

Human bocavirus in children with respiratory tract infection in Shanghai: a retrospective study

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Background: Human bocavirus (HBoV) was first reported in 2005. The worldwide presence of HBoV in children with acute respiratory tract infection (ARTI) has been confirmed. This study aimed to understand the prevalence and clinical features of HBoV in children with ARTI in Shanghai and explore the causative implication of HBoV in ARTI.

Methods: We retrospectively reviewed the medical records of 349 hospitalized children with ARTI between November 2006 and January 2007. From these children, 351 nasopharyngeal aspirate samples were collected; 325 of the samples were obtained from those with community-acquired ARTI and 26 from those with hospital-acquired ARTI. All samples were routinely screened for seven common respiratory viruses by immunofluorescence and further tested for HBoV by polymerase chain reaction.

Results: HBoV was detected in 16 (4.6%) of the 351 samples, and it was the second most commonly detected virus after respiratory syncytial virus. Three (19%) HBoV-positive samples were dual infection with respiratory syncytial virus or parainfluenza virus type 3. Of the 325 children with community-acquired ARTI, HBoV was identified to be positive in 11 (3.4%), of whom 6 were diagnosed with pneumonia with patchy or interstitial infiltrates in the lung indicated by chest radiography, 3 with bronchitis, and 2 with bronchial asthma exacerbation with attendant lung infection. Out of the 26 children with nosocomial ARTI, 5 (19.2%) had bronchitis which was found to be HBoV positive without co-detection of other viruses. The HBoV-positive children were aged 1.7 months to 43 months and their mean age

was 13.7 months. Sixteen (100%) children had cough, 11 (68.8%) had wheezing, and 10 (62.5%) had fever.

Conclusions: HBoV was circulating in Shanghai during the study period, and which was detected frequently in children with ARTI. HBoV was found to be associated with community-acquired ARTI and may play a pathogenic role in nosocomial ARTI.

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Key words: acute respiratory tract infection; children; community-acquired infection; hospital-acquired infection; human bocavirus

Introduction

Respiratory tract infection is a leading cause of morbidity and mortality for children.^[1] Viruses are the major causative agents for childhood acute respiratory tract illness.^[2] In 2005, human bocavirus (HBoV) was discovered in the respiratory samples from Swedish children with respiratory tract infection caused by other unknown agents.^[3] Based on the genetic sequence of HBoV, it is classified as bocavirus genus and belongs to the family of parvoviridae. To date, HBoV has been found to circulate worldwide.^[3-23] Serological studies have revealed that antibody seroconversion to HBoV is common in the early childhood.^[24-26] The pathogenic role of HBoV has not yet been proved by classical Koch's postulates because neither an *in vitro* culture of HBoV nor an animal mode of infection can be established. Two recent studies from Allander and coworkers^[23,27] suggested that HBoV significantly correlates with acute wheezing, and that HBoV-related respiratory infection can elicit B cell immune response.

Shanghai is a metropolitan city with a temperate climate where acute respiratory tract infection (ARTI) is endemic throughout the year. Usually respiratory virus season spans the winter and early spring annually and seven common respiratory viruses account for at least 24% of ARTI.^[28] HBoV, a newly identified

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virus, has been reported to be prevalent in the pediatric populations and is potentially associated with respiratory tract disease. Since epidemiology of respiratory viruses displays geographic and temporal characteristics, we investigated HBoV-associated respiratory tract infection among hospitalized children in Shanghai.

Methods

Patients and samples

We retrospectively studied HBoV infection between November 2006 and January 2007. Altogether 351 nasopharyngeal aspirates (NPAs) collected from 349 hospitalized children with ARTI were tested for HBoV. One sample was taken from each patient except two children from whom two samples were collected and tested at admission and during the occurrence of nosocomial infection. For children with community-acquired (CA) infection, samples were obtained within 48 hours after admission. For children with hospital-acquired (HA) infection, samples were collected within 2 days to 7 days after the occurrence of presumed nosocomial infection. Nosocomial ARTI was defined by the criteria that respiratory infection-associated symptoms such as cough, fever, and wheezing appeared 48 hours after hospitalization, and these symptoms could not be interpreted by primary infection.

