

Viruses in Community-Acquired Pneumonia in Children Aged Less Than 3 Years Old: High Rate of Viral Coinfection

Gustavo Cilla,¹ Eider Oñate,² Eduardo G. Perez-Yarza,² Milagrosa Montes,^{1,3} Diego Vicente,^{1,3} and Emilio Perez-Trallero^{1,3,4*}

¹Microbiology Department, Hospital Donostia, San Sebastián, Spain

²Pediatrics Department, Hospital Donostia, San Sebastián, Spain

³CIBERes/26, San Sebastián, Spain

⁴Universidad del País Vasco, San Sebastián, Spain

The occurrence of viral coinfections in childhood pneumonia has received little attention, probably because suitable detection methods have been lacking. Between November 2004 and October 2006, the presence of 14 respiratory viruses in children aged less than 3 years old with community-acquired pneumonia were investigated using molecular or immunochromatographic techniques and/or viral culture. A total of 315 children (338 episodes) were included, and hospitalization was required in 178 episodes. At least one virus was detected in 66.9% of the episodes and simultaneous detection of two or more viruses was frequent (27% of the episodes with viral detection). The most frequently detected virus was respiratory syncytial virus ($n=67$: 33 subgroup A, 33 subgroup B, 1 not typed), followed by human bocavirus ($n=48$), rhinovirus ($n=46$), human metapneumovirus ($n=39$: 13 genotype A2, 8 B1, 5 B2, 1 A1, 12 not genotyped) and parainfluenza viruses ($n=38$: 1 type 1, 3 type 2, 22 type 3, 11 type 4 and 1 not typed). The 14 viruses investigated were found in viral coinfections, which were more frequent in children aged less than 12 months. Except for adenovirus, the incidence of which was low, the percentage of viral coinfection ranged between 28.2% and 68.8%. Children with viral coinfection more frequently required hospital admission than those with single viral infection. It is concluded that viral coinfections are frequent in children aged less than 3 years old with community-acquired pneumonia and can be a poor prognostic factor. **J. Med. Virol.** 80:1843–1849, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: viral coinfections; human bocavirus; human metapneumovirus; parainfluenza virus type 4

INTRODUCTION

Almost 2 million children die each year from an acute respiratory infection, mainly pneumonia, and most of these children live in developing countries [Williams et al., 2002]. In developed countries, the incidence of lower respiratory tract infections is high, causing 19–27% of hospitalizations in children under the age of 5 years in the USA [Henrickson et al., 2004; Peck et al., 2005]. In Europe, there are an estimated 2.5 million cases each year [Ruuskanen and Mertsola, 1999].

Viral pathogens are increasingly recognized as playing a major role in the etiology of lower respiratory tract infections, and are considered the predominant pathogens in community-acquired pneumonia in preschool children [Sinaniotis and Sinaniotis, 2005]. Several studies have stressed the frequent occurrence of viral and bacterial coinfections in children with pneumonia [Juvén et al., 2000; Michelow et al., 2004; Templeton et al., 2005]. The occurrence of viral coinfections has received little attention, probably because previously available detection methods (cell culture, antigen detection, serological methods) lacked sensitivity. The advances made in the last few years in molecular diagnosis have improved the reliability and sensitivity of virologic diagnostic methods and have increased the number of viruses that can be included in the respiratory viral panel to be investigated. In the present study, we investigated the presence of 14 viruses associated with acute respiratory infection in a group of children with community-acquired pneumonia,

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*Correspondence to: Emilio Perez-Trallero, Servicio de Microbiología, Hospital Donostia, Paseo Dr. Beguiristain s/n, 20014 San Sebastián, Spain. E-mail: mikrobiol@terra.es

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with particular emphasis on the study of the newly discovered human metapneumovirus (HMPV) [van den Hoogen et al., 2001], coronavirus NL63 [van der Hoek et al., 2004], and human bocavirus (HBoV) [Allander et al., 2005], as well as viral coinfections.

METHODS

A prospective study was undertaken in the region that includes the areas of San Sebastián, Tolosa and the Urola Coast (in the province of Gipuzkoa in the Basque Country, northern Spain) with a population of 405,745 inhabitants, of which 11,696 are aged less than 3 years old. Hospital Donostia (in San Sebastián) is the only hospital in this region in the public health system and attends approximately 97% of pediatric hospitalizations.

