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Role of respiratory pathogens in infants hospitalized for a first episode of wheezing and their impact on recurrences

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ABSTRACT

In order to evaluate the infectious agents associated with the first episode of severe acute wheezing in otherwise healthy infants and to define the role of each of them in recurrences, 85 patients in Italy, aged <12 months, hospitalized because of a first acute episode of wheezing, were prospectively enrolled between 1 October 2005 and 31 March 2006. Upon enrolment, nasopharyngeal swabs were collected for the real-time PCR detection of respiratory syncytial virus (RSV) types A and B, influenza virus types A and B, adenovirus, parainfluenza viruses types 1, 2, 3 and 4, rhinovirus, human metapneumovirus, human coronavirus types 229E, OC43, NL63, and HKU1, bocavirus, enterovirus, and paraechovirus; nasopharyngeal aspirates were also obtained to detect atypical bacteria. At least one infectious agent was identified in 76 children (89.4%). RSV was the most frequently detected pathogen and its prevalence was significantly higher than that of the other pathogens in both age groups, and significantly higher in the children aged 3–12 months than in those aged <3 months. Only the children with RSV infection experienced recurrent wheezing. Viral load was significantly higher in children with than in those without recurrent wheezing. This study shows that RSV is the main reason for hospitalization during the first wheezing episode in infants, and that it appears to be the only pathogen associated with a high frequency of recurrences. A high viral load seems to be strictly related to the likelihood of recurrence.

Keywords Asthma, atypical bacteria, respiratory syncytial virus, respiratory viruses, wheezing

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INTRODUCTION

Wheezing episodes are a common reason for the hospitalization of infants and young children and can cause significant clinical and socio-economic problems [1–4]. Respiratory syncytial virus (RSV) has long been considered to be the main pathogen associated with wheezing, and it has been demonstrated that a lower airway illness caused by RSV in early childhood is an independent risk-factor for the development of subsequent wheezing episodes up to adolescence [5,6].

However, recently introduced, simple and more sensitive diagnostic techniques capable of identifying infectious agents in respiratory secretions have shown that a number of other pathogens can be detected in children with wheezing [7–11]. Of these, the most frequently found are viruses such as rhinoviruses, influenza viruses, human metapneumovirus (hMPV), human coronaviruses (hCoVs), and human bocavirus, and some atypical bacteria such as *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* [12–24].

The importance of each of these infectious agents as a cause of wheezing has not yet been

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precisely defined, because the studies carried out so far have not considered all of the pathogens together or have frequently led to opposite conclusions. In particular, it is still not clear which pathogens are really responsible for severe wheezing that requires hospitalization or are important in predisposing children to the subsequent development of recurrent wheezing and asthma.

Defining the role of pathogens more precisely seems to be particularly important in the case of children aged <1 year, because acute wheezing in early life often requires hospitalization, and the risk that early infection can be associated with a greater risk of further episodes seems to have been demonstrated in experimental models and has also been suggested in human studies [25–27].

The aim of this study was to evaluate the infectious agents associated with the first episode of acute wheezing that required the hospitalization of otherwise healthy infants during their first year of life, and to define the role of each in causing recurrences.

PATIENTS AND METHODS

Study design

This prospective study was carried out between 1 October 2005 and 31 March 2006, at the Institute of Paediatrics, University of Milan, Italy, and three associated hospitals (Legnano, Pavia and Imola). The study protocol was approved by the Institutional Review Boards of the four hospitals, and the written informed consent of a parent or legal guardian was obtained before the infants were enrolled.

Study population

The study involved otherwise healthy infants aged less than 12 months who were hospitalized in one of the participating hospitals because of a first acute episode of wheezing during the study period. The exclusion criteria were premature birth and the presence of a chronic disease increasing the risk of complications of a respiratory infection, including chronic disorders of the pulmonary or cardiovascular system, chronic metabolic diseases, neoplasias, kidney or liver dysfunction, haemoglobinopathies, immunosuppression, and genetic or neurological disorders. There was no refusal to participate.

