# Detection of Viruses Identified Recently in Children With Acute Wheezing

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The etiologic role of recently identified respiratory viruses for acute wheezing in children is not yet clear. The purpose of this study was to investigate the prevalence of recently identified viruses, including human metapneumovirus (hMPV), human bocavirus (hBoV), human coronavirus NL63 (hCoV-NL63), and human coronavirus HKU1 (hCoV-HKU1) in children with acute wheezing. Viral etiology was identified in 231 children hospitalized with acute wheezing, aged from 1 month to 5 years. Viral antigens for common respiratory viruses were detected by IFA or multiplex PCR. RT-PCR was used to detect respiratory rhinoviruses, hCoV-NL63, hCoV-HKU1, and hMPV. PCR assays for hBoV DNA were performed using the primer sets for non-capsid protein (NP1) and nonstructural protein (NS1) genes. Viruses were found in 61.5% (142/231) of the study population and a single virus was detected in 45.5% (105/231) of the study population. Rhinovirus (33.3%), human respiratory syncytial virus (hRSV; 13.8%), and hBoV (13.8%) were the most frequently detected viruses. hMPV and hCoV-NL63 were detected in 7.8% and 1.3% of wheezing children, respectively. HCoV-HKU1 was not detected. In 16.0% of the study population, more than one virus was detected. In children with acute wheezing, rhinovirus, hRSV, and hBoV were most frequently detected. Further studies including healthy control subjects are needed to define the clinical significance of hBoV in acute wheezing. *J. Med. Virol. 79:1238–1243, 2007.* © 2007 Wiley-Liss, Inc.

KEY WORDS: wheezing; human bocavirus; rhinovirus; RT-PCR

## INTRODUCTION

Viral respiratory infections are the most common cause of acute expiratory wheezing in children. Human respiratory syncytial virus (hRSV) and rhinovirus are known to be important triggers of acute expiratory wheezing in children [Sigurs et al., 2000; Lemanske et al., 2005], and recent studies using rhinovirus specific RT-PCR have indicated that rhinovirus is a major etiologic agent for acute bronchiolitis in infants [Papadopoulos et al., 2002; Jacque et al., 2006]. Recently, several new respiratory viruses have been identified, including human metapneumovirus (hMPV), and the human coronaviruses (hCoV-NL63 and hCoV-HKU1) [van den Hoogen et al., 2001; van der Hoek et al., 2005; Woo et al., 2005]. Of these new viruses, hMPV has been proposed to be an etiologic agent for acute wheezing in young children and adults, although some results have been contradictory [Rawlinson et al., 2003; Jartti et al., 2004; Williams et al., 2005]. Human bocavirus (HBoV) was recently identified as a member of the *Parvoviridae* and found to cause respiratory tract infections in people in Sweden [Allander et al., 2005]. Although the clinical significance of hBoV infection is still unclear despite frequent detection in respiratory specimens, hBoV has been detected in children with lower respiratory tract infection or asthma exacerbation [Arden et al., 2006; Arnold et al., 2006; Chung et al., 2006; Ma et al., 2006; Sloots et al., 2006]. The purpose of this study was to investigate the role of recently identified respiratory viruses in acute expiratory wheezing in children.

## MATERIALS AND METHODS

### Study Populations and Sample Collection

A total of 308 nasopharyngeal specimens were consecutively collected from 308 children under 6 years of

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age, who were hospitalized with acute wheezing at Sanggyepaik Hospital, from February 2006 to November 2006. A total of 231 specimens, which were sufficient for virus diagnostic evaluation, were tested with IFA (DAKO, Cambridgeshire, UK) or multiplex RT-PCR for common respiratory viruses. Acute bronchiolitis was defined as expiratory wheezing with or without tachypnea, air trapping, and substernal retraction in children aged <3 years, and bronchial asthma was diagnosed when acute wheezing occurred three or more times in children of any age or occurred one or more times in children aged >3 years [Mertsola et al., 1991]. All the samples were collected after informed consents were obtained from the parents. The study protocol was approved by the Ethics Committee of the Sanggyepaik Hospital, Inje University. On admission, a nasopharyngeal aspirate sample was collected using a disposable catheter and suction device after application of normal saline 2 ml into the nostril. Nasopharyngeal aspirates were centrifuged and the supernatants were stored at $-70°C$ until tested.

