Journal of Medical Virology 86:2011–2018 (2014)

Human Bocavirus Isolated From Children With Acute Respiratory Tract Infections in Korea, 2010–2011

Jong Gyun Ahn,[1,2] Seong Yeol Choi,[3] Dong Soo Kim,[2,3] and Ki Hwan Kim[2,3]*

[1]*Department of Pediatrics, School of Medicine, Ewha Womans University, Seoul, Korea*
[2]*Department of Medicine, The Graduate School of Yonsei University, Seoul, Korea*
[3]*Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, Seoul, Korea*

Human bocavirus (HBoV) was first recognized in respiratory samples in 2005. The clinical importance of HBoV infection remains unclear. This report describes the clinical features and molecular phylogeny of HBoV isolates in children with acute respiratory infections. Nasopharyngeal aspirates were obtained from 1,528 children with acute respiratory infections between 2010 and 2011. Respiratory samples were screened for HBoV by multiplex PCR. A phylogenetic analysis of the HBoV VP1/VP2 gene was also undertaken. HBoV was detected in 187 (12.2%) of the 1,528 patients with a peak incidence of infection observed in patients aged 12–24 months. Coinfection with other respiratory viruses was observed in 107 (57.2%) of the HBoV-positive children. The peak of HBoV activity occurred during the month of June in both 2010 and 2011. A higher previous history of wheezing ($P = 0.016$), a higher frequency of chest retraction ($P < 0.001$) and wheezing ($P = 0.022$), a higher respiratory symptom score ($P = 0.002$), and a longer duration of hospital stay ($P = 0.021$) were observed in HBoV-positive children compared with the HBoV-negative group. Phylogenetic analysis showed all 187 HBoV-positive isolates were identified as HBoV 1, indicating minimal sequence variations among the isolates. A single lineage of HBoV 1 was found to have circulated in children with acute respiratory infections between 2010 and 2011 and was associated with several clinical characteristics including age, seasonality, and clinical severity with retraction, wheezing, and longer hospitalization. The clinical relevance of the minimal sequence variations of HBoV remains to be determined. ***J. Med. Virol. 86:2011–2018, 2014.*** © 2014 The Authors. *Journal of Medical Virology* published by Wiley Periodicals, Inc.

KEY WORDS: human bocavirus; acute respiratory infections; phylogenetic analysis

INTRODUCTION

Over the past decade advancement of new molecular techniques has led to the detection of various potential respiratory viruses such as human metapneumovirus (HMPV) [van den Hoogen et al., 2001], human coronavirus (HCoV) NL63 [van der Hoek et al., 2004] and HKU1 [Woo et al., 2005], severe acute respiratory syndrome-coronavirus (SARS-CoV), [Drosten et al., 2003], and human bocavirus (HBoV) [Allander et al., 2005]. HBoV was first isolated in 2005 from nasopharyngeal specimens from children with respiratory tract infections [Allander et al., 2005]. HBoV, a small non-enveloped virus with an approximately 5 kb single-stranded DNA genome is classified in the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Bocavirus*. Since it was first identified, HBoV has been detected worldwide in respiratory samples. Several studies have shown that HBoV is associated with acute respiratory tract infections in young children, with a reported worldwide prevalence ranging from

Grant sponsor: Korea Centers for Disease Control and Prevention (KCDC) (partial support)

Conflicts of interest: none.

Ethical approval: Ethical clearance was obtained from Yonsei University Health System Institutional Review Board, Seoul, Korea (4-2010-0003), and parents or legal guardians provided informed consent.

*Correspondence to: Ki Hwan Kim, Department of Pediatrics, Severance Children's Hospital, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemungu, Seoul 120-752, Korea.
E-mail: khkim99@yuhs.ac

Accepted 12 December 2013

DOI 10.1002/jmv.23880
Published online 4 January 2014 in Wiley Online Library (wileyonlinelibrary.com).

1.5% to 19% [Bastien et al., 2006; Allander et al., 2007; Fry et al., 2007].

In recent years, three closely related variants of HBoV have been reported, designated as HBoV2, HBoV3, and HBoV4 [Arthur et al., 2009; Kapoor et al., 2009, 2010]. HBoV2 was discovered in the stool of children with non-polio acute flaccid paralysis in Pakistan [Kapoor et al., 2009]. Soon thereafter, HBoV3 and HBoV4 were identified in the stools of children with diarrhoea in Australia and the USA [Arthur et al., 2009; Kapoor et al., 2010]. To date, HBoV1 has been linked most frequently with respiratory tract illness, particularly in children [Bastien et al., 2006; Kesebir et al., 2006; Allander et al., 2007; Fry et al., 2007; Maggi et al., 2007], although it has also been detected in stool samples to a lesser extent [Albuquerque et al., 2007; Lau et al., 2007; Lee et al., 2007]. In contrast, HBoV2 occurs rarely in respiratory samples, and instead is found primarily in the stools of children, indicating an association between HBoV2 and gastrointestinal disease [Arthur et al., 2009; Chieochansin et al., 2009; Han et al., 2009; Kapoor et al., 2009; Chow et al., 2010; Santos et al., 2010]. Interestingly, the identification of HBoV3 and HBoV4 is so recent that the epidemiology and clinical characteristics associated with these viruses are only now being realized [Arthur et al., 2009; Chieochansin et al., 2009; Kapoor et al., 2010; Santos et al., 2010].

