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ORIGINAL ARTICLE

# Human bocavirus as an important cause of respiratory tract infection in Taiwanese children

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## KEYWORDS

Bocavirus;  
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infections

**Background:** Human bocavirus (HBoV), first described in September 2005, was considered a causative agent of previously unexplained respiratory tract diseases. However, only few reports provide the evidence for an association between HBoV and respiratory tract diseases. We conducted a prospective clinical and molecular study of HBoV in Taiwan.

**Methods:** We enrolled 705 children who visited our outpatient pediatric clinics in a medical center because of symptoms and signs of respiratory tract infections from November 2008 to October 2009. Throat swab was performed and HBoV polymerase chain reaction and viral culture were done simultaneously.

**Results:** Positive viral results were confirmed in 159 (22.6%) of the 705 children. HBoV was found in 35 samples and it was supposed to be as a single virus in 32 samples because viral isolation of these 32 samples did not identify other virus. The other three patients had coinfection with another virus. One child got HBoV reinfection 6 months after the first infection. Seventy-one percentage of these HBoV infections occurred between November and March. Of the 34 children with positive HBoV, 26 (76%) patients were younger than 5 years; their common symptoms were cough, rhinorrhea, and fever; the most common diagnoses were bronchitis (34%, 12/35) and sinusitis (31%, 11/35) followed by pharyngitis (29%, 10/35) and asthma exacerbation (26%, 9/35). Three of the 34 patients needed hospitalization.

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**Conclusion:** HBoV is an emerging human parvovirus that may cause respiratory tract infection in young children. Diseases associated with HBoV may range from pharyngitis, sinusitis, acute otitis media to bronchitis, asthma, and even pneumonia.  
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## Introduction

Human bocavirus (HBoV) was discovered by Allander et al.<sup>1</sup> in 2005. It is a novel member in the genus parvovirus, family Parvoviridae, and was identified in respiratory samples from infants in Sweden using a random amplification and cloning system. Afterward, HBoV was found worldwide including Australia, North America, Europe, Asia, and Africa,<sup>2–14</sup> and it is not only isolated from the respiratory tract, but also from the gastrointestinal tract.<sup>15–18</sup> By a specific polymerase chain reaction (PCR) assay, the virus was found to be present in 17 (3.1%) of 540 respiratory specimens collected from hospitalized children more than a 1-year period.<sup>1</sup> Among previous studies, HBoV was detected in 1.5%–18.3% of respiratory samples from individuals with acute respiratory tract illness, especially those from young children and infants.

The relative importance of HBoV as a causative pathogen for viral respiratory illnesses has not been determined yet, but it has been associated with respiratory diseases ranging from upper respiratory tract disease to bronchiolitis, and lower respiratory tract diseases, such as pneumonia.<sup>3,10,19–21</sup> So far it was controversially discussed whether HBoV is indeed a pathogen rather than an innocent bystander as it is frequently associated with coinfections and Koch's modified postulates cannot be fulfilled.<sup>22</sup> Although HBoV had been found in patients of all ages, most reports have suggested that children and infants are the most at risk for infection by HBoV. Currently, the methods of detecting HBoV include conventional PCR<sup>1,2,4,5,12,23,24</sup> and real-time PCR<sup>5,9,25–28</sup> because of the limited success of serological and viral culture techniques. To better understand the causative pathogen of respiratory tract infections of children in clinical practice and the relevance with the clinical presentations, we performed a prospective study of examining the specimens from pediatric patients with respiratory tract infections in Taiwanese children.

## Methods

### Patients and samples

From November 2008 to October 2009, 705 samples were collected from children aged between 5 months and 9 years, who were diagnosed with an acute respiratory tract infection. All specimens were collected at the Taiwan University Children Hospital, with informed consent from the children's parents. Two throat swab samples were collected from each patient and stored in a viral transport medium, and transported to a virology laboratory within 24 hours. The doctor who was involved in the study and performed the sampling remained the same throughout the

study. Furthermore, PCR of HBoV and viral culture were done simultaneously.

