Laboratory and Epidemiology Communications

Cocirculation of Influenza C Viruses with Distinct Internal Genome Constellations in Iwate Prefecture, Japan, in 2016

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Communicated by Takato Odagiri

The influenza C virus is a causative agent of mild upper respiratory tract illnesses. Additionally, it is known to affect the lower respiratory tract, causing bronchitis and pneumonia, particularly in children less than 2 years old (1–3). Seroepidemiological studies indicate that the virus is ubiquitously distributed in the human population (4,5). However, as only a few laboratories are actively performing surveys of the influenza C virus, its regional distribution is not fully understood. In this study, the unique genetic characteristics of the influenza C viruses isolated in the Iwate Prefecture, Japan, in 2016, have been described.

As part of the National Epidemiological Surveillance of Infectious Diseases, Japan, based on the Infectious Diseases Control Law, since 1999 we have been performing virus surveillance using nasopharyngeal swabs collected from pediatric patients who visited sentinel hospitals and clinics in the Iwate Prefecture. The collected swabs were transported to the Iwate Prefectural Research Institute for Environmental Sciences and Public Health. These were examined for the presence of the following viruses using cell culture and reverse transcription polymerase chain reaction (RT-PCR) techniques: the respiratory syncytial virus, human metapneumovirus, parainfluenza virus, rhinovirus, enterovirus, adenovirus, coronavirus, and influenza A and B viruses.

In January 2015, using real-time RT-PCR (6), we initiated a screening for the influenza C virus NP gene in the specimens wherein none of the above-mentioned viruses had been detected. Among the 141 specimens obtained between January 2015 and February 2018, 2 displayed positive results. One sample had been collected in March 2016, from an 18-month-old girl with symptoms of fever (38.9°C) and wheezing, and the other, in May 2016, from a 34-month-old girl who presented with a sore throat and erythema. The 2 samples were inoculated into MDCK cells and/or the amniotic cavity of 8-day-old embryonated chicken eggs and then passaged once in chicken eggs. The obtained amniotic fluids caused agglutination of chicken, but not guinea pig, erythrocytes (data not shown). A hemagglutination-inhibition (HI) test of the fluids using a panel of monoclonal antibodies against the hemagglutinin-esterase-fusion (HEF) protein (7) showed that the HEF antigenicity was identical to that of C/Sao Paulo/378/82 (data not shown). Thus, the isolated viruses were named as influenza C/Iwate/1/2016 and C/Iwate/2/2016. To our knowledge, these influenza C virus strains have been isolated in the Iwate Prefecture for the first time since the monitoring of the virus was initiated in 2015.

Next, the nucleotide sequences of the complete coding region of the individual RNA segments were determined. Data were analyzed using MEGA version 6.0 (8), and phylogenetic trees for each segment were constructed using the maximum-likelihood method (8), together with 26 representative strains isolated from 1981 to 2014. The HEF genes of both isolates belonged to the C/Sao Paulo lineage (Fig. 1), which was consistent with the results of the HI test. As shown in Fig. 2, the PB2, PB1, P3, NP, M, and NS genes of C/Iwate/1/2016 belonged to the C/Yamagata/81-related lineage (Y-lineage), Y-lineage, C/Mississippi/80-related lineage (M-lineage), M-lineage, Y-lineage, and Y-lineage, respectively. In contrast, those of C/Iwate/2/2016 belonged to the M-, Y-, M-, Y-, Y-,
and Y-lineages. Thus, a marked difference in the internal genome constellation was observed between the C/Iwate/1/2016 and C/Iwate/2/2016 isolates.

The influenza C viruses whose HEF gene belonged to the C/Sao Paulo lineage (C/Sao Paulo lineage viruses) were isolated in 1992, 1993, 1999, 2004, and 2005. Subsequently, these showed dominance in the years 2006, 2007, 2008, 2010, 2012, and 2014 (9). In Japan, the C/Sao Paulo lineage viruses with a constellation of Y-, Y-, M-, M-, Y-, and Y-lineages (identical to C/Iwate/1/2016) were first isolated in 2014, whereas the C/Sao Paulo lineage viruses with a constellation of M-, Y-, M-, Y-, Y-, and Y-lineages (identical to C/Iwate/2/2016) were isolated in 2005, 2006, 2008, 2010, and 2012, but not in 2014 (9). This finding suggests that the C/Iwate/2/2016-like viruses disappeared while the C/Iwate/1/2016-like viruses emerged in Japan in 2014. In the present study, however, 2 viruses (C/Iwate/1/2016 and C/Iwate/2/2016) with distinct internal genome constellations were successfully isolated for the first time in Japan, post-2015. Thus, the present findings indicate that the C/Iwate/2/2016-like viruses did not disappear and suggest that the C/Sao Paulo lineage viruses with distinct internal genome constellations were cocirculating in Japan from 2014 to 2016.

The nucleotide sequences have been submitted to the GenBank/DDBJ database and assigned the accession numbers LC327652 to LC327658 for C/Iwate/1/2016, and LC327659 to LC327665 for C/Iwate/2/2016.

Acknowledgments We thank Ms. Sumiko Yaegashi (Iwate Medical University) and the medical staff of Morioka Children’s Hospital for technical assistance. We also thank Editage (www.editage.jp) for English language editing. This work was partly supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (KAKENHI JP16K08816), Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labour and Welfare (H25-Shinko-Ippan-010), and the Japan Agency for Medical Research and Development (17fk0108105j0101, 17fk0108219j0202).

Conflict of interest None to declare.

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Fig. 2. Phylogenetic trees of the influenza C virus PB2, PB1, P3, NP, M, and NS genes. The nucleotide sequences of 6 internal genes of the 28 strains shown in Fig. 1 were analyzed using a maximum-likelihood method. The nucleotide positions of the genes were identical to those reported by Matsuzaki et al. (9). The names of the 2 lineages (Y, C/Yamagata/81-related lineage; M, C/Mississippi/80-related lineage) are indicated on the right side. For simplicity, only the following strains are depicted with symbols: C/Yamagata/26/81, □; C/Mississippi/80, □; C/Iwate/1/2016, ●; C/Iwate/2/2016, ○.