



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Human Coronavirus-NL63 infections in Korean children, 2004–2006

Tae Hee Han^a, Ju-Young Chung^{b,*}, Sang Woo Kim^b, Eung-Soo Hwang^c

^a Department of Diagnostic Laboratory Medicine, Sanggyepaik Hospital, College of Medicine, Inje University, Seoul, Republic of Korea

^b Department of Pediatrics, Sanggyepaik Hospital, College of Medicine, Inje University, 761-1 Nowon-Gu, Seoul, Republic of Korea

^c Department of Microbiology, College of Medicine, Seoul National University, Seoul, Republic of Korea

Received 30 May 2006; received in revised form 9 October 2006; accepted 19 October 2006

Abstract

Background: Human coronavirus-NL63 (*HCoV-NL63*) has been isolated from children with respiratory tract infections and its prevalence in Korea has not been reported.

Objectives: This study was designed to investigate the presence and the clinical features of *HCoV-NL63* during two winter seasons.

Study design: During April 2004–April 2006, nasopharyngeal specimens from children hospitalized with acute respiratory disease were tested for common respiratory viruses, including RSV, influenza A, influenza B, parainfluenza viruses, and adenovirus by IFA. *hMPV* infection was excluded by nested RT-PCR using primers for F-gene. To detect *HCoV-NL63*, previously described nested PCR assays for 1a and 1b were used. PCR products of the 1a gene for *HCoV-NL63* were sequenced.

Results: Out of 872 nasopharyngeal aspirate from children aged under 16 years, 14 (1.7%) were positive for *HCoV-NL63*. Most of the patients had croup (64.2%) or bronchiolitis (21.4%). The peak prevalence was found in November (28.5%). Most were collected between November 2004 and February 2005.

Conclusions: *HCoV-NL63* may be one of the causative agents of acute respiratory tract infection, especially croup.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *HCoV-NL63*; Croup; Acute bronchiolitis; Children

1. Introduction

Human respiratory syncytial virus (RSV), parainfluenza viruses, adenoviruses, influenza viruses, and recently discovered human metapneumovirus (*hMPV*) are the most commonly isolated viruses in acute respiratory infection in children (Peiris et al., 2003a,b; Selwyn, 1990). However, the etiologic agents are still unknown in a large proportion of respiratory infections. Human coronaviruses are positive sense RNA enveloped viruses (Nokso-Koivisto et al., 2000). *HCoV-229E* and *HCoV-OC43* are major causes of common colds.

In 2002, a novel human corona virus, *HCoV-SARS*, was identified as a cause of severe acute respiratory syndrome (SARS) (Nokso-Koivisto et al., 2000; Peiris et al., 2003a,b) and a new kind of group 1 human coronavirus-NL63 (*HCoV-*

NL63) was discovered in the Netherlands (van der Hoek et al., 2004). Another group from the Netherlands also identified *HCoV-NL* in a boy with pneumonia (Fouchier et al., 2004). Although *HCoV-NL63* has been associated with upper and lower respiratory tract infections in children, there are no published reports in Korea.

We used nasopharyngeal aspirate specimens from hospitalized children with acute respiratory disease to determine the prevalence and clinical features of *HCoV-NL63* during two winter seasons in Korea.

2. Methods

2.1. Patients and specimens

A total of 1005 nasopharyngeal specimens were consecutively collected from children under 16 years of age, who were admitted with acute respiratory disease at SanggyePaik

* Corresponding author. Tel.: +82 2 950 1073; fax: +82 2 950 1955.

E-mail address: pedchung@sanggyepaik.ac.kr (J.-Y. Chung).

Hospital from April 2004 to April 2006. A total of 827 specimens were tested with IFA (DAKO, Cambridgeshire, UK) for common respiratory viruses; RSV, influenza virus A, influenza B, parainfluenza viruses, and adenoviruses, were sought. The specimens were stored at -70°C until further tested. Informed consent was obtained at admission from parents. The possibility of *hMPV* infection was excluded by nested RT-PCR using specific primers to amplify a part of the F-gene as previously described (van den Hoogen et al., 2004). Clinical definitions were: croup was hoarseness of voice, barking cough, and inspiratory stridor due to laryngeal obstruction; tracheobronchitis was cough and rhonchi and no laryngeal obstruction or wheezing; bronchiolitis was expiratory wheezing with or without tachypnea, air trapping, and substernal retractions; pneumonia was rales or evidence of pulmonary consolidation on physical examination or radiograph; bronchial asthma was acute wheezing occurring three or more times in children of any age or occurring one or more times in children aged >3 years. The diagnoses of the patients were croup in 29, pneumonia in 290, bronchiolitis in 257, acute bronchitis in 40, upper respiratory tract infection in 21, and bronchial asthma in 190. The median age was 15 months (range = patients ranged from 1 to 192 months in age): children 5 years of age or younger constituted 92.7% (759/827) of the study population. The Ethics Committee of Faculty Medicine, Inje University, Seoul, Korea, approved the study.