Patients

Between October 2004 and September 2006, all infants and children aged between 1 and 35 months diagnosed with pneumonia in the Pediatric Emergency Department of Hospital Donostia or within 48 hr of admission were preselected. Infants and children with fever (temperature $>38^{\circ}\text{C}$) and chest X-ray and other signs compatible with a diagnosis of pneumonia (dyspnea and/or tachypnea and/or crackles on chest auscultation) were considered eligible for inclusion in the study. All X-rays were evaluated by an independent expert radiologist and the presence of consolidation, infiltrate or pleural effusion was considered to be significant pathology [WHO, 2001]. Infants and children with presumed nosocomial infections (hospital discharge in the previous 2 weeks or onset of pneumonia more than 48 hr after admission) were excluded. The patients were evaluated by the study pediatricians at least twice and, at the first visit, the parents were asked to sign an informed consent form before their offspring were definitively enrolled in the study. In the same patient, only new episodes occurring at an interval of at least 2 months were considered.

A standard medical history was taken, including demographic and epidemiological data, clinical antecedents and current disease. With hospital admissions, their duration, whether admission was to the Pediatric Intensive Care Unit and whether oxygen therapy was required was noted. The study was approved by the Ethics Committee for Clinical Research of the Hospital Donostia.

Samples and Laboratory Methods

A nasopharyngeal aspirate was obtained from all children in the first 24 hr of admission to the Pediatric Emergency Department. The samples were divided in two aliquots, one of which was stored at $2-8^{\circ}\text{C}$ and analyzed with diagnostic purposes within 48 hr of extraction, while the other was stored at -80°C for additional studies. In all nasopharyngeal aspirates, respiratory syncytial virus (RSV) antigen detection was performed, using a commercial immunochromatographic method (Binax, Portland, ME). RSV, influenza

A and B viruses, parainfluenza viruses types 1–4 and adenovirus were investigated through culture using rapid shell vial techniques on MDCK, A-549 and LLC-MK2 cell lines.

Viral DNA and RNA were obtained with an automatic BioRobot[®] M48 extractor (Qiagen GmbH, Hilden, Germany) using the MagAttact[®] Virus Mini M48 kit. Transcription of RNA to cDNA was performed with M-MuLV reverse transcriptase (Promega, Madison, WI) using random primers. Each PCR run included a negative control (water control) that was treated identically to the clinical samples throughout. All procedures were performed with the usual precautions to avoid contamination. Four multiplex nested PCR methods were used to detect: (1) virus influenza (using specific primers for the A, B, and C nucleoprotein genes [Coirás et al., 2004] and H1 and H3 hemagglutinin genes [Stockton et al., 1998]); (2) RSV subgroups A and B [Coirás et al., 2004]; (3) parainfluenza virus types 1–4 [Coirás et al., 2004]; and (4) coronaviruses (using broadly reactive primers for coronavirus [Drosten et al., 2003], and specific primers for coronavirus NL63 [Vabret et al., 2005]). HMPV [Kaida et al., 2006] and rhinovirus [Steininger et al., 2001] were investigated by nested PCR, while the presence of adenovirus [Xu et al., 2000], and the matrix gene of influenza A virus [Fouchier et al., 2000], were investigated using PCR methods. HBoV detection was performed through PCR using primers derived from the NP1 gene [Allander et al., 2005] with subsequent investigation of the presence of the VP1 gene in positive samples [Bastien et al., 2006]. Genetic analysis of the gene fragments amplified of HMPV and coronaviruses (broadly reactive method) were sequenced in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA), followed by comparison with sequences at the BLAST website (<http://www.ncbi.nlm.nih.gov/blast>).

On admission, a blood sample was taken for blood culture (Bactec 9240 system, Becton Dickinson, Sparks, MD) from every child. Detection of pneumococcal antigen was performed in pleural fluid using an immunochromatographic method (Binax). For serologic studies, paired serum samples were obtained in 236 patients during acute and convalescent phases of infection. Antibodies against *Mycoplasma pneumoniae* (*M. pneumoniae*) were detected using a microparticle agglutination assay (Fujirebio, Tokyo, Japan). For detection of IgG and IgM antibodies against *Chlamydia pneumoniae*, an indirect microimmunofluorescence assay was used (Focus Diagnostics, Cypress, CA). Manufacturer's instructions were followed in every assay. Seroconversion (increase of ≥ 4 -fold in antibody titer), and/or presence of IgM, or in the case of *M. pneumoniae* any titer $\geq 1/320$, were considered diagnostic for the corresponding microorganism.