### Extraction of the virus genome DNA and PCR

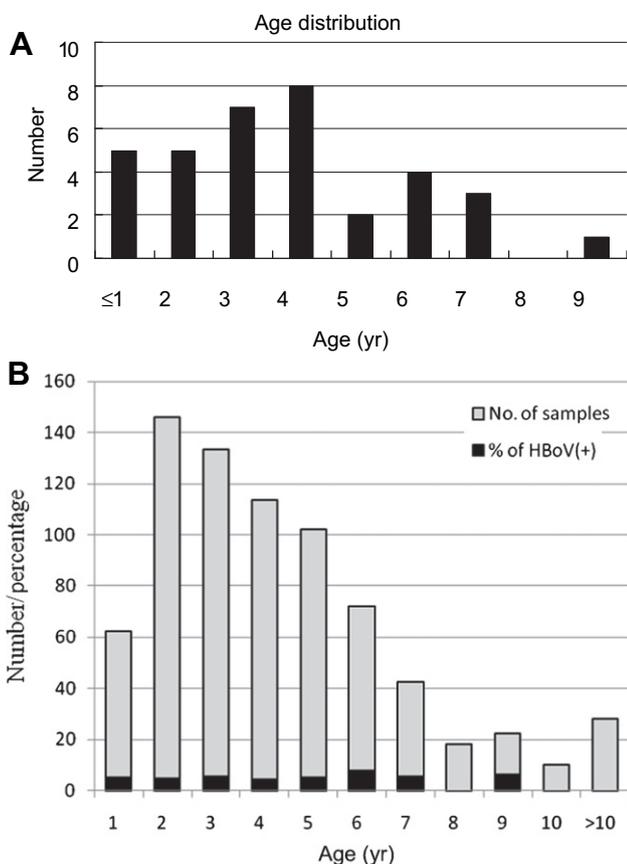
Viral nucleic acids were extracted by using a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. HBoV was detected by PCR using primers specific for two different regions of the genome. The screening primers: 188F (2281-GACCTCTGTAA GTACTATTAC-2301) and 542R (2634-CTCTGTGTTGACTGAAT ACAG-2614) were described previously by Allander et al.<sup>1</sup> and amplified a 354-bp fragment of the putative NP-1 gene.<sup>1,29</sup> HBoV DNA in the samples was confirmed and sequenced by using some newly designed PCR with the following primers: HBoV-Boca1F:5'-GCCGGCAGACATATTGGATTCC-3', HBoV-1139F:5'-CGTCTTCTGTTAGCACTAC-3', HBoV-2108R:5'-TCAG CACTATGAGGAAGG-3', HBoV-Boca2R:5'-CCATCAAGATCTGC GAGTTCAT-3', HBoV-Boca3F:5'-ACTCGCAGATCTTGATGGAA AT-3', HBoV-Boca3R: 5'-TGTACAACAACAACACATTA-3', and others were based on the published HBoV putative VP1/VP2 gene sequences (DQ000495).<sup>29,30</sup> Viral DNA was amplified by using a GeneAmp PCR system 9700 (Applied Biosystems, Foster, CA, USA) and the HotTaq DNA polymerase (QIAGEN) according to the manufacturer's recommendations. We used 5 µL of DNA in a volume of 25 µL containing 0.2 µM of each primer, and the applied thermocycler conditions were as follows: 94°C for 2 minutes for the activation of the DNA polymerase and denaturing the template, followed by 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1 minute, followed in turn by an extension of 10 minutes at 72°C. The PCR products were detected on an agarose gel with a size marker.<sup>12</sup>

The primer set for complete coding sequences amplification was designed based on the HBoV complete coding sequence available in the GenBank database. All primers were designed to have closely matching annealing temperatures so that virtually identical conditions could be applied for the PCR cycle.

## Results

### Demography

During a 1-year period, there were 705 patients with an age ranging from 5 months to 9 years whose throat swabs were obtained from the pediatric clinic. HBoV was found in 35 samples (5%) and it was supposed to be as a single virus in 32 samples because viral isolation of these 32 samples did not identify other virus. There was one patient who had positive results of HBoV twice with an interval of half a year during separate clinical visits.



**Figure 1.** Age distribution (A) of HBoV infection and number of samples and positive percentage of HBoV by age (B). Human bocavirus most infected children, especially in the group of patients who were younger than 5 years.

