Detection of Human Bocavirus in Canadian Children in a 1-Year Study

Nathalie Bastien,1 Natalie Chui,2 Joan L. Robinson,2 Bonita E. Lee,2 Kerry Dust,1 Laura Hart,1 and Yan Li1*

National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada,1 and Department of Pediatrics and Stollery Children’s Hospital, University of Alberta, Edmonton, Alberta, Canada2

Received 18 May 2006/Returned for modification 18 July 2006/Accepted 8 November 2006

Human bocavirus was detected by PCR in 65 (5.1%) of 1,265 respiratory specimens collected in 2002 and 2003 from the Stollery Children's Hospital from children <17 years of age. The spectrum of illness included upper respiratory infection, croup, bronchiolitis, and pneumonia with a prominence of cough and fever.

In 2005, a new human parvovirus, human bocavirus (HBoV), was identified in clinical specimens from infants and children suffering from respiratory tract illness (1). Phylogenetic analyses of the complete genome of HBoV revealed that the virus is most closely related to canine minute virus and bovine parvovirus, which are members of the Bocavirus genus of the Parvoviridae family (1). These viruses replicate in the nuclei of the cells and required the cell to go through S phase in order to replicate. Therefore, infection of respiratory and gut epithelium and hematopoietic cells and transplacental infection of fetuses are often observed in parvoviruses infection (4, 6). To date, the only Parvoviridae known to be pathogenic in human beings is the Erythrovirus genus, of which B19 (genotype 1), is the best characterized and is responsible for Fifth disease in children (4, 6). HBoV is currently being detected in patients with respiratory disease in several countries, suggesting that HBoV may be circulating worldwide (3–5, 8, 9). The relative importance of HBoV on viral respiratory tract illnesses is still not known, but it has been associated with respiratory illnesses ranging from upper respiratory tract disease (24%) to severe bronchiolitis (11 to 26%) and pneumonia (17 to 33%) (2, 3, 8).

Preliminary reports also have suggested that children and infants are most at risk for infection with HBoV. In the present study, we looked for HBoV in pediatric patients to assess the potential impact of HBoV infections on respiratory tract illnesses and to describe the clinical course of these illnesses in children.

Nasopharyngeal, throat, or tracheal swabs were collected from inpatients or outpatients younger than 17 years old at the Stollery Children’s Hospital from 12 November 2002 to 31 December 2003 and submitted in MICROTEST M5 viral transport media (Remel, Lenexa, KS) to the Provincial Laboratory for Public Health (Microbiology). Specimens that tested negative for influenza viruses A and B, parainfluenza viruses (PIV) types 1, 2, and 3, adenovirus and respiratory syncytial virus (RSV) by direct fluorescence assays and virus isolation and that had sufficient volume remaining were submitted to the National Microbiology Laboratory for the detection of HBoV. In addition, 41 specimens from which RSV (n = 39), influenza virus A (n = 1), and PIV (n = 1) had been detected were submitted. There were 2,597 respiratory specimens collected from children during the study months; 1,265 of these specimens were tested by PCR for the detection of HBoV. Health records were reviewed for all children with specimens that tested positive for HBoV. Demographic data, clinical features, and the results of investigations at the time of HBoV infection were recorded. For children admitted to the hospital with a nonrespiratory or chronic respiratory problem, only new signs or symptoms or new features on chest radiography that coincided with the HBoV infection were recorded. The absolute number and the percentage of specimens positive for HBoV per month were calculated. For this calculation, only one specimen per child obtained within a 14-day period was used unless repeat specimens were positive for different respiratory viruses, in which case both specimens were included.

HBoV was detected by PCR using primers specific for two different regions of the genome. The screening primers: 188F (2281-GACCTCTGTAAGTACTATTAC-2301) and 542R (2634-CTCTGTGTTGACTAATACAG-2641) were described in Allander et al. (1) and amplified a 354-bp fragment of the putative NP-1 gene. Primers used for confirmation and sequencing were based on the published HBoV putative VP1/VP2 gene sequences (DQ000495) (1). The forward primer HBoV VP1/VP2F (4492-CCAAACTCACTCCTCCTCAGGC-4509) and the reverse primer HBoV VP1/VP2R (4895-GCTCTCTCCCTCAAGTGACATGC-4875) used for confirmation amplified a 404-bp fragment of the putative VP1 and VP2 genes (3). The primer sets VP2-1017F 4055-GTGACTGCTCTCTCCTCCCAGTGACATGC-4076 were used to amplify an 842-bp fragment of the putative NP-1 gene. For sequence analysis, viral DNA was extracted from 285 μl of original samples using a BioRobot MDx with the QiAamp DNA Blood Kit (QIAGEN). Viral DNA was amplified by using a GeneAmp PCR system 9700 (Applied Biosystems) and the HotTaq DNA polymerase (QIAGEN) according to the manufacturer’s recommendations. We used 5 μl of DNA in a volume of 50 μl containing 20 pmol of each primer, and the thermocycler conditions used were as follows: 95°C for 15 min for the activation of the HotStartTaq DNA polymerase, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min, followed in turn by an extension of 10 min at 72°C. The PCR products were detected on a agarose gel with

