

Laboratory and Epidemiology Communications

Isolation of Influenza C Virus during the 1999/2000 – Influenza Season in Hiroshima Prefecture, Japan

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Although influenza C virus is considered to be an etiological agent for mild upper respiratory illness in humans (1), it can also cause lower respiratory tract infection (2). Seroepidemiological studies have revealed that the virus is prevalent worldwide and that infection occurs at an early stage in life (3,4). However, there is little information regarding its epidemiological and clinical features because the virus has only occasionally been isolated (2,5,6). According to the Infectious Agents Surveillance Report in Japan, while 5,699 influenza A(H1N1) virus isolates, 12,822 influenza A(H3N2) virus isolates and 5,232 influenza B virus isolates were reported in 1991-1996 in Japan, only 18 isolates of influenza C virus were reported during the same period (7). In this paper, we report eight isolated cases of influenza C virus from the 1999/2000 – influenza season in Hiroshima Prefecture, Japan.

From November 1999 to March 2000, viruses were isolated from 257 of 667 clinical specimens (throat swabs and nasopharyngeal aspirates), most of which were collected from pediatric patients with acute respiratory illness in Hiroshima Prefecture. Of these, 158 were influenza A(H3N2) virus, 79 influenza A(H1N1) virus, 1 influenza B virus, 9 were adenoviruses of different serotypes, 5 were coxsackievirus type 4, 1 was herpes simplex virus type 1, and 8 (0.2%) were influenza C virus. As shown in Figure and Table, the cases of influenza C virus had no relationship in terms of their location or the time of collection, i.e., they were sporadic infections.

Viruses were isolated by using MDCK cells and/or 7-day-old embryonated hen's eggs (Table). In MDCK cells, the viruses produced only weak cytopathic effects and grew very slowly. Several passages were necessary to attain a hemag-

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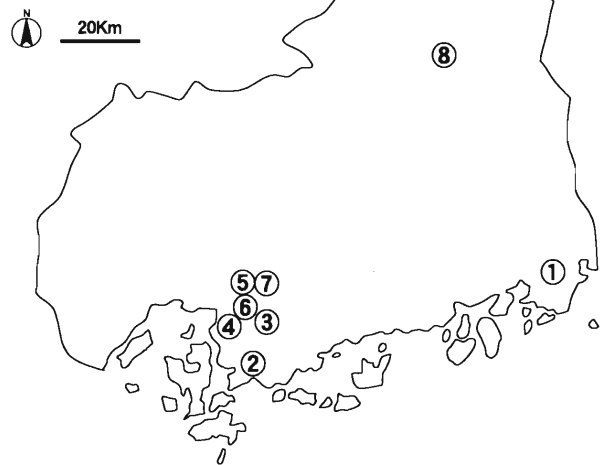


Figure. Geographical distribution of the patients infected by influenza C virus during the 1999/2000 – influenza season in Hiroshima Prefecture. The numbers indicate patient numbers, which also appear in Table.

Table. Characterization of the patients with isolated influenza C virus during the 1999/2000 – influenza season in Hiroshima Prefecture

Patient number	Sex	Age (year, month)	Specimen	Date of specimen collection	Clinical Symptoms	Code name of influenza C virus isolates
1	M	1y 5m	throat swab	Dec. 2, 1999	fever, upper respiratory illness, rash, myelodysplastic syndrome	C/Hiroshima/290/99 (C/H/290/99)
2	M	9m	throat swab	Dec. 21, 1999	fever (39°C), bronchitis, rhinorrhea	C/H/252/2000
3	M	6y 11m	throat swab	Jan. 24, 2000	fever (40°C), bronchitis	C/H/248/2000
4	M	4y 0m	throat swab	Feb. 21, 2000	fever (40°C), upper respiratory illness, rhinorrhea	C/H/249/2000
5	M	1y 2m	throat swab	Feb. 23, 2000	fever (38.4°C), upper respiratory illness,	C/H/246/2000
6	M	3y 1m	throat swab	Feb. 28, 2000	fever(38°C), upper respiratory illness	C/H/247/2000
7	M	3y 5m	throat swab	Mar. 8, 2000	fever (40.3°C), bronchitis, rhinorrhea	C/H/251/2000
8	F	3y 2m	throat swab	Mar. 28, 2000	fever (39°C), bronchitis	C/H/250/2000

M: male, F: female

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glutination (HA) titer over 4 units/25  $\mu$ l. Hemagglutination inhibition (HAI) and indirect immunofluorescence tests by using anti-C/Yamagata/8/89 resulted in the identification of eight influenza C virus isolates. The results were confirmed by RT-PCR assay using influenza C virus HA gene-specific primers (8). Among the eight isolates, seven were analyzed for antigenic characteristics with a set of monoclonal antibodies raised against hemagglutinin-esterase glycoproteins (9). It was found that the isolates could be divided into two antigenic groups: one similar to C/Yamagata/26/81 (9) that included C/H/290/99, C/H/247/2000, C/H/248/2000, and C/H/249/2000, and the other that included C/H/246/2000, C/H/250/2000, and C/H/251/2000. The data suggested that at least two antigenically different influenza C viruses circulated during the 1999/2000 – influenza season in Hiroshima Prefecture. A similar phenomenon was reported from Yamagata City for the years 1988-1990 (9). As the virus may circulate throughout the year (2), we are currently investigating the occurrence of the virus in non-influenza seasons.

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#### Laboratory and Epidemiology Communications

### Outbreaks of Heat Stable Enterotoxin-Producing *Escherichia coli* O169 in the Kinki District in Japan: Genotypic Comparison by Pulsed-Field Gel Electrophoresis of Isolates from Two Outbreaks in 2000 with Isolates from Four Outbreaks in 1997-1998

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An outbreak of enterotoxigenic *Escherichia coli* serotype O169:H41 infection was first reported in Japan in 1991 (1,2). From June 1997 to August 1998, we investigated four outbreaks (cases A-D) due to the same organism in Hyogo and neighboring Prefectures (3). In 2000, two more outbreaks occurred.

One outbreak occurred at the International Gardening and Landscaping Exhibition Japan Flora 2000 at Awaji Island in

Hyogo Prefecture in May (case E). Two hundred and sixty-six persons among three package-tour groups from neighboring Prefectures ate a Japanese-style box lunch prepared by a hotel near the site of the exposition. About half of the members (121 persons) experienced watery diarrhea and/or abdominal pain. We identified *E. coli* O169:H41 in fecal specimens from 22 patients and from one of 19 cooks at the hotel. The other outbreak occurred in April 2000 in a reformatory in Nara Prefecture (case F). Seventy-three of 88 boys aged 16-20 years became ill with symptoms including diarrhea, abdominal pain,

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