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Original Article

Respiratory adenoviral infections in Taiwanese children: a hospital-based study

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Background and purpose: Adenoviruses are a common etiology of respiratory tract infections in children, with several serotypes responsible for most epidemic respiratory infections. This study examined the molecular epidemiology and clinical features of pediatric adenoviral infections in a 1-year period.

Methods: From May 1999 to April 2000, virus specimens collected from children with respiratory tract infections were identified. The presence of adenovirus was confirmed by direct fluorescent staining, and viral types were determined by polymerase chain reaction sequencing.

Results: Adenoviruses were identified from 272 children (mean ± standard deviation age, 48.3 ± 30.5 months), 227 (83.5%) of whom were aged 6 years or younger. Inpatients were younger than outpatients (44.1 ± 30.7 months vs 53.0 ± 29.4 months; $p = 0.006$). The commonest serotype identified was serotype 3 (164 patients; 60.3%), 73.1% of which were identified between September 1999 and January 2000. Serotype 3 was more common in inpatients ($p = 0.015$), while serotypes 1, 2, 5, and 6 were more common in outpatients ($p = 0.021$). Patients with pneumonia were younger than those with other infections (31.8 ± 20.2 months vs 50.3 ± 31.0 months; $p = 0.001$). Most of the children (90.1%) had fever for a mean of 3.80 ± 2.65 days before seeing a doctor. The clinical manifestations were similar regardless of the serotype.

Conclusions: Adenovirus serotype 3 caused the most adenovirus infections in autumn and winter of 1999 to 2000. The children were mostly preschool age and required hospital admission.

Key words: Adenovirus infection; Respiratory tract infections; Taiwan

Introduction

Adenoviruses are responsible for a variety of infections, and cause approximately 2% to 9% of acute respiratory tract infections (RTIs) in children younger than 5 years [1,2]. Subgenus B1, including adenovirus serotypes 3 and 7, subgenus C (serotypes 1, 2, 5, and 6), and subgenus E (serotype 4) are common causes of RTI [3]. Serotypes 3 and 7 are responsible for most epidemics of lower RTI (LRTI) in children, although they are also identified in sporadic cases [4-6]. Identification of these subgroups or serotypes can be of both clinical and epidemiological value because more severe disease is associated with serotypes 1-7, 7a, 8, 19, 21, and 35 [1,5,6].

Adenovirus can be detected in viral culture, and by immunofluorescence and enzyme-linked immunosorbent assay. Typing and subtyping is done by immunofluorescence, neutralization tests, polymerase chain reaction (PCR), and restriction fragment length polymorphism [7,8]. However, most laboratories do not have the facilities to type adenoviruses routinely, making it difficult to identify outbreaks attributable to a specific serotype. In Taiwan, adenoviruses are endemic and can be isolated throughout the year. Only a few descriptions of the molecular epidemiology have been published [9-11].

This study was designed to provide epidemiological data of adenovirus infection in Taiwan, and to correlate the data with the clinical features of acute adenoviral RTI in children in northern Taiwan.

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Methods

All adenovirus samples isolated from pediatric patients between May 1, 1999 and April 30, 2000 in the clinical virology laboratory at Mackay Memorial Hospital, Taipei, Taiwan, were collected. The specimens had been obtained by throat swabs, nasopharyngeal aspirates, and conjunctival swabs from children with RTIs aged 15 years or younger.

The specimens were collected in transport buffer, placed on ice, and immediately transported to the clinical virology laboratory. Specimens were inoculated onto 4 different cell lines: MRC-5, Hep-2, A549, and MDCK. The presence of adenovirus was first identified by a cytopathic effect and then confirmed by direct fluorescent staining (DAKO Corp., Santa Barbara, CA, USA). Cultured supernatants were than stored at –70°C for further use. Adenoviruses isolated from several different specimens from the same patient were regarded as 1 isolate. The isolates were then typed using PCR, with primers designed to identify the adenovirus genus-specific VA RNA region. The upstream primer was VA3a 5'-CGGT(G/C)AGGCG(T/C)GCGCAGTC-3' and the downstream primer was VA6 5'-GCAGCCANGGATGCATCT-3' [3,7]. The primers were synthesized by MWG-Biotech AG (Ebersberg, Germany).

