High Prevalence of Human Bocavirus Detected in Young Children with Severe Acute Lower Respiratory Tract Disease by Use of a Standard PCR Protocol and a Novel Real-Time PCR Protocol*\textsuperscript{v}

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The human bocavirus (HBoV) was recently isolated from respiratory tract samples. Within a study collective of children with severe lower respiratory tract disease, the patients testing positive for HBoV (12.8%) had a higher rate of underlying cardiopulmonary disease. Viral loads in respiratory tract specimens varied from $10^2$ to $10^{10}$ genome equivalents/ml.

In addition to established pathogens for lower respiratory tract infections, such as respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, and adenoviruses, additional agents have been identified in recent years. This includes the human metapneumovirus (hMPV), coronaviruses, and the human bocavirus (HBoV). HBoV is a member of the family Parvoviridae, genus Bocavirus, and has been isolated from respiratory tract samples by large-scale molecular virus screening (1). So far, the virus has not been propagated in cell culture; there is no animal model. The incidence of viral detection in patients with lower respiratory tract infections is 3.1% to 10.3% (1, 4, 5, 6). The rate of coinfection with other viruses ranges from 17% to 55%. HBoV infections have been recorded worldwide in all age groups and show predominance in winter and spring. The patients may develop pneumonia and bronchiitis with fever, cough, and peribronchial infiltrates, detected on chest X-ray studies. Hospitalization of adults is rare.

Our study included 94 hospitalized patients of <36 months of age (mean, 6 months) with assumed severe lower respiratory tract disease as defined by a high rate of oxygen therapy (68%). An underlying cardiac and/or pulmonary disease was present in 18%. In most cases RSV infection was the most important differential diagnosis (53.2%) because of the seasonal epidemiology and the clinical symptomatology including marked airway obstruction in 62%. We chose this patient collective as young children represent reportedly the age group of HBoV genome-positive patients exhibiting the severest symptoms. Among 75 patients investigated by chest X-ray studies, 72% had peribronchial and 23% had pneumonia infiltrates. Respiratory tract specimens collected from these children between November 2005 and April 2006 (76% nasopharyngeal washes, 21% tracheal secretions, 3% bronchoalveolar lavage [BAL] samples) were studied for adenoviruses, influenza A virus, influenza B virus, parainfluenzavirus types 1 to 3, and RSV using antigen-specific immunoassays (IMAGEN respiratory screen; DAKO); for the hMPV using a PCR assay (2); and for HBoV using the PCR protocol described by Allender et al. (1). Specimens known to be positive for parvovirus B19 genotype 1 ($n = 5$), herpes simplex virus, human cytomegalovirus, Epstein-Barr virus, JC virus, adenovirus, papillomavirus, RSV, parainfluenza virus, influenza A virus, or influenza B virus ($n = 3$ for each virus) were analyzed as specificity controls.

Nucleic acid was extracted from 220 µl of each specimen by using a QIAamp virus Biorobot 9604 kit (QIAGEN, Hilden, Germany) and eluted with 60 µl of PCR-grade water. An aliquot of 5 µl was added to 15 µl of reaction mixture containing 3 mM MgCl$_2$, a 0.5 µM concentration of each primer (1888 F, 5′ GGTGAGTCATGGGAACGC-3′ for HBoV, 5′ TGCTGTGTCGATGACGCTC-3′ for parvovirus B19), 0.15 µM fluorescein hybridization probe (GGAAGAGACACTGGCAGACAAC-LC-Red 640, TIB Molbiol, Berlin, Germany), 0.15 µM LC-Red 640 probe (LC-Red 640-CATCACAGGAGCCGAG-3′, TIB Molbiol, Berlin, Germany), 0.15 µM LC-Red 640 probe (LC-Red 640-CATCACAGGAGCCGAG-3′, TIB Molbiol, Berlin, Germany), and 2 µl of enzyme mix (LightCycler FastStart DNA Master Hybridization Probes; Roche Applied Science, Mannheim, Germany). The experimental PCR protocol was as follows: an initial 10 min at 95°C for FastStart Taq polymerase activation, followed by 45 cycles of 2 s of denaturation at 95°C, 10 s of annealing at 54°C, and 15 s of extension at 72°C. Quantification of HBoV DNA was performed with a serial dilution of a plasmid standard containing the primer-spanning region of the N1 gene. The amplicon of 534 bp was generated by PCR using DNA from an HBoV-positive specimen as a template and subsequently cloned into the vector pCDNA3.1/V5-HisTOPO (Invitrogen, Carlsbad, CA), resulting in the plasmid pCDNA-HBoV.

