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Original Article

Epidemiological and clinical features of respiratory viral infections in hospitalized children during the circulation of influenza virus A(H1N1) 2009

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Setting: Two Pediatric Clinics at the University of Study of Milan, in Milan, Italy ('L. Sacco' and 'S. Paolo' Hospitals); Department of Public Health-Microbiology-Virology, University of Milan.

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Background Seasonal influenza viruses and respiratory syncytial virus (RSV) are primary causes of acute respiratory tract infections (ARTIs) in children. New respiratory viruses including human metapneumovirus (hMPV), human bocavirus (hBoV), and influenza 2009 A(H1N1) virus have a strong impact on the pediatric population.

Objectives To evaluate epidemiological and clinical features of ARTIs in hospitalized children.

Methods From December 1, 2008, to December 31, 2009, all children under age fifteen ($n = 575$) hospitalized for ARTIs were investigated for influenza A (subtype H1N1, H3N2, and 2009 H1N1) and B, RSV A and B, hMPV, and hBoV by PCR.

Results Fifty-one percent of samples were positive for these respiratory viruses. The frequencies of virus detection were RSV 34·1%, hBoV 6·8%, hMPV 5%, seasonal influenza A 5%, and seasonal influenza B 0%. From April 2009, 11·6% of collected samples were influenza 2009 A(H1N1) positive. Respiratory syncytial virus activity peaked in January, hBoV in February, and hMPV in April. Seasonal influenza A was detected only between January and April 2009, while influenza 2009 A(H1N1) peaked in November. Respiratory syncytial virus and hMPV were mainly associated with lower respiratory tract infections (LRTIs) and with necessity of O_2 administration. The 2009 pandemic influenza was more frequently detected in elder children ($P < 0·001$) and was associated with higher, longer-lasting fevers compared with other viral infections ($P < 0·05$).

Conclusions All considered viruses were involved in LRTIs. The primary clinical relevance of RSV and a similar involvement of both seasonal influenza and emerging viruses investigated were observed on the pediatric population.

Keywords Acute respiratory tract infections, emerging viruses, influenza virus A(H1N1) 2009, pediatric hospitalizations, viral respiratory infections.

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Introduction

Acute respiratory tract infections (ARTIs) are associated with significant morbidity worldwide; viruses are by far the most common causes of ARTIs, especially among young children.[1] In particular, seasonal influenza viruses (type A and B) and respiratory syncytial virus (RSV) are the main etiological agents during the epidemic period (between October and April in the northern hemisphere).[2,3] RSV infection is usually much more frequently identified than influenza in children; indeed, about 77% of infants have had an RSV infection before 5 years of age. This virus is the main cause of bronchiolitis, which is one of the major reasons of hospitalization in children under 2 years of age.[4] However, recent data show the important clinical role of seasonal influenza in children. In fact, the rate of hospitalization for seasonal influenza is not <3·6 per 10 000 child/year, and hospitalization can also regard previously healthy children and children older than 2 years.[4,5] The recent epidemiological scenario has

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been enlivened by the identification and emergence of several pathogens with an airborne transmission pathway such as human metapneumovirus (hMPV), first isolated in 2001,[6] human bocavirus (hBoV), discovered in 2005,[7] and influenza virus 2009 A(H1N1), identified in 2009, and responsible for the first pandemic of the new millennium.[8] HMPV and hBoV are isolated in 3·9–16% of children hospitalized for ARTIs. The first is associated with a large spectrum of clinical manifestations that range from mild upper respiratory tract disease to severe bronchiolitis and pneumonia,[9,10] and thus, it is considered to be one of the most important respiratory emerging viruses, while the second seems to have a marginal role as it causes mainly upper respiratory tract infections (URTIs), when detected alone.[11]

The 2009 pandemic influenza affected the pediatric population in 60% of cases[8] causing a significant number of recovery also in children aged over 5 years. It seems to cause generally mild disease, similar to those of seasonal influenza[12], while severe manifestations seem concentrated in patients with risk factors.