For PCR, 1 μL of cultured supernatant was added to each reaction tube. The composition of the PCR mixture (50 μL) was 50 mM potassium chloride, 10 mM trisaminomethane hydrochloride (pH, 8.3), 1.5 mM magnesium chloride, 10% glycerol, 200 μM deoxynucleotide triphosphate, 0.4 μM primers, and 1.25 U of Taq DNA polymerase (Qiagen, Hilden, Germany). The reaction tube was placed in a Perkin Elmer GeneAmp PCR System 2400 Thermal Cycler (Waltham, MA, USA) and the reaction was subject to 35 cycles of amplification (20 sec at 94°C, 30 sec at 45°C, and 50 sec at 72°C), followed by 7 min of extension at 72°C. All PCR products were electrophoresed on 2% agarose gel. Products were purified with GFX PCR DNA and Gel Band purification kit (Amersham Pharmacia Biotech, Inc., Uppsala, Sweden), and then sequenced using the ABI Prism® BigDye™ Terminator (Applied Biosystems, Inc., Foster City, CA, USA). The sequenced products were analyzed with an ABI Prism® 377 Genetic Analyzer (Applied Biosystems, Inc.). A National Center for Biotechnology Information Basic Local Alignment Search Tool was used to identify the serotype. This method is unable to differentiate types 1, 2, 5, and 6, so the designation serotype 1.2.5.6 was used to identify adenovirus isolates within that group.

The medical records of the patients with respiratory adenoviral infections were reviewed for epidemiological data, clinical presentation, diagnosis, and laboratory data, including total white blood cell (WBC) count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

Statistical analysis

Analysis for comparisons of proportion between 2 groups was performed with the chi-squared or Fisher's exact probability tests for categorical variables and Student's *t* test or Mann-Whitney *U* test for continuous variables. All statistical tests used were 2-sided, with p values <0.05 considered to be statistically significant.

Results

During the study period, 272 adenoviruses were isolated from children with RTIs; 226 (83.1%) strains were isolated from throat swabs, 37 from nasopharyngeal aspiration, and 9 from conjunctival swabs. Of the 272 isolates, 164 (60.3%) were serotype 3, with the next most common being serotype 1.2.5.6 (38; 14.0%). Other serotypes identified included type 4 in 27 children, type 7 in 3 children, type 14 in 5 children, type 21 in 2 children, and non-typeable in 33 children. No children had 2 serotypes identified. The numbers of isolates were high in autumn and winter, peaking in October (Fig. 1). Most serotype 3 isolates (73.1%) were identified between September and January, whereas serotypes 1.2.5.6 and 4 were not significantly associated with any particular time of year.

Significantly more boys than girls were infected with adenovirus (160 vs 112; male-to-female ratio, 1.43; $p = 0.004$). Most children (227; 83.5%) were younger than 6 years. Slightly more than half (154 children; 56.7%) were admitted to hospital, and had a mean ± standard deviation (SD) hospital stay of 5.5 ± 4.0 days. Children treated as inpatients were significantly younger than those treated as outpatients ($p = 0.006$; Table 1) and were significantly more likely to be infected with serotype 3 ($p = 0.015$; Fig. 2). Serotype 1.2.5.6 was more common in outpatients ($p = 0.021$).

The peak age range of children infected with adenovirus overall and with serotypes 3 and 4 was 3 to 6 years (Table 2). Serotype 1.2.5.6 was more common in children aged 1 to 3 years. The most com-

