The Pediatric Burden of Human Coronaviruses Evaluated for Twenty Years

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Background: The epidemiology of human coronaviruses (HCoVs) has not been established using reverse transcription polymerase chain reaction techniques in a specimen collection that spans decades.

Methods: We used real-time RT-PCR for 3 HCoVs, HCoV 229E, OC43, and NL63, to test nasal wash specimens that had been obtained from a cohort of children <5 years of age with upper or lower respiratory infection (URI, LRI) who were comprehensively followed during the period from 1977 to 2001. Prospectively collected clinical data and archival samples were analyzed.

Results: HCoV was detected in 92/1854 (5.0%) of available samples with no known viral etiology of which 9% were 229E, 59% OC43, and 33% NL63. This represented 10/119 (8.4%) of LRI samples and 82/1735 (4.7%) of URI samples. HCoV was not detected every year, but occurred episodically. The recently described HCoV-NL63 was detected as early as 1981. HCoV was associated with 11.4 LRI episodes/1000 child-years <5 years of age (all in children <2 years of age) and 67.3 URI episodes/1000 child-years <5 years of age.

Conclusions: HCoV-NL63 and OC43 are associated with a significant proportion of LRI in children less than 2 years of age and a substantial number of medically attended URI episodes.

Key Words: coronavirus, viral respiratory infections, bronchiolitis, croup, otitis media

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Human coronaviruses (HCoVs) were first identified as the cause of acute upper respiratory infection (URI) in the 1960s.[1,2] Two HCoVs designated OC43 and 229E were established as human pathogens, but the difficulty in culturing HCoV from clinical specimens hindered comprehensive epidemiologic studies. HCoVs OC43 and 229E have been associated with lower respiratory infection (LRI) in several populations, including young children, elderly persons, immunocompromised individuals, and military recruits using both culture and molecular methods.[3] These studies have been limited, however, by either small sample sizes or short periods of assessment since individual coronaviruses may not circulate yearly.[4,5] Recently, 3 new HCoV associated with LRI have been described: severe acute respiratory syndrome coronavirus (SARS-CoV),[6] HCoV-Netherlands (HCoV-NL63),[7,8] and HCoV Hong Kong (CoV-HKU1).[9] The discovery of these new HCoVs and improved molecular detection methods have renewed interest in the 2 previously identified HCoVs, OC43, and 229E.

The objectives of the current study were to use molecular tools to define the incidence and clinical features of URI and LRI associated with HCoV-NL63, OC43, and 229E during a 20-year period in a large cohort of otherwise healthy children followed prospectively during multiple respiratory seasons.

MATERIALS AND METHODS

Study Design and Population

This study used archival nasal wash specimens prospectively collected in the Vanderbilt Vaccine Clinic (VVC), a primary care clinic established in August 1972 to evaluate investigational vaccines in young children and to conduct surveillance for respiratory viruses.[10–14] Healthy, full-term infants were enrolled at birth and followed until 5-year of age. Children with comorbid conditions other than mild asthma were excluded. Pediatricians trained in infectious diseases and nurse practitioners conducted all sick- and well-child visits. History and physical examination findings were recorded on a standardized clinical form. Investigators were instructed to collect a nasal wash if a child presented with fever >38.3°C, acute otitis media, or LRI (defined below). Specimens were kept on ice and processed immediately for viral cultures with an aliquot snap-frozen and stored at −70°C. All samples were cultured for viral respiratory pathogens on human neonatal kidney, HEp-2, rhesus monkey kidney, human embryonic lung cell lines, and Madin-Darby canine kidney cells (during influenza season).

Case Definitions

Diagnoses were assigned during the visit by the clinical provider. LRI was defined as epiglottitis, laryngotracheobronchitis (croup), bronchiolitis, pneumonia, or asthma exacerbation. URI was defined as coryza, conjunctivitis, pharyngitis, otitis media, or otitis externa. A new episode of LRI or URI was defined as any visit with a diagnosis of LRI or URI that occurred at least 7 days after an earlier visit. All studies were conducted with the approval of the Vanderbilt Medical Center Institutional Review Board.

