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Pediatric Respiratory Virus Infections During the COVID-19 Pandemic in a Region Without Active Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Circulation

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ABSTRACT

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were not prevalent in Yonago and its vicinity during autumn 2020, and the relative frequencies of pathogen-induced respiratory infections during this period are unclear.

Methods We collected 109 nasopharyngeal swabs from 93 pediatric patients who visited Tottori University Hospital between October 1, 2020, and March 31, 2021. These samples were comprehensively tested for 18 pathogens with the FilmArray® respiratory panel test (v2.1) using nested real-time polymerase chain reaction, and the frequency of pathogens detected per month was calculated. In addition, we compared the duration of fever and the blood test results of patients infected with each pathogen or multiple pathogens.

Results Of the 109 samples, 42 were obtained from female patients and 67 from male patients (median age, 3 years; range, 0–15 years). Overall, 62 patients (56.9%) had a fever ≥ 38 °C at the time of examination, and the median duration of fever ≥ 38 °C was 2 days (1–12). During the study period, the highest number of samples (22) were collected in November 2020. Among samples that tested positive, the most common pathogens were rhino/enteroviruses (52 samples; 76.5%), followed by adenoviruses (7 samples; 10.3%), coronavirus NL63 (6 samples; 8.8%), coronavirus OC43, parainfluenza virus type 1, and parainfluenza virus type 2 (1 sample each; 1.5% each). The duration of fever was significantly longer in adenovirus-infected patients than in patients infected with other viruses ($P < 0.05$). Hemoglobin and sodium levels were also significantly lower among the adenovirus-infected patients. However, these variations were mostly within the normal range. No clinically meaningful differences were found between rhino/enterovirus-infected and non-rhino/enterovirus-infected cases, between coronavirus NL63-infected and non-coronavirus NL63-infected cases, and between cases with multiple- and single-pathogen infections.

Conclusion Rhino/enteroviruses were the most common viruses causing respiratory tract infections in areas without endemic SARS-CoV-2.

Key words adenovirus; common coronavirus; respiratory panel test; rhino/enterovirus; severe acute respiratory syndrome coronavirus 2

Since 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections have caused a pandemic.[1] The first case of SARS-CoV-2 infection in Japan was identified on January 16, 2020.[2] Since then, the SARS-CoV-2 epidemic has persisted in Japan. Similar to other countries worldwide, Japan has experienced an outbreak of severe respiratory infections due to SARS-CoV-2 coronavirus Disease-2019 (COVID-19).[1, 2] Although vaccination efforts are underway, they have not completely stopped the spread of the infection. Therefore, in addition to isolating patients with COVID-19, various medical facilities in Japan have also established nosocomial infection control measures such as disinfecting hands, wearing masks, monitoring body temperature, and restricting visits to hospitalized patients.[3]

Since October 2020, Tottori University Hospital has been implementing respiratory infection panel tests in addition to general infection control measures to prevent the entry and clustering of novel coronaviruses in hospitals. Respiratory infection panel testing with the FilmArray® respiratory infection panel is performed immediately prior to admission for pediatric emergency patients, preoperative patients, or patients whose attending physician determines that screening is necessary.[4, 5] In the FilmArray® panel, nested real-time polymerase chain reaction is used to detect multiple viral and

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Abbreviations: ALC, absolute lymphocyte count; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BUN, urea nitrogen; Cl, chloride; Cre, creatinine; CRP, C-reactive protein; Hb, hemoglobin; K, potassium; LD, lactate dehydrogenase; Na, sodium; PLT, platelet; RSV, respiratory syncytial virus; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SIADH, syndrome of inappropriate secretion of antidiuretic hormone; WBC, white blood cell

bacterial respiratory pathogens, and the test results are obtained in approximately one hour. Severe cases of COVID-19 are rare in pediatric patients.[5] Nevertheless, it is very important to prevent SARS-CoV-2 infection in pediatric patients because many immunocompromised children are hospitalized in the pediatric ward due to the administration of antineoplastic and immunosuppressive drugs.

The relatively large number of samples collected by respiratory panel testing in this study allowed us to investigate the frequency of transmission in children of a countermeasure respiratory infection virus in a special setting in which citizens were well infected, which was not known accurately before. More specifically, we were also able to determine the frequency of infection with rhinoviruses, coronaviruses other than SARS-CoV-2, adenoviruses, and other viruses that cause respiratory infections such as the common cold and acute pharyngitis.[6, 7]

In this study, we report on the frequency of infection by viruses that cause common respiratory tract infections, and compare clinical data for each virus type during a time in which SARS-CoV-2 was not circulating in the region. We also examined the duration of fever, a typical clinical manifestation of each pathogen, and laboratory data commonly measured on admission blood tests (abnormal blood cell counts, electrolytes, liver function, and renal function).

MATERIALS AND METHODS

The primary outcome of the study was the frequency of infection with respiratory infectious disease pathogens in a non-endemic area of the same virus under conditions of widespread infection control measures against novel coronaviruses. Secondary outcomes were characteristics of each viral infection and duration of fever, blood counts, liver function, renal function, electrolytes, inflammatory response, and the type and frequency of viruses that are infectious despite the absence of respiratory symptoms.

In this study, we used 109 nasopharyngeal swab samples from 93 patients (ages: 0–15 years) admitted to Tottori University Hospital between October 1, 2020, and March 31, 2021. Most tests were performed for screening purposes during hospitalization, and only a few procedures were performed in an outpatient clinic. Although the SARS-CoV-2 epidemic had not yet occurred in Yonago during this period, citizens had enhanced their infection control measures due to the outbreak in Japan. Although the study had an opt-out to allow patients or their guardians to refuse the study because it was a retrospective observational study, none of them expressed a refusal to participate in the study.

