

# Viruses and Atypical Bacteria Associated With Asthma Exacerbations in Hospitalized Children

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**Summary.** Objectives and Working Hypothesis: To evaluate the prevalence of respiratory viruses *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae* and gain insight into their seasonal circulation pattern in children with acute asthma exacerbations in a temperate southern hemisphere region. Study Design: Patients hospitalized between 3 months and 16 years of age were included in a 1-year prospective, observational, cross-sectional study. Respiratory secretions were collected and the presence of different viruses and atypical bacteria analyzed by immunofluorescence and polymerase chain reaction. Results: Two hundred nine patients (118 females) aged (mean  $\pm$  SD)  $4.4 \pm 4$  years were included. A potential causative agent was detected in 78% of the patients. The most frequently detected viruses were respiratory syncytial virus (HRSV) ( $n = 85$ ; 40%) and rhinovirus (HRV) ( $n = 52$ ; 24.5%); *M. pneumoniae* and *C. pneumoniae* were detected in 4.5% and 2% of the cases, respectively. Patients with HRSV (vs. HRV) were hospitalized for a longer time (6.7 vs. 5.2 days,  $P = 0.012$ ), required more days of oxygen supply (5.1 vs. 3.4,  $P = 0.005$ ), had a longer duration of the exacerbation before hospitalization (3.6 vs. 1.9 days,  $P = 0.001$ ) and were younger (3.7 vs. 5.1 years,  $P = 0.012$ ). Three peaks of admissions were observed. A first peak (early autumn) caused by HRV, a second peak (winter) caused mainly by HRSV and a third one (spring), caused by HRSV, an increase in HMPV together with a second outbreak of HRV. Conclusions: Children with an acute asthma exacerbation presented a high prevalence of respiratory viruses. Most hospitalizations corresponded to seasonal increases in prevalence of HRV and HRSV. **Pediatr Pulmonol.** 2010; 45:619–625. © 2010 Wiley-Liss, Inc.

**Key words:** rhinovirus; respiratory syncytial virus; wheezing illness.

## INTRODUCTION

Asthma is the most-frequent pediatric chronic disease, and its prevalence is increasing in numerous regions of the world.<sup>1</sup> The International Study of Asthma and Allergies in Childhood (ISAAC) has reported that in Argentina the current prevalence of asthma is 16.4% in children aged 6 years old and 10.9% in children aged 13.<sup>2</sup>

Respiratory exacerbations are the main source of morbidity, mortality, school absence, and health expenses associated with asthma. Infections associated with respiratory viruses and atypical bacteria are the main cause of asthma exacerbation. About 85–90% of the wheezing episodes in infants and 65–70% in children and adolescents are triggered by respiratory viruses.<sup>3</sup> Various viral agents, including the respiratory syncytial virus (HRSV), influenza A and B (FLUAV and FLUBV), parainfluenza (HPIV), adenovirus (HAdV), rhinovirus (HRV), enterovirus (HEV), coronavirus (HCoV), and the recently described metapneumovirus (HMPV)<sup>4</sup> and bocavirus (HBoV)<sup>5</sup> have been detected in patients presenting with asthma exacerbation. While HRSV is the main agent associated with wheezing in infants and preschool children HRV is the most-frequent agent

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detected in school children, adolescents, and adults.<sup>3</sup> Infections caused by atypical bacteria (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*) have been associated not only with asthmatic exacerbations but also with the development of chronic infections that may contribute to the persistence and severity of asthma.<sup>6,7</sup>

Numerous adequately treated asthmatic patients continue to experience wheezing episodes, so studies are necessary to understand the relationship between the inflammatory process triggered by various infectious agents and the events that lead to the loss of asthma control.<sup>8,9</sup> This knowledge will assist the development of prevention and treatment strategies to help decrease the morbidity caused by bronchial asthma.<sup>10–11</sup>

We performed a cross-sectional study to determine by means of immunofluorescence assay (IFA) and molecular techniques the prevalence of traditional and newly described respiratory viruses and atypical bacteria in children hospitalized due to an acute asthma exacerbation in Buenos Aires, a temperate southern hemisphere city.