Specimen Selection

The VVC database was queried to identify specimens obtained from children meeting the diagnosis of either LRI or URI from which a viral pathogen had not been identified at the time of

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the original clinic visit.[14,15] RNA was manually extracted from LRI nasal wash samples with the use of the QIAamp Viral RNA kit (Qiagen)[14] and from URI samples with a Qiagen BioRobot 9604 Workstation, using QIAamp Viral RNA kits (Qiagen).[15] Specimens were tested for HCoV OC43, 229E, and HCoV-NL63 as described below. In addition, specimens infected with respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza viruses (PIV), influenza, or adenovirus were tested for codetection with HCoV. These samples were chosen during years of high HCoV circulation.

Virus Detection

RNA was tested by real-time RT-PCR (QuantiTect Probe RT-PCR, Qiagen) using primers and probes for the nucleocapsid genes of the specific HCoV strains of interest.[7,16] To test for RNA integrity in archival samples, all URI specimens were tested for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using a commercial test (Applied Biosystems). LRI samples were not tested for GAPDH due to limited sample volumes.

Statistical Analysis

The proportion of LRI and URI episodes associated with HCoV in the specimens available for testing was determined. This proportion was then extrapolated to all episodes of URI and LRI without an identified etiology (including those with and without available specimens for testing) to determine the overall number of LRI and URI episodes associated with HCoV (Fig., Supplemental Digital Content 1, http://links.lww.com/A1288). Incidence was calculated by multiplying the number of LRI and URI episodes by the estimated proportion of LRI and URI episodes with detectable HCoV and then dividing by the total child-years of follow-up for the clinic population. Because some specimens were missing or depleted and some nasal wash specimens were never obtained for some episodes of LRI and URI, we examined the association between specimen availability and sex, race, age, follow-up time, examination year, and the presence of fever using multivariable logistic regression. To account for possible selection bias associated with specimen availability, we computed the proportion of LRI and URI with detectable HCoV weighted by the inverse of the probability of the specimen being available based on these covariates.[17] Details of these estimations, including calculation of confidence intervals are found in the Appendix. (Appendix, Supplemental Digital Content 2, http://links.lww.com/A1289).

Fisher exact test was used to compare URI and LRI diagnoses among HCoV and other common respiratory viruses. Data were analyzed using STATA version 9 (Statacorp, College Station, TX) and R version 2.4.1 (Vienna, Austria).

RESULTS

LRI Results

Subjects and Specimens Tested

Available LRI specimens were collected between July 1977 and December 2001. The study cohort during these 24 years included 1830 children with a total follow-up time of 3958 child-years (mean: 2.2 years per child). The clinic population was 51% male, 58% white, and 36% African-American. There were 1287 visits for 948 episodes of LRI (240 episodes per 1000 child-years). The population diagnosed with an LRI was 58% male, 59% white, and 36% African-American and the median age was 14.5 months. The incidence of LRI was 383 per 1000 child-years <6 months, 246 per 1000 child-years 6 to 23 months, and 154 per 1000 child-years 2 to 5 years of age.

Figure, Supplemental Digital Content 1, http://links.lww.com/A1288, outlines the availability of LRI nasal wash specimens. Of 948 LRI episodes, 553 (58%) specimens were obtained. Compared with children with LRI without samples, subjects with a specimen were similar ($P > 0.05$) with respect to sex, race, years of follow-up, and date of clinic visit, but tended to be younger and more likely to have had their temperature recorded ($P < 0.0001$ for both). Of the 553 nasal wash specimens originally obtained, 313 had no known viral etiology. Of these, 119 had residual aliquots available for testing. Unavailable specimens had been lost, discarded, or depleted. Subjects with available specimens tended to be younger ($P = 0.03$) and to have had a more recent clinic visit ($P = 0.01$).

HCoV Detection in LRI Specimens

Of the 119 LRI nasal wash specimens available for testing, 5 (4.2%) were positive for HCoV-NL63 RNA, 5 (4.2%) for OC43, and none were positive for 229E, yielding a total of 8.4% HCoV positivity in LRI samples tested. All children with identified HCoV were less than 2 years of age. Table 1 summarizes the clinical characteristics and diagnoses of the 10 children with HCoV-NL63 and OC43 LRI. Half of the HCoV-infected children were diagnosed with concomitant acute otitis media. Clinical characteristics did not differ between the 2 viruses, though the numbers were small. No child with LRI associated with either HCoV-NL63 or OC43 was hospitalized. One child with HCoV-NL63 had a chest radiograph performed that showed a diffuse infiltrate.

