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Influenza and respiratory syncytial virus in infants and children: relationship with attendance at a paediatric emergency unit and characteristics of the circulating strains

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Abstract A study was carried out on 2,696 Italian children, aged 0–14 years. The goals were: (1) to define the age-related impact of acute respiratory infections (ARI), measured as the risk of attendance at the Paediatric Emergency Room, (2) to better define the importance and proportion of influenza and respiratory syncytial virus (RSV) infections and (3) to acquire deeper knowledge of the influenza strains circulating in infants and children. A standardised emergency unit attendance risk (EUAR) was calculated, by age group for ARI. Specific EUARs were also calculated for the two pathogens. Pharyngeal swabs were tested by polymerase chain reaction (PCR) for influenza and RSVs. Isolation in Madine-Darby canine kidney cells (MDCK) and Hep cells, haemagglutination inhibition (HI) testing and HA1 gene sequence analysis were performed for influenza viruses. Most of the patients enrolled were aged 0–5 years, 1,139 (84.6%) and 1,061 (78.5%) in the two seasons, respectively. The most represented age class was that of 1 year olds (331 cases in 2001–2002 and 301 in 2002–2003). The highest EUAR for ARI was in patients aged 0–3 years (16.8 and 12.9 during the two seasons). The same was observed on calculating this risk by specific pathogens: 17.4 and 5.5 for influenza and 13.0 and 12.7 for RSV. Virological analysis was performed on 2,696 samples, 595 of which proved positive (22%). The highest number of isolates (326) came from patients aged 1–3 years. RSVs were more often identified than influenza viruses in infants aged up to 1 year (32 vs. 20 isolates). Of 265 strains isolated in 2001–2002, 103 were RSVs (87 type A, 16 B) and 162 were influenza (90 type A, 72 B). HI showed that influenza B viruses were related to two lineages, B/Victoria/2/87 (32%) and B/Yamagata/16/88 (68%). Of 330 strains isolated in 2002–2003, 102 were RSVs (91 type A, 11 B) and 228 were influenza viruses (220 type A, 8 B). A/H3N2 strains belonged to two clusters, A/Panama/2007/99-like and A/Fujian/411/02-like, a new variant. This paper discusses the possible role of the identified flu strains in determining EUARs among the population by age class.

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Introduction

Influenza A and B are two types of influenza virus that cause epidemic human disease [1]. Influenza A viruses are further classified into subtypes according to the characteristics of two surface glycoproteins: hemoagglutinin (H) and neuro-aminidase (N). Influenza B viruses are not subdivided into subtypes. After 1977 the epidemiological scenario was co-dominated by influenza A (H1N1 and H3N2) and influenza B strains. In 2001, influenza A (H1N2) viruses, which probably emerged after genetic re-assortment (antigenic shift) between A H3N2 and A H1N1 strains, began circulating widely. In the genome of influenza viruses, point mutations, which occur during viral replication, are also frequent. Point mutations generate new virus variants (antigenic drift).

Furthermore, partial or whole influenza gene sequencing enables viruses to be grouped into lineages or clades. Phylogenetic analysis of the influenza virus can help to clarify the

evolutionary mechanisms of the genome of influenza viruses and can lead to a better formulation of influenza vaccines [2–4].

Uncomplicated influenza illness is characterised by the abrupt onset of general and respiratory symptoms (e.g., fever, cough, etc.) [5]. Among children, otitis media, nausea and vomiting are also commonly associated with influenza illness [6–8]. Young children with influenza infection can present initial symptoms mimicking bacterial sepsis, including high fever [7, 9, 10]. Influenza infection has also been associated with encephalopathy, Reye's syndrome and other severe complications [11–13].

In children aged 0–4 years, influenza hospitalisation rates have been reported to range between 100/100,000 and 500/100,000 of the population, in healthy and at-risk subjects, respectively [14, 15]. Within this latter group, hospitalisation rates are highest in infants and are comparable to those reported in subjects aged ≥65 years [15, 16].

Respiratory illness caused by influenza is difficult to distinguish, on the basis of symptoms alone, from illness caused by other respiratory pathogens. Among these agents, the respiratory syncytial virus (RSV) plays an important role in respiratory tract diseases in infants.

Since RSV was identified in the late 1950s, it has been recognised as a major cause of lower respiratory tract infection in young children [17, 18]. RSV is a member of the paramyxovirus family and is an RNA virus. In the USA in 1991, disease caused by RSV was estimated to be responsible for the hospitalisation of 100,000 children, at a cost of US $300 million [19].

