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Human Bocavirus 2 in Children, South Korea

To the Editor: In 2009, Kapoor et al. and Arthur et al. published reports on the prevalence of the newly identified parvovirus, human bocavirus 2 (HBoV-2), in fecal samples (1,2). HBoV-1 had been discovered in 2005 (3), and reports indicate its possible role in respiratory diseases such as upper respiratory tract infections, lower respiratory tract infections (LRTIs), and in exacerbation of asthma (4); in these diseases, the virus co-infects with other respiratory viruses (5). Systemic infection with HBoV-1 and possible association of this virus with other diseases such as gastroenteritis, Kawasaki disease, and hepatitis have been reported (6–8). We looked for HBoV-2 in clinical samples from children with various diseases, including acute LRTIs, Kawasaki disease, Henoch-Schönlein purpura, and hepatitis.

During September 2008–January 2009, a total of 212 nasopharyngeal aspirates were collected from 212 children (median age 8 months, range 1–59 months) hospitalized with acute LRTIs at Sanggyepaik Hospital in Seoul, South Korea. Previously, during January 2002–June 2006, a total of 173 serum samples had been obtained from children (age range 1 month–15 years) with hepatitis (hepatitis B, 20 samples; hepatitis C, 11 samples; unknown hepatitis, 31 samples), Kawasaki disease (12 samples), and Henoch-Schönlein purpura (18 samples) and from healthy children (same age range, 81 samples) (9). The study was approved by the internal review board of Sanggyepaik Hospital.

DNA was extracted from serum samples, and RNA and DNA were extracted from nasopharyngeal aspirates by using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and a QIAamp DNA Blood Mini

Kit (QIAGEN GmbH), respectively. All nasopharyngeal aspirates were tested by PCR for common respiratory viruses such as respiratory syncytial virus, influenza viruses A and B, parainfluenza virus, and adenovirus, as described previously (10). PCRs to detect HBoV-1 were performed by using primers for the nonstructural (NS) 1 and nucleocapsid protein (NP) 1 genes, as described previously (10). Additional PCRs for rhinovirus, human metapneumovirus, human coronavirus (hCoV)-NL63, hCoV-OC43, hCoV-229E, hCoV HKU-1, WU polyomavirus, and KU polyomavirus were performed, as described, for HBoV-2–positive samples (10).

HBoV-2 was detected by performing first-round PCR with primers based on the NS gene, HBoV2-sf1, and HBoV2-sr1. Second-round PCR was performed by using primers HBoV2-sf2 and HBoVsr2, as described previously (1). The PCR products were sequenced by using an ABI 3730 XL autoanalyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were aligned by using BioEdit 7.0 (www.mbio.ncsu.edu/BioEdit/BioEdit.html) and presented in a topology tree, prepared by using MEGA 4.1 (www.megasoftware.net).

Of the 212 samples tested, the following viruses were detected: human respiratory syncytial virus (in 124 [58.4%] samples), human rhinovirus (24 [11.3%]), influenza virus A (18 [8.4%]), adenovirus (10 [4.7%]), and parainfluenza virus (8 [3.7%]). HBoV-1 was not detected in the study population. HBoV-2 DNA was found in 5 (2.3%) of the 212 samples collected; all positive samples had been obtained in October 2008. The age range of the children with HBoV-2–positive samples was 4–34 months (median 24 months), and all were male. The diagnoses were bronchiolitis for 3 children and bronchopneumonia for 2. The most frequently codetected virus was human respiratory syncytial virus, found in 4 (80%) of 5 samples. One

sample that was negative for respiratory syncytial virus and positive for HBoV-2 was negative for all other respiratory viruses.

Nucleotide sequences were determined for the NS-1 gene, and phylogenetic analyses, which included HBoV-3, a new lineage designated by Arthur et al. (2), showed that the NS-1 gene was relatively well conserved and that there were 2 major groups of the virus, the UK strain and the Pakistan strain. HBoV-2 strains isolated from South Korea belonged to the HBoV-PK2255 (FJ170279) cluster (Figure).

Recent studies have detected HBoV-1 in serum samples of children with Kawasaki disease and of an immunocompromised child with hepatitis (7,8). However, neither HBoV-1 nor HBoV-2 was detected in the 172 serum samples from 61 patients with hepatitis, 12 with Kawasaki disease, 18 with Henoch-Schönlein purpura, and 81 healthy children.

The absence of HBoV-1 in the samples examined was unexpected because HBoV-1 was detected in >10% of 558 respiratory samples collected from a demographically similar study population during the winter 2 years earlier (10). Future studies, with larger populations and over longer periods, are needed to delineate seasonal variations between HBoV-1 and HBoV-2.