Statistical Analysis

The Chi-square test was used to compare percentages (qualitative variables) with application of Yates' or

Fisher's corrections (two tailed) when required. The Mantel–Haenszel Chi-square test was used for stratified analyses. Analysis of variance was used to study quantitative variables. Analysis of hospitalization according to age and viral infection (single infection) was performed through binary logistic regression. A *P*-value of <0.05 was considered statistically significant.

RESULTS

Study Population and Relevant Clinical Antecedents

A total of 466 episodes of community-acquired pneumonia were diagnosed during the 2-year study period, of whom 338 episodes occurring in 315 children, were enrolled in the study. The remaining episodes were excluded due to lack of: family consent, complete clinical-epidemiological information or sufficient nasopharyngeal aspirate for virological studies. The age of patients and main course of the illness (hospitalization, admission to the Pediatric Intensive Care Unit) are showed in Table I and Figure 1. The mean hospital stay was 6.5 ± 3.4 days. In 46 episodes, the affected children required oxygen therapy (13.6%) and in 23 (6.8%) the children were admitted to the Pediatric Intensive Care Unit. In 15 episodes, there was pleural effusion (4.4%). None of the children required assisted ventilation or died. Eleven children (3.3%) had two ($n = 10$) or three ($n = 1$) episodes of community-acquired pneumonia. Of these, one patient had two episodes separated by 12 months in which parainfluenza virus type 3 was detected. One hundred children (113 episodes) had a history of lower respiratory tract infections or asthma, distributed into bronchiolitis in 55 episodes, pneumonia in 28 episodes, recurrent bronchitis in 23 episodes and asthma in 11 episodes. Nine children had underlying diseases (three children with congenital heart disease—one of whom had Down syndrome—three children with laryngotracheomalacia, one with bronchopulmonary

dysplasia, one with Klinefelter syndrome and one with neuroblastoma). At onset of the community-acquired pneumonia episode, 144 children (42.6%) had received at least one dose of the heptavalent pneumococcal conjugate vaccine. *Streptococcus pneumoniae* was detected in seven patients (2.1%), four of them with a positive blood culture, being in the other three the pneumococcal antigen detected in pleural fluid. In six patients *M. pneumoniae* infection was diagnosed. Due to the low number of bacterial infections stated, bacteria were excluded from subsequent analysis.

Viruses Detected

In 226 episodes (66.9%), at least one virus was detected; of these, two or more viruses were simultaneously detected in 61 (27.0%). Table I shows the viruses detected in the distinct age groups while Table II shows the viral coinfections identified. The most frequently detected virus was RSV, which was found in 67 episodes (33 subgroup A, 33 subgroup B, 1 not typed), followed by HBoV ($n = 48$), rhinovirus ($n = 46$), HMPV ($n = 39$: 13 genotype A2, 8 B1, 5 B2, 1 A1, 12 not genotyped) and parainfluenza viruses ($n = 38$: 1 type 1, 3 type 2, 22 type 3, 11 type 4 and 1 not typed). Of the 22 episodes with coronaviruses, 5 corresponded to type NL63, 8 to type OC43 and 3 to type 229E, being 6 not typed (insufficient sample). Of the 25 occasions in which the influenza viruses were detected, AH3 was found in 10, AH1 in 4, influenza B in 4 and influenza C in 7. Adenovirus was detected in 11 episodes. All the viral agents were detected on some occasion as forming part of viral coinfections.

Viral Coinfections and Viral Cocirculation

The virus associated most frequently with other viruses was HBoV (33/48, 68.8%), followed by influenza viruses (13/25, 52%; influenza A 57%) and RSV (34/67, 50.7%; Table I). The seasonal distribution of viral

TABLE I. Respiratory Viruses and Viral Coinfections Detected in 338 Episodes of Community-Acquired Pneumonia in Children Aged Less Than 3 Years Old in Gipuzkoa, Basque Country, Spain