RSVs are serologically subdivided into types A and B. These can be further differentiated into numerous subtypes and genotypes. Certain genotypes are associated with a serious disease course.

Profuse coryza, congestion and low-grade fever initially characterise the clinical syndrome in children. Sixty percent of primary RSV infections are confined to the upper airway [20]. During a period of 2 to 5 days, this may progress to lower respiratory tract involvement, with the development of cough, dyspnea, wheezing and feeding difficulties. Infants less than 1 month old may present hypothermia [21]. About 3% of each year's birth cohort is hospitalised for bronchiolitis every winter in Europe, Australasia and North America (20,000 infants in the UK, of whom 600 need ventilation) [22]. Traditionally, certain groups of infants (e.g., infants born prematurely) are considered to be at high risk of developing more severe RSV bronchiolitis.

The goals of the present study were: (1) to define the age-related impact of acute respiratory infections (ARI), measured as the risk of attendance at the paediatric emergency room, (2) to better define the importance and proportion of influenza and respiratory syncytial virus (RSV) infections and (3) to

acquire deeper knowledge of the influenza strains circulating in infants and children.

In the literature the epidemiological picture of paediatric influenza is almost always obtained from hospitalisation rates [15, 16, 23]. However, while such assessments enable us to evaluate the severity of influenza epidemics, they are not a very good indicator of morbidity. To obtain a more representative evaluation of the impact of influenza in subjects from 0 to 14 years, we considered attendance at an Italian emergency unit; attendance was not always followed by hospitalisation.

Because influenza and RSV viruses are the most important agents of respiratory diseases in infancy, RSV infections were also considered.

Materials and methods

Study population and emergency unit attendance risk

The study was approved by the Ethics Committee of the University of Milan. Patients aged 0 to 14 years requiring hospital access to the Paediatric Emergency Unit of the University Paediatric Clinic of Milan, Italy, for acute respiratory diseases were enrolled in the study. ARI was defined as any respiratory disease with sudden onset, fever higher than 38°C accompanied by at least one symptom indicating respiratory tract involvement and by at least one systemic symptom (malaise, headache, etc.).

The study period ran from October to May in the 2001–2002 and 2002–2003 seasons.

We assessed the emergency unit attendance risk (EUAR), because we assumed that this was a more comprehensive indicator of the impact of ARI among children. The EUAR was calculated by selected age group, arbitrarily assuming the EUAR of 14-year-old individuals as a reference (EUAR =1). To compare the EUAR among the different age groups, standardisation was necessary. Indeed, the crude rate cannot be used, because of the different age structure of the population. Population differences were adjusted by arbitrarily taking into account the age distribution of Milanese children and considering a standard population in which the number of individuals was the same (the mean number per year) [24]. The age-adjusted number of cases, as they emerged from the above-mentioned standardized population, was calculated. Four age groups were then identified (0–3, 4–5, 6–10 and 11–14 years) through analysis of the crude rates.

The difference in the denominator between influenza and RSV was based on the epidemiological trend of the two infections in the paediatric age; indeed, no RSV infections were found in children aged more than 11 years during the 2001–2002 winter, and from only one patient in the following year.

In practice, the EUAR showed how high the risk of attendance at the Paediatric Emergency Unit was for infants or children in comparison with 11–14 or 6–10-year-old children. Indeed, the attack rate in these two latter groups was assumed as the baseline incidence.

Virological surveillance

Pharyngeal samples were collected by Virocult swab (Medical Wire and Equipment, Corsham, UK) and divided into three equal fractions: two were inoculated into the Madine Darby canine kidney cells (MDCK) and Hep cell lines for isolation, while the other was used for detection, typing and sub-typing by gene amplification. Viral RNA was extracted by means of QIA techniques, following the manufacturer's instructions (RNeasy Minikit, Qiagen, Valencia, CA). Reverse transcription (RT) and polymerase chain reactions (PCR) were performed by using a multiplex-nested assay (influenza/RSV multiplex, Amplimedical, Torino, Italy) [25]. Subtyping of influenza viruses was performed by primer-specific PCR, as previously described [26].

Antigenic and molecular characterisation of influenza

The antigenic characterisation of isolates was carried out by means of the haemagglutination inhibition (HI) test, using ferret post-infection sera, at the WHO Influenza Centre, London, UK.

Molecular characterisation of a random number of isolates was performed by sequence analysis of the globular head region of the HA protein (HA1 subunit).

