Laboratory and Epidemiology Communications

Outbreak of Human Bocavirus 1 Infection in Young Children in Toyama, Japan

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Communicated by Makoto Takeda

Human bocavirus 1 (HBoV1), a member of the family Parvoviridae and genus Bocavirus, was first identified by Allander et al. in 2005 (1). HBoV1 has been detected worldwide in approximately 2% to 20% of patients with an acute respiratory infection (ARI), mainly in children younger than 3 years (y) during the winter and spring seasons (2). The impact of HBoV1 infection of the respiratory tract is often difficult to assess because of its frequent detection in asymptomatic children and co-infection with other respiratory viruses in symptomatic children, at a rate of up to approximately 80% in respiratory specimens (3,4). Although recent studies of serodiagnosis and quantification of the viral load in specimens have provided evidence that HBoV1 is a true respiratory pathogen and not a "bystander" (2), few reports are available regarding outbreaks of HBoV1 infection. Here we report the March 2014 outbreak of HBoV1 infection in young children in Toyama Prefecture, which is approximately 250 km northwest of Tokyo, Japan.

We conducted an investigation to identify respiratory viruses circulating in Toyama Prefecture between October 2013 and June 2014 (the winter and spring seasons in Japan). We studied 104 outpatients with ARIs who were shown to be negative for influenza by a rapid test kit: 67 patients at Yagi Pediatric Clinic located in Toyama City (the prefectural capital) and 37 patients at Oguri Pediatric Clinic located in Takaoka City (the second largest city in Toyama Prefecture). All of the patients were residents of one of these cities and were children under 12 y of age (range, 4 months (mo) to 12 y; median age, 1 y 5 mo). Signed informed consent was provided by the patients' guardian before the sampling. This study was approved by the Ethics Committee of the Toyama Institute of Health.

Nasopharyngeal swabs were collected using FLOQSwabs (Copan Flock Technologies, Brescia, Italy), which were immediately submerged in a conical centrifuge tube containing 2 mL of Nissui Nutrient Broth (Nissui Pharmaceutical, Tokyo, Japan) and stored at −80°C until use. Nucleic acid was extracted from samples using the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Duplex real-time reverse transcription (RT)-polymerase chain reaction (PCR) was performed targeting 21 respiratory viruses: human rhinovirus (HRV); respiratory syncytial virus (RSV) A and B; human parainfluenza virus (HPIV) types 1–4; human metapneumovirus (HMPV); influenza A–C viruses; human coronavirus (HCoV) OC43, 229E, NL63, and HKU1; human enterovirus (HEV); human adenovirus (HAdV) B, C, D, and E; and HBoV1. The PCR used previously described primers and probes (5–9) and the QuantiTect Multiplex RT-PCR Kit (Qiagen) according to the manufacturer’s instructions. Quantitative real-time PCR was also performed for HBoV1 using the above primer-probe set (6). A 10-fold serial dilution (10¹ to 10⁷ copies per reaction) of plasmid DNA containing a fragment of HBoV1 genomic DNA was used to generate a standard curve for the quantification of viral loads in specimens.

In addition, the variable region of the VP1/VP2 genes of HBoV1, corresponding to nucleotide positions 4,172–5,276 of strain ST2 (GenBank accession no. NC_007455), was amplified and sequenced from the HBoV1-positive specimens using the TaKaRa Ex Taq Hot Start Version (Takara Bio, Otsu, Japan) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The PCR and sequencing primers were as previously described (10). The sequence data were assembled and analyzed using SEQUENCER Win V4.10.1 (Hitachi Solutions, Tokyo, Japan) and GENETYX Ver. 10.1.4 (Genetyx, Tokyo, Japan).

Of the 104 tested specimens, one or more respiratory viruses were detected in 88 specimens (85%; 80 single infections, 8 co-infections). In total, 97 viruses were detected, which included the following: HRV (n = 18), RSV (n = 12), HPIV (n = 9), HMPV (n = 12), HCoV (n = 14), HEV (n = 2), HAdV (n = 9), and HBoV1 (n = 21). HBoV1 was thus the most frequently detected virus during the study period. HBoV1 was detected in specimens collected from November 2013 through June 2014: 1 in November, 1 in December, 3 in January, 1 in February, 7 in March, 2 in April, 4 in May, and 2 in June (Fig. 1). HBoV1 was the only virus detected in 14 specimens (67% of the HBoV1-positive specimens), whereas 1 or more respiratory viruses were detected in the remaining 7 HBoV1-positive specimens: co-infection with HCoV in 2 specimens, HMPV in 2, HPIV in 1, HAdV in 1, and HRV and HMPV in 1. Seven of the 14 HBoV1 single infection cases were found in March (Fig. 1), raising the possibility that the virus may have been circulating among children in March in Toyama.

The clinical characteristics of the 7 patients (5 males

Accepted April 17, 2015.

DOI: 10.7883/yoken.JJID.2015.046

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and 2 females) with an HBoV1 single infection in March 2014 are shown in Table 1. The patients were aged from 10 mo to 2 y 4 mo (6-11 mo in 3 patients, 12-23 mo in 3, >24 mo in 1). Of the 7 patients, 6 were diagnosed with bronchitis (Patients 2-7), whereas the seventh was diagnosed with an upper respiratory tract inflammation (Patient 1). Common symptoms among these patients were cough (in 6 patients), wheezing (6 patients), and fever (all patients). High fever over 38.5°C occurred in 6 patients. Rhinorrhea was observed in 3 patients, and diarrhea in 1 patient. No patients had underlying diseases.

