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An Outbreak of Human Coronavirus OC43 during the 2014–2015 Influenza Season in Yamagata, Japan

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Coronaviruses are enveloped, positive-sense, single-strand RNA viruses that belong to the subfamily *Coronavirinae* in the family *Coronaviridae* (1). Six coronaviruses are known to infect humans: human coronaviruses (HCoV-229E, -OC43, -NL63, -HKU1), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV). Four of these viruses (HCoV-229E, -OC43, -NL63, and -HKU1) are known to circulate continuously in the human population (1) and have been detected in the Japanese population while SARS-CoV and MERS-CoV have not. While HCoVs are associated with respiratory symptoms ranging from mild upper respiratory tract infections to severe lower respiratory tract infections including pneumonia and bronchiolitis (1,2), previous HCoV research has been hampered by a lack of observable cytopathic effects and poor growth in cell cultures. However, increasingly sensitive detection methods, such as reverse-transcription polymerase chain reaction (RT-PCR) assays, can now identify previously undetected cases (2–11).

According to the National Epidemiological Surveillance of Infectious Diseases (NESID) system, in Japan, only 196 HCoV-positive cases in 10 prefectures, including 66 cases from Yamagata, were reported in 2014 (12). In addition, few studies have examined the epidemiology of HCoV in Japan and the available literature is limited to descriptions representing a maximum of 1 year (6–9). Thus, we conducted a longitudinal survey between 2010 and 2013 in Yamagata, Japan, to clarify the epidemiology of HCoVs using RT-PCR methods (10) and address this gap in the literature. In 2011, we found that the monthly detection frequencies of HCoV-NL63 were higher than 20% in January and February (28.5% and 25.3%, respectively), whereas those of the other HCoVs (HCoV-229E, -OC43, -HKU1) did not exceed 20% (10). In light of these findings, we continued the HCoV surveillance and, thus, captured data from the largest outbreak of HCoV-OC43 reported in the last 6 years. During the 2014–2015 influenza season in Yamagata, Japan, the monthly detection frequencies

were approximately 30–40%. Therefore, in this study, we report the results of HCoV detection after 2013 and describe the characteristics of the HCoV-OC43 outbreak that occurred in the 2014–2015 influenza season.

Between January 2014 and March 2015, 1,187 throat and nasal swab specimens were collected from patients with upper or lower acute respiratory infections at the Yamanobe Pediatric Clinic. Swabs were collected in collaboration with the Yamagata Prefectural Institute of Public Health as part of the NESID. Specimens were transported to the Department of Microbiology at the Yamagata Prefectural Institute of Public Health for virus detection. Among the specimens, 800 (67.4%) were from patients ≤ 5 years old, 207 (17.4%) were from patients between 6 and 10 years old, 129 (10.9%) from patients between 11 and 15 years old, 31 (2.6%) from patients > 15 years old, and 20 (1.7%) from patients of unknown age.

Viruses were isolated using a microplate method (including HEF, HEp-2, Vero E6, MDCK, RD-18S, GMK, HMV-II, and LLC-MK2 cell-lines) to detect respiratory viruses, such as influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, adenovirus, enterovirus, rhinovirus, and parechovirus (13–15).

Furthermore, real-time RT-PCR assays were performed to detect the 4 HCoVs. Viral RNA was extracted from 200 μ L of specimen using a High Pure Viral RNA kit (Roche Diagnostics, Mannheim, Germany), and then transcribed into cDNA with a Prime ScriptTM RT Regent Kit (Takara Bio, Otsu, Japan) according to the manufacturers' instructions. Reaction mixtures were prepared using the TaqMan Fast Advanced Master Mix (Applied Biosystems, Carlsbad, CA, USA). Each 10 μ L reaction mixture contained primers, probes, nuclease-free water, and 1 μ L of cDNA. The primer and probe sequences are shown in Table 1 and were used as described elsewhere (4,5). Fluorogenic dyes were modified for the multiplex real-time RT-PCR for each of the 4 HCoVs (Table 1). Amplification was performed on an ABI Prism 7500 Fast Real-Time PCR system under the following cycling conditions: 1 cycle at 95°C for 10 min followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. Positive cases were those showing a viral load of more than 100 copies per reaction.