Sequencing

RNA was extracted by means of QIA techniques, following the manufacturer's instructions (RNeasy Minikit, Qiagen, Valencia, CA): RT and PCR were performed using standard methods. Amplicons were purified with a Micron micro-concentrator 100 (Amicon, Beverly, MA), sequenced by dye terminator chemistry according to the manufacturer's instructions (DNA sequencing kit, PE Biosystem, Foster City, CA) and then analysed by a ABI310 Genetic analyser (PE Biosystem). The HA1 coding region was retro-transcribed and amplified using primers 5′-AAT ATC CAC AAA ATG AAG GCA ATA-3′ and 5′-ATC ATT CCT TCC CAT CCT CCT TCC-3′. The above-mentioned primers, and primers 5′-AGA AAA GGC ACC AGG AGG ACC CTA-3′ and 5′-GGA ACC CCC AAA CAG TAA TTT GGT-3′, were used for HA1 sequencing (amino acid 1–334). Sequence assembly was carried out by using the Sequencer package, version 4.1, of the Gene Codes Corporation. Phylogenetic trees were constructed by means of the neighbor-joining method, using the MEGA package, version 1.01 of Pennsylvania State University (PA).

Statistical analysis

Statview software (SAS Institute Inc., 2nd edition, USA 1998) was used to calculate the median absolute median deviation (MAD) and mode and the chi-square test for comparison of the data.

Results

Study population and emergency unit attendance risk (EUAR)

During the 2001/2002 and 2002/2003 seasons, 1,345 and 1,351 paediatric patients were enrolled, respectively.

Table 1 reports the age-class distribution of the study population.

Most of the patients enrolled were aged 0–5 years: 1,139 (84.6%) and 1,061 (78.5%) subjects in the two seasons, respectively. The most represented age class was that of 1-year-old patients in both seasons monitored (331 and 301 cases in 2001–2002 and 2002–2003, respectively). The median age was 2 years (MAD=1 year) in 2001/2002 and 3 years (MAD=2 years) in 2002/2003. The mode of age was 1 year in both seasons.

Table 2 shows the absolute and adjusted numbers of cases, broken down according to age group, and the EUAR for ARI, with the risk for children aged 11–14 years set at 1. In each season, the highest EUARs (16.8 and 12.9) were seen among patients up to 3 years of age, particularly 1-year-old subjects (data not shown). The EUAR decreased in patients aged 4–5 years and fell further in the 6–10-year-old group.

Virological surveillance

A total of 2,696 samples underwent virological analysis and 595 samples (22%) proved positive. (Table 3).

Influenza-2001/2002 season

Of the 265 strains, 162 were influenza viruses (90 type A and 72 type B). The percentage of positive samples in each group increased with age (11.26, 13.11, 14.37 and 17.39% in the 0–3, 4–5, 6–10 and 11–14 age groups, respectively). Overall statistical comparison did not show any significant differences (chi-square=3.142; P=0.3703), nor did comparison between the 0–3 and the 11–14 positive subjects (chi-square=1.739; P=0.1872).

Table 1 Gender and age of the study population

Age (years)	Number of studied subjects 2001/2002			Number of studied subjects 2002/2003		
	F	M	Total	F	M	Total
0–12 months	37	37	74	59	91	150
1	156	205	361	130	171	301
2	130	135	265	91	113	204
3	91	104	195	91	85	176
4	65	92	157	70	81	151
5	32	55	87	37	42	79
6	32	21	53	26	34	60
7	21	22	43	28	36	64
8	12	11	23	21	20	41
9	10	14	24	23	18	41
10	4	13	17	15	14	29
11	10	6	16	9	9	18
12	9	9	18	9	10	19
13	4	3	7	2	7	9
14	2	3	5	4	5	9
Total	615	730	1,345	615	736	1,351

Out of 90 type A influenza viruses, 5 were A/H1, a strain closely related to the A/New Caledonia/29/99, and 85 were A/H3N2, lineage A/Panama/2007/99, which is closely related to the A/Toulouse/878/01 and A/Moscow/10/99 strains (Fig. 1). The HI test showed that in the 2001/2002 season influenza virus B strains were antigenically related to two lineages: B/Victoria/2/87 (32%) and B/Yamagata/16/88 (68%). These viruses showed wide heterogeneity (Fig. 2). B/Yamagata lineage strains were isolated more frequently than B/Victoria strains from 0–3-year-old subjects [23 vs. 12 (chi-square=5.714; P=0.0168)]. A similar number of viruses of the two lineages was obtained from children aged 4–7 years [10 B/Yamagata lineage strains vs. 15 B/Victoria strains (chi-square=1.280; P=0.2579)], while more strains of the B/Victoria lineage were isolated from children aged 8 to 14 years [9 vs. 3 (chi-square=4.167; P=0.0412)].

