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Detection of Four Human Coronaviruses in Respiratory Infections in Children: A One-Year Study in Colorado

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Lower respiratory tract infections are the leading cause of death in children worldwide. Studies on the epidemiology and clinical associations of the four human non-SARS human coronaviruses (HCoVs) using sensitive polymerase chain reaction (PCR) assays are needed to evaluate the clinical significance of HCoV infections worldwide. Pediatric respiratory specimens (1,683) submitted to a diagnostic virology laboratory over a 1-year period (December 2004–November 2005) that were negative for seven respiratory viruses by conventional methods were tested for RNA of four HCoVs using sensitive RT-PCR assays. Coronavirus RNAs were detected in 84 (5.0%) specimens: HCoV-NL63 in 37 specimens, HCoV-OC43 in 34, HCoV-229E in 11, and HCoV-HKU1 in 2. The majority of HCoV infections occurred during winter months, and over 62% were in previously healthy children. Twenty-six (41%) coronavirus positive patients had evidence of a lower respiratory tract infection (LRTI), 17 (26%) presented with vomiting and/or diarrhea, and 5 (8%) presented with meningoencephalitis or seizures. Respiratory specimens from one immunocompromised patient were persistently positive for HCoV-229E RNA for 3 months. HCoV-NL63-positive patients were nearly twice as likely to be hospitalized ($P = 0.02$) and to have a LRTI ($P = 0.04$) than HCoV-OC43-positive patients. HCoVs are associated with a small, but significant number (at least 2.4% of total samples submitted), of both upper and lower respiratory tract illnesses in children in Colorado. Our data raise the possibility that HCoV may play a role in gastrointestinal and CNS disease. Additional studies are needed to investigate the potential roles of HCoVs in these diseases. ***J. Med. Virol. 81:1597–1604, 2009.***

KEY WORDS: coronavirus; rhinovirus; respiratory infection; children

INTRODUCTION

Respiratory infections are the most common infectious disease worldwide. The World Health Organization (WHO) continues to rank lower respiratory tract infections as the leading cause of burden of disease in the world [WHO, 2008]. The burden of respiratory tract infections in children is particularly high. LRTIs are the leading cause of death in children younger than 5 years of age worldwide, and in all age groups in low income countries [WHO, 2003, 2008; Klig, 2004; Klig and Shah, 2005]. In the United States, acute respiratory infections (ARIs) are a significant cause of morbidity and hospitalization in children [Fendrick et al., 2003; Griffin et al., 2004]. Therefore, defining the etiologies of respiratory infections is a public health priority.

In our institution, the etiology of up to 40% of ARIs is unknown. The first two human coronaviruses (HCoVs), HCoV-229E and HCoV-OC43, were isolated in human cell cultures or fetal tracheal organ cultures in the 1960s. They were associated with up to 15–30% of common colds, and rarely with LRTIs [Holmes, 2001; Heikkinen and Jarvinen, 2003]. Interest in HCoVs was stimulated in 2002–2003 when the epidemic of severe acute respiratory syndrome (SARS) was found to be caused by a newly emerged HCoV, SARS-CoV [Drosten et al., 2003; Ksiazek et al., 2003; Rota et al., 2003]. After the SARS outbreak had ended and SARS-CoV was no

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longer circulating in humans, two additional HCoVs were discovered, HCoV-NL63 in 2004 and HCoV-HKU1 in 2005 [van der Hoek et al., 2004; Woo et al., 2005]. Based on genome sequences, HCoV-229E and HCoV-NL63 are classified as group 1 CoVs and HCoV-OC43 and HCoV-HKU1 are classified as group 2a CoVs [van der Hoek et al., 2004; Woo et al., 2005]. RNAs of HCoV-NL63 and HCoV-HKU1 are found in respiratory specimens throughout the world in association with both URTIs and LRTIs in children and adults [Arden et al., 2005; Bastien et al., 2005; Chiu et al., 2005; Ebihara et al., 2005; Esper et al., 2005, 2006; Kaiser et al., 2005; Moes et al., 2005; Vabret et al., 2005, 2006; Sloots et al., 2006; Gerna et al., 2007; Kuypers et al., 2007; Pyrc et al., 2007]. Ongoing investigations are uncovering other clinical associations for these HCoVs. HCoV-NL63 is associated with croup [van der Hoek et al., 2005], and HCoV-HKU1 is associated with febrile seizures [Lau et al., 2006]. HCoVs may also be implicated in enteric diseases [Vabret et al., 2006].

