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Short Communication

Detection of the Human Coronavirus 229E, HKU1, NL63, and OC43 between 2010 and 2013 in Yamagata, Japan

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SUMMARY: The available literature on human coronaviruses (HCoVs) in Japan is limited to epidemiological studies conducted over a maximum of 1 year. We conducted a 4-year study of HCoVs by analyzing 4,342 respiratory specimens obtained in Yamagata, Japan, between January 2010 and December 2013. A pan-coronavirus reverse transcription-PCR screening assay was performed, and all HCoV-positive specimens were subsequently confirmed by sequencing of the PCR products. We detected in 332 (7.6%) HCoV strains during the study period, comprising 133 (3.1%) HCoV-NL63, 83 (1.9%) HCoV-HKU1, 78 (1.8%) HCoV-OC43, and 38 (0.9%) HCoV-229E strains. HCoV detection per year ranged from 3.5% to 9.7%. HCoVs were detected mainly in winter, with January (28.5%) and February (25.3%) 2011 and December 2012 (14.6%) being the only months in which HCoV-NL63 detection per month exceeded 10.0%. HCoV-HKU1 displayed clear biennial peaks in January (18.3%) and February (10.7%) 2010 and in February (18.8%) and March (14.7%) 2012. The peak detection of HCoV-OC43 was 13.6% in November 2010, while that of HCoV-229E was 10.8% in March 2013. Our results indicated that there may be annual variations in the circulation of individual HCoV strains. Further long-term surveillance is necessary to clarify HCoV prevalence and circulation patterns in Japan.

Human coronaviruses (HCoVs) belong to the genus *Coronavirus* in the family *Coronaviridae* and include HCoV-229E, HCoV-OC43, and severe acute respiratory syndrome (SARS)-CoV (1). Recently, new HCoVs, such as HCoV-NL63, HCoV-HKU1, and Middle East respiratory syndrome (MERS)-CoV, have also been described (1). Four HCoVs (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) are associated with a wide range of respiratory illness, including common colds, and with high morbidity outcomes, such as pneumonia and bronchiolitis (1–3). These four HCoVs are found globally, although the frequency of detection of these HCoVs varies with geographical location (1–20). Poor growth and a lack of cytopathic effects on cell cultures have been major deterrents to HCoV research in the past (1). However, with the development of polymerase chain reaction (PCR) technology, there has been broad and rapid development in the field of coronavirology (1).

In Japan, according to the National Epidemiological Surveillance of Infectious Diseases (NESID) system, only 306 HCoV-positive cases were reported in 19 prefectures between 2010 and 2013 (21), which accounted for only 0.4% of the total number of reported cases of respiratory virus infection (306/68,332). Longitudinal studies of HCoV epidemiology are particularly lacking in Japan, with the available literature limited to descriptions representing a maximum of 1 year (4–7). Although we have conducted epidemiological surveys of acute viral respiratory infections based on virus isolation, we failed to isolate HCoVs in Yamagata. Thus, we performed a screening assay targeting HCoVs using reverse transcription-PCR (RT-PCR) to clarify the epidemiology of these viruses in Yamagata, Japan.

Between January 2010 and December 2013, 4,342 throat and nasal swab specimens were collected from patients with upper or lower acute respiratory infections at pediatric clinics in collaboration with the Yamagata Prefectural Institute of Public Health for the NESID. Specimens were transported to the Department of Microbiology at the Yamagata Prefectural Institute of Public Health for virus isolation. Among these specimens, 3,092 (71.2%) were from patients aged $\leq$5 years, 767 (17.7%) from patients aged 6–10 years, 326 (7.5%) from patients aged 11–15 years, 104 (2.4%) from patients aged $>$15 years, and 53 (1.2%) from patients of unknown age. We were able to isolate several respiratory viruses, including influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, adenovirus, enterovirus, rhinovirus, parechovirus, mumps virus, cytomegalovirus, and herpes simplex virus, from 2,355 specimens using a microplate method with the following cell lines: HEF, HEp-2, VeroE6, MDCK, RD18s, GMK, HMV-II, and LLC-MK2 (22–24). We next investigated the presence of HCoV in the above specimens by RT-PCR analysis.

