High Rate of Human Bocavirus and Adenovirus Co-infection in Hospitalized Israeli Children

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Abstract
We investigated co-infection of human bocavirus (HBoV) and other respiratory viruses in hospitalized children by real-time PCR. High rate of adenovirus infection was found among children infected with HBoV (69.2%). Such high HBoV/adenovirus co-infection has not been previously reported, underscoring the need to investigate HBoV contribution in patients' clinical presentations.

Word count: 50
The recent discovery of human bocavirus (HBoV) among Swedish children by Allander et al (1) in 2005 added a new member to the list of viruses that can cause respiratory tract infections. HBoV belongs to the *Parvovirinae* subfamily of the *Paroviridae* family (1). HBoV is a non-enveloped, single stranded DNA virus with a 5.2 kb length genome. Based on DNA sequencing, two groups of HBoV were identified, Stockholm 1 (st 1) and Stockholm 2 (st 2) (1). Since its discovery, HBoV has been found in several countries (2, 6, 8, 14-23, 25).

Allander et al (1) initially detected HBoV DNA in 3.1% of Swedish children and infants presenting with variable degrees of respiratory distress and fever. Moreover; 17.6% of positive HBoV patients were also positive for either adenovirus or RSV (1). Since then, the frequency of HBoV from several reports worldwide has ranged between 1.5% and 18.3%, and the rate of co-infection of HBoV and other respiratory viruses was as high as 91%, particularly co-infection with rhinovirus, or RSV or parainfluenza virus (9). The highest co-infection rate of HBoV and adenovirus reported to date was 37.1% in Korea (5). The high co-infection rate with other viruses and the difficulties in culturing HBoV could be in part due to its dependence on other respiratory viruses for its replication. This might be reminiscent of the *Dependovirus*, adeno-associated virus (AAV), which is dependent on adenovirus for its replication (4, 10, 24).

In this study we evaluated the rate of co-infection with HBoV and adenovirus in 231 Israeli children. These children were hospitalized between January 2006 and December 2006 with upper-respiratory infection and adenovirus was on the list of viruses to be checked or the patients had clinical symptoms most closely resembling adenovirus infection. The patients’ age ranged from a few days to less than 10 years. The male to female ratio was 1.4:1.0. The difference tended to be larger for those diagnosed with HBoV or adenovirus infection (male to female ratios 1.9 and 1.8, respectively).

Patient samples were obtained by well trained health care providers and transported to Israel Central Virology Laboratory (ICVL) in a timely manner. These included 97 (42%) nasal suction samples, 69 (30%) nose swab, 24 (10.4%) BAL, 23 (10%) throat swab, 12 (5%) sputum, 3 (1.3%) lung biopsy and 3 (1.3%) pleural fluid samples. All were examined for the presence of adenovirus. The presence of other viruses was tested according to physicians requests. HBoV was tested retrospectively in all samples.
The samples were tested for presence of adenovirus, HBoV, influenza A & B and RSV A & B, by real-time PCR using TaqMan chemistry as previously described (8, 11-13), while parainfluenza viruses 1, 2, 3, were tested by RT-PCR with gel detection as previously reported (7). Viral genomic DNA was extracted from patients samples using the QIAamp® DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany); and viral genomic RNA was extracted using High Pure Viral RNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany).

Of the 231 samples analyzed, 76 (32.9%) were positive for adenovirus DNA and 26 (11.3%) were positive for HBoV. The high adenovirus positivity rate found (32.9% versus 13.9% of all other viruses except HBoV together; p<0.0001, T-Test) likely reflects, in large part, pre-selection of patients suspected to be infected with this virus. The frequency of HBoV (11.3%) was higher than that found in Europe (2), equal to that reported from Korea and less than that from Jordan 18.3% (14). Eighteen (69.2%) of the 26 positive HBoV samples were co-infected with adenovirus, significantly above the frequency of adenovirus infection in the non-HBoV infected children (28.3%, p<0.001, T-Test). Even more meaningful, is the finding of a higher frequency of HBoV infection among adenovirus-infected children (23.7%) than in non-adenovirus infected (5.2%, p<0.0001, T-Test). Such high levels of association between HBoV and adenovirus have not been reported to date. The difference could in part be attributed to the use of highly sensitive molecular assays in the present study. Determining adenovirus serotype of 7 of 18 available co-infected samples revealed the presence of 3 different serotypes (T1 (3/7), T2 (3/7) and T3 (1/7)). None of the samples serotyped had multiple adenovirus co-infection. These were the most prevalent adenovirus serotypes found during the study period (each of them found in 28.5% (10/35) of samples tested; the other serotypes were T5, 3/35; and T7, 2/35). These results suggest that HBoV co-infection does not depend on a particular adenovirus serotype.

HBoV co-infections with other respiratory viruses have been previously reported to occur at rates in the range of 17-91% (1, 9). In particular, high HBoV co-infections (91%) with rhinovirus, RSV, or parainfluenza virus has been previously reported from hospitalized children less than 5 years of age with pneumonia (9). While rhinovirus infections was not investigated in our patient population, only 2 of the 8 HBoV positive, adenovirus negative, samples were co-infected with either RSV
(1/14 = 7.1%) or parainfluenza-3 virus (1/10 = 10%). Thus, the overall co-infection rate between HBoV and other evaluated respiratory viruses was 76.9%.

