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Influenza C Viruses Isolated during the 1999-2000 Influenza Season in Saitama Prefecture, Japan

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An influenza outbreak that occurred from November 1999 to March 2000 in Saitama Prefecture was caused by A(H1) and A(H3) viruses as were outbreaks throughout Japan. During this season, we isolated 293 influenza A viruses from 553 nasopharyngeal swab specimens mostly collected by pediatricians at private clinics or hospitals; 185 were A(H1) and 108 were A(H3) viruses. In addition, we obtained two influenza C virus isolates from throat swabs in separate clinical settings.

Influenza C viruses had been isolated sporadically throughout the year (1), including during the influenza season (2), but far less frequently than influenza A and B viruses. However, an outbreak at a nursery (3) and regional epidemics lasting for as long as 3 months (4) were reported in Japan.

The first influenza C virus isolate (obtained on 24 January 2000) was from a 1-year-old male with influenza-like symptoms. He had a fever of 38.5°C, a cough, runny nose, and malaise. A throat swab specimen was collected and placed in 2 ml of Veal Infusion Broth (Difco Laboratories, Detroit, Mich., USA) containing 0.5% bovine serum albumin, 500 U/ml of penicillin, 1 mg/ml of streptomycin, 0.06 mg/ml of gentamicin and 0.02 mg/ml of amphoterisin B. He received two shots of influenza A and B virus vaccine in November and December of 1999. He recovered in 2 days. No other members of his family had signs of respiratory infection when he got ill.

The second isolate (obtained on 29 February 2000) was from a 4-year-old female with a fever (37 - 40°C for 5 - 6 days), a cough and runny nose. She had no previous influenza vaccination. Her brother and some of her kindergarten mates had similar symptoms during this period, but clinical specimens for isolation of the virus were not obtained.

Cells such as MDCK, CaCo-2, LLC-MK2 and HeLa were inoculated with the specimens. Only the MDCK cells developed weak cytopathogenic effects (CPEs) at 5 to 6 days. The supernatants of the culture agglutinated chicken erythrocytes but not guinea pig erythrocytes. After passages in MDCK cells, the culture supernatants gave clearer CPEs, and HA titers rose up to 1:32 or 1:64. They were negative in an influenza A virus detection EIA assay, Directigen FluA (Becton Dickinson Co., Sparks, Md., USA), but positive in RT-PCR specific for the influenza C virus NS gene (5). Hemagglutination inhibition test using rabbit antiserum against C/Ann Arbor/1/50 (this test was kindly performed by Drs. H. Nishimura and A. Ohmi, Virus Research Center, Sendai National Hospital, Miyagi) confirmed that the isolates were influenza C viruses.

Causative agents of acute respiratory infections cannot be diagnosed clinically. Laboratory diagnosis should be conducted carefully so as to differentiate influenza viruses A, B and C, parainfluenza viruses, respiratory syncytial virus (RSV) and other respiratory viruses.

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