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## Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio

Brian D.W. Chow<sup>a,b,d</sup>, Yung T. Huang<sup>a,c</sup>, Frank P. Esper<sup>a,b,\*</sup>

<sup>a</sup> Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH 44106, United States

<sup>b</sup> Department of Pediatrics, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106, United States

<sup>c</sup> Department of Pathology, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106, United States

<sup>d</sup> Department of Internal Medicine, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106, United States

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### ABSTRACT

**Background:** Viral respiratory illness is a major cause of morbidity and mortality. The human bocavirus (HBoV) is a recently recognized parvovirus isolated from human respiratory secretions.

**Objectives:** To define the clinical and epidemiologic characteristics in adult and pediatric patients with evidence of HBoV.

**Study design:** From October 2005 through October 2006, we screened respiratory samples from children and adults negative for common respiratory pathogens for HBoV by PCR. Demographic and clinical characteristics were obtained from medical records of HBoV positive individuals.

**Results:** Of 2075 samples screened, 1826 (88.0%) represented distinct respiratory events: 1539 (84.3%) were pediatric (<18 years), and 273 (15.0%) adult (≥18 years). Forty (2.2%) patients had HBoV: 36 (2.3%) children and 4 (1.5%) adults. HBoV positive children had history of prematurity (31.3%) and cardiac disease (18.8%). Adults had underlying pulmonary (100%) and cardiac (50%) disease. Twenty-seven children (84.4%) were hospitalized; 9 (28.1%) required intensive care. All adults were hospitalized; none required intensive care. Nosocomial acquisition likely occurred in 3 patients.

**Conclusions:** HBoV circulates in Cleveland, OH, in children and adults with similar frequencies, and can warrant hospitalization and intensive care. Further study would clarify our understanding of this newly recognized human pathogen.

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### 1. Background

Respiratory illness is an important reason for adult and pediatric emergency department visits.<sup>1</sup> In the United States, the proportion of pediatric hospitalizations caused by asthma, pneumonia, and acute bronchitis is nearly 22%. Of all hospital discharges, 9.7% are attributed to diseases of the respiratory system.<sup>2</sup> In the United States, the annual burden to society for respiratory infections is estimated to be \$112 billion.<sup>3</sup>

Allander et al. (2005) described a novel parvovirus isolated from human respiratory secretions from patients with pneumonia named the human bocavirus (HBoV).<sup>4</sup> Since that time, its presence in both the pediatric and adult population has been confirmed throughout the world. The incidence of this pathogen has ranged from 1.8% to 19%, with a variety of clinical presentations.<sup>5–7</sup>

Human bocavirus has been found in patients with upper and lower respiratory tract disease,<sup>5,8,9</sup> as well as gastrointestinal illness.<sup>10–12</sup> Cases reported in children constitute the majority; adult cases are reported less frequently.<sup>7,13,14</sup> Common presentations include cough, wheezing, fever, and emesis.<sup>7,14,15</sup> Thai adults have been reported to have pneumonia associated with HBoV severe enough to warrant hospitalization.<sup>13</sup>

Multiple studies have reported frequent detection of other viral pathogens in HBoV positive samples.<sup>16</sup> It has been hypothesized that prolonged shedding or persistence may be a reason. Because of this, questions remain about the role of HBoV in respiratory infection.

We sought to further define the clinical and epidemiologic characteristics of HBoV in adult and pediatric patients in Cleveland, OH.

### 2. Methods

#### 2.1. Sample collection

From 1 October 2005 to 15 October 2006, respiratory samples were collected from the Core Laboratory at University Hospitals of

\* Corresponding author at: Division of Pediatric Infectious Diseases, Rainbow Babies and Children's Hospital, 11100 Euclid Avenue, Cleveland, OH 44106, United States. Tel.: +1 216 844 3645; fax: +1 216 844 8362.

E-mail address: [Frank.Esper@UHhospitals.org](mailto:Frank.Esper@UHhospitals.org) (F.P. Esper).

Cleveland. Samples were submitted to the Core Laboratory at the discretion of the primary medical teams. Submitted samples originated from the emergency department, inpatient wards, intensive care units and hospital-affiliated primary care outpatient clinics. We obtained all clinical specimens from children and adults that had negative results for RSV, parainfluenza viruses (1–3), influenza A and B, and adenovirus by direct immunofluorescence assay (DFA). For DFA, samples were processed, applied to slides by cytocentrifugation, stained with Respiratory Panel 1™ Direct immunofluorescence Assay (Millipore, Temecula, CA), and examined.

