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Arch Dis Child 2010 95: 35-41 originally published online October 11, 2009
doi: 10.1136/adc.2008.153361

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Accepted 15 September 2009
Published Online First
11 October 2009

ABSTRACT

Objective: To investigate the prevalence of 14 viruses in infants with bronchiolitis and to study demographic and clinical differences in those with respiratory syncytial virus (RSV), human bocavirus (hBoV) and rhinovirus (RV) infection.

Methods: 182 infants aged <12 months hospitalised for bronchiolitis were enrolled. Infants underwent nasal washing for the detection of RSV, influenza virus A and B, human coronavirus OC43, 229E, NL-63, HUK1, adenovirus, RV, parainfluenza 1–3, human metapneumovirus and hBoV. Demographic, clinical and laboratory data were obtained from parents and from patient medical files. Main outcome measurements were age, breastfeeding history, family smoking habits, family history for asthma and atopy, blood eosinophil count, chest radiological findings, clinical severity score and number of days of hospitalisation.

Results: A virus was detected in 57.2% of the 182 infants. The most frequently detected viruses were RSV (41.2%), hBoV (12.2%) and RV (8.8%). Infants with dual infections (RSV and hBoV) had a higher clinical severity score and more days of hospitalisation than infants with RSV, RV and hBoV bronchiolitis (mean ± SD: 4.7+2.4 vs 4.3 ± 2.4 vs 3.0 ± 2.0 vs 2.9 ± 1.7, $p<0.05$; and 6.0 ± 3.2 vs 5.3 ± 2.4 vs 4.0 ± 1.6 vs 3.9 ± 1.1 days; $p<0.05$). Infants with RV infection had higher blood eosinophil counts than infants with bronchiolitis from RSV and hBoV (307 ± 436 vs 138 ± 168 vs 89 ± 19 n/mm^3; $p<0.05$).

Conclusions: Although the major pathogen responsible for bronchiolitis remains RSV, the infection can also be caused by RV and hBoV. Demographic characteristics and clinical severity of the disease may depend on the number of viruses or on the specific virus detected.

What is already known on this topic

- Bronchiolitis is caused by various viruses including respiratory syncytial virus (RSV).
- It is unclear whether infants with RSV bronchiolitis and human bocavirus (hBoV) and rhinovirus (RV) bronchiolitis differ as regards their demographic and clinical characteristics.

What this study adds

- The prevalence of 14 viruses and the demographic and clinical characteristics of a group of infants aged <12 months with bronchiolitis are reported.
- Dual infection with RSV+hBoV is associated with increased severity of bronchiolitis.
- RVs cause a milder form of bronchiolitis than RSV.

Bronchiolitis is an airway disease primarily affecting the small, peripheral bronchioles of the lung and is the principal cause of hospital admission in infants under the age of 1 year.[1] Because bronchiolitis manifests in a typical seasonal pattern closely following the activity of respiratory syncytial virus (RSV), with yearly epidemic peaks during winter, RSV has attracted major interest.[2]

Over the past decade, detection of viral respiratory agents has improved due to new molecular techniques and the availability of monoclonal antibodies for numerous viral species.[3] These advances have allowed the role of the various respiratory viruses in the pathogenesis of acute bronchiolitis to be re-evaluated. However, despite the availability of more sensitive laboratory techniques, no causative agent can be identified in many infants with acute bronchiolitis.[4 5]

Rhinoviruses (RVs) are well-known causes of upper respiratory infections at all ages. Increasing evidence suggests that RVs also cause acute bronchiolitis. RV was identified in 21% and 29% of infants with bronchiolitis.[6 7] Also, RV respiratory infection appeared not to be significantly associated with any age group.[6]

A new respiratory virus, human metapneumovirus (hMPV), was isolated in 2001 from the nasopharyngeal aspirates of young children in the Netherlands.[8] hMPV is also a common and major causative agent in infants with bronchiolitis.[9 10] Human bocavirus (hBoV), a recently discovered parvovirus, is frequently detected in the respiratory tract of patients with acute respiratory diseases, but its prevalence in infants with acute bronchiolitis is still unclear.[11–16]