## MATERIALS AND METHODS

The study was performed at the Ricardo Gutiérrez children's hospital in Buenos Aires between January 1 and December 31, 2006. The study protocol was approved by the Institutional Review Board of the hospital. Legally authorized representatives of the children provided informed consent.

### Inclusion Criteria

Patients included in the study were between 3 months and 16 years of age, with a history of two or more previous wheezing episodes diagnosed by a physician and presenting with a new episode severe enough to require hospitalization.

Forty-two (20%) patients were under 12 months, an age group where it is difficult to establish a definite diagnosis of asthma. Because of the difficulty in differentiation of asthma from wheezing episodes of other origin in very young children,<sup>12</sup> to be included in the study patients under 3 years of age needed to have an index for the prediction of asthma according to the criteria of Castro-Rodríguez et al.<sup>13</sup> Children over three needed to comply with the diagnostic criteria of bronchial asthma according to the GINA guidelines.<sup>14</sup>

### Exclusion Criteria

The exclusion criteria were premature birth and the presence of a chronic disease of the pulmonary or cardiovascular system, metabolic disorders, immunosuppression, genetic or neurological disorders.

## Recording of Clinical and Epidemiological Data

We recorded number of weeks of gestation and weight at birth, familial history of asthma, allergic rhinitis or atopic eczema in first degree relatives, passive smoking, medications used to achieve asthma control, age at first wheezing episode, and hospitalizations in the last 12 months by interviewing the parents and reviewing medical records. During hospitalization, data on clinical evolution (days of hospitalization and oxygen requirement), treatment required (bronchodilators, systemic corticosteroids, antibiotics, mechanical respiratory assistance), and complications presented (pneumonia and atelectasis) were recorded.

The interval of time (days) between the onset of the respiratory exacerbation and admission and time between admission and the collection of the respiratory specimen were also recorded.

The results of the characterization of the 12 respiratory viruses studied (HRSV, HAdV, FLUAV and FLUBV, HPIV-1, 2, and 3, HMPV, HRV, HEV, HCoV, HBoV) as well as of the atypical bacteria *M. pneumoniae* and *C. pneumoniae* were registered.

Due to the wide range of age of the patients included, for statistical analysis of the different variables the patients were arbitrarily divided into three groups: children under 1 year of age, children between 1 and 4 years of age, and children between 4 and 16 years of age.

## Collection and Processing of Samples for Virological Testing

A nasopharyngeal aspirate (BSN 30<sup>®</sup>, Laboratorio Barcat, Buenos Aires, Argentina) was obtained from each patient on admission. On 12 patients in whom respiratory secretions could not be collected by this method, a nasal swab (Transport Swab<sup>®</sup>, Copan, Brescia, Italy) was obtained. The sample was kept at 4°C until submission to the laboratory.

The respiratory sample was processed the day it was collected by the IFA to detect HRSV, HAdV, HPIV-1, 2, and 3, FLUAV, and FLUBV (Light Diagnostics, Chemicon Int., Temecula, CA). A total of 500,000 cells per ml were considered adequate for IFA detection.

The secretions were kept at –20°C for further studies with polymerase chain reaction (PCR) and reverse transcription PCR (GoTaq<sup>®</sup>, Promega, Madison, WI; OneStep RT-PCR, QIAGEN, Valencia, CA). The viruses (HRSV, FLUAV, FLUBV, HPIV, HAdV, HRV, HEV, HCoV, HMPV, and HBoV) and atypical bacteria (*M. pneumoniae* and *C. pneumoniae*) were determined in separate reactions according to the protocols previously described.<sup>15</sup>

A positive case was defined as that in which at least one infectious agent was detected.

## Statistical Analysis

Descriptive statistics were performed using EPI 2000. For comparison of groups data were imported into R statistical programming language environment, version 2.6.2 (available from <http://www.r-project.org>).