## 2.2. RNA extraction, polymerase chain reaction (PCR) and sequencing

Nucleic acid from each respiratory specimen was extracted with the MagMAX™-96 Viral RNA Isolation Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. This product recovers both RNA and DNA from samples allowing us to screen for multiple respiratory pathogens. Samples were then screened by PCR for the presence of HBoV with Platinum Taq polymerase (Invitrogen) according to the manufacturer's specification.

Primers used in the screening of the respiratory specimens were based on sequences in published reports and targeted the HBoV NP-1 gene.<sup>15</sup> The forward primer, 5'-GACCTCTGTAAGTACTATTAC-3' and reverse primer, 5'-CTCTGTGTTGACTGAATACAG-3' produce a 353 base-pair amplicon that corresponds to nucleotides 2351–2704 of the HBoV NP-1 gene. Each set of PCR reactions contained appropriate negative controls. PCR amplification cycles were as follows: 95 °C for 3 min followed by 40 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 30 s and completed with a 10-min 72 °C cycle. Sequencing was performed on ABI Prism 3730 DNA Analyzer automated sequencer.

Isolates positive for HBoV were screened for common respiratory viruses by RT-PCR with published primer sets. Viruses screened include respiratory syncytial virus, human parainfluenza virus 3, human metapneumovirus, adenovirus, rhinovirus, influenza, WU polyomavirus, and the human coronaviruses NL63, HKU1, OC43, 229E.<sup>17–21</sup>

## 2.3. Phylogenetic analysis

The phylogenetic analysis included representative samples of Cleveland isolates in addition to isolates from published reports. The region of analysis corresponded to a 228-base pair region of the NP-1 gene spanning nucleotides 2426–2654 of the HBoV genome. ClustalW alignment was generated using Bioedit 7.0.5.2 alignment software. Five-hundred bootstrap data sets were created using the PHYLIP program SEQBOOT. Phylogenetic analyses were conducted using the PHYLIP program DNAML, with the default transition to transversion ratio of 2.0 and 1 jumble.

## 2.4. Clinical data

Available medical records for HBoV positive individuals were reviewed. Demographic data and clinical characteristics of each individual were recorded on a standard collection form. Pediatric cases were defined as patients whose age at the time of sample collection was less than 18 years; adult cases were defined as patient age at time of collection of 18 years and older.

## 3. Results

Of 2075 samples screened, 1826 (88.0%) were determined to be separate respiratory events. Samples collected greater than 28 days

apart from a single individual were considered separate respiratory events. Of these, 1539 (84.3%) were obtained from pediatric patients, and 273 (15.0%) from adult patients. Demographic information was unavailable for 14 (0.8%) patients.

Samples were collected throughout the year, with more samples obtained during the winter months in Cleveland. Monthly sample collections ranged from 63 (July) to 271 (March) (Fig. 1).

Forty samples (2.2%) tested positive for HBoV by PCR: 36 (90%) pediatric patients and 4 (10%) adult patients. Medical records were unavailable for 1 (2.5%) subject. Four (10.0%) samples had other respiratory pathogens identified by RT-PCR. These include rhinovirus (2) and coronaviruses HCoV-NL63 (1) and HCoV-229E (1). All co-infected samples originated from children and were excluded from clinical analysis.

HBoV samples were primarily identified in fall and winter, peaking in November where 6.8% of samples screened positive. HBoV continued to circulate until May. No HBoV was detected in the summer months of June, July, and August (Fig. 1).

The median pediatric age was 12 months, with the range of ages from the 24 days to 8 years 5 months. The median adult age was 58 years, ranging from 20 years to 86 years. The 12–18 month age group had the highest prevalence, with HBoV detected in 6.0% of respiratory samples screened (Fig. 2).

The clinical presentation of the pediatric populations is described in Table 1. Of pediatric patients testing positive, 66.7% were male, 53.1% were Caucasian, and 31.3% were African-American. Underlying conditions included prematurity (31.3%), cardiac disease (18.8%), immunodeficiency (9.3%), and pulmonary disease (9.3%). The most common symptoms reported included cough (75.0%), rhinorrhea (62.5%), and fever (53.1%). Gastrointestinal symptoms were reported in 11 (34.3%) patients, and 8 (25.0%) patients had wheezing.