Possible differences in the demographic characteristics of infants and in the clinical severity of bronchiolitis in RSV infections and bronchiolitis caused by viruses other than RSV remain controversial. In a study of infants with bronchiolitis, Papadopoulos *et al* reported that RV bronchiolitis is associated with more severe disease than RSV induced bronchiolitis.[7] Conversely, others found no differences in the clinical characteristics between RSV bronchiolitis and RV associated wheezing, but infants differed significantly as regards age, presence of atopic dermatitis, and eosinophilia during infection.[17] Others found similar clinical

characteristics in infants with hMPV bronchiolitis and RSV bronchiolitis.[10] No data are available on the demographic characteristics and clinical severity in infants with hBoV induced bronchiolitis. Nor have published studies described the demographic characteristics and disease severity in infants with acute bronchiolitis in whom no causative agent could be identified.

The main purpose of this study was to investigate the prevalence of 14 respiratory viruses in infants hospitalised for acute bronchiolitis during three consecutive annual epidemic periods. In addition, to test the hypothesis that infants with RSV bronchiolitis differ from infants with non-RSV bronchiolitis, we compared the demographic characteristics and clinical severity of RSV bronchiolitis and bronchiolitis caused by other viruses or virus negative bronchiolitis. To exclude infants with virus associated episodes of wheezing, we included only infants younger than 12 months presenting with their first episode of lower respiratory infection, who had diffuse crackles on auscultation. Having wheezing alone was not considered sufficient for inclusion in the study.[18]

METHODS

We prospectively enrolled 182 consecutive infants (mean ± SD age 2.5 ± 2.1 months, range 7 days–11 months, 104 (57% male) with acute bronchiolitis hospitalised in the paediatric emergency department of Sapienza University of Rome, during three consecutive annual epidemic periods from October 2004 through May 2005, October 2005 through May 2006, and October 2006 through May 2007.

Bronchiolitis was diagnosed according to the presence of a history of upper respiratory tract infection followed by acute onset of respiratory distress with cough, tachypnea, retraction and diffuse crackles on auscultation (having wheezing alone was not considered sufficient for inclusion in the study).[18] Exclusion criteria were underlying chronic diseases (eg, cystic fibrosis, chronic pulmonary disease, congenital heart disease, immunodeficiency) and recurrent (more than one) wheezing episodes.

Detailed demographic, clinical and laboratory data were obtained from parents with a structured questionnaire (appendix A) and from patients' medical files. Studied variables included age, gender, breastfeeding history, family smoking habits, school attendance by siblings, family history for asthma and atopy, blood eosinophil count, chest radiological findings and number of days of hospitalisation. In addition, a clinical severity score ranging from 0 to 8 was assigned to each infant on admission to the hospital according to respiratory rate (<45/min = 0, 45–60/min = 1, >60/min = 2), arterial oxygen saturation in room air (>95% = 0, 95–90% = 1, <90% = 2), presence of retractions (none = 0, present = 1, present+nasal flare = 2), and ability to feed (normal = 0, reduced = 1, intravenous = 2).[19] The parents of all infants were asked to participate in the study and gave informed consent. The study was approved by the research and ethics committee of the hospital.

Virus detection

From 1 to 3 days after hospitalisation, all infants underwent nasal washing obtained with 3 ml of sterile saline solution injected into each nostril and collected with a syringe. All samples were delivered on ice within 1–2 h to the virology laboratory and on arrival, if needed, were vortexed with beads to dissolve mucous. They were then divided into two aliquots: one was treated for nucleic acid extraction, and the second was split into equal aliquots and stored at −80°C. A 200 μl sample of respiratory specimens was subjected to nucleic acid extraction with the total nucleic acid isolation kit (Roche Diagnostics, Mannheim, Germany) eluting into 50 μl of the supplied elution buffer. A panel of reverse transcriptase-polymerase chain reaction (RT-PCR) or nested PCR assays was developed for detecting 13 respiratory viruses including RSV, influenza virus (IV) A and B, human coronavirus (hCoV) OC43, 229E, NL-63, HUK1, adenovirus, RV, parainfluenza (PIV) 1–3, and hMPV as previously described.[20] hBoV was detected using a PCR method described by Allander *et al*.[11] Most amplified fragments were purified and sequenced; amplification products not sequenced were confirmed as true positives by testing against an aliquot of the sample.