This study aimed to evaluate the frequency and the demographic and clinical features of ARTIs caused by known viruses (i.e., RSV and seasonal influenza) and newly identified viruses (i.e., influenza 2009 A(H1N1), hMPV, and hBoV) in children hospitalized for ARTI in Milan (Italy) from December 2008 to December 2009.

Materials and methods

From December 1, 2008, to December 31, 2009, we enrolled 575 children aged between 0 and 15 years hospitalized for an ARTI in two Pediatric Clinics at the University of Milan ('L. Sacco' and 'S. Paolo' Hospitals). After informed consent was obtained from the parents, an oropharyngeal swab (Plain Swabs; Copan, Brescia, Italy) was collected from each child within 24 hour after hospital admission and tested at the Department of Public Health-Microbiology-Virology, University of Milan, using PCR assays to detect viral respiratory pathogens (i.e., influenza type A, subtype H1N1, H3N2, and 2009 H1N1; influenza type B; RSV type A and B; hMPV; and hBoV).

A standardized datasheet was used to record sociodemographic data (age, gender, risk factors) obtained through parents interview and clinical information (duration of hospitalization, detailed disease signs and symptoms before and during hospitalization, prescribed drug therapy) by medical chart abstraction.

Clinical data interpretation

For this study, children <15 years of age hospitalized with symptoms of ARTI such as cough, rhinitis, sore throat, wheezing, panting, dyspnea, or apnea were enrolled.

Patients were assessed and categorized according to diagnosis of upper respiratory tract infections, wheezy bronchitis, bronchitis, bronchiolitis, and pneumonia on the basis of clinical and roentgenographic findings. The criteria proposed by Ruuskanen and Ogra[13] were used for definitions of bronchiolitis, pneumonia, and wheezy bronchitis.

Acute illnesses indicated as upper respiratory infections (URTIs) were characterized by cough, rhinorrhea, sore throat, and/or otitis media, with normal thoracic objectivity and X-ray. An acute illness characterized by cough, rhonchi, and diffuse expiratory wheezing upon thoracic auscultation was diagnosed as wheezy bronchitis, while a illness with the same characteristic but without expiratory wheezing in any phase of its course was diagnosed as bronchitis. Bronchiolitis was diagnosed when dyspnea, tachypnea, diffuse small crackles upon thoracic auscultation, and roentgenographic evidence of hyperinflation of the lung with or without areas of collapse were present in children younger than 2 years of age. Pneumonia diagnosis was based on auscultation of pathological breath sounds, such as small crackles or decrease/absence of vesicular sound, in a zone of the chest, and on radiographic findings of lung parenchymal involvement with interstitial-alveolar infiltrates and/or consolidation.

The term lower respiratory tract infections (LRTIs) was used to indicate acute illness with the presence of signs of lower airway involvement (tachypnea, dyspnea, wheezing, rhonchi, or rales) and/or a positive chest X-ray, so it includes bronchitis, wheezy bronchitis, bronchiolitis, and pneumonia.

Nucleic acid extraction and amplification

Nucleic acid extraction was conducted using a commercial method (NucliSENS®, miniMAG®; Biomérieux, Marcy L'Etoile, France). For RNA virus detection, cDNA was synthesized with pd(N)6 random hexamer primers (Amersham Biosciences, Little Chalfont, UK) using an MMLV reverse transcriptase (Invitrogen Tech-Line, Carlsbad, CA, USA). Viral detection was performed by PCR assays. To simultaneously detect and type seasonal A and B influenza viruses, a one-step real-time RT-multiplex-PCR assay was performed using primer/probe sets for two different genome regions: the matrix region of influenza type A virus and the nucleoprotein region of influenza type B virus.[14] Influenza A-positive samples were subtyped using an RT-multiplex-PCR assay with specific primers for the hemagglutinin gene of influenza A/H1 and A/H3 viruses.[15] Pandemic 2009 A(H1N1) influenza virus was detected using a one-step real-time RT-PCR, in accordance with the Centers for Disease Control and Prevention guidelines.[16] RSV A and RSV B were identified by multiplex nested PCR (fusion gene, 336 and 582 bp, respectively) using specific primer sets.[17] Two nested PCR assays were performed to detect a

151-bp (nt. 44–195) fragment of the matrix gene of hMPV and a 354-bp (nt. 2351–2704) fragment of the nucleoprotein gene of hBoV,[18] respectively. Appropriate positive and negative controls were included in any PCR assay.