Variables examined to compare patients with single agents versus co-detections and between HRSV and HRV single detections were age, gender, passive smoking, familial history of asthma or atopy, duration of exacerbation before hospitalization, days on oxygen supply, length of hospitalization and complications (atelectasis and pneumonia).

Chi-square and Fisher's exact tests were used for comparison of categorical variables. For numerical variables we used Student's *t*-test or Wilcoxon test when it was not possible to assume a normal distribution.

The values are expressed as mean  $\pm$  standard deviation. The probability level to determine statistical significance was 0.05.

## RESULTS

A total of 217 patients that fulfilled the inclusion criteria were admitted during the study period. Five patients, all less than 2 years of age, were excluded once studies were initiated to rule out differential diagnosis of asthma. Two patients declined to participate, and parents of another patient were not present to give the informed consent.

### Clinics and Epidemiology

A total of 42 (20%) out of the 209 patients studied were under 1 year of age, 77 (37%) between 1 and 4 years of age, and 90 (43%) between 4 and 16 years of age. Table 1 shows the demographic and clinical characteristics and complications present in the population studied.

Additional information on the first wheezing episode was obtained on 205 patients: 181 (88%) had their first wheezing episode in the first 3 years of life and 62% of these wheezed in their first year.

The interval of time between the onset of the respiratory exacerbation and admission was found to be (mean  $\pm$  SD):  $2.9 \pm 2.2$  days, between the onset of the respiratory exacerbation and the collection of the respiratory specimen  $4.4 \pm 2.4$  days, and between admission and the specimen collection  $1.5 \pm 1.1$  days.

A total of 69 (33%) patients had been prescribed an asthma controller therapy, but only 37 (18%) were complying with it adequately: 6 (14%) of the 42 children under 1 year of age, 7 (9%) of the 77 children between 1 and 4 years of age, and 24 (27%) of the 90 children between 4 and 16 years of age. Sixteen (44%) of those who were complying with therapy received budesonide, 13 (35%) fluticasone, 6 (16%) fluticasone plus salmeterol, and 2 (5%) montelukast.

In the physical exam carried out at admission, 199 (95%) of the patients presented cough, 177 (85%) rhinorrhea, 128 (61%) fever  $\geq 38^\circ\text{C}$ , 57 (28%) pharyngitis, and 24 (11%) conjunctivitis. In addition, 22 (10.5%) were diagnosed with acute otitis media. Overall, 197 (94%) were found to have an upper respiratory infection.

During hospitalization, all the patients received oxygen treatment through a nasal cannula or a facemask, short-acting bronchodilators (nebulized albuterol) and systemic corticosteroids (hydrocortisone, dexametasone, or methyl-prednisolone); but none of them required mechanical ventilation.

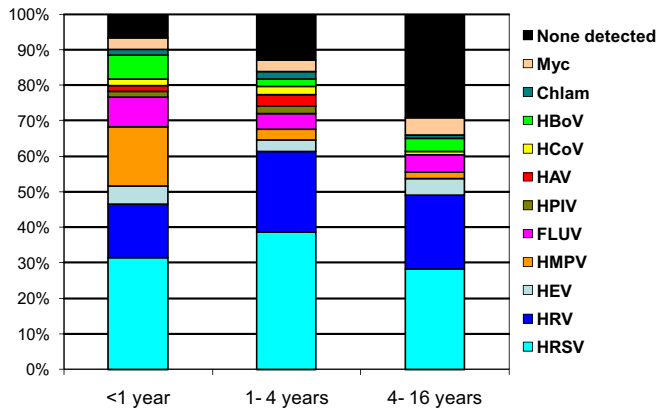
There were no significant differences when evaluating the presence of complications (atelectasis and pneumonia) when an infectious agent was detected or when co-detections occurred ( $P = 0.37$  and  $P = 0.75$ , respectively).