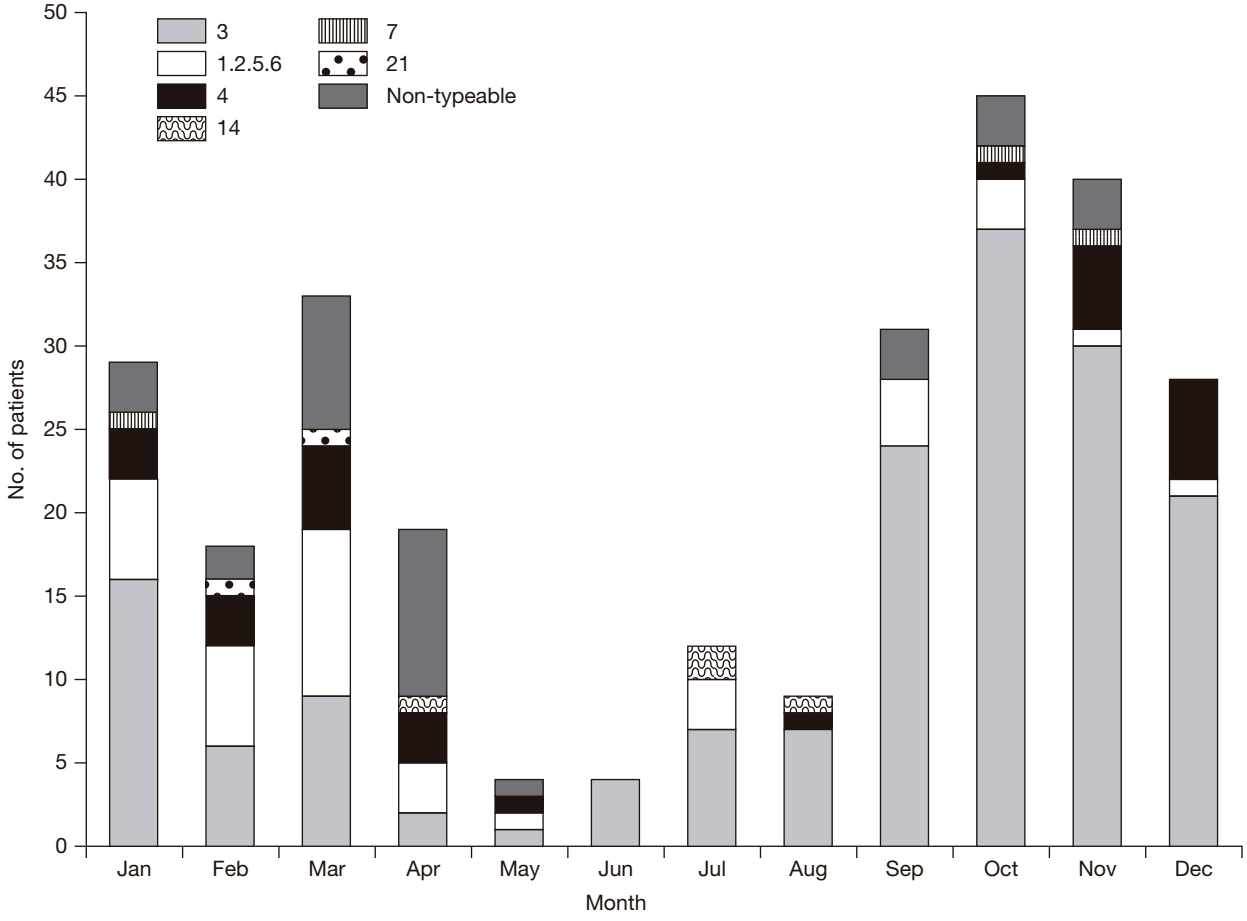

Fig. 1. Distribution of adenovirus serotypes by month.

mon clinical diagnoses were pharyngitis or tonsillitis (48.5%), bronchitis or bronchiolitis (25.7%), and pneumonia (12.9%) [Table 3]. Pharyngoconjunctival fever, a specific type of adenoviral infection, occurred in only 7.4% of children. Patients with LRTI, particularly pneumonia, were generally younger than those with upper RTI (URTI). Most children (90.1%) had fever, lasting for a mean of 3.8 days (SD, 2.7 days), with 50% being febrile for more than 3 days before they visited a doctor (Table 1). The symptoms did not differ significantly for the different serotypes (Table 4). Complete blood counts were performed for 171 children, mostly for those who were admitted to hospital. Only 1 patient had leukopenia (leukocytes, <4000/mm^3), while WBC counts >15,000/mm^3 were noted in 51 children (29.8%) and >20,000/mm^3 in 22 (12.9%). Serum CRP concentration was measured in 74 patients (mean ± SD, 5.7 ± 4.6 mg/dL) and was >4 mg/dL in 43 patients (58%). Neither the leukocyte count nor the CRP levels were correlated with specific serotypes. The ESR was tested in 123 patients (mean ± SD, 43.7 ± 26.0 mm/h) and was >20 mm/h in 104 patients (84.6%). ESR was significantly higher in patients infected with serotype 3 compared with serotype 1.2.5.6 ($p = 0.028$; Table 4).

