

Original Article

## Acute lower respiratory infections in hospitalized children over a 6 year period in Tokyo

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### Abstract

**Background:** Acute lower respiratory infections are major causes of hospitalization in children and are mainly caused by respiratory viruses. In the present study, we investigated the etiologic agents responsible for acute lower respiratory infections from the period November 1986 to October 1992 in order to determine the seasonal pattern and different characteristics of age distribution of respiratory infectious agents, mainly virus infections.

**Methods:** A total of 1521 patients with lower respiratory infections was hospitalized in Saiseikai Central Hospital, Tokyo, Japan. Nasopharyngeal secretions were obtained for virus isolation and paired sera in the acute and convalescent phases were obtained for serological examination.

**Results:** Etiological agents were identified in 668 of 1521 patients (43.9%) by serological antibody responses, virus isolation and/or detection of virus antigen: 240 (15.8%) with respiratory syncytial (RS) virus; 62 (4.1%) with influenza virus type A; 26 (1.7%) with influenza virus type B; 86 (5.7%) with adenovirus; 81 (5.3%) with parainfluenza virus; 32 (2.1%) with measles virus; 20 (1.3%) with enteroviruses or Herpes virus other than respiratory viruses; 75 (4.9%) with *Mycoplasma pneumoniae*; 10 (0.7%) with pertussis; and 36 (2.4%) with mixed infections. In the remaining 853 patients (56.1%), etiologic agents were not identified. Respiratory syncytial (RS) virus was a main causative agent of respiratory infections in patients younger than 3 years of age. Influenza virus and *M. pneumoniae* were two main causative agents in patients with acute respiratory illness over 5 years of age. Parainfluenza virus type 3 was frequently observed in infants from 9 to 12 months of age. A distinct seasonal pattern of viral infections was consistently observed in each year during the study period; RS and influenza viruses were prevalent in winter, parainfluenza virus was prevalent in spring and *M. pneumoniae* was prevalent in summer and autumn. However, adenovirus infections were observed in all seasons. Serological responses were poor in patients younger than 1 year of age and they were mainly diagnosed by virus isolation or detection of virus antigen.

**Conclusions:** Virological epidemiology provides useful information in daily clinical practice for the prediction of etiologic agents based on patient age and the seasonal distribution of agents. We should examine virus isolation and the detection of virus antigen, along with serological examinations in patients with respiratory infections, especially in infants younger than 1 year of age because of poor serological responses.

### Key words

acute lower respiratory infections, respiratory tract virus, virological epidemiology, virus isolation.

Acute lower respiratory infection is a major cause of hospitalization of children in developed countries as well as in developing countries. Most lower respiratory infections are caused by respiratory tract viruses and they are generally self-limiting illnesses.<sup>1</sup> Bacterial pneumonia or bronchitis is

rarely observed in developed countries, but impaired host defense mechanisms after some viral infections predispose to secondary bacterial mixed infection.<sup>2</sup> While viral–bacterial mixed infections are predominantly observed in pneumonia patients, concurrent dual viral infections are prominent in patients with bronchitis or upper respiratory infections during the winter season.<sup>3</sup> Respiratory virus infection is mainly diagnosed by conventional serological responses between sera obtained in the acute and convalescent phases, but the serological response is poor in young infants.<sup>4</sup> The most reliable diagnostic method is virus

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Received 3 August 1998; revised 18 February 1999; accepted 19 February 1999.

isolation or the detection of virus antigens, but viral etiologic diagnostic examinations are neglected in most patients because viral isolation is time consuming and has little advantage in the clinical setting. Several virus agents circulate concurrently in annual seasonal patterns, as reported by several authors<sup>5-15</sup> and it should be emphasized that the epidemiological investigation of etiologic agents in respiratory infections is essential for clinical study. In addition, the epidemiological data will provide useful information in daily practice for the prediction of pathological agents on the basis of patient age or the seasonal distribution of agents.

We investigated the epidemiology of respiratory viruses, *Mycoplasma pneumoniae* and pertussis infections in order to determine the seasonal pattern of circulating etiologic agents and the age distribution in lower respiratory infections.

