

Viral Findings in Children under the Age of Two Years with Expiratory Difficulties

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ABSTRACT. Korppi, M., Halonen, P., Kleemola, M. and Launiala, K. (Department of Paediatrics, University Central Hospital, Kuopio, Department of Virology, University of Turku, Turku and Department of Virology, National Public Health Institute, Helsinki, Finland). Viral findings in children under the age of two years with expiratory difficulties. *Acta Paediatr Scand* 75: 457, 1986.

Viral findings were prospectively studied in lower respiratory tract infections in small children with and without expiratory difficulties. On first admission, a viral aetiology was found in 71 of 127 children (56%). On re-admission, a viral etiology was found in only two of 31 cases (6%). Respiratory syncytial viruses (RSV) were responsible for 71% of the cases with viral diagnoses. A recently-developed method for the direct detection of viral antigens in nasopharyngeal specimens by radio-immunoassay was more sensitive than complement fixation serology, especially in patients aged less than six months. Viral diagnosis was reached using this new method alone in 43% of infections caused by RSV and in 27% of infections caused by other viruses. In children under six months, RSV were found in 89% by direct antigen detection and in 22% by serology. We suggest that direct antigen detection should be used as the primary virological method in small children with lower respiratory tract infections. The aetiological agents were the same in cases with and without expiratory difficulties, RSV being found in about 40% of children in both instances. It is concluded that host factors are critical to the development of expiratory difficulties. *Key words: respiratory syncytial virus, radio-immunoassay, bronchiolitis, wheezy bronchitis, breathing difficulties.*

An association between expiratory difficulties and respiratory viral infections in infants and small children is well documented. In children under the age of two years, the infectious agent most commonly reported is respiratory syncytial virus (RSV). Parainfluenza viruses and adenoviruses are also frequently found (1-3). In older children, and during repeated wheezing episodes, rhinoviruses have been most commonly found (1-3). In a recent study (4), coronaviruses were a frequent finding in small children with wheezy bronchitis. Why some infants and children wheeze during respiratory infections, especially those caused by RSV, is still obscure. Some authors emphasize the role of the virus itself in the production of airways damage (5-7), others believe in the importance of host factors, either bronchial hyper-reactivity (8-10), atopy (11-14) or both (15). Despite representative epidemiological studies on the incidence and aetiology of various lower respiratory syndromes (1, 16-20) viral discovery rates have been fairly low because of the low sensitivity of the serological and virus isolation methods used. Recently developed methods for direct viral antigen detection in clinical specimens using radio-immunoassay (RIA) (21-26) or enzyme immunoassay procedures (21-23, 25, 27-29) are more sensitive and have already proved useful, especially in small children.

The purpose of this study was to investigate viral aetiologies in lower respiratory tract infections in small children with and without expiratory difficulties. We compared the relative sensitivities of direct antigen detection in nasopharyngeal secretions using radio-immunoassay and standard serology, involving complement fixation (CF).

Table 1. Age and sex distribution of patients in the three clinical categories on first admission

ED = respiratory infection with expiratory difficulties; P+ED = pneumonia with expiratory difficulties; P = pneumonia without ED

Age (months)	ED		P+ED		P		All categories		
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Total
1-5	3	4	4	2	5	9	12	15	27
6-11	7	4	13	6	7	3	27	13	40
12-17	7	4	9	1	3	8	19	13	32
18-23	2	4	9	2	8	3	19	9	28
Total	19	16	35	11	23	23	77	50	127

MATERIAL AND METHODS

This prospective study included all children from the age of one month to two years who were hospitalized in the Department of Pediatrics, Kuopio University Central Hospital for respiratory tract infections with expiratory difficulties or pneumonia between 1st September 1981 and 31st August 1982. Children with infections acquired in hospital were excluded. The number of patients was 127 (77 boys and 50 girls) and that of admissions 158. Most (74%) of the 81 children with expiratory difficulties were wheezing for the first time.

The patients were divided into three clinical categories (Table 1): respiratory infection with expiratory difficulties (ED, 35 cases), pneumonia with expiratory difficulties (P+ED, 46 cases) and pneumonia without expiratory difficulties (P, 46 cases). Diagnoses of expiratory difficulties were based on auscultatory findings of prolonged expiratory phase and/or expiratory wheezing confirmed by one of us (MKo). Diagnoses of pneumonia were based on chest X-ray findings, evaluated by two radiologists without knowledge of the clinical signs.