Interestingly, of the patient sample types evaluated, HBoV DNA was mostly detected in throat swabs 26.1% (6 of 23) as compared to BAL 12.5% (3 of 24), nose swabs 10.1% (7 of 62) or NS 9.3% (9 of 88). On the other hand adenovirus DNA was detected at a similar rate in throat, BAL, and NS patient samples (32-39%). Adenovirus and HBoV were each detected from a patient pleural fluid, while no viral DNA was detected in either sputum or lung biopsy samples. HBoV and adenovirus co-infection was most frequently detected in nasal suctions 38.9% (7 of 18), followed by nose swabs 27.8% (5 of 18), and throat swabs 22.2% (4 of 18).

Stratifying patient samples analyzed by the month in which the infection occurred showed that adenovirus circulated throughout the year with major activity during the respiratory illness season, in particular during January and December 2006, while HBoV DNA was detected from January to May and then from September to December 2006 (Fig. 1a). The time distribution of HBoV positive samples was similar to that reported from the US, France and Germany (8, 15, 25). While, Bastien et al. from the colder Canada reported HBoV throughout the year 2003 with the exception to the month of August (3). In Israel, HBoV and adenovirus co-infection rate was the highest during December 2006 38.9% (7 of 18).

Adenovirus was detected in Israeli children up to 10 years of age, in particular, in those under the age of 3 (Fig. 1b). Of the patients positive for adenovirus, 15 (6.5%) was younger than 1 year, 30 (13%) was one year, 14 (6%) were 2 years and 8 (3.5%) were 3 years old. HBoV DNA was detected in children less than 6 years old, mainly those under 3 years (Fig. 1b). HBoV was detected in 5 (2.2%) patients who were under 1 year of age, 8 (3.5%) were 1 year, 7 (3.0%) were 2 years, and 4 (1.7%) were 3 years old. Adenovirus and HBoV co-infection was highest in 1 year old patients 33.3% (6 of 18) but was frequent also in patients under 1 year of age 22% (4 of 18) and in 2 years old 22% (4 of 18). The age distribution of HBoV infected children was similar to that reported from other countries (17, 25).

Phylogenetic analysis was performed on a part of the VP2 genes of 10 HBoV positive samples detected between January and December 2006. Briefly, primers sequences BocaSEQ3 (forward) and BocaSEQ4 (reverse) derived from the VP2 were used for this analysis of HBoV-positive patient samples and to confirm HBoV patient samples positive by the NP1 gene as described by Foulonge et al (8). The 514 bp
PCR products were gel purified using Qiagen® QIAquick Gel Extraction (Qiagen® GmbH, D-40724 Hilden, Germany) and sequenced using ABI PRISM Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). Reaction mixtures were analyzed on Applied Biosystems model 373 DNA automatic sequencing systems. The Sequencher program (Gencodes Corporation, Ann Arbor, MI) was used to compare 417 bp nucleotide sequences. Phylogenetic tree was prepared by nearest neighbor analysis using Clustal X with 1,000 bootstraps and trees were visualized using TreeView or NJ plot. Sequences from all 10 isolates (AM849098-AM849107) had high homology (99 to 100%) to HBoV sequences in the EMBL/Genbank/DDBJ databases (Fig. 2). The two main groups Stockholm 1 (DQ000495) and Stockholm 2 (DQ000496), which had been reported in several areas of the world, also circulated in Israel during 2006 as illustrated in 10 randomly selected HBoV positive samples from Israeli patients (Fig. 2).

Our study showed that both HBoV groups circulated in Israeli children less than 10 years of age, in particular those under 3, during the year 2006. The frequency in our selected patient population was 11.3%, at the upper end of what had been reported worldwide. This high HBoV frequency could be due to the evaluation of patients clinically suspected of having adenovirus. The high co-infection rate of adenovirus and HBoV in hospitalized Israeli children exemplifies the importance of examining the role that HBoV may play in the clinical presentations of the hospitalized patients diagnosed with adenovirus infection. In addition, it would be worthwhile investigating if HBoV replication is dependent in part on adenovirus or any other human respiratory virus.

HBoV sequences were assigned the following accession numbers (AM849098 - AM849107) by EMBL.

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Word count: 1546
References


Figure legends

Figure 1a. Histogram of the monthly distribution of clinical samples PCR positive for adenovirus and HBoV DNA among hospitalized Israeli patients during the year 2006. The number of patients tested in each age group is indicated on the top of each bar.

Figure 1b. Age distribution of patients samples positive for adenovirus and HBoV during the year 2006. The number of patients tested in each age group is indicated on the top of each bar.

Figure 2. Phylogenetic comparison of HBoV VP2 gene nucleotides detected during the year 2006 with sequences from EMBL/GenBank database. The Clustal X nearest Neighbor Joining method (bootstrap = 1000) was used to compare 417-nt sequences encoding VP2 genes with sequences from the EMBL/GenBank database.
Figure 1a

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Figure 1b
Figure 2