Statistical analysis

Data were expressed as median (interquartile range, IQR) and percentages (95% confidence intervals, 95% CI) as appropriate. Comparisons between groups were performed using the chi-square test or Fisher's exact test. A *P*-value < 0·05 was considered statistically significant (two-tailed test). All statistical analyses were performed using OpenEPI software, version 2.2.1.[19]

Results

Study population

From December 2008 to December 2009, 575 children hospitalized for ARTIs (338 boys and 237 girls; median age 9·0 months, IQR 3·0–24·0 months) were enrolled. Twenty-four children were not enrolled because guardian consent was lacking.

Children were divided into five age-groups: <6 months (n = 242; 42·1%, 95% CI: 38·1–46·2), 6–11 months (n = 77; 13·4%, 95% CI: 10·8–16·4), 12–23 months (n = 106; 18·4%, 95% CI: 15·4–21·8), 2–5 years (n = 99; 17·2%, 95% CI: 14·3–20·5), and 6–15 years (n = 51; 8·9%, 95% CI: 6·7–11·4).

Patients were more frequently affected by LRTIs than URTIs (67·8%, 95% CI: 63·9–71·5 versus 32·2%, 95% CI: 28·4–36·1; P < 0·0001). Bronchiolitis was diagnosed in 145 children (25·2%, 95% CI: 21·8–28·9), pneumonia in 151 (26·3%, 95% CI: 22·8–30·0), wheezy bronchitis in 57 (9·9%, 95% CI: 7·7–12·6), and bronchitis in 37 (6·4%, 95% CI: 4·6–8·7).

Frequency of viral respiratory infections

Molecular investigations revealed viral gene sequences in 293 (51·0%, 95% CI: 46·9–55·0) samples. Respiratory syncytial virus was detected in 196 (34·1%, 95% CI: 30·3–38·0) of collected samples. Of these, 67 (34·2%, 95% CI: 27·8–41·0) were RSV A positive and 129 (65·8%, 95% CI: 59·0–72·2) were RSV B positive (P < 0·05). HBoV was detected in 39 (6·8%, 95% CI: 5·0–9·1) and hMPV and seasonal influenza A in 29 (5·0%, 95% CI: 3·5–7·1) samples. Regarding seasonal influenza A-positive samples, 25 (86·2%, 95% CI: 70·0–95·5) were subtype H3 and 4 (13·8%, 95% CI: 4·5–30·0) were subtype H1 (P < 0·05). No influenza B virus cases were identified.

From April 2009 to December 2009, 224 samples were also tested for 2009 A(H1N1) viral sequences: 26 (11·6%, 95% CI: 7·9–16·3%) were 2009 A(H1N1) positive.

Mixed infections were detected in 8·2% of ARTIs (Table 1), and in particular, in 41·0% of samples positive for hBoV, 9·2% of samples positive for RSV, and 7·7% of samples positive for influenza 2009 A(H1N1) (hBoV co-infections versus RSV and influenza 2009 A(H1N1); P < 0·003). Single and co-infections were associated with LRTIs with similar percentage (83·3% versus 78·8%, P = 0·3).

Seasonal and age distribution of viral infections

Evidence of viral infections was present throughout the year and highest in December 2008 (81 positive of 99 samples) and in January 2009 (74/104). Virus detection fell steeply from March (30/61) to May (5/15) and further in June (3/5). No viruses were detected in July and August (0/7). A sharp rise in viral identification was observed between October and November (20/92), when influenza 2009 A(H1N1) peaked in Italy. The frequency of viral infection was higher in December 2008 than in December 2009 (81/99 versus 22/40; P < 0·05). The epidemic peaks of RSV (type A and B), hBoV, and hMPV were observed in January, February, and April, respectively. Seasonal influenza A/H1N1 and influenza A/H3N2 were detected only between December 2008 and April 2009, while their circulation was not registered in autumn or winter 2009. Influenza 2009 A(H1N1) was first detected in September and then peaked in November 2009 (Figure 1).

