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Coronavirus Infection and Hospitalizations for Acute Respiratory Illness in Young Children

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There is only limited knowledge on the burden of disease due to both new (HCoV-NL63 and HKU-1) and previously discovered coronaviruses (OC43 and 229E) in children. Respiratory specimens and clinical data were prospectively collected in an active, population-based surveillance study over a 2-year period from children aged <5 years hospitalized with acute respiratory symptoms or fever. These samples were retrospectively tested by real-time RT-PCR for HCoV-NL63, HKU1, OC43, and 229E. Human coronaviruses (HCoVs) were identified in 2.2% of study children <2 years of age. Rates of HCoV-associated hospitalization per 10,000 were 10.2 (95% CI 4.3, 17.6), 4.2 (95% CI 1.9, 6.9), and 0 (95% CI 0, 3.7) in children aged <6 months, 6–23 months, and 24–59 months, respectively. Coronaviruses were identified in a modest number of hospitalized children. ***J. Med. Virol. 81:853–856, 2009.***

KEY WORDS: acute respiratory infection; OC43; 229E; HCoV-NL63; HCoV-HKU

INTRODUCTION

Human coronaviruses (HCoV) were initially identified as causes of acute upper respiratory tract infections in the 1960s using classical tissue culture methods [Kendall et al., 1962; Hamre and Procknow, 1966]. Two of these HCoV strains, designated OC43 and 229E, were detected in individuals with common colds, and occasionally in young children, elderly persons, immunocompromised individuals, and military personnel with lower respiratory tract infection (LRI) [McIntosh et al., 1974; Monto, 1974; Wenzel et al., 1974; Riski and Hovi, 1980; Chany et al., 1982; Sizun et al., 1994, 1995; Folz and Elkordy, 1999; Falsey et al., 2002; Gagneur et al., 2002]. Recently, three new HCoV strains have been identified using both molecular and traditional culture methods: severe acute respiratory syndrome coronavirus (SARS-CoV) [Drosten et al., 2003], human coronavirus Netherlands (HCoV-NL63) [Fouchier et al., 2004; van der Hoek et al., 2004], and human coronavirus Hong Kong (CoV-HKU1) [Woo et al., 2005]. SARS-CoV was associated with an outbreak of severe pneumonia that spread from Asia to other parts of the world, but since then has not caused human outbreaks. HCoV-NL63 and CoV-HKU1 have been associated with LRI. Although strains of HCoV have been identified in children, the true burden of severe disease from these viruses is unknown.

The objectives of this study were to determine the incidence of hospitalization and clinical features in children aged <5 years with acute respiratory infections

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(ARIs) associated with four HCoV strains (HCoV-NL63, CoV-HKU1, OC43, and 229E) from two distinct geographic areas through active population-based surveillance.

MATERIALS AND METHODS

Study Design

The New Vaccine Surveillance Network (NVSN) is a prospective, population-based surveillance network established by the Centers for Disease Control and Prevention (CDC) in 1999 to assess the annual burden of acute respiratory illness or fever due to respiratory viruses in children <5 years of age in two counties; Davidson County (Nashville, Tennessee) and Monroe County (Rochester, New York) [Iwane et al., 2004]. The NVSN recruited subjects from inpatient, outpatient clinic, and Emergency Department settings and population-based rates were calculated for each of these settings separately. We chose to focus on inpatients for this study since they represent more severe disease. During each year of surveillance, the inpatient sample was population-based. Study nurses obtained informed consent from parents or guardians of children who were county residents, obtained nasal and throat swabs for viral culture and polymerase chain reaction (PCR), and collected demographic and clinical information, as well as discharge diagnoses. Details of the methodology have been published previously [Iwane et al., 2004].

Laboratory Methods

Archival nasal and throat samples obtained from October 1, 2001 through September 30, 2003 in children hospitalized in the two counties were evaluated. Samples were delivered to the laboratory in viral transport media within 2 hr of collection. Specimens were divided into aliquots and stored at -80°C prior to testing. RNA was extracted by a Qiagen BioRobot 9604 Workstation using QIAamp Viral RNA kits (Qiagen, Valencia, CA). RNA was tested by real-time RT-PCR (Quantitect Probe RT-PCR; Qiagen) using primers and probes for the nucleocapsid genes of 229E, OC43 [van Elden et al., 2004], and HCoV-NL63 [Fouchier et al., 2004] and for the replicase 1b gene of HKU1 [Dare et al., 2007]. Specimens were also tested by culture or RT-PCR for influenza virus, respiratory syncytial virus (RSV), parainfluenza viruses (PIV), human metapneumovirus (hMPV), and rhinovirus [Iwane et al., 2004; Mullins et al., 2004; Poehling et al., 2006].