Viruses and other pathogens were detected by swabbing the patient's nasopharynx with a sterile swab. These samples were analyzed with the FilmArray® Respiratory Panel v2.1 (bioMérieux, Marcy-l'Etoile, France).[8–10] The following microorganisms can be detected with this technique: adenovirus, conventional or common coronaviruses (HKU1, NL63, 229E, and OC43), SARS-CoV-2, human metapneumovirus, human rhino/enterovirus, influenza virus (types A and B), parainfluenza virus (types 1, 2, 3, and 4), respiratory syncytial virus (RSV), *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.[4] In this study, rhinovirus and enteroviruses were treated as the same pathogen because they were indistinguishable in the FilmArray® panel.

The monthly detection frequency of the viruses is represented as a bar graph (Fig. 1). Fever was defined as a maximum daily body temperature ≥ 37.5 °C. Fever duration was defined as the period from the day when fever was observed to the day before fever resolution.

The medians of fever duration and laboratory data were compared for each type of virus, and the Mann-Whitney *U* test was used to detect significant differences between the respective medians. The level of significance (*P*-value) was set at $P < 0.05$. Statistical analyses were conducted in R statistical software version 4.0.5 (2021-03-31).[11] This is an exploratory study and the *P*-values calculated are nominal. In addition, the issue of multiplicity was not considered.[12] The clinical and blood laboratory data reviewed included maximum body temperature, fever duration, white blood cell (WBC) count, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), Hb, platelet (PLT) count, and the levels of sodium (Na), potassium (K), chloride (CI), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), urea nitrogen (BUN), creatinine (Cre), and C-reactive protein (CRP).

This study was approved by the Ethical Review Committee of the Tottori University Faculty of Medicine (reference number: 21A040).

RESULTS

The demographic data are listed in Table 1. During the observation period, 109 patients underwent a FilmArray® examination at our hospital. Of these, 42 patients were female and 67 were male, and the median age was 3 years. Among the patients with fever ≥ 37.5 °C, the median time to reach a temperature of < 37.5 °C was one day. In total, 56 patients tested positive for some kind of virus by FilmArray® testing. Of these, 42

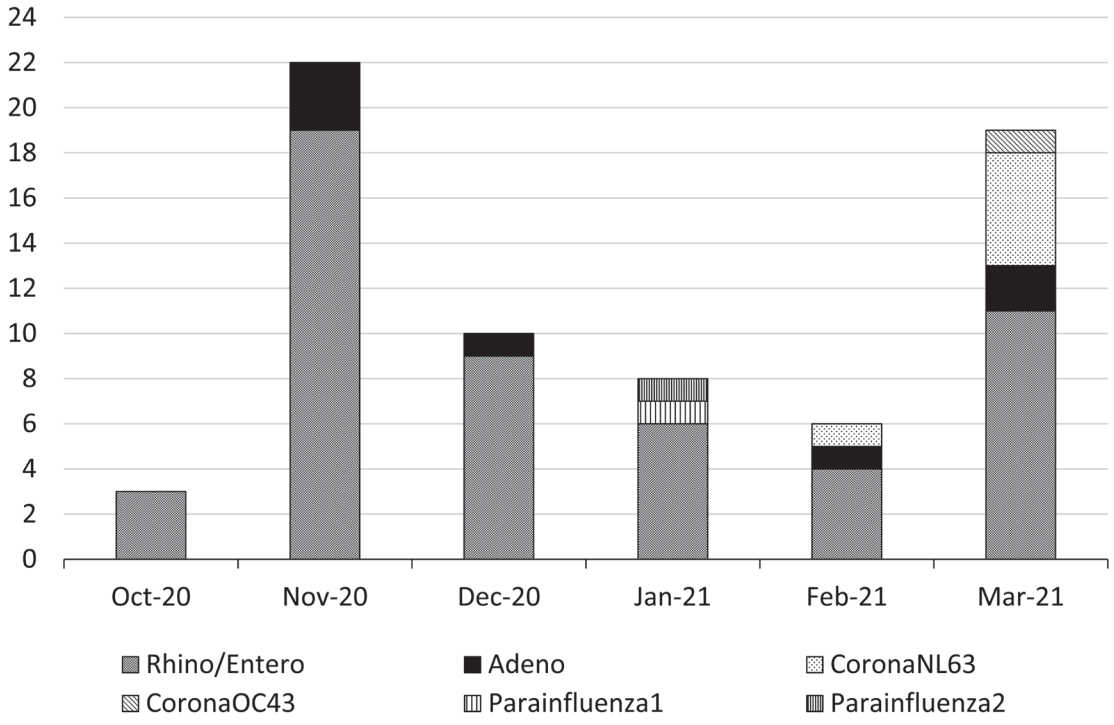

Fig. 1. Number of viruses detected in samples each month. The number of samples tested in October, November, and December 2020, and January, February, and March 2021 were 9, 25, 17, 17, 13, and 28, respectively.

were isolated rhinovirus/enterovirus infections, which constituted the highest number of viruses detected in this study. The other viruses detected included adenovirus (7 patients), coronavirus NL63 (6 patients), coronavirus OC43 (1 patient), and parainfluenza virus type 1 and 2 were detected simultaneously in one patient. Moreover, 10 patients tested positive for multiple viruses. SARS-CoV-2, RSV, and influenza viruses were not detected during the observation period. The most common reasons for consultation or hospitalization were febrile convulsions, bronchial asthma, acute pharyngitis, and acute bronchitis.