Nasopharyngeal aspirates were collected in a sterile container with 3 ml of sterile physiological saline, then transported to the virology laboratory for immediate processing. Each sample was pipetted and homogenized, and then 1 ml of the sample was removed into a sterile eppendorf tube and kept at -70°C for further HBoV DNA detection by polymerase chain reaction (PCR). The remaining sample was used for the routine test of respiratory syncytial virus (RSV), influenza virus (IV) A and B, parainfluenza virus (PIV) type 1-3, and adenovirus (ADV) by direct immunofluorescence assay (Respiratory Panel IFA Kit, Chemicon) according to the manufacturer's instructions.

Clinical symptoms and laboratory data of hospitalized children were reviewed from their medical records. The final diagnosis of these children conformed to the diagnosis on discharge. Diagnosis of pneumonia was confirmed by chest X-ray showing consolidation, patchy or interstitial infiltrates in the lungs. For patients, if moist rale was revealed on auscultation but chest X-ray did not show infiltrates, bronchitis or bronchiolitis was diagnosed. Bronchial asthma was defined on the basis of the guidelines of the Subspecialty Group of Respiratory Diseases of Chinese Pediatric Society.^[29] Our study was approved by the Ethics Committee of Fudan Children's Hospital,

which does not require patients' consent for laboratory analysis of routinely collected clinical specimens.

Detection of HBoV

The specimens were thawed and pipetted repeatedly and then centrifuged at 1500g for 5 minutes. 200 μl supernatant was used for DNA nucleic acid extraction with MinElute DNA Virus Spin Kit (Sangon, Shanghai) according to the manufacturer's procedures. One set of primers 188F 5'-GAC CTC TGT AAG TAC TAT TAC-3' and 542R 5'-CTC TGT GTT GAC TGA ATA CAG-3' was used to screen a 354-bp fragment of the putative NP-1 gene.^[3] If positive PCR product was visualized, positive template was amplified with another set of primers VP1/VP2F 5'-GCA AAC CCA TCA CTC TCA ATG C-3' and VP1/VP2F 5'-GCT CTC TCC TCC CAG TGA CAT-3' to produce a 404-bp fragment of the putative VP1 and VP2 genes.^[6] HBoV infection was ascertained on condition that both target PCR fragments

Table 1. Characteristics of the children investigated

	CA cases (n=325)	HA cases (n=26)	Total cases (n=351)*
Demographic characteristics			
Range of age (median), month	0.1-124 (6)	1.6-60 (6)	0.1-124 (6)
Mean \pm SD, month	15.8 \pm 22.0	12.8 \pm 14.4	15.5 \pm 21.4
Male:Female	197:128	18:8	215:136
Medical condition, n (%)			
Pneumonia	256 (78.8)	10 (38.5)	266 (75.8)
Bronchiolitis/bronchitis	43 (13.2)	13 (50.0)	56 (16.0)
Asthma attack with concomitant pneumonia or bronchitis	16 (4.9)	0 (0.0)	16 (4.6)
Croup	5 (1.5)	0 (0.0)	5 (1.4)
Upper respiratory tract infection	5 (1.5)	3 (11.5)	8 (2.3)
With acute wheezing	141 (43.4)	7 (26.9)	148 (42.2)
With underlying diseases	39 (12.0)	7 (26.9)	46 [†] (13.1)
Viruses detected, n (%)			
HBoV [‡]	11 (3.4)	5 (19.2)	16 (4.6)
RSV [§]	104 (32.0)	12 (46.2)	116 (33.0)
IV	10 (3.1)	1 (3.8)	11 (3.1)
ADV	9 (2.8)	0 (0.0)	9 (2.6)
PIV-1	3 (0.9)	0 (0.0)	3 (0.9)
PIV-2	2 (0.6)	0 (0.0)	2 (0.6)
PIV-3 [¶]	10 (3.1)	1 (3.8)	11 (3.1)

CA: community-acquired; HA: hospital-acquired; HBoV: human bocavirus; RSV: respiratory syncytial virus; IV: influenza virus; ADV: adenovirus; PIV: parainfluenza virus. *: 2 cases were tested for viruses and clinically analyzed twice on admission for CA pneumonia and during the occurrence of HA bronchitis, respectively; [†]: 39 had congenital heart disease, 3 had neuromuscular disease, 2 had multiple abnormality and 2 had laryngomalacia; [‡]: 3 HBoV positive samples were dual infection with 2 coinfection with RSV, 1 coinfection with PIV-3; [§]: 3 RSV positive samples were dual infection with 2 coinfection with HBoV, 1 coinfection with ADV; ^{||}: 1 ADV positive sample had coinfection with RSV; [¶]: 1 PIV-3 positive sample had coinfection with HBoV.

were positive.