Upon admission, the demographic characteristics and medical history of the children were systematically recorded, using standardized written questionnaires and, after a complete physical examination, children with a diagnosis of infectious wheezing, based on well-established criteria [28], were enrolled. The severity of the wheezing was defined on the basis of a global evaluation of the signs and symptoms. In particular, wheezing was considered to be severe in the presence of tachypnoea (≥60 breaths/min), tachycardia (≥180 beats/min), nasal flaring, retractions, a prolonged expiratory phase, pulse oximetry <92%, and dehydration (a reduction in body weight of >7%). All of the patients underwent chest radiography, and pneumonia was defined on the basis of the presence of a reticulo-nodular infiltrate, segmental or lobar consolidation, or bilateral consolidations [29]. Upon enrolment, Virocult (Medical Wire and Equipment, Corsham, UK) nasopharyngeal swabs were used to collect specimens for the detection of viruses, and nasopharyngeal aspirates were obtained for the detection of atypical bacteria. Acute serum samples were also obtained in order to assay antibodies to *M. pneumoniae* and *C. pneumoniae*.

During their hospital stay, the children's clinical signs and symptoms were monitored daily. They were treated with oxygen when saturation was <92%, and received bronchodilators, steroids, antibiotics and/or intravenous fluids, and chest physiotherapy as judged necessary by the attending paediatricians. They were discharged when they were able to maintain oximetry >95% without oxygen, but their parents were asked to bring them immediately to the study centre if there were any recurrent or worsening signs and symptoms.

The medical history, general physical condition and clinical symptoms of each patient were re-evaluated by investigators blinded to the aetiological findings 4–6 weeks and 3–6 months after enrolment, when the children's histories of wheezing and respiratory tract infections were carefully assessed. At the first of these visits, a second serum sample was obtained in order to assay *M. pneumoniae* and *C. pneumoniae* titres during the convalescent phase.

Identification of viral infections

The Virocult nasopharyngeal swabs were tested by means of previously described real-time PCR for RSV types A and B [19,29], influenza virus types A and B [19,29,30], adenovirus [31], parainfluenza virus types 1, 2, 3 and 4 [32,33], rhinovirus [32,33], hMPV [19,34–36], hCoV types 229E, OC43, NL63, and HKU1 genotype A [21,22,37,38], bocavirus [23,39], enterovirus [11,40,41] and paraechovirus [11,40,41] at the Department of Virology, Erasmus Medical Centre, Rotterdam, The Netherlands. Total nucleic acids were routinely isolated at the MagnaPureLC Isolation Station (Roche Applied Science, Penzberg, Germany). A universal internal control virus was used to monitor the whole process from nucleic acid isolation until real-time detection. The in-house real-time PCR for all of the tested viruses and the internal control phocine distemper virus was designed using Primer Express software (Applied Biosystem, Nieuwerkerk a/d Ijssel, The Netherlands).

RNA was amplified in a single-tube, two-step reaction using the Taqman reverse transcriptase reagents kit and PCR core reagent kit (Applied Biosystems) on an ABI 7700 or ABI 7500 sequence detection system (Applied Biosystems). As positive control for each assay, a cultured control virus was used, except in the case of HKU1 genotype A, for which a construct was synthesized on the basis of published sequences containing part of the nucleoprotein gene, and cloned. On the basis of proficiency testing data, the sensitivity of each assay was estimated to be less than 500–1000 copies/mL.

Identification of atypical bacterial infections

The nasopharyngeal aspirates were evaluated for the presence of *M. pneumoniae* DNA [7,17,42,43] and *C. pneumoniae* DNA [7,17,43,44], using a validated nested PCR for both pathogens

as previously described. Each assay included positive and negative controls. The MP-1 and MP-2 primer set was used for *M. pneumoniae*-specific amplification, followed by the MUH-1 and MUH-2 primers for *M. pneumoniae* nested PCR [7,17,42,43]. Touchdown nested PCR for the detection of *C. pneumoniae* DNA was performed using primers designed to detect the major protein of the outer-membrane [7,17,43,44].

Each serum sample was tested for IgM and IgG antibodies to *M. pneumoniae* (ELISA; Pantec, Italy) [7,13,17,43], and IgM and IgG antibodies to *C. pneumoniae* (microimmunofluorescence; Labsystems, Finland) [7,17,43,45], as previously described.