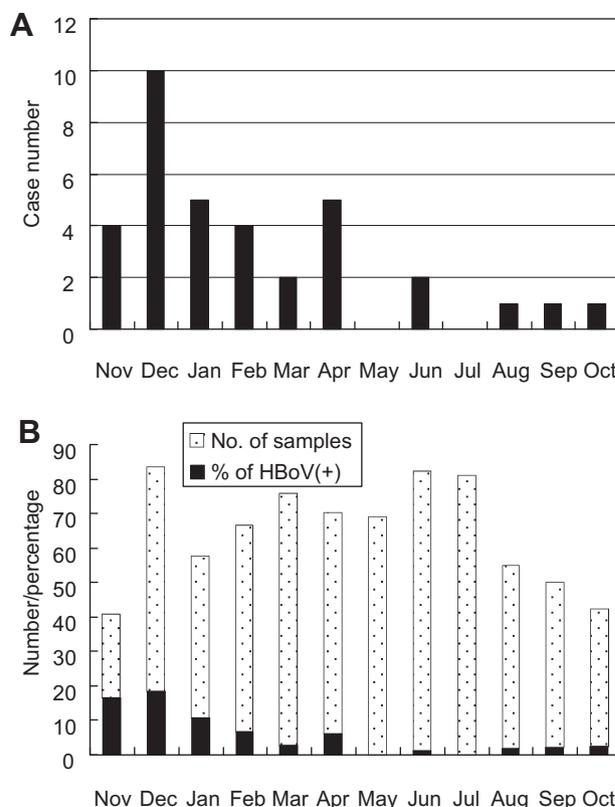
Of the 34 children with positive HBoV, the male to female ratio was 21:13. Twenty-six patients (76%) were less than 5 years old, and the mean age was 40 months. The age distribution is shown in Fig. 1. There were two patients who had underlying diseases, which were partial anomalous pulmonary venous return status postoperation and Kawasaki disease history. The seasonal distribution is demonstrated in Fig. 2A and B, which shows HBoV to be predominant in the winter and the spring.

**Symptoms**

Table 1 shows the clinical symptoms among the 35 episodes of HBoV infections. The common symptoms were productive cough (86%) followed by rhinorrhea (74%) and throat injection (7%). Fever occurred in two-thirds of patients with HBoV infection. One-third of the patients presented with wheezing breath sounds. Gastrointestinal symptoms such as vomiting, diarrhea, and decreased appetite appeared in four patients.

**Diagnosis**

The diagnoses of 35 episodes with HBoV infections are shown in Table 2. The most common clinical diagnosis was bronchitis (34%, 12/35), followed by sinusitis (31%, 11/35),



**Figure 2.** Monthly distribution (A) and the percentage (B) of HBoV infection from November 2008 to October 2009. The peak was between November and March, most in the winter and spring seasons. HBoV = human bocavirus.

pharyngitis (29%, 10/35), and asthma exacerbation (26%, 9/35). Lower respiratory tract infections including bronchitis, bronchiolitis, and pneumonia accounted for about 50% of the diagnoses. The definition of pneumonia in our study is lower respiratory tract disease characterized by lung infiltration or patch shown in the chest X-ray and fine crackles on auscultation.

Coinfection with another virus was observed in three (8.6%) of the HBoV-positive children. Among these three patients, one had adenovirus, one had influenza A virus, and the other one had coxsackie A virus. Another patient had a positive result of mycoplasma IgM. The individual clinical

**Table 1** Symptoms of 35 episodes associated with HBoV infection

Clinical symptoms	N (%)
Productive cough	30 (86)
Rhinorrhea	26 (74)
Wheezing	11 (31)
Shortness of breath	4 (11)
Injected eardrum	4 (11)
Fever	23 (66)
Injected throat <sup>a</sup>	27 (77)
Gastrointestinal symptoms	4 (11)

<sup>a</sup> Throat: there were exudates in two patients and ulcers in one patient.

**Table 2** Clinical diagnosis among 35 episodes of bocavirus infections

Clinical diagnosis	N (%)
Bronchiolitis	2 (6)
Bronchitis	12 (34)
Sinusitis	11 (31)
Pneumonia	3 (9)
Pharyngitis/tonsillitis	10 (29)
Pharyngoconjunctival fever	3 (9)
Herpangina	1 (3)
Gastritis	1 (3)
Acute otitis media	3 (9)
Asthma	9 (26)

diagnoses of those four patients were pharyngoconjunctival fever, bronchopneumonia, exudative tonsillitis with bronchitis, and mycoplasma pneumonia.

