Human bocavirus in Italian patients with respiratory diseases

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Abstract

Background: hBoV, a recently discovered parvovirus, can be present in the respiratory tract of patients with acute respiratory diseases (ARD), but its etiologic involvement in the underlying diseases is still uncertain.

Objective: To determine in a retrospective study, the prevalence of hBoV, compared with common respiratory viruses (RV), in respiratory specimens from patients with ARD.

Study design: A total of 335 specimens obtained over 7 years were examined. Two hundred were nasal swabs from infants hospitalized for ARD, 84 were nasal swabs or bronchoalveolar lavages from adults with pneumonia, bronchopneumonia or asthma, and 51 were nasal swabs from healthy children.

Results: The overall rate of hBoV detection in specimens from infants with ARD, which was 4.5%, varied slightly from year to year, except for the period 2000–2002, when no specimen was positive. Unlike other RV, no seasonal variation in hBoV incidence was noted. Infants with hBoV infection suffered either from bronchiolitis or from bronchopneumonia and 5 out of 9 cases yielded no co-infecting viral pathogen. Only one sample from an adult was hBoV positive. None of the nasal swabs from healthy subjects tested hBoV-positive.

Conclusions: The findings indicate that hBoV can cause ARD in infants.

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Keywords: hBoV; Respiratory diseases; Children; Respiratory viruses; Healthy controls

1. Introduction

A novel parvovirus has been recently suspected to cause ARD (Allander et al., 2005). This virus, still poorly characterized, has provisionally been named hBoV due to extensive genetic relatedness with the animal bocaviruses (Allander et al., 2005). Although studies are limited, worldwide distribution of hBoV has already been reported (Arden et al., 2006; Arnold et al., 2006; Bastien et al., 2006a,b; Choi et al., 2006; Chung et al., 2006; Foulongne et al., 2006a,b; Kesebir et al., 2006; Lu et al., 2006; Ma et al., 2006; Sloots et al., 2006; Smuts and Hardie, 2006; Weissbrich et al., 2006). Kesebir et al., 2006; Lu et al., 2006; Ma et al., 2006; Sloots et al., 2006; Smuts and Hardie, 2006; Weissbrich et al., 2006).

Using genome amplification methods, the only diagnostic approach thus far available, hBoV has been detected in 1.5–18.3% of patients with ARD, regardless of whether other putative etiologic agents were present (Bastien et al., 2006a,b; Choi et al., 2006; Kaplan et al., 2006; Qu et al., 2007; Sloots et al., 2006; Weissbrich et al., 2006). hBoV has been reported mainly in pediatric patients with severe forms of ARD (Foulongne et al., 2006a; Ma et al., 2006; Manning et al., 2006; Simon et al., 2007; Smuts and Hardie, 2006), but few studies have focused on individuals without respiratory symptoms (Kesebir et al., 2006; Manning et al., 2006), thus leaving open the possibility that hBoV might also be present in healthy, asymptomatic individuals. Indeed, molecular techniques have frequently detected viruses in the respiratory tract, such as TTV and TTMV, that have only an
indirect, if any, pathogenic role (Bendinelli and Maggi, 2005; Biagini et al., 2003; Maggi et al., 2003a).

In the current retrospective study, we examined respiratory specimens from 200 infants with ARD and 84 adults with pneumonia, bronchopneumonia or asthma, routinely submitted for virological testing to our Clinical Virology laboratory, and from 51 healthy pediatric controls.

2. Materials and methods

2.1. Specimens and routine tests

A total of 335 respiratory specimens obtained between January 2000 and May 2006 were studied. Specimens were transferred to the Department of Experimental Pathology where they were processed as described elsewhere (Maggi et al., 2003a,b), and aliquoted and stored at −80°C until use. Informed consent was obtained from the adult patients or from the parents of all children who provided specimens.