Estimated Proportion and Incidence of HCoV-Associated LRI

The estimated overall percentage of LRI associated with any HCoV was 4.8% (95% CI: 2.4%, 8.1%). The percentage of LRI associated with HCoV-NL63 was 2.4% (95% CI: 0.9%, 5.0%), with OC43 was 2.4% (95% CI: 0.9%, 5.0%), and with 229E was 0% (95% CI: 0%, 0.9%). These estimates assumed no coinfection and that the specimens not available for testing had a similar proportion of HCoV to those available. To examine the sensitivity of results to the latter assumption, we re-estimated the

TABLE 1. Summary of the Signs, Symptoms, and Diagnoses Seen in Patients With HCoV Lower Respiratory Infection

Percent of LRI Subjects With Indicated Characteristic	OC43 (n = 5) %	HCoV-NL63 (n = 5) %
Subject		
Mean age (mo)	9.5	13.8
Male	60	80
Symptoms		
Fever	60	20
Rhinorrhea	80	80
Cough	80	60
Wheezing	60	20
Rash	60	0
Signs		
Temperature ≥100	20	20
Rhinitis	80	80
Abnormal tympanic membranes	60	60
Pharyngeal erythema	60	20
Rhonchi	40	0
Wheezing	80	40
Diagnosis		
Bronchiolitis	60	40
Croup	20	20
Pneumonia	0	40
Asthma	20	0
AOM	60	40

Statistical difference was not noted between these 2 viruses.

TABLE 2. Estimated Percent and Incidence (95% Confidence Interval) of Upper and Lower Respiratory Infection Associated With HCoV by Age Group

Age Group	OC43	HCoV-NL63	229E	All HCoV
Percent of LRI associated with HCoV by age group (CI)				
<6 mo	1.5 (0.09, 6.5)	0.0 (0, 2.9)	0.0 (0, 2.9)	1.5 (0.09, 6.5)
6–23 mo	3.5 (1.1, 7.8)	4.4 (1.6, 8.9)	0.0 (0, 1.6)	7.8 (3.9, 13.3)
2–5 yr	0.0 (0, 5.5)	0.0 (0, 5.5)	0.0 (0, 5.5)	0.0 (0, 5.5)
Incidence of LRI per 1000 child-year associated with HCoV by age group (CI)				
<6 mo	5.9 (0.3, 24.8)	0 (0, 11.1)	0 (0, 11.1)	5.9 (0.3, 24.8)
6–23 mo	9.9 (3.1, 22.0)	12.3 (4.5, 25.3)	0 (0, 4.7)	22 (11.1, 37.7)
2–5 yr	0 (0, 8.4)	0 (0, 8.4)	0 (0, 8.4)	0 (0, 8.4)
Percent of URI associated with HCoV by age group (CI)				
<6 mo	1.9 (0.8, 3.6)	1.6 (0.6, 3.2)	0.3 (0.0, 1.1)	3.8 (2.2, 6.0)
6–23 mo	1.7 (1.2, 2.4)	0.7 (0.4, 1.2)	0.4 (0.1, 0.7)	2.8 (2.1, 3.6)
2–5 yr	1.9 (1.0, 3.1)	1.1 (0.4, 2.10.3)	0.2 (0.0, 0.7)	3.1 (2.0, 4.6)
Incidence of URI per 1000 child-year associated with HCoV by age group (CI)				
<6 mo	43.4 (18.8, 82.8)	37.2 (14.9, 74.4)	6.2 (0.3, 27.1)	86.4 (49.6, 138.1)
6–23 mo	54.2 (37.2, 75.6)	21.7 (11.7, 36.3)	10.8 (4.4, 21.9)	86.8 (64.9, 112.9)
2–5 yr	24.7 (13.3, 41.0)	14.4 (6.2, 27.7)	2.0 (0.1, 9.0)	41.2 (25.9, 61.2)

proportion of HCoV-associated LRI accounting for the differing patient characteristics between available and unavailable specimens. In these adjusted analyses, the estimated percentage of LRI associated with any HCoV, HCoV-NL63, or OC43, were 4.6%, 2.6%, and 1.9%, respectively, similar to the unadjusted estimates.