### Detection of Viruses

Viral antigens for adenovirus, influenza A, influenza B viruses, parainfluenza virus, and hRSV were detected by IFA (DAKO) or multiplex RT-PCR as described previously [Bellau-Pujol et al., 2005]. Viral RNA was extracted from each sample by QIAamp viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. RT-PCR was used to detect rhinoviruses, hMPV, hCoV-NL63, and hCoV-HKU1 as described previously using primers listed in Table I. Reverse transcription of 0.5 µg of each RNA was performed in a final volume of 20 µl containing 5 µM random hexadeoxynucleotides, 1 mM of each dNTP, two units of RNase inhibitor, and nine units of reverse transcriptase (Bioneer, Daejeon, Korea). All PCR assays were performed using 1 µl of cDNA and 0.6 µM of each primer.

DNA was extracted from the nasopharyngeal aspirates using the QIAamp DNA Blood Mini Kit (Qiagen GmbH). Two PCR assays using different primer sets were performed per sample to detect the NP1 gene or the NS1 gene of hBoV as described previously (Table I). The PCR product was size separated on a 2% agarose gel with ethidium bromide and visualized with ultraviolet light (UV) light.

### Statistical Methods

The Chi-square test was applied to compare results for children of different age and for specific virus groups. Statistical analyses were performed using MedCalc for Windows (MedCalc Software, Mariakerke, Belgium).

### RESULTS

Nasopharyngeal specimens taken from 231 children hospitalized with acute expiratory wheezing in the period of February 2006 to November 2006 were included in the study. The median age of the 231 children was 8.5 months (range 1 month to 5 years), and the gender ratio (M/F) was 1.6:1 (144:87). The age distribution of the study population was: 101 children were aged <12 months (43.7%); 94 children were aged 1–<3 years (40.7%); and 36 children were aged 3–5 years (15.6%). 125 children were diagnosed with bronchiolitis, and 106 were diagnosed with acute asthma exacerbation.

Viruses were found in 142 patients (61.5% of the study population) and, of these, single viral agent was detected in 105 patients (45.5% of the total study population; Table II). Rhinovirus (77 patients, 33.3%), hRSV (32 patients, 13.8%), and hBoV (26 patients, 11.3%) were the most commonly detected agents. hMPV was found in 18 (7.8%) of the study population, and parainfluenza virus was detected in 17 patients (7.4%). Human coronaviruses were detected in eight patients (3.4%); hCoV-OC43 in three, hCoV-NL63 in three, and hCoV-229E in two. Human adenovirus, influenza virus A, and influenza virus B were detected in three patients, in two, and in two, respectively. However, hCoV-HKU1 was not detected in the study population. The seasonal distribution of viruses associated with acute wheezing is shown in Figure 1. More than one virus was found in 37 patients (16.0% of the study population). hBoV and hRSV were co-detected in three patients (1.3%), hBoV and rhinovirus in seven patients (3.0%), hRSV and rhinovirus in nine patients (3.9%; Table II). Human adenovirus was co-detected with other virus; hMPV in 1 patient, rhinovirus in 1, and parainfluenza in 1.

Viruses were found in 34 patients (33.7%) of the infants group (patients aged <12 months), 55 patients (58.5%) of the group aged 1–<3 years, and 17 patients (47.2%) of the group aged 3–5 years. Rhinovirus was most frequently detected in all age groups. The rate of hRSV infection was significantly higher in infants group than in any other age group ($P < 0.05$), but the incidences of other viruses among age groups were not different significantly (Table III).

