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Coronavirus HKU1 and Other Coronavirus Infections in Hong Kong

Susanna K. P. Lau,[1,2,3]† Patrick C. Y. Woo,[1,2,3]† Cyril C. Y. Yip,[1] Herman Tse,[1,2,3] Hoi-wah Tsoi,[1] Vincent C. C. Cheng,[1] Paul Lee,[1] Bone S. F. Tang,[1] Chris H. Y. Cheung,[4] Rodney A. Lee,[4] Lok-yee So,[5] Yu-lung Lau,[6] Kwok-hung Chan,[1] and Kwok-yung Yuen[1,2,3]*

Department of Microbiology[1] and Research Centre of Infection and Immunology,[2] The University of Hong Kong, State Key Laboratory of Emerging Infectious Diseases (The University of Hong Kong),[3] Department of Microbiology, Pamela Youde Nethersole Eastern Hospital,[4] Department of Paediatrics and Adolescent Medicine, Pamela Youde Nethersole Eastern Hospital,[5] and Department of Paediatrics and Adolescent Medicine, The University of Hong Kong,[6] Hong Kong

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We have recently described the discovery of a novel coronavirus, coronavirus HKU1 (CoV-HKU1), associated with community-acquired pneumonia. However, the clinical spectrum of disease and the epidemiology of CoV-HKU1 infections in relation to infections with other respiratory viruses are unknown. In this 12-month prospective study, 4,181 nasopharyngeal aspirates from patients with acute respiratory tract infections were subjected to reverse transcription-PCRs specific for CoV-HKU1 and human coronaviruses NL63 (HCoV-NL63), OC43 (HCoV-OC43), and 229E (HCoV-229E). Coronaviruses were detected in 87 (2.1%) patients, with 13 (0.3%) positive for CoV-HKU1, 17 (0.4%) positive for HCoV-NL63, 53 (1.3%) positive for HCoV-OC43, and 4 (0.1%) positive for HCoV-229E. Of the 13 patients with CoV-HKU1 infections, 11 were children and 8 had underlying diseases. Similar to the case for other coronaviruses, upper respiratory infection was the most common presentation of CoV-HKU1 infections, although pneumonia, acute bronchiolitis, and asthmatic exacerbation also occurred. Despite a shorter duration of fever (mean, 1.7 days) and no difference in maximum temperature in children with CoV-HKU1 infections compared to patients with most other respiratory virus infections, a high incidence of febrile seizures (50%) was noted, which was significantly higher than those for HCoV-OC43 (14%), adenovirus (9%), human parainfluenza virus 1 (0%), and respiratory syncytial virus (8%) infections. CoV-HKU1 and HCoV-OC43 infections peaked in winter, although cases of the former also occurred in spring to early summer. This is in contrast to HCoV-NL63 infections, which mainly occurred in early summer and autumn but were absent in winter. Two genotypes of CoV-HKU1 cocirculated during the study period. Continuous studies over a longer period are warranted to ascertain the seasonal variation and relative importance of the different coronaviruses. Similar studies in other countries are required to better determine the epidemiology and genetic diversity of CoV-HKU1.

Since a significant proportion of patients with respiratory tract infections remain undiagnosed (14, 24), research has been conducted to identify novel causative agents. Of the four novel agents identified in the past 3 years, including human metapneumovirus (28), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) (21), human coronavirus NL63 (HCoV-NL63) (9, 29), and coronavirus HKU1 (CoV-HKU1) (32), three are coronaviruses. Based on serologic and phylogenetic characterization, coronaviruses were divided into three distinct groups, with human coronavirus 229E (HCoV-229E) and HCoV-NL63 being group 1 coronaviruses and human coronavirus OC43 (HCoV-OC43) and CoV-HKU1 being group 2 coronaviruses (12). SARS-CoV, which causes the most severe form of respiratory disease among coronaviruses that infect humans (3, 11, 19–21, 33, 34), represents an early split from group 2 coronaviruses (7, 15, 23, 26) and is believed to have originated from wild animals (10, 13).

While HCoV-229E and HCoV-OC43 were known to account for 5 to 30% of human respiratory tract infections (16), HCoV-NL63 was found to be present in 2 to 3.6% of respiratory specimens in several recent studies (1, 2, 5, 6, 17). After the recovery of CoV-HKU1 from two patients with pneumonia (32), we recently conducted a retrospective study on the prevalence of CoV-HKU1 in patients with community-acquired pneumonia during a 1-year period (31). CoV-HKU1 was found in the nasopharyngeal aspirates (NPAs) of 10 (2.4%) of 418 studied patients with community-acquired pneumonia. However, the clinical spectrum of illness of CoV-HKU1 infections and the epidemiology of this virus in relation to other coronaviruses remain undefined. In this study, we examined the epidemiology and clinical spectrum of disease of CoV-HKU1, HCoV-NL63, HCoV-OC43, and HCoV-229E infections in patients hospitalized for acute respiratory illness during a 1-year period. The clinical severity and incidence of febrile seizures in children hospitalized for CoV-HKU1 infections were analyzed and compared to those for children hospitalized for other respiratory virus infections. The molecular epidemiology of CoV-HKU1 was also analyzed.