We detected 154 (13.0%) HCoV strains from the 1,187 specimens collected during the study period. HCoV-OC43 accounted for 110 (9.3%) cases, HCoV-NL63 accounted for 31 (2.6%), HCoV-HKU1 for 11

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Table 1. Primers used for real-time polymerase chain reaction in this study

Virus	Final concentration nmol/L	Target region	Sequence (5'→3')
HCoV-229E ¹⁾		Nucleoprotein	
Forward	750		CAGTCAAATGGGCTGATGCA
Reverse	500		AAAGGGCTATAAAGAGAATAAGGTATTCT
Probe	50		TAMURA-CCCTGACGACCACGTTGTGGTTCA-BHQ2
HCoV-OC43 ¹⁾		Nucleoprotein	
Forward	500		CGATGAGGCTATTCCGACTAGGT
Reverse	750		CCTTCCTGAGCCTTCAATATAGTAACC
Probe	50		Cy5-TCCGCCTGGCACGGTACTCCCT-BHQ3
HCoV-NL63 ²⁾		Nucleoprotein	
Forward	250		GACCAAAGCACTGAATAACATTTTCC
Reverse	250		ACCTAATAAGCCTCTTTCTCAACCC
Probe ³⁾	50		FAM-AACACGCT“T”CCAACGAGGTTTCTTCAACTGAG
HCoV-HKU1 ²⁾		Replicase 1b	
Forward	100		CCTTGCGAATGAATGTGCT
Reverse	750		TTGCATCACCCTGCTAGTACCAC
Probe	50		HEX-TGTGTGGCGGTTGCTATTATGTTAAGCCTG-BHQ1

¹⁾: Primer and probe sequences for HCoV-229E and HCoV-OC43 are from van Elden et al. (4).

²⁾: Primer and probe sequences for HCoV-NL63 and HCoV-HKU1 are from Dare et al. (5).

³⁾: Labeled at the 5' end with FAM, internally quenched with Black Hole Quencher-1 (indicated by “T”), and labeled at the 3' end with a phosphate.