Statistical analysis

A one-way analysis of variance (ANOVA) and Student t test were used for the comparison of continuous variables. The χ^2 test was used to analyse categorical independent variables. Results for continuous data are expressed as mean ± SD. p Values of <0.05 were considered significant. Data analysis was carried out with SPSS v 1.3 for Windows.

RESULTS

All 182 consecutive children initially considered eligible for the study agreed to participate and completed the study.

Detection of respiratory viruses

A total of 121 viruses were detected in nasal wash specimens from 104 (57.2%) of the 182 infants with bronchiolitis: RSV in 75 (41.2%), hBoV in 22 (12.2%), RV in 16 (8.8%), PIV 1–3 and hMPV in 3 (1.7%), and hCoV and IVA in 1 (0.5%) (fig 1). Of the 75 RSV positive infants, 16 (21.3%) had co-infection with other viruses: 15 with hBoV and 1 with IVA. One of the hMPV positive infants had a co-infection with hBoV. No viruses could be detected in nasal wash specimens from 77 infants (42.3%).

When we studied the seasonal distribution of the 14 viruses during the three annual epidemics, we detected at least one virus in 21 of the 35 infants (60%) during the annual epidemic of 2004–2005, in 37 of 43 infants (86%) during the epidemic of 2005–2006, and in 47 of 104 (45%) during the epidemic of 2006–2007. The incidence of RSV infection peaked in February 2005, February 2006 and December 2006. The incidence of RSV infection differed during the three periods studied: 40% of RSV infections were detected during the first epidemic (2004–2005), 60.6% during the second (2005–2006), and 33.6% during the third (2006–2007) (fig 2). The incidence of RV and hBoV detection also differed during the three epidemics: 8.6% RV and 10.7% hBoV in 2004–2005, 23.3% RV and 9.3% hBoV in 2005–2006, and 3.8% RV and 16.5% hBoV in 2006–2007. The RV detection rate peaked in January 2005 and February 2006, with no clear peak during the third epidemic of 2006–2007. The incidence of hBoV detection peaked in January 2005 and January 2007, with no clear peak during the second epidemic in 2005–2006. Owing to the small numbers of other viruses isolated, we could not evaluate possible differences in incidence.

Demographic characteristics and clinical severity in infants with virus negative or virus positive bronchiolitis

When groups defined by virus identification (no virus and one or more virus detected) were compared, birth weight was significantly lower in infants with virus associated bronchiolitis than in infants with virus negative bronchiolitis (3.0 ± 0.5 vs 3.2 ± 0.5 kg; $p<0.05$ by Student t test). Significant differences