* Corresponding author. Mailing address: Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong. Phone: (852) 2855 4892. Fax: (852) 2855 1241. E-mail: hkumicro@hkucc.hku.hk.

† S.K.P.L. and P.C.Y.W. contributed equally to the manuscript.

MATERIALS AND METHODS

Patients and microbiological methods. All prospectively collected NPAs from patients with acute respiratory tract infections admitted to two public hospitals,

TABLE 1. Primers used in this study

Coronavirus	Primer name	Primer direction, sequence (5′–3′)	Gene target	Purpose
CoV-HKU1	LPW1926	Forward, AAAGGATGTTGACAACCCTGTT	*pol*	Detection of CoV-HKU1 from NPA
	LPW1927	Reverse, ATCATCATACTAAAATGCTTACA	*pol*	Detection of CoV-HKU1 from NPA
	LPW1465	Forward, GTTCAAGTGTCGCTGTTCA	*pol*	Sequencing of complete *pol* gene
	LPW1822	Reverse, CTATCATTATCACAATCCACAG	*pol*	Sequencing of complete *pol* gene
	LPW1467	Forward, GGGTATGAAGTATCATCCTA	*pol*	Sequencing of complete *pol* gene
	LPW1825	Reverse, GATAATCCCAACCCATAAGAAC	*pol*	Sequencing of complete *pol* gene
	LPW1826	Forward, CATCTTATAAAGGATGTTGAC	*pol*	Sequencing of complete *pol* gene
	LPW1829	Reverse, ACAAACAACACATGCACCTACAC	*pol*	Sequencing of complete *pol* gene
	LPW1887	Forward, TAGTGTATGGATACTGCCTTGT	N	Sequencing of complete N gene
	LPW1890	Reverse, GCTTTAACATTTCAGMATTACCA	N	Sequencing of complete N gene
	LPW1891	Forward, CAGTGTTTTGGTAAAAGAGGACC	N	Sequencing of complete N gene
	LPW1892	Reverse, TACCACCTAGTGTCGAATTAGG	N	Sequencing of complete N gene
	LPW1830	Forward, TTGCCTATTATTTTACAAGGT	S	Sequencing of complete S gene
	LPW1864	Reverse, ACACTACCTATAACTATAGTAC	S	Sequencing of complete S gene
	LPW1832	Forward, TATGTTAATAAWACTTTGTATAGTG	S	Sequencing of complete S gene
	LPW1866	Reverse, TACAATTGACAAGAACTAGAAG	S	Sequencing of complete S gene
	LPW1836	Forward, GATTTGCARTTGGGCAGTTCTGG	S	Sequencing of complete S gene
	LPW1868	Reverse, CCATTAGAATCATACAAAAGAT	S	Sequencing of complete S gene
	LPW1840	Forward, GGTATTTTTAAAGAAGTTTCTGC	S	Sequencing of complete S gene
	LPW1870	Reverse, AGCTTCAACAAAACCWACATCTG	S	Sequencing of complete S gene
	LPW1844	Forward, TAGGTYCACAMTGYGGTTCTTC	S	Sequencing of complete S gene
	LPW1872	Reverse, AMCCTTGYTTAGGTGCAATACCT	S	Sequencing of complete S gene
	LPW1848	Forward, TTAAAACTGTYTTAGTAAGTCC	S	Sequencing of complete S gene
	LPW1874	Reverse, TAGTAAAACTAGTTAYAACACC	S	Sequencing of complete S gene
HCoV-OC43	LPW3064	Forward, CTGGGATGATATGTTACGCCG	*pol*	Detection of HCoV-OC43 from NPA
	LPW3065	Reverse, TATTCTGTGACAAAGGTTG	*pol*	Detection of HCoV-OC43 from NPA
HCoV-229E	LPW2905	Forward, GTGTGATAGAGCTATGCCCTCA	*pol*	Detection of HCoV-229E from NPA
	LPW2906	Reverse, GTAACCAAGTCCAGCATAAGTT	*pol*	Detection of HCoV-229E from NPA
HCoV-NL63	LPW2907	Forward, AATAATATGTTGCGTACTTTA	*pol*	Detection of HCoV-NL63 from NPA
	LPW2908	Reverse, TCATTGAAAAATGTTTCCTA	*pol*	Detection of HCoV-NL63 from NPA

Queen Mary Hospital and Pamela Youde Nethersole Eastern Hospital, in Hong Kong during a 12-month period (April 2004 to March 2005) were included in the study. All NPAs were assessed for influenza A and B viruses, parainfluenza virus types 1, 2, and 3, respiratory syncytial virus (RSV), and adenovirus by direct immunofluorescence and for metapneumovirus by reverse transcription-PCR (RT-PCR) (22, 30). NPAs negative for these respiratory viruses were subject to RT-PCR for coronaviruses. Once coronaviruses were detected from NPAs, the corresponding patients were identified, and their clinical features, laboratory results, and outcomes were analyzed.

RNA extraction. Viral RNAs were extracted from NPAs by using a QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) within 10 h of receipt of specimens. The eluted RNAs (templates for RT-PCR) were stored immediately at −70°C until use.