Our central hospital provides care for all paediatric patients in a geographically defined area. Four children (five admissions) were from a town in which mild cases are treated in a regional hospital. The mean population of children between the ages of one month and two years was 5818 in the area primarily served by our hospital during the study period. Thus the total incidence of episodes of expiratory difficulties (clinical categories ED and P+ED combined) requiring hospital care was 17.7/1 000/year.

During the first hospital day, nasopharyngeal mucus specimens (NPS) were collected by suction via the nostrils, using a mucus extractor. All specimens, including small ones, were accepted. This practice may diminish positive findings in direct viral antigen detection but we wanted to ensure epidemiological representativeness of the data by including all cases. A portion of each sample was frozen at -20°C . NPS-RIA tests for virus antigens were undertaken in 93 cases on both fresh and frozen samples, in 27 cases on the fresh samples only and in 32 cases on the frozen samples only. Of these 93 double samples, results were positive in 18 cases in both samples, in seven cases in fresh samples alone and in eight cases in frozen samples alone. In the positive double samples, the virus was always the same. The methods used in the NPS-RIA tests for RSV and adenovirus have been described previously (21), as have those for influenza virus A and B (22) and parainfluenza virus 1, 2 and 3 (23).

Acute phase serum samples for viral serology were taken on admission and convalescent phase serum samples three to four weeks later. The standard CF test, on a microscale, was performed using the following antigen preparations: influenza A and B, parainfluenza 1, 2 and 3, adenoviruses, mumps virus, herpes simplex virus (HSV), cytomegalovirus (CMV), enteroviruses, respiratory syncytial virus (RSV), coronaviruses, *Mycoplasma pneumoniae* and *Chlamydiae*. A fourfold or greater rise in antibody titres between paired sera was considered diagnostic.

The chi-squared test was used in statistical analyses.

RESULTS

On first hospital admission, NPS for RIA were available for 122 of the 127 children (for 30 out of the 31 re-admissions) and paired sera for serology for 121 children (for 22 on re-admissions). We considered a virus to be an aetiological agent if the results of direct antigen detection and/or serology were positive. On first admission, a viral or mycoplasmal aetio-

Table 2. Viral findings in the three clinical categories in 127 children on first admission

Viruses were detected either by serology (CF), direct antigen detection (NPS-RIA) or both. *Mycoplasma pneumoniae* was detected by serology. ED = respiratory infection with expiratory difficulties; P+ED = pneumonia with expiratory difficulties; P = pneumonia without ED

Agents	Numbers of positive findings in the three clinical categories			
	ED (n=35)	P+ED (n=46)	P (n=46)	Total (n=127)
RSV	14 ^a	18 ^d	19 ^{b, e, c}	51
Adenovirus	5 ^f	0	6 ^b	11
Parainfluenza virus 1, 2 or 3	3 ^f	1	1	5
Other viruses	1 ^g	3 ^{d, g, h}	1 ^c	5
All viruses	23	22	27	72
<i>M. pneumoniae</i>	1 ^a	1	3 ^e	5
Patients with viral or mycoplasmal diagnosis	22	22	27	71

^{a, b, c, d} Double infections caused by RSV and ^a *M. pneumoniae*, ^b adenovirus, ^c CMV or ^d HSV.

^{e, f} Double infections caused by adenovirus and ^e *M. pneumoniae* or ^f parainfluenza virus.

^g Enterovirus only.

^h HSV only (acute stomatitis after respiratory infection).

logy was found in 71 of the 127 children (56%) (Tables 2 and 3), on re-admission in only two (pneumonia caused by RSV and parainfluenza virus, respectively) of the 31 cases (6%).

RSV, the most common aetiological agent, was found in 51 of the 127 children on first admission (40%) (Tables 2 and 3). It was responsible for 71% of the 73 cases with viral or mycoplasmal diagnoses. Two children with RSV finding in NPS-RIA had also significantly increased antibodies to adenovirus or *M. pneumoniae*. The cases were considered as double

Table 3. Viral findings in four age groups in 127 children on first admission

Viruses were detected either by serology (CF), direct antigen detection (NPS-RIA) or both. *Mycoplasma pneumoniae* was detected by serology

Agents	Age groups				Total (n=127)
	1-5 months (n=28)	6-11 months (n=40)	12-17 months (n=37)	18-23 months (n=28)	
RSV	20 ^{a, ***}	13	12 ^c	6 ^{b, d}	51
Adenovirus	0	4 ^{e, f}	2	5 ^d	11
Parainfluenza virus	0	3 ^f	0	2	5
Other viruses	0	1 ^h	2 ^{g, c}	2 ^{g, d}	5
All viruses	20*	21	15	15	72
<i>M. pneumoniae</i>	2 ^a	1 ^e	0	2	5
Patients with viral or mycoplasmal diagnosis	21*	20	15	15	71

^{a, b, c, d} Double infections caused by RSV and ^a *M. pneumoniae*, ^b adenovirus, ^c CMV or ^d HSV.