Of pediatric patients who screened positive for HBoV, 27 (84.4%) were admitted to the hospital, including 9 (28.1%) who required intensive care. For patients admitted to an intensive care unit, the median stay in intensive care was 2 days (range 1–5 days). Reasons for ICU admission included respiratory distress (66.6%), apnea (11.1%), cardiac arrhythmia (11.1%), and shock (11.1%).

Of the remaining pediatric patients, 18 (56.3%) were admitted to a pediatric ward. Disposition for one patient was unavailable. The median duration of hospitalization was 3 days (range 1–8 days). Admission diagnoses for patients not requiring intensive care include bronchiolitis (22.2%), dehydration (16.7%), exacerbation of asthma or reactive airway disease (16.7%), pneumonia (11.1%), apparent life threatening event (11.1%), hypoxia (11.1%), and bronchopulmonary dysplasia exacerbation (5.6%).

Of all pediatric patients admitted, oxygen therapy was initiated or increased over baseline requirements in 10 (31.3%) patients. One patient (3.1%) was discharged on oxygen therapy not previously required. Mean duration of oxygen therapy before return room air or baseline oxygen requirements was 4.7 days. In pediatric patients positive for HBoV, there were no deaths.

Three (9.4%) pediatric patients were already hospitalized when respiratory samples were obtained. One patient with hypoplastic left heart syndrome was admitted in respiratory failure with respiratory syncytial virus (RSV). Subsequent respiratory samples screened negative for RSV and HBoV. Thirty-one days into her admission a nasopharyngeal sample tested positive for HBoV and negative for other respiratory pathogens by RT-PCR. Two other cases involved newborn patients who had never been discharged from the hospital. One patient in the neonatal ICU was not previously on respiratory support, and presented with multiple drops in oxygen saturation. She required oxygen therapy for 20 days.

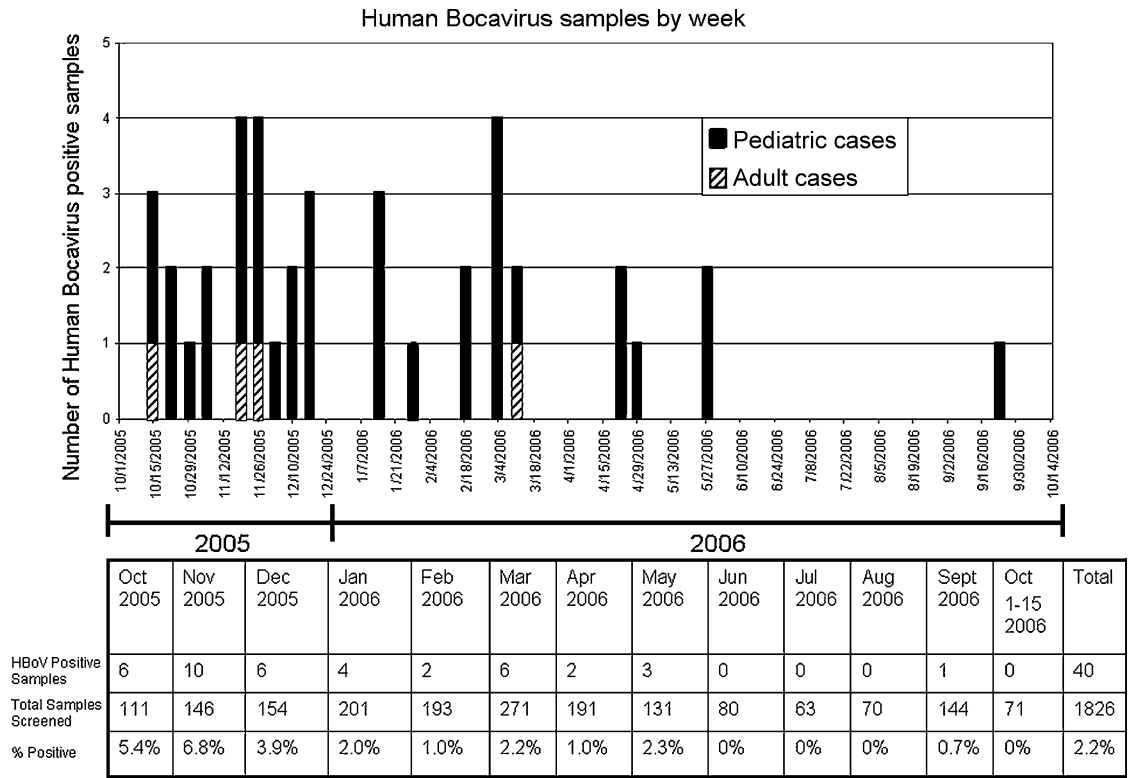


Fig. 1. Human bocavirus samples by week, with corresponding monthly totals tabulated below.