5 µl of extracted DNA templates were added into 50 µl master PCR reaction mixture containing 20 pmol each primer, 0.2 mmol/L dNTP, 2.5 U Taq DNA polymerase (Sangon, Shanghai), 2.5 mmol/L MgCl₂, and 1 × PCR buffer. PCR amplification thermocycles were performed at 95°C for 15 minutes followed by 35 cycles of PCR with denature at 94°C for 1 minute, annealing at 54°C for 1 minute, extension at 72°C for 1 minute, and a final 10-minute cycle at 72°C. A negative control consisting of sterile water and a positive control of HBoV capsid were included in each PCR run. 5 µl PCR product was mixed with 5 µl of loading buffer and subsequently separated on an ethidium bromide stained 1.5% agarose gel. Gels were run for 20 minutes at 100 V in 0.5 × TBE buffer and photographed under UV light with a digital camera.

Results

Frequencies of detection of HBoV and 7 common viruses

The demographic and clinical characteristics of patients are shown in Table 1. Among 351 nasopharyngeal aspirate samples obtained between 1 day and 25 days after onset of illness, 16 (4.6%) were ascertained to be HBoV positive through PCR assay and 3 (18.8%) were dual infection including 2 coinfection with RSV and 1 with PIV-3. In 16 HBoV-positive samples, 15 were obtained within 7 days after occurrence of respiratory illness and 1 was obtained at 14 days after onset of symptoms. In addition, routine immunofluorescence assay detected RSV in 116 (33.0%), IV in 11 (3.1%), PIV-3 in 11 (3.1%), ADV in 9 (2.6%), PIV-1 in 3 (0.9%), PIV-2 in 2 (0.9%), and RSV coinfection with ADV in 1 (0.3%). Obviously, HBoV was the second most prevalent virus in this study after RSV.

Among 351 NPA samples, 92 were collected in November 2006 and HBoV was detected in 6 (6.5%); 113 samples were collected in December 2006 and HBoV was detected in 6 (5.3%); 146 samples were collected in January 2007 and HBoV was detected in 4 (2.7%). Apparently there was a declining trend by month.

Clinical findings of HBoV-positive children

HBoV was found in 8 (14.8%) of 54 patients with bronchitis, in 2 (12.5%) of 16 patients with asthma attack, and in 6 (2.3%) of 266 patients with pneumonia. HBoV was detected in 10 (6.8%) of 148 children with acute wheezing and all 10 HBoV-positive children with wheezing were due to CA infection rather than HA infection. Among 16 HBoV-positive children, 11 were CA infection and 5 were HA infection; 6 children were

diagnosed with CA pneumonia (4 with sole infection and 2 coinfecting with RSV and PIV-3, respectively), 3 with CA bronchitis (2 with sole infection and 1 coinfecting with RSV), 2 with bronchial asthma exacerbation, and 5 with HA bronchitis without other seven viruses co-detected.

The HBoV-positive children were 1.7 to 43 months of age (mean: 13.7 months; median: 13 months). Five (31.3%) of 16 cases were <6 months old, 7 (43.8%) <12 months old, 13 (81.3%) <24 months old, 15 (93.8%) <36 months old, and all (100%) were younger than 48 months old.

All (100%) of the HBoV-positive children had a cough, 68.8% (11/16) had wheezing, and 62.5% (10/16) were febrile. 14 children were tested with white blood cell (WBC) and C-reactive protein (CRP) in peripheral blood. WBC was in the range of $4.6 \times 10^9/L$ and $25.5 \times 10^9/L$ and most patients had normal WBC. CRP ranged from <8 mg/L to 30 mg/L. Chest radiography revealed patchy or interstitial infiltrates in HBoV-positive patients with pneumonia (Table 2).

