

Clinical Disease in Children Associated With Newly Described Coronavirus Subtypes

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ABSTRACT

OBJECTIVES. Coronaviruses cause upper respiratory illness and occasionally lower tract disease in susceptible populations. In this study we examined the prevalence of 4 human coronaviruses, including subtypes OC43, 229E, and the recently described NL63 and HKU1 in a pediatric population presenting to a children's hospital.

PATIENTS AND METHODS. Specimens collected over a 1-year period from pediatric patients presenting with acute respiratory illness were analyzed for the presence of 4 coronavirus subtypes using consensus and subtype-specific real-time reverse-transcription polymerase chain reaction assays. The demographic and clinical characteristics associated with coronavirus infection were examined retrospectively.

RESULTS. Coronaviruses were detected in 66 of 1043 children. Eight, 11, 19, and 28 specimens were positive for subtypes 229E, NL63, OC43, and HKU1, respectively. Coronaviruses were detected throughout the study period; all 4 of the subtypes were present simultaneously in December. The acute clinical features were similar across subtypes. Of 32 children infected with a coronavirus as the sole respiratory pathogen, 13 had lower respiratory tract disease. Children whose only detectable respiratory virus was a coronavirus were more likely to have underlying chronic disease than were children coinfecting with another respiratory virus.

CONCLUSIONS. Although 4 subtypes of coronavirus were detected, the recently discovered coronavirus subtypes NL63 and HKU1 accounted for the majority of coronaviruses detected in our cohort of mostly hospitalized children with respiratory symptoms. New subtypes likely represent a substantial portion of previously unexplained respiratory illnesses.

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Key Words

coronavirus, epidemiology, respiratory viruses, children, real-time RT-PCR

Abbreviations

SARS—severe acute respiratory syndrome
RT-PCR—reverse-transcription polymerase chain reaction
FA—fluorescent antibody assay
RSV—respiratory syncytial virus
ALL—acute lymphocytic leukemia

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HUMAN CORONAVIRUS SUBTYPES 229E and OC43 cause upper respiratory illness, accounting for an estimated one third of common colds each winter¹ and occasionally causing lower tract disease in susceptible infants, elderly individuals, and immunocompromised adults.²⁻⁴ These viruses, belonging to Coronaviridae groups 1 and 2, respectively, were the only coronaviruses identified in humans until 2003, when identification of the severe acute respiratory syndrome (SARS) coronavirus⁵ led to renewed research in human coronaviruses.

In 2004, investigators in the Netherlands isolated a distinct human coronavirus, subtype NL63,^{6,7} from a child with bronchiolitis and conjunctivitis. This virus was subsequently detected in an additional 7 (1.6%) of 493 individuals with respiratory tract infections.⁶ Since these initial reports, NL63 has been detected in respiratory specimens collected in Canada, the United States, France, Japan, Hong Kong, and Australia.⁸⁻¹³ A second novel coronavirus, subtype HKU1, was isolated in 2005 from a 71-year-old man with a fever and productive cough.¹⁴ This subtype was found in 10 (2.4%) of 418 patients with community-acquired pneumonia in Hong Kong¹⁵ and was also identified in patients in Australia, France, the United States, Belgium, and Italy.¹⁶⁻²⁰ Knowledge of the distribution, epidemiology, and symptoms associated with non-SARS human coronaviruses remains limited.

To investigate the role of the human group 1 (229E and NL63) and group 2 (OC43 and HKU1) coronaviruses in childhood respiratory illnesses, we tested pediatric respiratory specimens using consensus and subtype-specific real-time reverse-transcription polymerase chain reaction (RT-PCR) assays. The clinical characteristics of children with coronavirus were further investigated by medical chart review. This article describes our real-time RT-PCR methods for detection and differentiation of 4 human coronavirus subtypes and the seasonality and clinical illnesses in children infected with these viruses.

METHODS

Clinical Specimens

From October 2003 through September 2004, ~4000 pediatric (0–19 years old) respiratory specimens were submitted to the University of Washington Virology Laboratory for respiratory virus fluorescent antibody assay (FA) testing or FA and culture. Of these, 1074 nasal wash specimens contained sufficient residual material for testing by real-time RT-PCR for the presence of respiratory viruses. Thirteen nasal washes that were collected as repeat specimens (>1 specimen from any patient) were excluded, leaving 1061 specimens for testing by RT-PCR. The mean age of the study patients was 35.3 months (range: 1 day to 19 years); 42.5% were <1 year old; 80.9% were <5 years old. Fifty-five percent of

samples were from male patients. There were no significant differences in mean age, gender distribution, specimen collection month, or FA results between patients whose samples were tested by RT-PCR and those whose samples had insufficient volume for testing. Of the 828 patients for whom we had information about location, 12, 179, and 637 were seen as outpatients, emergency department patients, and inpatients, respectively.