The overall incidence of HCoV-associated LRI was 11.4 episodes per 1000 child-years (95% CI: 5.8, 19.3), but varied substantially with age (Table 2) with HCoV not detected in any children with LRI ≥2 years of age. The incidence for children 0 to 23 months was 17.2 episodes of HCoV-associated LRI/1000 child-years (95% CI: 8.8, 28.9) and the peak incidence was in children 6 to 23 months at 22 episodes of HCoV-associated LRI/1000 child-years (95% CI: 11.1, 37.7).

Codetection

Sixty-nine LRI specimens previously positive for RSV, hMPV, PIV, influenza, or adenovirus were tested for HCoV-NL63 and 46 of these were tested for OC43 and 229E (23 specimens were depleted after HCoV-NL63 testing). One specimen was positive for HCoV-NL63, 1 for OC43, and none were positive for 229E. Therefore, including HCoV codetection and assuming similar HCoV codetection rates among untested specimens with a known viral etiology, the percentage of LRI associated with any HCoV increased slightly to 6.3%, corresponding to an overall incidence of 15.2 episodes per 1000 child-years.

URI Results

Subjects and Specimens Tested

Because of limited freezer storage, only URI specimens from January 1982 to December 2001 were available for testing. The study cohort during that period comprised 1481 children with 50% male, 59% white, and 35% African-American and included 6724 episodes of URI. Children with URI episodes were 53% male, 61% white and had a median age of 14.3 months. Of the 6724 URI episodes, 4080 had a nasal wash collected. A specimen was more likely to be collected if the patient was younger ($P = 0.04$), had longer follow-up ($P < 0.0001$), had a more recent clinic visit ($P < 0.0001$), was diagnosed with acute otitis media ($P < 0.0001$), or had a recorded temperature ($P = 0.0004$).

There were 2082 URI specimens (Fig., Supplemental Digital Content 1, http://links.lww.com/A1288) with no known viral etiology available for testing. These specimens did not differ statistically from the general clinic specimens with respect to age, length of follow-up, visit date, or race ($P > 0.05$), but specimens from males ($P = 0.01$), from children with acute otitis media ($P = 0.03$) and from children with a lower temperature ($P = 0.04$) were more likely to be available. Of available URI samples, 1735 (83%) had detectable GAPDH and were included in the analysis. Samples were less likely to have degraded if they had been collected more recently ($P < 0.0016$) or were from a child with acute otitis media ($P < 0.034$).

TABLE 3. Summary of the Signs and Symptoms Seen in Patients With HCoV Upper Respiratory Infection

Percent of URI Subjects With Indicated Characteristic	OC43 (n = 49) %	HCoV-NL63 (n = 25) %	229E (n = 8) %
Subject			
Mean age (mo)	18.4	17	16.4
Male	43	52	63
White	57	76	63
Symptoms			
Fever	37	20	38
Rhinorrhea	76	68	75
Cough	63	60	50
Earache	18	32	25
Anorexia	33	24	38
Irritability	33	40	38
Signs			
Temperature ≥100	31	28	50
Rhinitis	82	68	63
Abnormal tympanic membranes	63	64	63
Pharyngeal erythema	31	12	13
Diagnosis			
Coryza	45	40	38
Pharyngitis	6	4	0
AOM	49	52	63

Statistical difference was not noted among these 3 viruses.

HCoV Detection in URI Specimens

Of the 1735 URI samples available for testing with detectable GAPDH, 82 (4.7%) had detectable HCoV RNA, 49 (2.8%) with OC43 RNA, 25 (1.4%) with HCoV-NL63 RNA, and 8 (0.5%) with 229E. Table 3 highlights the signs, symptoms, and diagnoses of children with HCoV-associated URI. Clinical symptoms did not differ significantly among the 3 HCoV strains. Nearly two-thirds of all children with HCoV-associated URI had abnormal tympanic membranes and 51% were diagnosed with acute otitis media.

Proportion and Incidence of Coronavirus-Associated URI

The estimated proportion of URI associated with HCoV was 3.0%, (1.8% OC43, 0.9% HCoV-NL63, and 0.2% 229E). In contrast to HCoV-associated LRI, the percent of URI associated with HCoV was similar for each age group (Table 2). The overall incidence of HCoV-associated URI was 67.3/1000 child-years. HCoV circulated more frequently in the winter months, with the majority detected between November and March (Fig. 1). The burden of URI attributable to HCoV varied substantially from year to year (Fig. 2). A single virus strain circulated in some seasons, while in others, 2 or even 3 viral strains were present. HCoV was not detected in 2 of the study years. Of 139 URI specimens previously positive for other viruses that we tested for HCoV, 2 were positive for OC43, 2 for HCoV-NL63, and none for 229E.