Discussion

HBoV has been frequently found to circulate among children from the community while HBoV-related nosocomial infection has rarely been reported. In this study, we found HBoV is a common viral agent in the respiratory samples from symptomatic children. Kesebir et al^[11] reported HBoV was detected in 3 (14%) of 22 infants with presumed nosocomial respiratory infection. Similarly, our present study found HBoV was detected in 5 (19.2%) of 26 inpatients who developed nosocomial ARTI. In 5 HBoV-positive children with nosocomial infection, 7 common viruses were not detected coincidentally. Particularly 2 HBoV positive patients had HBoV detected only in their second samples collected during the episode of nosocomial infection; however, their first controlled samples obtained at admission were HBoV negative. Hence, it is reasonable to suggest that HBoV is very likely to play a pathogenic role in patients with nosocomial ARTI.

In our study, HBoV was positive in 4.6% of nasopharyngeal aspirates from hospitalized children with ARTI. The proportion of positive HBoV ranged from 1.5% to 19.3% in respiratory samples from symptomatic patients.^[6,30] Obviously, the incidence of HBoV infection varied with region and time. Among the viruses detected in this study, HBoV was the second most common virus after RSV. This finding is consistent with that reported by Weissbrich and Catalano-Pons who used the same protocol of virus detection in their studies.^[8,31] Nevertheless, we can not conclude that HBoV is more prevalent than IV, PIV and ADV because

Table 2. Demographic and clinical characteristics of 16 HBoV-positive children

Case No.	Type of infection	Time of infection	Sex	Age (mon)	Diagnosis on discharge	Days from sampling to onset of symptoms	HBoV & virus finding	Cough	Max. fever (°C)	Duration of fever (d)	Wheezing	Chest X-ray [†]	WBC (×10 ⁹ /L)	CRP (mg/L)
1	CA	Nov	M	5	Bronchitis	5	+	+	-	/	+	Hyperinflation	6.4	<8
2	CA	Nov	M	13	Bronchitis	4	+	+	39	3	+	Hyperinflation	6	<8
3	CA	Nov	M	43	Acute asthma exacerbation with bronchitis	3	+	+	38.5	3	+	Hyperinflation	7.1	14
4 [*]	CA	Nov	M	28	Acute status asthmaticus with bronchitis	6	+	+	38.7	1	+	Hyperinflation	8.7	<8
5	CA	Dec	M	15	Pneumonia	6	+	+	39.5	3	+	Patchy infiltrate	25.5	<8
6	CA	Dec	M	10	Bronchitis & shigellosis	4	+	+	-	/	+	Hyperinflation	8.6	<8
7	CA	Dec	M	5.7	Pneumonia with congenital heart disease	2	+	+	-	/	+	Patchy infiltrate; Hyperinflation	8.1	<8
8 [*]	CA	Dec	M	17	Pneumonia	2	+	+	-	/	+	Patchy & interstitial infiltrate; Hyperinflation	7.8	30
9	CA	Jan	M	14	Pneumonia	3	+	+	40.5	3	+	Patchy infiltrate	None	None
10	CA	Jan	M	5	Pneumonia	5	+	+	-	/	+	Patchy infiltrate	None	None
11 [§]	CA	Jan	M	1.6	Pneumonia (severe); Congenital heart disease; Respiratory & cardiac failure	14	+	+	-	/	+	Patchy infiltrate	4.9	<8
12	HA	Nov	M	5	CMV hepatitis; *HA-bronchitis	7	+	+	38.7	2	-	None	7.4	<8
13	HA	Dec	M	4	Congenital tuberculosis *HA-bronchitis	7	+	+	38	1	-	None	4.6	<8
14	HA	Dec	F	29	CA-pneumonia (RSV-related); *HA-bronchitis	3	+	+	39.2	2	-	Infiltrate resolved	None	None
15	HA	Dec	M	23	Acute hepatitis CA-pneumonia *HA-bronchitis HA-rotavirus diarrhea	2	+	+	40	4	-	Infiltrate resolved	7.8	<8
16	HA	Jan	F	1.6	CA pneumonia; Cerebral palsy; *HA-bronchitis	2	+	+	39.5	7	-	Infiltrate resolved	11.8	22

