Detection of Human Bocavirus in Japanese Children with Lower Respiratory Tract Infections

Xiaoming Ma,[1] Rika Endo,[1] Nobuhisa Ishiguro,[1*] Takashi Ebihara,[2] Hiroaki Ishiko,[3] Tadashi Ariga,[1] and Hideaki Kikuta[1]

Department of Pediatrics,[1] Department of Microbiology and Immunology,[2] Hokkaido University Graduate School of Medicine, Sapporo, and Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo,[3] Japan

* Corresponding author. Mailing Address: Department of Pediatrics, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan. Phone: 81-11-706-5954. Fax: 81-11-706-7898. E-mail: nishigur@med.hokudai.ac.jp

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ABSTRACT

Human bocavirus (HBoV), a newly cloned human virus of the genus *Bocavirus*, was detected by PCR from nasopharyngeal swab samples collected from children with lower respiratory tract infections (8 of 318, 5.7%). HBoV may be one of the causative agents of lower respiratory tract infections in young children. (48 words)

TEXT

The family *Parvoviridae* contains two subfamilies: *Parvovirinae*, which infects vertebrates, and *Densovirinae*, which infects insects. The subfamily *Parvovirinae* consists of five genera: *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus*, and *Bocavirus* (12). Parvovirus B19, which belongs to the genus *Erythrovirus*, is a well-known pathogen to humans (3, 12). A new human virus of the genus *Bocavirus*, provisionally named human bocavirus (HBoV), was recently cloned from pooled human respiratory tract samples and is considered to be pathogenic to humans (1). In this study, nasopharyngeal swab samples obtained from children with lower respiratory tract infections were investigated for the presence of HBoV.

From October 2002 to September 2003 and from January 2005 to July 2005, a total of 318 nasopharyngeal swab samples were collected from 318 children with lower respiratory tract infections at 4 hospitals in Sapporo, Japan. All of the samples were collected after excluding the possibility of infection with hRSV or influenza A or B by rapid antigen detection tests and after excluding the possibility of infection with human metapneumovirus (hMPV) by a reverse transcription polymerase chain reaction (RT-PCR) test (6). The median age of the children was 21.3 months. The male-to-female ratio was 1.4 to 1. All samples were collected after obtaining informed consent from the children's parents. RNA and DNA were extracted from each sample

by using the method by Chomczynski's protocol (5). RNA was used for the detection of hMPV (6) and DNA was used for the detection of HBoV as described below. The PCR primers and conditions used for detection of HBoV have been described previously (1). A forward primer with a sequence of 5'-GAGCTCTGTAAGTACTATTAC-3' and a reverse primer with a sequence of 5'-CTCTGTGTTGACTGAATACAG-3' were used for both PCR and sequencing. The PCR mixture consisted of 100 μmol of each deoxyribonucleotide, 1.0 U of Ampli*Taq* Gold, 50 mmol of potassium chloride/liter, 10 mmol of Tris-HCl (pH 8.3)/liter, 1.5 mmol of magnesium chloride/liter, 0.01% (wt/vol) gelatin, 10 pmol of each primer, and DNA in a volume of 25 μl. The PCR conditions were as follows: 94°C for 9 min, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min. Both sense and antisense strands of the PCR products were sequenced directly by using a BigDye terminator cycle sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Tokyo, Japan) with an ABI Prism 310 genetic analyzer (Perkin-Elmer Applied Biosystems).

DNA sequences of HBoV were detected in samples from 18 (5.7%) of the 318 patients with lower respiratory tract infections. Seventeen of the 18 HBoV-positive samples were collected during the period from January to May (one sample in January, three in February, one in March, four in April, eight in May and one in July). The ages of patients with HBoV-positive samples ranged from 7 months to 3 years (one patient of 7

to 9 months in age, five of 10 to 12 months in age, ten of 1 to 2 years in age and two of 2 to 3 years in age). Direct sequencing of PCR products of the 18 samples showed that 14 of the 18 sequences were completely identical to the published sequence of HBoV (GenBank accession numbers DQ000495 and DQ000496) (Group 1 in Fig. 1). Three of the 18 sequences had a single base pair substitution in the NP-1 gene, resulting in an amino acid exchange (Group 2 in Fig. 1). One of the 18 sequences had a single base pair substitution in the NP-1 gene without amino acid exchange (Group 3 in Fig. 1). The sequences were deposited at GenBank (accession numbers DQ296618 to DQ296635).

