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Human bocavirus (HBoV) in Thailand: Clinical manifestations in a hospitalized pediatric patient and molecular virus characterization

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Summary *Objective:* Human bocavirus (HBoV), a novel virus, which based on molecular analysis has been associated with respiratory tract diseases in infants and children have recently been studied worldwide. To determine prevalence, clinical features and perform phylogenetic analysis in HBoV infected Thai pediatric patients.
Methods: HBoV was detected from 302 nasopharyngeal (NP) suctions of pediatric patients with acute lower respiratory tract illness and sequenced applying molecular techniques.
Results: The incidence of HBoV infection in pediatric patients amounted to 6.62% with 40% co-infected with other respiratory viruses. There were no clinical specific manifestations for HBoV; however, fever and productive cough were commonly found. Generalized rales and wheezing were detected in most of the patients as well as perihilar infiltrates. The alignment and phylogenetic analysis of partial VP1 genes showed minor variations.
Conclusion: Our results indicated that HBoV can be detected in nasopharyngeal aspirate specimens from infants and children with acute lower respiratory tract illness.

Introduction

Acute respiratory tract infection is a major cause of pediatric morbidity and mortality worldwide. In most cases, viruses including influenza A and B, parainfluenza viruses, adenoviruses, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) are the causative agents. Recently, a new respiratory tract virus of the *Parvoviridae* family, Human Bocavirus (HBoV), has been discovered applying molecular analysis on pooled respiratory tract aspirations taken from children in Sweden. This virus is closely related to the bovine parvovirus and canine minute virus, which have been classified as members of the genus

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Bocavirus. The virus comprises two major open reading frames (ORFs) encoding a nonstructural protein (NS1) and at least two capsid proteins (VP1 and VP2), respectively. Moreover, the HBoV genome also contains a third middle ORF encoding a nonstructural protein (NP1) of unknown function.[1] The most conserved region of this virus is the NS1 and NP1 gene whereas the VP1/VP2 gene constitutes the variable region.[2] The induction of respiratory illness by HBoV is not clearly defined due to lack of propagation techniques in cell culture or animal models.[1] However, many studies have reported this virus infection to be associated with acute respiratory illness.[1,3,4] Upon discovery of HBoV in respiratory pools, its global prevalence has been reported to range from 1.5% to 19% and co-infection with other viruses was commonly found.[1,3–24] Moreover, in few studies were shown negative results for HBoV infection in nasopharyngeal (NP) swabs from healthy volunteers.[20,25,26] Additional epidemiological and clinical investigation will be essential in order to elucidate what exactly engenders HBoV related illness. Therefore, in the present study we applied polymerase chain reaction to detect HBoV from NP suctions collected from infants or children who had been admitted with respiratory tract illness.

Materials and methods

Clinical samples

Nasopharyngeal suction specimens were collected from 302 individual infants or children (age range: 5 days to 14 years) who were admitted and diagnosed as having acute lower respiratory illness during the period February 14, 2006 to February 28, 2007. All of the clinical samples were provided by the Department of Pediatrics, King Chulalongkorn Memorial Hospital, Thailand. Nasopharyngeal suction samples were collected in transport medium with antibiotics (0.5% BSA, penicillin G (2×10^6 U/L), streptomycin 200 mg/L) and stored at −70 °C until tested. The study was conducted after receiving approval by the Ethics Committee of the Faculty of Medicines, Chulalongkorn University. Prior to enrolment, all participating parent gave their written informed consent.

DNA and RNA extraction

DNA and RNA were extracted from 150 μl of NP suction using TRI REAGENT® LS (Molecular Research Center Inc., Cincinnati, OH) and solubilized in 20 μl of 8 mM NaOH or 12 μl of DepC treated water for DNA or RNA, respectively.