Sample Preparation for RT-PCR Assay

Total nucleic acids were isolated from each nasal wash as described previously.²¹ To ensure that negative results were not because of inefficient nucleic acid extraction or inhibition, an RNA specimen processing control (EXO)²² was added to the extraction buffer. One low positive control containing 200 to 1000 copies per reaction of coronavirus RNA harvested from cell culture and 1 negative control consisting of cultured, uninfected human epithelial cells were processed with each batch of clinical specimens.

Design of Primers and Probes

The coronavirus primer and probe sequences (Table 1) were designed using Primer Express software (Applied Biosystems, Foster City, CA) from aligned coronavirus complete genome sequences. GenBank accession numbers for the coronavirus sequences were: NC002645 and AF304460 for 229E; NC005147, AY903460, and AY391777 for OC43; NC005831, AY567487, and AY567487 for NL63; and NC006577 and AY597011 for HKU1. Eight primers and 3 TaqMan probes were designed to amplify 85- to 100-bp fragments of the polymerase 1b gene. Each gene sequence in the alignment matched ≥ 1 primer set with no more than 1 base mismatch per oligonucleotide. There were no mismatches for the probe binding regions. The probes were labeled on the 5' ends with the fluorescent dye 6FAM and on

TABLE 1 Sequences of Primers and Probes Used for Coronavirus Real-time RT-PCR Assays

Forward primers	
F1 ^a	TGGTGGCTGGGACGATATGT
F2	TTTATGGTGGTTGGAATAATATGTTG
F3	TGGCGGGTGGGATAATATGT
F-OC ^b	CCTTATTAAGATGTTGACAATCCTGTAC
Probes	
P1	ATAATCCCAACCCATRAG
P2	ATAGTCCCATCCATCAA
P-OC	CACACTTAGGATAGTCCCA
Reverse primers	
R1	GGCATAGCAGATCACACTTAGG
R2	GGCAAAGCTCTATCACATTTGG
R3	GAGGGCATAGCTCTATCACACTTAGG
R-OC	AATACGTAGTAGGTTTGGCATAGCAC

^a The 5' end of F1 is at base 15089 of coronavirus OC43 sequence (GenBank accession No. NC 005147).

^b The 5' end of F-OC is at base 15116 of coronavirus OC43 sequence (GenBank accession No. NC 005147).

the 3' ends with a minor groove binder nonfluorescent quencher (Applied Biosystems). A second primer set and VIC-labeled probe²² were added to a separate RT-PCR to amplify and detect EXO.

Real-Time RT-PCR Assays

All of the specimens were initially tested for coronaviruses using a consensus assay in which 6 primers (F1, F2, F3, R1, R2, and R3) and 2 probes (P1 and P2) were included. The RT-PCRs were performed using a 1-step reverse-transcription PCR master mix (RNA UltraSense One-Step Quantitative RT-PCR System, Invitrogen Life Technologies, Carlsbad, CA). Reaction mixtures were prepared using a 500-nM final concentration of F1; 250 nM of F2, F3, R1, R2, and R3; and 100 nM of P1 and P2. Ten microliters of extracted RNA were added to 30 μ L of master mix. RT-PCR was performed using the following cycling conditions: 50°C for 15 minutes, 95°C for 2 minutes, and 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds. Negative coronavirus results were considered valid only if EXO RNA was detected. RNA extraction (if sufficient sample volume was available) and RT-PCR were repeated on all of the samples that were negative for both coronavirus and EXO. Only specimens with satisfactory amplification of EXO were used in the analyses. This resulted in 1043 (98.3%) valid samples of the 1061 originally tested.

