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Address for correspondence: Jean-Marc Rolain, Université de la Méditerranée, URMITE UMR 6236 CNRS-IRD, Marseille, France; email: jean-marc.rolain@univmed.fr

Bocavirus in Children with Respiratory Tract Infections

To the Editor: Four species of human bocavirus (HBoV1–4) have been identified since 2005 (1–4). Several reports have documented that HBoV1 are prevalent in respiratory tract samples. Although there may be many asymptomatic carriers, HBoV1 has been shown to cause respiratory tract diseases (1,5,6). HBoV2 has mainly been detected in fecal samples and has been linked to gastroenteritis (3,7,8). HBoV3 and HBoV4 have

recently also been detected in fecal samples (3,4), although no link to disease has been established for these 2 species.

In this study, we identified and characterized 3 HBoV species (HBoV1–3) detected in respiratory samples. Nasopharyngeal aspirates were collected from 1,238 children (784 boys and 454 girls) with acute lower respiratory tract infections hospitalized in Beijing Children's Hospital from March 2008 through July 2010. Patients' ages ranged from 1 month to 9 years (median 10.0 months, mean 32.1 months).

Viral nucleic acid was extracted from nasopharyngeal aspirates by using the NucliSens easyMAG system (bioMérieux, Marcy l'Etoile, France). We screened for HBoV1–4 by nested PCR with touch-down procedure using primers targeting the viral proteins (VP) 1/2 region (4). For HBoV-positive samples, we then quantified viral loads by real-time PCR (online Technical Appendix, www.cdc.gov/EID/content/17/9/110078-Techapp.pdf). Additional viral infections were identified in all screened specimens as described (9). To avoid contamination, the PCR process (including master mixture preparation, nucleic acid extraction, reaction installation, and DNA amplifications) were performed in separate dedicated areas. Strict controls were used during the process of nucleic acid extraction and PCR analyses to monitor contamination. All PCR products were verified by sequence analysis.

We found 141 positive samples for HBoVs (11.4%) from patients ranging in age from 1 to 132 months (median 12.0 months, mean 19.8 months) (GenBank accession nos. HQ871520–HQ871650 and HQ871664–HQ871673). Among these samples, 131 (10.6%; patient ages 1–132 months, median 12 months, mean 19 months) were positive for HBoV1, 5 (0.4%; patients 1–113

months of age, median 7.2 months, mean 29.2 months) for HBoV2, and 5 (0.4%; 1–108 months of age, median 12.0 months, mean 30.4 months) for HBoV3 on the basis of sequence alignment and phylogenetic analysis of PCR amplicons. No specimens were positive for HBoV4. The number of samples positive for HBoV1, -2, and -3 in children ≤ 5 years old was 124/131 (94.7%), 4/5 (80%), and 4/5 (80%), respectively. Additional respiratory viruses were co-detected in 120/141 (85.1%) HBoV-positive patients (Table). An unanticipated finding was that real-time PCR only detected 1/5 positive sample for HBoV2 (viral load 4.87×10^9 copies/mL) and 2/5 positive samples for HBoV3 (viral load 2.59×10^4 and 4.1×10^2 copies/mL). In contrast, we detected viral loads of 8.35×10^4 to 1.28×10^9 copies/mL in 5 randomly selected HBoV-1-positive samples.

The clinical diagnoses of patients providing HBoV-positive samples included pneumonia (63.1%), bronchitis (14.9%), bronchopneumonia (12.8%), and acute asthmatic bronchopneumonia (9.2%). No clinically significant differences were found between the signs and symptoms of patients with HBoV1, -2, and -3 (cough, sputum production, fever, runny nose, wheezing, and diarrhea). For patients positive for HBoV3, the major diagnoses were pneumonia (2/5), bronchopneumonia (2/5), and acute asthmatic bronchopneumonia (1/5). Main signs and symptoms included cough (5/5), wheezing (4/5), sputum production (3/5), fever (3/5), runny nose (1/5), and diarrhea (1/5). For patients positive for HBoV2, the diagnoses were pneumonia (4/5) and bronchopneumonia (1/5), and main signs and symptoms included cough (5/5), sputum production (4/5), wheezing (3/5), fever (3/5), and runny nose (2/5) (Table).

HBoV2 and HBoV3 were detected sporadically during the study. HBoV3 was detected in samples collected in

Table. Distribution and clinical characteristics of HBoV species in 1,238 children with acute lower respiratory tract infections, Beijing, China, 2008–2010*