Viral RNA was extracted from 200 μL of each speci-

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men using the High Pure Virus RNA Kit (Roche Diagnostics, Mannheim, Germany) and was then transcribed into cDNA using the Prime Script™ RT Regent Kit (Takara Bio, Shiga, Japan), according to manufacturer's instructions. We next screened for the amplification of the four HCoVs (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) by nested PCR using the PanCoVfw, PanCoVfw2, PanCoVr, and PanCoVr2 primers, which target the polymerase gene specific to the HCoV family, as reported previously (2). However, in our experience, human genomes are sometimes detected in amplification products using these primers. We found that such human genome amplification products included TTTAAA, which is not included in the amplification products of HCoVs. We, therefore, digested the amplification products using the restriction enzyme *Dra*I, which recognizes TTTAAA. Using this step, we eventually succeeded in differentiating HCoV from human genomes. All HCoV-positive amplification products were then sequenced, and the four HCoVs were identified.

Figure 1 shows the monthly number and frequency of HCoVs detected. This report is the first to be based on a 4-year, long-term epidemiological study of HCoVs in Japan. We detected 332 (7.6%) HCoV strains during the study period, comprising 133 (3.1%) HCoV-NL63, 83 (1.9%) HCoV-HKU1, 78 (1.8%) HCoV-OC43, and 38 (0.9%) HCoV-229E strains. Our results also indicated that the frequency of HCoV detection per year ranged from 3.5% in 2013 to 9.7% in 2012. The detection frequencies of HCoVs in respiratory samples reported in the literature are highly variable, ranging from 1.6% to 16.0%, depending on the country, study period, study population, and detection modality (2–20). The most commonly observed HCoV in our survey was HCoV-NL63, whereas many previous studies reported a predominance of HCoV-OC43 (2,5–15). Generally, HCoVs display high detection rates in winter months, with little to no detection in summer months in temperate regions (2,3,8–10,14–16). We also mainly detected HCoVs in winter in Yamagata, Japan, located in a northern temperate region.

Our results showed an annual detection frequency of 0.6–6.4% for HCoV-NL63. A monthly detection frequency exceeding 10% was observed for NCoV-NL63 in January (28.5%) and February (25.3%) 2011 and in December 2012 (14.6%). However, HCoV-NL63 was detected almost throughout the entire period from July 2011 to March 2013. HCoV-NL63 was also detected in Niigata from December 2010 to January 2011 (6). Combining data of HCoV detection in Yamagata with those in Niigata suggested that HCoV-NL63 circulated in the winter 2010/2011 season in the Niigata–Yamagata area.

Our results also indicated that the annual detection frequency of HKU1 was 0.1–3.4%. HCoV-HKU1 displayed clear biennial peaks from January (18.3%) to February (10.7%) in 2010 and February (18.8%) to March (14.7%) in 2012. HCoV-HKU1 peaked primarily