To date, most studies have not found enough HCoV-positive samples to compare the clinical presentations of different HCoVs [Lau et al., 2006; Dare et al., 2007; Gerna et al., 2007; Vabret et al., 2008]. Furthermore, the epidemiology and clinical associations of the four non-SARS HCoVs in the Americas has been compared in only one cohort of patients [Kuypers et al., 2007]. Therefore, in order to compare the epidemiology and clinical associations of four HCoVs in children, we used sensitive RT-PCR assays to detect HCoV RNA in pediatric respiratory specimens submitted to the diagnostic virology laboratory at The Children's Hospital (TCH), Denver, CO, over a 1-year period (2004–2005) that were negative for other respiratory viruses.

PATIENTS, MATERIALS, AND METHODS

As part of an ongoing investigation of viral respiratory infections at The Children's Hospital in Denver, we archived at −70°C all (1,662 archived of 2,621 submitted) nasopharyngeal washes (NPWs) submitted from December 2004 to November 2005, that tested negative for respiratory syncytial virus (RSV), influenza viruses A (FLUAV) and B (FLUBV), parainfluenza viruses 1–3 (HPIV-1, -2, -3), and adenovirus (HAdV) by direct immunofluorescence (DFA). In addition, during influenza season NPWs (225 archived of 891 submitted) that were negative by a rapid immunoassay (IA) for FLUAV and FLUBV were also archived at −70°C. Use of the banked specimens and clinical data was approved by the Colorado Multiple Institutional Review Board. All samples were de-identified and coded at the TCH Virology Laboratory and then transferred to the Holmes lab for blinded analysis of HCoV RNA.

RNA was extracted using Qiagen EZ1 Virus Mini Kits (Valencia, CA) on a BioRobot EZ1 Extractor following the manufacturer's instructions. All of the specimens were initially screened using a modified quantitative real-time RT-PCR (qRT-PCR) assay that detects all four non-SARS HCoVs [Kuypers et al., 2007], in which we added 10 μl of RNA to 10 μl of master mix containing an additional 1.3 mM MgCl.

Specimens positive in the consensus coronavirus qRT-PCR assay were analyzed using virus-specific conventional RT-PCR assays using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) with random primers followed by PCR. Primer sets from previously published studies or designed based on consensus sequences for each HCoV were used (Supplemental Table I). Primers bound to unique regions within the HCoV-NL63 spike gene and nucleocapsid gene [Bastien et al., 2005; Moes et al., 2005], HCoV-HKU1 lb gene [Woo et al., 2005], HCoV-OC43 nucleocapsid gene, and HCoV-229E nucleocapsid gene. All positive specimens were further analyzed using a pancoronavirus nested RT-PCR assay [de Souza Luna et al., 2007]. A specimen was considered to be HCoV positive if it were positive in at least two different PCR assays that amplified different parts of the viral genome and the sequences of these amplicons confirmed that the bands were from HCoVs.

Because the initial DFA screens in the diagnostic laboratory did not test for human metapneumovirus (HMPV), human bocavirus (HBoV), or human rhinoviruses (HRVs), we also screened all HCoV-positive samples by RT-PCR assays specific for these viruses [Coiras et al., 2004; Maertzdorf et al., 2004; Lu et al., 2006]. The RT-PCR assay used for detection of HRVs detected HRV groups A, B, and the newly discovered HRV clade C [Dominguez et al., 2008]. Because samples submitted for IA were only tested for influenza viruses, HCoV-positive IA samples were also tested by multiplex RT-PCR for RSV, HPIV 1–3, and HAdV [Heim et al., 2003; Bellau-Pujol et al., 2005] to detect possible co-infection of these viruses with HCoVs. A table of all the primers used in this study is provided in Supplemental Table I.