Virus group	Age 1–11 months; no. of episodes = 78		12–23 months of age; no. of episodes = 116		Age >23 months; no. of episodes = 144		Total no. of episodes = 338	
	Total no. (%) ^a	Coinfections no. (%) ^b	Total no. (%) ^a	Coinfections no. (%) ^b	Total no. (%) ^a	Coinfections no. (%) ^b	Total no. (%) ^a	Coinfections no. (%) ^b
Respiratory syncytial virus	22 (28.2)	14 (63.6)	25 (21.6)	13 (52.0)	20 (13.9)	7 (35.0)	67 (19.8)	34 (50.7)
Human bocavirus	10 (12.8)	9 (90.0)	23 (19.8)	14 (60.9)	15 (10.4)	10 (66.7)	48 (14.2)	33 (68.8)
Rhinovirus	11 (14.1)	4 (36.4)	14 (12.1)	4 (28.6)	21 (14.6)	6 (28.6)	46 (13.6)	14 (30.4)
Human metapneumovirus	13 (16.7)	3 (23.1)	8 (6.9)	2 (25.0)	18 (12.5)	6 (33.3)	39 (11.5)	11 (28.2)
Parainfluenza viruses (1–4)	15 (19.2)	9 (60.0)	10 (8.6)	1 (10.0)	13 (9.0)	6 (46.2)	38 (11.2)	16 (42.1)
Influenza viruses (A, B, C)	8 (10.3)	4 (50.0)	12 (10.3)	6 (50.0)	5 (3.5)	3 (60.0)	25 (7.4)	13 (52.0)
Coronavirus ^c	4 (5.1)	2 (50.0)	7 (6.0)	3 (42.9)	11 (7.6)	4 (36.4)	22 (6.5)	9 (40.9)
Adenovirus	2 (2.6)	0 (—)	4 (3.5)	1 (25.0)	5 (3.5)	0 (0.0)	11 (3.3)	1 (9.1)
Episodes with viruses	60 (76.9) ^d	20 (33.3) ^e	81 (69.8) ^d	22 (27.2) ^e	85 (59.0) ^d	19 (22.4) ^e	226 (66.9)	61 (27.0)

^aPercentage among the total number of pneumonia episodes investigated in the corresponding age group.

^bPercentage of the total number of infections caused by this virus in the corresponding age group.

^cIncluding coronavirus NL63, OC43, and 229E and those not typed.

^dChi-square for linear trend = 7.87, *P* = 0.005 on comparing the results obtained in children aged <12 months, 12–23 months, and >23 months.

^eChi-square for linear trend = 3.50, *P* = 0.061 on comparing the results obtained in children aged <12 months, 12–23 months, and >23 months.

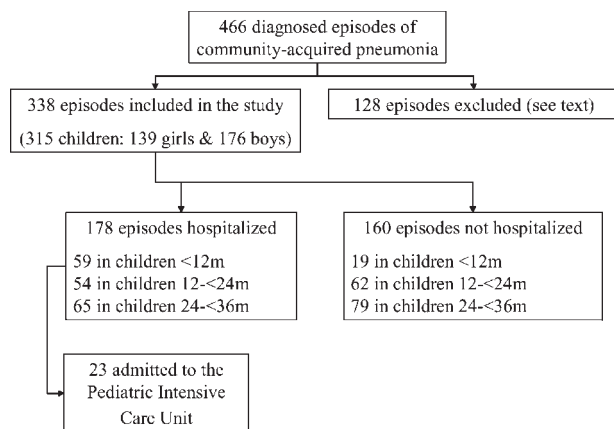


Fig. 1. Episodes of community-acquired pneumonia in children aged less than 3 years old in Gipuzkoa (Basque Country, Spain), October 2004 to September 2006.

detection is shown in Figure 2. Viral coinfection in HBoV, influenza viruses and RSV, showed greatest cocirculation in the cold months (November–April). HMPV circulated mainly at the end of winter and during spring, when circulation of RSV, HBoV and influenza viruses were already decreased. Adenovirus circulated singly (summer months) and the percentage of coinfection was low. Overall, viral coinfections were more frequent in cold months when the number of circulating respiratory viruses is highest than in the remaining months (35.7%, 56/157 vs. 7.2%, 5/69; Chi-square = 19.65, $P < 0.001$). The seasonality of the distinct viral infections in children with community-acquired pneumonia was similar to that detected in our region in children with other respiratory infections.