Influenza-2002/2003 season

Of the 330 viral strains isolated, 228 were influenza viruses, of which 220 and 8 were A and B type, respectively.

The percentage of positive samples in each group increased with age (14.07, 18.69, 21.27 and 32.72% in the 0–3, 4–5, 6–10 and 11–14 age groups, respectively). Analysis of the age-related percentages of positive samples in 2002/2003 proved to be significant: chi-square=18.271 (P=0.0004) for the overall comparison and chi-square=13.889 (P=0.002) for that of 0–3-year-old vs. 11–14-year-old subjects.

A total of 153 A virus isolates were H3N2, of which 95 were closely related to A/Egypt/130/02, belonging to the lineage A/Panama/2007/99. Of the 220 type A strains, 67 were H1 viruses, including 3 H1N2, which is closely related to A/Egypt/96/02. Although most H3N2 A virus strains were

Table 2 Age-adjusted risk of attendance at the Paediatric Emergency Room (EUAR) for children with acute respiratory diseases enrolled, assuming the risk for 11–14 year-old subjects to be =1

Age (years)	2001/02				2002/03			
	Absolute number of PERA*	Age-adjusted attendance per year of age	CI 95%	EUAR	Absolute number of PERA*	age-adjusted attendance per year of age	CI 95%	EUAR
0–3	895	204.3	181.2–227.1	16.8	831	188.6	164.3–211.2	12.9
4–5	244	121.2	101.3–142.5	9.9	230	114.7	94.4–134.9	7.8
6–10	160	33.5	22.3–44.3	2.7	235	48.3	35.5–62.5	3.3
11–14	46	12.1	5.2–19.1	1.0	55	15.1	7.3–22.1	1.0
Total	1,345				1,351			

*Paediatric Emergency Room attendance

Table 3 Incidence of different respiratory pathogens (influenza and RSV) in 2001/2002 and 2002/2003 seasons

Ages (years)	2001/2002					2002/2003				
	Influenza			RSV		Influenza			RSV	
	A/H1	A/H3	B	RSVA	RSVB	A/H1	A/H3	B	RSVA	RSVB
0–3	2	63	34	68	14	37	76	4	74	7
4–5	2	15	15	12	1	14	28	1	10	3
6–10	1	3	19	7	1	14	34	2	6	1
11–14	0	4	4	0	0	2	15	1	1	0
Total	5	85	72	87	16	67	153	8	91	11

closely related to the A/Moscow/10/99-like vaccine strain, 32 isolates were distinguishable from both A/Moscow/10/99 and A/Panama/2007/99 and were closely related to A/Fujian/411/02 (Fig. 1).

The B strains isolated or detected in 2002/2003 belonged to the B/Victoria/2/87 lineage, a branch closely related to B/Hong Kong/335/01 (Fig. 2).

RSV

Of the 265 strains isolated in 2001/2002, 103 were RSV (87 RSVA and 16 RSVB), while of the 330 viral strains isolated in 2002/2003, 102 were RSV (91 RSVA and 11 RSVB).

The percentage of positive samples in each group decreased with age during 2001–2002 (9.1, 5.3 and 5.0% in the 0–3, 4–5 and 6–10 age groups, respectively) and in 2002–2003, too (9.7, 5.6, 2.9 and 1.8% in the 0–3, 4–5, 6–10 and 11–14 age groups, respectively).

During the two periods studied, most of the isolates were obtained from 1–3-year-old subjects, and influenza viruses showed a moderately higher relative incidence than RSVs (data not shown). RSV was detected more often than influenza in infants up to 12 months old in both seasons [10 vs. 6 in 2001–2002 (chi-square=1.125; P=0.2890; not significant) and 22 vs. 14 in 2002–2003 (chi-square=1.722; P= 0.0983; not significant), respectively] (data not shown). As age increased, detection of the two pathogens decreased, particularly that of RSVs. In patients older than 6 years, isolation and/or identification of both pathogens showed a very low frequency.

