Journal of Medical Virology 78:1609–1615 (2006)

Impact of Human Coronavirus Infections in Otherwise Healthy Children Who Attended an Emergency Department

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This prospective clinical and virological study of 2,060 otherwise healthy children aged <15 years of age (1,112 males; mean age ± SD, 3.46 ± 3.30 years) who attended the Emergency Department of Milan University's Institute of Pediatrics because of an acute disease excluding trauma during the winter season 2003–2004 was designed to compare the prevalence and clinical importance of human coronaviruses (HCoVs) in children. Real-time polymerase chain reaction (PCR) in nasopharyngeal aspirates revealed HCoV infection in 79 cases (3.8%): 33 HCoV-229E (1.6%), 13 HCoV-NL63 (0.6%), 11 HCoV-OC43 (0.5%), none HCoV-HKU1 genotype A, and 22 (1.1%) co-detections of a HCoV and another respiratory virus. The HCoVs were identified mainly in children with upper respiratory tract infection; there was no significant difference in clinical presentation between single HCoV infections and HCoV co-infections. Diagnostic methods were used in a limited number of patients, and the therapy prescribed and clinical outcomes were similar regardless of the viral strain. There were a few cases of other members of the households of HCoV-positive children falling ill during the 5–7 days following enrollment. These findings suggest that HCoV-229E and HCoV-OC43 have a limited clinical and socioeconomic impact on otherwise healthy children and their household contacts, and the HCoV-NL63 identified recently does not seem to be any different. The quantitative and qualitative role of HCoV-HKU1 genotype A is apparently very marginal. ***J. Med. Virol. 78:1609–1615, 2006.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: human coronaviruses; epidemiology; respiratory tract infections; children

INTRODUCTION

Although they have been recognized since the mid-1960s, human coronaviruses (HCoVs) have received relatively little attention as human pathogens because the HCoV-229E and HCoV-OC43 strains were considered mainly as the etiological agents of common cold and only occasionally as the cause of severe lower respiratory tract infections in infants and immunocompromized hosts [Baker, 2004; Kahn and McIntosh, 2005; McIntosh, 2005]. However, their clinical importance has been re-evaluated following the identification of two apparently more virulent strains: one associated with severe acute respiratory syndrome (SARS) and the NL63 strain (HCoV-NL63) [Drosten et al., 2003; Ksiazek et al., 2003; Peiris et al., 2003; Fouchier et al., 2004; van der Hoek et al., 2004; Esper et al., 2005], which have been found in children with croup, bronchiolitis, and pneumonia, particularly in the first months of life [Arden et al., 2005; Bastien et al., 2005; Boivin et al., 2005; Chiu et al., 2005; Ebihara et al., 2005; Kaiser et al., 2005; Suzuki et al., 2005; van der Hoek et al., 2005]. A novel HCoV (HCoV-HKU1) has also been detected in adults with pneumonia and children with lower respiratory tract infection [Woo et al., 2005; Sloots et al., 2006; Vabret et al., 2006]. However, there are few data comparing the prevalence and clinical importance of

Appropriate informed consent was obtained and the study was conducted in accordance with the guidelines for human experimentation specified by the authors' institutions. No author has any commercial or other association that might pose a conflict of interest.

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Accepted 9 August 2006

DOI 10.1002/jmv.20745

Published online in Wiley InterScience (www.interscience.wiley.com)

the different HCoVs, and the real pediatric impact of each needs to be established better.

This prospective clinical and virological study of otherwise healthy children who attended the Emergency Department of Milan University's Institute of Pediatrics because of acute disease during the winter season of 2003–2004 was carried out in order to determine the prevalence and clinical features of the illnesses associated with infections due to different respiratory viruses, and the findings are described.

PATIENTS AND METHODS

Study Design and Study Population

This prospective study was carried out at the Institute of Pediatrics, University of Milan, Italy, between November 1, 2003 and March 31, 2004. The study protocol was approved by the University's Institutional Review Board and the written informed consent of a parent or legal guardian was required before the children were enrolled.