Statistical Analysis

Hospitalization rates were calculated as the weighted HCoV positive hospitalizations divided by US census county populations <5 years of age multiplied by 1,000. Weighting was based on percentage enrolled and surveillance days per week. Ninety-five percent confidence intervals were generated using 1,000 bootstrap samples, except for the rate in 24–59 month children, which used the exact Poisson method. Comparisons of characteristics in subjects with and without HCoV were performed using Pearson chi-square tests. All calculations were done using the Hmisc, epitools, and boot packages of R version 2.6.2 (Vienna, Austria).

RESULTS

Study Population

Of the 1,123 samples originally collected from hospitalized subjects during the 2 years of surveillance, 1,055 had sufficient archived aliquots for HCoV testing. Available and missing samples did not differ by gender, race, insurance, high-risk conditions, smoke exposure, siblings, daycare, or prematurity. However, compared to available samples, missing samples tended to be from older children ($P = 0.03$), living in Nashville ($P = 0.018$) and from the second study year ($P < 0.001$). All samples were tested, regardless of whether a virus had been identified previously. Of the samples tested, 47% were from children <6 months of age, 35% 6–23 months of age, and 18% 24–59 months of age; 55% were male, 49% white, and 34% black.

Virus Detection and Clinical Features

Nineteen samples (1.8%) were positive for HCoV: 4 HKU1, 12 HCoV-NL63, and 3 OC43. HCoV 229E was not detected in any sample. For comparison, influenza was detected in 5%, RSV 15.5%, hMPV 4%, and rhinoviruses 15.8% of the samples. All HCoV-positive specimens were from children <2 years of age and 47% were from children aged <6 months. More HCoVs were detected during the 2001–2002 than the 2002–2003 winter respiratory season (14/19 vs. 5/19) and most were HCoV-NL63 (9/14 and 3/5, respectively). Eleven of the 19 HCoV were detected in children with an underlying medical condition, while the other 8 were previously healthy. Symptoms associated with HCoV included cough (89%), fever (68%), nasal congestion (79%), poor appetite (68%), or shortness of breath (84%) in the majority of children, and diarrhea (32%), vomiting (21%), or wheezing (16%) in a minority of children.

The median duration of hospitalization for the 19 HCoV episodes was 2 days (range 1–7 days). Two children, one with asthma, were admitted to the ICU; nine required oxygen during their hospital stay; none of the children died. Three enrolled patients were diagnosed with Kawasaki syndrome and none of these tested positive for HCoV-NL63.

Discharge Diagnoses

Positive HCoV PCR samples were associated with several LRI diagnoses including pneumonia (n = 4), croup (n = 3), asthma (n = 2), and bronchiolitis (n = 3), but seven of the positives were assigned to other diagnoses such as seizure, apnea, otitis media, or ARI. All three children with croup had HCoV-NL63 isolated.

Viruses Co-Detected With HCoV

Of the 19 positive HCoV samples, 6 (32%) had one or more other viruses identified, including 4 RSV (1 HKU1 and 3 HCoV-NL63), 1 PIV (HCoV-NL63), and 1 hMPV (HCoV-NL63). None had co-detection of influenza virus or rhinovirus. One previously healthy child, with no underlying medical conditions, with both PIV and HCoV-NL63 detected was admitted to the ICU. Four of the six children diagnosed with bronchiolitis had more than one virus detected; three had RSV and one had hMPV. No difference was detected in days of illness or length of hospital stay for those patients found to have more than one virus than those with HCoV alone.

