Laboratory and Epidemiology Communications


Yohei Matoba¹, Yoko Aoki¹, Shizuka Tanaka¹, Maki Unno¹, Kenichi Komabayashi¹, Tatsuya Ikeda¹, Yoshitaka Shimotai², Yoko Matsuzaki², Tsutomu Itagaki³, and Katsumi Mizuta¹*

¹Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata 990-0031; ²Department of Infectious Diseases, Yamagata University Faculty of Medicine, Yamagata 990-9585; and ³Yamanobe Pediatric Clinic, Yamagata 990-0301, Japan

Communicated by Makoto Takeda

At present, 4 human coronaviruses (HCoVs), HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1, that cause upper and lower respiratory tract infections are known to be circulating worldwide. In temperate climates, HCoV infections predominantly occur in the winter and early spring (1). Our longitudinal observation of HCoVs in Yamagata, Japan supports the seasonality of these outbreaks (2,3). However, this region surprisingly experienced an outbreak of HCoV-OC43 in early summer of 2016. The main purpose of this paper is to describe the outbreak of HCoV-OC43 in June 2016 in Yamagata, Japan. This study was approved by the Ethics Committees of the Yamagata Prefectural Institute of Public Health (YPHIPCE17-05) and Yamagata University Faculty of Medicine (H28-482).

We performed a longitudinal epidemiological study of viral acute respiratory infections (ARIs) by cell culture using a microplate method (4) as part of the National Epidemiological Surveillance of Infectious Diseases (NESID), and began to target the 4 HCoVs mentioned above using reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR methods (2,3). The results for HCoVs circulating in 2010–2013 and between January 2014 and March 2015 have already been published (2,3). As a continuation of these previous studies, 1,380 nasopharyngeal specimens were obtained from patients diagnosed with ARIs at Yamanobe Pediatric Clinic between April 2015 and December 2016, and tested for HCoVs by real-time PCR (3). For evaluation of HCoV-229E, we also carried out virus isolation using HeLa-ACE2-TMPRSS2 cells (5).

Among the 1,380 specimens collected during the study period, 74 (5.4%) HCoV strains were isolated and detected, including 30 (2.2%) HCoV-NL63, 20 (1.4%) HCoV-OC43, 13 (0.9%) HCoV-229E, and 11 (0.8%) HCoV-HKU1.

The monthly numbers and frequencies of HCoV detection from January 2014 to December 2016, including the results from the previous study (3), are shown in Fig. 1. As reported previously, the monthly detection frequency of HCoV-OC43 was approximately 30–40% in January and February 2015 (3). Subsequently, HCoV-NL63 and HCoV-HKU1 showed peaks in April (21.4%, n = 15/70) and December (12.5%, n = 6/48) of 2015, respectively. In 2016, HCoV-229E showed a peak in March (9.5%, n = 7/74). Trends of HCoV-NL63 and HCoV-OC43 in 2016 were different from those detected in the previous seasons. HCoV-NL63 was detected sporadically without peaks between March and December. HCoV-OC43 showed a peak in June; 13 of the 40 (32.5%) specimens collected in June 2016 were positive for HCoV-OC43. The numbers of HCoV detections by age group are shown in Fig. 2. HCoV-OC43, HCoV-NL63, and HCoV-HKU1 were predominantly detected in children under 13 years old, and the greatest numbers of detections for HCoV-OC43, HCoV-NL63, and HCoV-HKU1 were all found in the age group of 0–1 years.

In general, in temperate climates, HCoVs display marked winter seasonality and are not detected in the summer months, which is similar to the pattern observed in temperate climates, HCoVs display marked winter seasonality and are not detected in the summer months, which is similar to the pattern observed...
for influenza viruses (6–10). In accordance with this general trend, we also mainly detected HCoVs in winter in Yamagata, Japan, which is located in a temperate region, between 2010 and 2015 (2,3). In particular, we observed the largest outbreak of HCoV-OC43 in Yamagata between January and February 2015 (Fig. 1), and we warned that HCoV-OC43 infection should be considered as a possible cause of outbreaks during the influenza season (3). However, several HCoV strains were also sporadically detected even in the summer season; HCoV-NL63 was detected between June and August in both 2011 and 2012, and HCoV-OC43 was detected between June and August in 2011 (2). In this study, the detection frequency of HCoV-OC43 in June 2016 was the second highest recorded between 2010 and 2016, reaching 32.5. This apparent outbreak of HCoV-OC43 in June 2016 was further analyzed because of this intriguing finding owing to its uncommon prevalent season.