Viral infections were detected more frequently in children younger than 24 months of age (84·3% versus 15·7%, P < 0·05). Respiratory syncytial virus (type A and B) accounted for 59·5% of infections in children younger than 6 months, while elder children experienced seasonal influenza, hBoV, RSV, and hMPV infections with similar frequencies (Figure 2).

The median age of children with a 2009 A(H1N1) influenza was significantly higher than that of children infected with the other viruses (median age: RSV: 3 months,

Table 1. Co-infections in positive samples

Viruses detected	No. of cases	Diagnosis
RSV B-Influenza A	5	2 URTI, 3 pneumonia
RSV A-hBoV	3	1 pneumonia, 2 wheezy bronchitis
RSV B-hBoV	5	2 pneumonia, 3 bronchiolitis
Influenza A-hBoV	2	2 URTI
Influenza A-RSV A-hBoV	2	2 bronchiolitis
RSV B-hMPV	3	1 bronchiolitis, 2 wheezy bronchitis
Influenza A(H1N1)2009-hBoV	2	2 pneumonia
hMPV–hBoV	2	2 bronchiolitis

hBoV, human bocavirus; hMPV, human metapneumovirus; RSV, Respiratory syncytial virus; URTI, upper respiratory tract infections.

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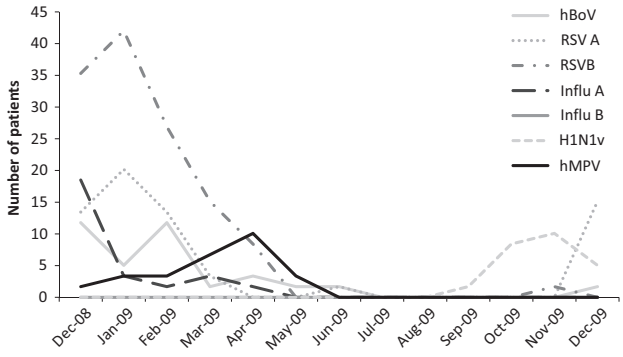

Figure 1. Monthly distribution of detected respiratory viruses in children hospitalized for respiratory tract infections between December 2008 and December 2009.

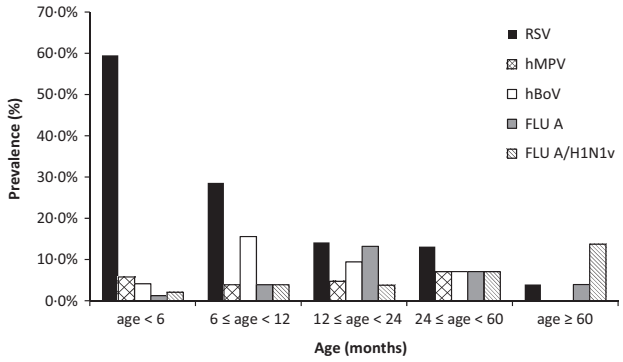

Figure 2. Age-group distribution of detected viruses in children hospitalized for respiratory tract infections.

hMPV: 6 months, hBoV: 10 months, seasonal influenza A: 15 months, 2009 A(H1N1) influenza: 27 months, $P < 0{\cdot}001$).

Socio-demographic and clinical features of children with viral respiratory infection

For analysis of the demographic and clinical features of children with viral respiratory infection, co-infected patients were excluded. Respiratory syncytial virus A and B were considered together because their clinical and socio-demographic features did not differ significantly ($P > 0{\cdot}05$).

Fever was present in all patients, except approximately one-third of RSV-infected children. The average duration of fever was significantly longer in children with 2009 pandemic influenza than in RSV-positive or hMPV-positive children (4·9 versus 3·6 and 2·8 days, respectively, $P < 0{\cdot}05$); 70·8% of children with 2009 pandemic influenza had high fever (≥39°C) (Table 2).