The study involved all of the patients aged less than 15 years who attended the Emergency Department on Wednesday and Sunday each week during the study period with an acute disease excluding trauma. The other exclusion criteria were chronic diseases increasing the risk of complications of viral respiratory infections, including premature birth; chronic disorders of the pulmonary or cardiovascular system including asthma; chronic metabolic diseases including diabetes mellitus; neoplasia; kidney or liver dysfunction; hemoglobinopathies; immunosuppression; diseases requiring long-term aspirin therapy; genetic or neurologic disorders. There were no refusals to participate.

Patient Enrollment and Evaluation

Upon enrollment, systematic records were made of the patients' demographic characteristics and medical history using standardized written questionnaires [Principi et al., 2004a; Bosis et al., 2005; Esposito et al., 2005]. The questions included detailed signs and symptoms of the acute disease; the required laboratory and/or radiological examinations; the prescribed drug therapy; any previous administration of influenza vaccine and/or of any RSV immune prophylaxis; family size and the number of siblings; parents' education and occupation; family living conditions; and information concerning child-care attendance. After a complete physical examination, including body temperature, the children were classified on the basis of the final diagnosis of the pediatrician-in-charge using well-established criteria [Feigin and Cherry, 1998]. Fever was defined as the presence of an axillary temperature of $\geq$37.8°C or a rectal temperature of $\geq$38°C.

Nasopharyngeal aspirates were collected upon enrollment by means of Virocult swabs (Medical Wire and Equipment, Corsham, UK), and were then tested by means of previously described real-time polymerase chain reaction (PCR) for adenovirus, influenza virus types A and B, respiratory syncytial virus (RSV) types A and B, parainfluenza viruses types 1, 2, 3, and 4, rhinoviruses, and human metapneumovirus (hMPV), HCoVs types 229E, OC43, NL63, and HKU1 genotype A, at the Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands [Heim et al., 2003; Fouchier et al., 2004; Kares et al., 2004; Maertzdorf et al., 2004; van der Hoek et al., 2004; Bosis et al., 2005; Woo et al., 2005]. Total nucleic acids were isolated routinely on the MagnaPureLC Isolation Station (Roche Applied Science, Penzberg, Germany). A universal internal control virus was used [Niesters, 2002] to monitor the whole process from isolation of nucleic acids until real-time detection. In-house real-time PCR for HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 as well for the internal control phocine distemper virus (PDV) was designed using the primer express software (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). For all HCoVs, the assay was designed in the nucleoprotein gene, for PDV in the hemagglutinin gene (Table I).

RNA was amplified in a single tube, two-step reaction using the Taqman reverse transcription reagents kit and PCR core reagent kit (Applied Biosystems) on an ABI 7700 or ABI 7500 sequence detection system (Applied Biosystems).

As positive control for each assay, a cultured control virus was used except for HKU1 genotype A. In the latter case, a construct was synthesized based on published sequences containing part of the nucleoprotein gene and cloned. Based on data from proficiency testing [Templeton et al., 2006], the sensitivity of each of the assays was estimated less than 500–1,000 copies per ml. No cross-reactivity with other HCoVs was observed.

The medical history of the study children was re-evaluated 5–7 days after enrollment and until the resolution of their illness by means of interviews and clinical examinations by trained investigators using standardized questionnaires [Principi et al., 2004a; Bosis et al., 2005; Esposito et al., 2005]. During these evaluations, information was also obtained regarding acute illnesses and related morbidity among household contacts. The children's parents or legal guardians were asked to answer a list of questions regarding the outcome of their child's disease (e.g., final diagnosis, administered medications, hospitalization, duration of signs and symptoms, medical visits, examinations, the number of lost school days) and the involvement of other members of the household (e.g., respiratory diseases in household contacts, medication, hospitalization, medical visits, the number of working days lost by parents to care for their ill children and their own respiratory diseases, the number of days of domestic help required to care for ill children).