### DISCUSSION

In this study, respiratory viruses were detected in 61.5% (142 patients) of children hospitalized with acute expiratory wheezing; this proportion of patients is relatively lower than those of previous studies, which detected viruses in 73–88% of patients [Freymuth et al., 1999; Andreoletti et al., 2000; Rawlinson et al., 2003; Thumerelle et al., 2003; Jartti et al., 2004]. This difference may be due to several factors such as lower sensitivity of antigen detection methods and no test for enterovirus. These results show that the most frequently detected viruses in children with wheezing were rhinovirus and hRSV, which is consistent with previous reports [Heymann et al., 2004; Jartti et al., 2004]. RT-PCR has been used to show that rhinoviruses are the most frequently detected viruses in school-aged children with wheezing and that they are also found in

TABLE I. Primers Used in PCR

| Virus | Primer | Sequence | References |
|---|---|---|---|
| hBoV | NP-1 188F | 5'-GACCTCTGTAAGTACTATTAC-3' | Sloots et al. [2006] |
| | 542R | 5'-CTCTGTGTTGACTGAATACA G-3' | |
| | NS-1 HBoV01.2 | 5'-TATGGCCAAGGCAATCGTCCAAG-3' | Sloots et al. [2006] |
| | HBoV02.2 | 5'-GCCGCGTGAACATGAGAAACAGA-3' | |
| hMPV | F-gene (forward) | 5'-GCAACAATTGAACTGATCTTCAGGAAAC-3' | Van den Hoogen et al. [2004] |
| | (Reverse) | 5'-GCAACATTGAACTGATCTTCAGGAA AC-3' | |
| | (Forward) | 5'-ACATGCCAACATCTGCAGGACAAATAAAAC-3' | |
| | (Reverse) | 5'-ACAT GCTGTTCACCTTCAACTTTGC-3' | |
| Rhinovirus | OL26 | 5'-GCACTTCTGTTTCCCC-3' | Gama et al. [1989] |
| | OL27 | 5'-CGGACACCCAAAGTAG-3' | Gama et al. [1989] |
| | JWA 1-b | 5'-CATTCAGGGGCCGGAGGA-3' | Johnston et al. [1993] |
| hCoV-NL63 | 1b gene (forward) | 5'-GTGATGCATATGCTAATTTG-3' (1st round) | Arden et al. [2003] |
| | (Reverse) | 5'-CTCTTGCAGGTATAATCCT-3' | |
| | (Forward) | 5'-TTGGTAAACAAAAGATAACT-3' (2nd round) | |
| | (Reverse) | 5'-TCAATGCTATAAACAGTCAT-3' | |
| | 1a gene (forward) | 5'-CTTTTGATAACGGTCACTATG-3' (1st round) | |
| | (Reverse) | 5'-CTCATTACATAAAACATCAAACGG-3' | |
| | (Forward) | 5'-GGTCACTATGTAGTTTATGATG-3'(2nd round) | |
| | (Reverse) | 5'-GGATTTTTCATAACCACTTAC-3' | |
| hCoV-HUK1 | LPW1465 (forward) | 5'-GTTCAAGTGTCGCTGTTCA-3' | Woo et al. [2005] |
| | LPW1822 (reverse) | 5'-CTATCATTATCACAATCCACAG-3' | |
| | LPW1467 (forward) | 5'-GGGTATGAAGTATCATCCTA-3' | |
| | LPW1825 (reverse) | 5'-GATAATCCCAACCCATAAGAAC-3' | |
| | LPW1826 (forward) | 5'-CATCTTATAAAGGATGTTGAC-3' | |
| | LPW1829 (reverse) | 5'-ACAAACAACCCATAAGAAC-3' | |
| hCoV-OC43 | MF1 (forward) | 5'-GGCTTATGTGGCCCCTTACT-3' | Bellau-Pujol et al. [2005] |
| | MF3 (reverse, 1st) | 5'-GGCAAATCTGCCCAAGAATA-3' | |
| | MF2i (reverse, 2nd) | 5'-CTCCAAAAACTTCCAGTTC-3' | |
| hCoV-229E | MD3 (reverse) | 5'-CCTGAACACCTGAAGCCAAT-3' | Bellau-Pujol et al. [2005] |
| | MD1 (forward, 1st) | 5'-TGGCCCCATTAAAAATGTGT-3' | |
| | MD2i (forward, 2nd) | 5'-CCGTATCAACACTCGTTATGTGG-3' | |