^{e, f} Double infections caused by adenovirus and ^e *M. pneumoniae* or ^f parainfluenza virus.

^g Enterovirus only.

^h HSV only (acute stomatitis after respiratory infection).

* $p < 0.05$. *** $p < 0.001$.

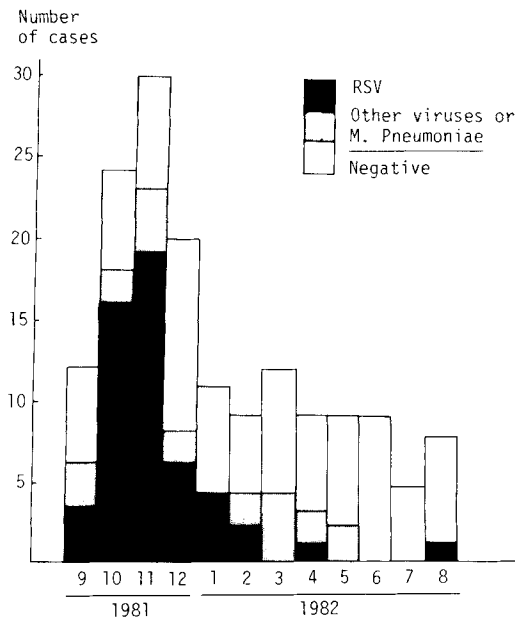


Fig. 1. Monthly distribution of 52 RSV-positive and 106 RSV-negative cases during the study period (September 1st, 1981–August 31st, 1982). Double infections caused by RSV and other viruses or *M. pneumoniae* have been recorded as RSV-positive cases.

infections as well as those four cases with significantly increased antibodies to two viruses (Tables 2 and 3). The second most common agent was adenovirus, found in 9% of cases on first admission. Parainfluenza viruses and *M. pneumoniae* were found in 4% of cases. No infections caused by influenza viruses or coronaviruses were found.

Direct antigen detection by NPS-RIA was compared with routine CF serology for diagnosing RSV infections (Table 4). Samples for both methods were available for 46 of the 52 RSV-positive children. Of these, 78% were positive by NPS-RIA and 57% by CF serology. Thus 43% of the RSV findings were based on NPS-RIA alone. Direct antigen detection was especially useful in infants below the age of six months by whom RSV was detected in 89%

Table 4. Comparison of serology and direct viral antigen detection in RSV-positive infections in four age groups

Only cases from whom both samples were available are included. Two cases in which NPS-RIA and CF-serology detected different viruses have been excluded. CF = complement fixation test, RIA = direct antigen detection by radioimmunoassay in nasopharyngeal specimens

Groups according to RIA and CF results	Age groups				Total
	1–5 months	6–11 months	12–17 months	18–23 months	
CF+ RIA+	2	5	7	2	16
CF+ RIA–	2	5	0	3	10
CF– RIA+	14***	3	3	0	20*
CF+	4	10	7	5	26
RIA+	16	8	10	2	36
Total	18	13	10	5	46

* $p < 0.05$. *** $p < 0.001$.

Table 5. RSV findings in patients experiencing expiratory difficulties for the first time as compared with those patients who had had one or more previous wheezing attacks

Double infections caused by RSV and *M. pneumoniae* or other viruses have been recorded as RSV-positive cases

Agents	History of previous wheezing episodes (+ or -)						Total
	Age 1-11 months		Age 12-23 months		All ages		
	-	+	-	+	-	+	
RSV	22	6	7	2	29**	3	32
Other viruses or <i>M. pneumoniae</i>	4	1	2	5	6	6	12
No virus	14	1	11	11	25	12	37
Total	40	3	20	18	60	21	81

** $p < 0.01$.

by RIA and in 22% by serology. In the older children, the two methods were more similar, with 71% of diagnoses given by RIA and 79% by CF. NPS-RIA performed well in all three clinical categories. The two methods were approximately equally efficient for detecting infections caused by the other viruses. In five cases of parainfluenza infection, serology was positive in three cases and RIA in four cases. In the 10 adenovirus infections, for which both samples were available, serology gave positive results in eight and RIA in five cases. None of these infections occurred in children below six months the age at which NPS-RIA proved superior to serology for demonstration of RSV.

