Role of Human Coronavirus NL63 in Hospitalized Children With Croup

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Background: Human coronavirus NL63 (HCoV-NL63) has recently been implicated as a common cause of croup in children. This study was performed to evaluate viruses associated with croup in children, with an emphasis on HCoV-NL63.

Methods: Nasopharyngeal aspirates were prospectively collected from 182 children hospitalized with croup at Seoul National University Bundang Hospital from January 2005 to June 2009. Multiplex reverse-transcriptase polymerase chain reaction was conducted for detection of 11 respiratory viruses, and medical records were reviewed.

Results: Viruses were identified in 147 (80.8%) of the 182 croup patients. The 3 most commonly detected viruses were parainfluenza virus type 1 (PIV1) in 44 (24.2%) patients, HCoV-NL63 in 30 (16.5%) patients, and influenza A virus in 25 (13.7%) patients. Other detected viruses were rhinovirus in 22 (12.1%) patients, PIV type 3 and respiratory syncytial virus in 15 (8.2%) patients, human bocavirus in 8 (4.4%) patients, and several others in a few patients. Coinfections with ≥2 viruses were found in 20 (11%) patients. HCoV-NL63 was identified primarily in winter, which coincides with the peak occurrence of croup. Patients with HCoV-NL63 infection were younger than those who were positive for PIV1 (median age 13 months versus 21 months, $P = 0.006$) and had shorter fever duration than influenza A virus infection (median 1.5 days versus 4 days, $P = 0.000$).

Conclusions: PIV1 and HCoV-NL63 were most commonly associated with patients who were hospitalized with croup. HCoV-NL63 is prevalent in winter and is associated with younger age and with shorter fever duration.

Key Words: human coronavirus NL63, croup, parainfluenza virus type 1

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Croup is a common respiratory tract infection that occurs in infants and children younger than 6 years,[1,2] with a peak incidence between 7 and 36 months of age. During the second year of life, approximately 5% of children develop croup.[3] It has been suggested that the annual incidence of croup in children younger than 6 years old ranges from 1.5% to 6%, with hospital admission rates of those affected ranging from 1.5% to 31%, depending on admission practices and severity of illness in the population.[1,4]

Viruses account for most cases of acute infectious croup, and parainfluenza viruses (PIVs) are the most common etiology. Several other respiratory viruses, including influenza A virus, rhinovirus, and respiratory syncytial virus (RSV), are also involved.

Human coronavirus NL63 (HCoV-NL63) was first identified in a child with bronchiolitis.[5] Studies in many countries have confirmed circulation of HCoV-NL63 in humans with respiratory symptoms, suggesting a worldwide distribution of this virus.[6] Prevalence of HCoV-NL63 varies according to geographic region, seasonality, and year.[6] Several reports have implicated HCoV-NL63 as a common cause of croup in children younger than 3 years of age,[7–10] and others have shown it to be associated with upper respiratory tract illness, bronchiolitis, and pneumonia. We sought to determine the role of HCoV-NL63 in children with croup compared with that of other respiratory viruses which were previously well recognized as etiologic agents of croup, namely, PIVs, influenza A virus, or RSV.

MATERIALS AND METHODS

Patients and Virus Specimens

The study population consisted of children admitted to Seoul National University Bundang Hospital with a diagnosis of croup, between January 2005 and June 2009. Nasopharyngeal aspirates were prospectively collected from children with croup at the time of presentation. The study protocol was approved by the Institutional Review Boards at Seoul National University Bundang Hospital. Because viral diagnosis for hospitalized patients with lower respiratory tract infections is a standard of patient care, only verbal consents were obtained from parents for collecting nasopharyngeal aspirates. The definition of croup in the inclusion criteria was hoarseness of voice, barking cough, or inspiratory stridor.[8] Croup patients were admitted when respiratory distress, uncontrolled high fever, lethargy, or poor oral intake were present. Fever was defined as axillary temperature over 38°C, and the duration of fever included the febrile period before the hospital days. Medical records were reviewed for evaluation of clinical manifestations and any underlying conditions.