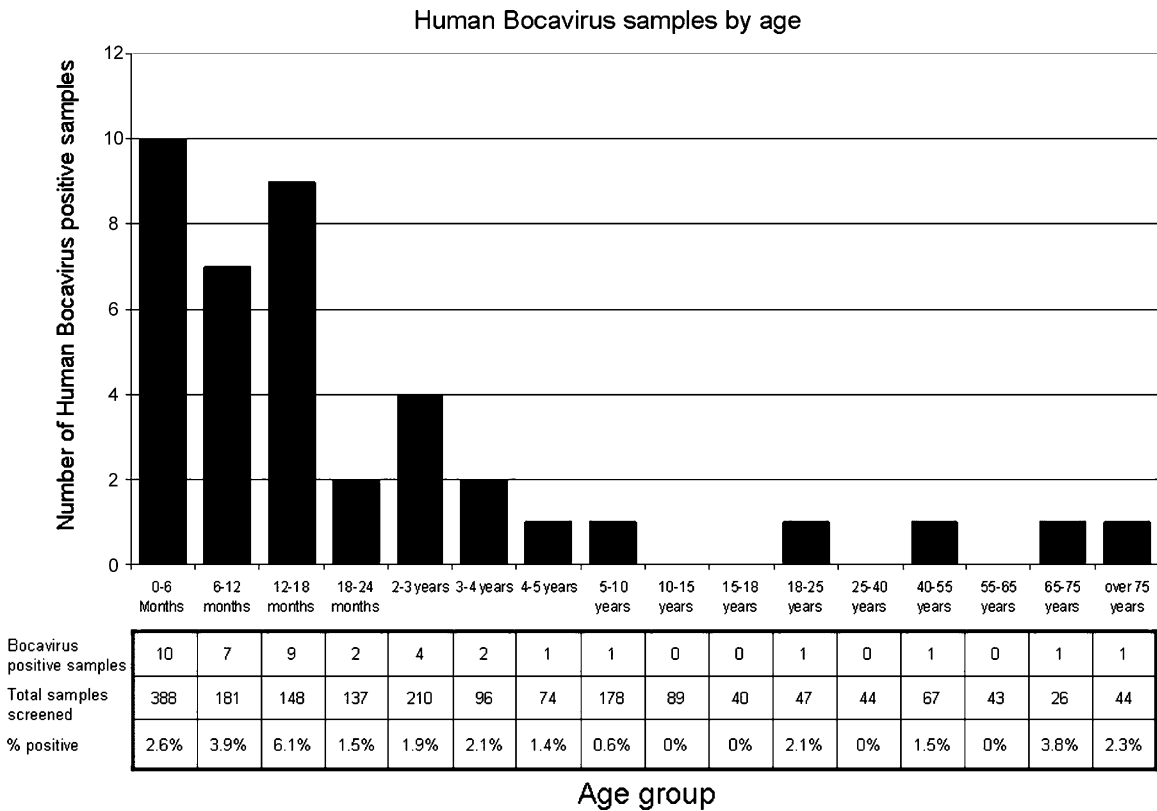


Fig. 2. Human bocavirus samples by age, with age group totals tabulated below.

Two subjects are twin siblings in the same household, both born prematurely with bronchopulmonary dysplasia, and were both admitted to the hospital sequentially with respiratory symptoms.

In the adult group, all patients had underlying pulmonary disease; half had underlying cardiac disease (Table 1). The most common presenting symptoms were rhinorrhea (50%), cough (50%), and wheeze (50%). All were hospitalized, with median hospitalization of 1.5 days (range 1–4 days). Admission diagnoses include change in mental status (50.0%) and asthma exacerbation (50.0%). None required oxygen therapy or intensive care, and there were no deaths. Adult chest radiographs, while abnormal, were unchanged from previous studies.