Two hundred nasal swab specimens were obtained from infants (mean age 1.1 ± 0.9 years; M/F: 120/80) with ARD admitted at the Department of Pediatrics, University Hospital of Pisa. Eighty-four specimens were obtained from adult patients (mean age: 48 ± 20 years; M/F: 48/36). These included bronchoalveolar lavage (BAL) from 62 consecutive individuals hospitalized for severe respiratory diseases (pneumonia or bronchopneumonia), 10 of whom were HIV-positive, and 22 nasal swabs from adult patients with persistent severe asthma. Fifty-one specimens were control nasal swabs obtained from 30 healthy infants (mean age: 0.5 ± 0.3; M/F: 20/10) and 21 pre-adolescent healthy children (mean age: 12.8 ± 0.6; M/F: 15/6) who had no signs of ARD and no history of asthma or wheezing.

The specimens that were submitted to systematic testing for RV by direct immunofluorescence (adenovirus, cytomegalovirus, influenza A and B, parainfluenza 1–3, RSV), enzyme immunoassay (RSV), rapid culture in shell vials (all above viruses), and in-house PCR assays (rhinoviruses and hMPV).

2.2. hBoV PCR

A 291 bp segment of the NS1 gene spanning nt 1475–1765 of the hBoV prototype st1 (Allander et al., 2005) was amplified by single-step PCR (Sloots et al., 2006). Briefly, viral DNA was extracted and amplified with primer sense hBoV01.2 (5′-TATGGCCAAAGGCAATCGTCCAAAG-3′; position 1475–1497) and primer antisense hBoV02.2 (5′-GCCGCGTGAAACATGAGAACAGA-3′; position 1743–1765) using the following conditions: initial PCR activation step at 95°C for 15 min and 45 subsequent PCR cycles with melting at 94°C per 20 s, annealing at 56°C for 20 s, and extension at 72°C for 30 s. Amplified products were then analyzed by electrophoresis on agarose gel after ethidium bromide staining and compared for size with standard molecular size markers. All samples were tested at least in duplicate, and specimens positive on the gel were confirmed by sequencing the amplification products. When the results were discordant, the samples were re-extracted and tested again in duplicate.

2.3. Sequencing

Amplicon sequencing was carried out on a 245 bp segment by using the Big Dye Terminator Cycle Sequencing kit and an automatic DNA sequencer (Applied Biosystems Foster City, CA). Alignment of nucleotide sequences with all the hBoV sequences in GenBank and branching pattern of the trees were obtained by the neighbour-joining algorithm included in the MEGA program (version 3.1). Bootstrap re-sampling was used to test the robustness of the tree.

3. Results

3.1. hBoV in infants with ARD and healthy controls

Most of the samples (161) were tested for common RV. As summarized in Table 1, RSV was the most common virus identified (31%), followed by rhinovirus (15%), hMPV (11%), influenza A (4%), and others (5%). The highest frequency of RSV detection was in 2004 with 51% of the swabs positive, and the lowest in 2003 with only 15% of the specimens positive. For the other RV, the maximum and lowest frequency tests was 78% in 2003 and 4% in 2005, respectively. Detection of these RV showed the expected seasonal distribution. Thus, RSV (Table 2) and hMPV detection rates (data not shown) peaked in January–March, while the other viruses were more uniformly distributed.

As also shown in Table 1, testing for hBoV yielded 9 positive results (4.5%). The rate of detection in the nasal swabs collected over the years 2003–2006 was similar, but none of 43 specimens were positive during the years 2000–2002. When the specimens were stratified by month of collection (Table 2), there was no seasonality in hBoV detection. The number of detections was highest in winter months, but this appeared solely due to the high number of specimens examined during these months. Stored nasal swabs obtained from 30 healthy infants in 2001 (9/200 versus 0/30 for infants, p = 0.60; Fisher’s exact test) and from 21 pre-adolescent children in 2003 were all hBoV negative.