RT-PCR for coronaviruses and DNA sequencing. RT was performed using random hexamers and a SuperScript II kit (Invitrogen, San Diego, CA) as described previously (31, 32). PCR for coronaviruses was performed using four sets of primers specifically designed to amplify CoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63, targeted to the same region of the RNA-dependent RNA polymerase (*pol*) gene (Table 1). Each PCR mixture (50 μl) contained cDNA, PCR buffer, a 200 μM concentration of each deoxynucleoside triphosphate, and 1.0 U *Taq* polymerase (Boehringer, Mannheim, Germany). The mixtures were amplified by 40 cycles of 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. To ensure the high specificities of the specific primers, RNAs of CoV-HKU1, HCoV-OC43, HCoV-229E, HCoV-NL63, and SARS-CoV and RNAs extracted from 200 NPAs positive for influenza A or B virus, parainfluenza virus 1 to 3, RSV, or adenovirus antigens were also subjected to RT-PCRs using specific primers. The amplified products were detected by agarose gel electrophoresis as described in our previous publication (32). Both strands of all PCR products were sequenced twice with an ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA), using the PCR primers. The sequences of the PCR products were compared with the sequences of the *pol* genes of coronaviruses in the GenBank database.

RT-PCR and sequencing of the complete RNA-dependent RNA polymerase, spike, and nucleocapsid genes of coronavirus-HKU1 and phylogenetic analysis. The complete *pol*, spike (S), and N genes of CoV-HKU1 from NPAs of the 13 patients with positive results were amplified and sequenced using the appropriate primers shown in Table 1 and the strategy described in our previous publication (32). The nucleotide and deduced amino acid sequences of the *pol*, S, and N genes were compared to those of CoV-HKU1 (32) and other group 2 coronaviruses. Phylogenetic tree construction was performed using the neighbor-joining method with GrowTree, using the Jukes-Cantor correction (Genetics Computer Group, Inc.).

Incidence of febrile seizures and clinical characteristics of children hospitalized for various respiratory virus infections. Since a high incidence of febrile seizures was noted for patients with CoV-HKU1 infection, the relative frequency of febrile convulsion, maximum temperature, duration of fever, and duration of hospitalization of children between 6 months and 5 years old (the age group at risk of febrile seizures) with different respiratory virus infections were analyzed by a retrospective review of clinical records, except for coronavirus infections, which were identified prospectively. Febrile seizure was defined as a seizure event occurring in association with fever but without evidence of intracranial infection or another definable cause. Seizure was defined as an involuntary generalized tonic, clonic, or tonic-clonic movement associated with impaired consciousness. Episodes in those with underlying seizure disorders were excluded.

Statistical analysis. Comparisons of maximum temperature, duration of fever, and duration of hospitalization among the various groups of patients with respiratory virus infections were performed using one-way analysis of variance. Comparison between CoV-HKU1 infections and other groups was performed using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. P values of <0.05 were regarded as statistically significant.

Nucleotide sequence accession numbers. The nucleotide sequences of the *pol* genes of the 13 strains of CoV-HKU1 have been lodged in the GenBank sequence database under accession no. DQ422728, DQ422729, DQ422730, DQ422731, DQ422732, DQ422733, DQ422734, DQ422735, DQ422736, DQ422737, DQ422738, DQ422739, and DQ422740. The nucleotide sequences of the spike

TABLE 2. Viruses identified in nasopharyngeal aspirate specimens obtained from 4,181 patients hospitalized for acute respiratory tract infections

Virus	No. (%) of patients infected
CoV-HKU1	13 (0.3)
HCoV-OC43	53 (1.3)
HCoV-229E	4 (0.1)
HCoV-NL63	17 (0.4)
Influenza A virus	545 (13.0)
Influenza B virus	120 (2.9)
Adenovirus	210 (5.0)
Human parainfluenza virus 1	31 (0.7)
Human parainfluenza virus 2	8 (0.2)
Human parainfluenza virus 3	183 (4.4)
Respiratory syncytial virus	420 (10.0)
Human metapneumovirus	117 (2.8)
None	2,460 (58.8)

genes of the 13 strains of CoV-HKU1 have been lodged in the GenBank sequence database under accession no. DQ437607, DQ437608, DQ437609, DQ437610, DQ437611, DQ437612, DQ437613, DQ437614, DQ437615, DQ437616, DQ437617, DQ437618, and DQ437619. The nucleotide sequences of the nucleocapsid genes of the 13 strains of CoV-HKU1 have been lodged in the GenBank sequence database under accession no. DQ437620, DQ437621, DQ437622, DQ437623, DQ437624, DQ437625, DQ437626, DQ437627, DQ437628, DQ437629, DQ437630, DQ437631, and DQ437632.