The types and numbers of viruses detected per month are shown in Fig. 1. Among samples that tested positive, the positivity rates of various viruses were as follows: 76.5% for rhino/enterovirus, 10.3% for adenovirus, 8.8% for coronavirus NL63, 1.5% for coronavirus OC43, and 1.5% for parainfluenza virus types 1 and 2. During the study period, the highest number of samples (22) were collected in November 2020.

To evaluate the relationship between viral infections and clinical data, we performed comparative analyses (Tables 2–5). Cases positive for adenovirus and those positive for other types of viruses were significantly different with respect to the duration of fever ≥ 38°C, Hb level, and Na level. Figure 2 shows the box plots for fever duration (Fig. 2a), Na level (Fig. 2b), and Hb level (Fig. 2c) among the groups. Fever duration and laboratory data were also compared between adenovirus-positive and adenovirus-negative cases (Table 2). As shown in Fig. 2, adenovirus-positive cases showed a long fever period, low sodium and low hemoglobin levels. Additionally, serum potassium K levels (Fig. 2d), aspartate aminotransferase AST levels (Fig. 2e) between patients infected with rhino/enterovirus and other pathogens and serum K levels (Fig. 2f) between patients infected with coronavirus NL63 and other pathogens.

Table 3 shows the comparisons of blood tests and fever duration between patients positive and negative for human rhinovirus/enterovirus among the cases where pathogens were detected. Serum K and AST levels were significantly lower in human rhinovirus/enterovirus-positive cases than in other virus-positive cases ($P < 0.05$). However, these differences were not clinically or morbidly significant.

Fever duration and blood test results were compared between patients positive and negative for coronavirus NL63 in cases where pathogens were detected (Table 4). Serum K levels were significantly higher in coronavirus NL63-positive cases than in other cases positive for other viruses ($P < 0.05$). However, this difference in serum K levels was not a clinically

Table 1. Demographic, diagnostic and clinical data of patients enrolled in the study

Demographic characteristics (n = 109)	
Sex	
Female	42 (38.5%)
Median age (years)	3 (0–15)
Admission	106 (97.2%)
Febrile cases (≥ 37.5°C)	73 (67.0%)
Febrile cases (≥ 38.0°C)	62 (56.9%)
Median Duration of fever (≥ 37.5°C)	2 (1–13)
Median Duration of fever (≥ 38.0°C)	2 (1–12)
Viruses (n = 56)	
Human rhinovirus/enterovirus	42
Adenovirus and Human rhinovirus/enterovirus	5
Coronavirus NL63 and Human rhinovirus/enterovirus	3
Coronavirus NL63	2
Adenovirus	1
Coronavirus OC43	1
Adenovirus, Coronavirus NL63 and Human rhinovirus/enterovirus	1
Parainfluenza 1, Parainfluenza 2 and Human rhinovirus/enterovirus	1
Diagnosis	n
Febrile seizure	16
Asthma	12
Pharyngitis	10
Bronchitis	9
Urinary tract infectious disease	6
Esophageal foreign objects	5
Acetonic vomiting	4
Pneumonia	4
Croup syndrome	4
Infectious gastroenteritis	3
Kawasaki disease	3
Food allergy	3
Intestinal obstruction	3
Unidentified fever	3
Juvenile idiopathic arthritis	2
Epilepsy	2
Head trauma	2
Other	18

The "Other" category includes one case each of the following diseases: cellulitis, fever after the vaccination, herpes zoster, neuroblastoma, rotavirus gastroenteritis, tibial fracture, vulvar hematoma, acute lymphatic leukemia, bacteremia, exanthema subitem, primary chordal rupture, soft palate wound, Yersinia infection, acute pancreatitis, laryngomalacia, pit viper bite, pulmonary edema, and systemic lupus erythematosus.

Table 2. Comparison of laboratory data and duration of fever between cases with and without adenovirus infection

	Detected [$n = 7$]	Undetected [$n = 46$]	P-value
Duration of fever (≥ 38.0°C)	4 (0–4) [5]	0 (0–6) [40]	0.0099*
WBC [*10^3/μL]	14.5 (7.9–25.7)	12.1 (3.8–24.1)	0.7327
ANC [*10^3/μL]	5.85 (4.51–16.7)	7.74 (1.22–20.3) [43]	0.9563
ALC [*10^3/μL]	5.22 (1.42–12.4)	3.05 (0.65–12.7) [43]	0.0928
Hb [g/dL]	11.5 (10.9–12.2)	12.4 (9.6–18.2)	0.0246*
PLT [*10^3/μL]	22.0 (15.0–61.7)	31.8 (4.09–65.2)	0.2424
CRP [mg/dL]	0.76 (0.02–18.4)	0.56 (0.01–29.3)	0.7324
Na [mmol/L]	134 (134–139)	138 (131–148) [45]	0.0100*
K [mmol/L]	4.5 (3.8–4.9)	4.2 (3.3-6.1) [45]	0.4923
Cl [mmol/L]	101 (98–103)	103 (96-110) [45]	0.0608
BUN [mg/dL]	9.0 (5.2–16.4)	11.1 (3.2-20.6) [45]	0.7476
Cre [mg/dL]	0.24 (0.17–0.26)	0.26 (0.15-0.92) [45]	0.1431
AST [U/L]	37 (25–60)	32 (15-168) [45]	0.3273
ALT [U/L]	18 (13–24)	17 (10-273) [45]	0.8931
LD [U/L]	308 (237–411)	304 (209-560) [45]	0.6389

In Table 2, 3, 4, and 5, the numbers represent the median, and values in parentheses represent the range. Numbers in square brackets indicate the number of samples in case of missing samples. *$P < 0.05$.

meaningful difference.