In the course of the study, an outbreak of infections caused by RSV occurred, between September 1981 and February 1982. The peak incidence was in October and November 1981, when about 2/3 of all infections were caused by RSV (Fig. 1). The majority of infections in infants under the age of six months (93%) and that of pneumonias (82%) occurred during this epidemic. During the RSV outbreak a viral diagnosis was established in 59% of 106 cases. After that it was established in only 19% of 52 cases (Fig. 1).

There were no differences in viral findings in the three clinical categories, RSV was the causative agent in 40, 39 and 41% of cases, respectively (Table 2). RSV was most commonly found in the youngest age group. It caused 71% of infections in infants under the age of six months (Table 3). The sex distribution in the cases of RSV infection was similar to that in the whole material (see Table 1). Before six months of age, the numbers of boys and girls were the same, thereafter boys predominated (2/3 of both RSV-positive and RSV-negative infections).

In children with expiratory difficulties, a causative virus was found in 58% of cases without a history of previous wheezing attacks and in 43% of those with such history (Table 5). No causative viruses were found on re-admission. RSV was demonstrated more frequently in children without previous wheezing episodes (48%) than in those who had had one or more such attacks (14%) (Table 5). This was also true in children over one year of age, in whom about half of the patients had a previous history of wheezing. The other viruses were divided equally between these two groups, although the number of positive findings was small. Nearly all RSV infections (91%) occurred in children without previous wheezing episodes (Table 5).

DISCUSSION

The percentages of positive virus identification in wheezing-associated respiratory infections have ranged from 14 to 49% in previous reports (11, 17, 19, 20, 24, 30, 31). In two representative population studies, an aetiological agent was found using viral and mycoplasmal culture in 21–24% of cases (1, 16) and using standard CF serology in 36% (16). In the present study, an aetiological agent was found more often, in 63% of respiratory infections and 48% of pneumonias with expiratory difficulties on first admission and in 46% and 37% on all admissions, respectively. There are at least two reasons for this. Firstly, an RSV outbreak occurred at the beginning of the study period. During two autumn months, a viral aetiology was found in 76% of infections, mostly involving RSV. After the RSV epidemic, a viral diagnosis was established in only 19% of cases. Secondly, direct viral antigen detection was used in addition to serology. A viral aetiology was established by NPS-RIA alone in 43% of RSV infections and in 27% of infections caused by other viruses. The antigen detection method was especially useful in infants aged below six months.

The sensitivity of viral antigen detection using RIA or enzyme immuno-assay methods has been found to range from 79 to 97% in RSV infections (21, 27–29) as compared with isolation or immunofluorescence methods. In two recent studies in which NPS-RIA was compared with a sensitive serological test involving virus specific IgG antibody, the sensitivity of NPS-RIA was 78% in RSV infections (26) and 80% in adenovirus infections (25). However, the relative sensitivities of the methods also depend on the age of the study population, as has clearly been shown in this study. Direct antigen detection is especially valuable in infants under six months of age, on the basis of the present data and that of previous studies (26). To improve the resolution of serology, the CF test used in this study should be supplemented by the newer sensitive radio-immunoassay or enzyme immunoassay methods which allow assay of antibodies of different immunoglobulin classes separately (25, 26).

In children with expiratory difficulties, a causative virus was detectable during first episodes but seldom during recurrences. We did not try to isolate rhinoviruses, which have been found to be the most frequent aetiological agent in repeated wheezing episodes (17, 19, 30–32). In a previous study (17), the percentage of cases in which a virus was found was higher on re-admission than on first admission because of a large number of rhinovirus infections.

Viral aetiologies were similar in the three clinical categories. In the whole material RSV was the causative agent in about 40% of cases, with or without expiratory difficulties. However in infants under six months of age, RSV caused 71% of all infections. Small infants suffered more often from pneumonia and less often from expiratory difficulties than the older children. This conflicts with the view that the RSV itself has a particular tendency to cause wheezing. We conclude that RSV infections start wheezing in those small children who are genetically predisposed to wheeze. It has been suggested that the constitutional factor concerned could be bronchial hyper-reactivity or atopy.

In conclusion, the majority of lower respiratory infections in children under two years of age are of viral origin. The causative agents can be detected in most cases using advanced virological methods. Direct antigen detection in nasopharyngeal mucus specimens should be used as the primary diagnostic method in small children. The causative viruses and the percentages of cases in which viruses were found was similar in lower respiratory infections with and without expiratory difficulties. Host factors therefore appear critical to the development of expiratory difficulties during respiratory viral infection episodes.

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