## Methods

### Patients

All patients were admitted in the Department of Pediatrics, Saiseikai Central Hospital, Tokyo, from November 1986 to October 1992 because of lower respiratory infections. They were all diagnosed by the attending physicians with clinical symptoms, physical examinations and chest radiographic findings. We enrolled 1521 patients with pneumonia, bronchitis, bronchiolitis or croup into the present study. Of these, 336 patients were younger than 1 year of age: 88 patients were <3 months, 83 patients were aged 3-6 months, 106 patients were aged 6-9 months and 59 patients were aged 9-12 months. In addition, 386 patients were 1 year of age, 258 were 2 years of age, 282 were 3 or 4 years of age, 136 were 5 or 6 years of age and 123 were over 7 years of age. Pathological agents were identified by serological response and/or positive results for virus isolation or detection of viral antigens.

### Samples and virus isolation

Nasopharyngeal secretion (NPS) samples were obtained from direct suctioning on the day of hospitalization.<sup>16,17</sup> A Nelaton catheter connected to a disposable syringe was inserted into the nasal cavities. Nasopharyngeal secretions were soaked in 1 mL minimum essential medium (MEM) supplemented with 5% calf serum and adequate antibiotics and were stored at -70°C until virus isolation and respiratory syncytial (RS) virus antigen detection. A 0.1 mL sample of NPS was inoculated on a monolayer of human embryonic lung (HEL), HEp-2, Vero and Madin-Darby canine kidney (MDCK) cells for virus isolation in a 24-well plate. The sample was inoculated into a pleuropneumonia-

like organism (PPLO) broth with methylene blue and phenol red for isolation of *M. pneumoniae* and a PPLO agar plate was used for identification of characteristic colonies. When measles was suspected from the clinical symptoms, B95a cells were used.<sup>18</sup> Plates were incubated at 37°C in 5% CO<sub>2</sub> until a cytopathic effect (CPE) was observed. Samples without a CPE or hemadsorption test after two passages were considered as negative for virus isolation. Isolates were identified by standard neutralization tests using antisera. As for the detection of virus antigens, a RS virus antigen detection kit (Ortho Diagnostics Systems, Raritan, NJ, USA) was used as per the manufacturer's instructions.

### Serology

Serum samples were obtained on the day of hospitalization and in the convalescent period, principally 7 days after admission, and were stored at -70°C. The complement fixation test was used for influenza A and B, adenovirus, RS virus and *M. pneumoniae*, the hemagglutination inhibition test was used for parainfluenza virus types 1, 2 and 3 and the measles virus and the agglutination test was used for pertussis. All patients were serologically examined and serological diagnosis of pathological agents was determined by a four-fold and greater increase in titers between the acute and convalescent phase sera.

## Results

### Etiological agents in lower respiratory infections

A total of 1521 patients were admitted to hospital during the 6 year period from November 1986 to October 1992. Pathogenic agents were determined in 668 patients (43.9%) and the details of the etiologic agents are summarized in Table 1. Respiratory syncytial virus infection was most frequently observed (240 patients; 15.8%), influenza A virus was seen in 62 patients (4.1%), influenza B virus was seen in 26 patients (1.7%), adenovirus was seen in 86 patients (5.7%), parainfluenza virus was seen in 81 patients (5.3%; type 1 was seen in seven patients, type 2 was seen in two patients, type 3 was seen in 66 patients and six patients were untypable because of serological cross-reaction) and the measles virus infection was seen in 32 patients (2.1%). Seventy-five patients (4.9%) were diagnosed with *M. pneumoniae* infection and 10 patients (0.7%) were diagnosed as having pertussis infection. Mixed virus infection was observed in 36 patients (detection of RS antigen and serological response to adenovirus in eight patients; detection of RS antigen and serological response to influenza virus A/B in six patients; isolation of adenovirus and serological response to parainfluenza virus in five patients; other different mixtures with respiratory viruses

**Table 1** Number of patients with lower respiratory tract infections and patients positive for isolation or antigen detection

Etiological agents	No. patients (%)	No. patients isolation or antigen positive (%)
RS virus	240 (15.8)	149 (62.1)
Influenza virus	88 (5.8)	41 (46.6)
–Type A	62 (4.1)	22 (35.5)
–Type B	26 (1.7)	19 (73.1)
Adenovirus	86 (5.7)	42 (48.8)
Parainfluenza virus	81 (5.3)	8 (9.9)
–I	7	
–II	2	
–III	66	
–Untypable	6	
Measles virus	32 (2.1)	32 (100)
Other viruses	20 (1.3)	
–(Herpes simplex, enteroviruses)		
Mixed viral infection	36 (2.4)	
–RS + adenovirus	8	
–RS + influenza A/B	6	
–Adenovirus + parainfluenza	5	
–Others	17	
<i>Mycoplasma pneumoniae</i>	75 (4.9)	12 (16)
Pertussis	10 (0.7)	2 (20)
Not identified	853 (56.1)	
Total	1521	

RS, respiratory syncytial.