The clinical significance and molecular characteristics of these newly identified HBoV species have not been completely assessed. The aim of this study was to determine the epidemiological and clinical profile of HBoV infection and to investigate the presence of HBoV1, HBoV2, HBoV3, and HBoV4 by analysing HBoV molecular phylogeny in the nasopharyngeal aspirates of children with acute respiratory tract infections between 2010 and 2011.

MATERIALS AND METHODS

Study Patients and Specimen Collection

Between January 2010 and December 2011, 1,528 nasopharyngeal specimens were collected from children hospitalized with acute respiratory tract infections at Severance Children's Hospital in Seoul, Korea. As part of the diagnosis, pneumonia, croup, bronchiolitis, asthma, acute pharyngotonsillitis, acute nasopharyngitis, and acute respiratory distress syndrome (ARDS) were included, which were diagnosed by the physician through physical examination and chest X-rays according to the World Health Organization (WHO) recommended guidelines [World Health Organization, 2005]. Patients who had immunocompromised conditions (e.g., current treatment of chemotherapy or immunosuppressive therapy, bone marrow transplantation or known primary immune deficiency) were all excluded from the study. Nasopharyngeal aspirates were obtained by suction using a fine, flexible plastic catheter and syringe and then immediately transported at 4°C to the laboratory, where they were stored at −70°C until use. Review of medical records, interviews at admission, and patient demographic and clinical data including medical history, detailed signs and symptoms, physical examination, and laboratory and radiology results were all collected. Disease severity was assessed according to a respiratory symptom scoring system based on a previously published metric. [Gern et al., 2002; Lemanske et al., 2005].

Multiplex PCR/RT-PCR for HBoV and Other Respiratory Viruses

Viral DNA or RNA from each nasopharyngeal sample was extracted with a QIAamp Viral Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. A one-step Multiplex PCR/RT-PCR kit (SolGent, Daejeon, Korea) was used to detect 12 respiratory viruses [Chun et al., 2009], including HBoV, respiratory syncytial virus (RSV), human rhinovirus (HRV), influenza A virus (IAV), influenza B virus (IBV), HMPV, adenovirus (ADV), HCoV 229E, OC43, and parainfluenza viruses (PIV)1–3. Amplification was performed according to the manufacturer's instructions, and products were run on a 2% agarose gel, stained with ethidium bromide, and visualized by UV light.

Sequencing of VP1/2 and Phylogenetic Analysis

HBoV-positive specimens were amplified with nested PCR using pan-bocavirus PCR primers targeting the VP1/VP2 gene as described previously [Kapoor et al., 2010]. Positive PCR samples were sequenced by SolGent Co. (Daejeon, Korea). VP1/VP2 sequences were aligned by CLUSTRAL W and phylogenetic analysis was conducted with MEGA 5.0 software. A topology tree was drawn by the neighbour-joining method and Kimura 2-parameter model bootstrapped with 1,000 replications.

Statistical Analysis

Statistical analysis was performed with SPSS (version 18.0, Chicago, IL). Values are expressed as percentages for discrete variables, or as the mean (m) ± standard deviation (SD) for continuous variables. Clinical features and laboratory variables were analyzed using the Pearson χ^2 test, Fisher's exact test, or Student's t-test. P-values less than 0.05 were considered statistically significant.

RESULTS

Epidemiologic Characteristics of HBoV

HBoV was detected in 187 (12.2%) of 1,528 nasopharyngeal aspirates. Overall, HBoV was the fourth most frequent pathogen identified in the study patients, following HRV (22.9%), ADV (20.9%), and RSV (17.4%) (Fig. 1). A total of 107 (57.2%) HBoV-positive

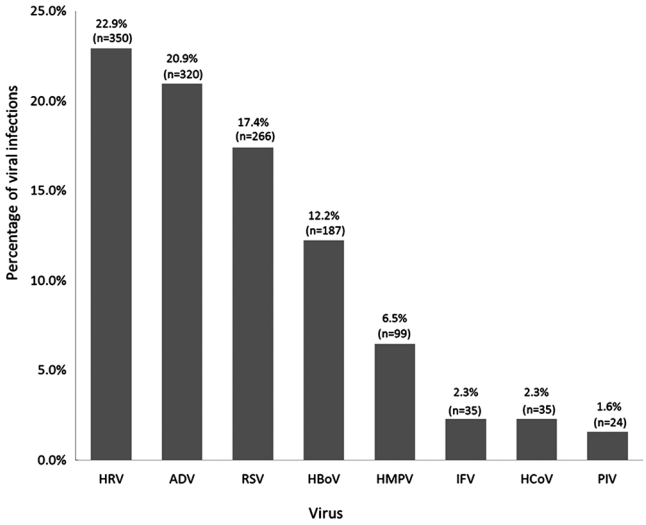

Fig. 1. Distribution of identified respiratory viruses. HRV, human rhinovirus; ADV, adenovirus; RSV, respiratory syncytial virus; HBoV, human bocavirus; HMPV, human metapneumovirus; IFV, influenza virus; HCoV, human coronavirus; PIV, parainfluenza virus.