Discussion

This study describes the epidemiological and clinical features of pediatric adenoviral RTIs during a 1-year period in Taiwan. These findings are notable for the identification of an epidemic involving serotype 3 in late 1999 and early 2000. Children infected with this serotype were more likely to be older and to be admitted to hospital than those with other serotypes. Adenovirus was the second commonest respiratory virus reported in Taiwanese children admitted to hospital between 1997 and 1999 [12], but specific serotypes were not reported. The higher overall incidence of adenoviral respiratory infection in these patients in autumn and winter is consistent with the data from the Centers for Disease Control in Taiwan [13].

Table 1. Comparison between pediatric outpatients and inpatients with respiratory adenoviral infections.

Variable	Total (n = 272)	Inpatients (n = 154)	Outpatients (n = 118)	*p*
Age (months; mean ± SD)	48.0 ± 30.3	44.1 ± 30.7	53.0 ± 29.4	0.006
Sex (male/female)	160/112	80/74	80/38	0.008
Serotype				
1.2.5.6	38	15	23	0.021
3	164	103	61	0.015
4	27	11	16	0.079
7	3	3	0	NS
14	5	3	2	NS
21	2	1	1	NS
Non-typeable	33	18	15	NS
Duration of fever before presentation (days; mean ± SD)	3.8 ± 2.7	3.7 ± 2.6	3.1 ± 2.9	0.005
≤3	133	59	74	<0.001
3-5	67	48	19	0.004
>5	45	29	16	0.246

Abbreviations: SD = standard deviation; NS = not significant.

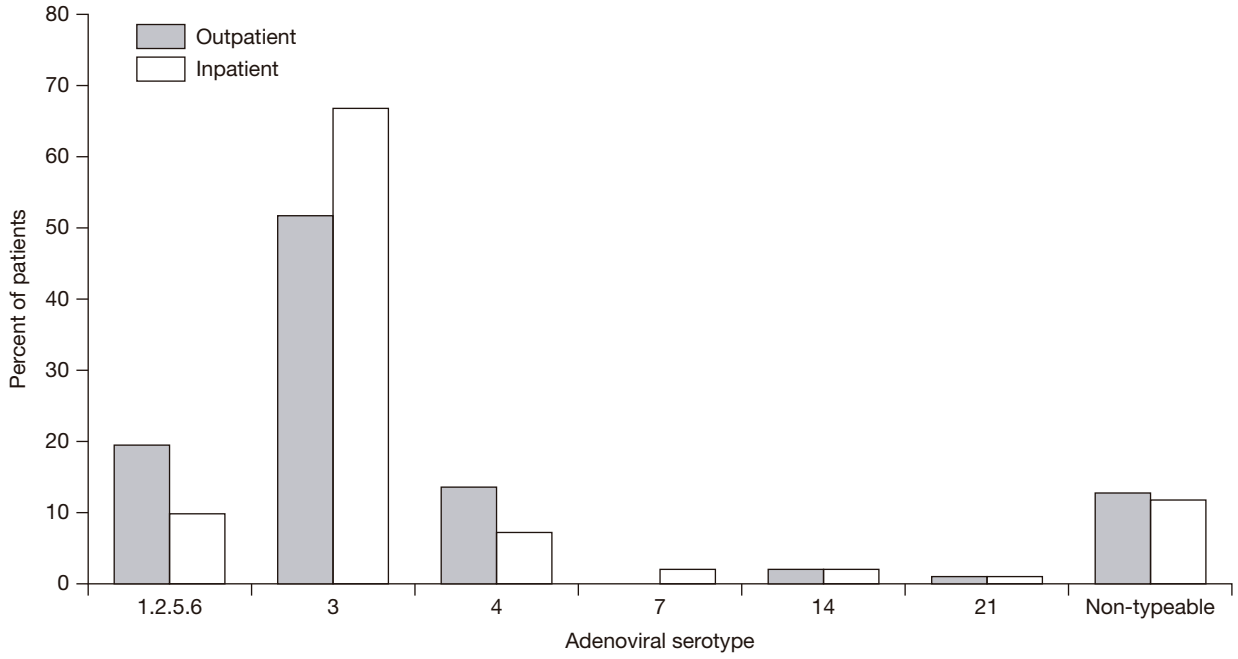

Fig. 2. Distribution of adenovirus serotypes by inpatient or outpatient treatment.