Correlation of the emergency unit attendance risk with viral isolations

EUAR caused by influenza viruses in children aged 0–3 years was higher in 2001/2002 (17.4) than in the following season (5.5 for the same age group) (Tables 4 and 5). EUAR due to

Fig. 1 The phylogenetic tree including some representative A/H3N2 viruses isolated during the 2001/02 and 2002/03 seasons along with reference strains
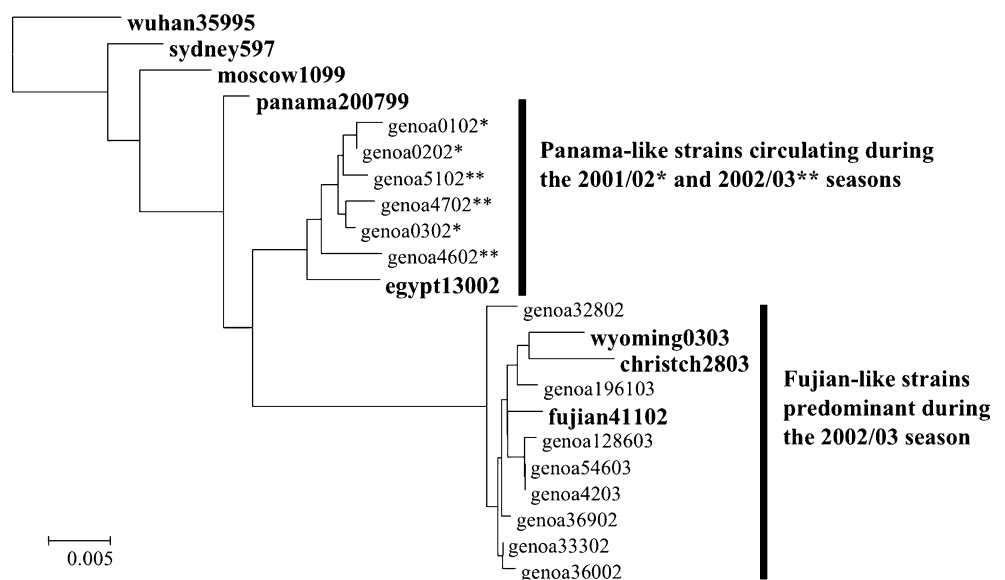
Fig. 2 The phylogenetic tree including some representative B viruses isolated during the 2001/ 2002 and 2002/2003 seasons along with reference strains
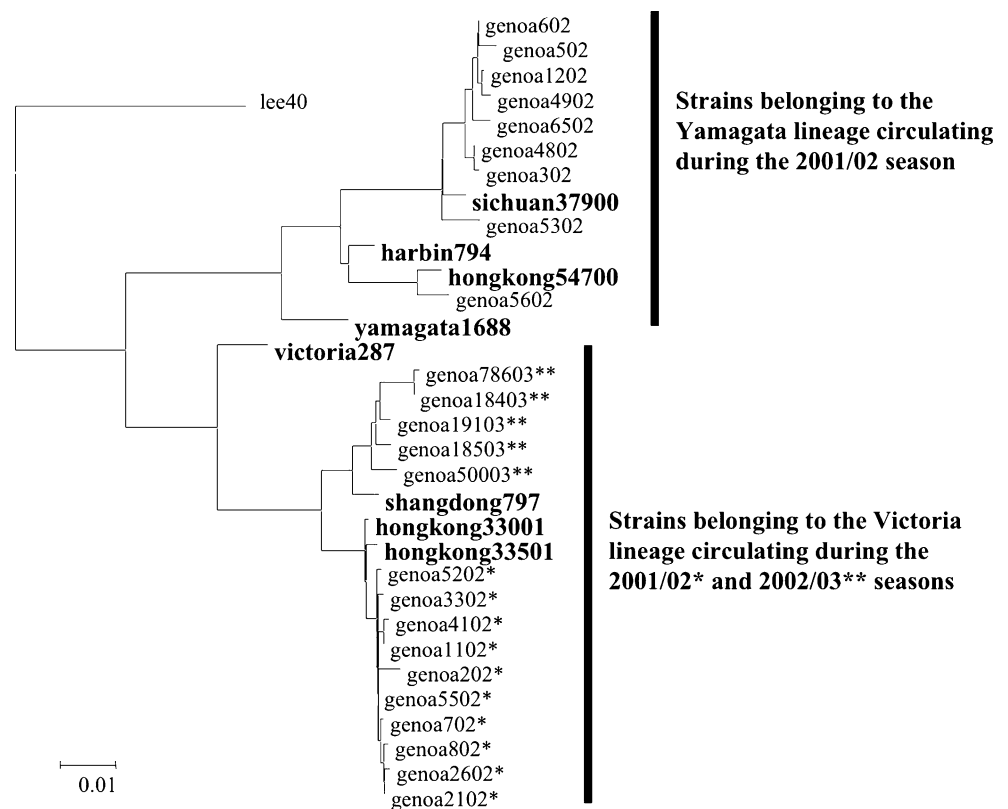
RSV was also higher among subjects aged 0–3 years than among 6–10-year-old children in both seasons (13.0 and 12.7, respectively). The risk of ER attendance for RSV-positive children was fairly similar in the two seasons, while it was 3.16 times greater for infants and toddlers positive for influenza in 2001/2002 than in 2002/2003.