Statistical Analysis

The data were analyzed using SAS Window v.12 (SAS Institute Inc., Cary, NC), and compared on the basis of the type of HCoV infection. The cases with co-detection

TABLE I. Primer Sequences and Concentrations of Real-Time PCR of HCoVs and Internal Control

Virus	Amplicon length (bp)	Sense primer (concentration/reaction, pmol)	Anti-sense primer (concentration/reaction, pmol)	Probe (concentration/reaction, pmol)
HCoV-229E	83	5′-CGCAAGAATTCAGAACCAGAG-3′ (10)	5′-GGGAGTCAGGTTCTTCAACAA-3′ (15)	5′-FAM-CCACACTTCAATCAAAAGCTCCCAAATG-3′ (5)
HCoV-OC43	67	5′-GCTCAGGAAGGTCTGCTCC-3′ (45)	5′-TCCTGCACTAGAGGCTCTGC-3′ (2,5)	5′-FAM-TTCCAGATCTACTTCGCGCACATCC-3′ (10)
HCoV-NL63	98	5′-AGGACCTTAAATTCAGACAACGTTCT-3′ (30)	5′-GATTACGTTTGCGATTACCAAGACT-3′ (15)	5′-FAM-TAACAGTTTTAGCACCTTCCTTAGCAACC-CAAACA-3′ (10)
HCoV-HKU1	64	5′-AGTTCCCATTGCTTTCGGAGTA-3′ (5)	5′-CCGGCTGTGTCTATACCAATATCC-3′ (15)	5′-FAM-CCCCTTCTGAAGCAA-MGB-3′ (5)
PDV	78	5′-CGGGTGCCTTTTACAAGAAC-3′ (30)	5′-TTCTTTCCTCAACCTCGTCC-3′ (40)	5′-FAM-ATGCAAGGGCCAATTCTTCCAAGTT-3′ (5)

HCoV, coronavirus; PDV, phocine distemper virus.

of HCoVs and other respiratory viruses were considered together as an extra group.

A P-value of <0.05 was considered statistically significant for all the tests. Parametric data were compared by analysis of variance (ANOVA); abnormally distributed or non-parametric data were analyzed using the Kruskal-Wallis test. Categorical data were analyzed using contingency analysis and the Chi-squared or Fisher's test.

RESULTS

A total of 2,060 children aged less than 15 years were enrolled (1,112 males; mean age ±SD, 3.46±3.30 years). The age distribution of the study population was: 41.6% aged <2 years, 33.1% aged 2–5 years, and 25.3% aged >5 years. The distribution of acute diseases for which the children attended the Emergency Department was: 56.9% respiratory tract infection, 14.2% gastrointestinal and intra-abddominal disease, 5.4% fever without source, 4.8% seizures with or without fever, 4.7% exanthematic disease, 4.4% nephritic or nephrosic syndrome, 3.4% skin and soft tissue infection, 2.0% bone or joint infection, 1.7% coagulation disorder, 1.0% meningitis/encephalitis, 0.9% sepsis, and 0.6% conjunctivitis.

Infection due to a HCoV strain was diagnosed in 79 cases (3.8%). It was significantly less frequent than infection due to influenza viruses (235 cases, 11.4%), RSV (171 cases, 8.3%), adenovirus (136 cases, 6.6%), and rhinoviruses (130 cases, 6.3%). HCoV infection was significantly more frequent than infection due to hMPV (48 cases, 2.3%) and parainfluenza viruses (29 cases, 1.4%). Fifty seven (72.2%) of the 79 HCoV-positive cases were single HCoV infections: 33 (1.6%) due to HCoV-229E, 13 due to HCoV-NL63 (0.6%), and 11 due to HCoV-OC43 (0.5%); none of the study children was positive for HCoV-HKU1 genotype A. The remaining 22 children (1.1%) showed co-infection of a HCoV and another respiratory virus (Table II), the most frequent association being HCoV and RSV infection. The frequency of the involvement of the three HCoV strains detected was similar: 40.9% HCoV-229E, 31.8% HCoV-NL63, and 27.3% HCoV-OC43. Two was the maximum number of detections per specimen.