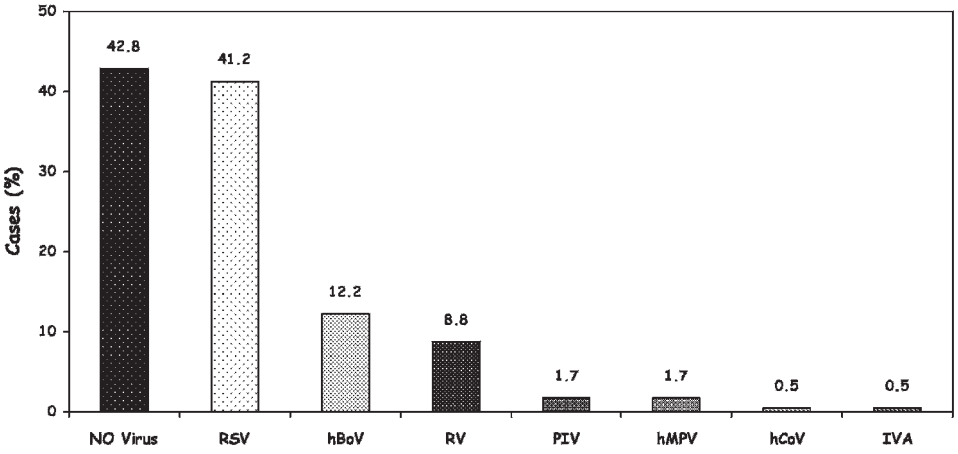

Figure 1 Distribution of identified viruses. The prevalence of the various respiratory viruses identified in the nasal wash specimens from the 182 infants with bronchiolitis is shown. hBoV, human bocavirus; hCoV, human coronavirus; hMPV, human metapneumovirus; IVA, influenza virus A; PIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.

between the two groups were observed also for chest *x* ray findings. Diffuse air trapping was significantly more common among infants with virus associated bronchiolitis, whereas patchy infiltrates were more common among infants in whom no virus could be detected ($p<0.02$ by χ^2 test) (table 1). There were no significant differences between groups in demographic characteristics (gender, age, breast feeding, exposure to smoking, school attendance, family history for asthma and atopy), clinical severity score, blood eosinophils and days of hospitalisation (table 1).

Demographic characteristics and clinical severity in infants with RSV, RV, hBoV and RSV+hBoV bronchiolitis

When patients were grouped according to the type of virus detected, several differences were identified among the groups. Infants with bronchiolitis from RSV alone were younger than infants with bronchiolitis from RV, hBoV and the combination RSV+hBoV ($p<0.003$ by one-way ANOVA) (table 2). Furthermore, infants with bronchiolitis from RSV and RSV+hBoV were breast fed for a shorter time ($p<0.04$). The percentage of infants with a positive family history for atopy was higher although not significantly higher in infants with RV bronchiolitis than in infants with bronchiolitis from RSV, hBoV and RSV+hBoV. No differences were observed in relation to gender, birth weight, exposure to smoking, school attendance or family history for asthma between groups.

Significant differences were observed also for the clinical severity score and the number of days of hospitalisation between infants with bronchiolitis from RSV+hBoV, RSV, RV and hBoV ($p<0.05$ by one-way ANOVA) (table 2, fig 3). In particular, infants with RSV bronchiolitis had a significantly higher clinical severity score at admission to hospital and number of days of hospitalisation than infants with RV bronchiolitis ($p<0.05$ by Student t test) and with hBoV bronchiolitis ($p<0.05$).

The frequency of abnormal chest radiography was similar in the four groups, but diffuse air trapping was significantly more common in infants with bronchiolitis from RSV and RSV+hBoV than among patients with hBoV and RV (table 2). Conversely, patchy infiltrates were significantly more common in infants with bronchiolitis from RV and hBoV than among infants with bronchiolitis from RSV and RSV+hBoV ($p<0.02$ by χ^2 test). Finally, the blood eosinophil count was significantly higher in infants with bronchiolitis from RV than among infants with bronchiolitis from RSV, hBoV and RSV+hBoV ($p<0.01$ by one-way ANOVA) (table 2).

DISCUSSION

In this prospective study we found that the prevalence of the 14 respiratory viruses we detected in a well characterised cohort of infants hospitalised with acute bronchiolitis differed during the three consecutive annual epidemic periods studied.

