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Detection of Human Coronavirus NL63 in Young Children With Bronchiolitis

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HCoV-NL63, the fourth human coronavirus, has been isolated recently from children with respiratory tract infections, including upper respiratory infection, bronchiolitis, and pneumonia. The virus has been also detected in immunocompromised adults with respiratory tract infections. A total of 118 nasopharyngeal swab samples from 118 hospitalized young children aged less than 2 years with bronchiolitis who were not infected with human respiratory syncytial virus, influenza A or B, or human metaneumovirus were selected. Three (2.5%) of the 118 samples were positive for HCoV-NL63 by reverse transcription-polymerase chain reaction tests. HCoV-NL63 may be one of the causative agents of bronchiolitis in young children. ***J. Med. Virol. 75:463–465, 2005.***

KEY WORDS: HCoV-NL63; acute bronchiolitis; acute laryngotracheobronchitis

INTRODUCTION

Coronaviruses, a genus of the Coronaviridae family, have been identified in humans and animals and are divided genetically and serologically into four groups [Holmes and Lai, 1996]. Three strains of coronaviruses, HCoV-229E, HCoV-OC43, and severe acute respiratory syndrome-associated coronavirus (SARS-CoV), have been associated with various respiratory illnesses in humans ranging from common cold to severe pneumonia. HCoV-229E and HCoV-OC43, which were discovered as causes of the common cold in the 1960s [Holmes and Lai, 1996], belong to group 1 and group 2, respectively. Both strains have been identified occasionally as agents of lower respiratory tract infections in infants, older adults, and immunocompromised hosts [McIntosh et al., 1974; Nicholson et al., 1997; Glezen et al., 2000]. SARS-CoV, which was identified as a pathogen causing severe respiratory illnesses in 2003, belongs to group 4 [Ksiazek et al., 2003].

A new human coronavirus, NL63 (HCoV-NL63), was discovered first from a 7-month-old Dutch child with bronchiolitis and conjunctivitis by van der Hoek et al. [2004]. Fouchier et al. [2004] also identified the new human coronavirus (HCoV-NL) from an 8-month-old boy with pneumonia. Since no official name has been given to the new human coronavirus by the International Committee on Taxonomy of Viruses yet, the name "HCoV-NL63" was used tentatively to designate the virus in this study. Since their sequence studies showed that HCoV-NL63 was related most closely to HCoV-229E, it was classified into group 1 [Fouchier et al., 2004; van der Hoek et al., 2004]. These two studies showed that the virus seemed to be associated with non-fatal upper and lower respiratory tract infection in young children and immunocompromised adults.

In this study, nasopharyngeal swab samples obtained from hospitalized young children with bronchiolitis were investigated for the presence of HCoV-NL63.

MATERIALS AND METHODS

Patients

From October 2002 to September 2003, a total of 118 nasopharyngeal swab samples were collected from 118 children aged less than 2 years who were diagnosed as having bronchiolitis and admitted to hospitals in Sapporo, Japan. All of the samples were collected after excluding the possibility of infection with hRSV or influenza A or B by rapid antigen detection tests and excluding the possibility of infection with human metaneumovirus (hMPV) by a reverse transcription-polymerase chain reaction (RT-PCR) test [Ebihara et al., 2004]. The median age of the children was 11.5 months. The male-to-female ratio was 1.7 to 1. All samples were

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TABLE I. Detection of HcoV-NL63 in 2002–2003

Periods	Positive no./no. tested
October 2002–November 2002	0/20
December 2002–January 2003	0/26
February 2003–March 2003	3/22
April 2003–May 2003	0/38
June 2003–July 2003	0/8
August 2003–September 2003	0/4

collected after obtaining informed consent from the children's parents.

RT-PCR Test and Sequencing

Total RNA was extracted from each sample by using the RNAzolTMB (TEL-TEST, Inc., Friendswood, TX) method according to the manufacturer's protocol. cDNA was synthesized from total RNA by using a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Piscataway, NJ). The PCR primers used for detection of HCoV-NL63 have been published previously [van der Hoek et al., 2004]. Two primer pairs were used for a nested PCR to detect a 169-nucleotide replicase 1b gene fragment of HCoV-NL63. A forward primer with a sequence of 5′-GTGATGCATATGCTAATTTG-3′ and a reverse primer with a sequence of 5′-CTCTTGCAGG-TATAATCCTA-3′ were used for the first PCR. The second PCR was performed by using a forward primer with a sequence of 5′-TTGGTAAACAAAAGATAACT-3′ and a reverse primer with a sequence of 5′-TCAATGC-TATAAACAGTCAT-3′. Sequencing of the PCR products was performed by using a BigDye terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Foster, CA) with an ABI Prism 310 genetic analyzer (Perkin-Elmer Applied Biosystems).