2.2. RT-PCR and sequencing

Viral RNA was extracted from each sample by QIAamp viral mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Reverse transcription of 0.5 μg of each RNA sample was performed in a final volume of 20 μL containing 5 μM of random hexadeoxynucleotides, 1 mM of each dNTP, 2 units of RNase inhibitor, and 9 units of reverse transcriptase (Bioneer, Daejeon, Korea). After incubation at 42°C for 1 h, the samples were heated for 5 min at 94°C . To detect HCoV-NL63, previously described nested PCR assays for 1a and 1b were used (Arden et al., 2005). All PCR assays were performed using 1 μL of cDNA and 0.6 μM of each primer. To validate the amplification process and to exclude the presence of carryover contamination, positive and negative controls were run on each PCR, and positive samples were verified against an independent RNA extraction.

PCR products of the 1a gene were sequenced. Amplicon was purified using QIAquick (Qiagen GmbH, Hilden, Germany) and sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing products were resolved with an ABI 3730 XL autoanalyzer (Applied Biosystems, Foster City, CA, USA). Phylogenetic trees were constructed using MEGA version 3.0 (Kumar, Tamura, Nei 2004).

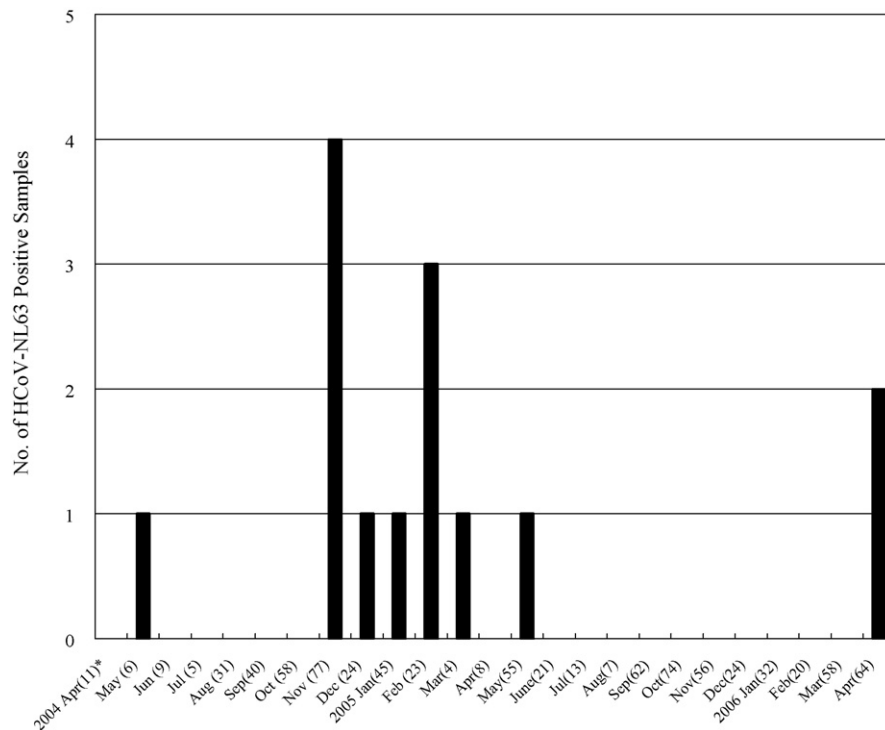


Fig. 1. Seasonal distribution of HCoV-NL63 positive samples. The number in parenthesis after each month gives the number of the samples tested.

Table 1
Clinical characteristics of *HCoV NL-63* positive patients

Patient	Age (months)	Sex	Date (sample collection)	Clinical manifestations	Duration of symptoms (days)	Underlying disease	Clinical diagnosis
1	6	M	May 2004	Cough, sputum	3	None	Croup
2	18	F	Nov 2004	Cough, sputum	4	None	BA exacerbation
3	13	M	Nov 2004	Cough, Fever, dyspnea	2	None	Croup
4	9	F	Dec 2004	Cough	1	None	Croup
5	23	M	Jan 2005	Cough, dyspnea	2	None	Croup
6	15	M	Feb 2005	Cough, dyspnea	1	None	Croup
7	11	M	Feb 2005	Cough, dyspnea	2	None	Croup
8	17	F	Feb 2005	Cough, Fever, dyspnea	4	None	Croup
9	5	M	Mar 2005	Cough, fever	3	None	Bronchiolitis
10	22	M	Nov 2004	Cough, Fever, dyspnea	2	None	Croup
11	7	M	Nov 2004	Cough, sputum	10	None	Bronchiolitis
12	33	M	May 2005	Cough, sputum	2	None	Bronchiolitis
13	5	F	Apr 2006	Cough	2	None	Croup
14	58	F	Apr 2006	Cough, fever	2	None	Pneumonia

BA, bronchial asthma.