### Detection of Respiratory Viruses and Atypical Bacteria

Samples of respiratory secretions were obtained through a nasopharyngeal aspirate from 197 (94%) patients. In 12 (6%) patients respiratory secretions could not be obtained by this method, so a nasal swab was

**TABLE 1—Demographic and Clinical Characteristics of the Population Studied**

	<1 year	1–4 years	4–16 years
n	42	77	90
Age in years (mean $\pm$ SD)	0.58 $\pm$ 0.20	2 $\pm$ 0.85	8.41 $\pm$ 3
Gender (female)	16 (38%)	45 (62.5%)	57 (63%)
Familial atopy (n, %)	35 (83%)	63 (82%)	67 (74%)
Personal atopy (n, %)	28 (67%)	46 (60%)	62 (69%)
Passive smoking	23 (55%)	41 (53%)	34 (38%)
Length of hospital stay (mean $\pm$ SD)	6.6 $\pm$ 2.5	6.1 $\pm$ 3.5	5.4 $\pm$ 2
Days on oxygen (mean $\pm$ SD)	4.7 $\pm$ 2.3	4.4 $\pm$ 3	3.7 $\pm$ 2
Complications (n, %)	9 (21.4%)	21 (27.6%)	24 (26.7%)
Pneumonia	6	11	6
Atelectasis	3	10	18



**Fig. 1. Distribution of the different respiratory viruses, *M. pneumoniae*, and *C. pneumoniae*, grouped by age.**

performed. A higher number of infectious agents were detected in younger patients, and the detection rate decreased as age increased. The detection of positive cases was 91% (38/42), 84.5% (65/77), and 66% (59/90) in each of the three groups, respectively ( $P = 0.001$ ). The samples were first processed by IFA, which allowed the detection of 59 different respiratory viruses: HRSV ( $n = 51$ ), FLUAV ( $n = 3$ ), HAdV ( $n = 2$ ), HPIV-1 ( $n = 2$ ), and HPIV-3 ( $n = 1$ ). The respiratory samples then were processed using the PCR technique. This allowed the detection of 47 other “traditional” respiratory viruses that were not detected by IFA: HRSV ( $n = 34$ ), FLUAV ( $n = 10$ ), HAdV ( $n = 2$ ), and FLUBV ( $n = 1$ ). PCR detected 107 other agents: HRV ( $n = 52$ ), HMPV ( $n = 16$ ), HEV ( $n = 11$ ), HBoV ( $n = 10$ ), HCoV ( $n = 4$ ), *M. pneumoniae* ( $n = 10$ ), and *C. pneumoniae* ( $n = 4$ ). The

distribution of detected organisms by age groups is presented in Figure 1.

Overall, 162 (78%) positive cases were detected: 115 (78%) patients presented a single agent, while 43 (20%) presented a double co-detection; and 4 (2%) a triple co-detection. The 43 double co-detections were as follows: HRSV-HRV (10), HRSV-HEV (5), FLUAV-HRV (5), HRV-*M. pneumoniae* (4), HRSV-HMPV (3), HRSV-HBoV (3), HRV-HCoV (2), HRSV-*M. pneumoniae* (2), HRSV-FLUBV (1), HRV-HMPV (1), HRV-HBoV (1), HEV-HMPV (1), HCoV-HBoV (1), HMPV-*M. pneumoniae* (1), FLUAV-*M. pneumoniae* (1), HRSV-*C. pneumoniae* (1), and HRV-*C. pneumoniae* (1). The four triple co-detections were as follows: HRSV-FLUAV-HEV (1), HRSV-HMPV-HCoV (1), FLUAV-HRV-HBoV (1), and HRSV-HRV-*C. pneumoniae* (1). The co-detections presented a different distribution according to the age of the patient: 16/42 (38%) in the group under 1 year of age, 16/77 (21%) in the group between 1 and 4 years of age, and 15/90 (17%) in the group between 4 and 16 years of age (Table 2). Patients with co-detections were significantly younger than those without co-detections (3.1 vs. 4.8 years of age;  $P = 0.008$ ).