After all specimens had been analyzed and confirmed in duplicate assays, HCoV-positive specimens were decoded, and medical chart review performed using a standardized form. Lower respiratory tract illness (LRTI) was defined as having one or more of the following: requirement for supplemental oxygen or mechanical ventilation, or a chest radiograph showing infiltrates. Statistical computations were conducted with SAS software, version 9.1.3. Significance was determined using the Wilcoxon Signed Rank test or the Fisher's Exact Test.

RESULTS

Virus Detection

Sufficient sample was available for analysis from 1,683 (89%) of the 1,887 archived pediatric respiratory specimens that had previous been shown to be negative for other viruses by either DFA or IA. We detected HCoV RNA using the consensus qRT-PCR assay in 84 (5.0%) of these 1,683 specimens. In the 3,512 consecutive NPWs from children with respiratory symptoms submitted to the viral diagnostic laboratory during the same time period, the prevalence of RSV was 14.5%, parainfluenza

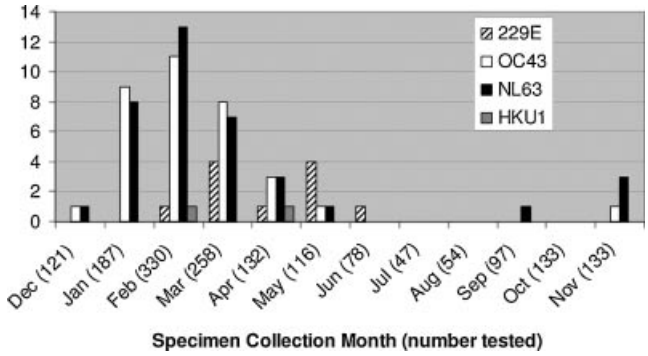

Fig. 1. Number of coronavirus positive respiratory specimens (n = 84) detected each month during a 1-year period.

viruses, 9.0%, influenza virus type A, 7.7%, adenoviruses, 4.2%, and influenza virus type B, 2.8%. The majority of the 84 HCoV-positive infections occurred during the winter months, with the maximum number 26 (30%) in February 2005 (Fig. 1). Of the 84 specimens positive for HCoV RNA, 37 (44%) were HCoV-NL63, 34 (40%) were HCoV-OC43, 11 (13%) were HCoV-229E, and 2 (2%) were HCoV-HKU1. One specimen was positive for both HCoV-NL63 and HCoV-229E.

Eight of the 84 HCoV-positive specimens were collected at different times from three patients (four specimens from one patient and two specimens each from two other patients). PCR tests for other respiratory viruses showed that 5 of the 84 HCoV-positive specimens were also positive for HMPV RNA, but none were positive for HBoV. Of the HCoV-positive samples that had only been tested by influenza IAs, one was also positive for RSV RNA, one for HPIV-1 RNA, and for HAdV DNA. We excluded from analysis all of the HCoV positive specimens found to be co-infected with RSV, HPIV-1–3, HAdV, HMPV, or another HCoVs, and all but the first specimens from each of the three patients with multiple specimens (Tables I–IV). Clinical data were available from 64 (90%) of the 71 remaining patients, including 17 (27%) who were also positive for HRV RNA. Because of the large percentage of specimens positive for both an HRV and an HCoV, and to determine if co-infection with HRVs affected the clinical presentation of HCoV infections, HCoV-positive patients co-infected with an HRV were included in the clinical analysis.

Clinical Epidemiology

The demographic and clinical characteristics of the HCoV-infected patients are shown in Table I. Most of the patients were young (median age 15 months), previously healthy (62%) children. The most common clinical finding in the HCoV-positive patients was fever (61%). The majority of patients presented with upper respiratory tract symptoms of cough (53%), rhinorrhea (47%), and congestion (45%). Twenty-four (38%) patients were hypoxic requiring admission for oxygen support, and 26 (41%) patients had evidence of a LRTI. Seventeen (27%) HCoV-positive patients had accompanying gastrointestinal symptoms (Table I) and 5 (8%) presented with meningoencephalitis or seizures (Table II). Of the patients with CNS disease, we were able to locate frozen CSF on two of these patients. The CSF was negative for coronavirus RNA by RT-PCR on both these samples. The diagnoses given HCoV-positive patients by their treating physicians are shown in Table II. Although there

TABLE I. Demographic and Clinical Characteristics of Patients With a Human Coronavirus Positive Respiratory Specimen