Burden of Illness

Because inpatient surveillance was population-based, the rate of HCoV-associated hospitalizations could be calculated. In children <2 years of age the overall hospitalization rate per 10,000 was 5.7 (95% CI 3.3–8.2). Rates were 10.2 (95% CI 4.3, 17.6) in those <6 months of age, 4.2 (95% CI 1.9, 6.9) in those 6–23 months of age, and 0 (95% CI 0, 3.7) for those 2–5 years of age (Table I).

DISCUSSION

HCoVs have long been associated with the common cold, but recently have been implicated in more severe disease. This study provided a unique opportunity to evaluate the relationship between HCoV and disease in young hospitalized children and to define the incidence of hospital admission associated with HCoV. Chiu et al. [2005] reported hospitalizations associated with HCoV-NL63 to be 22.4/10,000 children ≤6 years of age in Hong Kong. Rates of hospitalization associated with all four identified human HCoV in our study were much lower than rates of HCoV-NL63 alone reported in Hong Kong. This mirrors the much higher hospitalization rate for influenza in children in Hong Kong than in the US [Chiu et al., 2002]. An additional reason for the discrepancy in rates between the two studies may be regional and year-to-year variation in HCoV epidemiology. Interestingly, HCoV-associated hospitalizations in our study for ARI were only detected in children <2 years and the highest rates were in children <6 months of age. This could reflect greater susceptibility due to immunological immaturity of young children and/or a lack of maternal antibody in the younger children. The rates of HCoV-associated ARI hospitalization were lower than the reported rates of both influenza and RSV-associated hospitalizations from the same cohort during the same years [Poehling et al., 2006; Hall et al., 2008].

TABLE I. Burden of Illness for HCoV by Age Group

Age groups (months)	Hospitalizations per 10,000 child years (CI)
0–5	10.2 (4.3, 17.6)
6–23	4.2 (1.9, 6.9)
24–59	0 (0, 3.7)
0–24	5.7 (3.3, 8.2)
0–59	2.3 (1.3, 3.3)

HCoVs exhibit variable circulation from year-to-year [Dare et al., 2007] and hence this prospective study spanning 2 years may not represent the true spectrum of illness and disease burden for HCoV. The samples from the first year of this study are the same time period of samples tested by Chiu et al. [2005] who also detected more HCoV-NL63 than OC43 or 229E during that year. However studies done in 2003–2004 showed very different results. Dare et al. [2007] identified much higher percentages of disease associated with OC43 than HCoV-NL63 in Thailand, and a study by Esposito showed greater proportions of children with 229E than OC43 or HCoV-NL63 in Italy [Esposito et al., 2006]. Hence there may be regional and yearly variation in the circulation of individual coronaviruses.

The clinical significance of co-detection of other viruses is unclear. PCR methodology is very sensitive and can detect low viral titers resulting from recent prior infections, although the duration of detection for different viruses has not been established. Smuts et al. [2008] showed that dual infection occurred in 2.5% of respiratory tract infections. One study found higher rates of hospital admission in children with more than one virus detected [Drews et al., 1997]. While detection of HCoV does not prove a causal relationship with ARI, the finding of HCoV in the absence of other respiratory viruses, and the differential concentration of these infections in very young hospitalized children suggests a primary role for HCoV. Despite the large number of children enrolled in the study, the small numbers with HCoV limits our ability to determine if the morbidity is higher for children with multiple viruses detected than for those with a single virus. In addition, the lack of specimens from healthy children limits the inference of a disease association.

Although our sample was small, we found no association between any of the four tested HCoV infections and Kawasaki syndrome, consistent with other studies that have failed to confirm this suggested association [Rowley, 2006]. However, we did find subjects with HCoV-NL63 discharged with a diagnosis of croup as previously described [van der Hoek et al., 2005; Ko et al., 2007; Wu et al., 2008].

This study has several limitations. We did not assess bacterial co-infection or antibiotic use. In addition, we did not test specimens from asymptomatic control subjects. Currently it is not known how long children shed HCoV, or how long they would test positive by RT-PCR. However, the majority of HCoV-infected children (68%) did not have another virus detected using sensitive RT-PCR assays.

In conclusion, HCoVs are associated with a relatively low incidence of ARI hospitalizations among preschool children. The spectrum of clinical disease in children hospitalized with HCoV resembles the clinical features from other respiratory viruses. Additional population-based studies are needed to complement our data and to characterize HCoV variability over time and location.

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