610
a size marker, purified by using QIAquick PCR purification kit (QIAGEN), and sequenced on an ABI 377 Sequencer using 150 ng of DNA and a fluorescent dye-terminator kit (Applied Biosystems). The DNA sequences were assembled and analyzed with the SEQMAN, EDITSEQ, and MEGALIGN programs in Lasergene (DNASTAR, Madison, WI). To avoid cross-contamination, we conducted the specimen processing, DNA extraction, PCR amplification, and reverse transcription-PCR analyses in three different rooms. For DNA extraction and PCR procedures, we included a total of 12 of negative controls per 96-well plate. A HBoV-positive nasopharyngeal specimen from a previous study was used as positive control (3).

HBoV DNA was detected in 65 of the 1,265 specimens (5.1%) obtained from 56 children. Seven positive specimens were considered to be duplicate specimens from the same episode of respiratory tract infection since they were obtained within 14 days of another specimen that was positive for HBoV (range, 0 to 7 days). There were two patients who had two positive HBoV specimens obtained 131 and 154 days apart, with specimens testing negative for HBoV during the interval. Each of those four positive specimens was considered to be a separate episode of HBoV infection, accounting for 58 episodes in 56 children. HBoV was detected every month of the year except August (Fig. 1), with the percentage of total specimens per month being tested for HBoV ranging from 26.4% (March 2003) to 82.1% (July 2003).

There were 58 episodes of HBoV infection (Table 1). Virus was detected in 39 (67%) males and 19 (33%) females ranging in age from 35 days to 12.5 years (median, 1.2 years). Seven of the patients were seen in the emergency department, primarily with cough (100%), fever (86%), and rhinitis (71%). Five of those seven patients were previously well children, one was preterm, and one had congenital heart disease (CHD). All had mild respiratory illness with one patient also having erythema multiforme.

In 23 episodes of HBoV infection, detection of virus occurred during an admission for noncardiorespiratory problems (n = 11), CHD (n = 8), or chronic respiratory problems (n = 4). Typically, a nasopharyngeal aspirate was submitted as the child had new onset of cough and/or rhinitis. Only seven of the children (32%) developed a clinical picture compatible with a lower respiratory tract infection (LRTI) (bronchiolitis in five and pneumonia in two). Two patients required ventilation 3

![FIG. 1. Number of specimens tested for HBoV and number of specimens positive for HBoV per month.](http://jcm.asm.org/)

### TABLE 1. Features of 58 episodes in which HBoV was detected

<table>
<thead>
<tr>
<th>Patient group (n)</th>
<th>Gender&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Age range in yr (median)</th>
<th>Diagnosis (no. of cases)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CXR findings (no. of cases)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>WBC range (median)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outpatients (7)</td>
<td>4 M, 3 F</td>
<td>0.9–2.2 (0.9)</td>
<td>URI (4), croup (1), bronchiolitis (3)</td>
<td>ND (4), normal (2), PHT and HI (1)</td>
<td>8.5–12.7 (10.0)</td>
</tr>
<tr>
<td>Inpatients admitted for nonrespiratory or chronic respiratory problems (23)</td>
<td>15 M, 8 F</td>
<td>0.1–12.5 (0.9)</td>
<td>URI (16), bronchiolitis (5), pneumonia (2)</td>
<td>ND (7); normal (3); A (4); PHT (1); PBT (1); A and HI (1); PHT, HI, and A (1); A and PE (1); A and increased interstitial markings (1); PC (3)</td>
<td>3.7–38.7 (8.6)</td>
</tr>
<tr>
<td>Inpatients admitted for acute respiratory problems (28)</td>
<td>20 M, 8 F</td>
<td>0.3–3.4 (1.6)</td>
<td>URI (1), croup (1), bronchiolitis (15), pneumonia (11)</td>
<td>ND (2); normal (2); HI (1); A (2); PHT (3); PHT and A (1); HI and A (1); PHT and HI (1); PBT and HI (1); PHT, HI, and A (3); PBT, HI, and A (1); PC (5); LC (5)</td>
<td>2.4–17.4 (9.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup> M, male; F, female.

<sup>b</sup> URI, upper respiratory tract infection.

<sup>c</sup> A, atelectasis; CXR, chest radiograph; HI, hyperinflation; LC, lobar consolidation; LP, lobar pneumonia; ND, not done; PBT, peribronchial thickening; PC, patchy consolidation; PE, pleural effusion; PHT, perihilar thickening.

<sup>d</sup> WBC, white blood cell count.
and 6 days prior to the collection of the specimen that was positive for HBoV, but both had CHD with congestive heart failure so it is not clear whether this deterioration was due to their LRTI. Nosocomial infection was very likely in 12 of these 23 patients since HBoV was first detected >10 days after admission. It was also possible in several other patients who had virus detected upon transfer from another hospital or several days after admission to this hospital. Specimens from two patients obtained 1 day after the one that was positive for HBoV, were, respectively, positive for adenovirus and both adenovirus and influenza virus A. Human coronavirus NL63 was detected in a specimen from another patient obtained 8 days prior to the study specimen.