Many studies have demonstrated a positive correlation between ARI and high copy numbers of HBoV1 DNA in respiratory secretions, and that an HBoV1 single infection is associated with a higher viral load than co-infection with other respiratory viruses (2,11). We thus performed quantitative real-time PCR to measure the viral load in nasopharyngeal specimens obtained from Patients 1–7. In our system, the minimum viral load that would enable reproducible quantification was 10 copies per reaction, corresponding to 10^3 copies per swab. The viral loads ranged from <1 × 10^3 (detection threshold) to 4.3 × 10^8 copies of HBoV1 genome per swab (Table 1). Only Patient 6 had a low viral load, possibly because of the initial phase of an incipient infection or technical issues such as a loss during sampling. The highest viral load among all 7 patients with co-infections was 1.1 × 10^9 copies per swab (5 of 7 cases were < 1 × 10^3). These data suggest that HBoV1 was the causative agent of ARI in these patients with a single infection of the virus.

Patients 1 to 6 had visited Yagi Pediatric Clinic. Patients 2, 3, 4, and 6 had been in the same daycare center located near the clinic. In addition, the onsets of the disease in Patients 2, 3, 4, and 5 occurred around the same time (March 14 to 17, 2014). To evaluate the circulating viruses among these children, we performed direct sequencing of PCR amplicons for the VP1/VP2 genes. In the case of Patient 6, the target genes were not successfully amplified, probably because the PCR for the VP1/VP2 genes appeared to be less sensitive than the real-time PCR assay used in this study. Direct sequencing of PCR amplicons in the 6 cases other than Patient 6 showed that all nucleotide sequences were completely identical in the variable region of the VP1/VP2 genes of the viruses (data not shown). These data suggest that an outbreak of HBoV1 infection occurred among young children in March 2014 in Toyama, Japan, and that the virus was transmitted through children in a daycare center and nearby. Several studies demonstrated that a serologic analysis is a more precise approach for the diagnosis of HBoV1 infections.

Table 1. Clinical characteristics and viral loads associated with an HBoV1 single infection in children in March 2014

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Onset date</th>
<th>Sample date</th>
<th>Clinical diagnosis</th>
<th>Symptom</th>
<th>Body temperature (°C)</th>
<th>Viral load (copies/swab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 y 3 mo</td>
<td>M</td>
<td>Mar. 4, 2014</td>
<td>Mar. 5, 2014</td>
<td>URTI</td>
<td>Fever, cough, wheezing</td>
<td>38.0</td>
<td>4.2 × 10^7</td>
</tr>
<tr>
<td>2</td>
<td>11 mo</td>
<td>M</td>
<td>Mar. 17, 2014</td>
<td>Mar. 17, 2014</td>
<td>Bronchitis</td>
<td>Fever, cough, wheezing, rhinorrhea, diarrhea</td>
<td>40.0</td>
<td>2.3 × 10^7</td>
</tr>
<tr>
<td>3</td>
<td>10 mo</td>
<td>M</td>
<td>Mar. 14, 2014</td>
<td>Mar. 17, 2014</td>
<td>Bronchitis</td>
<td>Fever, cough</td>
<td>38.5</td>
<td>3.4 × 10^4</td>
</tr>
<tr>
<td>4</td>
<td>1 y 0 mo</td>
<td>F</td>
<td>Mar. 16, 2014</td>
<td>Mar. 17, 2014</td>
<td>Bronchitis</td>
<td>Fever, cough, wheezing, rhinorrhea</td>
<td>39.0</td>
<td>4.3 × 10^4</td>
</tr>
<tr>
<td>5</td>
<td>1 y 1 mo</td>
<td>M</td>
<td>Mar. 15, 2014</td>
<td>Mar. 17, 2014</td>
<td>Bronchitis</td>
<td>Fever, cough, wheezing</td>
<td>39.0</td>
<td>2.6 × 10^7</td>
</tr>
<tr>
<td>6</td>
<td>2 y 4 mo</td>
<td>M</td>
<td>Mar. 31, 2014</td>
<td>Mar. 31, 2014</td>
<td>Bronchitis</td>
<td>Fever, cough, wheezing, rhinorrhea</td>
<td>38.5</td>
<td>&lt;1.0 × 10^3</td>
</tr>
<tr>
<td>7</td>
<td>11 mo</td>
<td>F</td>
<td>Mar. 27, 2014</td>
<td>Mar. 28, 2014</td>
<td>Bronchitis</td>
<td>Fever, wheezing</td>
<td>39.3</td>
<td>4.6 × 10^7</td>
</tr>
</tbody>
</table>

y, year; mo, month; M, male; F, female; URTI, upper respiratory tract inflammation.
(12,13). However, no serum samples were available in the present study.

Although in the present study we were unable to investigate whether HBoV1 had circulated to other regions in Toyama Prefecture, the present data indicate the possibility that ARIs caused by this virus were prevalent in the spring of 2014 in Toyama Prefecture, Japan. This virus is commonly detected in young children with ARIs and is often associated with severe disease requiring hospitalization (14,15). Therefore, careful monitoring of the prevalence of HBoV1 infection is necessary for the surveillance of ARIs, especially lower respiratory tract infections among young children in the winter and spring seasons.

Acknowledgments We thank Dr. Harutaka Katano, National Institute of Infectious Diseases, Tokyo, Dr. Hidekazu Nishimura, Sendai Medical Center, Miyagi, Dr. Atsushi Kaida, Osaka City Institute of Public Health and Environmental Sciences, Osaka, and Dr. Hiroyuki Tsukagoshi, Gunma Prefectural Institute of Public Health and Environmental Sciences, Gunma, Japan for providing positive control-viral cDNAs or RNAs for the real-time RT-PCR assay. This work was supported in part by a Grant-in-Aid for Research on Emerging and Re-emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan (H24-Shinko-Ippan-014).

Conflict of interest None to declare.

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