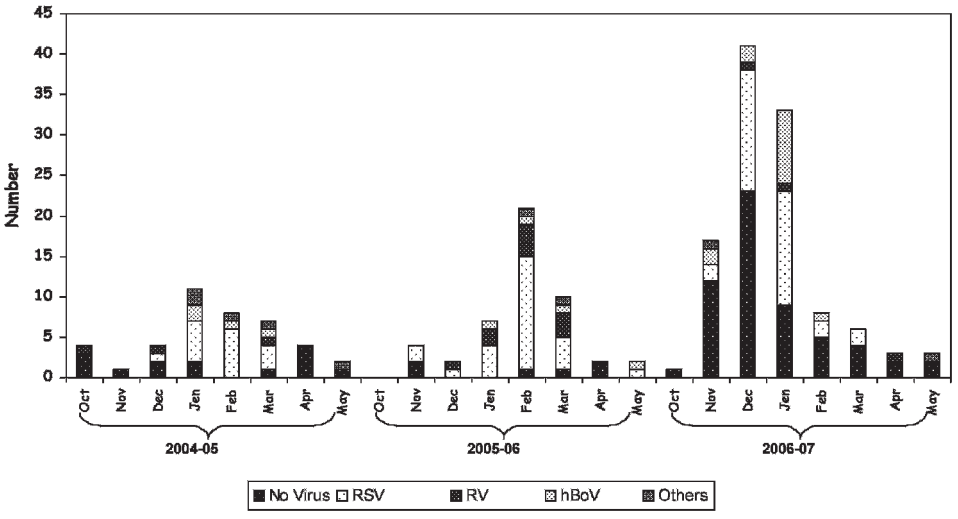

Figure 2 Seasonal distribution according to type of identified virus. Note that respiratory syncytial virus was the most frequently detected virus and the incidence peaked in February 2005, February 2006 and December 2006, followed by rhinovirus and human bocavirus. hBoV, human bocavirus; RSV, respiratory syncytial virus; RV, rhinovirus.

Table 1 Comparison of demographic characteristics and clinical severity in infants with acute bronchiolitis with and without identified viruses

Variable	No virus (n = 78)	Virus detected (n = 104)	p Value
Gender (male)	46 (59.0%)	58 (55.8%)	NS
Age (months)	2.4 ± 1.8	2.5 ± 2.4	NS
Birth weight (kg)	3.2 ± 0.5	3.0 ± 0.5	0.01
Breast feeding (months)	1.3 ± 1.6	1.7 ± 2.0	NS
Exposure to smoking	38 (51.4%)	44 (44%)	NS
School attendance	48 (62.3%)	56 (54.9%)	NS
Family history for asthma	22 (28.2%)	21 (20.2%)	NS
Family history for atopy	26 (33.3%)	36 (34.6%)	NS
Clinical severity score	3.8 ± 2.5	3.9 ± 2.3	NS
Radiographic findings	(n = 75)	(n = 94)	
Normal	12 (16.0%)	9 (9.6%)	
Diffuse air trapping	17 (22.7%)	41 (43.6%)	**0.02**
Patchy infiltrates	46 (61.3%)	44 (46.8%)	
Eosinophils (n/mm^3)	149 ± 153	153 ± 229	NS
Days of hospitalisation	4.8 ± 2.0	5.0 ± 2.4	NS

Data are expressed as number of positive cases and per cent, and as mean ± SD.

The strength of this study is that we prospectively considered a well-characterised cohort of infants aged <12 months admitted to hospital with acute bronchiolitis and analysed a comprehensive panel of respiratory viruses, whereas other studies retrospectively investigated the results of virological examinations in patients who differed in age and had various respiratory diseases.[6 7 9]

Our results confirm that RSV is the major pathogen in infants with bronchiolitis.[5] RT-PCR detected RSV in 41.2% of our infants. RSV bronchiolitis followed the typical seasonal pattern with a peak incidence during the winter months (February 2005, February 2006 and December 2006).[21 22] After RSV, the other viruses most frequently detected among infants with acute bronchiolitis in our study were hBoV and RV. The 12.2% prevalence of hBoV is the highest so far reported in infants hospitalised for bronchiolitis.[12–14] Clavo *et al* found an incidence of 13.9% in children with respiratory infections. They also reported an hBoV incidence of 33% in children with bronchiolitis, but their definition of bronchiolitis differed from ours and their children were older.[15] In agreement with previous studies, co-infection of hBoV with other viruses was very common.[12–16] Although nearly all the co-infections detected in our study (93.8%) were with RSV, this high percentage of RSV leaves the pathogenic potential of hBoV unchanged.