Table III shows the enrollment characteristics of the study children diagnosed as having HCoV infection. There was no significant difference in gender distribution, but the mean age of the children in the HCoV-229E and HCoV+other respiratory viruses groups was significantly higher. The majority of the children (55.7%) had fever at enrollment but only few of them were diagnosed as patients with fever without source. HCoVs were mainly identified in children with respiratory tract infections (with upper respiratory tract infections being more common than lower respiratory tract infections), but a significant number of children in all of the groups were affected by gastrointestinal infections (defined as infectious diarrhea with or without vomit). There were no significant differences in clinical presentation

TABLE II. Co-Detections of a Coronavirus and Another Respiratory Virus in the Study Population

Co-detection	Number of cases (%)
HCoV + RSV	7 (31.8)*
HCoV-229E + RSV	4
HCoV-NL63 + RSV	2
HCoV-OC43 + RSV	1
HCoV + adenovirus	5 (22.7)
HCoV-NL63 + adenovirus	3
HCoV-OC43 + adenovirus	2
HCoV + hMPV	4 (18.2)
HCoV-229E + hMPV	2
HCoV-OC43 + hMPV	2
HCoV + influenza	3 (13.6)
HCoV-229E + influenza	2
HCoV-NL63 + influenza	1
HCoV + rhinovirus	2 (9.1)
HCoV-NL63 + rhinovirus	1
HCoV-OC43 + rhinovirus	1
HCoV + parainfluenza virus	1 (4.6)
HCoV-229E + parainfluenza virus	1
Total	22 (100.0)

HCoV, coronavirus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus.
*$P < 0.05$ versus HCoV + parainfluenza virus; no other significant differences between the groups.

between the individual HCoV strains, or between single HCoV infection and HCoV co-detections.

Table IV summarizes the data concerning diagnostic methods at enrollment, therapeutic approaches, and clinical outcomes among the study children with demonstrated HCoV infection. Further diagnostic methods were used in a limited number of cases regardless of the HCoV strain or the presence of HCoV co-detections, and prescribed therapy and clinical outcome were similar in all four groups, with marginal rates of hospital admissions.

Table V shows the socioeconomic impact of HCoV infections on the household contacts of the study children. In the 5–7 days following enrollment, household members suffered a limited number of diseases similar to those of the infected children, with a significantly higher frequency of illnesses among the households of HCoV-229E- and HCoV-OC43-positive children. In line with these findings, additional medical visits and antipyretic prescriptions were significantly more common among the households of the HCoV-229E- and HCoV-OC43-positive children than among those who were HCoV-NL63 positive or infected by HCoV and other respiratory viruses. There were no between-group differences in terms of antibiotic prescriptions or lost working days by mothers or fathers. The impact of the HCoV infection of the study children on household hospitalization rates seemed to be marginal: only one sibling of a child with HCoV and other respiratory viruses required hospital admission.

DISCUSSION

This study suggests that HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 genotype A play a limited role in the acute diseases of otherwise healthy infants and children who attended an Emergency Department. Only 3.8% of the study population was infected by one of these viruses, and the importance of HCoVs in attendance to the Emergency Department was significantly less than that of other common respiratory viruses including influenza viruses, RSV, adenovirus, and rhinoviruses. As HCoVs are one of the main causes of the common cold and circulate widely during the winter [Baker, 2004; Kahn and McIntosh, 2005; McIntosh, 2005], their low prevalence in this study population can be explained by their limited pathogenicity and the consequent fact that most infected children are treated by general practitioners rather than in hospitals. Differences could also be explained by the fact that the majority of previous

TABLE III. Characteristics at Enrollment of the Study Children in Whom Coronavirus Infection Was Diagnosed