3. Results

From April 2004 to April 2006, a total of 827 nasopharyngeal specimens from children, hospitalized with acute respiratory disease, were tested for common respiratory viruses. IFA diagnosis indicated that 151 (18.2%) specimens were positive for RSV, 9 (1.1%) for parainfluenza virus, 5 (0.6%) for influenza A virus, 2 for adenoviruses, and 1 for influenza B virus. RT-PCR detected 83 (10.0%) samples containing *hMPV*, and 14 (1.7%) containing *HCoV-NL63*. *HCoV*

NL-63 was detected during May (1 specimen), November (4 specimens), and December (1 specimen) in 2004; January (1 specimen), February (3 specimens), and May (2 specimens) in 2005; April (2 specimens) in 2006 (Fig. 1). Most of *HCoV NL-63* positive specimens were collected between November 2004 and February 2005. None were detected during the winter season of 2005–2006. *HCoV-NL63* was detected in one case with RSV and in one case with RSV and *hMPV*.

The *HCoV NL-63* positive patients were 5–58 months old (median, 11.5 months) and the ratio of males to females was

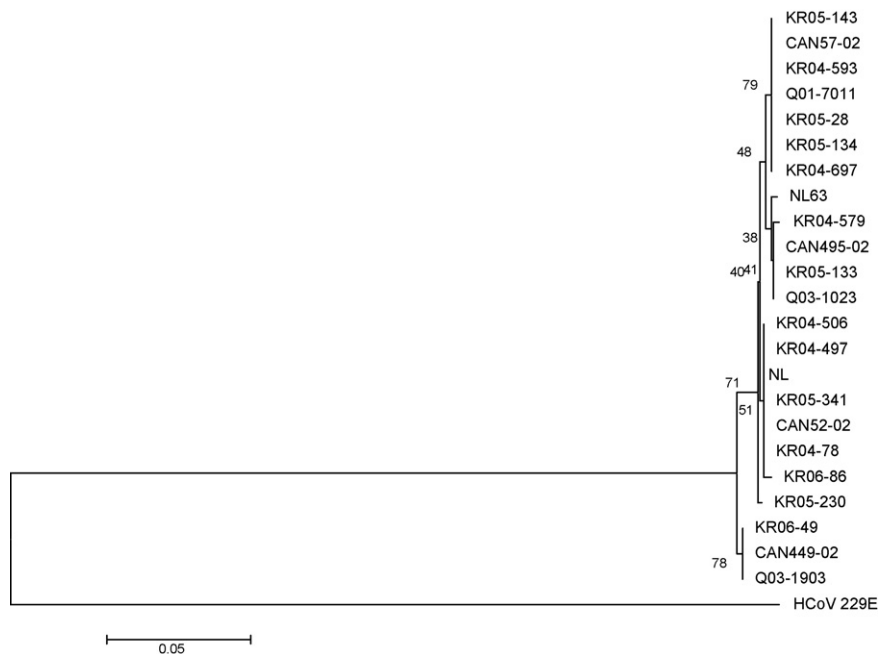


Fig. 2. Phylogenetic analysis of Korean (KR), Canadian (CAN), and Queensland (Q) *Ia* gene sequences from *human coronavirus NL63* strains presented on a topology tree prepared in MEGA3. Nucleotide alignment of a 425 bp portion of the *Ia* gene was prepared using BioEdit V7.0. The nucleotide distance matrix was generated using the Kimura two-parameter estimation. Nodal confidence values indicate the results of bootstrap resampling ($n = 1000$). Two main sequence clusters are apparent and all Korean strains belong to the upper cluster. GenBank accession numbers: KR (DQ093116–DQ093123, DQ351988, DQ453793–DQ453795, DQ534705–DQ534706), CAN (AY675543, AY675550, AY675542, AY675547), Q (AY746455, AY746458, AY746451), NL63 (NC_005831), and NL (AY518894).