Symptoms and viral isolation

Table 6 shows data on symptoms among influenza and RSV-positive children during the 2001–2002 and 2002–2003 seasons. In terms of clinical presentation, the differences between influenza and RSV-positive children were maintained in all age groups.

Comparison between clinical symptoms due to influenza and those due to RSV showed significant differences in both years with regard to fever, pharyngitis, acute bronchitis, wheezing, pneumonia and croup (Table 6). In particular, acute bronchitis, wheezing and pneumonia were typical of RSV infections.

Seasonal trend of viral infections in the two winters studied

During the first year, RSV was primarily detected between the end of December and the end of February, peaking slightly between the end of January and the beginning of February (Fig. 3a,b). During the second year, most of the RSV detections were recorded between the end of November and

Table 4 Risk of attendance to the emergency unit for children with a laboratory diagnosis of influenza, with the risk for subjects aged 11–14 years set at 1

Age (years)	2001/2002			2002/2003		
	Age-adjusted number of isolates per age class	CI 95%	EUAR	Age-adjusted number of isolates per age class	CI 95%	EUAR
0–3	22.7	14.9–30.5	17.4	26.5	17.7–35.3	5.5
4–5	15.9	8.5–23.3	12.9	21.4	12.8–29.9	4.4
6–10	4.7	0.8–8.9	3.6	10.3	3.7–16.3	2.1
11–14	1.3	0.03–2.8	1.0	4.8	0.0–9.2	1.0

Table 5 Risk of attendance to the emergency unit for children with a laboratory diagnosis of RSV infection, with the risk for subjects aged 6–10 years set at 1

Age (years)	2001/2002			2002/2003		
	Age-adjusted number of isolates per age class	CI 95%	EUAR	Age-adjusted number of isolates per age class	CI 95%	EUAR
0–3	18.8	15.9–25.8	13.0	18.3	11.2–25.4	12.7
4–5	6.5	1.8–11.2	4.5	6.5	1.7–11.2	4.5
6–10	1.4	0.0–3.2	1.0	1.4	0.0–3.8	1.0
11–14	0.0	-	-	0.2	0.0–0.7	-

the beginning of January. Peak influenza virus circulation was reached between January and February in 2001–2002 and between February and March in 2002–2003. During the winter of 2001–2002, B viruses were responsible for the first impact of the influenza epidemic; thereafter, A/H3N2 virus became more important in March. During 2002–2003, the peak of influenza was recorded in February, owing to the co-circulation of A/H3N2 and A/H1N1 or A/H1N2 subtypes. The RSV infections occurred in January, before the influenza epidemics.

Discussion

The results obtained revealed clear differences between influenza and RSV infections in terms of clinical findings. In our study, fever, pharyngitis and croup were more often associated with influenza, while acute bronchitis, wheezing and pneumonia were more frequent in infants and children with RSV infections. These findings are consistent with the literature data [27].

In the Lombardy region, clinical-epidemiological surveillance data indicate that the morbidity rate for influenza-like illness (ILI) was higher in the 2001/2002 season than in the 2002/2003 season. This is consistent with our data [28]. In the

two seasons, the median ages of the affected subjects were: 2 years (MAD 1 year) in 2001/2002 and 3 years (MAD 2) in 2002/2003. The mode was 1 for both seasons; of the patients aged 0–3 years who particularly suffered from severe respiratory diseases, most were infants younger than 1 year. In children aged 0–3 years influenza viruses and RSV were almost equally represented; by contrast, most of the viruses isolated or identified in infants up to 1 year of age were RSVs.

Iwane et al. [29] have reported that subjects younger than 5 years, and particularly those younger than 1 year, have a high burden of hospitalisation due to RSV, and Schanzer et al. [30] have found that RSV rates are highest for infants less than 6 months of age.