Of the 24 patients who had a complete blood count with differential drawn, 44.4% were abnormal. Abnormalities include immature granulocytes (37.5%), atypical lymphocytes (29.1%), and leukocytosis (20.8%). Twenty-six (81.3%) pediatric patients had chest radiographs taken, with 76.9% of chest radiographs reported as abnormal. Abnormalities included perihilar/peribronchial thickening or central inflammatory changes (42.3%), infiltrate (19.2%), ground glass disease (7.7%), pleural effusion (7.7%), hyperinflation (7.7%), and atelectasis (7.7%).

Phylogenetic analysis confirmed that a single strain of HBoV circulates in Cleveland, OH, and is similar to strains described in various studies reported worldwide. There was

**Table 1**  
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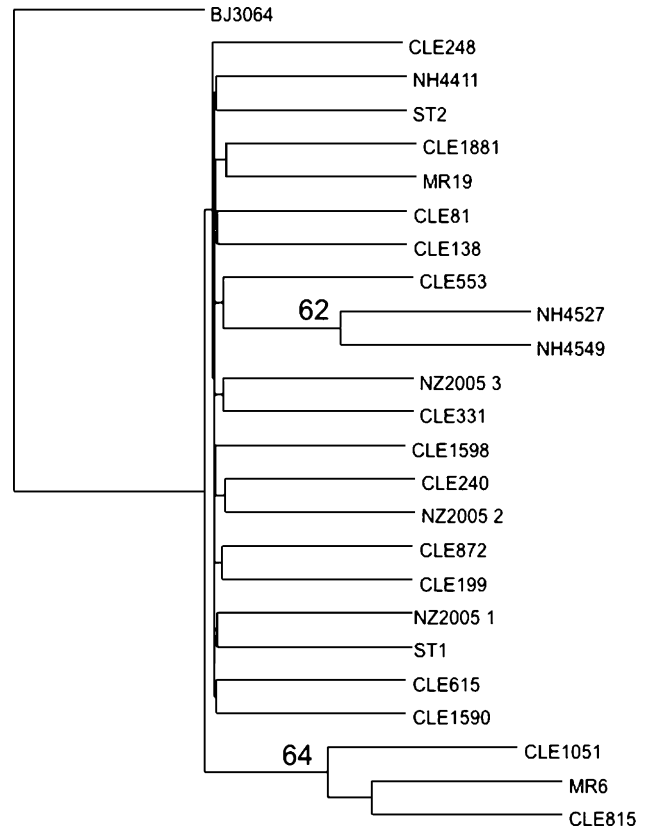
	Pediatric patients no. (%)	Pediatric patients no. (%)
<b>Demographics</b>		
Number of subjects	32 (88.8%)	4 (12.2%)
Median age (range)	12 months (24 days to 8 years)	58 years (20–86 years)
Male	22 (66.7%)	2 (50%)
African-American	10 (31.3%)	2 (50%)
Caucasian	17 (53.1%)	2 (50%)
<b>Underlying conditions</b>		
Prematurity (<37 weeks)	10 (31.3%)	N/A
Cardiac disease <sup>a</sup>	6 (18.8%)	2 (50%)
Immunodeficiency <sup>b</sup>	3 (9.3%)	0
Pulmonary disease <sup>c</sup>	3 (9.3%)	4 (100%)
<b>Clinical signs and symptoms</b>		
Respiratory findings <sup>d</sup>	28 (87.5%)	3 (75%)
Cough	24 (75.0%)	2 (50%)
Rhinorrhea	20 (62.5%)	2 (50%)
Fever > 38.0 °C	17 (53.1%)	1 (25%)
Tachycardia	13 (40.6%)	0
Gastrointestinal symptoms	11 (34.3%)	0
<b>Hospitalization</b>		
Hospitalized	27 (84.4%)	4 (100%)
Median hospital days (range)	3 days (1–8 days)	1.5 days (1–4 days)
Admitted to ICU	9 (28.1%)	0
Median ICU days (range)	2 days (1–5 days)	N/A
Oxygen therapy	10 (31.3%)	0

<sup>a</sup> Pediatric diagnoses include multifocal atrial tachycardia, ventricular septal defect, atrial septal defect, patent ductus arteriosus, and hypoplastic left heart. In adults, diagnoses include heart failure, atrial fibrillation/flutter, and prosthetic mitral and tricuspid valves.

<sup>b</sup> Include Kippel–Feil syndrome with asplenia, neutropenia secondary to chemotherapy, and common variable immune deficiency.