RESULTS

Results of respiratory virus detection in nasopharyngeal aspirates. During the 12-month study period, NPAs from 4, age [mean ± standard deviation], 22 ± 30 years) with acute respiratory tract infections for the detection of respiratory virus antigen were identified in two hospitals. Coronavirus infections were detected in 87 (2.1%) of the 4,181 patients, with 13 patients positive for CoV-HKU1, 53 positive for HCoV-OC43, 4 positive for HCoV-229E, and 17 positive for HCoV-NL63 by RT-PCR. During the same period, influenza A virus was detected in 545 (13%) patients, influenza B virus was detected in 120 (2.9%) patients, adenovirus was detected in 210 (5%) patients, human parainfluenza virus 1 was detected in 31 (0.7%) patients, human parainfluenza virus 2 was detected in 8 (0.2%) patients, human parainfluenza virus 3 was detected in 183 (4.4%) patients, respiratory syncytial virus was detected in 420 (10%) patients, and human metapneumovirus was detected in 117 (2.8%) patients (Table 2). None of the 200 NPAs positive for influenza A and B virus, parainfluenza virus 1 to 3, RSV, or adenovirus antigens were RT-PCR positive for coronaviruses. The PCRs were also specific for the corresponding coronaviruses. CoV-HKU1 and HCoV-OC43 infections mainly occurred in the fall and winter months, whereas

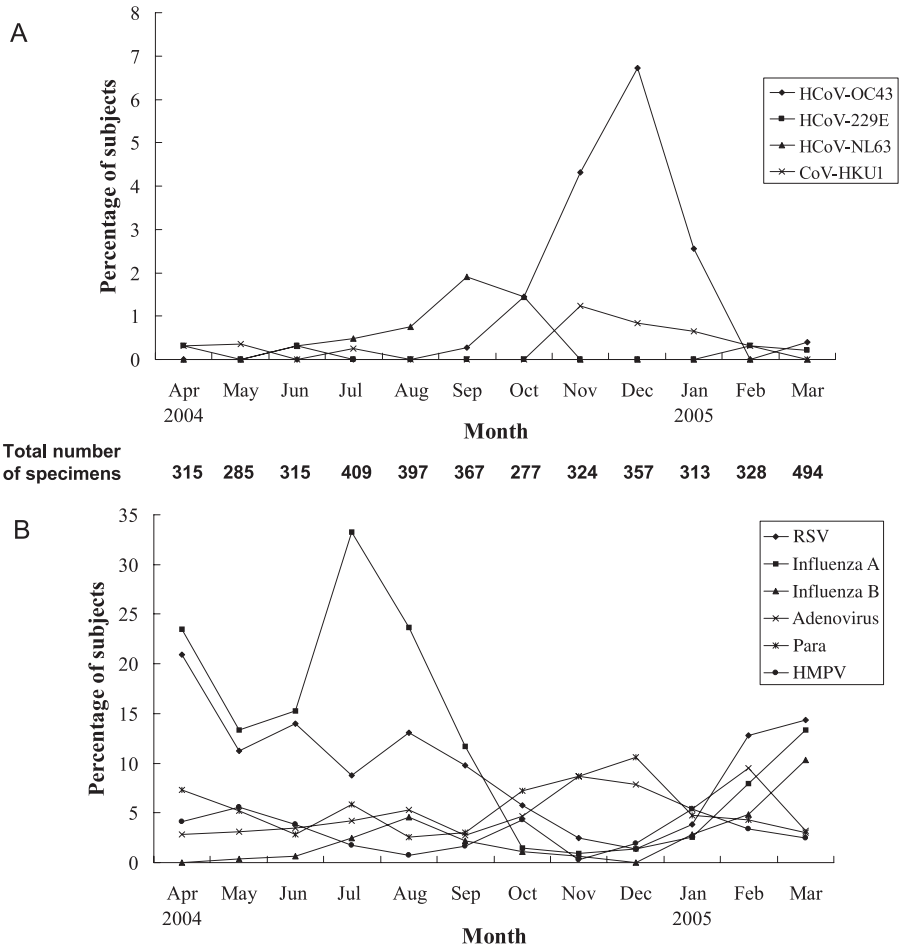

FIG. 1. Seasonality of coronavirus (A) and other respiratory virus (B) infections in Hong Kong, Chinapage.

TABLE 3. Clinical characteristics of patients with CoV-HKU1 infections

Characteristic	Description or value for patient[b]:					
	1	2	3	4	5	6
Sex/age (yr)[a]	F/2	F/7	M/84	M/3	M/87	F/4
Ethnic origin	Chinese	Chinese	Chinese	Chinese	Chinese	Chinese
Underlying condition(s)		Prematurity, cerebral palsy, epilepsy	COPD, old PTB, IHD, BPH, ex-smoker	Recurrent febrile exanthema, SMA carrier	MVP, AF, HT, hypercholesterolemia, ex-smoker	Febrile convulsion
Presenting symptoms						
Fever	+	+	+	+	+	+
Chills			+	+	+	
Rigor			+	+	+	
Cough			+		+	+
Sputum			+		+	
Dyspnea			+			
Rhinorrhea	+	+	+	+		
Vomiting					+	+
Convulsion	+	+				+
Other symptom(s)	Diarrhea	Skin rash	Myalgia	Skin rash, joint swelling		Abdominal pain
Physical examination finding(s)	Congested throat, shotty generalized lymphadenopathy	Generalized maculopapular rash, shotty cervical lymphadenopathy		Limb and joint swelling, generalized rash, shotty cervical lymphadenopathy	Bilateral basal crackles	Congested throat
Chest radiograph finding(s)	Not taken	Clear	Old tuberculosis	Not taken	Bilateral lower-zone haziness	Clear
Diagnosis	URTI, febrile seizure	URTI, epilepsy	URTI	URTI, angioedema	Pneumonia	URTI, febrile seizure
Duration of hospitalization (no. of days)	3	5	7	2	5	3

