Short Communication

Surveillance of Adenovirus Respiratory Infections in Children from Osaka, Japan

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SUMMARY: Human adenovirus (HAdV) strains isolated from respiratory specimens of 139 children were analyzed to evaluate the endemic situation of HAdV infections in Osaka, Japan, between 2008 and 2015. The cases increased during spring and winter, and the infections were confirmed mainly in children aged ≤ 5 years, comprising 91.9% of the total population examined. Molecular typing of the isolates revealed that the most common types belonged to HAdV-B and -C. Co-infection of HAdV-C1 and -C2 was also confirmed in a case. The median age of HAdV-E cases was higher than that of the HAdV-B and -C cases. These results revealed age and seasonal distribution of respiratory HAdV infections in children from Osaka, and indicate that majority of these children might have acquired immunity through endemic HAdV infection before reaching school age.

Human adenoviruses (HAdVs) are DNA viruses belonging to the genus *Mastadenovirus* of the family *Adenoviridae*, and > 70 types have been grouped into species A–G based on their biological and genetic differences (1,2). Majority of respiratory HAdVs belong to the species B, C, and E, and the most common types are HAdV-C2 and -B3, which cause respiratory illness and pharyngoconjunctival fever (PCF) (3,4).

HAdV infections are monitored by the Japanese surveillance system, and the isolates are analyzed by the prefectural or designated city public health institutes. Given the paucity of the surveillance report, this study aimed to retrospectively evaluate the endemic situation of HAdVs associated with respiratory infections circulating among children in Osaka.

Respiratory specimens (pharyngeal swab) and information (age and sex) were collected from children who visited 19 sentinel hospitals in 6 areas of Osaka prefecture as part of routine infectious disease surveillance, from 2008 to 2015. The inclusion criteria were children aged 0–15 years diagnosed with PCF (cases with fever, pharyngitis, and conjunctivitis), or those diagnosed with suspected HAdV infection and presenting at least one of the PCF symptoms. A total of 303 swabs were collected from the children, and the samples were only tested for HAdV. Two children were excluded due to sample inadequacy. Consequently, 301 cases were retrospectively enrolled, and the children positive for HAdV isolation were evaluated. Statistical analyses (Kruskal-Wallis and Mann-Whitney tests) of age differences between the groups (by species) were performed using the SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and Bonferroni adjustment was applied. The surveillance was performed

under the Infectious Diseases Control Law of Japan. This study was approved by the ethics committee of the Osaka Prefectural Institute of Public Health.

Virus isolation was conducted using A549 cells as described previously (5), and viral DNA was extracted from 200 μ L of the isolates using a MagDEA viral DNA/RNA kit (Precision System Science, Chiba, Japan). In the routine surveillance, a partial hexon gene (loop 1 or C4 region) was amplified by PCR using the Platinum PCR SuperMix (Invitrogen, Carlsbad, CA, USA) with methods described elsewhere (6,7). The partial penton base, hexon, and fiber genes were amplified for type classification of the HAdV-D strains as described elsewhere (5–8). Nucleotide sequences of the amplicons were determined using an Applied Biosystems 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Analyses of nucleotide sequences were performed using the BLAST search of the National Center for Biotechnology Information database and Molecular Evolutionary Genetics Analysis version 5 software (9). Nucleotide sequence analysis of one isolate implied that a 2008 case was co-infected with HAdV-C1 and -C2, and the isolated virus was cloned in the presence of 10 antibody units/mL of anti-HAdV-C1 or anti-HAdV-C2 antisera (Denka Seiken, Tokyo, Japan) using the A549 cells.

From 2008 to 2015, a total of 139 children were positive for HAdV based on viral isolations. The ages of the children ranged from one month to 12 years, with a mean age of 34.5 months and median of 24.0 months. The proportion of children aged $\leq$ one year was 47.5% (66 cases) and that of children aged ≤ 5 years was 92.8% (129 cases). The HAdV isolation positive rate was lower among school-aged children, particularly those aged > 9 years (Fig. 1). There were 70 (50.4%) and 67 (48.2%) male and female children, respectively, with sex unknown in 2 cases.