With regard to the distribution of influenza by age, our results differ from those of other authors. Indeed, Neuzil et al. [14, 15] found the highest influenza hospitalisation rates in 0–1-year-old infants. However, our data refer to ER attendance. Our perspective is therefore different, and it is consistent with the fact that the severity of illness in our children did not always warrant subsequent hospitalisation. Furthermore, it is possible that the EUAR values are overestimated in infants and young children, as parents and paediatricians often adopt a more cautious attitude towards babies than towards older children. Regarding other possible confounding factors, socioeconomic levels were similar among all the

Table 6 Correlation of symptoms and viral isolation

Symptoms	2001–2002 Isolates (N° 256)				2002–2003 Isolates (N° 330)			
	Influenza N° 162	RSV N° 103	χ^2	P	Influenza N° 228	RSV N° 162	χ^2	P
Axillary temperature ≥38	144 (89%)	51 (50%)	48.9	<0.001	205 (90%)	53 (52%)	99.5	<0.001
Common cold	23 (14%)	9 (9%)	1.1	0.292	34 (15%)	10 (10%)	1.1	0.225
Pharyngitis	76 (47%)	13 (13%)	30.9	<0.001	109 (48%)	16 (16%)	29.2	<0.001
Acute otitis media	16 (10%)	11 (11%)	0	>0.999	23 (10%)	12 (12%)	0.1	0.699
Acute bronchitis	10 (6%)	18 (18%)	8.1	0.004	14 (6%)	13 (13%)	3.3	0.051
Wheezing	5 (3%)	20 (20%)	18.9	<0.001	7 (3%)	22 (22%)	27.8	<0.001
Pneumonia	5 (3%)	15 (15%)	9.9	0.001	7 (3%)	18 (18%)	19.3	<0.001
Croup	24 (15%)	1 (1%)	12.8	<0.001	34 (15%)	2 (2%)	10.8	<0.001
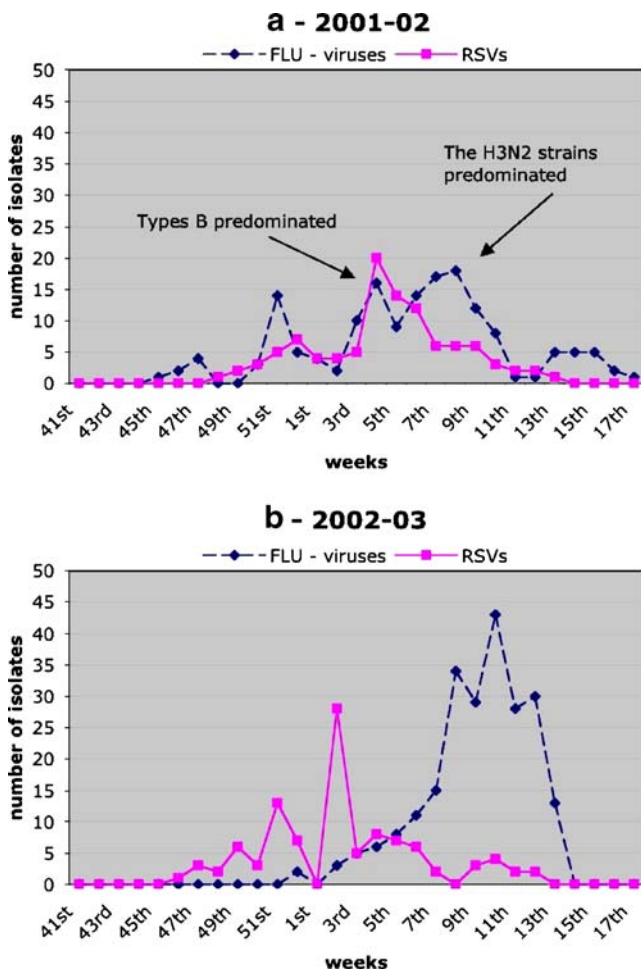
Fig. 3 Relative incidence of different respiratory pathogens (influenza and RSV) in the two studied seasons

patients, and the non-selective recruitment probably gave rise to sampling errors. Nevertheless, the EUAR appears to be a good index by which to evaluate the impact and risk of severe influenza or moderate-severe ARI in infants and children.

Differences in the percentages of positive samples in the various age classes could be explained by the fact that infants are particularly susceptible to several respiratory pathogens, such as parainfluenza virus, rhinovirus, methapneumovirus, adenovirus, and so on. Moreover, the tendency of parents to request ER admittance for infants could have partially influenced our results.

Regarding the correlation between EUAR and influenza virus isolation, in 2002/2003 EUARs were lower than in 2001/2002, probably because of the circulation of new variants of the H3N2 subtype, such as A/Fujian/411/02.

With regard to the risk of ER attendance for children with a laboratory diagnosis of RSV infection, comparison of the two years revealed very similar results. This could be explained by the greater genetic stability of RSVs and better cross-protection after infection, which would tend to restrict narrow RSV circulation to a narrower age range, namely that of infants.