[a] F, female; M, male.
[b] +, present; URTI, upper respiratory tract infection; COPD, chronic obstructive pulmonary disease; PTB, pulmonary tuberculosis; IHD, ischemic heart disease; BPH, benign prostatic hyperplasia; SMA, spinal muscular atrophy; MVP, mitral valve prolapse; AF, atrial fibrillation; HT, hypertension; PBSCT, peripheral blood stem cell transplant.

HCoV-NL63 infection mainly occurred in the summer and fall (Fig. 1).

Clinical and laboratory characteristics of patients with CoV-HKU1 infections. No epidemiological linkage was identified among the 13 cases. Ten cases occurred in autumn or winter (November to February), and three occurred in spring or summer (April to July) (Table 3). The median patient age was 3 (range, 19 months to 87 years). Eight patients were male, and five were female. Eight patients had underlying diseases. Apart from one patient (patient 12) who acquired the infection during hospitalization for a peripheral blood stem cell transplant, all cases were community acquired. Two patients had recent travel histories to Shenzhen, China (patient 10), and Korea (patient 11). Two patients were smokers. Fever, runny nose, and cough, with or without sputum, were common presenting symptoms. Two patients (patients 5 and 7) had lower respiratory tract infections, while others had upper respiratory tract infections. Chest radiographs were performed for seven patients, and two showed abnormalities, with bilateral lower-zone haziness in one (patient 5) and perihilar haziness in the other (patient 7). Five children had febrile seizures, and two others with underlying epilepsy had breakthrough seizures. None of the children with febrile seizures had neurological sequelae. One patient (patient 9) had concomitant rotavirus gastroenteritis. Bacterial or mycobacterial pathogens were not detected in any of the sputum samples from the patients. Direct antigen detection for influenza A and B viruses, parainfluenza viruses 1 to 3, RSV, and adenovirus and RT-PCR for metapneumovirus were negative for all NPAs. All 13 patients survived.

Characteristics of patients with other coronavirus infections and comparison with CoV-HKU1 infections. The characteristics of patients with other coronavirus infections are summarized in Table 4. Among the four coronaviruses studied, HCoV-OC43 infections had the highest incidence during the study period. All four coronaviruses mostly infected children, although old persons or those with underlying diseases, and occasionally immunocompetent adults, were also infected. The duration of fever was usually brief for all four viruses, and the median duration of hospitalization was 3 days for all four groups. Most patients infected with coronaviruses had upper respiratory tract infections, although some presented with acute exacerbations of underlying airway diseases, acute bronchiolitis, or pneumonia. Croup was diagnosed in two patients

TABLE 3—*Continued*

Description or value for patient[b]:						
7	8	9	10	11	12	13
M/2	M/19 mo	M/3	F/9	F/3	M/5	M/4
Chinese	Arabian	Chinese	Chinese	Chinese	Chinese	Chinese
			Asthma, allergic rhinitis, eczema		Neuroblastoma, 3 mo postautologous PBSCT	Epilepsy, right parietal cavernous hemangioma
	+	+	+	+	+	+
			+			
+	+	+		+		
+	+					
			+			
+	+		+		+	+
+		+	+	+		
	+	+		+		+
		Abdominal pain, diarrhea		Right ear pain		
Tachypnea, diffuse wheezing, occasional crackles	Inflamed throat, shotty cervical lymphadenopathy		Tachypnea, moderate insucking, diffuse wheezing			Congested throat
Perihilar haziness	Not taken	Not taken	Hyperinflated	Not taken	Not taken	Clear
Acute bronchiolitis	URTI, febrile seizure	URTI, febrile seizure, rotavirus gastro-enteritis	URTI, asthma	URTI, febrile seizure	URTI	URTI, epilepsy
2	3	3	3	2	Hospital acquired	2

with HCoV-NL63 infections. Both patients were 1-year-old male infants who presented with stridor and shortness of breath. One patient also had pneumonic changes over the right lower zone on a chest radiograph. These two patients recovered with nebulized bronchodilator treatment, with or without steroid. Kawasaki disease was diagnosed in one patient with HCoV-OC43 infection. The patient was a 4-year-old girl who presented with fever, runny nose, maculopapular rash, conjunctivitis, cervical lymphadenopathy, and elevated liver parenchymal enzymes. An echocardiogram showed a dilated left coronary artery with aneurysmal changes. Her nasopharyngeal aspirate was positive for HCoV-OC43 but negative for other respiratory viruses. She recovered with intravenous immunoglobulin and high doses of aspirin. Febrile seizures occurred in five (38%) patients with CoV-HKU1 infections, three (18%) patients with HCoV-NL63 infections, and three (6%) patients with HCoV-OC43 infections. Breakthrough seizures in patients with preexisting epilepsy were also noticed for patients with CoV-HKU1 and HCoV-NL63 infections. Both febrile and breakthrough seizures were more common in patients with CoV-HKU1 infections than in those with HCoV-OC43 infections ($P < 0.05$).