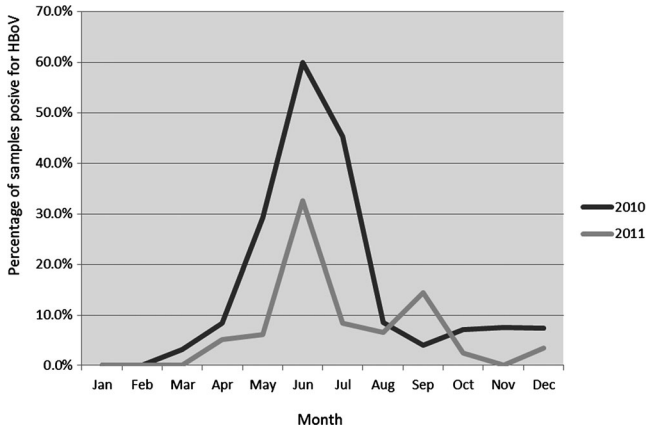

Fig. 2. Distribution of HBoV-positive cases by month, 2010–2011.

samples were co-detected with other respiratory viruses. One additional virus was found in 73.8%, two in 22.4%, and three in 3.7%. HRV, ADV, and HMPV were the most frequently co-detected (Table I).

Seasonal distribution of HBoV was noted between March and December, with a peak occurring in the month of June in both 2010 and 2011 (Fig. 2). Of the 187 HBoV-positive patients, 112 (59.9%) were male and 75 (40.1%) were female, with no significant difference between HBoV positive and negative patients (Table II). The mean ± SD age of HBoV infected children (24.3 ± 15.9 months) was significantly less than that of the HBoV-negative group (31.5 ± 30.5 months) (Table II). Further, the group aged 12 to <24 months had the highest rate of HBoV infection (43.9%), while the group >60 months of age had the lowest rate (3.2%) (Fig. 3).

Clinical Features of HBoV-Positive Patients

Of the 187 cases of HBoV infection, the main diagnosis was pneumonia (65.8%), followed by bronchiolitis (20.3%), croup (5.9%), asthma (3.7%), acute pharyngotonsillitis (2.7%), acute nasopharyngitis (1.1%), and ARDS (0.5%).

Previous wheezing history ($P = 0.016$), frequency of chest retraction and wheezing ($P < 0.001$ and $P = 0.022$, respectively), respiratory symptom score

TABLE I. Distribution of Detected HBoV

Type of infection	Virus				No. of patients (%)	Subtotal (%)
Single infection	HBoV(only)				80 (42.8)	80 (42.8)
Co-infection	HBoV	HRV			39 (20.9)	107 (57.2)
	HBoV	HMPV			16 (8.6)	
	HBoV	ADV			10 (5.3)	
	HBoV	ADV	HRV		10 (5.3)	
	HBoV	RSV			7 (3.7)	
	HBoV	PIV3			4 (2.1)	
	HBoV	ADV	RSV		3 (1.6)	
	HBoV	HRV	HMPV		3 (1.6)	
	HBoV	PIV1			2 (1.1)	
	HBoV	ADV	PIV3		2 (1.1)	
	HBoV	ADV	RSV	HRV	2 (1.4)	
	HBoV	ADV	HMPV		1 (0.5)	
	HBoV	ADV	HCoV (OC43)		1 (0.5)	
	HBoV	HMPV	HCoV (229E)		1 (0.5)	
	HBoV	HCoV (OC43)			1 (0.5)	
	HBoV	HRV	IBV		1 (0.5)	
	HBoV	PIV3	HRV		1 (0.5)	
	HBoV	RSV	HCoV (OC43)		1 (0.5)	
	HBoV	ADV	HRV	HMPV	1 (0.5)	
	HBoV	ADV	HRV	PIV1	1 (0.5)	
Total						187 (100)

HBoV, human bocavirus; HRV, human rhinovirus; HMPV, human metapneumovirus; ADV, adenovirus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HCoV, human coronavirus; IBV, influenza virus type B.

