Quantification of human bocavirus in lower respiratory tract infections in China

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Published: 31 January 2007
Received: 30 October 2006
Accepted: 31 January 2007

Infectious Agents and Cancer 2007, 2:3 doi:10.1186/1750-9378-2-3
This article is available from: http://www.infectagentscancer.com/content/2/1/3
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Abstract

A quantitative PCR method was established to quantify human bocavirus (HBoV) genomic copies in clinical specimens from children with lower respiratory tract infections (LRTI) in China. A total of 257 respiratory tract specimens were tested, and 7 (2.7%) of these (all sputum samples) were positive, with genomic copies that ranged from $8.0 \times 10^3$ to $8.0 \times 10^9$ in the samples. The main clinical symptom of patients who were positive for HBoV DNA was a pneumonia-like syndrome represented by high fever and cough. Our results suggest that HBoV may be an important etiological agent of LRTI in children in China.

Finding

Virus infection is the major cause of lower respiratory tract infections (LRTI) in children worldwide, and the most important viral agent is arguably respiratory syncytial virus (RSV) [1]. Others viruses such as influenza viruses, parainfluenza viruses, adenoviruses, rhinoviruses, coronaviruses, and human metapneumovirus are also frequently reported to cause LRTI [2-5]. Human bocavirus (HBoV) was first cloned from pooled human respiratory tract samples collected in Sweden, and was provisionally classified into the genus Bocavirus based on sequence comparisons [6]. HBoV has been reported worldwide to be present in between 1.5% to 11.3% of respiratory samples tested from individuals with acute respiratory illness [7-10], and it appears to be associated with LTRI [11-13].

To date, there have been no studies reporting the detection of HBoV DNA in children with LRTI from China. Currently, detection of HBoV in children with LRTI mainly relies on DNA amplification by regular PCR. Because these assays are not quantitative, positive results are hard to interpret. Recently, a reliable quantitative PCR (Q-PCR) method has been developed to detect HBoV genomic copies in clinical samples, and this has demonstrated a presence of HBoV DNA in children with pneumonia-like symptoms in Thailand [14]. In this study, we used a Q-PCR with the amplicon targeted to the NS coding region of HBoV to detect the presence of HBoV DNA in children with respiratory tract infection in China. Our results suggest that HBoV may be an important etiological agent of LRTI in children in China.
A total of 257 specimens were collected from December, 2005 to February, 2006 from infants or children with LRTI hospitalized in Wenling First Hospital, Zhejiang Province, China. The specimens included throat swab, nasopharyngeal aspirate, sputum and aspirated sputum, together with blood samples, on the day of hospitalization. All the blood samples tested negative for antibodies directed against influenza virus A and B, respiratory syncytial virus (RSV), parainfluenza virus and adenovirus by commercially available ELISA kits. All these clinical samples were taken after informed consent was obtained from parents or other legal guardians.

DNA extraction from clinical specimens was performed as follows. Throat swab and nasopharyngeal aspirates were diluted in 2 ml of PBS, and were centrifuged at 12,000 rpm at 4°C for 10 min. The pellets were resuspended in 200 µl PBS. The sputum and aspirated sputum specimens were digested with 3 volumes of 4% NaOH, and were centrifuged at 8,000 rpm at 4°C for 5 min. Pellets were further washed with PBS and resuspended in a final volume of 200 µl PBS. All these resuspended pellets were extracted DNA using QIAamp blood mini kit (QIAGEN). A plasmid (pskHBoV) containing the HBoV sequence (nts 1-5299) was synthesized by extension of PCR fragments with primers designed based on the ST2 sequence of HBoV [Genbank: DQ000496], and this was subsequently inserted into pBluescript vector (Stratagene). This plasmid was used as a control (1 genomic copy = 5 × 10^-12 µg) to establish the standard curve for absolute quantification using TaqMan technology with an Applied Biosystems 7500 system (Foster City, Calif.) as a quantitative PCR method [15,16]. The amplicon and the TaqMan probe for this HBoV specific quantitative PCR were designed by Primer Express 2.0.0 software (Applied Biosystems) in the conserved regions of the NS coding region among HBoV genome sequences deposited in GenBank. Their conserved regions of the NS coding region among HBoV isolates. In addition, lower copy numbers of viral genomes were not detected in our assay, perhaps because of limitations in the extraction of DNA from specimens or inhibitors of the Q-PCR in the clinical specimens. Further investigation with different paired primers and probes to quantify the HBoV genome in clinical samples are under way for probing the etiology of HBoV in LTRI in children, using, in addition, normal controls. Nevertheless, this quantitative PCR method provides a reliable means to screen samples with high titers of genomic copies for virus isolation and to begin to address the relationship between HBoV and LRTI in children in China.

**Abbreviations**
HBoV (Human bocavirus); LTRI (Lower respiratory tract infection); Q-PCR (Quantitative polymerase chain reaction).

**Table 1: Clinical characteristics of 7 patients positive for HBoV DNA by Q-PCR**

| No. specimens | Type of samples | Sex | Age  | Symptom                        | Genomic copies (gc/ml)* | Clinical Diagnosis   |
|---------------|-----------------|-----|------|--------------------------------|-------------------------|----------------------|
| WL102         | Aspirated sputum| F   | 13 mo| Fever with cough for 10 day    | 1.46 × 10⁷              | Pneumonia            |
| WL108         | Aspirated sputum| F   | 1 yr | Fever with cough for 5 day, seizure once | 3.95 × 10⁹              | Pneumonia            |
| WL109         | Sputum          | F   | 7 yr | Fever for 2 days,             | 4.01 × 10³              | Bronchiolitis        |
| WL160         | Aspirated sputum| F   | 7 mo | Fever for 5 days and cough for 3 days | 4.51 × 10⁴              | Bronchiolitis        |
| WL221         | Aspirated sputum| M   | 2 yr | Fever and cough for 2 days    | 6.95 × 10⁵              | Bronchiolitis        |
| WL223         | Sputum          | F   | 3 yr | Fever for 3 days              | 6.94 × 10⁵              | Bronchiolitis        |
| WL226         | Sputum          | M   | 3 yr | Fever and cough for 5 days    | 5.45 × 10⁴              | Bronchiolitis        |

*The number of genomic copies was converted to per ml in the original collected sample (2 ml).
Competing interests
The author(s) declare that they have no competing interests.

Authors’ contributions
FL planned the study, collected clinical samples in conformation with all the human subject consents and participated in work of ELISA, DNA extraction and Q-PCR performance. AZ and HL performed ELISA, extracted DNA and Q-PCR. NY, EY and SW developed and standardized the Q-PCR method. DP and IQ coordinated the study, constructed plasmid, designed primers and probe, initiated Q-PCR and wrote the manuscript.

Acknowledgements
The work was partially supported by internal funds from Wenling First Hospital, Wenling, Zhejiang Province, China, and was partially supported by PHS grants ROI AI46458, ROI AI56310, and ROI AI12302 from NIAID to DJP. This work also was made possible by NIH Grant Number P20 RR016443 from the COBRE program of the National Center for Research Resources to QJ.

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