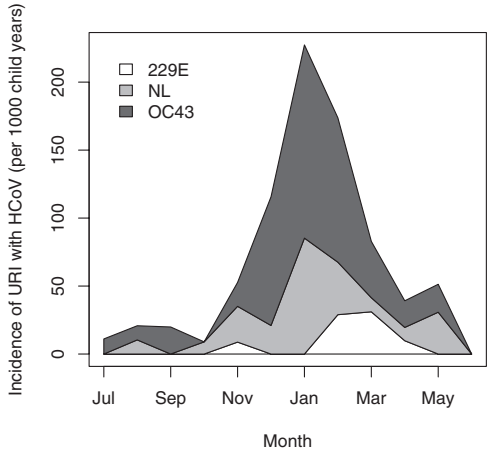

FIGURE 1. Cumulative monthly incidence of HCoV infections from 1981 to 2001 in the VCC. The y-axis shows the incidence of HCoV URI during the month accounted for by each virus.

DISCUSSION

Utilizing prospectively collected specimens and data from a unique longitudinal cohort, we determined the association between URI and LRI in otherwise healthy children <5 years of age with the previously described and new HCoVs. Using molecular tools, we assessed the 2 to 3 year periodicity of HCoV circulation suggested by earlier serologic studies.[4,5] Although our study did not confirm this periodicity, it did show substantial year-to-year variation and highlighted how studying samples from just 1 or 2 years is problematic in determining overall HCoV burden.

The combined incidence of LRI associated with these HCoVs was 17.2/1000 child-years for children less than 2 years of age, with a substantial burden of disease associated with both OC43 and HCoV-NL63. The greatest incidence of LRI associated with these viruses was between 6 to 23 months of age, older than the peak age of <6 months with RSV-associated LRI.[10] The biologic reason for this observation is unknown, but may relate to maternal antibody titers or differences in immunopathogenesis.

Previous studies of this same prospective cohort have used culture techniques and antigen testing to describe LRI caused by RSV, influenza, and PIV, and RT-PCR to describe hMPV.[10–14] These prior studies spanned slightly different time periods, but showed that RSV was detected in more LRI than other viruses, with 37.8% of LRI episodes associated with RSV.[10] Influenza virus had an incidence of 8 LRI episodes per 1000 child-years[11] and PIV (including PIV 1, 2, and 3) was associated with 12 LRIs/1000 child-years in children 3 to 15 months of age,[12] comparable to the incidence of LRI associated with HCoV (17.2 episodes/1000 child-years for children less than 2 years of age). Since several of these earlier studies used only culture techniques, they may have underestimated detection rates compared with RT-PCR methods, making direct comparisons of incidence difficult. More recently, Williams et al[14] detected hMPV in 12% of all LRI in this cohort using RT-PCR methods comparable to the techniques that were employed in the present study. Our data