We detected RV in the nasal washes of 16 infants (8.8%), and none of the infants infected with RV had co-infections with other viruses. Our 8.8% infection rate was considerably lower than the 29% reported by Papadopoulos *et al*.[7] Again, the differences between the two studies could be due to age because Papadopoulos *et al* included in their study infants aged > 12 months, some of whom could have had viral associated episodes of wheezing. The other respiratory viruses we tested were detected far less frequently: hMPV and PIV 1–3, 7%; influenza virus and hCoV OC43, 0.5%. These findings confirm that acute bronchiolitis is not a synonym for RSV and can also be caused by other viruses.

Although the sensitive and comprehensive PCR method we used detected a viral pathogen in 57.2% of the infants, no aetiological agent could be identified in the remaining 42.8%. The large percentage of patients in whom the causative pathogen remained undetected is in agreement with previous studies in bronchiolitis[4 5] and is a major cause of concern. The large number of virus negative cases could be partly due to technical problems related to collecting and storing samples and partly to the fact that bronchiolitis may arise from other as yet unknown viral agents. Because we took all possible precautions to reduce technical problems, including the presence of inhibitors in the samples and the risk of PCR false-positive

Table 2 Comparison of demographic characteristics and clinical severity in infants with acute bronchiolitis by type of identified virus

Variable	RSV (n = 60)	RV (n = 16)	hBoV (n = 7)	RSV+hBoV (n = 15)	p Value
Gender (male)	37 (61.7%)	7 (43.8%)	3 (42.9%)	8 (53.3%)	NS
Age (months)	2.0 ± 1.8	3.1 ± 2.4	4.9 ± 3.4	3.5 ± 3.1	0.003
Birth weight (kg)	3.1 ± 0.6	2.9 ± 0.6	3.0 ± 0.4	2.9 ± 0.4	NS
Breast feeding (months)	1.4 ± 1.6	2.6 ± 2.8	3.2 ± 3.2	1.6 ± 1.6	0.04
Exposure to smoking	25 (43.1%)	9 (56.3%)	2 (33.3%)	6 (42.9%)	NS
School attendance	29 (50.9%)	11 (68.7%)	3 (50.0%)	8 (54.3%)	NS
Family history for asthma	11 (18.6%)	3 (18.8%)	1 (14.3%)	4 (26.7%)	NS
Family history for atopy	23 (38.3%)	8 (50.0%)	2 (28.6%)	2 (13.3%)	NS
Clinical severity score	4.3 ± 2.4	3.0 ± 2.0	2.9 ± 1.7	4.7 ± 2.4	0.05
Radiographic findings	(n = 55)	(n = 15)	(n = 6)	(n = 13)	
Normal	7 (12.5%)	0 (0.0%)	0 (0.0%)	2 (15.4%)	0.02
Diffuse air trapping	27 (48.2%)	4 (26.7%)	2 (33.3%)	6 (46.1%)	(RSV vs others)
Patchy infiltrates	22 (39.3%)	11 (73.3%)	4 (67.7%)	5 (38.5%)	
Eosinophils (n/mm^3)	138 ± 168	307 ± 436	89 ± 1119	60 ± 84	0.014
Days of hospitalisation	5.3 ± 2.4	4.0 ± 1.6	3.9 ± 1.1	6.0 ± 3.2	0.05

Data are expressed as number of positive cases and per cent and as mean ± SD.

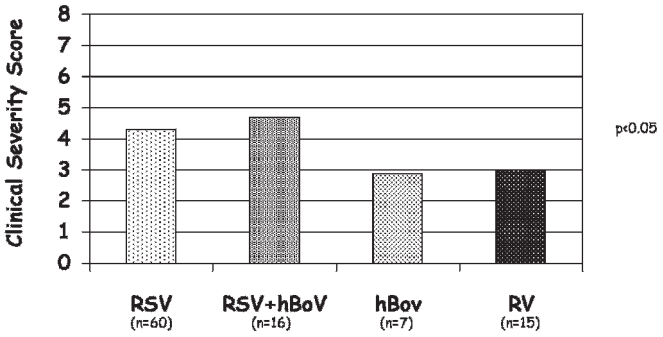

Figure 3 Clinical severity score by different viruses. hBoV, human bocavirus; RSV, respiratory syncytial virus; RV, rhinovirus. Data are reported as means ± SD ($p<0.05$ by one-way analysis of variance (ANOVA)).

findings, we attribute most of these cases of virus negative bronchiolitis to undetected pathogens.