Fever duration and blood test data were also compared between fever between cases with multiple- and single-pathogen infections (Table 5). ALC and serum K levels were higher in cases positive for multiple viruses and lower in cases positive for a single virus, although the differences were not significant. Therefore, there was no significant difference in fever duration or laboratory data between cases infected with multiple pathogens compared to those infected with only a single pathogen.

In some cases, the virus was detected despite the absence of respiratory symptoms (Fig. 3). By far the most common virus was rhino/enterovirus, followed by adenovirus and coronavirus NL63.

DISCUSSION

Here, we reviewed previously unreported epidemiological data and clinical outcomes of respiratory pathogens in children and their impact on blood test results in and around a rural, pre-endemic region, while measures to prevent the spread of SARS-CoV-2 were already in place. We used the FilmArray® Respiratory Panel v2.1 to determine the frequency of infection by various pathogens in respiratory tract infections. In this study, there were 10 rhino/enterovirus-detected cases without respiratory symptoms and it was the most frequent. Interestingly, human rhinovirus/enterovirus was also detected in patients not directly affected by respiratory tract infections. This included patients who presented with snakebites, urinary tract infections, accidental ingestion of foreign objects, convulsions without fever, head trauma, ileus, acute pancreatitis, and juvenile idiopathic arthritis. In addition, coronavirus NL63 (two cases) and adenovirus (one case) were detected in patients without respiratory symptoms. This suggests a high frequency of asymptomatic rhino/enterovirus carriers. In fact, this result is consistent with reports that rhino/enterovirus infections cause about 4% of deaths, while more than half of the pediatric cases are asymptomatic or mildly ill.[13] Among adult patients who test positive for viruses that cause respiratory infections (such as rhinoviruses or conventional coronaviruses), 57.7–93.3% have been reported to be asymptomatic.[14] Tomlinson et al. also reported that respiratory viruses—except for rhinoviruses—were not frequently detected in asymptomatic healthy adults, and suggested that this generally indicates a current or recent symptomatic infection.[15]

In Yonago City, where Tottori University Hospital is located, the prevalence of COVID-19 is lower than that in larger cities such as Tokyo and Osaka. During the period of this study, there were no lockdowns or other restrictions on the movement of Japanese citizens. In particular, Tottori Prefecture, where Yonago is located, has the smallest population in Japan, and due to