Viral Diagnosis

Samples of nasopharyngeal aspirates were kept frozen at −70°C. In accordance with the manufacturer's instructions, viral ribonucleic acid in nasopharyngeal aspirates was extracted using a QIAamp Viral Ribonucleic acid Mini kit (QIAGEN GmbH, Hilden, Germany). Complementary deoxyribonucleic acid was synthesized using random hexamers and superscript reverse transcription (Invitrogen, Gaithersburg). Multiplex reverse-transcriptase polymerase chain reaction (RT-PCR) assays were performed as described previously with minor modifications,[11,12] for detection of 11 respiratory viruses, including PIVs type 1 (PIV1) and 3 (PIV3), influenza A virus, influenza B virus, RSV, rhinovirus, HCoV-NL63, HCoV OC43/229E, human metapneumovirus, hu-

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man bocavirus (HBoV), and adenovirus. Positive band for HCoV OC43/229E was further analyzed by sequencing. The detection sensitivity of the multiplex RT-PCR was determined previously.[11]

RESULTS

Patient Characteristics

A total of 225 infants or children were hospitalized for management of croup during the study period. Among those, nasopharyngeal aspirates were obtained from 182 (80.9%) infants or children who were hospitalized with croup. Almost half (18/43, 41.9%) of the missed cases were from the beginning of the study project in 2005 when study protocol had not been widely known to hospital staffs. Among the 182 patients, 115 (63.2%) were male. Median age was 18 months, with a range of 4 to 87 months. These demographic data were not so different for the missed cases; median age was 17 months, and 65.4% were male. Fourteen (7.7%) patients had underlying medical conditions, including preceding lower respiratory tract infections or recurrent wheezing in 6 (3.3%) patients, congenital heart disease in 4 (2.2%) patients, and prematurity in 3 (1.6%) patients. Ten (5.5%) patients had more than 1 episode of croup. Most patients were admitted by way of the emergency room.

TABLE 1. Viruses Identified in 182 Nasopharyngeal Aspirates Obtained From Children With Croup

Virus Identified	No. (%) Positive Specimens
Parainfluenza virus 1	44 (24.2)
Human coronavirus NL63	30 (16.5)
Influenza A virus	25 (13.7)
Rhinovirus	22 (12.1)
Respiratory syncytial virus	15 (8.2)
Parainfluenza virus 3	15 (8.2)
Human bocavirus	8 (4.4)
Human metapneumovirus	5 (2.7)
Adenovirus	2 (1.1)
Influenza B virus	1 (0.5)
Human coronavirus OC43	1 (0.5)
Total virus positive	147/182 (80.8)*

*A total of 168 viruses identified from 147 specimens, with more than 1 virus detected in 20 specimens (11%).

Prevalence of Respiratory Viruses

Of the 182 samples tested, 147 (80.8%) showed a positive result for any of 11 respiratory viruses. A total of 168 viruses were identified from 147 specimens, with simultaneous detection of more than 1 virus in 20 specimens (11%). The most common virus identified was PIV1 in 44 (24.2%) patients. The second and the third most common viruses were HCoV-NL63 in 30 (16.5%) patients and influenza A virus in 25 (13.7%) patients. Other detected viruses are shown in Table 1.

Among samples that were positive for more than 1 virus, PIV1 in 11 patients, rhinovirus in 8 patients, and HCoV-NL63 in 6 patients were the most frequently codetected viruses by number (Fig. 1). However, the proportion of codetected isolates among total positive isolates was highest in adenovirus and HBoV (both 50%), although the total number of isolates was small. There were 6 cases of HCoV-NL63 codetection. With regard to codetection of HCoV-NL63, influenza A virus was found simultaneously with HCoV-NL63 in 2 cases. Other viruses involved in the 4 remaining cases of HCoV-NL63 codetection were PIV1, rhinovirus, RSV, and adenovirus. HCoV-NL63, influenza A virus and HBoV were simultaneously identified in 1 case.

