

Human Bocavirus in Hospitalized Children, South Africa

To the Editor: In recent years, several novel respiratory viruses have been identified. These include human metapneumovirus (HMPV) (1), severe acute respiratory syndrome-associated coronavirus (2), human coronavirus (HCoV) NL63 (3,4), HCoV HKU1 (5), and, most recently, human bocavirus (HBoV) (6). The latter belongs to the *Parvoviridae* family and is most closely related to bovine parvovirus and canine minute virus (CnMV), which are members of the genus *Bocavirus* (6). Parvovirus B19 and HBoV are the only 2 parvoviruses known to be pathogenic to humans, but the relevance of HBoV infection in the clinical setting is not known.

In this retrospective study, 341 nasopharyngeal and bronchoalveolar lavage samples were taken from children (age 2 days–12 years) hospitalized with respiratory tract infections in 2004 in the Red Cross War Memorial Children's Hospital, Cape Town, South Africa. Samples were originally screened by using an indirect immunofluorescence assay (Light Diagnostics, Chemicon International, Temecula, CA, USA) for common respiratory viruses, including respiratory syncytial virus; influenza virus A and B; parainfluenza viruses 1, 2, and 3; adenovirus; and cytomegalovirus. Subsequently, HMPV and HCoV NL63 were detected by using reverse transcription-PCR (1,3).

Samples were also screened for HBoV DNA. DNA was extracted by using the QIAamp DNA blood mini kit according to the manufacturer's instructions (Qiagen Inc., Valencia, CA, USA). PCR amplification of a region of the NP-1 gene and the 3' portion of the VP1/2 capsid gene of HBoV was performed. Briefly, 10 μ L

DNA was added to a 50- μ L PCR mix containing 2 IU Supertherm polymerase (JMR Holdings, Kent, UK), 1.5 mmol/L MgCl₂, 200 μ mol/L each dNTP, and 0.2 μ mol/L primers NP-1 s1 (5'-TAACTGCTCCAGCAAGTC-CTCCA) and NP-1 as1 (5'-GGA-AGCTCTGTGTTGACTGAAT). To improve sensitivity, a second seminested reaction with 2.5 μ L outer product and NP-1 as1 primer and NP-1 s2 (5'-CTCACCTGCGAGCTCTG-TAAGTA) primer was performed at an annealing temperature of 55°C. Negative controls were used, and appropriate measures were taken to prevent contamination (7). Samples with an NP-1-specific PCR product of 368 bp were confirmed by amplifying a 980-bp product of the VP1/2 capsid gene in a similar seminested PCR amplification protocol (primers VP s1 5'-GCACTTCTGTATCAGAT-GCCTT, VP as1 5'-CGTGGTATG-TAGGCGTGTAG, and VP s2 5'-CTTAGAACTGGTGAGAGCACTG). A selection of the inner VP1/2 amplicons obtained from samples taken over the year were sequenced directly and aligned in ClustalX, and a phylogenetic tree was constructed with the Kimura 2-parameter neighbor-joining method with 1,000 bootstrap resamplings. Comparative sequences were obtained from GenBank and included HBoV isolate st1 (DQ000495), HBoV isolate st2 (DQ000496), and a CnMV isolate (NC_004442). Nucleotide sequences from this study were deposited into GenBank (DQ317539–DQ317561).

HBoV DNA was detected in 38 (11%) samples from 35 children, all <2 years of age. Infections occurred throughout the year, although more positive results were found in the autumn/winter season from April to August (63%) than during the rest of the year (37%). A diagnosis of pneumonia or lower respiratory tract infection was made for 30 (86%) children. Thirteen (37%) HBoV-positive children required admission to the inten-

sive care unit. Comorbid conditions were present in 22 children: cystic fibrosis (1), spinal muscular atrophy type 1 (4), Down syndrome (4), cardiac abnormalities (5), and HIV infection (8). Co-infection with a range of viral and bacterial organisms was a common feature in HBoV-positive children and was found in 14 (37%) samples. These organisms included cytomegalovirus (4), respiratory syncytial virus (2), adenovirus (1), HCoV NL63 (1), parainfluenza 3 (1), *Staphylococcus aureus* (1), *Streptococcus pneumoniae* (1), *Klebsiella pneumoniae* (1), and *Pneumocystis jirovecii* (2). However, in the remaining 24 (63%) samples, no other infectious agent was identified.

HBoV was detected in serial samples from 2 children during a 2-day (V04/2591 and V04/2613) and 7-day (V04/2599 and V04/2631) period. In both, sequences were identical and clustered within the proposed subgroup B. In a third child, HBoV sequences were detected in 2 samples taken 2 months apart; in these samples, the isolates were different (V04/1159 and V04/2062) (Figure).