	Total (N = 64)	
	#	%
Median age in days (range)	462 (9–6,856)	
Male	39	60.9
Hospitalized	40	62.5
Admitted to PICU	14	21.9
Median length of stay (range)	4 (0–47)	
Antibiotics	30	46.9
Albuterol	9	14.1
Steroids	8	12.5
Positive chest X-ray	11	17.2
Fever	39	60.9
Hypoxia	24	37.5
Wheezing	4	6.3
Crackles	6	9.4
Retractions	9	14.1
Cough	34	53.1
Rhinorrhea	30	46.9
Congestion	29	45.3
Conjunctivitis	4	6.3
Pharyngitis	4	6.3
Rash	6	9.4
Vomiting	17	26.6
Diarrhea	7	10.9
Apnea	4	6.3
Seizures	5	7.8
Underlying condition[a]	24	37.5
Immunocompromised	8	12.5
LRTI	26	40.6

PICU, pediatric intensive care unit; LRTI, lower respiratory tract infection.
[a]Underlying disease conditions were defined as significant pulmonary, cardiac, genetic, central nervous system, renal and/or hepatic conditions, malignancy, or prematurity defined as gestational age <37 weeks.

TABLE II. Most Common Clinical Diagnoses of Patients With Human Coronavirus Positive-Respiratory Specimens

Diagnosis	Number (n = 64)	%
Viral syndrome or URI	13	20.0
Pneumonia	12	18.5
ALTE or apnea	6	9.2
Croup	4	6.2
Fever and neutropenia	4	6.2
Meningoencephalitis	3	4.6
Fever in young infant	3	4.6
Bronchiolitis	3	4.6
Asthma exacerbation	2	3.1
Seizures	2	3.1

URI, upper respiratory tract infection; ALTE, acute life threatening event.

TABLE III. Comparison of Clinical and Demographic Characteristics of Patients with Respiratory Specimens Positive for a Single Coronavirus (and No Other Detectable Viruses) Versus Those Positive for Both a Coronavirus and a Rhinovirus

	Coronavirus only (N = 47)		Coronavirus + HRV (N = 17)		
	#	%	#	%	*P*-value[a]
Median age in days (range)	550 (9–6856)		438 (22–5,250)		0.44[b]
Male	31	66.0	8	47.1	0.14
Hospitalized	29	61.7	11	64.7	0.22
Admitted to PICU	12	25.5	2	11.8	0.15
Median length of stay (range)	4 (0–47)		4 (2–16)		0.29[b]
Antibiotics	22	46.8	8	47.1	0.22
Albuterol	5	10.6	4	23.5	0.13
Steroids	5	10.6	3	17.6	0.24
Positive chest X-ray	8	17.0	3	17.6	0.29
Fever	30	63.8	9	52.9	0.17
Hypoxia	17	36.2	7	41.2	0.21
Wheezing	2	4.3	2	11.8	0.23
Crackles	4	8.5	2	11.8	0.32
Retractions	6	12.8	3	17.6	0.27
Cough	23	48.9	11	64.7	0.13
Rhinorrhea	20	42.6	10	58.8	0.13
Congestion	19	40.4	10	58.8	0.11
Conjunctivitis	3	6.4	1	5.9	0.43
Pharyngitis	2	4.3	2	11.8	0.24
Rash	6	12.8	0	0.0	0.14
Vomiting	10	21.3	7	41.2	0.10
Diarrhea	3	6.4	4	23.5	0.08
Underlying condition	19	40.4	5	29.4	0.31
Immunocompromised	7	14.9	1	5.9	0.24
LRTI	19	40.4	7	41.2	0.59

HRV, rhinovirus; PICU, pediatric intensive care unit; LRTI, lower respiratory tract infection.
[a]Fisher's exact *T*-test unless otherwise noted.
[b]Wilcoxon signed-rank test.