Specimens positive in the consensus coronavirus assay were analyzed using subtype-specific assays. Coronavirus subtypes 229E and NL63 were detected using primer/probe sets F3/P2/R3 and F2/P1/R2, respectively. Subtypes OC43 and HKU1 were both detected using the primer/probe set F1/P1/R1. Specimens positive in this assay were further tested using primers F-OC and R-OC and probe P-OC, which were specific for amplification of subtype OC43. All of the specimens were also tested by real-time RT-PCR for the presence of respiratory syncytial virus (RSV); human metapneumovirus; influenza virus type A; parainfluenza virus types 1, 2 and 3; and adenovirus using methods described previously.²³

Assay Validation

The specificity of the coronavirus RT-PCR consensus assay was assessed by testing RNA or DNA purified from ≥ 2 culture isolates of 19 viruses that might be found in respiratory specimens: RSV; parainfluenza virus types 1, 2, 3, and 4; influenza virus types A and B; rhinovirus; enterovirus; coxsackie B virus; adenovirus; coronavirus types OC43 and 229E; and herpesvirus types 1 through 8. Only RNA extracted from the coronavirus strains gave positive signals in the RT-PCR. The sensitivity of the assays was determined using 10-fold serial dilutions of previously quantified specimens containing known coronavirus subtypes. The sensitivity of the consensus assay for each coronavirus subtype was equal to the sensitivity of each subtype-specific assay for its intended

target. The assays could reliably detect 10 viral copies per reaction of each subtype in both the consensus and subtype-specific reactions. To confirm the detection of all 4 coronavirus subtypes, PCR amplicons from a subset of positive specimens were cloned, and the plasmid inserts were sequenced.

Demographic and Clinical Data

Institutional review board approval was received before reviewing any records. Data were abstracted from medical charts using a standardized form. Temperature, respiratory rate, radiographic findings, antibiotic use, bronchodilator use, supplemental oxygen use, and ventilator requirements were assessed for the 24 hours preceding and after each positive sample. Lower respiratory illness was defined as having ≥ 1 of the following: supplemental oxygen requirement, mechanical ventilation requirement, or a chest radiograph showing possible or definite infiltrates. The chart data were supplemented by demographic data, information from progress notes, and admission and discharge codes. *International Classification of Diseases, Ninth Revision*, codes were used to ascertain illness diagnoses and to determine the presence of underlying conditions. Underlying diseases or conditions were categorized as pulmonary, malignancy, cardiac, genetic, renal and/or hepatic, central nervous system, asthma, and prematurity (defined as a gestational age of < 36 weeks). The number of days between admission and a positive coronavirus sample and the length of any symptomatic illness were examined for evidence of nosocomial acquisition.

Statistical Analysis

The univariate associations among coronavirus subtypes, coronavirus subgroups, or coinfections and clinical correlates were evaluated using Pearson's χ^2 for binary variables (with Fisher's exact method when appropriate), the Mann-Whitney U rank sum test for comparisons of continuous variables between subgroups, and the Kruskal-Wallis test for comparisons of continuous variables between subtypes. The association between the presence of coinfections and clinical correlates was further evaluated while controlling for the presence of underlying conditions using logistic regression for categorical variables and linear regression of continuous variables. All of the statistical analyses were performed by using Stata 8.0 (Stata Corp, College Station, TX).

RESULTS

Viral Detection

Coronaviruses were detected in 66 (6.3%) of 1043 specimens using the consensus RT-PCR assay. By comparison, the prevalence of RSV, parainfluenza viruses, influenza virus type A, human metapneumovirus, and

adenovirus by RT-PCR was 23%, 9%, 12%, 7%, and 13%, respectively, among all of the specimens.²³ Coronaviruses were detected in every month except July, with a maximum number in December (23 [34.8%] of 66 positive specimens; Fig 1), although this may be because of the large number of specimens tested from December. Specimens collected in December and April had the highest proportion of coronavirus-positive specimens (10.6% and 9.1%, respectively), reflecting a proportionately large number of HKU1-positive and NL63-positive specimens from December and April, respectively. Coronavirus-positive specimens were collected from children ranging in age from 23 days to 17 years, with a median age of 19.4 months. When tested for other respiratory viruses by RT-PCR, 30 (45.5%) of the 66 coronavirus-positive specimens also had another respiratory virus detected. RSV was the most common additional respiratory virus detected, accounting for 20 (66.7%) of the 30 coinfections. Other copathogens included 2 parainfluenza viruses, 2 influenza type A viruses, 3 metapneumoviruses, and 3 adenoviruses.

Of the 66 specimens positive for a coronavirus, 19 (28.8%) were group 1 subtypes (8 were 229E and 11 were NL63), and 47 (71.2%) were group 2 subtypes (19 were OC43 and 28 were HKU1). The RT-PCR amplicons from ≥ 4 positive specimens of each subtype were sequenced and compared with the sequences of the prototype strains, confirming the specificity of the subtype-specific assays. No specimen contained >1 coronavirus subtype. Group 2 coronavirus subtypes OC43 and HKU1 were detected from September through April, whereas group 1 coronavirus subtypes 229E and NL63 were detected from December through August (Fig 1).