50% of the adults with acute wheezing [Rawlinson et al., 2003; Thumerelle et al., 2003; Heymann et al., 2004; Jartti et al., 2004]. In the present study, influenza A viruses were detected infrequently in the study population, which may be due to the low rate of influenza virus infection during the study period. In previous reports, co-detection of viruses was reported for 18–20% of children hospitalized with asthma, and hRSV and rhinovirus or hRSV and enterovirus were the most

TABLE II. Detection of Viruses in 231 Children With Acute Wheezing

| Detection of virus | Number of positive (%) |
|---|---|
| Single detection | 105 (45.4) |
| Rhinovirus | 56 (24.2) |
| hRSV | 17 (7.4) |
| hBoV | 13 (5.6) |
| hMPV | 9 (3.9) |
| Others | 10 (4.3) |
| Mixed detection | 37 (16.0) |
| hRSV + rhinovirus | 9 (3.9) |
| Rhinovirus + hBoV | 7 (3.0) |
| hRSV + hMPV | 4 (1.7) |
| hRSV + hBoV | 3 (1.3) |
| Others | 14 (6.1) |

hRSV, human respiratory syncytial virus; hBoV, human bocavirus; hMPV, human metapneumovirus.

frequently detected virus combinations [Jartti et al., 2004; Nagayama et al., 2006]. In this study, of the 137 children in whom viruses were detected, 33 children were infected with more than one virus. Rhinovirus and hRSV, rhinovirus and hBoV, and hRSV and hMPV were the most common co-infections.