Of the 94 specimens included in this study, 26 (27.6%) were negative in all assays (Table 1); 50 (53.2%) were positive for RSV; 7 (7.4%) were positive for adenoviruses, influenzaviruses, or parainfluenzaviruses; and 5 (5.3%) were positive for the human metapneumovirus. HBoV could be detected in 12 (12.8%) specimens (83.3% were nasopharyngeal washes, 8.3%...

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TABLE 1. Clinical and virological findings in 94 young children with assumed viral lung infections

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. of children</th>
<th>chest X-ray findings</th>
<th>tracheal secretion samples</th>
<th>BAL samples</th>
<th>No. of children with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>26</td>
<td>6.1 ± 6.6</td>
<td>8.8 ± 1.3</td>
<td>None</td>
<td>Oxygen therapy</td>
</tr>
<tr>
<td>RSV</td>
<td>44</td>
<td>5.1 ± 4.4</td>
<td>8.1 ± 2.0</td>
<td>2.5</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>7.2 ± 6.3</td>
<td>8.0 ± 1.5</td>
<td>1.5</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>hMPV</td>
<td>17</td>
<td>8.1 ± 4.6</td>
<td>8.0 ± 2.0</td>
<td>2.5</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>HBoV</td>
<td>7</td>
<td>9.6 ± 6.8</td>
<td>8.0 ± 1.5</td>
<td>1.5</td>
<td>2 (18%)</td>
</tr>
</tbody>
</table>

The data available so far indicate that HBoV may be responsible for at least 3% of all respiratory tract infections in young children, particularly in those with underlying cardiopulmonary disease. The severity of clinical symptoms or patient's age was observed.

The prevalence of HBoV found in this study is the highest reported so far. This result may be explained by the high prevalence of viral infections in young children and the high prevalence of HBoV in respiratory samples. The correlation between the severity of clinical symptoms and HBoV genome detection was also observed. The correlation between the severity of clinical symptoms and the chest X-ray findings was also observed. The correlation between the severity of clinical symptoms and the BAL samples was also observed.

The share of HBoV genome-positive tracheal secretions (8.7%) was low compared to the share of tracheal secretions (8.3%) and BAL samples (8.7%). This may indicate that tracheal secretion is less suitable for detection of HBoV than nasopharyngeal washes.

No correlation was observed between the severity of clinical symptoms and the chest X-ray findings. No correlation was observed between the severity of clinical symptoms and the BAL samples. No correlation was observed between the severity of clinical symptoms and the tracheal secretions.

The data available so far indicate that HBoV may be responsible for at least 3% of all respiratory tract infections in young children, particularly in those with underlying cardiopulmonary disease. The severity of clinical symptoms or patient's age was observed. The correlation between the severity of clinical symptoms and HBoV genome detection was also observed. The correlation between the severity of clinical symptoms and the chest X-ray findings was also observed. The correlation between the severity of clinical symptoms and the BAL samples was also observed. The correlation between the severity of clinical symptoms and the tracheal secretions was also observed.
pulmonary disease, narrowing the gap of infections with unknown etiology. Lu et al. (3) described an HBoV-specific real-time protocol using the exonuclease probe format. No absolute quantification of viral loads was given. The LightCycler PCR assay developed by us using the hybridization probe format allows the accurate detection and quantitative assessment of HBoV DNA in human specimens. Sensitivity and specificity of the assay are high. The specimens studied are dominated by low viral loads; only 25% of specimens had loads exceeding $10^4$ genome equivalents/ml. Two hypotheses may explain the lack of correlation between the viral load and the clinical data. First, the nasopharyngeal washes, tracheal aspirates, and BAL samples were not collected by means of a standardized protocol for virus quantification but during routine procedures aiming at qualitative detection of viral particles, which may result in variable viral loads. Second, viral loads may change rapidly in the course of a disease, resulting in variable quantification results. The samples, however, had not been taken at predefined time points. Prospective studies based on quantitative HBoV detection are needed to specify the clinical impact of HBoV and to define the kinetics of the HBoV viral load.

REFERENCES


FIG. 1. (A) Correlation between the crossing point and the log template concentration of an HBoV-specific real-time PCR using pcDNA-HBoV as a template. (B) HBoV viral load in respiratory specimens from 12 children with respiratory disease.