Comparison of frequency of febrile seizure, maximum temperature, duration of fever, and duration of hospitalization for children with different respiratory virus infections. A total of 629 children aged 6 months to 5 years were hospitalized for acute respiratory virus infections during the study period. Febrile seizures occurred in 5 (50%) of 10 children with CoV-HKU1 infections, 3 (14%) of 22 children with HCoV-OC43 infections, 4 (29%) of 14 children with HCoV-NL63 infections, 35 (25%) of 142 children with influenza A virus infections, 6 (21%) of 28 children with influenza B virus infections, 9 (9%) of 103 children with adenovirus infections, 1 (33%) of 3 children with human parainfluenza virus 2 infections, 23 (28%) of 82 children with human parainfluenza virus 3 infections, 15 (8%) of 191 children with respiratory syncytial virus infections, 5 (25%) of 20 children with human metapneumovirus infections, and none of those with HCoV-229E or human parainfluenza virus 1 infections (Table 5). The incidence of febrile seizures was significantly higher for children with CoV-HKU1 infections than for those with HCoV-OC43, adenovirus, human parainfluenza virus 1, and respiratory syncytial virus infections ($P < 0.05$). There was no significant difference in maximum temperature and duration of hospitalization between CoV-HKU1 infections and other viruses. However, children with other respiratory virus infections, except HCoV-OC43 infections, had longer durations of fever than did those infected with CoV-HKU1 ($P < 0.05$).

RT-PCR and sequencing of the complete RNA-dependent RNA polymerase, spike, and nucleocapsid genes of coronavi-

TABLE 4. Comparison of clinical characteristics of patients with CoV-HKU1, HCoV-NL63, HCoV-OC43, and HCoV-229E infections

Parameter	Value for patients infected with:			
	CoV-HKU1	HCoV-NL63	HCoV-OC43	HCoV-229E
Total no. of patients	13	17	53	13
Male-to-female ratio	8:5	8:9	28:25	1:3
Age				
Range	19 mo–87 yr	6 mo–86 yr	1 mo–88 yr	2 yr–75 yr
Median (yr)	4	2	9	8.5
Duration of fever (days)				
Range	1–4	1–7	1–8	1–4
Median	1	1	1	2.5
Duration of hospitalization (days)				
Range	2–7	1–9	1–20	3–14
Median	3	3	3	3
No. (%) of patients with underlying diseases	8 (62)	10 (59)	34 (64)	2 (50)
No. (%) of patients with diagnosis of[a]:				
URTI[c]	11 (85)	12 (71)	41 (77)	2 (50)
Asthma/COPD exacerbation[c]	1 (8)	2 (12)	4 (8)	0 (0)
Acute bronchiolitis	1 (8)	1 (6)	3 (6)	0 (0)
Pneumonia	1 (8)	3 (18)	8 (15)	2 (50)
Croup	0 (0)	2 (12)	0 (0)	0 (0)
Febrile convulsion	5 (38)[b]	3 (18)	3 (6)[b]	0 (0)
Breakthrough seizure	2 (15)[b]	1 (6)	0 (0)[b]	0 (0)
Aseptic meningitis	0 (0)	0 (0)	1 (2)	0 (0)
Kawasaki disease	0 (0)	0 (0)	1 (2)	0 (0)
No. (%) of patients who died	0 (0)	0 (0)	1 (2)	0 (0)

[a] Percentages add up to >100% because some patients had more than one diagnosis.
[b] $P < 0.05$.
[c] URTI, upper respiratory tract infection; COPD, chronic obstructive pulmonary disease.

rus-HKU1 and phylogenetic analysis. The complete *pol*, S, and N genes of CoV-HKU1 isolates from NPAs of 13 patients were amplified and sequenced. The phylogenetic trees are shown in Fig. 2. Seven of the 13 S or N genes (patients 1, 2, 3, 7, 8, 12, and 13) showed similar nucleotide sequences to previous genotype A sequences, and those of the other 6 (patients 4, 5, 6, 9, 10, and 11) showed similar nucleotide sequences to previous genotype B sequences (Fig. 2B and C) (32). For the *pol* gene, the nucleotide sequences of the seven CoV-HKU1 isolates of genotype A were also clustered together (Fig. 2A). This suggests the presence of at least two genotypes which are better distinguished based on S and N genes than on the *pol* gene.