For what concerns viral isolation, comparison of the frequency of symptoms showed that acute bronchitis, wheezing and pneumonia were more frequent when RSVs were isolated. This could be explained in terms of the anatomy and development of the respiratory system.

Since children aged 6–23 months have a greater risk of influenza-related hospitalisation, the American Academy of Pediatrics and the American Academy of Family Physicians recommend that they be vaccinated [31], which can trigger a "herd immunity effect" [32].

In the present study, two different lineages and four sub-lineages of the B strains, namely B/Victoria/2/87 and B/Yamagata/16/88, were shown to have co-circulated in 2001–2002. High susceptibility to a re-emerging B variant, as well as to a variety of the B/Yamagata/16/88 virus lineage, was responsible for the high influenza morbidity rate. Viruses of the B/Victoria lineage were regularly isolated from subjects of various ages. Indeed, the fact that B/Yamagata lineage strains were more frequently isolated in infants, while more B/Victoria lineage strains were isolated in 8- to 14-year-olds, confirms that 0–3-year-old subjects are susceptible to all influenza virus variants, while children over 8 years already have an immune memory of the strains circulating in previous years.

In subjects aged less than 3 years, we also observed co-circulation of a wide variety of viruses antigenically similar to those in circulation in previous years. It is also possible that, in children, adolescents and young adults, random mutations may give rise to new B influenza strains with the potential to circulate in other age groups, too.

The 2002–2003 season was characterised by the co-circulation of influenza A, H3N2 and H1N1 subtypes. The sporadic occurrence of H1N2, a new subtype, emphasises the constant need for virological surveillance. Levels of influenza B were lower than in the previous season. We observed circulation of the A/Fujian/411/02 virus, which was previously unreported in Italy. Unfortunately, matching between circulating viruses and vaccine strains was sub-optimal at that time, and vaccination with the A/Moscow/10/99 (H3N2)-like virus strain was recommended.

For children, innovative influenza vaccines, such as multivalent vaccines, vaccines obtained by molecular biology techniques or live-attenuated vaccines, could be particularly advantageous.

RSV, which had circulated throughout the 2001–2002 season, showed its usual epidemiological pattern, with a peak around Christmas. Bronchitis and pneumonia morbidity rates, as in 2001–2002, were highest in children younger than 3 years.

Our data are consistent with the opinion of Hall and McCarthy [17], who maintain that the annual peak of RSV infection tends to occur in the absence of other respiratory viral pathogens. Influenza epidemics tend to occur when

RSV decreases, although RSV and influenza epidemics may coincide [18]. Nonetheless, we also identified RSV strains during the peak of influenza infections. Moreover, Zambon et al. [33] identified individuals (<3% of the total) in all age groups with dual infections, most commonly RSV A and RSV B, or RSV A and influenza A/H3N2 [33]. In our study, RSV type A prevailed, as reported worldwide [33–35]. The high rate of ER attendance of our patients is in agreement with the observation that RSV type A strains are associated with a more severe course of infection than RSV type B. However, the spread of RSVs in outpatients was not studied. For this reason our results should not be interpreted as indicating that RSV A prevailed in the general population, too.

In conclusion, the EUAR appears to be a good indicator of the morbidity of respiratory infections in subjects <14 years of age. However, in order to obtain a clearer picture of the epidemiology of influenza and RSV infections in this age group, the rates of hospitalisation, outpatient visits and the results of paediatric sentinel surveillance should be evaluated together. The EUAR for acute respiratory diseases was very high for subjects aged 0–3 years. While hospitalisation rates indicate the impact on very young infants (0–6 months) or infants at risk, the EUAR provides an epidemiological picture of cases of influenza infections that have a severe or moderately severe course. With regard to the severity of illness, the EUAR is therefore an intermediate indicator between the rates of hospitalization and outpatient visits.

This study, based on virological samples, provides additional information on the aetiology and epidemiology of ARI and the healthcare impact of influenza and RSV infections in Italy. Indeed, our results indicate that the greater risk observed for 0–3-year-old subjects for severe or moderately severe influenza and RSV infections causes a risk of ER attendance from 5.5 to 17.4 times higher than the baseline group for influenza and equally from 12.7 to 13.0 higher for RSV infections. These findings must be taken into account by decision-makers when free influenza vaccination is made available to at-risk subjects. Furthermore, our results confirm that a vaccine against RSVs has a good chance of modifying the natural history of lower respiratory tract infections.

Infants and children have been shown to be a very important target of influenza viral strains, which have ample opportunity to circulate, co-circulate and vary their genes in these subjects; strengthening virological surveillance, particularly in this age group, could contribute to the early identification of new, potentially pandemic strains.

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