**Table 2** Number of patients diagnosed by virus isolation or serology

Pathogens	V(+), S(+)	No. patients (%)	
		V(+), S(–)	V(–), S(+)
RS virus ( <i>n</i> = 262)	101 (38.5)	61 (23.3)	100 (38.2)
Influenza A ( <i>n</i> = 72)	9 (12.5)	17 (23.6)	46 (63.9)
Influenza B ( <i>n</i> = 30)	7 (23.3)	15 (50.0)	8 (26.7)
Adenovirus ( <i>n</i> = 102)	16 (15.7)	36 (35.3)	50 (49.0)
Parainfluenza ( <i>n</i> = 95)	0 (0)	8 (8.4)	87 (91.6)

V, virus isolation or detection of viral antigen; S, serological response; RS, respiratory syncytial.

and enteroviruses in 17 patients). Enteroviruses and Herpes simplex virus were isolated in 20 patients. Etiologic agents were not identified in 853 patients. We also demonstrated the number of positives for isolation of pathogens or detection of viral antigens. The rate of isolation of measles virus (100%) was the highest, while that of parainfluenza virus (9.9%) was the lowest. Isolation of *M. pneumoniae* (12/75; 16%) and pertussis (2/10; 20%) were relatively low in comparison with the rate of viral isolation.

#### **Virus isolation and serological response**

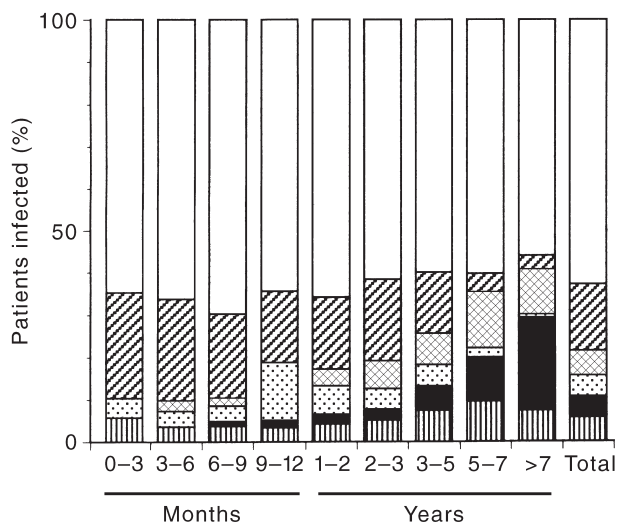
The main pathological viral agents of respiratory illness were RS, influenza, adenovirus and parainfluenza viruses.

Respiratory syncytial virus infection was diagnosed in 262 patients, influenza A virus in 72, influenza B virus in 30, adenovirus in 102 and parainfluenza virus in 95, including mixed infection. The diagnostic methods for pathological agents by virus isolation, detection of antigens or serological responses are summarized in Table 2. In 262 RS infections, 101 (38.5%) were diagnosed by both serology and detection of antigens or virus isolation, 100 (38.2%) were diagnosed with serology only and 61 (23.3%) were diagnosed with virus isolation or detection of antigens. Virus isolation increased the diagnostic rate in 23.6% of influenza A virus infection, 50% of influenza B virus infection, 35.3% of adenovirus infection and 8.4% of parainfluenza virus infection while showing negative serological responses.

