore, our results may reflect quite accurately the relative contribution of each respiratory virus to severe acute wheezing episodes in hospitalized children.

Our data confirm that RSV and rhinovirus play a key role in acute wheezing episodes in children; RSV was the most frequent respiratory virus detected, closely followed by rhinovirus, in infants <2 years of age; whereas rhinovirus was the most prevalent in older children. However, unlike the observations of Khetsuriani et al.¹³ other viruses including HBoV, were also frequently identified (16%) in severe wheezing episodes. In fact, HBoV was the third most frequent virus found in infants and the fourth in older children. Our findings confirm that HBoV is a common virus in hospitalized children with acute wheezing and are consistent with those obtained by Allander et al.² (19% of HBoV), Smuts et al.⁸ (7.4%), and Chung et al.¹⁹ (13.8%). Adenovirus, which has been largely associated with obliterans bronchiolitis,²⁰ has also been identified in hospitalized children with an acute wheezing episode.^{21,22} However, the high frequency of adenovirus amongst our wheezy patients deserves to be highlighted. In our series, we found adenovirus in 17.8% of all patients, being the second most frequent virus in older children, only after rhinovirus. As far as we know, this is the first report of such a high frequency of adenovirus in acute wheezing episodes, which were also related with some distinctive clinical features; adenovirus-positive patients presented frequently with abnormal

chest radiographs and leukocytosis, so were prescribed antibiotics more often than other wheezy hospitalized children. Our data confirm that adenovirus can mimic bacterial infection in previously healthy children that often results in inappropriate antibiotic therapy. Identifying adenovirus infections would affect care favorably.²³ In this study, HMPV was detected in 4.7% of the total study population and was the fifth respiratory virus in both, infants and children. Although initial reports questioned the role of HMPV in wheezing episodes, our results and those of other authors^{5,8,24–27} have clarified this issue, showing clearly that HMPV is involved, at least, in the most severe wheezing episodes, requiring hospital admission. Coronaviruses were identified in 1.4% of wheezing episodes. As it has been previously suggested,²⁸ our data confirms that coronaviruses may be associated with more severe symptoms in young children. However, no conclusion can be drawn on the clinical role played by coronaviruses on the basis of our data. Only larger studies would provide some information at these low prevalences. Noticeably, it does not appear that influenza was a substantial contributor to severe wheezing episodes in Spain from 2005 to 2008, as it was one of the less frequent viruses detected in our wheezy hospitalized group. Similar results have been reported by Chung et al.¹⁹ in Korea.

According to our results there was a clear pattern of fall/winter increase and summer decrease for wheezing admissions in Spain in children older than 2 years of age, as has been previously described, in many northern hemisphere countries.^{29–34} In fact, the epidemics of wheezing hospitalization had two peaks in the last 3 years; the first in April and May, and the second one started in September. These results parallel rhinovirus circulation in Spain, with two peaks of maximum activity in April/May and September/December. Furthermore, other viruses such as HMPV may play a significant role in wheezing admissions occurred in February/April; also adenovirus, quite frequent in our series, reached its maximum activity in spring and fall and may contribute to the epidemics of wheezing admissions occurring in these periods of time.

Proof of viral causality is extremely difficult, especially for the newly described virus; however, despite the relatively high coinfection rate, our results suggest a potential etiologic role for respiratory viruses in acute wheezing episodes. In fact, the finding that up to 75.5% of children with a wheezing episode had evidence of a viral infection, in comparison with only 20% in a healthy control group published by our group elsewhere,³⁵ suggests that many respiratory viral infections may result in wheezing disease, although the absence of a control group enrolled during the same period as children with wheezing is a major limit. Two recent studies have also studied the presence of respiratory viruses in control

subjects and have demonstrated an association between respiratory virus infection and acute wheezing episodes.^{2,13} On the other hand, single and dual infections were associated with similar clinical features. Taken together, these data strongly suggest an etiologic role of respiratory viruses in severe wheezing episodes in children.

One limitation of this study is that outpatients with wheezing were excluded, but it has been reported that rates of respiratory virus detection are higher in hospitalized children than in children not requiring hospitalization.³ Nevertheless, no hypothesis on outpatient cases can be done based in our study.

In summary, wheezing episodes in Spanish children severe enough to require hospitalization are very frequently associated with viral infection and follow seasonal patterns. In Spain the epidemics of wheezing hospitalization takes place in April/May and September/December, especially in children >2 years old, following the circulation of rhinovirus. RSV and rhinovirus are the most prevalent viruses in acute wheezing in infants, while adenovirus is the second most frequent after rhinovirus in older children. However, other emerging viruses as HMPV and especially HBoV, seem to play an important role in wheezing episodes. Despite the importance given to influenza viruses, it does not appear that influenza is a substantial contributor to severe asthma exacerbation in Spanish children.

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