Parameters	HBoV1	HBoV2	HBoV3
No. (%) positive	131 (10.6)	5 (0.4)	5 (0.4)
Age range, mo	1–132	1–113	1–108
No. (%) patients ≤5 y of age	124 (94.7)	4 (80)	4 (80)
Age mean/median, mo	19/12	29.2/7.2	30.4/12
M/F	72/59	3/2	4/1
Clinical manifestations, no. (%)			
Fever	78 (59.5)	3 (60)	3 (60)
Cough	131 (100)	5 (100)	5 (100)
Sputum production	115 (87.8)	4 (80)	3 (60)
Wheezing	65 (49.6)	3 (60)	4 (80)
Runny nose	46 (35.1)	2 (40)	1 (20)
Diarrhea	9 (6.9)	0 (0)	1 (20)
Mean ± SD leukocyte count, ×10 ⁹ /L	9.4 ± 5.3	7.56 ± 1.4	13.45 ± 6.1
Co-detection in samples, no. (%)			
Rhinovirus	58 (44.3)	1 (20)	2 (40)
Respiratory syncytial virus	42 (32.1)	2 (40)	2 (40)
Parainfluenza virus	24 (18.3)	0 (0)	1 (20)
Influenza virus	11 (8.4)	1 (20)	2 (40)
Coronavirus	15 (11.5)	0	0
Adenovirus	14 (10.7)	0	1 (20)
Human metapneumovirus	16 (12.2)	0	0
Enterovirus	2 (1.5)	1 (20)	0

*Detection rate for HBoV species, $\chi^2 = 234.1$, $p < 0.01$. No HBoV4 was found. HBoV, human bocavirus.

July and October 2008, December 2009, and March and April 2010, whereas HBoV2 was detected in May, June, and October 2008, March 2009, and January 2010. The prevalence of HBoV1, which was responsible for 92.9% of the HBoV cases, was highest in January 2009 and April 2010.

Multiple-alignment analysis of sequences obtained in this study and reference sequences from GenBank (accession nos. FJ948861, NC_012564, EU918736, HM132056, and HQ152935) by using MEGA 4.0 (10) showed that amino acids in HBoV VP1/2 regions were 94.8%–100% identical among the strains of same species and 76.3%–100% identical among strains of different species. No obvious nucleotide and amino acid differences were found for the HBoV3 strains detected in respiratory samples and those in stool samples.

In summary, we report detection of genomic DNA of HBoV1, -2, and -3 in children with lower respiratory tract infections in China. The predominant HBoV species identified

in our study was HBoV1. HBoV2 and HBoV3 appear to be present in much fewer positively identified cases and their viral load seems very low. For these latter viruses, however, low level mucosal contamination from the gastrointestinal tract cannot be entirely excluded in all cases. Further investigations are needed to confirm potential associations of HBoV2 and HBoV3 with acute lower respiratory tract infections, to determine their replication in the respiratory tract, and the viruses' role in human disease.

Acknowledgments

We thank Yaowu Yang for his assistance with designing the real-time PCR.

This study was supported in part by grants from the International Science and Technology Cooperation Program of China (2010DFB33270) and National Major Science & Technology Project for Control and Prevention of Major Infectious Diseases of China (2009ZX10004-206).

Li Guo, Richard Gonzalez, Zhengde Xie, Hongli Zhou, Chunyan Liu, Chao Wu, Gláucia Paranhos-Baccalà, Guy Vernet, Kunling Shen, Qi Jin, and Jianwei Wang

Author affiliations: State Key Laboratory for Molecular Virology and Genetic Engineering, Beijing, People's Republic of China (L. Guo, Q. Jin, J. Wang); Institute of Pathogen Biology, Beijing (L. Guo, R. Gonzalez, H. Zhou, C. Wu, Q. Jin, J. Wang); Fondation Mérieux, Lyon, France (R. Gonzalez, G. Paranhos-Baccalà, G. Vernet); and Beijing Children's Hospital, Beijing (Z. Xie, C. Liu, K. Shen)

DOI: <http://dx.doi.org/10.3201/eid1709.110078>

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Address for correspondence: Jianwei Wang, 9# Dong Dan San Tiao, Dongcheng District, Beijing 100730, People's Republic of China; email: wangjw28@163.com

Highly Virulent *Escherichia coli* O26, Scotland

To the Editor: Hemolytic uremic syndrome (HUS) is a rare disorder characterized by microangiopathic hemolytic anemia, microthrombi, and multiorgan injury. HUS is one of the commonest causes of acute renal failure in children worldwide and is most frequently precipitated by infection with verotoxin-producing *Escherichia coli* (VTEC) such as *E. coli* O157 (1). However, non-O157 VTEC serotypes have been increasingly found in the development of HUS (2–4).

Although previous surveillance of childhood HUS in Scotland identified *E. coli* O157 in >90% of cases, non-O157 serotypes have

also been associated with HUS (5). In 2010, several particularly severe cases of HUS were reported to Health Protection Scotland by a consultant pediatric nephrologist. Subsequent tests identified the pathogen in these cases as *E. coli* O26. However, in a recent study of pediatric HUS cases in Europe, children infected with *E. coli* O26 did not exhibit different clinical signs and symptoms from patients infected with other VTEC serotypes (6). To establish whether the host pathophysiologic responses to *E. coli* O157 and *E. coli* O26 strains differed, we analyzed a cohort of children with HUS who were infected with these VTEC serotypes.