<sup>c</sup> In pediatric patients, excludes lung disease associated with prematurity. Includes asthma and Hunter's disease. In adults, includes asthma, chronic obstructive pulmonary disease, transudative pleural effusion, and restrictive lung disease.

<sup>d</sup> Specific respiratory findings (number, %) -Pediatric patients respiratory distress (13, 40.6%), hypoxia (9, 28.1%), ronchi (10, 31.2%), wheeze (8, 25.0%), tachypnea (8, 25.0%), rales (7, 21.8%), cyanosis (4, 12.5%), apnea (3, 9.3%). Adult patients: tachypnea (3, 75%), wheeze (2, 50%), rales (1, 25%), and hypoxia (1, 25%).



**Fig. 3.** Phylogenetic analysis. Phylogenetic tree of representative pediatric and adult human bocavirus strains from Cleveland, OH using a 229 bp region of the HBoV NP-1 gene. Additional strains from Sweden (ST2 [GenBank accession no. DQ000496]), ST1 [DQ000495], New Zealand (NZ2005.1 [EF686011]; NZ2005.2 [EF686012]; NZ2005.3 [EF686013]), CT, USA (NH4441 [DQ652164], NH4549 [DQ652165], NH4527 [DQ652167]), Beijing (BJ3064 [DQ988933]) and England (MR6 [AB257721], MR19 [AB257722]) are included. Bootstrap values are displayed at major branch points.

no difference in strains circulating among children and adults (Fig. 3).

**4. Discussion**

This study demonstrates that HBoV circulates in Cleveland, OH. In our study, HBoV predominates in the winter months, and extends into the late spring and early fall. The predominate age group affected are children under 18 months. In our study, HBoV is present in many patients who have cardiac or pulmonary disease.

In many institutions, respiratory sample collection and viral screening occur infrequently in adults. However, it is notable that HBoV was found at similar rates in both pediatric and adult patients in our population. We found HBoV at slightly higher rates in adults than other reports.<sup>6,7</sup> This underscores that viral respiratory disease leading to hospitalization in the adult population may be underappreciated.

Like prior studies, we find a substantial number of patients with gastrointestinal symptoms including emesis and diarrhea. Gastrointestinal symptoms are reported with other respiratory viruses, but generally with lower frequency.<sup>19,22</sup> By screening respiratory samples, this study is biased towards finding respiratory tract disease. Further investigation of patients with gastrointestinal illness will improve our understanding of this virus in gastrointestinal pathology.



Our data suggests nosocomial acquisition of HBoV in three cases, and close household contact in another. For the two patients who screened HBoV positive in the NICU, vertical transmission cannot be ruled out. Siblings with sequential hospitalization both screening positive for HBoV does not prove that the children infected each other although it seems likely their infections originated from close contact. These cases highlight the importance of universal caregiver and visitor hygiene. Further study on modes of transmission are needed to elucidate specific precautions needed for patients with HBoV.

HBoV was the only identified pathogen in 36 (90%) isolates. This differs from other reports which demonstrate a higher rate of viral co-infection.<sup>7</sup> This discrepancy is likely due to our selection of samples that are negative for common respiratory pathogens routinely screened at this institution. As such, the co-infection rate seen in this study cannot be compared to those of other studies. Screening of samples positive for other respiratory pathogens will likely increase the number of HBoV positive samples. However, this report suggests that clinical disease associated with HBoV alone may be severe enough to require admission to the hospital in both adults and children and to the intensive care unit in children.

Limitations of our study include its retrospective nature and lack of a control group. By screening samples of patients who present to a tertiary care center, there is likely an overrepresentation of patients with severe disease. In our study all 80–90% of samples screened were collected from patients who were hospitalized. Other reports show that many patients with HBoV can have either mild or severe respiratory tract disease.<sup>23</sup> The sensitivity of the nucleic acid extraction and primer set used has not been established. Using different primer sets or alternate DNA isolation techniques may identify further HBoV positive samples.

We find that HBoV is circulating in Cleveland, OH, in children and adults. Disease associated with HBoV is severe enough to warrant hospitalization and intensive care support. Our study suggests that HBoV may be transmitted between close household contacts and within the hospital. Further study on the prevalence in adult patients, both in hospital settings and outpatient settings, would clarify our understanding of this virus.

#### Conflict of interest

None.

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