Age and Prevalence of Infection

Overall, community-acquired pneumonia secondary to respiratory viral infections was more frequent in

TABLE II. Associations Among 14 Respiratory Viruses in 338 Episodes of Community-Acquired Pneumonia in Children Aged Less Than 3 Years Old (Gipuzkoa, Basque Country, Spain)

Viruses detected	Number	Percentage of the total number of episodes	Percentage of virus-positive episodes
Single infections	165	48.8	73.0
Viral coinfections, two viruses	52	15.4	23.0
HBoV + RSV	14	4.1	
HBoV + rhinovirus	5	1.5	
RSV + PIV (types 1, 2, 3, 4 and non-typed)	5	1.5	
HBoV + influenza A virus	4	1.2	
RSV + influenza A virus	2	0.6	
RSV + coronavirus	2	0.6	
HBoV + PIV (types 3 and 4)	2	0.6	
PIV type 3 + rhinovirus	2	0.6	
PIV (types 3 and 4) + HMPV	2	0.6	
HBoV + HMPV	2	0.6	
RSV + influenza B virus	1	0.3	
RSV + influenza C virus	1	0.3	
RSV + rhinovirus	1	0.3	
RSV + HMPV	1	0.3	
PIV type 3 + coronavirus	1	0.3	
HBoV + coronavirus	1	0.3	
Rhinovirus + adenovirus	1	0.3	
Rhinovirus + coronavirus	1	0.3	
Rhinovirus + HMPV	1	0.3	
HMPV + influenza A virus	1	0.3	
HMPV + coronavirus	1	0.3	
Coronavirus + influenza C	1	0.3	
Viral coinfections, three viruses	9	2.7	4.0
HBoV + RSV + rhinovirus	2	0.6	
HBoV + RSV + PIV type 4	1	0.3	
HBoV + RSV + influenza C virus	1	0.3	
HBoV + influenza A virus + PIV type 3	1	0.3	
RSV + HMPV + rhinovirus	1	0.3	
RSV + HMPV + coronavirus	1	0.3	
RSV + PIV type 3 + coronavirus	1	0.3	
HMPV + influenza B virus + PIV type 3	1	0.3	
Total number of viral coinfections	61	18.0	27.0
Total number of episodes with viral detection	226	66.9	
No virus detected	112	33.1	
Total number of episodes	338		

HBoV, human bocavirus; RSV, respiratory syncytial virus; PIV, parainfluenza viruses; HMPV, human metapneumovirus.

Mixed viral infections were detected in 22 RSV subgroup A, 11 RSV subgroup B and 1 RSV not typed.

Coronavirus NL63 was found in three coinfections: with RSV (2) and with influenza C (1). Coronavirus OC43 participated in three coinfections: with HBoV (1), with RSV + HMPV (1) and with RSV + PIV type 3 (1). Coronavirus 229E was found in mixed infection with rhinovirus (1), and finally, coronaviruses not typed were found in dual viral infection with PIV type 3 (1) and HMPV (1).

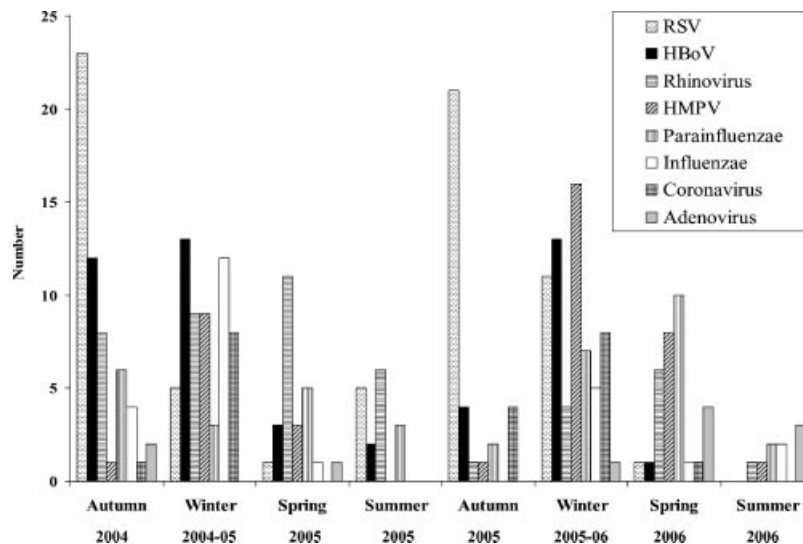


Fig. 2. Seasonal distribution of the most frequently detected viruses in 338 episodes of community-acquired pneumonia in Gipuzkoa (Basque Country, Spain) between October 2004 and September 2006.