Characteristics	HCoV-229E (n = 33)	HCoV-NL63 (n = 13)	HCoV-OC43 (n = 11)	HCoV + other respiratory viruses (n = 22)
Demographic data				
Gender, males (%)	21 (63.6)	6 (54.6)	6 (46.2)	10 (45.4)
Mean age ± SD (years)	3.43 ± 3.02*	2.02 ± 1.45	1.42 ± 1.12	3.85 ± 3.42*
Clinical presentation				
Presence of fever (%)	20 (60.6)	7 (53.8)	6 (54.6)	11 (50.0)
Respiratory tract infection (%)	25 (75.8)**	10 (76.9)**	9 (81.8)**	18 (81.8)**
Common cold (%)	9 (27.3)	3 (23.1)	3 (27.3)	6 (27.2)
Pharyngitis (%)	12 (36.4)	3 (23.1)	4 (36.4)	7 (31.8)
Acute otitis media (%)	1 (3.0)	1 (7.7)	2 (18.2)	3 (13.6)
Croup (%)	1 (3.0)	1 (7.7)	0 (0)	0 (0)
Wheezing (%)	1 (3.0)	1 (7.7)	0 (0)	1 (4.5)
Pneumonia (%)	1 (3.0)	1 (7.7)	0 (0)	1 (4.5)
Gastrointestinal tract infection (%)	5 (15.2)	3 (23.1)	2 (18.2)	3 (13.6)
Conjunctivitis (%)	1 (3.0)	0 (0)	0 (0)	0 (0)
Fever without source (%)	2 (6.0)	0 (0)	0 (0)	1 (4.6)

HCoV, coronavirus;
*$P < 0.05$ versus HCoV-NL63 and versus HCoV-OC43.
**$P < 0.05$ versus gastrointestinal tract infection, versus conjunctivitis, and versus fever without source; no other significant differences between the groups.

TABLE IV. Diagnostic Methods at Enrollment, Therapeutic Approaches, and Clinical Outcomes Among the Study Children Diagnosed as Having Coronavirus Infection

Characteristics	HCoV-229E (n = 33)	HCoV-NL63 (n = 13)	HCoV-OC43 (n = 11)	HCoV + other respiratory viruses (n = 22)
Diagnostic methods				
Routine blood examinations (%)	4 (12.1)	1 (7.7)	1 (9.1)	3 (13.6)
Microbiological examinations (%)	4 (12.1)	1 (7.7)	1 (9.1)	3 (13.6)
Chest radiography (%)	1 (3.0)	1 (7.7)	0 (0)	0 (0)
Therapeutic approaches				
Antipyretic prescriptions (%)	19 (57.6)	6 (46.2)	6 (54.5)	15 (68.2)
Antibiotic prescriptions (%)	11 (33.3)	3 (23.1)	5 (45.5)	6 (27.3)
Bronchodilator prescriptions (%)	1 (3.0)	1 (7.7)	0 (0)	2 (9.1)
Steroid prescriptions (%)	1 (3.0)	1 (7.7)	0 (0)	1 (4.5)
Clinical outcome				
Hospitalization (%)	3 (9.0)	1 (7.7)	1 (9.1)	2 (9.1)
School absence, median days (range)	4 (2–5)	4 (2–5)	4 (2–5)	4 (2–5)

No significant differences between the groups.
HCoV, coronavirus.

studies were retrospective, mainly tested specimens that were negative for other pathogens, and included only children with respiratory symptoms [Arden et al., 2005; Bastien et al., 2005; Chiu et al., 2005; Kaiser et al., 2005; Suzuki et al., 2005]. Moreover, considering the peak age of HCoVs infection according to the HCoV literature [Bastien et al., 2005; Chiu et al., 2005; Suzuki et al., 2005], also the low mean age of the children enrolled in this study may explain the low prevalence of HCoV infection. Furthermore, taking into account that classical HCoV infections are known to occur in 2- to 3-year epidemic cycles and scattered outbreaks [Kahn and McIntosh, 2005], the study population could be from a season of low HCoV activity. Finally, despite their high sensitivity and specificity [Fouchier et al., 2004; van der Hoek et al., 2004; Woo et al., 2005], the assays used in this study may not have detected all HCoV strains.