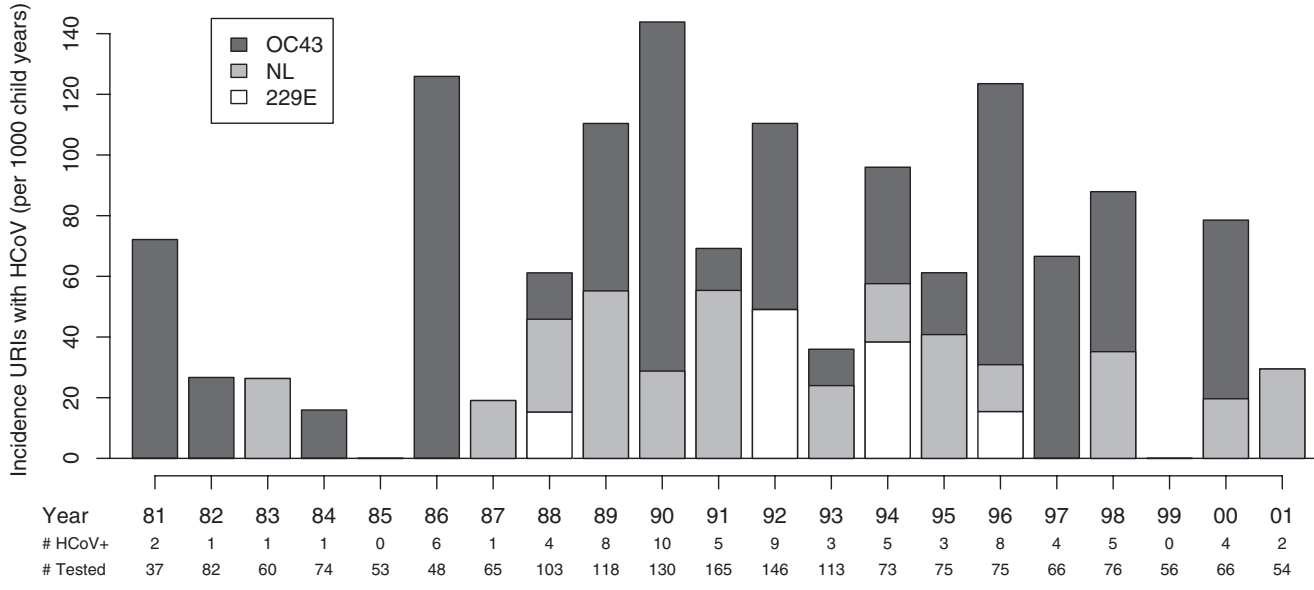

FIGURE 2. Incidence by year of OC43, HCoV-NL63, and 229E. Data are from 1981 to 2001 in the VCC. Each year has been defined from July to June. Hence 1981 is July 1981 through June 1982.

suggest that HCoVs are associated with a substantial proportion (approximately 8%) of LRI in children 6 to 23 months of age.

OC43 was detected in 2.8% of LRI with an incidence of 8.6 episodes/1000 child-years for children less than 2 years of age. While earlier studies described OC43 as a cause of URI, this strain appears also to be associated with LRI, similar to the newly discovered HCoV-NL63. None of the children with HCoV infection were hospitalized. Because of the exclusion of children born prematurely, infants with cardiopulmonary disease, and those with immunodeficiency from our surveillance population, the impact of the disease on these populations remains unknown.

HCoV-NL63 was originally described in 2004. Although samples detecting HCoV-NL63 date back to 1988 in the literature,[7] our samples clearly showed that HCoV-NL63 has circulated since 1981 in Nashville, TN. To our knowledge, this is the oldest reported case of HCoV-NL63. Our report demonstrates HCoV-NL63 has been identified in children who have had URI and acute otitis media. Interestingly, 229E was not detected in the LRI specimens, although it has been associated with LRI in infants and older adults[18–20] and with less severe illness than OC43.[21] 229E had the lowest prevalence in the URI specimens, which may be a result of collection of specimens primarily from children with acute otitis media, fever, or LRI. Thus, we likely underestimated the burden of 229E-associated URI.

Notably, half of the HCoV-infected children with LRI were diagnosed with acute otitis media while overall only 19% of children with LRI in the cohort were diagnosed with acute otitis media ($P = 0.03$). Similarly, 51.2% of the children with HCoV-associated URI had acute otitis media. Thus, our data support earlier reports that HCoV is associated with acute otitis media.[22]

The detection rate of HCoV in healthy children has not been extensively studied and the samples obtained in our clinic during well visits are no longer available. One study done in atopic children found similar rates of detection of 229E and OC43 in children with respiratory illness (5.5%) and those who were asymptomatic (4.4%).[23] Recently Dare et al[24] tested specimens obtained from a group of healthy control patients for different human coronaviruses. HCoVs were not detected in the 34 healthy children aged less than 1 year and in only 1 of the 51 healthy children aged 1 to 4 years. Boivin et al, found HCoV-NL63 in 3.0% of children hospitalized for acute respiratory illness and 1.7% of asymptomatic children. Interestingly, the mean age of symptomatic children was 10.1 months and 24.3 months for asymptomatic children ($P = 0.04$).[25] These findings suggest that asymptomatic infection may be more common in older children. Additionally, codetection of other viruses is also more common with the use of increasingly sensitive diagnostic tools. One recent study showed 27% of viral respiratory episodes were associated with the detection of more than one virus.[26] In that study, children with more than one virus detected were more likely to require hospitalization and were more likely to be <12 months of age.