infants aged less than 1 year old, being detected one or more viruses in 76.9% of the episodes (Table I). Viral coinfections were also more frequent among these infants, with 25.6% (20/78) of the episodes occurring in infants aged less than 12 months and 15.8% (41/260) occurring in children aged more than 12 months (Chi-square = 3.95, $P = 0.047$). The prevalence of HBoV was low in infants aged less than 6 months (5.3% [1/19]), increased to 15.3% (9/59) and 25.0% (13/52) in those of 6 to <12 and 12 to <18 months of age, respectively (Chi-square for linear trend = 4.13, $P = 0.042$), and decreased after the age of 18 months, being 15.6% (10/64), 14.3% (10/70), and 6.8% (5/74) in those of 18 to <24, 24 to <30, and 30 to <36 months of age, respectively (Chi-square for linear trend = 7.62, $P = 0.006$). The prevalence of RSV decreased with age as did that of influenza viruses in the third year of life ($P < 0.05$). HMPV, coronavirus, rhinovirus and adenovirus remained stable throughout the first 3 years of life ($P = \text{NS}$). The age of onset of parainfluenza virus infection depended on the type of virus: 5/22 (22.7%) of patients with type 3 were aged more than 18 months compared with 10/11 (90.1%) of those with type 4 (Yates 11.14, $P < 0.001$).

Severity Indicators, Clinical Antecedents and Viral Coinfections

Analysis of episodes grouped by the type of virus detected showed no significant differences in hospitalization when total infections, single infections and viral coinfections were compared. No differences were observed when other severity-related parameters such as length of hospital stay, Pediatric Intensive Care Unit admission, O_2 saturation <95% and the need for oxygen therapy (data not shown) were compared. However, considered all together, children coinfecting with two or more viruses were hospitalized more frequently than those infected with a single virus (67.2% [41/61] vs.

46.1% [76/165], Chi-square = 7.98, $P = 0.005$). These relative frequencies persisted after the episodes were stratified by age; thus 65% (26/40), 37.3% (23/59), and 42.4% (28/66) respectively of patients aged less than 12 months, 12–23 months, and >23 months with single infections were hospitalized compared with 90.0% (18/20), 63.6% (14/22), and 47.4% (9/19) of those with viral coinfections (Mantel–Haenszel Summary Chi-square = 5.87, $P = 0.015$). The binary logistic regression analysis confirmed that age and viral coinfection were independent risk factors for hospitalization ($P < 0.001$, OR = 1.058, 95% CI [1.026–1.090] and $P = 0.012$, OR = 2.24, 95% CI [1.026–4.230], respectively).

One or more respiratory viruses were detected in 63.7% (72/113) of the episodes of community-acquired pneumonia in children with a history of lower respiratory tract infections or asthma compared with 68.5% (154/225) of those with no prior history of these diseases (Chi-square = 0.76, $P = \text{NS}$). In the first group, there were 26 (23.0%) coinfections compared with 35 (15.6%) in the second group (Chi-square = 2.83, $P = \text{NS}$). During episodes of recurrent pneumonia ($n = 37$), the frequency with which viral infections (54.1%) and coinfections (16.2%) were documented was similar to that observed in the initial episodes (68.4% and 18.3% respectively) (Chi-square = 3.08, $P = \text{NS}$).

DISCUSSION

In this prospective study, at least one respiratory virus was detected in a large percentage of cases, supporting the findings of recent studies of patients with community-acquired pneumonia that used molecular detection methods in children [Juvén et al., 2000; Tsolia et al., 2004] and in adults [Templeton et al., 2005].

This study, like others that investigated viral pneumonia, has two major limitations: detection of viruses in nasopharyngeal aspirate provides only indirect

evidence of the etiology of pneumonia, and, also the study lacked a control group. However, obtaining pulmonary or lower airways specimens from these patients would not have been ethical, as invasive procedures would have been required. Moreover, the procedures currently available to diagnose bacterial infections are unsuitable because, unlike viruses, bacteria are part of normal flora and distinguishing between commensal bacteria of upper respiratory tract and bacteria causing respiratory illnesses is often unfeasible. In children, currently available techniques for the diagnosis of bacterial respiratory infections have a low diagnostic yield, for example, blood or pleural isolation of *S. pneumoniae* has low sensitivity, while pneumococcal antigenuria—the most sensitive method in adults—has low specificity. For these reasons, we have refrained from discussing bacteriological results and have focused the present study on virological results. In addition, knowledge about the biology and clinical relevance of some of the recently identified respiratory viruses, like the HBoV, is limited [Schildgen et al., 2008]. Therefore, their detection may represent asymptomatic persistence, prolonged shedding, or other situations. Nevertheless, although some cases may have been the result of a concurrent viral isolation in the presence of bacterial pneumonia and others may have been mixed bacterial and viral infections, it is believed that overall this study highlights the important role played by viruses in children aged less than 3 years old with community-acquired pneumonia.