Seasonal Distribution

Monthly distribution of samples during the study period is shown in Figure 2. The virus-positivity rate varied by month with

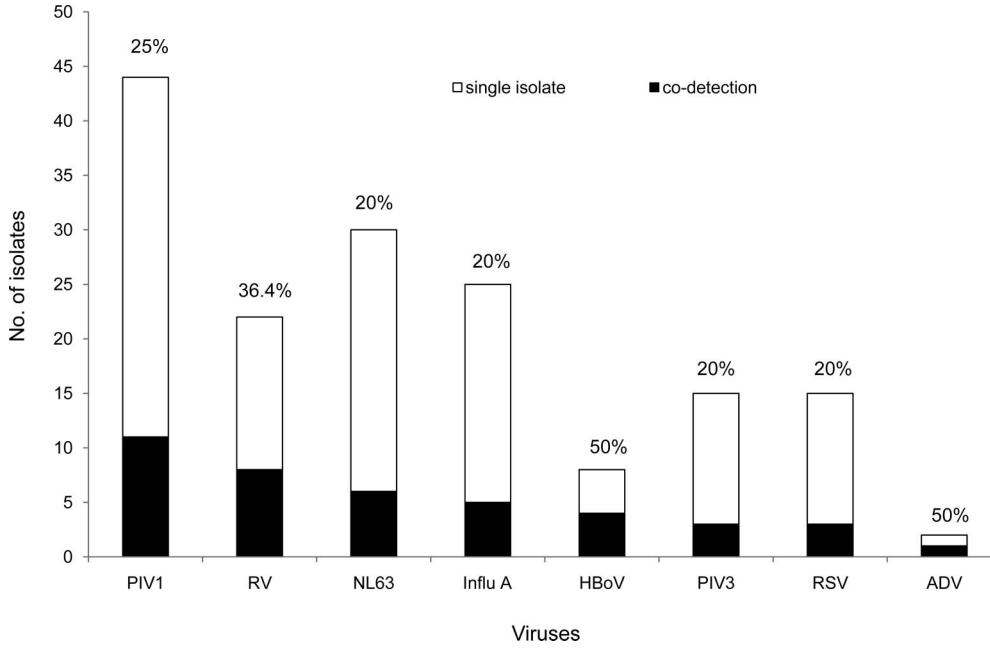

FIGURE 1. Viruses involved in codetection; the number of single isolates and codetection, with the proportion of codetection among total positive isolates in percentage. PIV1, parainfluenza virus type 1; NL63, human coronavirus-NL63; RV, rhinovirus; Influ A, influenza A virus; PIV3, parainfluenza virus type 3; HBoV, human bocavirus; RSV, Respiratory syncytial virus; ADV, adenovirus % above the bars: % of codetection isolates among total positive isolates.

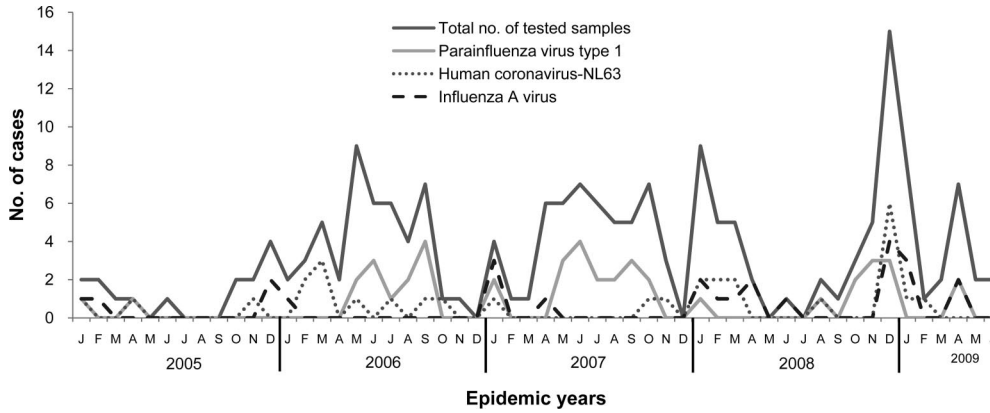

FIGURE 2. Monthly distribution of tested samples, parainfluenza virus type 1, human coronavirus-NL63, and influenza A virus in children with croup.