Phylogenetic analysis of the 3' region of the VP1/2 capsid gene (Figure) showed that the Cape Town strains of HBoV were most closely aligned with the HBoV st2 prototype strain. The nucleotide sequence homology was 98% with 1 amino acid change, N474S. The HBoV st2 branch could be separated into 2 lineages, A and B, with a 3-nucleotide change at positions 4615 (A/G), 4756 (A/C), and 4888 (G/A) on the basis of the numbering of the HBoV st2 sequence.

These results suggest that HBoV infection occurs predominantly during the winter season and that children <2 years of age are most at risk. The study by Sloots et al. (8) also found HBoV infections mainly during the winter months (61%) in children <2 years. Although co-infections were found, the proportion (63%) of children in whom only HBoV was detected was

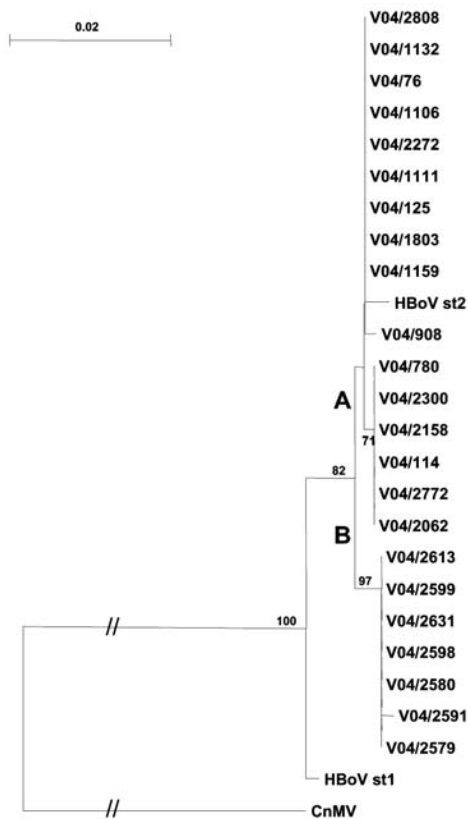


Figure. Phylogenetic analysis of a 980-bp region of the human bocavirus (HBoV) VP1/2 capsid gene from South African children with respiratory tract disease. The tree was constructed by using the neighbor-joining method with 1,000 bootstrap resamplings. All nucleotide sequences were submitted to GenBank (accession nos. DQ317539–DQ317561). CnMV, canine minute virus.

Shigella sonnei Outbreak among Homosexual Men, London

To the Editor: In the summer of 2004, genitourinary medicine clinics in London reported cases of *Shigella sonnei* with a novel phage type pattern (later designated PTQ). Outbreak case finding involved local laboratories and genitourinary medicine physicians in London, as well as the national reference laboratory. A case was considered confirmed if *S. sonnei* PTQ was isolated from January 2004 through April 2005, and the patient had not traveled outside the country the week before illness. Possible cases were defined as for confirmed cases but were so designated when patient had a history of foreign travel in the week before illness or when travel history was unknown. From October 2004, when we became aware of the outbreak, until December 2004, we conducted telephone interviews with newly identified case-patients. For cases that occurred before October 2004, and from January 2005 through April 2005, information was obtained from laboratory records only.

Strains were phage typed by using the scheme described by Hammerstrom, Kallings, and Sjoberg, according to a protocol supplied by R. Wollin (1,2). The scheme consists of 11 phages and is based on the typing of the rough phase II variant of *S. sonnei*. The scheme comprises defined phage types (PT) 1–100 and provisional PTs A–P. Cultures were grown overnight on MacConkey agar, and a rough colony was placed in nutrient broth and grown for 18 hours at 37°C. The broth culture was then used to flood a nutrient agar plate and, once dry, spotted with the 11 phages and incubated at 37°C for 5 hours. The patterns of lysis were recorded and compared with those indicated on the typing chart. All isolates were

substantial. These findings suggest that HBoV may play a role in respiratory tract infections in young children who require hospitalization.

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References

- van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001;7:719–24.
- Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet*. 2003;361:1319–25.
- van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, et al. Identification of a new coronavirus. *Nat Med*. 2004;10:368–73.
- Fouchier RAM, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, Simon JH, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci U S A*. 2004;101: 6212–6.
- Woo PCY, Lau SKP, Chu C-M, Chan K-H, Tsoi H-W, Huang Y. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol*. 2005;79:884–95.
- Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by screening of respiratory tract samples. *Proc Natl Acad Sci U S A*. 2005;102:12891–6.
- Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature*. 1989;339:237–8.
- Sloots TP, McErlean P, Speicher DJ, Arden KE, Nissen MD, Mackay IM. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol*. 2006;25:99–102.

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