Acute *M. pneumoniae* and/or *C. pneumoniae* infection was diagnosed if the child showed a significant antibody response to one of the pathogens in paired sera (for *M. pneumoniae*, an IgM-specific titer of ≥1:100 or a four-fold increase in IgG antibody; for *C. pneumoniae*, an IgM-specific titer of ≥1:16 or a four-fold increase in IgG antibody) and/or if the nasopharyngeal aspirates were PCR-positive according to previously established criteria [7,13,17,43].

Statistical analysis

The data were analyzed using SAS Windows v.12 (SAS Institute, Cary, NC, USA); a p value of <0.05 was considered to be statistically significant. Comparisons were made between infants aged <3 months and those aged 3–12 months. Parametric data were evaluated by means of analysis of variance (ANOVA); abnormally distributed or non-parametric data were analyzed using the Kruskal–Wallis test. The categorical data were analyzed by means of contingency analysis and the chi-squared or Fisher's test.

RESULTS

The study enrolled 85 infants aged less than 12 months who were hospitalized because of a first acute episode of wheezing (57 males; mean age ± standard deviation, 2.95 ± 2.21 months). Fifty-three patients (62.3%) were aged less than 3 months, and 32 (37.7%) were aged 3–12 months. All were full-term newborns (61.2% born by means of an eutocic delivery) and all had a physiological neonatal history. Twenty-two (25.9%) had a family history of asthma, all lived in an urban area, and none had attended a day-care centre before enrolment. There were no differences in the demographic characteristics of the two age groups.

Table 1 shows the infectious agents detected in the study population. At least one agent was identified in 76 of the 85 children (89.4%). Sixty-one patients (71.8%) were infected with a single pathogen; 15 were co-infected with different viruses (17.6%; p <0.0001 vs. patients infected with a single pathogen); and no pathogen was detected in nine cases (10.6%; p <0.0001 vs. patients infected with a single pathogen). There were no significant differences in the prevalence of the single infections, co-infections or absence of pathogen in the two age groups.

Table 1. Infectious agents detected in otherwise healthy infants hospitalized for a first episode of wheezing

Infections	Age	
	<3 months (n = 53)	3–12 months (n = 32)
Patients infected with a single pathogen, no. (%)	38 (71.8)[a]	23 (71.8)
RSV, no. (%)	30 (56.6)[b,c]	22 (68.7)[c]
Influenza viruses, no. (%)	3 (5.7)	0 (0.0)
hCoVs, no. (%)	3 (5.7)	0 (0.0)
hMPV, no. (%)	1 (1.9)	0 (0.0)
Bocavirus, no. (%)	1 (1.9)	0 (0.0)
Mycoplasma pneumoniae, no. (%)	0 (0.0)	1 (3.1)
Patients infected with more than one pathogen, no. (%)	8 (15.1)	7 (21.9)
RSV + hCoVs, no. (%)	2 (3.7)	1 (3.1)
RSV + adenovirus, no. (%)	0 (0.0)	2 (6.3)
RSV + paraechovirus, no. (%)	0 (0.0)	2 (6.3)
RSV + influenza viruses, no. (%)	1 (1.9)	1 (3.1)
RSV + rhinovirus, no. (%)	1 (1.9)	1 (3.1)
Rhinovirus + enterovirus, no. (%)	1 (1.9)	0 (0.0)
Rhinovirus + parainfluenza virus, no. (%)	1 (1.9)	0 (0.0)
Influenza + adenovirus, no. (%)	1 (1.9)	0 (0.0)
Influenza + bocavirus, no. (%)	1 (1.9)	0 (0.0)
Patients not infected with any of the studied pathogens, no. (%)	7 (13.2)	2 (6.2)

RSV, respiratory syncytial virus; hCoVs, human coronaviruses; hMPV, human metapneumovirus.
[a]p <0.0001 vs. patients infected with more than one pathogen and vs. patients not infected with any of the studied pathogens.
[b]p <0.05 vs. RSV in infants aged 3–12 months.
[c]p <0.0001 vs. patients infected with a single pathogen other than RSV.