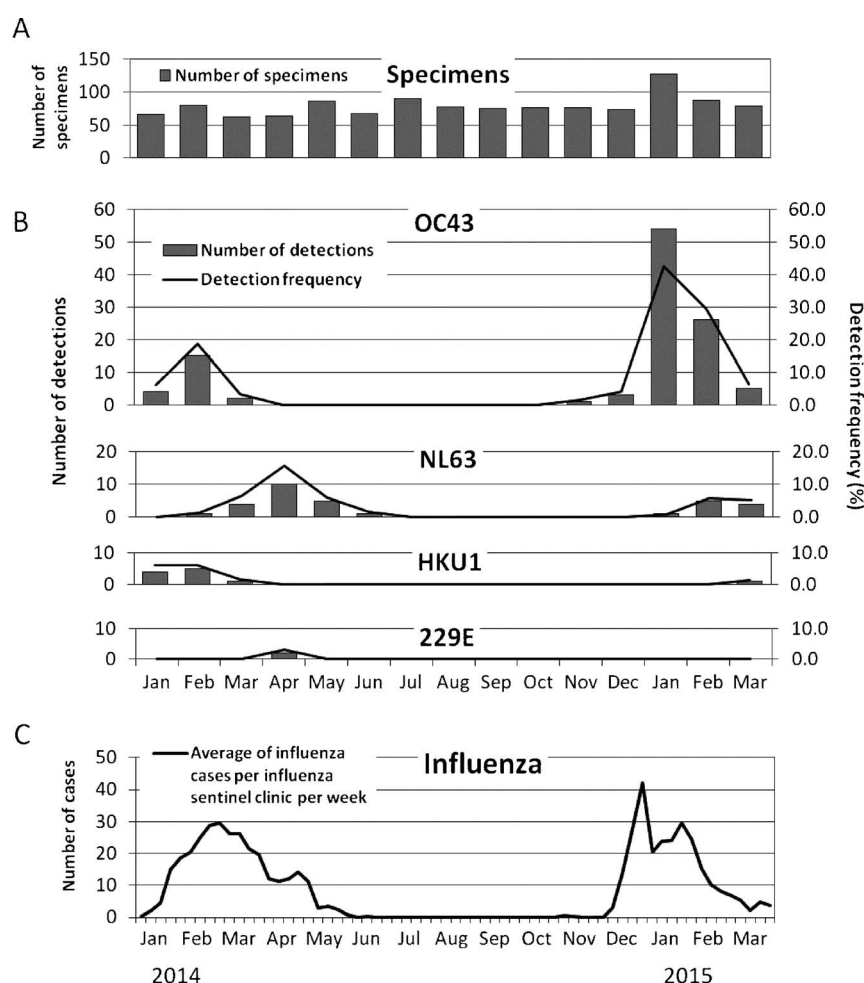


Fig. 1. Monthly distribution of clinical specimens (A) and HCoVs (B) detected from patients with acute respiratory infections between January 2014 and March 2015, in Yamagata, Japan. Weekly distribution of an average of influenza cases per influenza sentinel clinic reported in Murayama district of Yamagata Prefecture (C) (17).

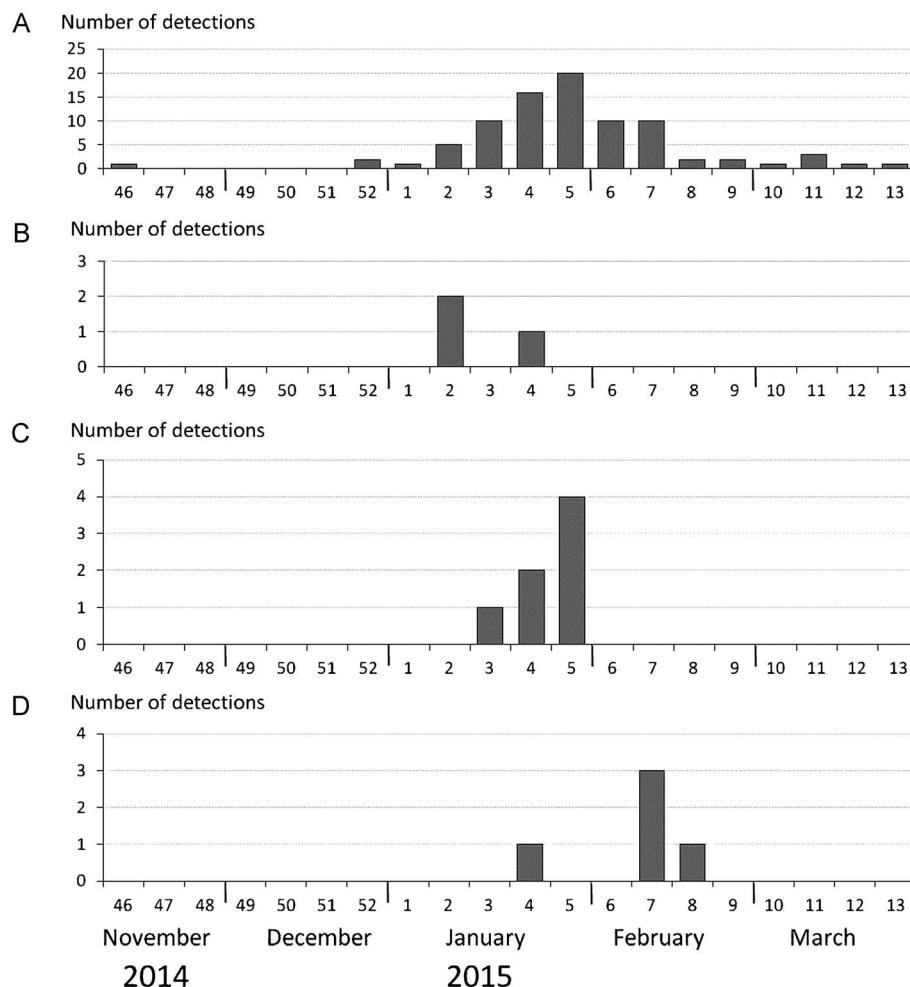


Fig. 2. Weekly distribution of HCoV-OC43 detected in Yamagata, Japan, between November 2014 and March 2015 (A). Weekly distributions are also shown for 3 nurseries located Ooe-town (B), Asahi-town (C), and Yamanobe-town (D).