TABLE II. Clinical and Laboratory Characteristics of HBoV Positive Patients

Clinical data	HBoV (+), n = 187	HBoV (−), n = 1,341	*P*-value
Age (months)	24.3 ± 15.9	31.5 ± 30.5	<0.001*
Gender (male%)	59.9	60.3	0.925
Prematurity Hx. (%)	10.7	8.4	0.303
Congenital heart disease Hx. (%)	4.8	5.7	0.606
Neurologic disorder Hx. (%)	10.7	7.6	0.144
Previously wheezing Hx. (%)	19.8	13.3	0.016*
Chest retraction (%)	24.1	11.9	<0.001*
Wheezing (%)	40.1	31.7	0.022*
Tachypnea (%)	2.7	1.2	0.103
Respiratory symptom score	8.5 ± 4.1	7.5 ± 3.4	0.002*
WBC ($\times 10^3$ cells/mm)	11.4 ± 5.0	11.1 ± 5.3	0.501
Haemoglobin (g/dl)	12.0 ± 1.1	12.0 ± 1.1	0.986
Platelet count ($\times 10^3$ cells/mm)	380.4 ± 131.2	373.4 ± 139.4	0.544
Neutrophils (%)	53.2 ± 16.6	51.7 ± 19.4	0.289
Lymphocyte (%)	34.7 ± 15.0	36.0 ± 17.3	0.342
CRP (mg/L)	21.3 ± 33.7	26.9 ± 40.1	0.088
ESR (mm/hr)	32.8 ± 26.4	36.0 ± 25.7	0.137
Total protein (g/dl)	6.5 ± 0.5	6.5 ± 0.5	0.103
Albumin (g/dl)	4.2 ± 0.4	4.2 ± 0.5	0.149
ALT (IU/L)	17.8 ± 16.6	21.6 ± 23.9	0.011*
AST (IU/L)	32.9 ± 11.5	36.4 ± 20.6	0.001*
Length of hospital stay (days)	4.7 ± 5.2	3.8 ± 4.6	0.021*

*$P < 0.05$.

($P = 0.002$), and length of hospital stay ($P = 0.021$) were significantly higher in the HBoV-positive group than in HBoV-negative children (Table II). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in both groups were within normal ranges, although there was a significant difference between them ($P = 0.011$ and $P = 0.001$, respectively) (Table II). Aside from ALT and AST, no significant differences were observed with respect to underlying history such as prematurity, congenital heart disease and neurologic disorder, frequency of tachypnea, and laboratory findings between HBoV positive and negative patients (Table II).

Between children in which HBoV was the only specimen detected and those co-infected with both HBoV and other viruses, there were no significant differences in clinical and laboratory data other than gender ratio ($P = 0.017$), the percentage of neutrophils in white blood cells (WBCs) ($P = 0.037$), and total protein ($P = 0.036$) (Table III). However, the neutrophil ratio in WBCs and total protein were within the normal range in both groups.

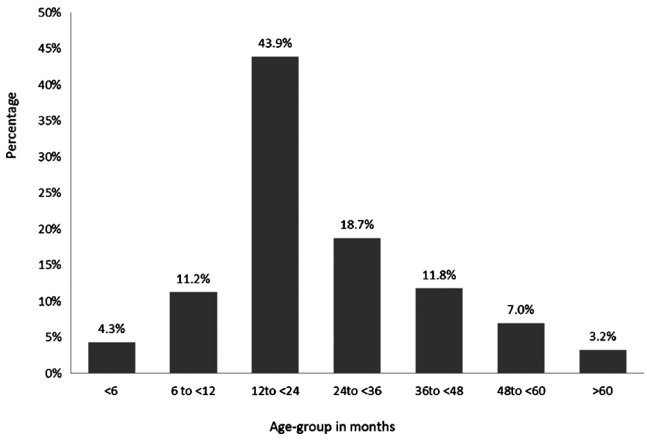

Fig. 3. Age distribution of HBoV-positive patients.

Phylogenetic Analysis of HBoV

To investigate the divergence of the HBoV genome, a 576-bp fragment of the VP1/VP2 gene (nucleotides 3328–3903) was sequenced for all HBoV-positive samples. Phylogenetic analysis demonstrated that all of 187 HBoV-positive strains were HBoV 1, and HBoV 2, HBoV3, and HBoV4 were not detected (Fig. 4). There was a high nucleotide identity (98.78–100%) between all HBoV sequences. As most of the nucleotide variations were conservative at the amino acid level, there was a 98.96–100% sequence identity among all strains. Overall, when compared to the Swedish reference strain (accession number NC_007455), 17 of the 576 nucleotide positions were found to be variable, with 15 (88.2%) transitions and 2 (11.8%) transversions. No significant differences were observed in clinical and laboratory data between the group with nucleotides variations and those with wildtype HBoV1.

DISCUSSION

Since the first report of HBoV in 2005, it has been detected frequently worldwide in respiratory samples, with a reported prevalence ranging from 1.5% to 19% [Schildgen et al., 2008; Tran et al., 2013]. In Korea, several reports on the presence of HBoV have been published, with a frequency range of 4.7–19.1% [Choi et al., 2006, 2007, 2011; Chung et al., 2006; Kim et al., 2011]. In the present study, HBoV was detected in 187 (12.2%) of 1,528 respiratory samples. The difference in frequency in the present study and

TABLE III. Comparison of Clinical and Laboratory Characteristics Between HBoV Single Infection and Co-infection With Other Viruses