Reverse transcription

Reverse transcription (RT) was performed at 37 °C for 2 h using 200 units of M-MLV reverse transcriptase (Promega, Madison, WI), 5 μl of 5× M-MLV reaction buffer (Promega), 5 μl of 10 mM dNTP (Promega), 25 units of RNasin® Ribonuclease Inhibitor (Promega) and 0.5 μg/μl of Random Primer (Promega), 12 μl of RNA heating to 70 °C for 5 min then cooling on ice, and adding nuclease-free water to a final volume of 25 μl.

Detection of HBoV and other respiratory viruses

For HBoV detection we amplified the NP1 gene by conventional PCR modified from a previous study.[1] The reaction mixture contained 2 μl DNA, 0.5 μM 188F primer and 0.5 μM 542R primer, 10 μl 2.5× Eppendorf masterMix (Eppendorf, Hamburg, Germany), and nuclease-free water to a final volume of 25 μl. The amplification reaction was performed in a thermocycler (Eppendorf) under the following conditions: Initial denaturation at 94 °C for 3 min, followed by 35 amplification cycles consisting of 94 °C for 30 s (denaturation), 55 °C for 30 s (primer annealing), and 72 °C for 1 min (extension), and concluded by a final extension step at 72 °C for 7 min. Another set of primers specific for the VP1 gene, VPF2 forward primer (5′-TTCAGAATGGT CACCTCTACA-3′: nt 3639—3659) and VPR2 reverse primer (5′-CTGTGCTTCCGTTTTGTCTTA-3′: nt 4286—4266), were used in a separate PCR reaction to exclude false positives. After 2% agarose gel electrophoresis stained with ethidium bromide, the expected products of 354 and 648 bp representing the NP1 and VP1 gene, respectively, were visualized on a UV transilluminator.

Influenza A virus detection was performed by conventional PCR using 1 μl of cDNA, 0.5 μM of FluA_M_F: 5′-RGGCCCCCTCAAAGCCGA-3′ (nt 76—93), 0.5 μM of FluA_M_R: 5′-ACTGGGCACGGTGAGYGT-3′ (nt 235—218), 10 μl of 2.5× Eppendorf masterMix, and nuclease-free water to a final volume of 25 μl. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of each sample was amplified in one additional reaction mixture of identical volume with primers GAPDH_F: 5′-GTG AAGGTCGGAGTCAACGG-3′ (nt 112—131) and GAPDH_R: 5′-GTTGTCATGGATGACCTTGGC-3′ (nt 603—583) at a 0.5 μM concentration, each. The amplification reaction was performed in a thermocycler (Eppendorf) under the following conditions: Initial denaturation at 94 °C for 3 min, followed by 40 amplification cycles consisting of 94 °C for 30 s (denaturation), 55 °C for 30 s (primer annealing), and 72 °C for 1 min (extension), and concluded by a final extension step at 72 °C for 7 min. After 2% agarose gel electrophoresis, the expected products were influenza A virus (160 bp) and the housekeeping gene (492 bp).

Parainfluenza virus was detected by real-time PCR using SYBR green I carried out in a Rotor-Gene 3000 (Corbett Research, New South Wales, Australia). The reaction mixture contained 1 μl of DNA sample, 10 μl 2.5× Eppendorf masterMix, 0.5 μM ParaF: 5′-GCTAAATACTGTCTTMAH TGGAGAT-3′ (nt 11,254—11,278), 0.5 μM ParaR: 5′-GTAAGG ATCACCWACATADAWTGTA-3′ (nt 11,392—11,370), 2 μl 1× SYBR Green and nuclease-free water to a final volume of 20 μl. The amplification reaction consisted of a preincubation step at 95 °C for 3 min followed by 35 cycles of amplification including 95 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s. The fluorescent signal was detected once per cycle upon completion of the extension step. After amplification, melting curve analysis was performed by heating to 95 °C then cooling to 60 °C for 15 s, followed by a temperature increase to 95 °C, while continuously collecting the fluorescent signal data.

Adenovirus, influenza B virus, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) were detected