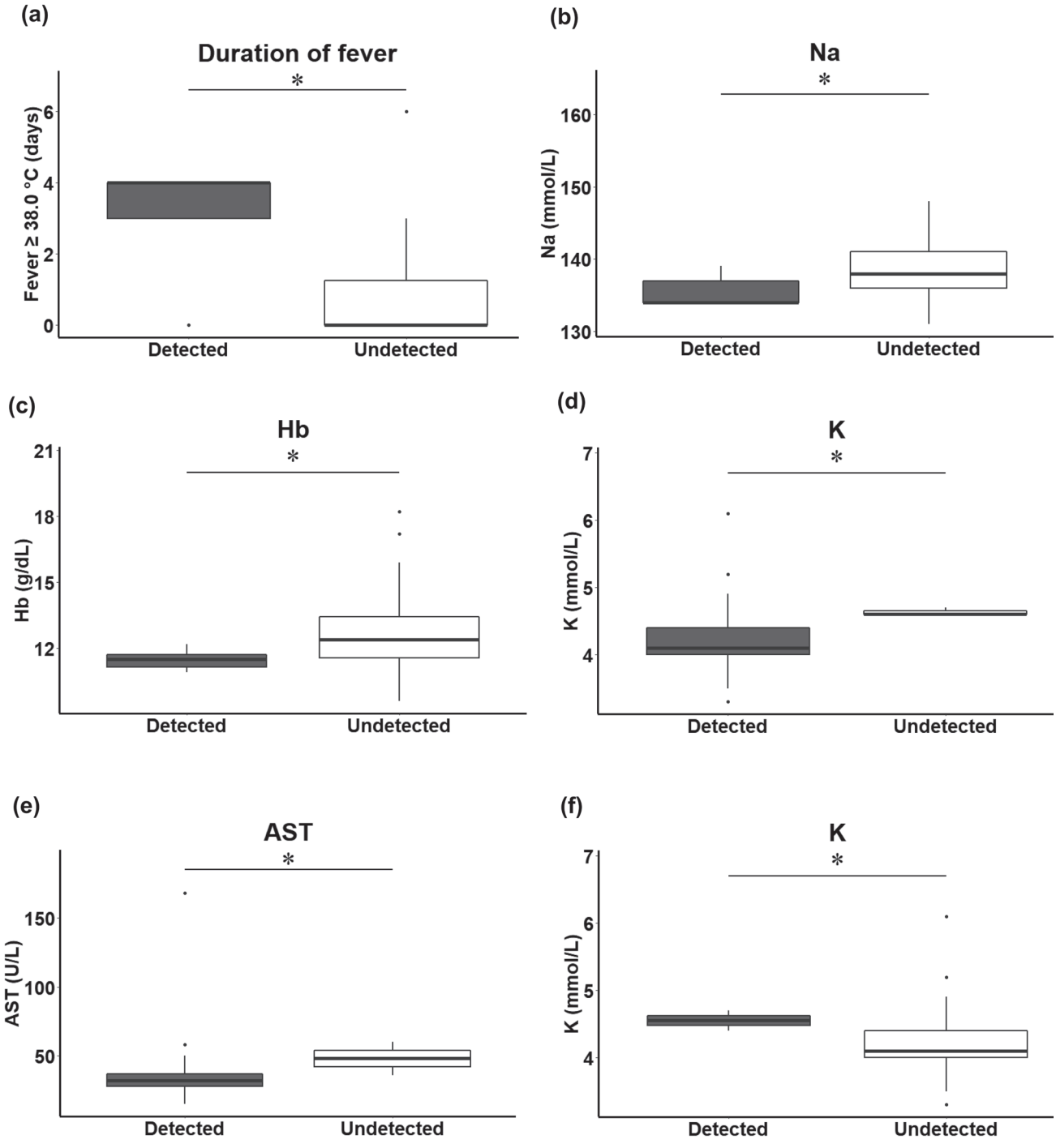

Fig. 2. Box-and-whisker plots of fever duration (**a**), sodium (Na) levels (**b**), and hemoglobin levels (Hb) (**c**) for adenovirus-positive and negative cases are shown. Comparison of serum potassium (K) levels (d) and aspartate aminotransferase (AST) levels (e) between patients infected with rhino/enterovirus and other pathogens, and serum K levels (f) between patients infected with coronavirus NL63 and other pathogens are also illustrated. Statistically significant differences ($P < 0.05$) were observed in these (**a**), (**b**), (**c**), (**d**), (**e**) and (**f**).

its geographical characteristics, there is less traffic from outside the prefecture than in other prefectures. Perhaps because of this situation, none of the subjects in this study tested positive for the novel coronavirus.

One of the findings of statistical significance in this study was a longer duration of fever in patients infected with adenovirus. Adenoviral infections are known to cause higher temperatures and more prolonged fever compared with other viruses.[16] In addition, the analysis of blood test results show leukocytosis and elevated

Table 3. Comparison of laboratory data and duration of fever between cases with and without human rhinovirus/enterovirus infection

	Detected [*n* = 50]	Undetected [*n* = 4]	*P*-value
Duration of fever (≥ 38.0°C)	0 (0–6) [41]	2 (0–4)	0.1848
WBC [*10^3/μL]	12.2 (3.8–25.7)	10.6 (9.2–12.4) [3]	0.3456
ANC [*10^3/μL]	7.74 (1.22–20.3) [47]	4.51 (1.61–7.94) [3]	0.1612
ALC [*10^3/μL]	3.19 (0.65–12.7)	4.23 (2.85–8.34)	0.3854
Hb [g/dL]	12.1 (9.6–18.2)	11.0 (10.2–12.8) [3]	0.2106
PLT [*10^3/μL]	29.6 (4.09–65.2)	29.5 (15.0–48.8) [3]	0.9846
CRP [mg/dL]	0.6 (0.01–29.3)	0.08 (0.02–0.28) [3]	0.0731
Na [mmol/L]	138 (131–148) [49]	138 (134–139) [3]	0.5149
K [mmol/L]	4.1 (3.3–6.1) [49]	4.6 (4.6–4.7) [3]	0.0247*
Cl [mmol/L]	103 (96–110) [49]	103 (100–103) [3]	0.5939
BUN [mg/dL]	11.1 (3.2–20.6) [49]	9.0 (6.8–13.8) [3]	0.6516
Cre [mg/dL]	0.25 (0.15–0.92) [49]	0.2 (0.19–0.24) [3]	0.1568
AST [U/L]	32 (15–168) [49]	48 (36–60) [3]	0.0373*
ALT [U/L]	17 (10–273) [49]	22 (19–24) [3]	0.1568
LD [U/L]	304 (209–560) [49]	307 (280–368) [3]	0.7387

*$P < 0.05$.

Table 4. Comparison of laboratory data and duration of fever between cases with and without coronavirus NL63 infection

	Detected [*n* = 4]	Undetected [*n* = 49]	*P*-value
Duration of fever (≥ 38.0°C)	0.5 (0–1)	0 (0–6) [41]	0.648
WBC [*10^3/μL]	11.6 (9.4–22.1)	12.1 (3.8–25.7)	0.9866
ANC [*10^3/μL]	6.51 (4.98–12.4)	7.63 (1.22–20.3) [46]	0.9024
ALC [*10^3/μL]	4.17 (2.85–7.07)	3.12 (0.65–12.7) [46]	0.3674
Hb [g/dL]	11.7 (10.2–12.4)	12.2 (9.6–18.2)	0.2382
PLT [*10^3/μL]	40.4 (26.0–48.8)	29.3 (4.09–65.2)	0.2319
CRP [mg/dL]	0.51 (0.28–1.0)	0.56 (0.01–29.3)	1.0000
Na [mmol/L]	137 (134–139)	138 (131–148) [48]	0.3335
K [mmol/L]	4.55 (4.4–4.7)	4.1 (3.3–6.1) [48]	0.0311*
Cl [mmol/L]	101 (98–103)	103 (96–110) [48]	0.1419
BUN [mg/dL]	9.7 (6.8–11.9)	11.1 (3.2–20.6) [48]	0.4295
Cre [mg/dL]	0.23 (0.19–0.27)	0.25 (0.15–0.92) [48]	0.3802
AST [U/L]	36.5 (32–42)	32 (15–168) [48]	0.2289
ALT [U/L]	20 (12–25)	17 (10–273) [48]	0.7307
LD [U/L]	328 (255–411)	305 (209–560) [48]	0.6064

*$P < 0.05$.