Clinical and laboratory features of the 18 HBoV-positive patients are shown in Table 1. All of the 18 patients suffered from fever, cough, and various degrees of respiratory distress. Maximum temperature ranged from 37.5 to 40.2°C. The duration of fever (temperature of > 37.5°C) ranged from 1 to 8 days. Eight of the 18 patients showed abnormal findings on a chest x-ray (4 patients with lung infiltration, 3 patients with peribronchial infiltration, and one patient with hyperinflation). The clinical diagnoses of the HBoV-positive patients were pneumonia (6 patients), wheezy bronchitis (6 patients), bronchitis (2 patients), bronchiolitis (2 patients), asthma attack (1 patient), and laryngotracheitis (1 patient). Sixteen of the 18 patients were admitted to hospital for 3 to 9 days.

Bovine parvovirus (BPV) and canine minute virus (MVC) are members of the *Parvoviridae* family, subfamily *Parvovirinae*, genus *Bocavirus*. BPV caused mild diarrhea in calves when inoculated *per os*, and it caused diarrhea and mild respiratory symptoms when inoculated intranasally (11). MVC was first described as an isolate from a healthy dog in the U.S.A (2). Although it is likely that most infections with MVC are subclinical, diseases associated with virus infection include fetal infections leading to reproductive failure and neonatal respiratory disease (4, 7, 8). The virus may also be associated with some cases of enteritis in puppies or older dogs (2). HBoV was first cloned from pooled human respiratory tract samples collected in Sweden and was provisionally classified into the genus *Bocavirus* from resemblance of the sequences (1). HBoV has recently been found in Australian children with respiratory tract infection (10). HBoV was detected from samples collected during the period from winter to spring (1, 10). The detection rate of HBoV in respiratory tract infections has been reported to be 3.1% to 5.6% (1, 10), which is consistent with our data (5.7%). It should be noted that the possibility of infection with other viruses (parainfluenza viruses, rhinoviruses and coronaviruses) in our bocavirus positive specimens could not be excluded and that the possibility of influenza virus infection could not be totally excluded because of the limited sensitivity (60-80%) of rapid antigen tests for influenza

virus infection (9). The sequences of the amplified NP-1 region showed limited variation (1), as our data also showed (Table 1). The HBoV genome has been detected in samples from patients aged between 5 and 17 months (1) and aged between 6 month and 2 years (10). In our study, the ages of HBoV-positive patients ranged from 9 months to 2 years and 7 months (Table 1). The antibody against HBoV derived from the mother might protect infants under 5 months of age from HBoV infection, and primary HBoV infection might occur early in life. Serological study is needed to validate this hypothesis. Clinical findings in HBoV-positive patients are indistinguishable from those caused by other respiratory viruses. Our study showed that pneumonia and wheezy bronchitis were the major diagnoses in HBoV-positive patients. Carefully controlled studies are needed to clarify the full spectrum of diseases associated with HBoV. Five nasopharyngeal samples from five patients were inoculated on LLC-MK2 cells. However, the HBoV genome was not detected in DNA extracted from cells after a 3-week culture period (data not shown), suggesting that LLC-MK2 cells might not be appropriate for initial isolation of HBoV.

To our knowledge, this is the first report of detection of HBoV in patients with lower respiratory tract infections in Asia. This study suggests that HBoV may be widespread throughout the world and that it is one of the causative agents of lower respiratory tract infections in young children. To clarify the clinical impact of HBoV,

further surveillance in various age groups and various clinical groups is needed.