In the present study, the prevalence of viral infection was greater in males than females (M/F, 1.6:1), which is similar to those of previous studies suggesting male susceptibility to viral infection due to gender-specific immune response [Johnston et al., 2005; Vabret et al., 2006]. In previous studies [Thumerelle et al., 2003; Weissbrich et al., 2006], most cases of asthma hospitalization occurred in September, and viral respiratory infections, especially rhinovirus, were considered to be associated with asthma exacerbation. In this study, peak rate of rhinovirus detection in wheezing children was in September, but hBoV and hMPV was in peak in May. It is an interesting finding that hBoV was detected most frequently during spring season, because it was found throughout the year in previous study [Chung et al., 2006]. This finding is different from those of previous studies, which show year-round detection of hBoV and mostly in winter months [Arden et al., 2006; Arnold et al., 2006; Weissbrich et al., 2006]. Additional year-round studies over a longer period are needed to know the exact seasonality of hBoV infection.
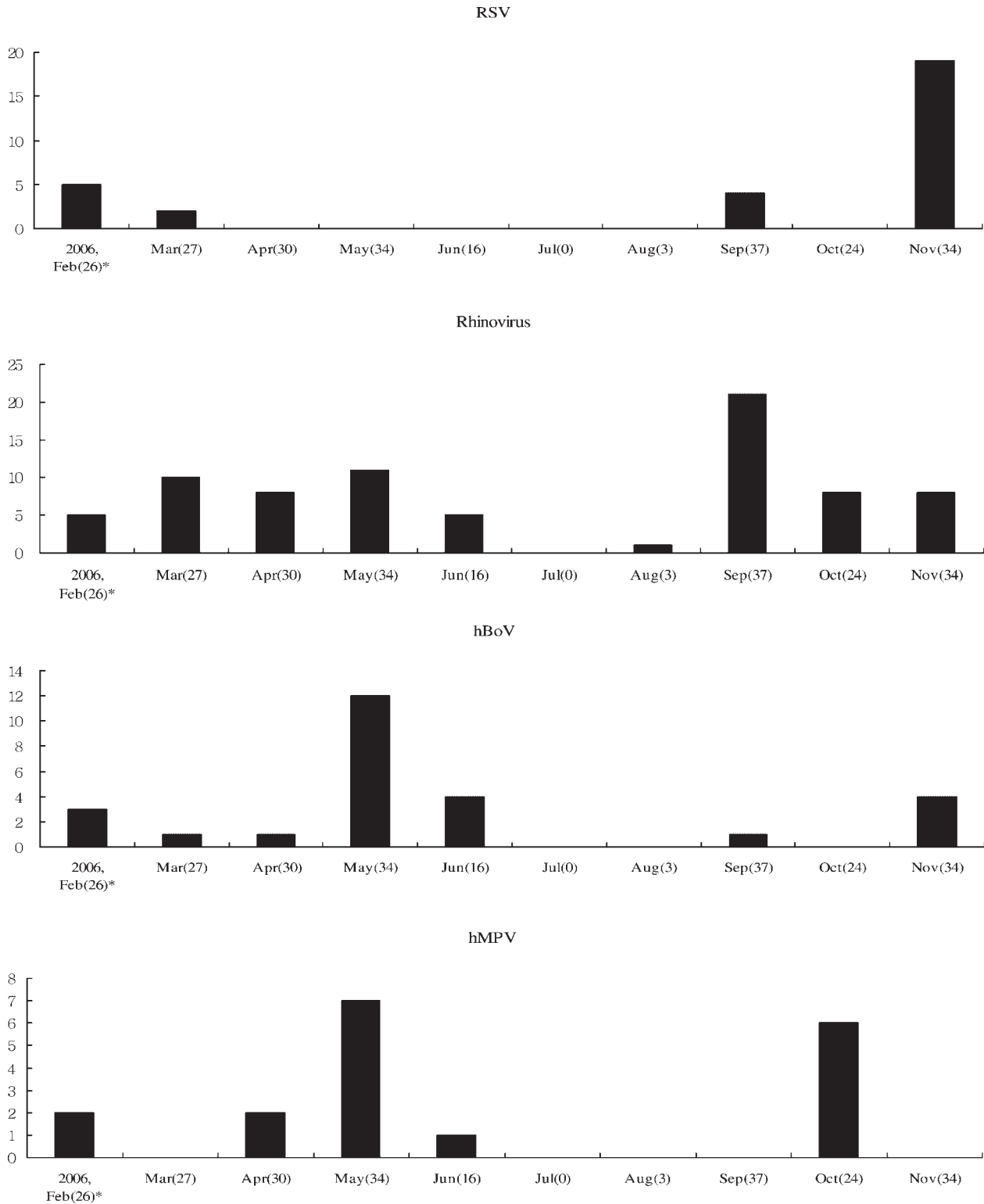
RSV

Rhinovirus

hBoV

hMPV

Fig. 1. Seasonal distribution of hRSV, rhinovirus, hBoV, and hMPV during the study period. hRSV, human respiratory syncytial virus; hBoV, human bocavirus; hMPV, human metapneumovirus. *The number in parenthesis after each month gives the number of the samples tested.

In previous studies, coronaviruses were detected in 0–13% of patients with asthma exacerbation [Freymuth et al., 1999; Papadopoulos et al., 2002; Thumerelle et al., 2003; Jartti et al., 2004; Nagayama et al., 2006]. Recently identified viruses, such as hCoV-NL63 or hCoV-HKU1, are known to be associated with croup or lower respiratory tract infections in children, but these infections are not thought to be highly prevalent

