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Detection of Human Coronavirus NL63 and OC43 in Children with Acute Respiratory Infections in Niigata, Japan, between 2010 and 2011

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Human coronavirus (HCoV) is a member of the respiratory viruses that includes HCoV-229E, HCoV-OC43, and the severe acute respiratory syndrome (SARS) CoV (1). Recently, new types of HCoVs, such as NL63 and HKU-1, have also been described (1). Historically, HCoV research has been hampered by poor growth and lack of cytopathic effect in cell culture (1). The development of polymerase chain reaction (PCR) technology had allowed the field of coronavirology to develop widely and rapidly (1). However, according to the Infectious Agents Surveillance Report, only 54 and 59 HCoV-positive cases, including 18 cases from Niigata, were reported in Japan in 2010 and 2011, respectively (2). Although we have succeeded in isolating HCoV-229E viruses from children with nasopharyngitis using CaCo-2 cells in March 2008 and April 2010, we failed to isolate any further HCoVs thereafter (3). We therefore used reverse-transcription PCR (RT-PCR) methods in a screening analysis for HCoV in order to clarify the epidemiology of this virus in Niigata, Japan.

Between August 2010 and July 2011, 507 throat and nasal swab specimens were collected from patients with upper or lower acute respiratory infections at pediatric clinics working in collaboration with the Niigata Prefectural Health authorities as part of the national surveillance of viral diseases in Japan. Specimens were transported to the Virology Section of the Niigata Prefectural Institute of Public Health and Environmental Sciences for virus isolation. We were able to isolate respiratory viruses, including influenza virus, parainfluenza virus, RS virus, human metapneumovirus, rhinovirus, adenovirus, and enterovirus, from 376 specimens using 6 cell lines (MDCK, LLC-MK2, CaCo-2, HEp-2, Vero9013, and RD-18S). Therefore, we investigated the presence of HCoV in the 131 specimens from which no other respiratory virus was isolated.

Viral nucleic acid was extracted from the specimens using the QIAamp Viral RNA Mini Kit (Qiagen, Germantown, Md., USA), suspended in AVE buffer, and applied to RT reactions using a PrimeScript™ RT Reagent Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Then, we screened for the amplification of 4 HCoVs (HCoV-229E, HCoV-OC43, NL63, and HKU-1) by performing multiplex PCR using outer sense and antisense primers and by heminested PCR, using inner sense and outer antisense primers, as a previously reported method (4) with some modifications. Direct sequencing was used to determine the nucleic acid sequence of the 443-bp and 328-bp PCR products for identifying HCoV-OC43 and NL63, respectively. When the screening identified a HCoV-positive sample, we also amplified a portion of the spike glycoprotein region by performing PCR using our original primers to construct a phylogenetic tree. We prepared the following primer pairs: 1st PCR primers (OC-SP1F: 5’-ATGGTGGATAATGTTACTAGGCT-3’ and OC-SP1R: 5’-TAGTACCTGCAGGACAAGTGCC-3’) and 2nd PCR primers (OC-SP2F: 5’-ATAATGTTACCTGCAGGACAAGTGCC-3’ and OC-SP2R: 5’-CAGGACAAGTGGATCCTGAACAGCACTA-3’), and for HCoV-OC43 based on the reference strain (AY391777) and 1st PCR primers (NL-SP1F: 5’-TGAGTTTGGATTAAGAGTGTTAGG-3’ and NL-SP1R: 5’-CAAAGTGAATGGTGGTACGCTA-3’), and 2nd PCR primers (NL-SP2F: 5’-GATTAAAGTGGATCCTGAACAGCACTA-3’ and NL-SP2R: 5’-GCGGCATCCTA-3’).
Gaunt et al. (4) and Kaida et al. (5), the detection rate of OC43-positive cases were observed later. According to cases were all observed in winter, whereas the HCoV-

From year to year (1). Interestingly, the NL63-positive cases were observed from midwinter to early spring, the seasonality varies respectively. Although HCoV infections are generally seen February, March, April, May, June, and July, respectively. We attempted to isolate 16 of the 17 HCoV-positive specimens using the CaCo-2 cell line, with which we had previously isolated HCoV-229E (3), and the LLC-MK2 cell line, which is commonly used to isolate HCoV (7). Crystal trypsin (final concentration, 0.5 μg/ml) was added to the maintenance media and cells were passaged 5 times. However, we failed to observe any cytopathic effect or to amplify the HCoV genome. HCoVs tend to induce subtle cytopathic effects and many cell types are not susceptible (7). We noticed that HCoV-229E does not grow in the CaCo-2 cell line if trypsin is not present in the maintenance media (3); this finding was a milestone in our attempts to isolate one of the HCoVs. Further study will be required to elucidate the cell lines and the proper maintenance media necessary to isolate different HCoVs.

According to the national surveillance in Japan (2), HCoV detection has been reported from only 8 prefectures, including Niigata, Osaka City, and Mie (5).
Fig. 1. Phylogenetic trees of the partial spike glycoprotein gene of the human coronavirus NL63 (A) and OC43 (B) strains. The trees of the NL63 (A) and HCoV-OC43 (B) strains were based on 576 bp and 1488 bp nucleotides, respectively. Evolutionary distance was calculated using the Maximum Composite Likelihood method, and the trees were plotted using the neighbor-joining method. Numbers at each branch indicate the bootstrap values of the clusters supported by that branch. The scales of NL63 (A) and HCoV-OC43 (B) indicate 10^z and 1^z nucleotide differences, respectively. The present strains are represented in bold type.

Therefore, the epidemiology of HCoV in Japan is not well understood and HCoV surveillance may have been ineffective. In this study, we failed to isolate HCoV, but succeeded in detecting the HCoV genome in 13% of specimens (17/131) using RT-PCR. These findings suggest that the difficulties associated with HCoV isolation underscore the importance of HCoV as a causative agent of acute respiratory infections in the national surveillance. Together with parainfluenza virus type 4 and rhinovirus, HCoV is considered one of the most difficult respiratory viruses to identify, despite the fact that these viruses are responsible for a large proportion of viral respiratory tract infections (8). The use of molecular methods may assist in the study of HCoV epidemiology, and our primers designed for sequencing the HCoV-OC43 and NL63 spike glycoprotein gene will be useful for future molecular epidemiological analyses. This does not diminish the need to develop virus isolation technologies and to stock clinical isolates in public health laboratories in order to develop such viral disease control strategies as vaccine development and longitudinal epidemiological studies (8). To conclude, we should continue to clarify the etiology and epidemiology of HCoV infection using a combination of virus isolation techniques and molecular methods such as RT-PCR.

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Conflict of interest None to declare.

REFERENCES