CRP levels in these patients, indicating a strong inflammatory response.[17, 18] Although this is speculated to be related to hypercytokinemia, the details are unknown. Sodium was significantly lower in the adenovirus-infected cases. This difference may be due to syndrome of inappropriate secretion of antidiuretic hormone (SIADH). Other statistically significant differences shown in Tables 2–5, such as Hb, K, and AST are unlikely to be due to viral infection and may be related to factors unrelated to infection, such as hemolysis during

Table 5. Comparison of laboratory data and duration of fever between cases with multiple- and single-pathogen infections

	Detected [$n = 9$]	Undetected [$n = 44$]	P-value
Duration of fever (≥ 38.0°C)	1 (0–4) [7]	0 (0–6) [38]	0.3139
WBC [*10^3/μL]	10.8 (7.9–25.7)	12.2 (3.8–24.1)	0.7944
ANC [*10^3/μL]	5.63 (1.53–16.7)	7.94 (1.22–20.3) [41]	0.4078
ALC [*10^3/μL]	4.86 (1.42–12.4)	2.87 (0.65–12.7) [41]	0.0729
Hb [g/dL]	11.7 (10.9–12.4)	12.4 (9.6–18.2)	0.1177
PLT [*10^3/μL]	23.5 (11.3–61.7)	31.8 (4.09–65.2)	0.3255
CRP [mg/dL]	0.61 (0.02–18.4)	0.56 (0.01–29.3)	0.6868
Na [mmol/L]	136 (134–141)	138 (131–148) [43]	0.0886
K [mmol/L]	4.4 (3.8–4.9)	4.2 (3.3–6.1) [43]	0.5197
Cl [mmol/L]	102 (98–105)	103 (96–110) [43]	0.1282
BUN [mg/dL]	10.4 (5.2–16.4)	11.1 (3.2–20.6) [43]	0.6987
Cre [mg/dL]	0.24 (0.17–0.3)	0.25 (0.15–0.92) [43]	0.3507
AST [U/L]	37 (25–168)	32 (15–60) [43]	0.1952
ALT [U/L]	18 (12–273)	17 (10–58) [43]	0.9613
LD [U/L]	308 (237-560)	304 (209-450) [43]	0.4905

*$P < 0.05$.

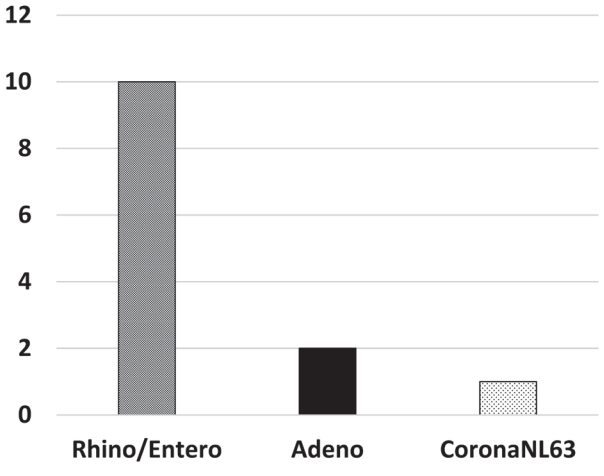

Fig. 3. Type of virus detected in patients without respiratory symptoms and number of cases. A particularly large number of patients with entero/rhinoviruses were not accompanied by respiratory symptoms.

blood collection. Hemoglobin may also be affected by patient factors unrelated to viral infection, such as iron deficiency.

Although RSV and influenza virus infections are usually prevalent between fall and spring, they were not detected in this study. A recent study from Yokohama, which is one of the largest cities in Japan, during a SARS-CoV-2 epidemic reported that incidences of rhinovirus infections increased among children below 10 years of age. In contrast, other viral infections—such as influenza and infections caused by parainfluenza virus, RSV, and adenovirus—generally decreased.[19] This may be because of behavioral changes among citizens due to the COVID-19 pandemic, including hand washing with detergents, disinfection with alcohol, wearing masks, and maintaining social distance when speaking.[20] Another possible explanation for this is that viruses without envelopes including rhinoviruses/enteroviruses are less effective to disinfection methods, including disinfection with ethanol than enveloped viruses.[21, 22] However, coronaviruses, RSVs, influenza viruses, and parainfluenza viruses have envelopes, and are thus susceptible to detergents or alcohol.[20, 23–26] One study reported that alcohol-based disinfectants are more effective against Coxsackie viruses (a type of enterovirus). This may lead to a decrease in the frequency of infections, as observed in this study.[27] Moreover, because rhinoviruses can be transmitted via aerosols and droplets, surgical masks cannot completely prevent transmission.[28, 29] In contrast, there have been no reports of aerosol transmission of adenoviruses in the natural environment. Therefore, it is possible that the frequency of rhino/enterovirus infections did not decrease even after behavioral changes in citizens. Another potential reason for the scarcity of RSV and influenza infections may be viral interference. For example, viral interference has been reported between

rhinoviruses and influenza type A viruses, suggesting that rhinoviruses inhibit the proliferation of influenza type A viruses by activating the immunological defense system in the mucosal epithelium.[30]