Cough and rhinitis were less associated with seasonal influenza than with other infections (Table 2). Dyspnea, hypoxia, and the resulting necessity of O_2 administration were significantly associated with RSV ($P < 0{\cdot}05$). The 2009 A(H1N1) influenza-positive and hMPV-positive children had feeding difficulties compared with children with the other infections (hMPV: 100%; influenza 2009 A(H1N1): 87·5%; RSV: 68·0%; hBoV: 56·5%; seasonal influenza A: 50·0%; $P < 0{\cdot}05$) and received antibiotics more frequently than RSV-positive children (hMPV: 100%; influenza 2009 A(H1N1): 100%; RSV: 79·8%; $P < 0{\cdot}05$). No patients required intensive care (Table 2).

All viruses considered in this study were involved in LRTIs with different frequencies. Lower respiratory tract infections were associated with 91·6% of RSV, 66·6% of hMPV, 56·5% of hBoV, 50·0% of seasonal influenza A, and 41·7% of 2009 A(H1N1) influenza infections (RSV-LRTI versus other viruses-LRTI, $P < 0{\cdot}05$), (Table 2). In particular, bronchiolitis was more frequently associated with RSV than with hMPV or hBoV infections (55·6% bronchiolitis-RSV versus 20% bronchiolitis-hMPV and 8·7% bronchiolitis-hBoV, $P < 0{\cdot}05$). No cases of bronchiolitis were observed in association with seasonal or 2009 pandemic influenza.

RSV-positive and hMPV-positive patients required use of bronchodilators more frequently than children infected by the other viruses (RSV: 84·8%; hMPV: 79·2%; hBoV: 43·5%; influenza A: 35·0%; 2009 A(H1N1) influenza: 37·5%; $P < 0{\cdot}05$) (Table 2). No influenza antivirals were administered to any patient during the study period.

The main risk factors for ARTIs observed in patients with a viral infection were exposure to environmental tobacco smoke (25·3%), family history of atopic disease (23·9%), and the presence of at least one sibling (15·3%) (Table 2).

Discussion

This report describes the frequencies, the demographic features, and the clinical features of viral infections identified following surveillance for ARTIs in children hospitalized for ARTI in Milan (Italy) from December 2008 to December 2009.

Fifty-one percent of children hospitalized for ARTIs had an infection caused by either known viruses (i.e., RSV and seasonal influenza) or newly identified viruses (i.e., 2009 A(H1N1) influenza, hMPV, and hBoV). Probably, most of samples that resulted negative for the considered viruses could have been positive for other viruses not included in this study, such as parainfluenza viruses, coronaviruses, adenoviruses, and rhinoviruses.

The data revealed that children younger than 2 years of age, particularly boys, were at high risk of hospitalization for ARTIs compared with older patients.[20,21]

Respiratory syncytial virus was the main agent associated with ARTI in children, especially in patients younger than

Table 2. Socio-demographic and clinical characteristics in children hospitalized for a single viral respiratory infection