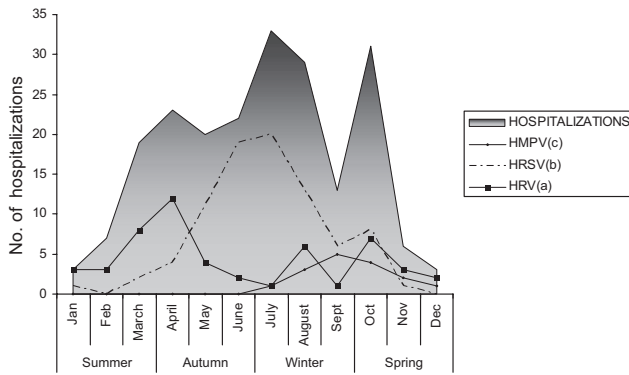
No HPIV-2 was detected. The four cases of HCoV and the only case of FLUBV were present in co-detections.

The importance of the two more-prevalent respiratory viruses (HRSV and HRV) as single detection, as well as that of the viral co-detections, was analyzed related to the main variables studied. We found that the patients with HRSV were hospitalized later in the course of their disease (3.6 vs. 1.9 days,  $P = 0.001$ ), experienced a longer hospitalization (6.7 vs. 5.2 days,  $P = 0.012$ ), required more days of oxygen supplementation (5.1 vs. 3.4,

**TABLE 2—Total of Respiratory Viruses and Atypical Bacteria Present Either Alone or in Co-Detections**

Agent	<1 year (n: 42)		1–4 years (n: 77)		4–16 years (n: 90)		Total (%)
	n	Co-detection %	n	Co-detection %	n	Co-detection %	
HRSV	19	37	36	28	30	37	85 (40)
HRV	9	89	21	38	22	45	52 (25)
HMPV	10	40	3	67	3	34	16 (7)
FLUAV	5	100	3	34	5	40	13 (6)
HEV	3	100	3	67	5	40	11 (5)
HBoV	4	75	2	100	4	25	10 (4.5)
<i>M. pneumoniae</i>	2	100	3	100	5	60	10 (4.5)
HAdV	1	0	3	0	0	0	4 (2)
HCoV	1	100	2	100	1	100	4 (2)
<i>C. pneumoniae</i>	1	100	1	100	1	100	4 (2)
HPIV-1	0	0	2	0	0	0	2 (1)
FLUBV	0	0	1	100	0	0	1 (0.5)
HPIV-3	1	0	0	0	0	0	1 (0.5)
Total	56	61%	80	40%	76	42%	213

HRSV, respiratory syncytial virus; HRV, rhinovirus; HMPV, metapneumovirus; FLUAV, influenza A; HEV, enterovirus; HBoV, bocavirus; HAdV, adenovirus; HCoV, coronavirus; HPIV-1, parainfluenza 1; FLUBV, influenza B; HPIV-3, parainfluenza 3.



**Fig. 2. Seasonal circulation integrated by the most frequent respiratory viruses and records of hospitalizations during the study period. Three peaks of admissions are observed. A first peak (early autumn) caused by HRV, in coincidence with the beginning of the school year. A second peak (winter) caused mainly by HRSV and a third one (spring), caused by HRSV, an increase in HMPV together with a second outbreak of HRV.**

$P = 0.005$ ), and were younger (3.7 vs. 5.1 years,  $P = 0.012$ ) than the patients infected with HRV.

The number of hospitalizations varied seasonally and was associated with the circulation of the more frequently isolated viruses (Fig. 2).

## DISCUSSION

In the present study, a potential causative agent of an acute asthma exacerbation was identified in 78% of the patients studied. This rate of detection is in agreement with those reported in the literature, which vary between 65% and 90% of positive cases according to the age of the population studied, the number of infectious agents evaluated, and the diagnosis techniques used.<sup>16,17</sup> The PCR technique allowed the detection of HRV, HEV, HMPV, HBoV, HCoV, *M. pneumoniae*, and *C. pneumoniae*, and also contributed to the identification of cases that had not been detected by IFA. However, it should be pointed out that while the IFA technique detects viral antigens in patients that present an active viral infection, the higher sensitivity of the PCR technique allows the detection of nucleic acids that may correspond either to a current infection or to a past infection.<sup>18</sup> In effect, Nokso-Koivisto et al.<sup>19</sup> described the presence of positive picornavirus RNA in respiratory samples obtained from children without concurrent respiratory symptoms. In our study, 94% of the patients had an active upper respiratory infection at the time of hospital admission, thereby lowering the risk of diagnosing false positive cases.