Clinical Epidemiology

Clinical data were available for 56 (84.8%) of the 66 coronavirus-positive children. The age, gender, and subtype distributions of the 56 were similar to those observed in all 66 of the positive samples. Seventy-five percent of coronavirus-positive children were inpatients

with a median stay of 2 days; 23.2% were treated in the emergency department and not hospitalized overnight. A majority (54%) of the coronavirus-infected children were treated with antibiotics within 24 hours of specimen collection. The proportion of children treated with antibiotics was not significantly affected by a concurrent infection with RSV as documented by FA. The majority of children (60%) did not have a temperature $>38^{\circ}\text{C}$ within 24 hours of the time the diagnostic specimen was collected. Lower respiratory tract disease was noted in 34% of coronavirus-positive children; 4 children (7%) required intensive care unit support. Based on hospital admission and discharge diagnoses, 73% and 9% had evidence of respiratory or gastrointestinal illness, respectively. The majority of children with coronavirus detected (64%) also had ≥ 1 underlying chronic condition. We did not find any evidence of nosocomial acquisition of coronavirus in our patients. All but 3 of the coronavirus-positive samples were collected within 1 day of admission. Of the 3 children who had positive samples collected 2 to 5 days after admission, all had symptoms of respiratory illness at admission. No statistically significant differences were observed for any of the demographic, laboratory, or clinical characteristics examined between subtypes or between group 1 and 2 coronaviruses.

A coronavirus was the sole respiratory viral pathogen detected in 32 (57%) of the 56 children for whom clinical data were available, including 14 HKU1, 11 OC43, 5 NL63, and two 229E infections. Three children with coronavirus as the only identified respiratory virus (1 patient each with 229E, OC43, and HKU1) were treated in the intensive care unit. None of these 3 had other viral, bacterial, or fungal coinfections identified. Mechanical ventilation was required for 2 of these children, both of whom had underlying conditions, including Aicardi syndrome with recurrent seizures in 1 child and congenital cardiac disease and trisomy 21 in the other. Both children had a history noting respiratory symptoms and fever before admission. Few differences in clinical

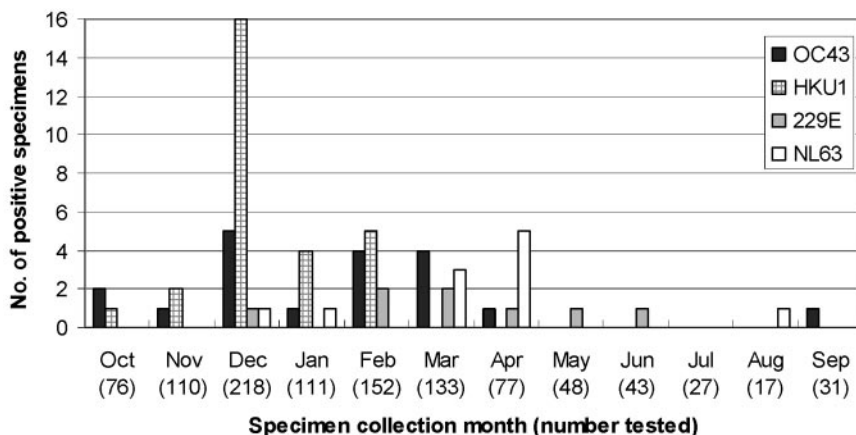


FIGURE 1
Number of coronavirus-positive specimens detected each month over a 1-year period according to coronavirus subtype.

characteristics were found between children infected only with coronavirus and children with multiple respiratory virus infections (Table 2). Children with only coronavirus were more likely than children with >1 respiratory virus infection to have an underlying condition ($P < .01$). By univariate analysis, children with only coronavirus were marginally more likely to be admitted to the hospital, and their length of stay was significantly longer compared with those with mixed infection. However, differences in hospital admission and length of stay were no longer statistically significant after controlling for underlying disease.