Characteristics	RSV *n* = 178	hBoV *n* = 23	hMPV *n* = 24	Seasonal influenza A *n* = 20	Influenza A(H1N1) 2009 *n* = 24	*P*-value*
Demographic data						
Male/female ratio	1·5	1·8	0·75	0·7	1·8	$P < 0·001$
Median age (months)	3[a]	10[a]	6[a]	15[a]	27[b]	
IQR (months)	2·0–6·0	5·0–17·0	3·0–19·0	12·0–30·0	8·0–57·5	
Risk factors						
No. of children in household ≥3 (%)	27 (15·2)[a]	4 (17·4)	9 (37·5)[a]	0 (0)[b]	5 (20·8)[a]	$P < 0·05$
Passive smoking (%)	45 (25·3)	8 (34·8)	5 (20·8)	7 (35·0)	9 (37·5)	
Personal atopy** (%)	3 (1·7)[a]	0 (0)	0 (0)	5 (25·0)	5 (20·8)[b]	$P < 0·05$
Family history of atopic disease*** (%)	36 (20·2)	10 (43·5)	10 (41·7)	2 (10·0)[a]	14 (58·3)[b]	$P < 0·05$
Chronic illness†	3 (1·7)[a]	0 (0)	0 (0)	0 (0)[a]	5 (20·8)[b]	$P < 0·05$
Prematurity (<37 weeks) (%)	15 (8·4)[a]	2 (8·7)	5 (20·8)[b]	2 (10)	3 (12·5)	$P < 0·05$
Birth weight < 10th percentile (%)	15 (8·4)[a]	4 (17·4)	5 (20·8)[b]	2 (10)	2 (8·3)	$P < 0·05$
Attending of community†† (%)	24 (13·5)[a]	8 (34·8)	5 (20·8)	7 (35·0)	15 (62·5)[b]	$P < 0·0001$
Duration of hospitalization mean (days)	5·6	4·9	5·4	5·3	5·6	
Clinical symptoms						
Fever (≥37·5°C, axillary) (%)	127 (71·3)[a]	23 (100)[b]	24 (100)[b]	20 (100)[b]	24 (100)[b]	$P < 0·05$
Duration of fever (days)	3·6[a]	4·0	2·8[a]	4·3	4·9[b]	$P = 0·001$
High fever (≥39°C, axillary) (%)	39 (21·9)[a]	13 (56·5)	14 (58·3)	7 (35·0)[a]	17 (70·8)[b]	$P < 0·05$
Cough (%)	178 (100)[a]	21 (91·3)	24 (100)[a]	15 (75·0)[b]	22 (91·7)	$P < 0·05$
Duration of cough (days)	8·0	6·3	6·2	7·5	7·0	
Rhinitis (%)	178 (100)[a]	23 (100)[a,c]	24 (100)[a,c]	12 (60·0)[b]	21 (87·5)[b,c]	$P < 0·05$
Duration of rhinitis (days)	8·8	7·2	6·6	6·7	7·0	
Dyspnea††† (%)	63 (35·4)[a]	6 (26·1)	5 (20·8)	2 (10·0)[b]	5 (20·8)	$P < 0·05$
Hypoxia (O_{2sat} < 95% in aria) (%)	45 (25·3)[a]	4 (17·4)[a]	5 (20·8)[a]	0 (0)[b]	5 (20·8)[a]	$P < 0·05$
Feeding difficulties (%)	121(68·0)[b]	13 (56·5)[b]	24 (100)[a]	10 (50·0)[b]	21 (87·5)[a]	$P < 0·05$
Clinical diagnosis						
URTI (%)	15 (8·4)[a]	10 (43·5)[c]	8 (33·4)[c]	10 (50·0)[c]	14 (58·3)[c]	$P < 0·05$
Bronchiolitis (%)	99 (55·6)[a]	2 (8·7)[c]	5 (20·8)[c]	0 (0)[c]	0 (0)[c]	$P < 0·05$
Pneumonia (%)	39 (21·9)	4 (17·4)	7 (29·2)	5 (25·0)	5 (20·8)	
Wheezy bronchitis (%)	17 (9·6)	5 (21·7)	2 (8·3)	2 (10·0)	3 (12·5)	
Bronchitis (%)	8 (4·5)	2 (8·7)	2 (8·3)	3 (15·0)	2 (8·4)	
Therapy						
Oxygen administration‡ (%)	51 (28·6)[a]	2 (8·7)[c]	5 (20·8)[a,b]	0 (0)[c]	3 (12·5)[b,c]	$P < 0·05$
Corticosteroids (%)	124 (69·7)	10 (43·5)	14 (58·3)	7 (35·0)	14 (58·3)	
Bronchodilators (%)	151 (84·8)[a]	10 (43·5)[c]	19 (79·2)[a]	7 (35·0)[c]	9 (37·5)[c]	$P < 0·05$
Antibiotics (%)	142 (79·8)[a]	21 (91·3)	24 (100)[c]	17 (85·0)	24 (100)[c]	$P < 0·05$

IQR, interquartile range; RSV, Respiratory syncytial virus; URTI, upper respiratory tract infections.
*Chi-square test or Fisher's exact test or Student's *t*-test.
**Asthma, atopic dermatitis, allergic rhinitis, or anaphylaxis.
***Asthma, atopic dermatitis, allergic rhinitis, or anaphylaxis in first-degree relatives.
†Pulmonary, cardiac, or neurological diseases, chronic kidney insufficiency, or immunodeficiency
††Attending day nursery or school.
†††Conditions characterized by tachypnea, use of accessory respiratory muscles, presence of costal retraction, nasal flare, and grunting.
‡Criteria to supplement oxygen were <92% oxygen saturation or severe tachypnea.
Different primes ([a,b,c]) between groups mean a statistically significant difference.