Clinical data	Single infection, n = 80	Co-infection, n = 107	*P*-value
Age (months)	25.6 ± 16.3	23.2 ± 15.6	0.34
Gender (male%)	50	67.3	0.017*
Prematurity Hx. (%)	10	11.2	0.79
Congenital heart disease Hx. (%)	3.8	5.6	0.735
Neurologic disorder Hx. (%)	11.3	10.3	0.832
Previously wheezing Hx. (%)	16.3	22.4	0.294
Chest retraction (%)	30	19.6	0.101
Wheezing (%)	32.5	45.8	0.066
Tachypnea (%)	3.8	1.9	0.653
Respiratory symptom score	8.6 ± 3.8	8.5 ± 4.4	0.881
WBC ($\times 10^3$ cells/mm^3)	11.6 ± 5.6	11.3 ± 4.5	0.693
Haemoglobin (g/dl)	12.1 ± 1.2	12.0 ± 1.1	0.236
Platelet count ($\times 10^3$ cells/mm^3)	382.1 ± 120.5	379.0 ± 140.3	0.881
Neutrophils (%)	56.1 ± 14.9	50.8 ± 17.6	0.037*
Lymphocyte (%)	32.4 ± 13.7	36.7 ± 15.9	0.066
CRP (mg/L)	19.0 ± 26.3	23.3 ± 38.9	0.413
ESR (mm/hr)	34.3 ± 27.1	31.6 ± 25.9	0.509
Total protein (g/dl)	6.6 ± 0.5	6.5 ± 0.5	0.036*
Albumin (g/dl)	4.2 ± 0.4	4.2 ± 0.4	0.438
ALT (IU/L)	18.8 ± 18.6	17.0 ± 14.7	0.486
AST (IU/L)	34.2 ± 13.4	31.8 ± 9.5	0.192
Days in hospital (days)	5.1 ± 7.0	4.4 ± 2.8	0.352

*$P < 0.05$.

those previously reported may be related to the different study populations and periods. Nevertheless, this study confirmed that HBoV is circulated frequently in Korean children with acute respiratory tract infections, and was the fourth most prevalent virus among the 12 respiratory viruses which were tested.

Previous studies have reported that the seasonal preference of HBoV infections varies among different counties [Schildgen et al., 2008]. These differences are thought to be due to differences in climates and various factors affecting the prevalence of respiratory infections in each country. In this study, the peak of HBoV infections occurred during the month of June in both 2010 and 2011. These results were similar to those of two earlier studies [Choi et al., 2006; Kim et al., 2011] in Korea that reported HBoV infections peaked in May and June between 2000 and 2005 and between April and June in 2006. In close accordance with previous studies, this study suggested that HBoV was especially prevalent in the late spring and early summer seasons in Korea.

The age distribution data in this study showed that the mean ± SD age was significantly younger in HBoV-positive patients compared with HBoV-negative patients, with a peak incidence of HBoV observed in children aged 12 to <24 months and lowest incidence in children older than 60 months. These results are in close agreement with numerous previous studies [Kesebir et al., 2006; Fry et al., 2007; Maggi et al., 2007], which showed that most patients with HBoV infections are infants or children below 5 years of age. A previous seroepidemiologic study of HBoV showed that the seropositive rate in children aged under 3 months is 90.5%, but drops to 5.6% in children 6–8 months old, likely due to waning levels of maternally-acquired antibodies [Endo et al., 2007]. The percentage of HBoV-seropositive patients rebounds and increases with age, such that all children are exposed to HBoV by the age of six. One study [Kahn et al., 2008] showed that although 91.8% of infants ≤2 months old are HBoV-seropositive, the seropositive rate is lowest (25.0%) in children aged 4 months, but is increased to >85% in children ≥ 48 months old. Together, these data suggest that infants younger than 4–6 months of age may be protected from HBoV infection by maternal antibodies, and that the first HBoV infection begins after the loss of maternal antibodies, with the majority of HBoV infections occurring very early in childhood. In addition, the lower rate of detection of HBoV in older children and adults, as reported in several studies [Allander et al., 2005, 2007; Fry et al., 2007; Lau et al., 2007; Han et al., 2009; Noh et al., 2013], may be due to life-long immunity acquired from an infection early in childhood.

With respect to the clinical features of HBoV infection, the present study showed that HBoV-positive children had a higher prevalence of prior wheezing history, a higher frequency of chest retraction and wheezing, higher respiratory symptom scores, and a longer duration of hospital stay compared with the HBoV-negative group. These results are in agreement with previous studies reporting an association of HBoV with acute wheezing in children [Choi et al., 2006; Chung et al., 2006; Allander et al., 2007; Fry et al., 2007; Deng et al., 2012] as well as respiratory distress and hypoxia as main clinical characteristics of HBoV infection [Moriyama et al., 2010]. In addition, several studies have shown that HBoV is a cause of severe exacerbation of asthma in children [Naghipour et al., 2007; Vallet et al., 2009]. Although