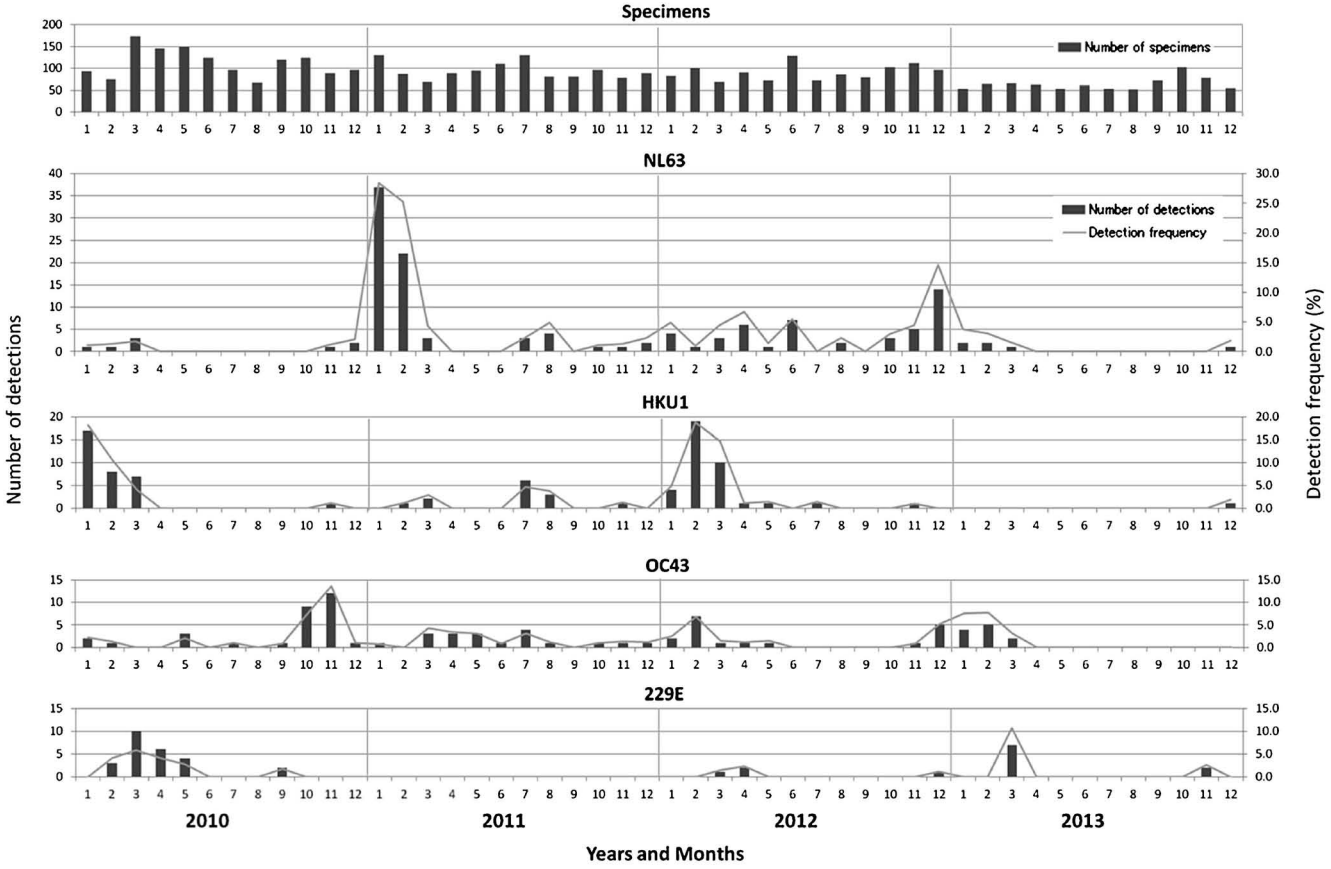

Fig. 1. Monthly distribution of human coronaviruses detected from patients with acute respiratory infections in Yamagata, Japan between 2010 and 2013. Bar indicates the number of detections. Detection frequency (%) per month is shown by the line.

Table 1. Coinfection of respiratory specimens with human coronaviruses and other viruses between 2010 and 2013 in Yamagata, Japan