6 months, and was responsible for almost all LRTIs. The primary clinical relevance of RSV in children is well characterized. Serologic evidence indicates that nearly all children have been infected by RSV within the first 2 years of life.[22] Hospitalization is required in approximately 0·5–2% of cases and 80% of these occur in the first year of life.[23] In Europe, RSV accounts for 42–45% of hospital admission for LRTIs in children younger than 2 years; in particular, in studies on hospitalized children, RSV is associated with bronchiolitis in 60–90% of cases and with pneumonia in 25–50% of cases.[24] No vaccines against this virus are available so far, but prophylaxis with palivizumab (a human,

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mouse monoclonal antibody directed against the F protein of RSV) is available for vulnerable infants, in whom the course of RSV infection could be worse, with a 1–5% mortality rate.[25]

The two most important studies on RSV epidemiology in Italy have shown that the epidemic starts in October–November and ends in April–May, with peak incidence in February.[3,26–29] In this study, RSV activity peaked in January; however, in contrast with previous studies, it rose gradually from October 2009.

Seasonal influenza viruses were identified in 5% of samples and between December 2008 and April 2009 only. No influenza B viruses were detected during the study period. The absence of seasonal influenza viruses at the end of 2009 in our study is in agreement with data from both the Italian Influenza Surveillance Network (INFLUNET)[30] and the European Centre for Disease Prevention and Control.[31]

In this study, hMPV was detected in 5% of patients. These data are in agreement with published literature, in which frequencies of hMPV infections ranged from 3·9% to 16%. Some authors have claimed a clinical similarity between hMPV and RSV infections.[32,33] In the population analyzed, hMPV was associated with LRTIs, dyspnea, and hypoxia less frequently than RSV.

HBoV infection was involved in 6% of ARTIs and in 41% of all co-infections identified. These data are in agreement with studies investigating hBoV infection in ARTIs, which have found a prevalence of 1·5–18·3%,[11,34] with a 42·5% mean percentage of co-infections with other viral pathogens.[34] In most cases, hBoV had a marginal clinical importance when identified alone, causing an URTI in 43·5% and requiring oxygen in 8·7% of cases. However, according to other studies, this study raises the possibility that hBoV is also associated with severe ARTIs such as bronchiolitis,[35,36] asthma exacerbations,[37,38] and pneumonia.[39] The seasonal peak of hBoV varies among studies, but is usually described in the early winter.[34] In this study, hBoV peak was observed in February, and its circulation was low at the end of 2009. In agreement with data registered in European countries,[30,31,40] the present study demonstrates that the 2009 pandemic influenza virus peaked in November 2009; In agreement with published literature,[41,42] our data indicate that children with high hospitalization risk for pandemic influenza were not only those younger than 2 years of age or with chronic disease (as usually observed for seasonal influenza), but also children older than 5 years of age, accounting for more of 25% of recovery from 2009 pandemic influenza. Finally, 2009 pandemic influenza caused a clinical manifestation similar to seasonal influenza with mild severity, although these children had bad general conditions because of fever.

In conclusion, all viruses considered in this study circulated in Italy and were involved in LRTIs in children. These findings confirm the primary clinical relevance of RSV, and a similar involvement of both the seasonal influenza and the emerging viruses investigated in ARTIs among hospitalized children.

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Author contributions

Study concept and design: G Zuccotti, E Tanzi, M Giovannini, E Riva, A Amendola. Acquisition of data: D Dilillo, E Galli, F Salvini. Analysis and interpretation of data: A Zappa, M Martinelli, E Galli, A Amendola, E Pariani. Drafting of the manuscript: E Galli, A Zappa. Critical revision of the manuscript for important intellectual content: D Dilillo, A Amendola, A Zappa. Statistical analysis: E Galli, A Zappa, M Martinelli. Final approval of the version to be published: G Zuccotti, E Tanzi, M Giovannini, E Riva.

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