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Figure Legend

Sequences of PCR products of HBoV-positive samples. A of the ATG initiator methionine codon of NP-1 protein is denoted as nucleotide +1. The sequences of the 18 samples were divided into three groups and compared with that of the published sequence of HBoV (GenBank accession number DQ000495) as a reference. Group 1 (JPBS03-112, JPBS03-217, JPBS03-219, JPBS03-261, JPBS03-298, JPBS05-7, JPBS05-18, JPBS05-28, JPBS05-29, JPBT03-5, JPBT03-12, JPBT03-43, JPBT05-46, and JPBT05-52) was completely identical to the reference. Group 2 (JPBS03-182, JPBS03-207, and JPBS03-208) had a single base pair substitution (176G to A) in the NP-1 gene, resulting in an amino acid exchange (Ser to Asn). Group 3 (JPBS03-98) had a single base pair substitution (201G to A) in the NP-1 gene without amino acid exchange.

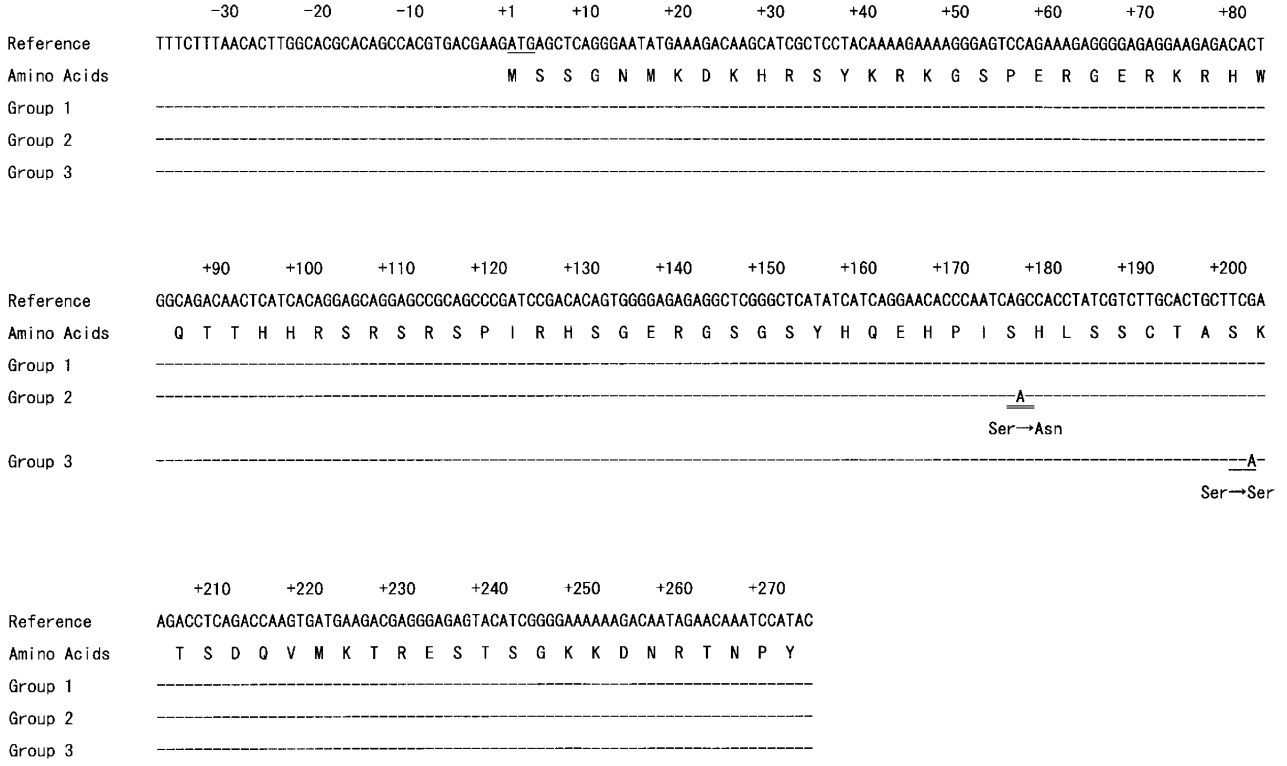
-30 -20 -10 +1 +10 +20 +30 +40 +50 +60 +70 +80
Reference
TTTCTTTAACACTTGGCACGCACAGCCACGTGACGAAGATGAGCTCAGGGAATATGAAAGACAAGCATCGCTCCTACAAAAGAAAAGGGAGTCCAGAAAGAGGGGAGAGGAAGAGACACT
Amino Acids
M S S G N M K D K H R S Y K R K G S P E R G E R K R H W
Group 1
Group 2
Group 3
+90 +100 +110 +120 +130 +140 +150 +160 +170 +180 +190 +200
Reference
GGCAGACAACTCATCACAGGAGCAGGAGCCGCAGCCCGATCCGACACAGTGGGGAGAGAGGCTCGGGCTCATATCATCAGGAACACCCAATCAGCCACCTATCGTCTTGCACTGCTTCGA
Amino Acids
Q T T H H R S R S R S P I R H S G E R G S G S Y H Q E H P I S H L S S C T A S K
Group 1
Group 2
-A-
Ser→Asn
Group 3
-A-
Ser→Ser
+210 +220 +230 +240 +250 +260 +270
Reference
AGACCTCAGACCAAGTGATGAAGACGAGGGAGAGTACATCGGGGAAAAAAGACAATAGAACAAATCCATAC
Amino Acids
T S D Q V M K T R E S T S G K K D N R T N P Y
Group 1
Group 2
Group 3