Immunocompromised Children

Six immunocompromised children, including 5 with acute lymphocytic leukemia (ALL) and one 15-year-old renal transplant recipient, had coronavirus detected as the only respiratory pathogen. All of the patients initially presented with rhinorrhea and/or nasal discharge; 2 children had cough as a presenting symptom, and 2 had sore throats. Five patients had a fever within 24 hours of detection of coronavirus that lasted between 1 and 7 days. A 12-year-old girl, who was admitted with nasal congestion, bruising, and suspected sepsis, was diagnosed with ALL the same day the respiratory sample was collected. She was afebrile at presentation but developed a fever the next day to 39.2°C. Blood culture the day after presentation was positive for *Streptococcus viridans*. Three other children, ages 20 months to 6 years, had symptoms including fever, rhinorrhea, nasal congestion, and cough while receiving outpatient maintenance che-

motherapy. One of these, a 5-year-old receiving consolidation chemotherapy for ALL, presented with a 1-day history of rhinorrhea and fever, a temperature of 39.9°C, and an abnormal oxygen saturation on room air of 93%. The 15-year-old renal transplant recipient, who had been on chronic immunosuppression for 2 years after transplantation, presented with rhinorrhea, cough, shortness of breath, left-sided chest pain, and diarrhea for 3 days.

Of the 6 immunocompromised patients, 4 (3 with ALL and 1 renal transplant recipient) were admitted to the hospital and stayed between 2 and 12 days. Intravenous broad-spectrum antibiotics were administered to 3 children with ALL who were neutropenic at the time of presentation, as well as the renal transplant recipient. Chemotherapy or immunosuppressive regimens were altered in only 1 patient. Of the 3 children who had radiographs taken, 2 had lower lobe infiltrates (1 child with ALL on maintenance chemotherapy and the renal transplant recipient). One child had severe neutropenia (50 neutrophils per mm^3) and an oxygen requirement at the time of coronavirus detection. This patient underwent a bronchoalveolar lavage, and no other respiratory organism was identified by viral, fungal, or bacterial cultures and staining or by antigen detection.

DISCUSSION

Although coronaviruses are considered an important cause of upper respiratory tract disease,¹ the lack of reliable detection methods has hampered studies of the epidemiology of the viral subtypes in this family. The

TABLE 2 Clinical Characteristics of Coronavirus Infected Patients by Coinfection Status

Clinical Correlate	Coronavirus as Sole Respiratory Pathogen (n = 32)	Coronavirus Coinfected With Other Respiratory Virus (n = 24)	P ^a
Inpatient, n (%)	27 (84)	15 (63)	.51
Admitted to ICU, n (%)	3 (9)	1 (4)	.90
Bronchodilators, n (%)	14 (44)	14 (58)	.24
Mechanical ventilation, n (%)	2 (6)	0 (0)	— ^b
Supplemental oxygen, n (%)	8 (25)	5 (21)	.93
Antibiotics, n (%)	20 (63)	10 (42)	.21
Temperature >38°C, n (%)	14 (44) ^c	10 (42)	.40
Length of stay, median (IQR), d	2 (1–7)	1 (0–2)	.30
Lower respiratory disease, n (%)	13 (41)	6 (25)	.38
Gastrointestinal symptoms, n (%)	4 (13)	1 (4)	.41
Underlying conditions or diseases, n (%)	26 (81)	10 (42)	<.01
Pulmonary	6 (19)	1 (4)	.22
Malignancy	5 (16)	1 (4)	.22
Genetic	8 (25)	1 (4)	.06
Cardiac	4 (13)	2 (8)	.69
Renal/hepatic	5 (16)	0 (0)	.06
CNS	3 (9)	0 (0)	.25
Asthma	6 (19)	6 (25)	.57
Prematurity	1 (3)	1 (4)	.9995

IQR indicates interquartile range; CNS, central nervous system.

^a All P values were adjusted for the presence of underlying conditions except for those testing underlying conditions themselves.

^b All of the children on a ventilator had only coronavirus infection and an underlying condition.

^c One missing value.

RT-PCR method used in this study enabled the sensitive and relatively inexpensive detection of 4 coronavirus subtypes and allowed subtype-specific epidemiological characterization of this infection. Important findings of our study include the seasonality of the virus, the potential for cocirculation of multiple subtypes, and the patient populations infected by the virus. Coronaviruses, including all 4 of the known non-SARS subtypes, were detected in 6.3% of specimens from children with acute respiratory illnesses who were either hospitalized or evaluated in the emergency department at a children's hospital.