In five cases of pharyngitis, one case of croup syndrome, four cases of bronchitis, three cases of pneumonia, and two cases of febrile convulsions, the FilmArray® panel did not detect any pathogens. Although the FilmArray® panel can comprehensively detect multiple respiratory pathogens (as described above), they do not detect all pathogens that cause respiratory infections. Viruses such as the human bocavirus, human parechovirus, and influenza virus type C, as well as bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* cause respiratory infections, but are not detected by the FilmArray®.[31–34] In particular, parechoviruses and bocaviruses do not have envelopes, and may have caused a small epidemic. Furthermore, it is important to consider the possibility of false-negative results. We could not find any published reports on the sensitivity of the FilmArray® Respiratory Panel v2.1 for all 18 detectable pathogens within the scope of our search. However, for SARS-CoV-2, the sensitivity of this testing panel is not inferior to that of quantitative reverse transcriptase polymerase chain reaction.[10] We speculate that if there had been false negative results, it is likely to have been due to poor specimen collection.

This study had some limitations. The study period was short (6 months), the data were collected in a relatively small area of Yonago City and its vicinity, and the frequency of virus detection was not high. Therefore, this was quite a small study. It is possible that the investigation of a small number of cases, for example, coronavirus NL63, may not reflect actual clinical practice. However, this study provides important information, as there have been no previous studies examining the frequency of infection and clinical data of pathogen-caused respiratory tract infections in children during the time window preceding the COVID-19 pandemic. In addition, because this was a retrospective and exploratory study, the division into groups infected and uninfected with each pathogen in Tables 2 through 5 was unavoidably biased.

In conclusion, rhino/enteroviruses were the most common cause of pediatric respiratory tract infections (including asymptomatic carriers) in an area where SARS-CoV-2 control measures had been implemented before the epidemic.

AUTHOR CONTRIBUTIONS

SK and YM analyzed and interpreted the collected data analysis and wrote the first draft and contributed equally; KO was the correponding author and described the first draft; AM and DK were involved in data accumulation and analysis; NN critically examined and revised the first draft.

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REFERENCES

1 Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5:536-44. DOI: 10.1038/s41564-020-0695-z, PMID: 32123347

2 Song P, Karako T. The strategy behind Japan's response to COVID-19 from 2020-2021 and future challenges posed by the uncertainty of the Omicron variant in 2022. Biosci Trends. 2021;15:350-2. DOI: 10.5582/bst.2021.01560, PMID: 34980786

3 Dzinamarira T, Murewanhema G, Musuka G. Different SARS-CoV-2 variants, same prevention strategies. Public Health in Practice. 2022;3:100223. DOI: 10.1016/j.puhip.2021.100223, PMID: 34927108

4 Nagakubo Y, Hirotsu Y, Maejima M, Shibusawa M, Hosaka K, Amemiya K, et al. Non-pharmaceutical interventions during the COVID-19 epidemic changed detection rates of other circulating respiratory pathogens in Japan. PLoS One. 2022;17:e0262874. DOI: 10.1371/journal.pone.0262874, PMID: 35061862

5 Brendish NJ, Malachira AK, Clark TW. Molecular point-of-care testing for respiratory viruses versus routine clinical care in adults with acute respiratory illness presenting to secondary care: a pragmatic randomised controlled trial protocol (ResPOC). BMC Infect Dis. 2017;17:128. DOI: 10.1186/s12879-017-2219-x, PMID: 28166743

6 Monto AS, Sullivan KM. Acute respiratory illness in the community. Frequency of illness and the agents involved. Epidemiol Infect. 1993;110:145-60. DOI: 10.1017/S0950268800050779, PMID: 8432318

7 Jain N, Lodha R, Kabra SK. Upper respiratory tract infections. Indian J Pediatr. 2001;68:1135-8. DOI: 10.1007/BF02722930, PMID: 11838568

8 Eckbo EJ, Locher K, Caza M, Li L, Lavergne V, Charles M. Evaluation of the BioFire® COVID-19 test and Respiratory Panel 2.1 for rapid identification of SARS-CoV-2 in nasopharyngeal swab samples. Diagn Microbiol Infect Dis. 2021;99:115260. DOI: 10.1016/j.diagmicrobio.2020.115260, PMID: 33340934

9 Creager HM, Cabrera B, Schnaubelt A, Cox JL, Cushman-Vokoun AM, Shakir SM, et al. Clinical evaluation of the BioFire® Respiratory Panel 2.1 and detection of SARS-CoV-2. J Clin Virol. 2020;129:104538. DOI: 10.1016/j.jcv.2020.104538, PMID: 32650276