TABLE IV. Comparison of Patients With Respiratory Specimens Positive for HCoV-NL63 Versus HCoV-OC43

	HCoV-NL63 (N = 26)		HCoV-OC43 (N = 29)		
	#	%	#	%	*P*-value[a]
Median age in days (range)	285 (21–6631)		455 (9–6856)		0.20[b]
Male	17	65.4	17	58.6	0.41
Hospitalized	**19**	**73.1**	**13**	**44.8**	**0.02**
Admitted to PICU	3	11.5	8	27.6	0.09
Median length of stay (range)	4 (0–21)		3.5 (2-23)		0.40[b]
Antibiotics	**15**	**57.7**	**10**	**34.5**	**0.05**
Albuterol	4	15.4	4	13.8	0.29
Steroids	2	7.7	5	17.2	0.19
Positive chest X-ray	6	23.1	3	10.3	0.13
Fever	15	57.7	19	65.5	0.18
Hypoxia	12	46.2	8	27.6	0.08
Wheezing	1	3.8	2	6.9	0.40
Crackles	2	7.7	3	10.3	0.34
Retractions	4	15.4	5	17.2	0.28
Cough	**11**	**42.3**	**20**	**69.0**	**0.02**
Rhinorrhea	**9**	**34.6**	**19**	**65.5**	**0.01**
Congestion	**10**	**38.5**	**18**	**62.1**	**0.04**
Conjunctivitis	3	11.5	1	3.4	0.23
Pharyngitis	2	7.7	2	6.9	0.39
Rash	4	15.4	1	3.4	0.13
Vomiting	8	30.8	7	24.1	0.47
Diarrhea	3	11.5	3	10.3	0.61
Underlying condition	10	38.5	7	24.1	0.20
Immunocompromised	**7**	**26.9**	**1**	**3.4**	**0.02**
LRTI	**14**	**53.8**	**8**	**27.6**	**0.04**
HRV positive	6	23.1	6	20.7	0.54

Bold font indicates a statistically significant association.
PICU, pediatric intensive care unit; HRV, human rhinovirus; LRTI, lower respiratory tract infection.
[a]Fisher's exact *T*-test unless otherwise noted.
[b]Wilcoxon signed-rank test.

were no statistically significant clinical differences between patients infected with a single HCoV and those co-infected with a HRV, none of the co-infected patients presented with rash (Table III).

The majority of the HCoV-positive specimens were positive for either the group 1 virus, HCoV-NL63 (26, 41%) or the group 2 virus, HCoV-OC43 (29, 45%), which permitted comparison between the clinical presentations associated with these two viruses (Table IV). HCoV-NL63-positive patients were almost twice as likely as HCoV-OC43-positive patients to be hospitalized ($P = 0.02$), have evidence of a LRTI ($P = 0.04$), or receive antibiotics ($P = 0.05$), and nine times more likely to be immunocompromised ($P = 0.02$) (Table IV). HCoV-OC43-positive patients were more likely to have URTI symptoms of cough ($P = 0.02$), rhinorrhea ($P = 0.01$), and congestion ($P = 0.04$) (Table IV). Except for the clinical symptom of congestion, these findings remained statistically significant even when the 17 specimens co-infected with HRV were excluded from the analysis (data not shown). Because of the small number of cases in our data set, we did not have enough power to conduct a highly informative multivariate analysis. Nevertheless, a binary logistic regression analysis showed that rhinorrhea (URTI symptoms) was independently correlated with a higher likelihood of HCoV-OC43 infection (data not shown).

Because more HCoV-NL63 patients than HCoV-OC43 patients were immunocompromised ($P = 0.02$), we performed a separate analysis on patients who were not immunocompromised to see if this could account for the differences in clinical presentations. Limiting our analysis to immunocompetent children did not affect the results of our analysis, except that the use of antibiotics became nonsignificant (data not shown).

Three immunocompromised patients in our study had multiple, serial respiratory samples positive for the same HCoV. The first was a 4-month-old male with hepatoblastoma who presented in January 2005, with a 1-day history of fever, fussiness, rhinorrhea, and congestion. He was admitted for 2 days with a diagnosis of fever and neutropenia. He was readmitted 22 days later for 4 days with a second episode of fever and neutropenia. NPWs collected on both admissions were positive for HCoV-NL63 RNA. The second was a 9-year-old female with severe aplastic anemia who had received a bone marrow transplant in September 2004. In January 2005, due to persistent respiratory symptoms, she had two NPW collected 12 days apart both of which were positive for HCoV-NL63 RNA. The third patient was a 4-year-old male with a rhabdoid brain tumor who underwent an autologous bone marrow transplant in March 2005. He was admitted to the hospital for 16 days in April 2005 due to severe mucositis, fever, diarrhea, and increased oxygen requirement. He was readmitted to the hospital in mid-May for 43 days. On day 6 of that admission he developed fevers and increasing respiratory compromise, and a chest CT revealed bilateral pulmonary infiltrates. He was transferred to the PICU for a diagnostic bronchoalveolar lavage (BAL) and remained intubated for 13 days. Four respiratory specimens collected from late April to early June (a total of 37 days) were persistently culture positive for HRV. He was also persistently positive for HCoV-229E RNA, with four positive respiratory specimens (three NPWs and one BAL) over a 77-day period.