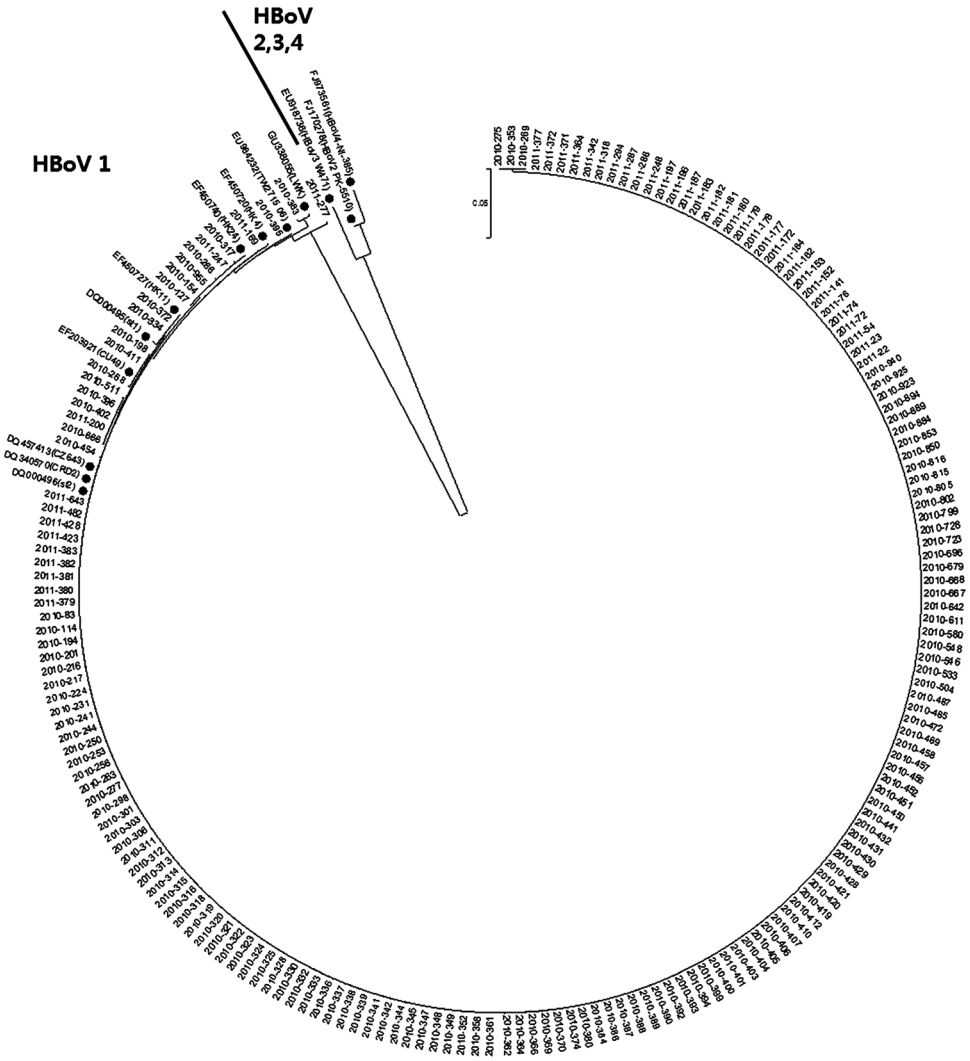

Fig. 4. Phylogenetic analysis of the VP1/VP2 gene junction sequence in HBoV isolates. The topology tree was constructed by the neighbour-joining method using MEGA 5.0. Black dots designate reference strains; other sequences were analyzed in the present study. GenBank accession nos. HBoV2 PK-5510 (FJ170278); HBoV3 W471 (EU918736); HBoV4-NI-385 (FJ973561); LWK (GU338055); TW2715_06 (EU984232); HK4 (EF450720); HK24 (EF450740); HK11 (EF450727); st1 (HBoV strain st1, DQ000495); CU49 (EF203921; CZ643 (DQ457413); CRD2 (DQ340570); st2 (HBoV strain st2, DQ000496).

the impact of co-infection with other viruses should be considered, results of this study showed that there were no significant differences in clinical features between children with HBoV infection alone and those co-infected with another virus, implying that HBoV-positive patients experience clinical severities such as respiratory distress, wheezing, and longer hospitalization regardless of co-infection status. Deng et al. [2012] suggested that a high HBoV viral load could lead to longer duration of wheezing and hospitalization in children with severe lower respiratory tract infection, while co-infection does not influence clinical severity and outcome of HBoV infection.

In the present study, HBoV2, HBoV3, and HBoV4 in respiratory samples was not detected. HBoV1 is well described in association with respiratory illness. Although recent reports have described an HBoV2-4 prevalence of 0.4–4.3% in respiratory specimens children [Han et al., 2009; Song et al., 2010; Koseki et al., 2012], the roles of HBoV2-4 in respiratory disease remain unclear. HBoV2-4 occurs rarely in respiratory samples but is fairy common in stools of children [Arthur et al., 2009; Chieochansin et al., 2009; Han et al., 2009; Kapoor et al., 2009, 2010; Chow et al., 2010; Santos et al., 2010], suggesting an association between HBoV2-4 and gastrointestinal disease. Results of the present study indicate that HBoV2-4 is not directly involved in respiratory tract infections. Further, it is presumed that HBoV2-4 is detected rarely in respiratory secretions originating from gastrointestinal mucosa due to contamination with oral secretions at the time of sampling, although

further studies are needed to determine if HBoV2-4 are associated with clinical illness outside of the gastrointestinal tract.