10 Hirotsu Y, Maejima M, Shibusawa M, Amemiya K, Nagakubo Y, Hosaka K, et al. Analysis of a persistent viral shedding patient infected with SARS-CoV-2 by RT-qPCR, FilmArray Respiratory Panel v2.1, and antigen detection. J Infect Chemother. 2021;27:406-9. DOI: 10.1016/j.jiac.2020.10.026, PMID: 33183963
11 The R Project for Statistical Computing [Internet]. Vienna, Austria: The R Foundation [updated 2021 Mar 31; cited 2021 May 26]. Available from: https://www.R-project.org/
12 Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology. 1990;1:43-6. DOI: 10.1097/00001648-199001000-00010, PMID: 2081237
13 Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, Aarons EJ, et al. Patient characteristics and severity of human rhinovirus infections in children. J Clin Virol. 2013;58:216-20. DOI: 10.1016/j.jcv.2013.06.042, PMID: 23886500
14 Shaman J, Morita H, Birger R, Boyle M, Comito D, Lane B, et al. Asymptomatic summertime shedding of respiratory viruses. J Infect Dis. 2018;217:1074-7. DOI: 10.1093/infdis/jix685, PMID: 29300926
15 Tomlinson RH, Harrison LG, Meals EA, DeVincenzo JP. Do asymptomatic respiratory viral infections occur? J Clin Virol. 2018;102:93-4. DOI: 10.1016/j.jcv.2018.03.002, PMID: 29529485
16 Ruuskanen O, Meurman O, Sarkkinen H. Adenoviral diseases in children: a study of 105 hospital cases. Pediatrics. 1985;76:79-83. PMID: 2989764
17 Putto A, Meurman O, Ruuskanen O. C-reactive protein in the differentiation of adenoviral, Epstein-Barr viral and streptococcal tonsillitis in children. Eur J Pediatr. 1986;145:204-6. DOI: 10.1007/BF00446066, PMID: 3021463
18 Mistchenko AS, Diez RA, Mariani AL, Robaldo J, Maffey AF, Bayley-Bustamante G, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. J Pediatr. 1994;124:714-20. DOI: 10.1016/S0022-3476(05)81360-5, PMID: 8176557
19 Takashita E, Kawakami C, Momoki T, Saikusa M, Shimizu K, Ozawa H, et al. Increased risk of rhinovirus infection in children during the coronavirus disease-19 pandemic. Influenza Other Respir Viruses. 2021;15:488-94. DOI: 10.1111/irv.12854, PMID: 33715290
20 Vittucci AC, Piccioni L, Coltella L, Ciarlitto C, Antilici L, Bozzola E, et al. The disappearance of respiratory viruses in children during the COVID-19 pandemic. Int J Environ Res Public Health. 2021;18:9550. DOI: 10.3390/ijerph18189550, PMID: 34574472
21 Kampf G. Efficacy of ethanol against viruses in hand disinfection. J Hosp Infect. 2018;98:331-8. DOI: 10.1016/j.jhin.2017.08.025, PMID: 28882643
22 Savolainen-Kopra C, Korpela T, Simonen-Tikka ML, Amiryousefi A, Ziegler T, Roivainen M, et al. Single treatment with ethanol hand rub is ineffective against human rhinovirus-hand washing with soap and water removes the virus efficiently. J Med Virol. 2012;84:543-7. DOI: 10.1002/jmv.23222, PMID: 22246844
23 Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2019;16:69. DOI: 10.1186/s12985-019-1182-0, PMID: 31133031
24 Luo M. Influenza virus entry. Adv Exp Med Biol. 2012;726:201-21. DOI: 10.1007/978-1-4614-0980-9_9, PMID: 22297515
25 Branche A, Falsey A. Parainfluenza virus infection. Semin Respir Crit Care Med. 2016;37:538-54. DOI: 10.1055/s-0036-1584798, PMID: 27486735
26 Heumann R, Duncan C, Stobart CC, Kaschner S. Dynamical differences in respiratory syncytial virus. Bull Math Biol. 2022;84:11. DOI: 10.1007/s11538-021-00971-8, PMID: 34850293
27 Iwasawa A, Niwano Y, Kohno M, Ayaki M. Virucidal activity of alcohol-based hand rub disinfectants. Biocontrol Sci. 2012;17:45-9. DOI: 10.4265/bio.17.45, PMID: 22451431
28 Leung NHL, Chu DKW, Shiu EYC, Chan KH, McDevitt JJ, Hau BJP, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat Med. 2020;26:676-80. DOI: 10.1038/s41591-020-0843-2, PMID: 32371934
29 Dick EC, Jennings LC, Mink KA, Wartgow CD, Inborn SL. Aerosol transmission of rhinovirus colds. J Infect Dis. 1987;156:442-8. DOI: 10.1093/infdis/156.3.442, PMID: 3039011
30 Wu A, Mihaylova VT, Landry ML, Foxman EF. Interference between rhinovirus and influenza A virus: a clinical data analysis and experimental infection study. Lancet Microbe. 2020;1:e254-62. DOI: 10.1016/S2666-5247(20)30114-2, PMID: 33103132
31 Petrarca L, Nenna R, Frassanito A, Pierangeli A, Di Mattia G, Scagnolari C, et al. Human bocavirus in children hospitalized for acute respiratory tract infection in Rome. World J Pediatr. 2020;16:293-8. DOI: 10.1007/s12519-019-00324-5, PMID: 31776891
32 Sharp J, Bell J, Harrison CJ, Nix WA, Oberste MS, Selvarangan R. Human parechovirus in respiratory specimens from children in Kansas City, Missouri. J Clin Microbiol. 2012;50:4111-3. DOI: 10.1128/JCM.01680-12, PMID: 23015672
33 Katagiri S, Ohizumi A, Homma M. An outbreak of type C influenza in a children's home. J Infect Dis. 1983;148:51-6. DOI: 10.1093/infdis/148.1.51, PMID: 6309999
34 Xu Q, Wischmeyer J, Gonzalez E, Pichichero ME. Nasopharyngeal polymicrobial colonization during health, viral upper respiratory infection and upper respiratory bacterial infection. J Infect. 2017;75:26-34. DOI: 10.1016/j.jinf.2017.04.003, PMID: 28412081