TABLE III. Age Distribution of Viruses in Children With Acute Wheezing

| Viral agents | Number (%) | | | |
|---|---|---|---|---|
| | <1 year | 1–<3 years | 3–5 years | Total |
| Rhinovirus | 32 (41.6) | 34 (44.2) | 11 (14.3) | 77 (100) |
| hRSV | 23 (71.9) | 9 (28.1) | 0 (0) | 32 (100) |
| hBoV | 13 (50.0) | 10 (38.5) | 3 (11.5) | 26 (100) |
| hMPV | 7 (38.9) | 8 (44.4) | 3 (16.7) | 18 (100) |
| Other viruses | 15 (51.7) | 11 (37.9) | 3 (12.5) | 29 (100) |
| No. positive | 90 (49.4) | 72 (39.6) | 20 (11.0) | 182 (100) |

hRSV, human respiratory syncytial virus; hBoV, human bocavirus; hMPV, human metapneumovirus.

[Woo et al., 2005; van der Hoek et al., 2005; Vabret et al., 2006]. In Korea, the prevalence of hCoV-NL63 was 1.7% in hospitalized children with acute respiratory disease, especially high in croup [Han et al., 2007]. In this study, coronaviruses were detected in 3.4% of children with wheezing and hCoV-NL63 was detected in 0.8% of hospitalized children with wheezing, which indicates that they play a minor role in provoking acute wheezing in children. HCoV-HKU1 was not found in children with acute wheezing, which is different from recent studies showing high prevalence of hCoV-HKU1 in lower respiratory tract infections. This difference of hCoV-HKU1 prevalence may be due to several factors such as size of study population, low sensitivity of primers used, geographical variance, and cyclic pattern of hCoV-HKU1 infections.

In a Korean study, the detection rate of hBoV in children with acute lower respiratory tract infection was 7.5% [Chung et al., 2006]. In this study, hBoV infections are frequently detected in children with acute wheezing, which suggest that hBoV may have an important role in acute wheezing. However, others reported that rhinovirus was the only virus significantly associated with exacerbations of asthma and hBoV was not detected in the study population [Khetsuriani et al. 2007]. Although it is possible that the detection of hBoV in nasopharyngeal specimens from children with acute wheezing can be an incidental finding, hBoV was not detected in healthy control children in a recent study [Kesebir et al., 2006]. Further studies are needed to clarify the clinical role of hBoV-only infections and infections with hBoV and other respiratory viruses. In particular, these should include the prevalence of hBoV infection in healthy control subjects, assessment of disease severity by other clinical variables, and determination of immunologic effects.

HMPV has previously been reported to have a role in exacerbation of asthma in children [Rawlinson et al., 2003; Bosis et al., 2005; Williams et al., 2005] and the median ages of children with hMPV or rhinovirus infections are known to be greater than those of children infected with hRSV [Bosis et al., 2005]. In this study, hMPV was detected in 7.8% of the total study population, which is in agreement with those of a recent study showing similar prevalence of hMPV (8.9%) infection in children with wheezing and indicating a role of hMPV in children aged <3 years [Williams et al.,

2005]. In infants, hRSV, influenza virus, and hMPV are known to be associated with wheezing [Chiu et al., 2002; Heymann et al., 2004; Williams et al., 2005]. Viral infections are still important triggering factors in asthmatic children aged >2 years, and rhinoviruses are most frequently involved [Johnston et al., 1995; Rawlinson et al., 2003].

One limitation of this study is that outpatients with wheezing were excluded, but it has been previously reported that rates of respiratory virus infection are higher in hospitalized children than in children not requiring hospitalization [Rakes et al., 1999; Thumerelle et al., 2003; Heymann et al., 2005]. Other limitations of this study are not performing PCR for enteroviruses which were frequently detected in young children with asthma in previous studies [Rawlinson et al., 2003; Jartti et al., 2004], and diagnostic tests for *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, both of which are known to be associated with the exacerbation of wheezing. However, it has previously been reported that *M. pneumoniae* infections are not often detected in patients with exacerbated asthma (1–5% of patients are infected) and *C. pneumoniae* infections are more commonly associated with prolonged asthmatic symptoms than with acute exacerbation [Cunningham et al., 1998; Freymuth et al., 1999; Thumerelle et al., 2003]. There are some other limitations including low specimen numbers which could account for no detection of hCoV-HKU1, the absence of specimens in some months, and mixed use of antigen detection methods and PCR testing for common respiratory viruses, which could have a different sensitivity or specificity.