Six of the 200 infant patients tested had laryngitis, 15 had bronchitis, 59 had bronchiolitis, and 120 had bronchopneumonia. The hBoV-positive infants (4 males, 5 females; mean age: 16 ± 13 months) had been diagnosed with bronchiolitis with mono- or bilateral interstitial lung infiltration (5 cases) or bronchopneumonia (4 cases). Two of these tested positive for RSV and 1 each for RSV plus rhinovirus and for influenza A plus hMPV. The other 5 had no other virus detected beyond...
Table 1
hBoV and other RV in nasal swabs of the children with ARD, grouped by year of sampling

<table>
<thead>
<tr>
<th>Year</th>
<th>hBoV Examined</th>
<th>Positive no. (%)</th>
<th>RV Examined</th>
<th>RSV positive no. (%)</th>
<th>Other RV positive no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000–2002</td>
<td>43</td>
<td>0 (0)</td>
<td>30</td>
<td>9 (30)</td>
<td>19 (63)</td>
</tr>
<tr>
<td>2003</td>
<td>46</td>
<td>2 (4)</td>
<td>40</td>
<td>6 (15)</td>
<td>31 (78)</td>
</tr>
<tr>
<td>2004</td>
<td>41</td>
<td>3 (7)</td>
<td>35</td>
<td>18 (51)</td>
<td>4 (11)</td>
</tr>
<tr>
<td>2005</td>
<td>37</td>
<td>3 (8)</td>
<td>28</td>
<td>9 (32)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>2006</td>
<td>33</td>
<td>1 (3)</td>
<td>28</td>
<td>8 (28)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>9 (4)</td>
<td>161</td>
<td>50 (31)</td>
<td>57 (35)</td>
</tr>
</tbody>
</table>

a Included in the total number of specimens examined for hBoV in each respective year. The reduced numbers of tests examined reflect the fact that some specimens were frozen immediately after collection and, therefore, unsuitable for RV detection by direct immunofluorescence or rapid culture in shell vials.
b One cytomegalovirus, two rhinovirus, and six hMPV were present also in 9 nasal fluid specimens positive for RSV.
c Including rhinovirus (25), hMPV (18), influenza A virus (6), cytomegalovirus (4), parainfluenza 1-3 viruses (3), and adenovirus (1). The number of viruses detected exceeded the number of virus-positive samples because 7 patients yielded two RV.
d Significantly different from year 2004, 2005, and 2006 at \( p < 0.0001 \), \( p < 0.0001 \), and \( p < 0.0001 \), respectively (Chi-square test).
e Significantly different from year 2004 at \( p < 0.001 \) (Chi-square test).
f Significantly different from year 2004, 2005, and 2006 at \( p < 0.0001 \), \( p < 0.0001 \), and \( p < 0.0001 \), respectively (Chi-square test).
g Specimens collected until May, 2006.

Table 2
hBoV and other viruses in nasal swabs of the children with ARD, grouped by month of sampling

<table>
<thead>
<tr>
<th>Month</th>
<th>hBoV Examined</th>
<th>Positive no. (%)</th>
<th>RV Examined</th>
<th>RSV positive no. (%)</th>
<th>Other RV positive no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January–March</td>
<td>107</td>
<td>5 (5)</td>
<td>78</td>
<td>32 (41)</td>
<td>25 (32)</td>
</tr>
<tr>
<td>April–June</td>
<td>33</td>
<td>2 (6)</td>
<td>31</td>
<td>8 (26)</td>
<td>11 (35)</td>
</tr>
<tr>
<td>July–September</td>
<td>12</td>
<td>1 (8)</td>
<td>11</td>
<td>0 (0)</td>
<td>1 (9)</td>
</tr>
<tr>
<td>October–December</td>
<td>48</td>
<td>1 (2)</td>
<td>41</td>
<td>10 (24)</td>
<td>20 (49)</td>
</tr>
</tbody>
</table>

a See footnotes (a) and (c) in Table 1, respectively.
b Significantly different from July–September at \( p = 0.007 \) (Chi-square test).
c Significantly different from October–December at \( p = 0.017 \) (Chi-square test).

hBoV. Three of these 5 patients had bronchopneumonia diagnosed by X-ray.

3.2. hBoV in paired blood and feces of one infant

For three infants whose nasal swabs tested hBoV-positive, we examined additional clinical specimens collected simultaneously with the nasal swab. These included plasma and stools of a 6-month-old boy followed for neurological problems who had presented with diarrhea and bronchopneumonia. Both specimens were positive for hBoV and antigen negative for rotaviruses, adenoviruses, astroviruses, and calicivirus 1 and 2. The plasma of two patients who had ARD symptoms only tested negative for hBoV.

