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Epidemiology Communication

Detection of Respiratory Viruses during the Early Phase of the Coronavirus Disease 2019 Pandemic in Ibaraki and Gunma Prefectures, Japan

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ABSTRACT: Respiratory infections are common, and the most common causative agent is a virus. Therefore, routine surveillance of respiratory viruses is useful in the case of novel viral diseases such as coronavirus disease 2019 (COVID-19). In this study, to clarify the kind of virus involved in suspected cases of COVID-19 in the early stages of the pandemic, we attempted to detect various respiratory viruses in 613 specimens that tested negative for severe acute respiratory syndrome coronavirus 2 using reverse transcription polymerase chain reaction. As a result, viruses were detected in 59 (9.6%) patients. In addition, human rhinovirus (HRV), human metapneumovirus (HMPV), human respiratory syncytial virus, and human parechovirus were detected in 29, 25, 3, and 2 patients, respectively. Although this study was conducted over a short period of time and not all specimens were tested, these results indicate that various respiratory viruses, especially HRV and HMPV, can be detected even during the early stages of the COVID-19 pandemic. Because various respiratory viruses maintain a constant effect during the outbreak of the newly emerged pandemic, systematic surveillance of respiratory viruses is needed during the normal period to make good use for clinical and public health.

Many pathogens can cause respiratory infections, which have symptoms such as fever, upper respiratory inflammation (URI), and lower respiratory inflammation (LRI), and viruses are the most common causative agents (approximately 69% of cases) (1). Unfortunately, many types of viruses cause respiratory infections, and it is difficult to identify the cause based on clinical signs and symptoms (2). However, identification of the causative virus is important for treatment, infection control, and public health. In addition, routine surveillance of respiratory viruses is especially useful for monitoring novel viral pandemics, such as coronavirus disease 2019 (COVID-19), and has the potential to reduce healthcare costs (3).

The COVID-19 outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in Wuhan, China, in December 2019 and rapidly spread to become a major pandemic. As of October 2021, there have been 1,716,813 cases of COVID-19 and 18,260 COVID-19-related deaths in Japan (4). The initial symptoms of respiratory viral infections, including COVID-19, can be very similar, including fever, cough, and URI. However, in some cases, the infection can become severe and require hospitalization (5).

In this study, to clarify the viruses involved in cases of suspected SARS-CoV-2 infection in the early stages of the COVID-19 pandemic, we conducted respiratory virus surveillance. We obtained nasopharyngeal swabs or saliva specimens from 5,480 patients with suspected COVID-19 from January to June 2020 as part of the National Epidemiological Surveillance of Infectious Diseases and the Active Epidemiological Investigation for COVID-19 in Japan. All specimens were collected from the Gunma or Ibaraki Prefecture. SARS-CoV-2 was detected in 260 of the 5,480 patients. In this study, we only included specimens that were negative for SARS-CoV-2 using real-time reverse transcription polymerase chain reaction (RT-PCR), as previously described (6). To identify the causative agent of respiratory infection in these cases, we randomly selected specimens from symptomatic patients (fever and URI or LRI) without bias concerning age or sex. A total of 613 specimens were included in this study. Viral ribonucleic acid (RNA) was purified using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA). We used RT-PCR assays to detect common respiratory viruses, such as human rhinovirus (HRV)/

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Table 1. Subject data in this study

No. of patients	Sex (male/female)	Age[1] (Yrs)	Total no. of detections	Sex (male/female)	Virus	No. of detections	Age[1] (Yrs)	Type	Symptoms	
									URI	LRI
613	347/266	39.7 ± 20.5	59	28/31	HRV	29	25.0 ± 22.4[2]	A	4	13
								C	5	7
					HMPV	25	42.7 ± 20.6[2]	A2b	4	8
								B2	4	9
					RSV	3	43.3 ± 36.9	A		3
					HPeV	2	1.5 ± 2.1	3		1
								6		1

[1]: Mean ± standard deviation (SD).
[2]: $P < 0.05$, HRV vs. HMPV.
HRV, human rhinovirus; HMPV, human metapneumovirus; RSV, respiratory syncytial virus; HPeV, human parechovirus.