RSV was the most frequently detected pathogen and its prevalence was significantly higher than that of the other pathogens in both age groups, and significantly higher in the children aged 3–12 months than in those aged <3 months. With the exception of the children with RSV co-infections, all of the other viruses were found only in the youngest age group, whereas *M. pneumoniae* was found in a 9-month-old child. Among the patients infected with a single pathogen, the RSV cases were due to RSV A in 13 infants (25.0%) and RSV B in 39 (75.0%), the influenza cases were due to influenza A virus in two infants (66.7%) and influenza B virus in one (33.3%), and the hCoV cases were due to hCoV OC43 in two infants (66.7%) and hCoV HKU1 genotype A in one (33.3%). No single infection due to adenovirus, rhinovirus, parainfluenza viruses (types 1, 2, 3 and 4), hCoV 229E and hCoV NL63, enterovirus or paraechovirus was observed; these viruses were only detected in infants with co-infections. *C. pneumoniae* was never detected.

Table 2. Clinical presentation of infants infected with a single pathogen

	Age						
	<3 months					3–12 months	
Characteristic	RSV (n = 30)	Influenza (n = 3)	hCoVs (n = 3)	hMPV (n = 1)	Bocavirus (n = 1)	RSV (n = 22)	*Mycoplasma pneumoniae* (n = 1)
Onset, no. (%)							
Gradual	25 (83.3)	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	14 (63.6)	1 (100.0)
Acute	5 (16.7)	1 (33.3)	1 (33.3)	1 (100.0)	1 (100.0)	8 (36.4)	0 (0.0)
Rectal temperature ≥38°C, no. (%)	9 (30.0)	2 (66.7)	1 (33.3)	0 (0.0)	1 (100.0)	9 (40.9)	1 (100.0)
Severe symptoms, no. (%)							
Tachypnoea ≥60 breaths/min	20 (66.7)	1 (33.3)	3 (100.0)	0 (0.0)	1 (100.0)	10 (45.5)	0 (0.0)
Tachycardia ≥180 beats/min	11 (36.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	6 (27.3)	0 (0.0)
Nasal flaring	23 (76.7)	1 (33.3)	3 (100.0)	0 (0.0)	1 (100.0)	15 (68.2)	1 (100.0)
Prolonged expiratory phase with wheezes	30 (100.0)	3 (100.0)	3 (100.0)	1 (100.0)	1 (100.0)	22 (100.0)	1 (100.0)
Pulse oximetry <92%	10 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)
Dehydration	20 (66.7)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	11 (50.0)	0 (0.0)
X-ray with evidence of pneumonia, no. (%)	17 (56.7)	1 (33.3)	1 (33.3)	0 (0.0)	1 (100.0)	8 (36.4)	1 (100.0)

RSV, respiratory syncytial virus; hCoVs, human coronaviruses; hMPV, human metapneumovirus. There were no significant differences between the pathogens or age groups.

Table 3. Outcome of infants infected with a single pathogen.

	Age						
	<3 months					3–12 months	
Variable	RSV (n = 30)	Influenza (n = 3)	hCoVs (n = 3)	hMPV (n = 1)	Bocavirus (n = 1)	RSV (n = 22)	*Mycoplasma pneumoniae* (n = 1)
Treatment, no. (%)							
Bronchodilators	30 (100.0)	3 (100.0)	3 (100.0)	1 (100.0)	1 (100.0)	22 (100.0)	1 (100.0)
Steroids	8 (26.7)	1 (33.3)	0 (0.0)	0 (0.0)	1 (100.0)	8 (36.4)	0 (0.0)
Antibiotics	16 (53.3)	2 (66.7)	1 (33.3)	0 (0.0)	0 (0.0)	7 (31.8)	1 (100.0)
Intravenous fluids	20 (66.7)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	11 (50.0)	0 (0.0)
Supplemental oxygen	10 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)
Chest physiotherapy	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)
Duration of hospitalization, mean days ± SD	5.96 (2.43)	5.0 (1.0)	5.33 (1.15)	4.0	5.0	5.47 (2.15)	6.0
Recurrent wheezing in the following 6 months, no. (%)							
At least one episode	18 (60.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (63.6)	0 (0.0)
More than one episode	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (13.6)	0 (0.0)

RSV, respiratory syncytial virus; hCoVs, human coronaviruses; hMPV, human metapneumovirus. There were no significant differences between the pathogens or age groups.

Tables 2 and 3 show the clinical presentations and outcomes, respectively, of the infants infected with a single pathogen. The onset of the first wheezing episode was gradual in most of the children, and not all of them presented with fever. All were hospitalized because of severe symptoms; chest examination showed that all had a prolonged expiratory phase with wheezes, and some had a chest radiograph showing signs of pneumonia.