1.8:1. HCoV-NL63 positive patients were diagnosed as croup in 9 (64.2%), bronchiolitis in 3 (21.4%), bronchial asthma exacerbation in 1, and pneumonia in 1 (Table 1). HCoV-NL63 was positive in 31.0% (9/29) of children with croup in this study population. Of the 20 cases of croup that were not associated with HCoV-NL63, RSV was detected in 5 patients, hMPV in 3 patients, and parainfluenza virus in 3 patients. The clinical presentations of patients were cough (100%), fever (35.7%), sputum production (28.5%), and dyspnea (35.7%). No patients had predisposing factors or underlying diseases.

The positive PCR products were confirmed by sequencing and the partial –sequences of the 1a gene were deposited in GenBank (DQ093116–DQ093123, DQ351988, DQ453793–DQ453795, DQ534705–DQ534706). Direct sequencing of the PCR products of the 1a gene revealed that seven isolates had the same sequences and others limited variation of sequence (Fig. 2).

4. Discussion

This is the first report of HCoV-NL63 infections in Korean children hospitalized with acute respiratory disease. HCoV-NL63 circulated in Korean children during 2004–2006. Coronaviruses are divided into three different groups: group 1 (HCoV-229E and HCoV-NL63); group 2a (HCoV-OC43 and HCoV-HKU1); group 2b (SARS-CoV); group 3, but human pathogens are only found in groups 1 and 2. Coronavirus HCoV-NL63 infections have been reported in the Netherlands, Australia, Japan, Canada, USA, France, and Hong Kong (Arden et al., 2005; Bastien et al., 2005; Chiu et al., 2005; Ebihara et al., 2005; Esper et al., 2005; Vabret et al., 2005; van den Hoogen et al., 2004), which suggest that this newly discovered human coronavirus has a worldwide distribution. Although, we did not include a healthy control group, HCoV-NL63 presence in the nasal aspirates of children with acute respiratory disease, in the absence of an alternate etiology, suggests that it may have a role in the illness. However, the causal association HCoV-NL63 cannot be established without including a healthy control group. The incidence of HCoV-NL63 in patients with respiratory disease of unknown etiology is reported to be 1.2–9.3% (Esper et al., 2005; Moes et al., 2005; Suzuki et al., 2005; Vabret et al., 2005). In this study, the positive rate of HCoV-NL63 (1.7%) was comparable to that of Australia and Japan (Arden et al., 2005; Suzuki et al., 2005). The differences in HCoV-NL63 positivity rates among several studies may be due to the characteristics of the study populations and the collection time of respiratory specimens. In a Japanese study, HCoV-NL63 was detected in 2.5% of hospitalized children with acute bronchiolitis suggesting that it plays an etiological role in bronchiolitis (Ebihara et al., 2005). In this study, HCoV-NL63 was associated with croup in most of the positive children (9/14, 64.1%), which is consistent with a recent report showing strong association between HCoV-NL63 infection and croup (Moes et al.,

2005). However, others have reported that mild and nonspecific symptoms are frequent in HCoV-NL63 positive patients and that detection occurred occasionally in healthy children (Boivin et al., 2005; Moes et al., 2005; Suzuki et al., 2005). In this study, HCoV-NL63 infections occurred in previously well patients which is consistent with what was found in previous studies (Arden et al., 2005; Chiu et al., 2005; Ebihara et al., 2005), although others have reported that many children with HCoV-NL63 infection had an underlying disease such as prematurity or cardiac disease (Esper et al., 2005; Moes et al., 2005).

To elucidate, exactly, the clinical spectrum and significance of HCoV-NL63 infection, further population-based studies are needed. In this study, most of the HCoV-NL63 positive patients (85.7%, 12/14) were under 24 months old, and 42.8% (6/14) were under 12 months old. These results are similar to a recent report, which showed that HCoV-NL63 causes lower respiratory tract symptoms in early life and that it is one of the important etiologic agents of acute respiratory infection in young children (Kaiser et al., 2005).

Two out of 14 sequences of HCoV-NL63 detected in this study were identical to that of HCoV-NL63 (NC-005831) and 5 sequences were identical to that of HCoV-NL (AY518894). These results indicate that the 1a gene of HCoV-NL63 is a highly conserved region which aids in its detection by RT-PCR. We do not think that our results are false positives because cross contamination controls were negative and all positive RT-PCR results were confirmed by a second run. Although some reported high frequency (75%, 9/12) of mixed infection (Boivin et al., 2005), we detected co-infections with HCoV-NL63 and another respiratory virus in only two cases.