**Table 3** Number of patients diagnosed only by serological responses in different age groups

Virus	No. patients diagnosed by serological response (%)			Total
	< 1 year	1–3 years	> 3 years	
RS virus ( <i>n</i> = 262)	40/79 (50.6)	111/128 (86.7)	50/55 (90.9)	201/262 (76.7)
Influenza A ( <i>n</i> = 72)	0/2 (0)	15/28 (53.6)	40/42 (95.2)	55/72 (76.4)
Influenza B ( <i>n</i> = 30)	0/2 (0)	5/11 (45.5)	10/17 (58.8)	15/30 (50)
Adenovirus ( <i>n</i> = 102)	8/20 (40)	17/36 (47.2)	41/46 (89.1)	66/102 (64.7)
Parainfluenza ( <i>n</i> = 95)	15/19 (78.9)	45/48 (93.8)	27/28 (96.4)	87/95 (91.6)

RS, Respiratory syncytial.



**Fig. 1** Percentage of patients infected with pathological agents in different age groups. Columns demonstrate the percentage of the patients infected with (▨) respiratory syncytial virus, (⊠) influenza virus, (⊞) parainfluenza virus, (■) *Mycoplasma pneumoniae*, (▮), adenovirus or (□) other pathologic agents.

The serological response in different age groups (< 1 year of age, from 1 to 3 years of age, and > 3 years of age) is summarized in Table 3. A serological response was observed in 40 of 79 patients < 1 year of age (50.6%) with RS infection. None of the four patients diagnosed by virus isolation had a serological response to influenza virus. Serological response was demonstrated in 40% of adenovirus infections and in 78.9% of parainfluenza virus infections in patients < 1 year of age.

#### Age distribution

Of the 1521 patients, 336 were < 1 year of age and 386 were 1 year of age, 258 were 2 years of age, 282 were 3 or 4 years of age, 136 were 5 or 6 years of age and 123 were over 7 years of age. The pathological agents in the different age groups are shown in Fig. 1. In patients under 5 years of age, RS virus was most frequently related to pathological agents of lower respiratory infections and influenza viruses

were common pathogens in young children over 5 years of age. Parainfluenza virus was frequently observed in infants aged 9–12 months. *Mycoplasma pneumoniae* was a dominant agent in children over 7 years of age and adenovirus had no characteristic age distribution.

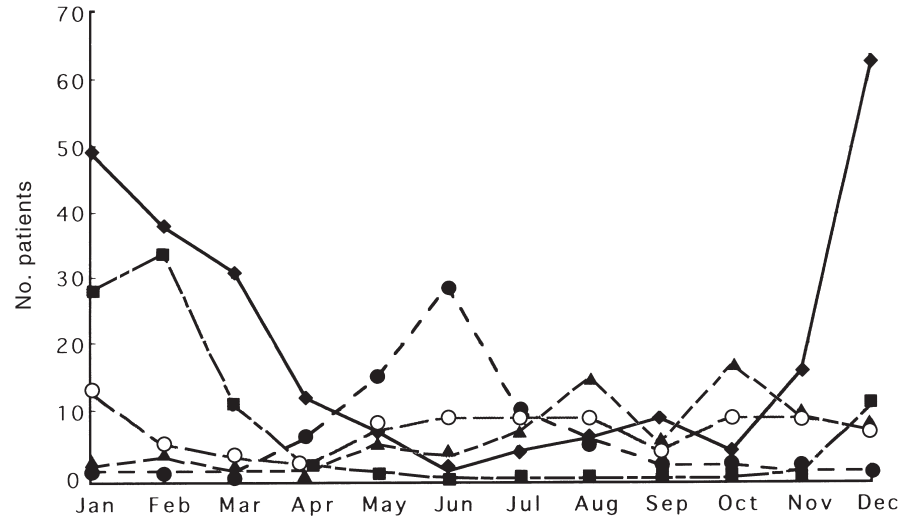
#### Seasonal pattern of circulating agents

During the 6 year period in Tokyo, 1521 patients with lower respiratory infections were shown to have a seasonal pattern of respiratory pathogens. A seasonal pattern for etiological agents was consistently observed each year (data not shown) and the results of the cumulative number of patients in each month are shown in Fig. 2. Respiratory syncytial virus emerged in November and faded in April. However, several patients presented in August or September with RS infection. Influenza virus infections were detected in December, January and February in each year of the investigation. Parainfluenza virus infections were mainly detected in May and June and *M. pneumoniae* was detected from July to October, but no seasonal prevalence was observed for adenovirus infections.