Coinfecting isolated virus	HCoV-NL63 (n = 133)	HCoV-HKU1 (n = 83)	HCoV-OC43 (n = 78)	HCoV-229E (n = 38)	All HCoV (n = 332)
Enterovirus	8 (6.0)	2 (2.4)	2 (2.6)	2 (5.3)	14 (4.2)
HPIV	7 (5.3)	0	2 (2.6)	3 (7.9)	12 (3.6)
Adenovirus	4 (3.0)	2 (2.4)	4 (5.1)	1 (2.6)	11 (3.3)
Rhinovirus	7 (5.3)	2 (2.4)	1 (1.3)	0	10 (3.0)
HMPV	2 (1.5)	4 (4.8)	0	3 (7.9)	9 (2.7)
Influenza virus	3 (2.3)	2 (2.4)	0	0	5 (1.5)
RSV	1 (0.8)	1 (1.2)	1 (1.3)	1 (2.6)	4 (1.2)
Parechovirus	0	0	1 (1.3)	0	1 (0.3)
Mumps virus	0	1 (1.2)	0	0	1 (0.3)
CMV	6 (4.5)	1 (1.2)	3 (3.8)	0	10 (3.0)
HSV	0	1 (1.2)	0	0	1 (0.3)
HPIV, Rhinovirus	0	0	1 (1.3)	0	1 (0.3)
HPIV, Parechovirus	0	0	1 (1.3)	0	1 (0.3)
HMPV, CMV	0	0	0	1 (2.6)	1 (0.3)
Total	38 (28.6)	16 (19.3)	16 (20.5)	11 (28.9)	81 (24.4)

Data are no. (%) of patients.
HPIV, human parainfluenza virus; HMPV, human metapneumovirus; RSV, respiratory syncytial virus; CMV, cytomegalovirus; HSV, herpes simplex virus.

in winter and spring in 2010 and 2012, whereas it was detected only sporadically in summer and/or autumn in 2010, 2011, and 2012. This is the first report on HCoV-HKU1 circulation in Japan in the literature.

The annual detection frequency of HCoV-OC43, according to our data, was 1.4–2.2%. HCoV-OC43 detection showed a peak in November 2010 (13.6%) and was mainly detected from autumn to winter. However, HCoV-OC43 was detected almost throughout the period from May 2010 to May 2012. At the same time, HCoV-OC43 was also detected in Niigata from February to July 2011 (6). Our data of virus detection in Yamagata and Niigata suggest that HCoV-OC43 circulated in late winter, spring, and early summer in 2011 in the Niigata–Yamagata area. HCoV-OC43 was detected at a rate of 37.2% (29/78) in Mie from January to March 2013 (7). At the same time, the detection frequency was 7.5% in January and 7.8% in February in Yamagata. These data of virus detection in Yamagata and Mie suggest the possibility that HCoV-OC43 circulated in different areas of Japan from January to March 2013.

This study also showed that the annual detection frequency of HCoV-229E was 0–1.9%. HCoV-229E detection peaked in March 2013 (10.8%); however, it was detected at low levels throughout the study period. Many previous studies have also reported low frequencies of HCoV-229E compared with the frequencies of other HCoVs (2,3,5–11,14–17). HCoV-229E was mainly detected in spring, although the seasonal pattern of 229E could not be determined because of the small number of positive cases.

Our result and those of previous reports (3–10,12,14–16,19,20) suggest that HCoVs peak mainly in winter, although they can be detected throughout the year.

Table 1 shows respiratory specimens with coinfection of HCoVs and other viruses, which were identified by virus isolation using a microplate method between 2010 and 2013 in Yamagata, Japan. Of all 332 patients with HCoV infections, 24.4% were coinfected with other viruses. The respiratory viruses most frequently detected with HCoVs were enterovirus (4.2%), parainfluenza virus (3.6%), adenovirus (3.3%), and rhinovirus (3.0%). The coinfection rates of HCoVs in respiratory samples reported in the literature vary, ranging from 30.5% to 70.0% (2,3,12–20). Previous studies have demonstrated a high rate of HCoV coinfection with respiratory syncytial virus, influenza virus, and rhinovirus (2,3,13–19). However, as detection methods, PCR primers, and study designs often differ from one study to another, direct comparisons of data are difficult.

This study revealed that HCoVs were a significant cause of acute respiratory infections, accounting for 3.5–9.7% of such cases in Japan. Therefore, further long-term surveillance is necessary to more clearly evaluate the prevalence and circulation patterns of HCoVs in Japan.

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Conflict of interest None to declare.

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