This study has several limitations, most importantly the use of archival specimens and the unavailability of some specimens. Vigorous statistical attempts were made to control for these missing samples. In addition, another HCoV strain, HCoV-HKU1, was discovered recently and was not included in this study due to further depletion of samples. Hence the overall incidence of HCoV determined in this analysis likely underestimates the true burden because it does not include HCoV-HKU1. Despite the fact that HCoV strains were associated with 67 medically attended URI visits/1000 child-years and a modest amount of LRI, it is still unclear if these viruses cause a significant burden of disease to warrant vaccine development. The exclusion of immunocompromised children or those with other chronic diseases likely underestimates the burden and severity of disease.

In summary, we have shown in a prospective longitudinal cohort study of young children that HCoV-NL63 and HCoV OC43 strains are associated with a substantial burden of LRI and medically attended URI in previously healthy outpatient children. The LRI clinical syndromes associated with HCoV infection include bronchiolitis and pneumonia and are similar to disease caused by other respiratory viruses. A high proportion of HCoV infections are associated with acute otitis media. Future studies in normal healthy children and in high-risk pediatric populations will further elucidate the impact of these viruses.

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REFERENCES

1. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med*. 1966;121:190–193.
2. Kendall EJ, Bynoe ML, Tyrrell DA. Virus isolations from common colds occurring in a residential school. *Br Med J*. 1962;5297:82–86.
3. McIntosh K. Coronavirus infection in acute lower respiratory tract disease of infants [comment]. *J Infect Dis*. 2004;190:1033–1041.
4. Hambre D, Beem M. Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. *Am J Epidemiol*. 1972;96:94–106.
5. Monto AS. Medical reviews: coronaviruses. *Yale J Biol Med*. 1974;47:234–251.
6. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med*. 2003; 348:1967–1976.
7. Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci USA*. 2004;101:6212–6216.
8. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med*. 2004;10:368–373.
9. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*. 2005;79:884–895.
10. Fisher RG, Gruber WC, Edwards KM, et al. Twenty years of outpatient respiratory syncytial virus infection: a framework for vaccine efficacy trials. *Pediatrics*. 1997;99:E7.
11. Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. *J Infect Dis*. 2002;185:147–152.
12. Reed G, Jewett PH, Thompson J, et al. Epidemiology and clinical impact of parainfluenza virus infections in otherwise healthy infants and young children <5 years old. *J Infect Dis*. 1997;175:807–813.
13. Edwards KM, Thompson J, Paolini J, et al. Adenovirus infections in young children. *Pediatrics*. 1985;76:420–424.
14. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004;350:443–450.
15. Williams JV, Wang CK, Yang CF, et al. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. *J Infect Dis*. 2006;193:387–395.
16. van Elden LJ, van Loon AM, van Alphen F, et al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. *J Infect Dis*. 2004;189:652–657.
17. Horvitz DG, Thompson DJ. A generalization of sampling without replacement from a finite universe. *J Am Stat Assoc*. 1952;96:440–488.
18. Falsey AR, McCann RM, Hall WJ, et al. The "common cold" in frail older persons: impact of rhinovirus and coronavirus in a senior daycare center. *J Am Geriatr Soc*. 1997;45:706–711.

19. McIntosh K, Chao RK, Krause HE, et al. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis*. 1974;130:502–507.
20. Pohl-Koppe A, Raabe T, Siddell SG, et al. Detection of human coronavirus 229E-specific antibodies using recombinant fusion proteins. *J Virol Methods*. 1995;55:175–183.
21. Hendley JO, Fishburne HB, Gwaltney JM Jr. Coronavirus infections in working adults. Eight-year study with 229 E and OC 43. *Am Rev Respir Dis*. 1972;105:805–811.
22. Pitkaranta A, Virolainen A, Jero J, et al. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics*. 1998;102:291–295.
23. Kusel MM, de Klerk NH, Holt PG, et al. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J*. 2006;25:680–686.
24. Dare RK, Fry AM, Chittaganpitch M, et al. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis*. 2007;196:1321–1328.
25. Boivin G, Baz M, Cote S, et al. Infections by human coronavirus-NL in hospitalized children. *Pediatr Infect Dis J*. 2005;24:1045–1048.
26. Cilla G, Onate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: high rate of viral coinfection. *J Med Virol*. 2008;80:1843–1849.