Notable findings were the wide variety of viruses detected and the high frequency with which the new respiratory viruses, HBoV and HMPV, were detected. One or both of these new viruses were detected in one out of every four episodes of pneumonia. The most frequent virus in this series was RSV, a result consistent with those of other large series of community-acquired pneumonia in children aged less than 3 years old [Juvé et al., 2000; Henrickson et al., 2004; Sinaniotis and Sinaniotis, 2005]. The second most frequent virus was HBoV (14.2%), an important finding of this study. This result confirms those of Fry et al. [2007] who recently detected HBoV in 11.9% of children aged less than 5 years old with pneumonia compared with 2.4% in children without respiratory infection. HMPV has frequently been associated with a diagnosis of pneumonia in children [Williams et al., 2004; Choi et al., 2006; Wolf et al., 2006]. In the present series, HMPV also occupied a prominent place, being detected in 11.5% of the episodes studied. The new coronavirus NL63, which has shown a strong association with croup [van der Hoek et al., 2005], was found in 1.5% of pneumonia cases, this being one of the few series of childhood lower respiratory tract infections in which its presence has been reported. Another relevant finding of this study was the frequency of parainfluenza virus type 4 detection (11 patients, 2.9%). Parainfluenza virus type 4 is a relatively unknown and uncharacterized virus, which has a low recovery rate in cell culture, having been therefore underreported in other studies. The findings of this

study, together with those of other authors [Garcia Garcia et al., 2002; Lau et al., 2005], suggests that parainfluenza-4 should be included in the panel of viruses investigated in the diagnosis of childhood lower respiratory tract infections. The results of this series, looking at the combination of a great number of viruses with sensitive virus-detection assays, re-enforce previous descriptions obtained in studies that have focused on one virus or a minor number of viruses.

In the present study, 27% of the episodes of childhood community-acquired pneumonia in which viruses were detected corresponded to viral coinfections, a percentage that increased to 35% in the cold months of the year. To our knowledge, this is the series of community-acquired pneumonia in children that has documented a higher rate of viral coinfection. However, some recent studies that included other lower acute respiratory infections, mainly acute expiratory wheezing in children, reported viral coinfections of 20% [Aberle et al., 2005] and 34% respectively [Allander et al., 2007]. The virus producing the highest percentage of coinfections was HBoV. Numerous publications have reported that HBoV is commonly detected together with other viruses in distinct types of infection [Choi et al., 2006; Allander et al., 2007; Fry et al., 2007; Vicente et al., 2007]. In this study, however, other viruses circulating in the cold months also showed high percentages of coinfection (RSV 51%, influenza A virus 57%, etc.). The viruses most frequently detected as single infections were those whose circulation coincided least with that of the most prevalent viruses (HBoV and RSV), whether circulating throughout the year (rhinovirus), or towards the end of winter, spring and beginning of summer (parainfluenza, HMPV), or even showing isolated circulation in the summer (adenovirus). These results suggest that the percentage of coinfection of each virus is determined by the greater or lesser possibility of its seasonal circulation coinciding with that of another virus.

Viral coinfections were most frequently observed in younger children, probably as a result of the greater incidence of respiratory viruses, as a whole, in this age group [Drews et al., 1997]. Therefore, although we cannot definitively rule out preferential interactions among specific viruses, apart from the greater susceptibility in younger children, the frequency of coinfections seemed to be mainly related to the time of year in which viral circulation occurs. Some authors have pointed out that viral coinfections may have a worse prognosis than single infections [Drews et al., 1997; Greensill et al., 2003; Aberle et al., 2005; Foulongne et al., 2006], but this association has not been found by others [Choi et al., 2006; Wolf et al., 2006]. The present study indicates greater severity in viral coinfections than in single viral infections due to the greater frequency of hospitalization in coinfecting patients. This association persisted after an age-stratified analysis was performed and other possible variables such as the type of infecting virus were included.

In conclusion, this study shows that viral coinfection is frequent in preschool children with community-

acquired pneumonia, and it can be a factor of poor prognosis. New investigations based on quantification of viral load could provide key information on the role of each of these viruses in the future.

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