TABLE 2. Comparison of Demographic Data and Clinical Characteristics of Croup Patients Associated With PIV1, HCoV-NL63, and Influenza A Viruses

Characteristics	PIV1 (n = 33)	HCoV-NL63 (n = 24)	Influenza A (n = 20)	*P*
Age (mo), median (range)	21 (4–72)	13 (4–29)	17.5 (4–87)	0.006*
Boys	22 (66.7)	15 (62.5)	12 (60.0)	0.865
Underlying condition	4 (12.1)	0 (0)	1 (5.3)	0.183
Hospital stay (d), median (range)	3 (0–7)	3 (1–6)	4 (2–5)	0.323
Fever >39°C	14 (42.4)	6 (25.0)	11 (57.9)	0.090
Median fever duration (d) (range)	3 (1–7)	1.5 (0–5)	4 (1–7)	0.000†
Dexamethasone (0.6 mg/kg/dose) use	18 (54.5)	15 (62.5)	12 (63.2)	0.768
Intravenous antibiotics use	6 (18.2)	1 (4.2)	4 (21.1)	0.213
Supplemental oxygen	1 (3.0)	0 (0)	1 (5.3)	0.554
Combined acute otitis media	1 (3.0)	1 (4.2)	1 (5.3)	0.922
Combined pneumonia/bronchiolitis	1 (3.0)	2 (8.3)	2 (10.5)	0.528

Data are presented in number of episodes (%) unless otherwise specified. Episodes of infection that tested positive for a single viral agent are included. PIV1, parainfluenza virus type 1; HCoV-NL63, human coronavirus-NL63.

*PIV1 versus HCoV-NL63, using The Mann-Whitney *U* test.

†HCoV-NL63 versus influenza A virus, using The Mann-Whitney *U* test.

a range from 0% to 100% (mean 82.2%, $P = 0.000$). Differences in the virus-positivity rates may be explained by the epidemiology of each virus. Unrecognized viruses including PIV2 and HCoV-HKU1 which might be associated with seasonal outbreak, can contribute to the months demonstrating lower positivity rate. Croup specimens were prevalent during May and July in 2006 and 2007; however, the prevalence changed to winter (December to February) in 2008 and during the 2008 to 2009 season. Because there were only a few samples from 2005, determination of the epidemic in 2005 was difficult.

PIV1 was prevalent from May to September in 2006 and 2007, from October to December in 2008, and in April in 2009. The prevalence of HCoV-NL63 peaked during the winter season (mainly in December to February). Influenza A virus showed an epidemic curve similar to that of HCoV-NL63 in winter, with a slightly earlier curve than that of HCoV-NL63 during the 2005 to 2006 season.

Demographic Data and Clinical Characteristics

Demographic data and clinical characteristics of croup patients with the 3 most commonly identified viruses (PIV1, HCoV-NL63, and influenza A virus) were compared (Table 2). HCoV-NL63 was associated with younger children, particularly when compared with PIV1. Fever duration was shorter in HCoV-NL63—positive patients than either PIV1-positive or influenza A virus-positive patients.

Management of croup was not significantly different between the virus groups. Of the 38 cases of intravenous antibiotics used, 15 cases had acute otitis media or other lower respiratory infections (pneumonia or bronchiolitis). A total of 22 (12.1%) patients had other diagnoses in addition to croup. Pneumonia, bronchiolitis, and acute otitis media were the most frequent comorbidities. Seven patients required supplemental oxygen; however, none of the patients needed intensive care unit admission or ventilator care, and all patients recovered without serious complications.

We analyzed the clinical characteristics of those with HCoV-NL63 infection with and without coinfection by another virus. Median age in the HCoV-NL63 codetection group was 17.5 months (range 14–28 months), which was older than in the single isolation group (median age 13 months); however, the difference was not statistically significant ($P = 0.073$). Mean fever duration was 3.8 days, and all 6 (100%) patients had fever greater than 39°C in the codetection group, compared with a mean of 1.5 days of fever duration and 6 (25%) of 24 patients with fever greater than 39°C in the single isolation group; the differences were statistically significant ($P = 0.005$ and $P = 0.002$, respectively).

DISCUSSION

In this study, we applied multiplex RT-PCR of nasopharyngeal aspirate samples for prospective evaluation of respiratory viruses associated with hospitalized croup patients during a 4-year period. The 3 most commonly detected viruses were PIV1, HCoV-NL63, and influenza A virus. Comparison of clinical characteristics of PIV1, HCoV-NL63, and influenza A virus revealed a younger age at the onset of illness and shorter duration of fever in HCoV-NL63 associated croup patients.