During the acute episode of wheezing, all of the patients were treated with bronchodilators, many received antibiotics, and a minority received steroids. Most of the RSV and hCoV cases required intravenous fluids, but supplemental oxygen was administered to only some of them. Chest physiotherapy was required because of atelectasis in a small number of patients with RSV infection. The duration of hospitalization varied from 1 to 8 days.

There were no significant differences in clinical presentation, drug administration or duration of hospitalization between the patients, with respect to pathogen, or between the two age groups. However, after the initial episode of wheezing that led to enrolment, only infants with RSV infection experienced at least one further episode in the following 6 months.

Table 4 summarizes the relationships between RSV and the clinical data. Clinical presentations and outcomes were similar regardless of the type of RSV. However, although viral load did not correlate with the severity of disease, the numbers of RSV copies/mL were significantly higher in the children with recurrent wheezing than in those without.

Table 4. Relationship between respiratory syncytial virus (RSV) and clinical data in children with RSV infection

Characteristic	RSV viral load (copies/mL)	RSV A (n = 13)	RSV B (n = 39)
Rectal temperature			
≥38°C	$(5.50 \times 10^5) \pm (2.01 \times 10^6)$	3 (25.0)	11 (28.2)
<38°C	$(4.20 \times 10^5) \pm (7.89 \times 10^5)$	10 (75.0)	28 (71.8)
Severe symptoms			
Tachypnoea ≥60 breaths/min	$(3.53 \times 10^5) \pm (8.17 \times 10^5)$	8 (61.5)	22 (56.4)
Breath rate <60 breaths/min	$(6.59 \times 10^5) \pm (2.39 \times 10^6)$	5 (38.5)	17 (43.6)
Pulse oximetry <92%	$(7.06 \times 10^5) \pm (1.42 \times 10^6)$	3 (23.1)	9 (23.1)
Pulse oximetry ≥92%	$(4.75 \times 10^5) \pm (1.74 \times 10^6)$	10 (76.9)	30 (76.9)
X-ray			
Evidence of pneumonia	$(6.03 \times 10^5) \pm (1.79 \times 10^6)$	6 (46.2)	19 (48.7)
No evidence of pneumonia	$(5.40 \times 10^5) \pm (2.10 \times 10^6)$	7 (53.8)	20 (51.3)
Drug administration			
Antibiotics	$(5.49 \times 10^5) \pm (3.10 \times 10^6)$	5 (38.5)	18 (46.2)
No antibiotics	$(5.10 \times 10^5) \pm (1.49 \times 10^6)$	8 (61.5)	21 (53.8)
Intravenous fluids	$(4.17 \times 10^5) \pm (1.76 \times 10^6)$	6 (46.2)	20 (51.3)
No intravenous fluids	$(5.12 \times 10^5) \pm (1.82 \times 10^5)$	7 (53.8)	19 (48.7)
Duration of hospitalization			
<4 days	$(6.77 \times 10^5) \pm (2.40 \times 10^6)$	6 (46.2)	19 (48.7)
≥4 days	$(4.27 \times 10^5) \pm (9.98 \times 10^5)$	7 (53.8)	20 (51.3)
Recurrent wheezing in the following 6 months			
At least one episode of wheezing	$(8.27 \times 10^5) \pm (2.10 \times 10^6)$*	8 (61.5)	24 (61.5)
No wheezing	$(9.88 \times 10^2) \pm (2.41 \times 10^3)$	5 (38.5)	15 (38.5)

Percentages in parentheses. RSV, respiratory syncytial virus.
*p <0.05 vs. no wheezing.

DISCUSSION

Our findings indicate that RSV is the main pathogen associated with the first episode of wheezing requiring hospitalization in infants younger than 1 year, and that the other respiratory pathogens recently suggested to play a significant pathogenic role [7,8,10–24] are only marginally important at this stage. More than 60% of the infants in our study sample were infected with RSV, whereas the prevalence of the other pathogens never exceeded 6%. The absolute relevance of RSV was particularly evident among the 23 infants aged 3–12 months infected with a single pathogen, 22 of whom (95.7%) had RSV infection. However, even in the first 3 months of life, an association between wheezing and respiratory infections due to pathogens other than RSV was uncommon, occurring in only eight (21.1%) of the 38 children in whom a single pathogen was detected. The importance of RSV in the infants' first wheezing episode was also clear in the co-infected cases, because 11 of the 15 cases in whom more than one pathogen was detected (73.3%) had RSV infection.