Table 2. Detections by month

Virus	Type	Month					
		February	March	April	May	June	Total
HRV	A	3	2	8	3	1	17
	C	1	7	3		1	12
HMPV	A2b	2	4	6			12
	B2		6	7			13
RSV	A		2		1		3
HPeV	3		1				1
	6	1					1
	Total	7	22	24	4	2	59

enterovirus (HEV), human metapneumovirus (HMPV), human parechovirus (HPeV), human parainfluenza virus (HPIV) 1, 2, 3, and 4, and respiratory syncytial virus (RSV), as previously described (7–9). In this study, adenovirus and influenza virus were excluded because they were diagnosed using rapid diagnostic kits at their respective medical institutions. The amplicons were confirmed using agarose gel electrophoresis and sequencing. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (SPSS for Windows, version 10.0). Comparisons between groups were performed using the Mann–Whitney U test and Pearson's chi-squared test. Statistical significance was set at $P < 0.05$.

Viral detection was performed on 613 specimens that tested negative for SARS-CoV-2. Respiratory viruses were detected in 59 (9.6%) patients. The viruses detected were HRV in 29, HMPV in 25, RSV in 3, and HPeV in 2 cases (Table 1). In respective order, the average patient's age was 25.0 ± 22.4, 42.7 ± 20.6, 43.3 ± 36.9, and 1.5 ± 2.1 years (Table 1). Patients infected with HRV were significantly younger than those infected with HMPV. HRV-A and HRV-C were detected in 17 and 12 cases, respectively. The HMPV subgroups detected were A2b in 12 and B2 in 13 patients. RSV-A was detected in 3 cases in the RSV subtype. The HPeV serotypes detected were 3 and 6 (Table 1). There were no statistically significant associations between the detected virus and the clinical symptoms of fever and URI or LRI. HRV was detected throughout the study period, whereas HMPV was detected only from February to April (Table 2). In addition, we compared the clinical data of patients infected with each of these viruses and those infected with SARS-CoV-2. We analyzed 203 cases with complete data out of 260 COVID-19 confirmed cases. The average age of patients with HRV (25.0 ± 22.4 years) was significantly lower ($P < 0.05$) than that of patients with COVID-19 (51.9 ± 23.0 years). Next, we compared the clinical symptoms in patients with COVID-19. In patients with COVID-19, 75.4% (153/203) had a fever, and URI incidence was substantially higher than in those with HRV or HMPV.

Although this study was conducted over a short period and not all specimens were tested, these results indicate that various respiratory viruses were present in the early stages of the COVID-19 pandemic. HRV and HMPV are major respiratory viruses in both children and adults and are prevalent mainly in spring (10). In this study, HRV and HMPV were the most frequently detected viruses, suggesting that these viruses should be considered even in the early stages of the COVID-19 epidemic. RSV and parainfluenza were less frequently implicated in suspected COVID-19 specimens.

HRV has been suggested to be important in children, while HMPV has been suggested to be important in adults. Although HRV is generally thought to be

associated with the mild common cold in children, it can also cause lower respiratory tract infections and should be treated with caution (11). Similarly, HMPV is also thought to be a pediatric infection, but caution is needed in adult infections (12). HRV infection has significantly increased in children during the COVID-19 pandemic. It is suggested that HRV continues to be detected during pandemics (13). Understanding the dynamics of the virus according to the patient's age would be useful in the event of a pandemic. Among the viruses detected in the present study, HRV-A and -C were the most prevalent HRVs, and A2b and B2 were the most prevalent HMPV subgroups. Similar to recent reports, there is no association between clinical symptoms and virus type (14,15). It is important to diagnose or exclude HRV and HMPV in outbreaks of respiratory infections because they can also cause URI and LRI. In general, it is difficult to identify the causative agent of respiratory infections based on clinical symptoms alone. Many factors contribute to the prevalence of viruses, including seasonality and patient's age (16). Our data indicate that various other respiratory viruses circulated and caused infections during the SARS-CoV-2 outbreak. From a clinical and public health perspective, systematic surveillance of respiratory viruses during both epidemic and non-epidemic periods is needed.

Due to the lack of written informed consent, the study protocols were considered and approved by the ethics committee on Human Research of the Gunma Prefectural Public Health and Environmental Sciences. All experiments were performed in accordance with approved guidelines.

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Conflict of interest None to declare.

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