This is the first study comparing the detection of each coronavirus subtype in the same cohort in North America and the first demonstration of all 4 subtypes in clinical samples submitted during a single month. Our study also demonstrated that although these viruses were more prevalent in the winter months, they were present throughout the year. This finding is distinct from classical coronavirus epidemiology, which relied heavily on serology and showed epidemics of 229E or OC43 occurring every few years, with uncommon overlap.¹ However, recent studies using sensitive PCR methods have reported detection of coronavirus in all seasons and detection of >1 subtype in the same season.^{12,19,20,24} The recently described HKU1 was the most common subtype detected in our study, with more cases detected than those of coronavirus "classical" subtypes 229E and OC43 combined. Coronaviruses were detected in both previously healthy children and those with underlying medical conditions and were frequently noted to be copathogens with other respiratory viruses, particularly RSV. Similar findings of frequent viral coinfections have been reported for NL63^{13,25} and HKU1.¹⁶

Detection of a coronavirus was associated with severe respiratory outcomes. Lower respiratory tract disease, including pneumonia, was reported in 34% of all coronavirus-positive patients and in 41% of patients infected only with coronavirus. Seven percent of all coronavirus-positive patients required intensive care unit support, and all of the children who required assisted mechanical ventilation were infected with coronavirus as the sole respiratory pathogen. Our data are consistent with previously reported findings of upper and lower respiratory tract disease in patients of all ages infected with subtypes NL63^{7,10-13,26} and HKU1¹⁵⁻¹⁷ and associations between coronavirus infection and severe lower respiratory tract disease in hospital settings.^{9,15} The high incidence of lower respiratory tract disease, especially in high-risk children, including the immunocompromised population, indicates that availability of rapid and reliable viral detection methods for coronavirus may be of use in the identification of the etiology of pneumonia in children.

Children who had only coronavirus detected were more likely to have underlying chronic diseases than children who had dual respiratory infections. We spec-

ulate that even relatively mild disease, such as upper respiratory tract disease, can result in substantial problems for children with underlying medical conditions. The more frequent detection of coronaviruses in this patient population may be the result of more frequent visits to physicians in specialty care clinics who request viral testing or more frequent hospital admission by concerned caretakers.

Gastrointestinal illness was documented in only 9% of our coronavirus-positive patients. Stool samples were not obtained for coronavirus in our study and were not systematically collected or tested for viral pathogens. Although it is possible that the absence of diarrhea can be related to inadequate reporting, we were able to document the presence of rotavirus in 2 patients, and an additional immunocompromised patient presented with symptoms of diarrhea and dehydration in addition to cough. Although nosocomial infection with NL63 has been suggested in an infant intensive care unit,⁹ our positive samples came from children who clearly demonstrated community acquisition of their illness. We speculate that patient-to-patient spread of coronavirus may be possible but may be more limited in a hospital setting where hospital stays are brief, respiratory precautions may be posted for coinfections such as RSV, and follow-up of patients is frequently conducted offsite.

Few data have been reported to enable adequate comparisons of clinical outcomes between infection with the newly described group 1 and 2 coronaviruses, NL63 and HKU1. The results of our study are similarly limited because of the small numbers of patients infected with each viral subtype. Woo et al¹⁵ reported that 2 adult patients died of HKU1-associated pneumonia, whereas a study of NL63 in children¹² did not document any fatalities. In our study, the clinical, demographic, and laboratory correlates of coronavirus infection were similar across all of the subtypes and between coronavirus groups, and the use of antibiotics and bronchodilators was similarly high in both groups.

Limitations of our study include the retrospective study design, the reliance on samples collected in a hospital-based setting, and the lack of an asymptomatic control group. We note that the detection of a coronavirus as the sole pathogen is suggestive but does not prove causation of disease. Although we looked extensively for other viral pathogens, it is possible that other undetected or as yet unknown pathogens could contribute to the symptoms of some patients. This study was designed to detect coronaviruses in hospitalized children with respiratory symptoms who had samples sent for viral diagnostic tests and, therefore, does not permit an estimate of the prevalence or overall burden of disease in the general pediatric population.

Additional prospective case-control studies of coronavirus infections in patients of all ages using sensitive detection methods will ultimately demonstrate the true

epidemiology and disease manifestations of these ubiquitous viruses. The detection of a coronavirus in asymptomatic control subjects²⁵ demonstrates the need for additional prospective studies of the clinical manifestations of coronavirus infection and duration of shedding in patients. Meanwhile, the use of standard infection control measures for hospitalized patients with suspected viral respiratory and gastrointestinal disease is recommended as we continue to detect and characterize respiratory viruses that are associated with presentations to hospitals by ill children.

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Clinical Disease in Children Associated With Newly Described Coronavirus Subtypes

Jane Kuypers, Emily T. Martin, Judson Heugel, Nancy Wright, Rhoda Morrow and Janet A. Englund

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