The most frequently detected agent was HRSV, in both the group of children between 1 and 4 years of age and the group of children between 4 and 16 years of age. This finding supports previous observations that HRSV can cause acute exacerbations of chronic diseases at any age.<sup>20</sup>

Community-based studies in school children<sup>21</sup> have found that HRV is the most prevalent virus, responsible for up to 70% of the asthma exacerbations. In our study, we detected HRV in 25% of the patients. This difference could be that our patients were not only hospitalized, but also younger. Some concerns could be regarded about the sample size that could lack sufficient statistical power, specially in smaller subgroups of analysis. However, several differences between groups reached statistical significance. Patients with exacerbations caused by HRV had a significantly shorter number of days from the beginning of the respiratory symptoms to hospital admission, and also shorter hospitalization and less oxygen requirement days than those with exacerbations caused by HRSV. Although the children affected by HRV were older and thus could be expected to have a wider airway caliber, this difference may be because the exacerbations triggered by HRV show a better response to treatment than those triggered by other respiratory viruses due to different pathogenic mechanisms of airway obstruction.<sup>22</sup> Thus, it was not possible to determine if the difference in clinical outcome observed might be related to the age of the patients' group or to the different pathogenic mechanisms of HRV and HRSV.

In our cohorting, other common respiratory viruses, such as FLUV, HBoV, HCoV, HMPV, and HPIV, exhibited a much lower prevalence than HRSV and HRV. It is well known that FLUV triggers asthma exacerbations less frequently than other respiratory viruses.<sup>23</sup> Due to its frequent presence in co-detection and its long period of excretion after an active infection, the role of HBoV as a causative agent of asthmatic exacerbations requires further studies. The detection of HMPV, HCoV, HPIV, and HAdV, while higher in infants and school children, was significantly lower than that of HRSV and HRV.<sup>24</sup>

In asthmatic hospitalized children, studies have reported prevalence of 20% for *M. pneumoniae* and 5–15% for *C. pneumoniae*,<sup>25</sup> which is higher than that found by our group (4.5% for *M. pneumoniae* and 2% for *C. pneumoniae*). This difference may be due to a lower circulation of these agents during the year in which the study was performed or to a lower detection rate, as we did not use serological methods. Another possible explanation could be the preponderance of young children sampled, as *M. pneumoniae* is usually less frequent under 4 years of age than in school age children. Therefore, treatment with macrolides is not justified in all patients presenting an asthma exacerbation but should be considered in children presenting persistent symptoms that cannot be controlled with conventional treatment.<sup>26</sup>

In almost a quarter of patients (22%) we found evidence of a co-detection between respiratory viruses and/or atypical bacteria. We did not find significant differences when analyzing the occurrence of co-detections between the different agents involved and the hospitalization or

oxygen requirement days and the development of complications.<sup>27,28</sup> However, the presence of such a high index of co-detection points to the need to revise the concept of “cohortization” or grouping patients in hospital rooms. Co-detections in which any of the viral agents recently described is involved can favor the development of nosocomial infections.<sup>29</sup>

Although previously described in other regions of the world, to our best knowledge, this is the first report of respiratory viruses and atypical bacteria associated to wheezing in children described in the southern cone of America. In the near future, when antiviral therapies become more readily available, to know the regional patterns of circulation of viral agents might be useful to treat specific viral respiratory infections, helping to prevent asthmatic exacerbations.<sup>30–31</sup>

In conclusion, the present study confirms the high prevalence of respiratory viruses in hospitalized children with an acute asthma exacerbation, highlights the importance both of HRSV and HRV in all age groups, and describes their seasonal pattern in a temperate Southern Hemisphere location.

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