All described clinical data were extracted from the medical records. "+": presence, "-": absence. *: HBoV-related HA illness; †: For patients with CA pneumonia and CA bronchitis, chest X-ray was taken before or after admission; for inpatients diagnosed with nosocomial respiratory tract infection, chest X-ray was repeated if the first report indicated pneumonia. ‡: These two patients were presented to intensive care for treatment; §: This patient was admitted to intensive care center and finally died from respiratory failure and cardiac failure due to severe congenital heart disease (ventricular septal defect and aortic stenosis) and pneumonia; ||: These two patients had two nasopharyngeal aspirates obtained. The first samples from both were HBoV-negative at admission and the second samples from both were HBoV-positive after occurrence of nosocomial infection.

the sensitivity of methods for viral diagnosis is different and PCR is more sensitive than immunofluorescence assay.^[32]

Previous studies reported that HBoV coinfection with other respiratory viruses was common with a frequency of 18% to 90%.^[3,15] In this study, 16.7% of HBoV-positive samples were coinfecting with RSV and PIV-3, which is lower than the previous report. The difference of enrolled subjects, study period, geographic

area, disease spectrum, and diagnostic assay may lead to varied results. Our study may underestimate the overall HBoV coinfection. A few known respiratory viruses such as human metapneumovirus and rhinovirus were not tested in our study. On the other hand, less sensitive immunofluorescence assay was used to screen seven common viruses.

Seasonal variation of HBoV prevalence has been shown. A higher prevalence of HBoV infection

occurred in the winter and early spring in Germany, Sweden, South Africa, United Kingdom, Hong Kong, Thailand and United States, and in the late spring and early summer in Korea.^[3,8,9,11,12,14,16,20,33] Two studies from Italy and Canada did not observe seasonal activity of HBoV.^[6,19] Because our study spanned only a winter season in Shanghai, we cannot describe the seasonality of HBoV in Shanghai. The investigation throughout the year is ongoing.

HBoV can be found in respiratory samples from pediatric and adult patients, but mainly in children.^[6,16,19,34] Most HBoV-infected patients are younger than 5 years old.^[6,16,20,21,35] In this study, HBoV-positive inpatients were younger than 4 years, as reported previously. Studies from Japan and America revealed that the HBoV-seropositive rate increases with age after 6 months and most children have been exposed to HBoV before school-age.^[24,25] We speculate that infants and young children are most susceptible to HBoV infection, but none of the neonates with pneumonia in our study was found to be HBoV-positive. Kahn et al.^[25] found 91.8% of serum specimens from infants in the first month of life were HBoV seropositive. Maternal IgG could aid to prevent neonates from acquiring HBoV infection.

HBoV has been detected in children with upper and lower respiratory tract infections and acute wheezing.^[10,16,20-21,23,33,35,36] Since a few children had upper respiratory tract infection in our study cohort, we only observed that HBoV is associated with lower respiratory tract infection. Most of HBoV-positive children with CA infection suffered from acute wheezing, whereas all HBoV-positive children with nosocomial ARTI did not present wheezing. Whether other viruses especially rhinovirus we had not tested were co-pathogenic for our patients with acute wheezing deserves further investigation. Allander and colleagues^[23] reported that HBoV was prevalent in 19% of children with acute wheezing but only 5% of the children had HBoV detected alone. We are not sure that HBoV is a major virus triggering acute wheezing for our children.

The role of HBoV in respiratory tract diseases is still controversial. Some studies compared HBoV detection in symptomatic children and asymptomatic controls to demonstrate its pathogenic significance. The existing literatures reported the frequency of HBoV detection in asymptomatic children was 0%-5%, which was significantly lower than that in symptomatic children.^[3,11,16,19,21] These findings, however, is challenged by a recent study from Canada which reported that HBoV was detected in 13.8% of symptomatic children and 43% of asymptomatic children.^[34] It is necessary to understand persistent HBoV shedding or asymptomatic carriage in the

respiratory tract for rationally explaining the clinical implication of HBoV.

In conclusion, our study suggests that HBoV circulating in Shanghai might play a pathogenic role in young children with ARTI in the community and hospital settings.

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Competing interest: None declared.

Contributors: Zeng M designed the study, performed the experiment and wrote the paper. Zhu QR supervised the study. Wang XH and Yu H gave advice about data analysis and revised the paper critically. Shen J collected part of specimens.

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