In the remaining 28 episodes of HBoV infection, the children were admitted to hospital with primarily acute respiratory symptoms, including cough (100%), fever (57%), rhinitis (50%), and vomiting (32%) and had a median hospital stay of 3 days. Nine of these children (32%) were previously healthy, whereas the others had a history of asthma (n = 6), prematurity (n = 4), tracheostomy (n = 3), previous LRTI (n = 3), previous croup (n = 1), prematurity and CHD (n = 1), or prematurity and short-gut syndrome (n = 1) as their primary underlying medical condition. Twenty-five of these patients (89%) required 1 to 24 days of oxygen therapy. Four of the children (14%) required admission to the pediatric intensive care unit with respiratory problems, and all of the patients survived. Detection of another respiratory virus occurred within 14 days of detection of HBoV in 4 patients (parainfluenza virus 4 days prior, influenza virus B 2 days later, and RSV 2 and 14 days later.

Nucleotide sequences were determined for nucleotides 4121 to 4831 of the HBoV genome representing the partial VP1/VP2 genes for 55 of 65 positive specimens that had enough amplicon to allow for DNA sequencing (GenBank accession numbers DQ839263 to DQ839317). Sequence comparison with published Swedish (GenBank accession numbers DQ000495 to DQ000496) (1) and French (GenBank accession numbers AM160609 to AM160614) (5) isolates showed that the VP1 and VP2 genes were relatively well conserved with nucleic acid identity between specimens ranging from 97.7 to 100%. The phylogenetic tree of the HBoV isolates showed the existence of two major groups or clusters (Fig. 2). Canadian, Swedish, and French HBoV strains were found in both clusters. Viruses from the two patients who had two separate HBoV episodes were closely related in one case (CAN544-03 and CAN1275-03) and belonged to the same cluster, whereas in the other case the viruses were more divergent (CAN234-03 and CAN596-03) and belonged to each cluster.

The present study has shown that HBoV circulated in infants and young children in Canada during 2002 and 2003. The detection rate of HBoV of 5.1% and the male predominance is similar to that reported from previous studies (1, 3, 5, 8, 9). A control group of healthy children would be required to demonstrate that HBoV is pathogenic. Such a control group was not added to the present study due to the difficulty of obtaining willing participants. However, it seems possible that HBoV was the cause of the respiratory signs and symptoms of the children in this study. The spectrum of illness with HBoV infection appears to be similar to that described from other respiratory viruses, including upper respiratory infection, croup, bronchi-

![Fig. 2. Phylogenetic analysis of HBoV isolates. Nucleotide sequences were determined for nucleotides 4121 to 4831 of the HBoV genome representing the partial VP1/VP2 genes. The corresponding gene sequences from previously reported Sweden and French HBoV isolates were also included. Phylogenetic analysis was performed by using the neighbor-joining method of the MEGA program (7). The Swedish and French isolates are indicated by HBoV-ST and HBoV-MPT, respectively, followed by the isolate number (i.e., HBoV-ST1). The isolates presented here are indicated by CAN, followed by the isolate number and the year (i.e., CAN162-03).](http://jcm.asm.org/)
olitis, and pneumonia with the prominence of cough and fever being consistent with previous studies (8).

Risk factors for severe disease with HBoV appear to be similar to those for RSV (prematurity, congenital heart disease, and asthma). However, based on the present and previous studies, hospitalization of children <6 months of age with HBoV appears to be rarer than would be expected with RSV, with only one of the 28 children admitted with acute respiratory distress being under 6 months of age (1, 3, 4, 8). The occurrence of two separate HBoV episodes in two of the patients suggests that reinfection may play a role in the epidemiology of HBoV infection, similar to RSV.

The seasonality of HBoV infection remains to be established. In Sweden, virus was primarily identified from December to February (1), but in our study the positive specimens were distributed fairly uniformly throughout the first, second, and fourth quarters of the year, which is comparable to the absence of apparent seasonal prevalence reported in a previous Canadian study (3).

The role of coinfection with other viruses may be important, with rates as high as 55.6% reported in a previous study (9). The variability in the incidence of coinfection in different studies may be related to the sensitivity of the methods used for viral detection. The present study adds limited information about coinfection since HBoV was tested primarily in specimens that had already tested negative for other respiratory viruses.

Phylogenetic analysis based on the VP1 and VP2 genes shows the cocirculation of two distinct genetic clusters. Close clustering of HBoV isolates recovered from Canada, France, and Sweden suggests that the evolutionary pattern of HBoV does not correlate with geographic variation.

In summary, our data suggest that HBoV may play a role in acute respiratory illness in young children. More comprehensive studies, including data on prevalence, risk factors, and the use of health services, are needed to determine the importance of HBoV in acute respiratory illness and its impact on the health care system.

REFERENCES