Another distinctive finding, so far addressed in only one study,[7] is the lack of differences in the demographic characteristics of bronchiolitis in infants who tested negative or positive for the 14 viruses studied. Nor did we find differences between the two groups in the clinical severity score or number of days of hospitalisation. The only differences we observed between the two groups were related to chest *x* ray findings. In particular, radiological findings of diffuse air trapping were more frequent in infants with bronchiolitis in whom at least one virus was detected, whereas patchy infiltrates were more frequent in infants in whom no viruses were detected. This observation is difficult to explain because we found no differences in the clinical severity of bronchiolitis in the two groups. Our results therefore confirm that the presence or absence of virus has no effect on the clinical severity of bronchiolitis.[7]

By comparing demographic characteristics and clinical severity between infants with RSV bronchiolitis and infants with bronchiolitis associated with RV, hBoV and hBoV+RSV, we obtained clinically interesting findings. Even though we found no differences between virus positive and virus negative bronchiolitis, the presence in the nasal washes of a specific virus distinguished specific subgroups of patients within the group of bronchiolitis as regards both demographic characteristics and clinical severity of disease. Most importantly, infants with RSV bronchiolitis were younger than infants with bronchiolitis caused by other viruses and had been breast fed for a shorter time. They also had a more severe form of bronchiolitis with prevalent chest *x* ray findings of diffuse air trapping. Finally, their blood eosinophil counts were lower than those of infants with RV bronchiolitis, but higher than those of infants with hBoV bronchiolitis. These findings are difficult to interpret. Because they were the youngest group (mean age 2 months) they should have had the highest blood eosinophil counts,[23] but being the group with more severe disease we expected them to have the lowest concentration.[24] Conversely, infants with hBoV bronchiolitis were the oldest, had been breast fed for longer and had the lowest blood eosinophil counts; they also had the mildest form of bronchiolitis with chest *x* ray findings of patchy infiltrates. The low blood eosinophil counts in this group was an unexpected finding because they had less severe disease. These findings suggest that blood eosinophil counts in infants with acute bronchiolitis may depend in part on the severity of the disease, but mainly on the specific viral infection. Because no other published study has to our knowledge determined eosinophil counts in infants less than 12 months old with a first episode of acute bronchiolitis from different viruses, we unfortunately could not compare our findings with those from other studies. Further studies are warranted to evaluate blood eosinophil responses after viral bronchiolitis. In addition, the 15 infants with hBoV and RSV co-infection had a more severe form of bronchiolitis than infants with RSV bronchiolitis alone. The association of dual viral infections with more severe bronchiolitis has already been reported by Semple *et al*.[25] The clinical findings in our series of infants suggest that since hBoV increased the severity of bronchiolitis in infants co-infected with RSV, hBoV is a viral pathogen rather than an occasional virus. This conclusion receives support from the demographic, clinical and radiological differences we observed in infants with hBoV bronchiolitis and bronchiolitis related to other respiratory viruses. The absence or infrequency of hBoV detection in healthy children also supports the pathogenic role of this virus in respiratory diseases in children.[26]