In conclusion, rhinovirus, hRSV, and hBoV were the most frequently identified respiratory viruses in children hospitalized with acute wheezing. However, further studies including healthy control subjects and in other geographic areas are needed to clarify the possible association of hBoV with asthma exacerbation in children.

## REFERENCES

Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci USA 101:12891–12896.

Andreoletti L, Lesay M, Deschildre A, Lambert V, Dewilde A, Wattre P. 2000. Differential detection of rhinoviruses and enteroviruses

RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. J Med Virol 61:341–346.

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.

Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. 2006. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. J Med Virol 78:1232–1240.

Arnold JC, Singh KK, Spector AA, Sawyer MH. 2006. Human bocavirus: Prevalence and clinical spectrum at a children's hospital. Clin Infect Dis 43:283–288.

Bellau-Pujol S, Vabret A, Legrand L, Dina J, Gouarin S, Petitjean-Lecherbonnier J, Pozzetto B, Ginevra C, Freymuth F. 2005. Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses. J Virol Methods 126:53–63.

Bosis S, Esposito S, Niesters HG, Crovari P, Osterhaus AD, 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, Lau YL, Chan KH, Wong WH, Peiris JS. 2002. Influenza related hospitalizations among children in Hong Kong. N Engl J Med 347:2097–2103.

Chung JY, Han TH, Kim CG, Kim SW. 2006. Bocavirus infection in hospitalized children, South Korea. Emerg Infect Dis 12:1254–1256.

Cunningham AF, Johnston SL, Julious SA, Lampe FC, Ward ME. 1998. Chronic *Chlamydia pneumoniae* infection and asthma exacerbations in children. Eur Respir J 11:345–349.

Freymuth F, Vabret A, Brouard J, Toutain F, Verdon R, Petitjean J, Duhamel JF, Guillois B. 1999. Detection of viral, *Chlamydia pneumoniae* and *Mycoplasma pnumoniae* infections in exacerbations of asthma in children. J Clin Virol 13:131–139.

Gama RE, Horsnell PR, Hughes PJ, North C, Bruce CB, al-Nakib W, Stanway G. 1989. Amplification of rhinovirus specific nucleic acids from clinical samples using polymerase chain reaction. J Med Virol 28:73–77.

Han TH, Chung JY, Kim SW, Hwang ES. 2007. Human coronavirus-NL63 infections in Korean children. 2004–2006. J Clin Virol 38:27–31.

Heymann PW, Carper HT, Murphy DD, Plat-Mills TA, Patrie J, McLaughlin AP, Erwin EA, Shaker MS, Hellems M, Peerzada J, Hayden FG, Haltey TK, Chamberlain R. 2004. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 114:239–247.

Heymann PW, Platts-Mills TA, Johnston SL. 2005. Role of viral infections, atopy, antiviral immunity in the etiology of wheezing exacerbation among children and young adults. Pediatr Infect Dis J 24:S217–S222.

Jacque J, Bouscambert-Duchamp M, Moret H, Carquin J, Brodard V, Lina B, Motte J, Andreoletti L. 2006. Association of respiratory picornaviruses with acute bronchiolitis in French infants. J Clin Virol 35:463–466.

Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, Ruuskanen O. 2004. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 10:1095–1101.

Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, Lambden PR, Tyrell DA, Holgate ST. 1993. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J Clin Microbiol 31:111–117.

Johnston SL, Pattermore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, Tyrell DA. 1995. Community study of the role of viral infections in exacerbations in 9–11 years old children. BMJ 310:1225–1229.