The study was carried out during the late autumn and winter periods, when RSV and influenza viruses are often associated with epidemic attacks [18,19,46], which may be a limitation, as it has been found that some other respiratory pathogens (including recently identified viruses and atypical bacteria) [10–24] can be isolated in children throughout the year. It is therefore possible that, if the study had extended over a longer period, the proportions of wheezing episodes associated with the different infectious pathogens may have been slightly different. However, as most cases of wheezing in the first year of life occur during late autumn and winter [1–4], it is unlikely that these differences would significantly change the outcome of the current study.

Our knowledge of the real importance of different respiratory pathogens in causing wheezing in the first year of life is relatively poor because most of the published studies of infectious wheezing in children considered only a single pathogen and/or did not consider the subjects aged less than 1 year as a separate group. In addition, most of the papers do not specify whether the studied wheezing episode was the first, and, in some cases, the disease for which each child was admitted is not precisely defined. However, the results of the few studies comparing different infectious agents (but not atypical bacteria) and their association with wheezing in early life have also highlighted the primary role of RSV [9,11,14,15].

The clinical relevance of wheezing episodes does not seem to depend on the associated infectious agent. Although only a small number of children in the current study had infections due to a pathogen other than RSV, all of the data regarding the onset and severity of the disease, drug administration and duration of hospitalization are similar regardless of aetiology. These findings suggest that all of the respiratory pathogens can cause wheezing, and that all of them lead to a similar clinical presentation in hospitalized infants. However, this does not mean that the clinical impact of the studied pathogens was similar, because it is possible that respiratory pathogens other than RSV may cause more frequent, although less severe, wheezing in children not requiring hospitalization.

Recurrences were observed only in patients infected with RSV. The finding that RSV remains the most important pathogen associated with the first episode of wheezing during the first year of life, and that only subjects infected with this virus are at risk of developing long-term airway hyperresponsiveness, confirm the data suggesting that

RSV induces age-related changes in lung function [5,25–27]. Mouse studies have shown that the probability of a chronic alteration in lung function is greater the earlier the first RSV infection occurs, as is the probability that a subsequent infection will be followed by severe wheezing [25,47]. Earlier infection seems to induce a polarized Th2 response which, in the case of re-infection, causes exacerbated disease, as indicated by enhanced pulmonary resistance, mucus hyperproduction, eosinophilia, and high Th2 cytokine levels [48–50]. Our data suggest that, in humans, the period during which RSV infection can lead to a greater risk of further wheezing episodes extends at least until the end of the first year of life. The proportion of children in the current study infected by RSV who experienced a new episode of wheezing in the 6 months following the first episode was virtually the same in both age groups, whereas none of the children whose first wheezing episode was associated with an infectious agent other than RSV experienced a further episode.

This study also demonstrates for the first time that RSV viral load predicts the tendency to recur, but not disease severity, in children hospitalized because of a first wheezing episode. On the contrary, the type of RSV does not seem to be related to the clinical presentation, outcome or recurrence. Recent studies have assessed the role of host and viral factors in influencing RSV illness, but their results are controversial. A relationship between RSV genotypes and clinical data has not been completely established [51–53], and RSV viral load appears to correlate with disease severity in some studies [54,55] but not in others [51].

Further studies comparing RSV viral load in outpatients and hospitalized infants with wheezing, as well as in paediatric patients with different types of respiratory tract infection, are required to clarify its role in acute illnesses. However, this is the first study correlating RSV viral load with the development of subsequent recurrent wheezing, which is the primary concern with RSV infections acquired in early life.

In conclusion, our findings show that RSV is the main cause of hospitalization during a first wheezing episode in the first year of life, and that it is the only pathogen associated with a high frequency of recurrence. A high RSV viral load appears to be strictly related to recurrence. These results suggest that every effort should be made to prevent RSV infection and to contain RSV replication in the first year of life to reduce the risk of subsequent episodes of wheezing.

TRANSPARENCY DECLARATION

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