DISCUSSION

This prospective study represents the first to describe the clinical spectrum of respiratory illness associated with the newly discovered coronavirus CoV-HKU1. In our previous

TABLE 5. Characteristics of various acute respiratory virus infections in children aged 6 months to 5 years

Virus	Total no. of children	Maximum temp (°C)[a]	Duration of fever (days)[a]	Duration of hospitalization (days)[a]	No. (%) of children with febrile convulsion
CoV-HKU1	10	39 ± 0.6	1.7 ± 0.5	2.3 ± 0.7	5 (50)
HCoV-OC43	22	38.7 ± 0.6	2 ± 1.9	3.1 ± 1.8	3 (14)[b]
HCoV-229E	2	39.4 ± 0.5	3.5 ± 0.7[b]	3	0 (0)
HCoV-NL63	14	39.3 ± 0.7	3.2 ± 2.3[b]	3.4 ± 2.6	4 (29)
Influenza A virus	142	39.6 ± 0.8	5 ± 2.9[b]	3.1 ± 1.9	35 (25)
Influenza B virus	28	39 ± 0.9	5.2 ± 2.6[b]	3 ± 1.2	6 (21)
Adenovirus	103	39.5 ± 0.8	6.4 ± 2.7[b]	3.2 ± 1.5	9 (9)[b]
Human parainfluenza virus 1	12	39.1 ± 0.9	4.5 ± 2.6[b]	2.8 ± 1.3	0 (0)[b]
Human parainfluenza virus 2	3	39.7 ± 1.6	5.5 ± 0.7[b]	3.7 ± 2.9	1 (33)
Human parainfluenza virus 3	82	39.3 ± 1.1	4.7 ± 3.2[b]	3.2 ± 2.3	23 (28)
Respiratory syncytial virus	191	39 ± 0.8	5.2 ± 2.5[b]	3.8 ± 2.5	15 (8)[b]
Human metapneumovirus	20	38.9 ± 1.1	3.7 ± 1.9[b]	3.6 ± 1.8	5 (25)

[a] Data are means ± standard deviations.
[b] $P < 0.05$.

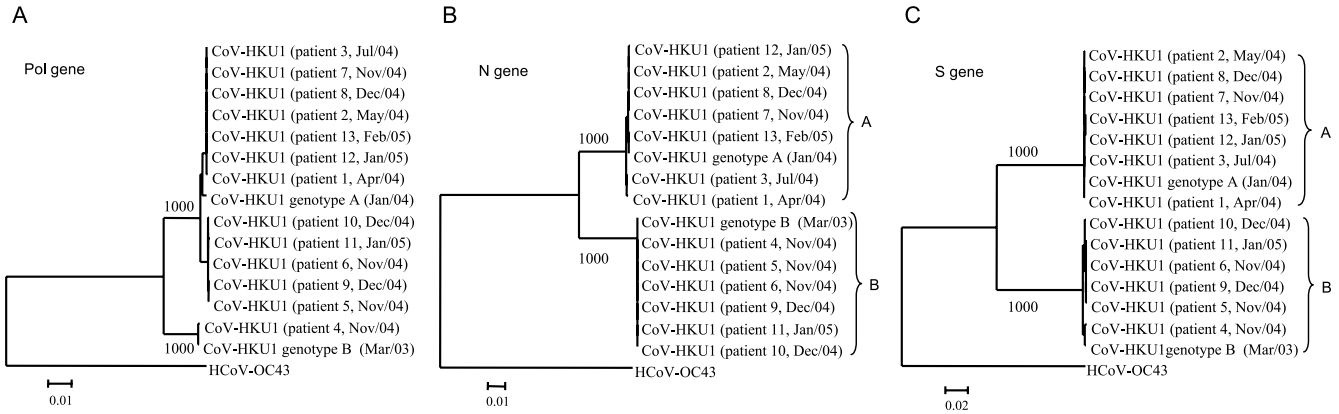

FIG. 2. Phylogenetic trees of complete *pol*, S, and N gene sequences of CoV-HKU1 isolates from 13 patients. The trees were inferred from *pol* (A), S (B), and N (C) gene data by the neighbor-joining method, with bootstrap values calculated from 1,000 trees. The trees were rooted using *pol*, S, and N gene sequences of HCoV-OC43. Sequences for 2,784 nucleotide positions in each *pol* gene, 4,071 nucleotide positions in each S gene, and 1,326 nucleotide positions in each N gene were included in the analysis. Bars, estimated numbers of substitutions per 100 (A and B) or 50 (C) bases, using Jukes-Cantor correction.

study, CoV-HKU1 was shown to be responsible for 2.5% of community-acquired pneumonia cases, using a retrospective collection of nasopharyngeal aspirates. The clinical significance of CoV-HKU1 was confirmed by the presence of a specific antibody response in patients for whom serum samples were available (31). In the present study, CoV-HKU1 was found to be responsible for only 0.3% of patients with acute respiratory illness. The apparent higher incidence in our previous study may have been due to the differences in clinical practice in the collection of nasopharyngeal aspirates. Our previous study was performed when collection of nasopharyngeal aspirates was more frequently performed for diagnosis of SARS-CoV infections during the SARS epidemic. Unlike CoV-HKU1 pneumonia, which mainly affects old people with major underlying diseases (31), upper respiratory tract infection due to CoV-HKU1 affects mainly young children, with or without underlying diseases, and was the most common presentation of CoV-HKU1 acute respiratory tract infections in the present study. While other coronaviruses, such as HCoV-229E, HCoV-OC43, and more recently, HCoV-NL63, have been found to be causative agents of bronchiolitis, patients with CoV-HKU1 infections also occasionally presented with acute bronchiolitis and asthmatic exacerbation.