Three of the 34 patients needed to be hospitalized for further treatment of intravenous fluid supplement and symptomatic medication. Of these three patients, two had bronchiolitis and one had herpangina. Among the two bronchiolitis patients, one had positive result of respiratory syncytial virus antigen in sputum specimen. The patient who was diagnosed clinically as herpangina was because of the finding of the only one ulcer in the throat and the viral culture didn't yield enterovirus. The RSV bronchiolitis patient may not be infected only by HBoV, and the relationship of these two viruses would need further clarification. The mean age of three inpatients was 11 months, the hospital stay was around 5–7 days, and viral cultures of the throat swabs were all negative.

## Discussion

The report was to evaluate HBoV infection in children's clinical practice. In our study, 77% of patients with HBoV infections were younger than 5 years. Most studies found that HBoV infection occurred throughout the year with peak occurrences in the winter and spring.<sup>29,31</sup> On the other hand, there were studies showing that there was no obvious seasonality for HBoV infections.<sup>12</sup> In our cohort, the seasonal distribution showed that 71% of HBoV infections were between November and March.

Most patients in our study presented with symptoms of upper respiratory tract infections including fever, cough, rhinorrhea, and throat injection. Some reports described that the patients had to be hospitalized because of bronchiolitis,<sup>32</sup> asthma exacerbation,<sup>26,33</sup> and wheezing.<sup>34</sup> One report found that as many as 18% of children hospitalized with pneumonia were positive for HBoV.<sup>7</sup> Most HBoV reports were retrospective analyses based on hospital settings, and up to 6.6% of patients required stays in intensive care units, and up to 40% required oxygen therapy at some point during their hospitalization.<sup>11,31,35</sup> These reports would potentially exaggerate the true role of HBoV in respiratory diseases and overestimate the severity of disease. We recruited patients while they had respiratory symptoms at our outpatient department, so our results might show more realistic presentations in pediatric groups. In fact, there

were studies that suggested that HBoV occurred rarely in asymptomatic children<sup>4,19</sup> and supported its role in lower respiratory tract illness.<sup>36</sup>

There were only three (8.6%) patients who showed coinfection phenomenon. It was uncertain whether HBoV exacerbated other viral illnesses, or whether the other viral pathogens were causative symptoms. Fifty percentage of the patients presented with lower respiratory tract involvement. The frequent occurrence of lower respiratory tract symptoms, especially wheezing, led to the speculation that HBoV may be a significant cause of asthma exacerbation.<sup>3,4,7–9,11–13,26,28,37–41</sup> In addition to respiratory diseases, one study found that 9.1% of children with HBoV infections had gastroenteritis in Spain, and the another study in Korea showed that only 0.8% of HBoV patients were hospitalized for gastroenteritis.<sup>15,16,42</sup> There was one study in France, which screened children with Kawasaki disease for bocavirus, and 31.2% of the samples were found to be positive,<sup>43</sup> however, further investigation was needed to find the association. Moreover, prolonged detection of HBoV in nasopharyngeal aspirates of immunocompetent children with respiratory tract disease was found recently.<sup>44</sup> HBoV DNA was proved to be slowly cleared during a period of 4.5 months in a five series of samples in the same patient. In our study, we also identified one patient who had two HBoV infection episodes with different clinical diagnoses, bronchitis and sinusitis, respectively, in the period of half a year without other concurrent pathogen. During the interval between the two different clinical visits in our hospital, no repetitive respiratory tract infections were noted. The observations could be explained by several hypotheses including persistent HBoV infection, reactivation induced by other infections, or reinfection. Therefore, more examinations are needed. Serologic testing to diagnose primary infection, detection of HBoV viremia, or high viral loads in quantitative PCR may be more useful predictors of HBoV-associated clinical disease than only qualitative detection of HBoV DNA in respiratory tract samples. Beside, simultaneous examination of the other viruses by PCR may be also needed to exclude coinfection. There was currently no specific treatment available for HBoV infection because of the lack of cultivability and relatively benign clinical course in most patients. Supportive care played a large role in the management and further copathogens should be investigated because of some degree of coinfections appearance.

In conclusion, HBoV is an emerging human parvovirus that may cause respiratory tract infections in young children and it appears more in the winter and spring time in Taiwan (Fig. 2A and B). Diseases associated with HBoV range from pharyngitis, sinusitis, acute otitis media to bronchitis, asthma, and even pneumonia.

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