In southern Taiwan, between 1981 and 2001, 7 serotypes (1, 2, 3, 4, 7, 8, and 14) were reported, with serotypes 3 and 7 being most often isolated from November 1999 to March 2000 [10]. These data, along with the increased incidence of serotype 3 infections from September 1999 to January 2000 found in this study, indicate an outbreak of adenovirus serotype 3 in both northern and southern Taiwan during this period. In Korea, from 1990 to 1998, serotypes 7, 3, and 2 were the most common adenovirus serotypes causing pediatric LRTI leading to hospital admission [14]. Again, this is consistent with the data from this study showing that adenovirus serotype 3 caused more severe RTIs and was more likely to result in hospital admission. In this series, serotypes 1.2.5.6 caused more URTIs than LRTIs, a finding that differs from that of a study from southern Taiwan in 2001 to 2002 [9]. Similar to this study, Lin et al found serotype 3 clustered in northern Taiwan in the winter of 2004 [11].

Table 2. Age distribution of 272 children with respiratory adenoviral infections.

Adenoviral serotype	Age (months)				
	Mean ± SD	≤12 No. (%)	13-36 No. (%)	37-72 No. (%)	>72 No. (%)
1.2.5.6	38.0 ± 32.8	4 (10.5)	20 (52.6)	9 (23.7)	5 (13.2)
3	48.8 ± 29.4	16 (9.8)	37 (22.6)	85 (51.6)	26 (15.9)
4	57.1 ± 29.3	2 (7.4)	4 (14.8)	15 (55.6)	6 (22.2)
7	67.0 ± 31.5	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)
14	41.4 ± 27.8	1 (20.2)	1 (20.0)	3 (60.0)	0 (0)
21	56.5 ± 16.3	0 (0)	0 (0)	2 (100)	0 (0)
Non-typeable	46.4 ± 33.2	5 (15.2)	10 (30.3)	11 (33.3)	7 (21.2)
Total	48.0 ± 30.3	28 (10.3)	73 (26.8)	136 (46.3)	45 (16.5)

Abbreviation: SD = standard deviation.

Table 3. Clinical diagnoses of 272 children with respiratory adenoviral infections.

Variable	Pharyngitis/ tonsillitis No. (%)	Croup No. (%)	Pharyngoconjunctival fever No. (%)	Bronchitis/ bronchiolitis No. (%)	Pneumonia No. (%)
Age (months; mean ± SD)	51.4 ± 29.2	51.8 ± 45.3	51.6 ± 38.4	43.6 ± 30.2	31.8 ± 20.2
Serotype					
1.2.5.6	22 (57.9)	3 (7.9)	1 (2.6)	8 (21.1)	4 (10.5)
3	76 (46.3)	2 (1.2)	13 (7.9)	49 (29.9)	24 (14.6)
4	14 (51.9)	0 (0)	3 (11.1)	6 (22.2)	4 (14.8)
7	1 (33.3)	0 (0)	0 (0)	2 (66.7)	0 (0)
14	0 (0)	0 (0)	2 (40.0)	2 (40.0)	1 (20.0)
21	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Non-typeable	19 (57.6)	10 (30.3)	1 (3.0)	1 (3.0)	2 (6.1)
Total	132 (48.5)	15 (5.5)	20 (7.4)	70 (25.7)	35 (12.9)

Abbreviation: SD = standard deviation.

The incidence of adenoviral infection peaks in infants and children aged between 6 months and 5 years [1]. Close contact and droplets appear to be the main routes of spread from one person to another [1]. The mean age of these patients was 48 months, and approximately four-fifths were younger than 6 years, similar to findings from Korea [14] and the UK [15]. The peak age range among the patients in this study who were infected with serotype 3 was 3 to 6 years, which may reflect close contact among children in nursery schools, making it easier for the virus to spread. Boys are more frequently infected than girls by all serotypes [14,15], and a similar sex difference was found in this study. Inpatients were younger than outpatients. Inpatients also had a longer duration of fever than outpatients, which may, in part, be explained by their having more disseminated infections. This is consistent with a previous study showing that children with disseminated adenovirus disease were significantly younger than those without disseminated infections [15,16].