Etiology of viral croup includes many respiratory viruses, most commonly PIVs. However, no pathogen could be defined in 36% to 62% of croup cases.[4] Denny et al[1] studied 951 children with croup and, using viral culture, found 360 (34%) virus-positive children. In this study, viruses were identified by multiplex RT-PCR in 147 (80.8%) specimens. This figure is higher than the positivity associated with less-sensitive methods. Other studies using PCR assays have reported similar figures.[13,14]

The reported rate of HCoV-NL63 infection in children with respiratory infection ranged from 1.3% to 9.3% in studies from many countries.[7,9,15–20] The reasons for the discrepancy in rates may be regional, year-to-year variation in epidemiology, and the characteristic of the study populations, because HCoV-NL63 is more prevalent in the out-patient group than in the in-patient group.[8]

HCoV-NL63 is usually identified in younger children, primarily those less than 2 years of age, which could reflect greater susceptibility because of immunologic immaturity of young children and/or a lack of maternal antibody in younger children.[21,22] Median age of children with HCoV-NL63 in this study was 13 months, younger than that of croup patients as a whole, or children with other viruses, such as PIV1 and influenza A virus.

Association of HCoV-NL63 infection with croup has been demonstrated in previous studies.[8–10] In Korea, Han et al[6] reported the positive rate of HCoV-NL63 in children hospitalized with acute respiratory disease as 1.7%, and croup was the most common diagnosis for HCoV-NL63 positive children. Another Korean study[11] found that despite the small number of children identified with HCoV-NL63 infection (1.6%), when it was found, it was frequently associated with croup (3 of 6 cases).

The study conducted over a 20-year period revealed more frequent circulation of HCoV during the winter months, with the majority detected between November and March.[21] The seasonality of HCoV-NL63 is usually October to February, mainly in autumn, in Taiwan[9] and Hong Kong,[10] and winter in temperate countries.[16–20] This study also showed peak prevalence of HCoV-NL63 during the winter season. In a previous study of HCoV-NL63 infection in Korean children by Han et al,[6] HCoV-NL63 was detected in spring and winter of 2004 and spring of 2006; however, it was not detected during the winter in 2005 to 2006.

Although the outcome of coup is usually excellent, some reports have shown that influenza virus may be associated with more severe manifestation of croup.[23,24] In a study comparing clinical presentation of croup caused by influenza virus and PIVs, the clinical picture of croup caused by influenza viruses was substantially more severe than that caused by PIVs in hospitalized children.[25] A study of HCoV-NL63 in Hong Kong also showed that duration of fever was shorter with HCoV-NL63 infection compared with influenza A virus and RSV infection.[7] Leung et al[10] recently reported that HCoV-NL63-infected patients were more likely to have febrile convulsion and acute gastroenteritis. However, none of the patients with HCoV-NL63 identification in this study group had such findings.

This study is subject to several limitations. Because samples were taken from patients who were admitted to hospital, croup patients with less severe symptoms who did not require hospital admission were not properly investigated. Therefore, interpretation of results is limited to croup that required hospitalization. There were only 15 samples from 2005, compared with those from other study years; 2006 (46), 2007 (51), 2008 (48), and first half of 2009 (22), respectively. Because of the small number of samples, evaluation of the epidemiology of viruses in 2005 was difficult. Considering the viral shedding period and the sensitivity of the PCR assays, which can detect even very low viral load, the issue of a causal relationship between the detected virus and the disease remains. Quantitative analysis of the virus might have revealed data that could not be evaluated in this study, particularly in cases involving codetection. Another limitation of the study was that the samples were not tested for PIV2 or HCoV-HKU1. Although not as common as PIV1, PIV2, along with more recently discovered HCoV-HKU1, are known to be closely associated with croup. However, those 2 viruses were not tested in this study because their local prevalence seemed quite low from the pilot study. Despite these limitations, this study compared prevalence, epidemiology, and clinical manifestations of common etiologic viruses in croup patients; results were interesting.

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