HCoV-229E was detected more frequently than HCoV-OC43 or HCoV-NL63. In fact, the most striking difference between these and other recently published data [Arden et al., 2005; Bastien et al., 2005; Chiu et al., 2005; Kaiser et al., 2005; Suzuki et al., 2005] is the marginal epidemiological and clinical importance of HCoV-NL63 in this study, despite the fact that it has been associated with croup [van der Hoek et al., 2005], asthma exacerbation [Arden et al., 2005; Chiu et al., 2005; Ebihara et al., 2005; Esper et al., 2005; Suzuki et al., 2005], febrile seizures, and high fever [Bastien et al., 2005; Chiu et al., 2005] leading to hospital admission as well as upper respiratory tract infection [Bastien et al., 2005; Boivin et al., 2005; Chiu et al., 2005; Esper et al., 2005]. In this prospective study of a large group of otherwise healthy children evaluated for acute diseases in the Emergency Department, HCoV-NL63 was mainly found in patients with mild diseases and its clinical impact was no different from that of HCoV-229E and HCoV-OC43. Only one child infected by HCoV-NL63 was admitted to hospital, and a similar number of children infected by the different HCoVs strains required laboratory, microbiological, and radiological diagnostic investigations.

It is interesting to note that the various HCoVs can be co-detected with different types of respiratory viruses, although it is difficult to establish whether these are true co-infections or whether one of the pathogens represents continued shedding from a previous infection [Kaiser et al., 2005]. Nevertheless, the clinical pictures of cases with co-infection with HCoV-229E, HCoV-NL63, or HCoV-OC43, and other respiratory viruses appeared to be similar to those observed in cases of single HCoV infection.

No case of HCoV-HKU1 genotype A infection was detected in this study, and as only a few such patients have so far been described [Woo et al., 2005; Sloots et al., 2006; Vabret et al., 2006], its real importance is impossible to define. Moreover, no conclusion could be drawn on the presence of HCoV-HKU1 genotype B or C because the HKU1 primers used in this study could detect only genotype A. However, the fact that all of the reported HCoV-HKU1 cases experienced a lower respiratory tract infection requiring hospital admission suggests that its clinical impact may be substantial [Woo et al., 2005; Sloots et al., 2006; Vabret et al., 2006], but the fact that there was no case among a large study population suggests that its circulation and quantitative importance may be marginal.

The limited pathogenicity of HCoVs is further demonstrated by their restricted socioeconomic impact on household contacts. Only a small number of the family members of the HCoV-positive cases fell ill during the 5–7 days following the children's enrollment, and, consequently, received few antipyretics and antibiotics, and lost a limited number of working and school days. In comparison with previous findings concerning influenza and hMPV infection [Principi et al., 2004a,b; Bosis et al., 2005; Esposito et al., 2005], it seems that HCoVs only involve marginally the family. Furthermore, the socioeconomic impact of HCoV-NL63 was even less than that of HCoV-229E and HCoV-OC43.

In conclusion, the data show that HCoV-229E and HCoV-OC43 have a limited clinical impact in otherwise healthy children, and are rarely involved in causing diseases requiring attendance at the Emergency

TABLE V. Socioeconomic Impact of Infections Due to Coronaviruses on the Household Contacts of the Study Children