DISCUSSION

In this study, coronavirus RNA was detected in 5% of 1,683 specimens from children with acute respiratory illnesses submitted to our diagnostic virology laboratory in 2004–2005, that were negative for other respiratory pathogens by conventional clinical laboratory methods. This is the second study in North America that simultaneously analyzed the prevalence of the four non-SARS HCoVs in a single pediatric cohort. In the previous study [Kuypers et al., 2007], coronavirus RNA was detected in 66 (6.3%) of 1,043 samples, but 24 (36%) were co-infected with a second respiratory virus. Those HCoV-positive samples were found predominantly during the winter, with the largest number of samples positive in December. The majority of positive samples were HCoV-HKU1 [Kuypers et al., 2007]. In contrast, we detected only a few HCoV-HKU1 infections, but a large number of HCoV-NL63 and HCoV-OC43 infections, also predominantly in the winter, but with the peak incidence in February. These differences may reflect our different geographical regions (Pacific Northwest vs. Rocky Mountain region) and the different years of collection (2003–2004 vs. 2004–2005). Comparing current PCR data from other parts of the world, the prevalence of the different HCoVs appears to vary by year and location [Lau et al., 2006; Dare et al., 2007; Gerna et al., 2007; Vabret et al., 2008]. During the same time period as our study, HCoV-OC43 was the most frequently detected strain in Hong Kong [Lau et al., 2006], whereas in France there was relatively equal prevalence of HCoV-NL63, HCoV-OC43, and HCoV-HKU1 [Vabret et al., 2008]. In Italy, in 2005–2006, HCoV-229E was the most frequently detected strain [Gerna et al., 2007]. In a 2-year study in Thailand in 2003–2004, HCoV-OC43 was the major circulating strain with a small quantity of HCoV-NL63 also present, but the following year HCoV-HKU1 predominated [Dare et al., 2007]. Serological data in the 1970s showed that the prevalence of HCoV-OC43 and HCoV-229E infections peaked in alternating years [Holmes, 2001]. However, serologic tests may not have discriminated between the four HCoVs currently recognized. Our PCR data combined with those from other regions, suggest there may be significant yearly and geographical variation in circulation in all four HCoVs. Additional multiyear studies on the prevalence of HCoVs in the same location are needed.

In addition to URTIs, HCoVs in our study were also associated with more severe respiratory infections. LRTIs, including pneumonia, were reported in 40% of HCoV-positive specimens, and 22% required admission to the PICU. One of the immunocompromised patients

had a BAL sample that was positive for HCoV-229E, suggesting that the virus was in the lower airways. In contrast to other studies [Lau et al., 2006; Gerna et al., 2007; Kuypers et al., 2007], the majority (62%) of our 64 HCoV-positive patients had no underlying predisposing chronic medical conditions. We did not find an association between HCoV-NL63 and croup [van der Hoek et al., 2005]. This most likely reflects the differences in study design between our study and that of van der Hoek et al. which analyzed specimens collected from patients with a diagnosis of croup. The majority of patients with croup in our emergency department are treated empirically and no respiratory specimens are collected.

Gastrointestinal symptoms were reported in 27% of HCoV-positive patients. In this study, no stool samples were obtained for analysis of HCoV RNA. Numerous animal coronaviruses are pneumoenteric, causing both respiratory and enteric disease [Saif, 1996]. CoV-like particles were previously detected in human diarrheic stools [Caul et al., 1977; Resta et al., 1985] and SARS-CoV and HCoV-HKU1 RNA were detected in stools of patients with diarrhea [Leung et al., 2003; Vabret et al., 2006]. These findings suggest that HCoVs, like coronaviruses of animals, might also cause enteric disease. Further studies of fecal specimens from patients with gastroenteritis are needed to determine the role of HCoVs in enteric disease.