Finally, our study provides evidence that infants with RV bronchiolitis are a third subgroup of patients who have a milder form of bronchiolitis than infants with RSV bronchiolitis, but a more severe form than those with hBoV bronchiolitis, with chest *x* ray findings of patchy infiltrates. In contrast, when Papadopoulos *et al* assessed the severity of RV bronchiolitis by clinical scoring they came to the opposite conclusion, namely that RV increased the severity of the disease.[7] Again, we presume that the discrepancies arose because the two studies selected different populations of patients. Unlike the present study, Papadopoulos *et al* included infants older than 12 months, some of whom presumably had virus associated episodes of wheezing and not true bronchiolitis. They also found no significant differences in the family history for atopy between the various viruses. In a group of children aged less than 3 years with respiratory symptoms, Manoha *et al* also showed that hMPV, RSV and RV infections led to similar clinical manifestations. Unfortunately, they did not compare clinical severity between the infants and provided no data on the family history for asthma and atopy.[27] In a group of infants hospitalised for acute respiratory symptoms, Korpi *et al* found that infants with RV and RSV bronchiolitis, despite having similar clinical characteristics, differ significantly in age, presence of atopy and eosinophilia during the infection.[17] In a group of infants in a high-risk birth cohort for asthma, followed from birth to 6 years of age, Jackson *et al* found that viral wheezing illnesses caused by RV infection were the most significant predictors of the subsequent development of asthma at age 6 years.[28] Also in our series, infants hospitalised with RV bronchiolitis had a more frequent family history for atopy and higher blood eosinophil counts than other infants with bronchiolitis. Collectively these findings suggest that RV preferentially infects infants with a genetic predisposition to atopy. Infants with RV bronchiolitis could be those in whom reactive airway diseases and atopy will probably develop in the future.[29] Our hypothesis is confirmed by Lemanske *et al* who showed in a population of infants at increased risk of developing allergies and asthma that the most significant risk factor for the development of preschool childhood wheezing was a history of symptomatic RV illness during infancy.[30] Similarly, Ehlenfield *et al* have shown that eosinophilia at the time of bronchiolitis predicts the development of wheezing persisting into later childhood.[31] Hence the association between bronchiolitis and childhood asthma may reflect an immunological anomaly

disclosed for example by RV infection rather than structural airway damage caused by viral replication.

Our findings in this prospective study of infants hospitalised with bronchiolitis suggest that bronchiolitis is a well-characterised clinical entity that can be associated with various viral pathogens. Variations in disease severity might therefore depend either on genetic differences in the population infected by these viruses or on the lung damage caused by a specific virus.

In conclusion, although the major pathogen responsible for bronchiolitis remains RSV, bronchiolitis can be caused also by other "old" viruses such as RV and by "new" viruses such as hBoV. The demographic characteristics and clinical severity of the disease may depend on the specific virus detected. Infants with bronchiolitis related to RV seem to be those predisposed to atopy, whereas infants with RSV bronchiolitis have more severe disease. The main aims of future research should be to seek new viruses, and to study the immunopathogenesis of the various respiratory viruses during acute bronchiolitis and their influence on the long-term consequences of the disease.

Funding: This work was supported in part by a grant to FM from Sapienza University (Fondi Progetto di Ricerca Universitaria).

Competing interests: None.

Ethics approval: The study was approved by the research and ethics committee of the hospital.

Patient consent: Parental consent obtained.

Provenance and peer review: Not commissioned; internally peer reviewed.

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APPENDIX A

Questionnaire

Case Number_____

Date___/___/___

Diagnosis_____

Date of Birth___/___/___ Date of hospitalization and discharge___/___/___

Gestational Age ___ week Birth weight _____ g

Breast-feeding	__ yes	___no	# months _____ (exclusive: #months ____)
School Attendance by siblings			
Brothers/Sister	__ yes	___no	#___
Familiarity for asthma	__ yes(*)	___no	
Mother	__ yes	___no	
Father	__ yes	___no	
Brothers/Sister	__ yes	___no	#___
Familiarity for rhino-conjunctivitis	__ yes(*)	___no	
Mother	__ yes	___no	
Father	__ yes	___no	
Brothers/Sister	__ yes	___no	#___
Familiarity for eczema	__ yes(*)	___no	
Mother	__ yes	___no	
Father	__ yes	___no	
Brothers/Sister	__ yes	___no	#___
Smoking habit			
Mother	__ yes	___no	cig per day ____
Mother during pregnancy	__ yes	___no	cig per day ____
Father	__ yes	___no	cig per day ____
Other house living	__ yes	___no	cig per day ____

(*)Yes at least one parent