3.3. hBoV infection in adults with severe pulmonary diseases

One of the 62 BAL specimens from adult patients with pneumonia or bronchopneumonia was positive for hBoV. This was from a 31-year-old HIV-negative woman with a history of lymphoma who had developed bronchopneumonia with bilateral, mainly interstitial lung infiltration at the time of BAL collection; her BAL was also positive for *Pseudomonas aeruginosa* and cytomegalovirus. Twenty-two nasal swabs from adults with severe symptomatic asthma were negative for hBoV.

3.4. Genetic analysis of the hBoV isolates

All the amplicons from the hBoV PCR assays were sequenced and confirmed detection of hBoV. No differences in nucleotide sequence were detected among the 10 Italian isolates, regardless of the source (nasal swab, BAL, plasma, or stool). The Italian isolates were also identical or differed little from the ones already in GenBank (Fig. 1).

4. Discussion

The discovery of hBoV inspired the present retrospective investigation on respiratory specimens collected from infants and adults in Pisa, Italy. Among the 200 nasal swabs from infants with ARD collected over a 7-year period that were examined, 9 (4.5%) were found to be hBoV positive, and 5 of these yielded no other RV that might have been responsible for the ARD. This rate of hBoV detection is similar to that reported previously for pediatric patients from...
Europe, USA, Canada, Asia and Australia (Allander et al., 2005; Arden et al., 2006; Arnold et al., 2006; Bastien et al., 2006a,b; Foulongne et al., 2006a,b; Ma et al., 2006; Manning et al., 2006; Sloots et al., 2006), thus indicating that hBoV is evenly distributed in developed countries. Similar to previous observations (Allander et al., 2005; Bastien et al., 2006a), we found no seasonal differences in the prevalence of hBoV. Also, no major differences were observed in the yearly rates of hBoV detection during the years 2003–2006. The latter finding, however, contrasted with the absence of hBoV detection in the swabs collected in the period 2000–2002, which might suggest that the prevalence of hBoV varies from year to year, although this difference was not significant (0/43 versus 9/148, p = 0.20; Fisher’s exact test). Clinical diseases we observed in association with hBoV detection in infants were severe forms of bronchiolitis and bronchopneumonia, in agreement with previous reports that this virus could be associated with the more serious forms of ARD (Allander et al., 2005; Foulongne et al., 2006a; Manning et al., 2006; Smuts and Hardie, 2006).

Notably, none of the nasal swabs obtained from 51 healthy infants and children yielded a positive hBoV test. The finding suggests that hBoV is not a frequent commensal virus inhabiting the respiratory tract in the absence of symptomatic disease. It should be noted, however, that only the 21 nasal swabs from older children were obtained in a period when, as discussed above, hBoV was detected in the infants with ARD studied in parallel. The swabs from healthy infants were harvested in 2001, i.e. in the middle of a 3-year period during which no hBoV was detected in the infants with ARD. Thus, there is some uncertainty about our failure to detect asymptomatic carriage of hBoV, although a recent report has failed to identify hBoV in 96 healthy children (Kesebir et al., 2006).

Of special interest was the demonstration of hBoV DNA in the plasma and feces of one of the infants who harbored hBoV in the nose. The finding might indicate that, similar to RSV and hMPV (Maggi et al., 2003b; Rohwedder et al., 1998), hBoV may not remain restricted to the respiratory tract. It is noteworthy that the infant in whom the observation was done was suffering of diarrhea as well of bronchiolitis, and that his feces were negative for the major viruses that cause gastroenteritis in infants. The study of 84 BAL specimens from adult patients with severe pulmonary diseases or asthma resulted in a single positive test in a specimen that contained other viral and bacterial pathogens, indicating that hBoV infection is rare as a cause of serious ARD in adult patients. This confirms the report of Allander et al. (2005), who found an hBoV prevalence rate of 3.1% in children, but not a single positive case in 112 adults. It contrasts, however, with the results of Bastien et al. (2006a,b) who found an overall hBoV positivity rate of 1.5% with no significant differences between age groups. Thus, conclusions about age-specific and disease-specific prevalence of hBoV infection will require additional prospective studies that will include patients with mild respiratory disease. This determination may also benefit from convalescent serological testing in addition to the molecular diagnostic methods.

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References