Coronavirus infections were found to be responsible for 2.1% of admissions for all acute respiratory tract infections in all age groups. HCoV-OC43 was the most prevalent among the four coronaviruses during the study period. This was followed by HCoV-NL63 and CoV-HKU1, with similar incidences, and HCoV-229E, with the least importance. These findings are in keeping with a previous study of children showing that HCoV-NL63, HCoV-OC43, and HCoV-229E contribute to a significant proportion of hospitalization cases due to respiratory illness in our locality (5). The incidence of coronavirus infections reported here appears to be lower than those in some previous reports (1, 2, 5, 6, 17), which may be related to geographical variation or the recruitment of only children and cases occurring in winter in some studies. The clinical spectra of diseases caused by the different coronaviruses appear to be similar, with most manifesting as acute respiratory tract infections, as in previous studies. Kawasaki disease was diagnosed in one patient with HCoV-OC43 infection but in none of those with HCoV-NL63 infection, which has been associated with Kawasaki disease in one study (8). Our results are in line with a subsequent report which argued against such an association (25).

The present study demonstrated differences in the seasonal epidemiology of the different coronaviruses. While HCoV-OC43 and HCoV-229E infections are well known to be winter diseases in temperate regions, the seasonality of HCoV-NL63 was found to have different patterns in different geographical areas. While HCoV-NL63 infections peaked in winter in The Netherlands, a previous study in our locality, a subtropical city, showed a spring-summer predominance (5, 27, 29). In the present study, HCoV-NL63 infections appeared in early summer and peaked in autumn but were absent in winter. Cases of CoV-HKU1 infections, in parallel with HCoV-OC43 infections, rose in autumn and peaked in winter. However, unlike HCoV-OC43 infection, where no cases were observed in the other seasons, a few cases of CoV-HKU1 infections occurred in spring to early summer. The seasonal pattern of HCoV-229E infection, however, cannot be determined because of the small number of cases. Continuous studies carried out over a number of years would be required to ascertain the seasonal and any possible interyear variation in the relative incidences of the different coronaviruses.

Febrile seizures were particularly common in children with CoV-HKU1 infections. Although the clinical severity of CoV-HKU1 infection in children is relatively mild, with a shorter duration of fever than that for infections due to most other respiratory virus infections, CoV-HKU1 infection is associated with a high incidence of febrile and breakthrough seizures. In fact, half of the affected children had febrile seizures, which is the highest rate among all the studied respiratory viruses. In those with HCoV-NL63 infections, 29% also had febrile seizures. However, only one of the previous clinical studies on HCoV-NL63, which was carried out in our locality, had mentioned the occurrence of febrile seizures, which occurred in 4 (27%) of 15 children (5). Although there is no significant

difference among the maximum temperatures of fevers for infections due to CoV-HKU1 and other respiratory viruses, there is a trend toward lower temperatures with the coronaviruses than with influenza A virus and adenovirus. Therefore, it is unlikely that the high incidence of febrile seizures associated with CoV-HKU1 and HCoV-NL63 infections can be explained by differences in fever magnitude. This is further supported by another study, which demonstrated an overall higher incidence of febrile seizures in children hospitalized for influenza A virus (19.5%) than in those hospitalized for parainfluenza virus (12.2%) and adenovirus (9%), despite adjustment for peak temperature and duration of fever (4). The figures were in line with the present findings, which demonstrated febrile seizure rates of 25% and 9% for children hospitalized for influenza A virus and adenovirus, respectively. In addition, the present study analyzed the three parainfluenza viruses separately and showed differences in their propensities to cause febrile seizure. While 1 (33%) of the 3 children with parainfluenza virus 2 and 23 (28%) of 82 children with parainfluenza virus 3 infections developed febrile seizures, none (0%) of the 12 children with parainfluenza virus 1 infections did. According to a recent report, a novel amino acid substitution in the hemagglutinin gene of influenza A virus was found to correlate with acute encephalopathy (18). Further studies are warranted to investigate the presence of specific neurotropic or epileptogenic factors in CoV-HKU1 and those respiratory viruses with a propensity to cause febrile seizures. The availability of rapid laboratory diagnosis tests for coronavirus infections, especially CoV-HKU1 infections, may be useful to clinicians as a clue to anticipate febrile seizures in children with acute respiratory tract infections.

Similar to our previous study, sequencing and phylogenetic analysis showed that the 13 CoV-HKU1 isolates belonged to two distinct genotypes based on both S and N genes, while the *pol* gene was less discriminative for such classification (Fig. 1). Seven of the 13 strains belonged to genotype A, and the other 6 belonged to genotype B (31). The two genotypes cocirculated during the winter, a phenomenon similar to that for HCoV-NL63, which was demonstrated in different geographical areas (2, 5, 29). Although no cases of CoV-HKU1 infections have been reported in other countries, the travel history of two of our patients suggests that CoV-HKU1 may exist outside Hong Kong. Studies of CoV-HKU1 infection in other areas are warranted to elucidate the distribution of CoV-HKU1 and its genetic diversity.

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