In Scotland, most patients with pediatric thrombotic microangiopathy are referred to a specialist pediatric hospital, which immediately reports cases of HUS to Health Protection Scotland as part of national surveillance. To test the hypothesis that *E. coli* O26 was more virulent than *E. coli* O157, we performed an age-matched, nested case–case study of HUS patients and compared host clinical markers, treatment, and outcomes from pediatric cases in 2010. Data collection has been described elsewhere (5). The statistical significances of associations between categorical variables were investigated by using χ^2 , Fisher exact, Mann-Whitney, or *t* tests. All analyses were performed by using SPSS version 11 (SPSS Inc., Chicago, IL, USA) with a significance level of 5%.

Although initial signs and symptoms were similar for both sets of cases, i.e., bloody diarrhea and abdominal pain, statistical analysis showed that children with O26-HUS were more likely to have neurologic complications and diabetes mellitus and require admission to the intensive care unit than O157-HUS patients ($p = 0.02$ for neurologic complications and diabetes and $p = 0.04$ for admission to an intensive treatment unit; Table).

All patients with HUS were oligoanuric, and the 2 groups did not differ with respect to this parameter. However, O26-HUS patients had significantly longer periods of anuria than O157-HUS patients ($p = 0.04$; Table) and were more likely to require treatment with hemofiltration than with peritoneal or hemodialysis ($p = 0.001$; Table). One patient with O26-HUS also experienced cardiomyopathy resulting in reduced left ventricular function.

Our study illustrates the potential for increased severity of *E. coli* O26 infection in children. In Scotland, HUS is more commonly associated with *E. coli* O157 infection, and the outcome for children infected with this pathogen is much better than that reported in other studies (7,8). In this study, the clinical severity and outcomes for the children with O26-HUS were worse than for children requiring treatment for O157-HUS. We investigated the prehospital management of *E. coli* O157 and O26 patients in this cohort and found no difference in pharmacologic intervention or duration of delay in admission to hospital.

In our cohort, *vtx*₁ and *vtx*₂ genes were detected in isolates from 2 of 3 patients. A diagnosis was made in the third patient by detection of *E. coli* O26 lipopolysaccharide-specific immunoglobulin M in serum; it was therefore not possible to confirm the presence of *vtx* genes. However, it is not unusual for VTEC to be undetectable in stool samples from patients with HUS, most likely because of intrahost bacteriophage lysis. Therefore, serodiagnosis of VTEC is considered a necessary adjunct to bacteriological confirmation of infection (9). A recent study suggests *E. coli* O26 exists as a highly dynamic group of organisms that can undergo verotoxin gene loss and be transferred during infection in humans, resulting in new pathogenic clones (10). Therefore, *vtx*₂ gene acquisition by *E. coli* O26 may have contributed to increased virulence.

Bocavirus in Children with Respiratory Tract Infections

Technical Appendix

Real-time PCR for Human Bocavirus

To quantify the viral loads of human bocaviruses (HBoVs), a universal Taqman real-time PCR that can detect HBoV1–4 was performed. The primers/probe of the Taqman real-time PCR were designed to target the conserved regions of the viral protein (VP) 1/2 gene segment of HBoV1–4, amplifying a 113-bp fragment. The sequence of the forward primer was 5'-TGGMATTATTGGMTCMAGTTT-3', corresponding to the HBoV1 st1 strain (GenBank accession no. DQ000495) nt 3316–3326, and that of the reverse primer was 5'-CACCTTTATTTGAGTTDGCA-3', corresponding to the HBoV1 st1 strain nt 3309–3328. The sequence of the probe was 5'-HEX-AAGCGCGCCGTGGCTCCTGCTCT-BHQ-1-3', corresponding to the HBoV1 st1 strain nt 3247–3269. Each 25- μ L reaction mixture consisted of 2 μ L of viral DNA, 0.5 μ M each of the forward and reverse primers, and 0.3 μ M of the probe labeled at its 5' end with a reporter dye (HEX) and at its 3' end with a nonfluorescent quencher. PCR was conducted for 10 cycles at 95°C for 15 s, 50°C for 30 s, and 72°C for 1 min, followed by 40 cycles of 10 s each at 95°C and 40 s each at 60°C. The real-time PCR was performed on a CFX 96 (Bio-Rad Laboratories Inc., Hercules, CA, USA). The baseline fluorescence threshold was manually adjusted on the basis of background fluorescence of the no template control reaction by using the Baseline Subtracted Curve Fit analysis mode in Bio-Rad CFX Manager Software. Positive was defined as an amplification curve exceeding baseline fluorescence with a corresponding cycle threshold value (not exceeding a 40-cycle run). A real-time fluorescence amplification curve lower than the baseline fluorescence was considered negative. Serial

dilutions of the pGEMT Easy Vector (Promega, Madison, WI, USA) containing the HBoV VP1/2 gene segment were used as quantification standards. The housekeeping gene GAPDH was detected as the internal control to monitor the quality of the specimens. The limit of detection of this assay was 10 copies/reaction.