Phylogenetic analysis in this study confirmed the results of previous findings [Allander et al., 2005; Chung et al., 2006; Kesebir et al., 2006; Lau et al., 2007; Neske et al., 2007] showing that HBoV1 is highly conserved with a very low degree of genetic variability. Based on these results, Lau et al. [2007] predicted that HBoV infections may occur only once because of the subsequent development of life-long immunity conferred by neutralizing antibodies. In addition, it is postulated that HBoV1-associated respiratory infections might have a homogenous pattern of clinical and epidemiologic features because of the low genetic diversity of HBoV1. In support of this possibility, the study results indicated that there were no significant differences with respect to both clinical and laboratory data between children with and without minor HBoV1 variations. Specifically, even though 23 strains with nucleotide variations out of 187 HBoV isolates was identified, only 2 of the 23 strains resulted in an amino acid substitution. Thus, the 21 strains with nucleotide variations were thought to have the same biologic characteristics as non-mutated HBoV. In addition, the two strains with an amino acid substitution did not exhibit specific clinical features, suggesting they may not influence the biological action of HBoV. In contrast, several novel types of HBoV2-4, which were not detected in this study, exhibit a high degree of genetic diversity relative to HBoV1 [Arthur et al., 2009; Kapoor et al., 2009, 2010]. These findings suggest that HBoV2-4 and HBoV1 have different clinical impacts and support the above proposal that HBoV2-4 are not directly involved in respiratory disease.

This study had several limitations. First, the study patients were limited to children hospitalized with respiratory infections at a single-centre. Second, quantification of viral loads, which would be useful to analyze co-infection cases more clearly, was not measured. Third, there is the lack of an asymptomatic control group. Fourth, the evaluation of the causative agents of acute respiratory infections covered only 12 respiratory viruses, and other bacterial or viral pathogens which can cause acute respiratory illnesses were not investigated. Despite these shortcomings, these data should aid in the understanding of the role that HBoV plays in respiratory illness.

In summary, HBoV1 with minimal sequence variations circulated in children with acute respiratory infections between 2010 and 2011, and was associated with several clinical characteristics including age, seasonality, and clinical severity with retraction, wheezing, and longer hospitalization.

It is concluded that HBoV1 is an important respiratory pathogen in young children and exhibits a homogenous pattern of clinical and epidemiologic features due to the relatively minor degree of genetic variation. Further studies are needed to clarify the clinical relevance of the minimal sequence variations within HBoV1 and to define the clinical roles of HBoV2-4.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance rendered by Ms. Kim Heui Og.

REFERENCES

Albuquerque MC, Rocha LN, Benati FJ, Soares CC, Maranhao AG, Ramirez ML, Erdman D, Santos N. 2007. Human bocavirus infection in children with gastroenteritis, Brazil. Emerg Infect Dis 13:1756–1758.

Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, Vuorinen T, Waris M, Bjerkner A, Tiveljung-Lindell A, van den Hoogen BG, Hyypia T, Ruuskanen O. 2007. Human bocavirus and acute wheezing in children. Clin Infect Dis 44:904–910.

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 102:12891–12896.

Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. 2009. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog 5:e1000391.

Bastien N, Brandt K, Dust K, Ward D, Li Y. 2006. Human Bocavirus infection, Canada. Emerg Infect Dis 12:848–850.

Chieochansin T, Kapoor A, Delwart E, Poovorawan Y, Simmonds P. 2009. Absence of detectable replication of human bocavirus species 2 in respiratory tract. Emerg Infect Dis 15:1503–1505.

Choi CS, Park CH, Jung K, Lee G, Sun KK, Kim EY, Kim KSK, Kim YW, Seo J-J, Chung Y-S. 2007. Clinical manifestation of human bocavirus infection in children. Korean J Pediatr Infect Dis 14:136–144.

Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, Lee JH, Song EK, Kim SH, Park JY, Sung JY. 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clin Infect Dis 43:585–592.

Choi JH, Paik JY, Choi EH, Lee HJ. 2011. Epidemiologic characteristics of human bocavirus-associated respiratory infection in children. Korean J Pediatr Infect Dis 18:61–67.

Chow BD, Ou Z, Esper FP. 2010. Newly recognized bocaviruses (HBoV, HBoV2) in children and adults with gastrointestinal illness in the United States. J Clin Virol 47:143–147.

Chun JK, Lee JH, Kim HS, Cheong HM, Kim KS, Kang C, Kim DS. 2009. Establishing a surveillance network for severe lower respiratory tract infections in Korean infants and young children. Eur J Clin Microbiol Infect Dis 28:841–844.

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

Deng Y, Gu X, Zhao X, Luo J, Luo Z, Wang L, Fu Z, Yang X, Liu E. 2012. High viral load of human bocavirus correlates with duration of wheezing in children with severe lower respiratory tract infection. PloS ONE 7:e34353.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova 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.

Endo R, Ishiguro N, Kikuta H, Teramoto S, Shirkoohi R, Ma X, Ebihara T, Ishiko H, Ariga T. 2007. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. J Clin Microbiol 45:3218–3223.

Fry AM, Lu X, Chittaganpitch M, Peret T, Fischer J, Dowell SF, Anderson LJ, Erdman D, Olsen SJ. 2007. Human bocavirus: A novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. J Infect Dis 195:1038–1045.