Table 1. Clinical characteristics of 18 patients positive for HBoV DNA by PCR

Case	Sample	Sex	Age	Diagnosis	Max. Temp (°C)	Duration of Fever >37.5°C (days)	Cough	Wheezing	Chest x-ray	Max. WBC (/μl)	Max. CRP (mg/dl)	Days hospitalized
1	JPBS03-98	F	1yr 6mo	Bronchitis	39.4	8	+	-	No abnormality	14,300	<0.20	4
2	JPBS03-112	F	10mo	Bronchiolitis	37.9	3	+	+	Peribronchial infiltration	6,400	<0.20	7
3	JPBS03-182	M	1yr 3mo	Pneumonia	40.2	2	+	+	Bilateral lung infiltration	21,900	3.80	8
4	JPBS03-207	M	1yr 10mo	Wheezy Bronchitis	39.1	2	+	+	No abnormality	13,980	0.20	5
5	JPBS03-208	M	1yr 0mo	Pneumonia	40.0	4	+	+	Right lung infiltration	11,800	1.58	7
6	JPBS03-217	M	1yr 3mo	Wheezy Bronchitis	38.8	3	+	+	No abnormality	21,980	2.40	5
7	JPBS03-219	M	10mo	Pneumonia	40.0	6	+	-	Peribronchial infiltration	20,260	1.80	5
8	JPBS03-261	F	1yr 1mo	Wheezy Bronchitis	38.0	3	+	+	No abnormality	6,590	0.40	7
9	JPBS03-298	F	1yr 9mo	Wheezy Bronchitis	38.1	1	+	+	No abnormality	15,190	<0.20	4
10	JPBS05-7	M	9mo	Bronchiolitis	37.9	3	+	+	Hyperinflation	12,400	0.20	9
11	JPBS05-18	M	1yr 11mo	Laryngotracheitis	39.6	5	+	-	No abnormality	14,000	1.02	7
12	JPBS05-28	M	11mo	Pneumonia	38.8	3	+	-	Right lung infiltration	17,000	4.48	7
13	JPBS05-29	F	11mo	Pneumonia	37.7	3	+	-	Right lung infiltration	10,220	0.38	6
14	JPBT03-5	M	2yr 7mo	Asthma attack	37.5	1	+	+	No abnormality	15,000	2.57	3
15	JPBT03-12	M	2yr 4mo	Pneumonia	39.0	2	+	+	Peribronchial infiltration	4,800	0.94	0
16	JPBT03-43	M	1yr 3mo	Bronchitis	39.5	3	+	+	No abnormality	9,400	0.77	4
17	JPBT05-46	M	1yr 3mo	Wheezy Bronchitis	38.8	3	+	+	No abnormality	11,700	0.27	7
18	JPBT05-52	M	1yr 9mo	Wheezy Bronchitis	39.0	1	+	+	No abnormality	10,800	0.23	0