RESULTS

RNA sequences of HCoV-NL63 were detected in samples from 3 (2.5%) of the 118 children with bronchiolitis. Three (13.6%) of 22 samples collected in February and March were positive for HCoV-NL63 (Table I). Direct sequencing of the PCR products of the three samples showed that the sequences were identical to the sequence of HCoV-NL63 (GenBank accession number AY567487). Clinical and laboratory features of the three patients positive for HCoV-NL63 are shown in Table II. The three patients had no underlying disease. All three patients suffered from expiratory wheezing, fever, cough, and nasal discharge. Two patients presented with tachypnea. One girl (case 2) developed hoarseness and barking cough after admission, and she was diagnosed as also having laryngotracheobronchitis. Maximum temperature ranged from 39.0 to 40.3°C. The duration of fever and expiratory wheezing ranged from 1 to 4 days and from 3 to 7 days, respectively. Radiographs of the chest showed emphysematous changes in two patients.

DISCUSSION

Bronchiolitis is an acute, inflammatory respiratory illness in children occurring mainly in the first 2 years of life. hRSV is the virus detected most frequently detected virus in patients with bronchiolitis, reported detection rates being 50%–60% [Andreoletti et al., 2000; Papadopoulos et al., 2002; Jartti et al., 2004]. Respiratory picornavirus is the second-most frequent agent, and has been detected in 20%–40% of bronchiolitis cases [Andreoletti et al., 2000; Papadopoulos et al., 2002; Jartti et al., 2004]. hMPV, which has been recognized recently as a causative agent as bronchiolitis, was present in 11%–16% of bronchiolitis cases [Jartti et al., 2004; Xepapadaki et al., 2004]. The detection rate of HCoV-229E and HCoV-OC43 in bronchiolitis cases has been reported to be only 2% [Papadopoulos et al., 2002; Jartti et al., 2004]. However, there are other causative agents of bronchiolotis that remain to be discovered. van der Hoek et al. [2004] reported an infant with bronchiolitis caused by HCoV-NL63. In this study, HCoV-NL63 was detected in 2.5% of the bronchiolitis cases, indicating that HCoV-NL63 may be one of the causative agents of bronchiolitis.

TABLE II. Clinical and Laboratory Features of Three Patients Positive for HcoV-NL63

	Case 1	Case 2	Case 3
Sampling date	14 February, 2003	19 March, 2003	28 March, 2003
Age	1 year 11 months	1 year 6 months	1 year 3 months
Sex	F	F	F
Presenting symptoms	Rhinorhoea, cough, wheezing, fever (max: 39.6°C)	Rhinorhoea, barking cough, wheezing, tachypnea, hoarseness, fever (max: 40.3°C)	Rhinorhoea, cough, wheezing, tachypnea, fever (max: 39.0°C)
Duration of fever (days)	1	4	4
Duration of wheezing (days)	3	3	7
Clinical signs	Expiratory wheezing	Inspiratory and expiratory wheezings, subcostal retraction	Expiratory wheezing
Chest X ray	No abnormality	Emphysematous change	Emphysematous change
WBC (leukocytes/μl)	7,900	16,200	12,140
CRP (mg/dl)	0.26	0.3	1.04

F, female; WBC, white blood cells; CRP, C reactive protein.

Fouchier et al. [2004] reported that HCoV-NL63 was detected in 4 (2.9%) of 139 patients with respiratory tract infections of unknown etiology. Since the incidence of HCoV-NL63 in patients with respiratory tract infections varies depending on several factors such as age, season, year, and clinical diagnosis, it is difficult to determine the true incidence of the virus. Previous studies showed that outbreaks of HCoV-229E and HCoV-OC43 occur every 2–3 years [McIntosh et al., 1970; Hambre and Beem, 1972]. If outbreaks of HCoV-NL63 occur every 2–3 years and this 1-year study was performed in non-outbreak year, there is the possibility of underestimation in this data. In studies by the two Dutch groups, all of the samples positive for HCoV-NL63 were collected in winter. In this study, all the three positive samples were collected in February and March, which is a winter season in Sapporo. This suggests that HCoV-NL63 may have a seasonal peak in winter in Japan as well as in The Netherlands. Although van der Hoek et al. [2004] suggested the cocirculation of multiple subgroups of HCoV-NL63, the sequences of three HCoV-NL63 detected in this study were completely identical to that of HCoV-NL63 (GenBank accession number AY567487).

Three nasopharyngeal samples from the three patients were inoculated on LLC-MK2 cells. However, no cytopathic effect was observed during a 3-week culture period (data not shown). The two Dutch groups initially carried out virus isolation of HCoV-NL63 on tertiary monkey kidney cells, and LLC-MK2 cells or Vero cells were used for subsequent virus propagation [Fouchier et al., 2004; van der Hoek et al., 2004]. Tertiary monkey kidney cells may be more sensitive than LLC-MK2 cells for initial isolation of HcoV-NL63.

To our knowledge, this is the first report on HCoV-NL63 infections and the incidence of HCoV-NL63 infection in children with bronchiolitis in Asia. This study suggests that HCoV-NL63 may be widespread throughout the world and is one of the causative agents of acute respiratory wheezing in young children. To clarify the clinical impact of HCoV-NL63, further surveillance in various age groups and various clinical groups is needed.

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