The median age of the 13 HCoV-OC43-positive patients in June 2016 was one year (range: 0–2 years). Interestingly, the age distribution in 2016 was different from that found in our previous study (3). The frequency of HCoV-OC43 detection in infants aged 0–1 years reached 92.9% (13/14) from June (week 23) to July (week 28) of 2016, while the frequency in this same age group from December (week 52) 2014 to March (week 13) 2015 was only 35.2% (31/88). Regarding the clinical diagnosis of the 13 HCoV-OC43-positive cases without dual infection (one case was also positive for adenovirus), 10 were diagnosed with nasopharyngitis, bronchitis, or post-vaccinal fever.

The weekly distribution of HCoV-OC43 during the 2016 outbreak is shown in Fig. 3. This HCoV-OC43 outbreak started in week 23 (June) and continued till week 28 (July) of 2016, although 2 cases were detected in week 21 (May) of 2016 (Fig. 3A). In June 2016, we detected HCoV-OC43 at 2 nurseries (3 and 5 cases, respectively) located within a distinct community (Fig. 3B, C). These observations suggested that HCoV-OC43 infection should be considered as a possible case of outbreaks in nurseries, as observed during the 2014–2015 influenza season (3).

With regard to the other viruses detected in June 2016, apart from HCoV-OC43, we isolated 7 strains of parainfluenza virus type 3, 3 strains of rhinovirus, 3 strains of adenovirus, 2 strains of coxsackievirus B4, one strain of influenza B virus (Yamagata lineage), and one HCoV-NL63 strain among 40 specimens examined using the microplate method and real-time PCR. Thus, HCoV-OC43 was the most frequently detected virus in June 2016.

We carried out PCR amplification and sequence analysis of the S gene for representative HCoV-OC43 strains detected in 2015 and 2016 using the primers shown in Table 1. Sequence data of the 26 HCoV-OC43 strains were registered under GenBank accession numbers LC331068–331093. The nucleotide homologies among the 11 strains in 2015, one strain in 2016 (HCoV-OC43 Yamagata.JPN 23.16-0980), and genotype D strain 12694 (KF572833) (13) were high at 99–100. The homologies among the 14 strains in 2016 and genotype B strain 2145A (KF572810) (13) were also high at 99–100. Thus, there was a genotype shift between 2015 and 2016. These preliminary results suggest that it is necessary to conduct a longitudinal molecular epide-

### Table 1. Primers used for PCR and sequencing of the S genes of the 26 HCoV-OC43 between 2015 and 2016 in Yamagata, Japan

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer direction, sequence (5'–3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>For PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23522F</td>
<td>CTACCAGTTATTTTGGTTGTCGCC</td>
<td>This study</td>
</tr>
<tr>
<td>LPW2094R</td>
<td>GCCCAAATTTACCCACTTAGTCAG</td>
<td>Lau et al. 2011 (11)</td>
</tr>
<tr>
<td>LPW2095F</td>
<td>TGATGCTGCTAAGATATATGG</td>
<td>Lau et al. 2011 (11)</td>
</tr>
<tr>
<td>S-26126R</td>
<td>AATCACCACAGAAATGCAC</td>
<td>Kin et al. 2015 (12)</td>
</tr>
<tr>
<td>For sequencing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24220F</td>
<td>GATACAGGTGTTGTTGTTCTTG</td>
<td>This study</td>
</tr>
<tr>
<td>24326R</td>
<td>TAGCATGAAAGATGACATTACCA</td>
<td>This study</td>
</tr>
<tr>
<td>S-25372F</td>
<td>AATCACCACAGAAAT GCAC</td>
<td>Kin et al. 2015 (12)</td>
</tr>
<tr>
<td>S-24569R</td>
<td>AACCTCAACAAAAATGCCTTGG</td>
<td>Kin et al. 2015 (12)</td>
</tr>
</tbody>
</table>
miological study to clarify the epidemiology of HCoV-OC43.

In conclusion, the HCoV-OC43 outbreak in June 2016 could indicate the spread of HCoV-OC43 among infants and young children, such as in nurseries. HCoV-OC43 commonly shows peak activity in winter but also in early summer in Yamagata, Japan. According to the NESID system, only 557 HCoV-positive cases in 14 prefectures in Japan, including 228 cases from Yamagata, were reported in 2014–2016 (14). Therefore, further surveillance, including molecular analysis, is necessary to more clearly evaluate the circulation patterns of HCoVs in Japan.

Acknowledgments We thank the medical staff and people of Yamagata Prefecture for their collaboration in specimen collection for NESID, Japan based on the Infectious Diseases Control Law.

Conflict of interest None to declare.

REFERENCES