Gern JE, Martin MS, Anklam KA, Shen K, Roberg KA, Carlson-Dakes KT, Adler K, Gilbertson-White S, Hamilton R, Shult PA, Kirk CJ, Da Silva DF, Sund SA, Kosorok MR, Lemanske RF, Jr. 2002. Relationships among specific viral pathogens, virus-induced interleukin-8, and respiratory symptoms in infancy. Pediatr Allergy Immunol 13:386–393.

Han TH, Chung JY, Hwang ES. 2009. Human bocavirus 2 in children, South Korea. Emerg Infect Dis 15:1698–1700.

Kahn JS, Kesebir D, Cotmore SF, D'Abramo A, Jr., Cosby C, Weibel C, Tattersall P. 2008. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. J Infect Dis 198:41–50.

Kapoor A, Simmonds P, Slikas E, Li L, Bodhidatta L, Sethabutr O, Triki H, Bahri O, Oderinde BS, Baba MM, Bukbuk DN, Besser J, Bartkus J, Delwart E. 2010. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis 201:1633–1643.

Kapoor A, Slikas E, Simmonds P, Chieochansin T, Naeem A, Shaukat S, Alam MM, Sharif S, Angez M, Zaidi S, Delwart E. 2009. A newly identified bocavirus species in human stool. J Infect Dis 199:196–200.

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.

Kim JS, Lim CS, Kim YK, Lee KN, Lee CK. 2011. Human bocavirus in patients with respiratory tract infection. Korean J Lab Med 31:179–184.

Koseki N, Teramoto S, Kaiho M, Endo Gomi R, Yoshioka M, Takahashi Y, Nakayama T, Sawada H, Konno M, Ushijima H, Kikuta H, Ariga T, Ishiguro N. 2012. Detection of human bocavirus 1-4 from nasopharyngeal swab samples collected from patients with respiratory tract infections. J Clin Microbiol 50:2118–2121.

Lau SK, Yip CC, Que TL, Lee RA, Au-Yeung RK, Zhou B, So LY, Lau YL, Chan KH, Woo PC, Yuen KY. 2007. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. J Infect Dis 196: 986–993.

Lee JI, Chung JY, Han TH, Song MO, Hwang ES. 2007. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. J Infect Dis 196:994–997.

Lemanske RF, Jr., Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Gern JE. 2005. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. J Allergy Clin Immunol 116:571–577.

Maggi F, Andreoli E, Pifferi M, Meschi S, Rocchi J, Bendinelli M. 2007. Human bocavirus in Italian patients with respiratory diseases. J Clin Virol 38:321–325.

Moriyama Y, Hamada H, Okada M, Tsuchiya N, Maru H, Shirato Y, Maeda Y, Hirose Y, Yoshida M, Omura Y, Honda T, Muto A, Hayashi K, Terai M. 2010. Distinctive clinical features of human bocavirus in children younger than 2 years. Eur J Pediatr 169:1087–1092.

Naghipour M, Cuevas LE, Bakhshinejad T, Dove W, Hart CA. 2007. Human bocavirus in Iranian children with acute respiratory infections. J Med Virol 79:539–543.

Neske F, Blessing K, Tollmann F, Schubert J, Rethwilm A, Kreth HW, Weissbrich B. 2007. Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. J Clin Microbiol 45:2116–2122.

Noh JY, Song JY, Cheong HJ, Choi WS, Lee J, Lee JS, Wie SH, Jeong HW, Kim YK, Choi SH, Han SB, So BH, Kim H, Kim WJ. 2013. Laboratory surveillance of influenza-like illness in seven teaching hospitals, South Korea: 2011–2012 season.PloS ONE8: e64295.

Santos N, Peret TC, Humphrey CD, Albuquerque MC, Silva RC, Benati FJ, Lu X, Erdman DD. 2010. Human bocavirus species 2 and 3 in Brazil. J Clin Virol 48:127–1130.

Schildgen O, Muller A, Allander T, Mackay IM, Volz S, Kupfer B, Simon A. 2008. Human bocavirus: Passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev 21:291–304, table of contents.

Song JR, Jin Y, Xie ZP, Gao HC, Xiao NG, Chen WX, Xu ZQ, Yan KL, Zhao Y, Hou YD, Duan ZJ. 2010. Novel human bocavirus in children with acute respiratory tract infection. Emerg Infect Dis 16:324–327.

Tran DN, Nguyen TQ, Nguyen TA, Hayakawa S, Mizuguchi M, Ushijima H. 2013. Human bocavirus in children with acute respiratory infections in Vietnam. J Med Virol, doi: 10.1002/jmv.23789, in press.

Vallet C, Pons-Catalano C, Mandelcwajg A, Wang A, Raymond J, Lebon P, Gendrel D. 2009. Human bocavirus: A cause of severe asthma exacerbation in children. J Pediatr 155:286–288.

van den Hoogen BG, de Jong JC, 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 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.

Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, Wong BH, Poon RW, Cai JJ, Luk WK, Poon LL, Wong SS, Guan Y, Peiris JS, Yuen KY. 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 79:884–895.

World Health Organization. 2005. Pocket book of hospital care for children: Guidelines for the management of common illnesses with limited resources. Geneva, Switzerland: WHO.