A total of 140 HAdV strains were isolated containing adenovirus co-infection. The strains involved in the co-infection case were classified as HAdV-C1 and -C2, and homologous recombination was not detected by analyzing the partial penton base, hexon, and fiber genes

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of the cloned viruses (data not shown). HAdV-B3 was the most common type, followed by HAdV-C2 (Table 1). HAdV-B and -C infections were predominant in the age group of < one to 7 years, and 4 of 5 children aged ≥ 8 years were infected with HAdV-E4 (Fig. 1). The median age of children with HAdV-E (4.4 years) was higher than that of children infected with other species, and differed significantly ($P < 0.05$) compared to that of children with HAdV-C (1.6 years). Annual distribution showed that HAdV-C2 and -B3 were detected throughout the study period, while HAdV-E4 was isolated from 2012

Fig. 1. Age distribution of 139 children with respiratory HAdV infections in Osaka (bar graph). The line graph indicates number of HAdV isolation negative cases.

Fig. 2. Seasonal distribution of HAdV isolates per month during 2008–2015.

Table 1. Annual distribution of HAdV types in Osaka from 2008 to 2015

Type	2008	2009	2010	2011	2012	2013	2014	2015	Total
C1	1	6			1	5	2		15
C2	7	2	4	3	4	6	8	10	44
B3	7	1	2	6	2	12	13	13	56
E4					4	2	2	4	12
C5		3	1	1		1		2	8
C6						1	2		3
D37								1	1
D53			1						1
Total	15	12	8	10	11	27	27	30	140

onward (Table 1). The proportion of HAdV-C1, -C5, and -C6 differed every year. HAdV-D37 and -D53 were isolated in 2015 and 2010, respectively. Distribution was seasonal, with the number of cases increasing from April to July, and in December. The number of HAdV-B isolates peaked during summer, while HAdV-C isolates peaked during the spring (Fig. 2).

The molecular typing of respiratory HAdVs in children in Osaka revealed that HAdV-C2 and -B3 are the most common types, and a case of adenovirus co-infection was also confirmed. Although HAdV-B7 associated with respiratory disease was endemic in the 1990s in Japan (10), it was not detected in this study. Epidemic levels of HAdV-B7 have been low in the recent years in Japan, and detection of both HAdV-B14 and -B55 has not been reported thus far (<http://www.nih.go.jp/niid/en/iasr-e.html>). Acute respiratory diseases are typically caused by HAdV-E4, -B7, -B14, and -B55 (11–13). HAdV-D has occasionally been reported as a cause of respiratory infections (14), and molecular epidemiological study of HAdVs associated with epidemic keratoconjunctivitis showed circulation of HAdV-D37 and -D53 in Osaka (5). In this study, as the patient infected with HAdV-D37 and -D53 had conjunctivitis and eye discharges, respectively, the strains might have sporadically and continuously circulated as causative agents of conjunctivitis in Osaka. The number of male and female children infected was closely similar, which contrasts with the previous reports of a higher proportion of infected males than females (15–17). As this study involved a smaller number of cases than the previous studies, further long-term surveillance is required to clarify the male-to-female ratio.

Age distribution of the cases showed that HAdV infections mainly occur among young children, and the results are consistent with previous reports from other countries (16,17). A study in the UK reported that immunity against HAdV-C and -F was established early in life (15). Meanwhile, HAdV-E infection predominated among children aged ≥ 8 years in the present study, and in addition to the routine surveillance, HAdV-E was isolated from all 5 samples of children in an event of class closure due to the spread of respiratory illness at an elementary school in Osaka in 2012 (data not shown). These results support the previous report that HAdV-E4 was associated with respiratory infection in adults and children and can cause outbreaks (11).

Therefore, the present study identified HAdV isolates in its surveillance of respiratory HAdV infections in Osaka, and determined that majority of children may have acquired immunity to endemic HAdV-B and -C before reaching school age. The cases increased during spring and winter, and seasonal distribution was different depending on the species. The possibility of adenovirus co-infection should be closely monitored in the surveillance because natural recombination could occur between the same species (13,18). These findings provide insights into the epidemiology of HAdV infections in Japan. As HAdVs are important pathogens that can cause severe diseases, surveillance should be continued by future studies.

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Conflict of interest None to declare.

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