#### Discussion

The role of virus infections in acute respiratory illnesses is common world-wide. Denny *et al.*<sup>6</sup> reported that infants usually had seven or eight respiratory illnesses each year, on average, and the frequency decreased by age in the day-care setting in the US. The incidence of infections peaked at 7–12 months of life and less than 10% of infections involved the lower respiratory tract. Viral pathogens were recovered in 31% of cases and the frequent isolates were RS virus, parainfluenza virus (particularly type 3), adenovirus, enterovirus, rhinovirus and influenza virus with less frequency. The number of patients hospitalized for respiratory infections is limited and, thus, the results of the pathogens responsible may depend on the study design. Anderson *et al.*<sup>19</sup> reported that RS virus was isolated as yearly outbreaks; parainfluenza virus 1 and 2 infections

**Fig. 2** Seasonal pattern of etiological agents. (—◆—), Respiratory syncytial virus; (—■—), influenza virus; (—●—), parainfluenza virus; (—▲—), *Mycoplasma pneumoniae*; (—○—), adenovirus.



occurred every other year in the autumn; parainfluenza virus type 3 isolation occurred throughout the year; and influenza virus isolation occurred as yearly winter outbreaks. A strong correlation between RS virus outbreaks and lower respiratory tract infection deaths was reported in patients aged 1–11 months; influenza virus infections were correlated with lower respiratory deaths of infants aged 24–59 months. However, no relationship was reported between parainfluenza virus infections and lower respiratory deaths. Respiratory syncytial virus is the most important viral lower respiratory tract pathogen in infants and children in developed countries and it usually circulates from late autumn to early spring in temperate climates. Viral agents in lower respiratory infections are common in developed and developing countries. Epidemiological studies have been conducted in hospitalized children in Bangladesh, The Philippines, Thailand and Uruguay.<sup>11–14</sup> However, these reports were limited to a survey of only a few years. De Arruda *et al.*<sup>20</sup> reported the role of respiratory viruses in a 29 month household-based follow up of 175 children under 5 years of age in north-east Brazil. Respiratory tract viruses were isolated in 35% and, of these isolates, 45.6% were rhinoviruses, 16% were parainfluenza viruses, 15.8% were enteroviruses, 9.9% were adenoviruses, 7.0% were Herpes simplex viruses and 5.7% were influenza virus. Furthermore, these authors demonstrated that the peaks of influenza and parainfluenza virus activity were related to rainfall, but that rhinovirus activity was not. We investigated a longitudinal hospital-based survey of respiratory infectious diseases in Tokyo. During our 6 years study, RS and influenza virus infections were observed in the winter season, parainfluenza virus type 3 was observed in the spring, *M. pneumoniae* was observed in summer and autumn, but adenovirus circulated without seasonal differences and the seasonal pattern was slightly different from that in the US, depending on climate

differences. Constant seasonal patterns of viral respiratory infection have been reported in developed countries.<sup>5,6,19,21</sup> Essentially the same seasonal pattern has been reported for the Northern hemisphere. As for the distribution of agents in different age groups, RS was mainly responsible for pathogenic agents in children under 5 years of age, parainfluenza was observed in infants 9–12 months of age, influenza virus and *M. pneumoniae* were dominant agents in children over 5 years of age.

As for the diagnostic methods, we confirmed that the serological response was poor in young infants, especially in those less than 1 year of age, as reported previously.<sup>4</sup> A serological response was demonstrated in 40 of 79 patients (50.6%) infected with RS virus under 1 year of age, none of four patients with influenza A and B viruses and eight of 20 patients (40%) with adenovirus, as shown in Table 3. The rate of isolation of parainfluenza viruses was not sufficient in the present study, but we are uncertain for the reason for this. When virus isolation or detection of viral antigens was not used, etiological agents of the respiratory illness remained unidentified in approximately more than half the cases in children under 1 year of age.

In the present study, we intended to isolate RS, influenza, adenovirus, parainfluenza and measles viruses, *M. pneumoniae* and pertussis, but we did not intend to isolate bacteria other than pertussis or chlamydias or rhinoviruses and coronaviruses. It is possible that these agents may be related to the etiological agents in the 853 patients who were not etiological diagnosed. We have demonstrated a seasonal pattern and age distribution of viruses related to lower respiratory infections. The epidemiology of pathogens determined from the present investigation provides useful information about the main causative agent for use in our daily outpatient clinical setting.

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