Adenoviruses in children cause URTIs, including the common cold, conjunctivitis, pharyngitis, tonsillitis, and croup, and LRTIs, including bronchitis and pneumonia. Severe pneumonia occurs most commonly in young children aged 3 to 18 months [1]. While tonsillitis or pharyngitis was the most common illness in this study, nearly 40% of the children had LRTIs, almost half of which were caused by serotype 3. One-third of LRTIs were pneumonia, and this occurred in the youngest group in the study. Three-quarters of the LRTIs were caused by serotype 3, which is consistent with the findings that this particular serotype tends to be associated with more serious illness. Serotype 1.2.5.6 was more commonly associated with tonsillitis or pharyngitis. Pharyngoconjunctival fever epidemics usually occur in the summer months in school-aged children and are mostly associated with serotype 3 infections [1]. Less than 10% of these patients had pharyngoconjunctival fever, perhaps because the patients were younger and were mostly seen in the winter months.

Table 4. Comparison of laboratory data and clinical manifestations in 272 children with respiratory adenoviral infections.

Variable	Serotype			
	1.2.5.6 (n = 38) No. (%)	3 (n = 164) No. (%)	4 (n = 27) No. (%)	All (n = 272) No. (%)
Fever	34 (89.5)	148 (90.2)	22 (81.5)	245 (90.1)
Infected throat	29 (76.3)	134 (81.7)	24 (88.9)	219 (80.5)
Cough	25 (65.8)	117 (71.3)	20 (74.1)	195 (71.7)
Rhinorrhea	19 (50.0)	108 (65.9)	19 (70.4)	178 (65.4)
Sore throat	4 (10.5)	19 (11.6)	5 (18.5)	35 (12.9)
Exudates	8 (21.1)	46 (28.0)	8 (29.6)	73 (26.8)
Headache	2 (5.3)	6 (3.7)	0 (0)	11 (4.0)
Abdominal pain	2 (5.3)	12 (7.3)	4 (14.8)	22 (8.1)
Vomiting	8 (21.1)	36 (22.0)	5 (18.5)	52 (19.1)
Diarrhea	7 (18.4)	26 (15.9)	2 (7.4)	42 (15.4)
White blood cells (/mm^3; mean ± SD)	13,004 ± 6692	12,850 ± 6405	14,592 ± 4990	12,851 ± 6170
>15,000	5/17 (29.4)	35/115 (30.4)	7/14 (50.0)	51/171 (29.8)
C-reactive protein (mg/dL; mean ± SD)	2.6 ± 2.6	5.4 ± 4.0	6.1 ± 7.8	5.7 ± 4.6
>4	1/4 (25.0)	33/54 (61.1)	3/6 (50.0)	43/74 (58.1)
Erythrocyte sedimentation rate (mm/h; mean ± SD)	27.7 ± 16.4[a]	45.6 ± 23.3[a]	37.6 ± 27.6	43.7 ± 26.0
>20	4/7 (57.1)	80/91 (87.9)	8/11 (72.7)	104/123 (84.6)

[a] $p < 0.05$.
Abbreviation: SD = standard deviation.

Leukocytosis and elevated CRP are often used to help distinguish between bacterial and viral infections, but they can be elevated in the latter and are high in adenoviral infections [17,18]. A serum CRP concentration of 70 mg/L is suggested as the cut-off point for the identification of clinically undetectable serious bacterial infections [19]. However, adenovirus is clearly associated with a higher mean CRP level than are other viral RTIs [20], suggesting that adenovirus causes more severe inflammation than do other respiratory viruses. Approximately one-third of these patients had leukocytosis (WBC, >15,000/mm^3) and two-thirds had a CRP level of >40 mg/L, a finding similar to that of earlier studies [9,19]. Therefore, neither the WBC count nor the CRP is helpful for distinguishing adenoviral disease from bacterial infections. A rapid adenovirus diagnostic tool that can provide quick and reliable confirmation of infection is therefore needed.

In conclusion, this study has demonstrated that an outbreak involving adenovirus serotype 3 occurred in northern Taiwan in the autumn and winter of 1999 to 2000. This particular serotype 3 had a propensity for infecting preschool-age children and causing illness requiring hospital admission.

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