Five of the patients with HCoV-positive respiratory specimens presented with seizures, meningitis, or encephalitis. Six additional patients presented with a diagnosis of apnea or an acute life threatening event (ALTE). Although many animal coronaviruses cause central nervous system (CNS) disease [Murray et al., 1992; Cabirac et al., 1994; Houtman and Fleming, 1996; Holmes, 2001], to date there is no proof that CoVs cause CNS disease in humans. Febrile seizures have been significantly associated with HCoV-HKU1 infections [Lau et al., 2006]. There is one report of an ALTE in an infant with HCoV-229E infection [Simon et al., 2007]. CoV RNA has been detected in both normal and diseased human brain tissue, and HCoV-229E and HCoV-OC43 can infect human neural cell cultures, indicating that these human respiratory viruses may also be neurotropic [Arbour et al., 2000]. In patients with encephalitis, using sensitive PCR techniques, an etiologic agent is detected in only about 40% of cases [Glaser et al., 2003]. Therefore, further investigation of the potential HCoV-associated CNS disease is warranted.

Only a few studies have had enough HCoV-positive specimens to permit a statistically significant comparison of the clinical outcomes of infections with different HCoVs [Lau et al., 2006; Dare et al., 2007; Gerna et al., 2007; Vabret et al., 2008]. The large number of positive samples for HCoV-NL63 and HCoV-OC43 in our study permitted us to compare the clinical features of these two HCoVs. Patients with HCoV-NL63-positive respiratory specimens were nearly twice as likely as patients with HCoV-OC43 to present with a LRTI and to be hospitalized. Thus, although these two HCoVs were equally prevalent during the 2004–2005 year, HCoV-NL63 was associated with more serious respiratory disease.

It is unclear how long coronavirus shedding can occur in the respiratory tract following natural infection. RT-PCR analysis of serial respiratory specimens showed that a normal infant shed HCoV-NL63 for 3 weeks [Kaiser et al., 2005], and a 3-year-old child undergoing hematopoietic stem cell transplantation shed HCoV-HKU1 for 38 days [Gerna et al., 2007]. Here we report detection of HCoV-229E RNA in respiratory specimens for at least 11 weeks, demonstrating that immunocompromised children may be persistently infected with respiratory HCoVs for up to 3 months.

HRVs are the most common cause of URTIs in both children and adults. Recently, HRVs have been associated with more serious LRTIs [Kusel et al., 2006; Miller et al., 2007]. In addition, we and others have reported a new clade of HRV (HRV-C) associated with both URTIs and LRTIs [Lau et al., 2007; McErlean et al., 2007; Briese et al., 2008; Dominguez et al., 2008]. Although our study was not designed to study HRVs, we found that 27% of our HCoV-positive specimens were co-infected with an HRV. We found no statistically significant differences in clinical presentation between these two groups. Thus, HRVs apparently did not exacerbate the disease presentation in co-infected patients.

We studied children with respiratory symptoms primarily in a hospital setting (inpatient and emergency department). Therefore, our data do not necessarily reflect the overall burden of HCoVs respiratory disease in the general pediatric population. In addition, we did not attempt to detect HCoV RNA in NPWs that were previously found to be positive for another respiratory virus. This probably underestimated the number of HCoV-positive specimens in our total study populations, as co-infections of HCoV with other respiratory viruses have been reported. As we were unable to obtain NPWs from healthy children, we did not include an asymptomatic control group, and we acknowledge that HCoV infection does not prove etiology of reported signs and symptoms.

In conclusion, we found that in Colorado the four non-SARS HCoVs are associated with both URTIs and LRTIs in both healthy children and children with predisposing medical conditions. In addition, our data suggest that HCoV-OC43 and HCoV-NL63 may also contribute to gastrointestinal and CNS disease. Further investigations of the potential roles of HCoVs in these diseases are warranted. Multiplex PCR technology now entering diagnostic clinical virology laboratories will provide new insights into the geographical and seasonal variations in HCoVs infections and their clinical associations.

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