The prevalence of HCoV-NL63 varies according to the geographical region, seasonality, and year. In the Netherlands, Canada, Japan and Belgium, HCoV-NL63 was mostly detected during winter (Bastien et al., 2005; Fouchier et al., 2004; Moes et al., 2005; van der Hoek et al., 2005), but some have been detected in spring (Chiu et al., 2005; Esper et al., 2005). In this study HCoV-NL63 was detected in spring and winter of 2004, and spring of 2006. Although a comparable number of specimens were included, we could not detect HCoV-NL63 in the respiratory specimens during winter 2005–2006. It is known that outbreaks of other coronaviruses including HCoV-229E and HCoV-OC43 occur every second year (Monto and Lim, 1974). Our results show that outbreaks of HCoV-NL63 infections do not occur every year: further studies are needed to confirm the pattern of periodicity of outbreaks in Korea. Phylogenetic analysis showed that different strains are cocirculating in Korea, which is similar to findings in other countries (Arden et al., 2005; Bastien et al., 2005; Moes et al., 2005; Vabret et al., 2005).

In conclusion, we confirmed the presence of HCoV-NL63 infection in Korean children hospitalized with acute respiratory tract disease. Although the prevalence of HCoV-NL63 is not high, it is one of the important etiologic agents of respiratory tract infections (especially croup) in hospitalized children.

Acknowledgements

We thank to SJ Kim of the research center of Sanggyepaik hospital for excellent technical assistance.

References

- Arden KE, Nissen MD, Sloots TP, Mackay IM. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. *J Med Virol* 2005;75:455–62.
- Bastien N, Anderson K, Hart L, Van Caeseele P, Brandt K, Milley D, et al. Human coronavirus NL63 infection in Canada. *J Infect Dis* 2005;191:503–6.
- Boivin G, Baz M, Cote S, Gilca R, Defrasnes C, Leblanc E, et al. Infections by human coronavirus-NL in hospitalized children. *Pediatr Infect Dis J* 2005;24:1045–8.
- Chiu SS, Chan KH, Chu KW, Kwan SW, Guan Y, Poon LL, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin Infect Dis* 2005;40:1721–9.
- Ebihara T, Endo R, Ma X, Ishiguro N, Kikuta H. Detection of human coronavirus NL63 in young children with bronchiolitis. *J Med Virol* 2005;75:463–5.
- Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. *J Infect Dis* 2005;191:492–8.
- Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci USA* 2004;101:6212–6.
- Kaiser L, Regamey N, Roiha H, Defferenz C, Frey U. Human coronavirus NL63 associated with lower respiratory tract symptoms in early life. *Pediatr Infect Dis J* 2005;24:1015–7.
- Moes E, Vijgen L, Keyaerts E, Zlateva K, Li S, Maes P, et al. A novel pancoronavirus RT-PCR assay: frequent detection of human coronavirus NL63 in children hospitalized with respiratory tract infections in Belgium. *BMC Infect Dis* 2005;5(1):6.
- Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974;129:271–6.
- Nokso-Koivisto J, Pitkaranta A, Blomqvist S, Kilpi T, Hovi T. Respiratory coronavirus infections in children younger than two years of age. *Pediatr Infect Dis J* 2000;19:164–6.
- Peiris JS, Tang WH, Chan KH, Khong PL, Guan Y, Lau YL, et al. Children with respiratory disease with metapneumovirus in Hong Kong. *Emerg Infect Dis* 2003a;9:628–33.
- Peiris JS, Yuen KY, Osterhaus AD, Stohr K. The severe acute respiratory syndrome. *N Engl J Med* 2003b;349:2431–41.
- Selwyn BJ, Coordinated Data Group of BOSTID Researchers. The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. *Rev Infect Dis* 1990;12:S870–88.
- Suzuki A, Okamoto M, Ohmi A, Watanabe O, Miyabayashi S, Nishimura H. Detection of human coronavirus NL-63 in children in Japan. *PIDJ* 2005;24:645–6.
- Vabret A, Mourez T, Dina J, van der Hoek L, Gouarin S, Petitjean J, et al. Human coronavirus NL63, France. *Emerg Infect Dis* 2005;11:1225–9.
- van den Hoogen BG, Herfst S, Sprong L, Cane PA, Forleo-Neto E, de wart RL, et al. Antigenic and genetic variability of human metapneumoviruses. *Emerg Infect Dis* 2004;10:658–69.
- van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, et al. Identification of a new human coronavirus. *Nat Med* 2004;10:368–73.
- van der Hoek L, Sure K, Ihorst G, Stang A, Pyrc K, Jebbink MF, et al. Croup is associated with the novel coronavirus NL63. *PRoS Med* 2005;2:e240.