Characteristics	Households of HCoV-229E-positive children (n = 94)	Households of HCoV-NL63-positive children (n = 45)	Households of HCoV-OC43-positive children (n = 38)	Households of children with HCoV+other respiratory viruses (n = 76)
Disease similar to that of the infected children, no. (%)	14/94 (14.9)*	0/45 (0)	5/38 (13.2)*	6/76 (7.9)
Mothers, no. (%)	6/33 (18.2)	0/13 (0)	4/11 (36.4)*	2/22 (9.1)
Fathers, no. (%)	5/33 (15.2)	0/13 (0)	1/11 (9.1)	1/22 (4.5)
Siblings, no. (%)	3/28 (10.7)	0/19 (0)	0/16 (0)	3/32 (9.4)
Additional medical visits, no. (%)	9/94 (9.6)*	0/45 (0)	4/38 (10.5)*	3/76 (3.9)
Mothers, no. (%)	3/33 (9.1)	0/13 (0)	3/11 (27.3)	0/22 (0)
Fathers, no. (%)	2/33 (6.1)	0/13 (0)	1/11 (9.1)	0/22 (0)
Siblings, no. (%)	4/28 (14.3)	0/19 (0)	0/16 (0)	3/32 (9.4)
Antipyretic prescriptions, no. (%)	9/94 (9.6)*	0/45 (0)	4/38 (10.5)*	3/76 (3.9)
Mothers, no. (%)	4/33 (12.1)	0/13 (0)	3/11 (27.3)	1/22 (4.5)
Fathers, no. (%)	3/33 (9.1)	0/13 (0)	1/11 (9.1)	0/22 (0)
Siblings, no. (%)	2/28 (7.1)	0/19 (0)	0/16 (0)	2/32 (6.3)
Antibiotic prescriptions, no. (%)	3/94 (3.2)	0/45 (0)	1/38 (2.6)	1/76 (1.3)
Mothers, no. (%)	1/33 (3.0)	0/13 (0)	1/11 (9.1)	0/22 (0)
Fathers, no. (%)	1/33 (3.0)	0/13 (0)	0/11 (0)	0/22 (0)
Siblings, no. (%)	1/28 (3.6)	0/19 (0)	0/16 (0)	1/32 (3.1)
Hospitalization, no. (%)	0/94 (0)	0/45 (0)	0/38 (0)	1/76 (1.3)
Mothers, no. (%)	0/33 (0)	0/13 (0)	0/11 (0)	0/22 (0)
Fathers, no. (%)	0/33 (0)	0/13 (0)	0/11 (0)	0/22 (0)
Siblings, no. (%)	0/28 (0)	0/19 (0)	0/16 (0)	1/32 (3.1)
Lost working days by mothers, median (range)	2 (1–3)	0 (0)	2 (1–4)	2 (1–3)
Lost working days by fathers, median (range)	3.5 (2–5)	0 (0)	4 (4–4)	0 (0)
Lost school days by siblings, median (range)	2 (1–2)	0 (0)	0 (0)	2 (1–5)

*$P < 0.05$ versus households of HCoV-NL63-positive children; no other significant differences between the groups.
HCoV, coronavirus.

Department. The demonstration of a marginal socioeconomic impact of the infected patients on their households confirm further the limited role of HCoVs. The recently identified HCoV-NL63 does not seem to be any different in terms of clinical or socioeconomic importance, and HCoV-HKU1 genotype A seems to play a marginal quantitative and qualitative role. Further community-based studies involving different countries and children with chronic underlying conditions should make it possible to determine the role of HCoVs more precisely, and identify the children in whom these viruses can lead to severe clinical problems.

REFERENCES

Arden KE, Nissen MD, Sloots TP, Mackay IM. 2005. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. J Med Virol 75:455–462.

Baker SC. 2004. Coronaviruses. From common colds to severe acute respiratory syndrome. Pediatr Infect Dis J 23:1049–1050.

Bastien N, Robinson JL, Tse A, Lee BE, Hart L, Li Y. 2005. Human coronavirus NL-63 infections in children: A 1-year study. J Clin Microbiol 43:4567–4573.

Boivin G, Baz M, Coté S, Gilca R, Deffrasnes C, Leblanc E, Bergeron MG, Déry P, De Serres G. 2005. Infections by human coronavirus-NL in hospitalized children. Pediatr Infect Dis J 24:1045–1048.

Bosis S, Esposito S, Niesters HGM, Crovari P, Osterhaus ADME, Principi N. 2005. Impact of human metapneumovirus in childhood: comparison with respiratory syncytial virus and influenza viruses. J Med Virol 75:101–104.

Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LLM, Peiris JSM. 2005. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clin Infect Dis 40:1721–1729.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Rabenau H, Panning M, Kolesnikona L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1967–1976.

Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. 2005. Detection of human coronavirus NL63 in young children with bronchiolitis. J Med Virol 75:463–465.

Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. 2005. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. J Infect Dis 191:492–498.

Esposito S, Gasparini R, Bosis S, Marchisio P, Tagliabue C, Tosi S, Bianchi C, Crovari P, Principi N. 2005. Clinical and socioeconomic impact of influenza and respiratory syncytial virus (RSV) infection on healthy children and their households. Clin Microbiol Infect 11:933–936.

Feigin RD, Cherry JD, editors. 1998. Textbook of pediatric infectious diseases. 4th edition. Philadelphia, PY: W.B. Saunders Company.

Fouchier RA, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, Osterhaus AD. 2004. A previously undescribed coronavirus associated with respiratory disease in humans. Proc Natl Acad Sci USA 101:6212–6216.

Heim A, Ebnet C, Harste G, Pring-Akerblom P. 2003. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. J Med Virol 70:228–239.

Kahn JS, McIntosh K. 2005. History and recent advances in coronavirus discovery. Pediatr Infect Dis J 24:S223–S227.

Kaiser L, Regamey N, Roiha H, Deffernez C, Frey U. 2005. Human coronavirus NL63 associated with lower respiratory tract symptoms in early life. Pediatr Infect Dis J 24:1015–1017.

Kares S, Lonnrot M, Vuorinen P, Oikarinen S, Taurianen S, Hyoty H. 2004. Real-time PCR for rapid diagnosis of entero- and rhinovirus infections using LightCycler. J Clin Virol 29:99–104.

Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, DeRisi J, Yang JY, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ. 2003. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348:1953–1966.

Maertzdorf J, Wang CK, Brown JB, Quinto JD, Chu M, de Graaf M, van den Hoogen BG, Spaete R, Osterhaus AD, Fouchier RA. 2004. Real-time reverse transcriptase PCR assay for detection of human metapneumovirus from all known genetic lineages. J Clin Microbiol 42:981–986.

McIntosh K. 2005. Coronaviruses in the limelight. J Infect Dis 191: 489–491.

Niesters HG. 2002. Clinical virology in real time. J Clin Virol 25:S3–S12.

Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yee WK, Yan WW, Cheung MT, Cheng VC, Chan KH, Tsang DN, Yung RW, Ng TK, Yuen KY, SARS Study Group. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361: 1319–1325.

Principi N, Esposito S, Bosis S. 2004a. Human metapneumovirus in otherwise healthy infants and children. N Engl J Med 350:1788–1790.

Principi N, Esposito S, Gasparini R, Marchisio P, Crovari P, for the Flu-Flu Study Group. 2004b. Burden of influenza in healthy children and their households. Arch Dis Child 89:1002–1007.

Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. 2006. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. J Clin Virol 35:99–102.

Suzuki A, Okamoto M, Ohmi A, Watanabe O, Miyabayashi S, Nishimura H. 2005. Detection of human coronavirus-NL63 in children in Japan. Pediatr Infect Dis J 24:645–646.

Templeton KE, Forde CB, Loon AM, Claas EC, Niesters HG, Wallace P, Carman WF. 2006. A multi-centre pilot proficiency programme to assess the quality of molecular detection of respiratory viruses. J Clin Virol 35:51–58.

Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. 2006. Detection of the new human coronavirus HKU1: A report of 6 cases. Clin Infect Dis 42:634–639.

van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. 2004. Identification of a new human coronavirus. Nat Med 10:368–373.

van der Hoek L, Sure K, Ihorst G, Stang A, Pyrc K, Jebbink MF, Petersen G, Forster J, Berkhout B, Uberla K. 2005. Croup is associated with the novel coronavirus NL63. PLoS Med 2:e240.

Woo PCY, Lau SKP, Chu C, Tsoi HW, Chan KH, Yuen KY. 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 79:884–895.