Johnston NW, Johnston SL, Duncan JM, Greene JM, Kebadze T, Keith PK, Roy M, Waserman S, Sears MR. 2005. The September epidemic of asthma exacerbation in children: A search for etiology. J Allergy Clin Immunol 115:132–138.

Kesebir D, Vazquez M, Weibel C, Shapiro ED, Ferguson D, Landry ML, Kahn JS. 2006. Human bocavirus infection in young children in the United States: Molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis 194:1276–1282.

Khetsuriani N, Kazerouni NN, Erdman DD, Lu X, Redd SC, Anderson LJ, Teaque WG. 2007. Prevalence of viral respiratory tract infections in children with asthma. J Allergy Clin Immunol 119:314–321.

Lemanske RF, Jr., Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. 2005. Rhinovirus illness during infancy predicts subsequent childhood wheezing. J Allergy Clin Immunol 116:571–577.

Ma X, Endo R, Ishiquro N, Ebihara T, Ishiko H, Ariga T, Kikuta H. 2006. Detection of human bocavirus in Japanese children with lower respiratory tract infections. J Clin Microbiol 44:1132–1134.

Mertsola J, Ziegler T, Ruuskanen O, Vanto T, Koivikko A, Halonen P. 1991. Recurrent wheezy bronchitis and viral respiratory infections. Arch Dis Child 66:124–129.

Nagayama Y, Tsubaki T, Nakayama S, Swada K, Taguchi K, Tateno N, Toba T. 2006. Gender analysis in acute bronchiolitis due to respiratory syncytial virus. Pediatr Allergy Immunol 17:29–36.

Papadopoulos NG, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. 2002. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 165:1285–1289.

Rakes GP, Arruda E, Ingram GM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA, Heymann P. 1999. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. Am J Respir Crit Care Med 159:785–790.

Rawlinson WD, Waliuzzaman Z, Carter JW, Belessis YC, Gilbert KM, Morton JR. 2003. Asthma exacerbation in children associated with rhinovirus but not human metapneumovirus infection. J Infect Dis 187:1314–1318.

Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. 2000. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. Am J Respir Crit Care Med 161:1501–1507.

Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, MacKay IM. 2006. Evidence of human coronavirus HKU-1 and human bocavirus in Australian children. J Clin Virol 35:99–102.

Thumerelle C, Deschildre A, Bouquillon C, Santos C, Sardet A, Scalbert M, Delbecque I, Debray P, Dewilde A, Turck D, Leclerc F. 2003. Role of viruses and atypical bacteria in exacerbations of asthma in hospitalized children: A prospective study in the Nord-Pas de Calais region (France). Pediatr Pulmonol 35:75–82.

Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. 2006. Detection of the new human coronavirus HKU-1. Clin Infect Dis 42:634–639.

van den Hoogen BG, de Jong JC, Groen J, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med 7:719–724.

van den Hoogen BG, Herfst S, Sprong L, Cane PA, Forleo-Neto E, de Swart RL, Osterhaus AD, Fouchier RA. 2004. Antigenic and genetic variability of human metapneumoviruses. Emerg Infect Dis 10:658–666.

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

Weissbrich BB, Neske F, Schubert J, Tollmann F, Blath K, Blessing K, Kreth W. 2006. Frequent detection of bocavirus DNA in German children with respiratory tract infections. BMC Infect Dis 6:109.

Williams JV, Tollefson SJ, Heymann PW, Carper HT, Patrie J, Crowe JE. 2005. Human metapneumovirus infection in children hospitalized for wheezing. J Allergy Clin Immunol 115:1311–1312.

Woo PC, Lau SK, Tsoi HW, Huang Y, Poon RW, Chu CM, Lee RA, Luk WK, Wong GK, Wong BH, Cheng WC, Tang BS, Wu AK, Yung RW, Chen H, Guan Y, Chan KH, Yuen KY. 2005. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. J Infect Dis 192:1898–1907.