(0.9%), and HCoV-229E for 2 (0.2%). The monthly numbers and frequencies of HCoV detection during the study period are shown in Fig. 1. In 2014, HCoV-OC43 and HCoV-NL63 showed peaks in February (18.8%, $n = 15/80$) and April (15.6%, $n = 10/64$), respectively. However, in 2015, HCoV-OC43 was the most frequently detected HCoV with monthly detection frequencies reaching approximately 30–40% in January and February (42.5%, $n = 54/127$ and 29.5%, $n = 26/88$, respectively). These detection frequencies were higher than those found between 2010 and 2013 (maximum detection rate 13.6%) (10).

This large HCoV-OC43 outbreak started in week 52 (December) in 2014 and continued to week 13 (March) in 2015, although a single case was detected in week 46 (November) in 2014 (Fig. 2A). HCoV-OC43-positive cases in 2015 were distributed in 4 cities and 5 towns across the Murayama district where the Yamanobe Pediatric Clinic is located. The temporal distribution of HCoV-OC43 detection partially coincided with the influenza epidemic in this district (Fig. 1C). On average, more than 10 cases of influenza infection per week were reported from each influenza sentinel clinic from week 50 (December) in 2014 to week 7 (February) in 2015. In addition, the number of patients diagnosed with influenza increased and 2 peaks were observed: 1 in week 52

(December) and 1 in week 4 (January) (16). These data revealed that the influenza virus and HCoV-OC43 co-circulated simultaneously in the Murayama district of Yamagata Prefecture.

Among the 444 specimens collected between November 2014 and March 2015, 89 were positive for HCoV-OC43. In addition, 4 of these were also positive for influenza virus A, adenovirus, and cytomegaloviruses. The median age of the HCoV-OC43-positive patients was 2 years (range: 0–39 years). Among the 85 HCoV-OC43-positive cases without dual infection, 83 were diagnosed with upper respiratory infection (45 nasopharyngitis, 33 pharyngitis/tonsillitis, and 5 laryngitis), 1 with a lower respiratory infection, and 1 with acute gastroenteritis. Of these 85 HCoV-OC43-positive cases, 67 (78.8%) had been previously found negative for influenza A and B viruses on rapid antigen tests. This suggests that HCoV-OC43-infected children were clinically suspected of having influenza at the time of the first visit to the clinic.

During the study period, we detected HCoV-OC43 at 3 nurseries (3, 7, and 5 cases, respectively) located within a distinct community (Fig. 2B, 2C, and 2D). HCoV-OC43 infections among the children at each nursery were concentrated over a 2 to 3-week period between January and February. These observations sug-

gest that HCoV-OC43 infection should be considered as a possible cause of outbreaks in nurseries during the influenza season. In addition, at 1 nursery (Fig. 2D), only 1 case was identified in week 4 (January). This case was also found to be positive for influenza A virus by both a cell culture and a rapid antigen test. These findings suggest that outbreaks of HCoV-OC43 and influenza virus might co-occur in nurseries.

As described above, the largest outbreak of HCoV-OC43 was observed during the 2014–2015 influenza season in Yamagata, Japan. These results indicate that we should pay attention to HCoV-OC43 during influenza season, especially in cases that test negative for influenza virus. Furthermore, HCoV-OC43 was also detected in the Mie prefecture between January and March 2013 (i.e., during the influenza season) (9) and a small outbreak of HCoV-OC43 was also observed in Yamagata during the 2013–2014 influenza season (Fig. 1). These findings suggest that HCoV-OC43 infections overlap seasonally with the influenza infections. Therefore, continuous surveillance for HCoVs, particularly HCoV-OC43, at the national level is needed to distinguish HCoVs infections from influenza infections in a clinical setting.

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Conflict of interest None to declare.

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