We demonstrated HBoV-2 DNA in the respiratory tract secretions of children with acute LRTIs. In most positive samples, the virus was found in addition to other respiratory viruses. A limitation is that the study did not consider health control measures and other clinical disease such as gastroenteritis and was conducted for a short time. The role of HBoV-2 in LRTIs remains unclear; further studies are needed to clarify whether this virus is only shed from the respiratory tract or whether it replicates in the gastrointestinal tract.

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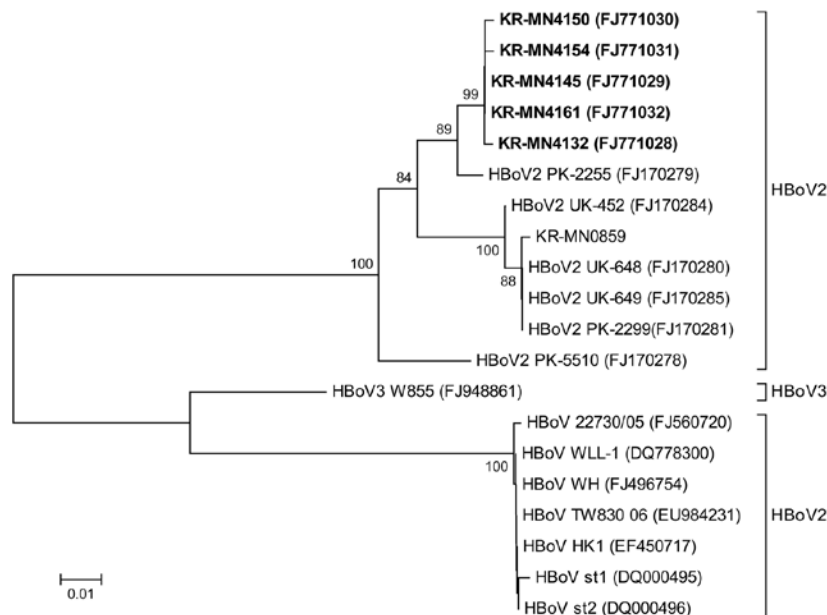


Figure. Phylogenetic analysis of nonstructural (NS) 1 gene sequences from human bocavirus 2 strains from Korea (KR), United Kingdom (UK), and Pakistan (PK), presented on a topology tree prepared by using MEGA 3.1 (www.megasoftware.net). Nucleotide alignment of a 417-bp portion of the NS-1 gene was prepared by using BioEdit 7.0 (www.mbio.ncsu.edu/BioEdit/BioEdit.html). The nucleotide distance matrix was generated by using the Kimura 2-parameter estimation. Nodal confidence values indicate the results of bootstrap resampling ($n = 1,000$). Five strains from South Korea (FJ771028–32) are in **boldface**. Scale bar indicates estimated number of substitutions per 10 bases.

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Nontuberculous Mycobacterium Infection and Tumor Necrosis Factor- α Antagonists

To the Editor: *Mycobacterium haemophilum* is an aerobic, slow-growing microorganism with optimal growth at 30°C to 32°C. It has a unique requirement for ferric iron-containing compounds (1), from which it acquired its name (i.e., *haemophilum*). Infections with *M. haemophilum* are rare, but cervicofacial lymphadenitis caused by *M. haemophilum* has been described in children (2). Besides cervicofacial lymphadenitis, extrapulmonary signs of *M. haemophilum* disease include subcutaneous noduli, arthritis, and osteomyelitis, which generally affect immunocompromised patients (3). Recently, 2 cases of cutaneous *M. haemophilum* infections after alemtuzumab treatment were reported (4). A small number of pulmonary *M. haemophilum* infections associated with AIDS or solid organ or bone marrow transplantation have been described (1). We report pulmonary *M. haemophilum* infection in a woman who had been immunosuppressed

by tumor necrosis factor- α antagonist (TNF- α) (adalimumab) treatment for rheumatoid arthritis.

A 72-year-old woman with a history of rheumatoid arthritis and obstructive sleep apnea syndrome had signs and symptoms of fatigue, mild fever episodes, and a nonproductive cough 9 months after treatment for rheumatoid arthritis had begun with methotrexate (MTX) and TNF- α . Physical examination was unremarkable except for a body temperature of 38.9°C. Laboratory testing showed an increased erythrocyte sedimentation rate (ESR) (77 mm/h), an increased C-reactive protein (CRP) level (60 mg/L), a normal leukocyte count (8,500 cells/ μ L), and relative monocytosis (12%). HIV serologic testing results were negative. Chest radiograph showed an infiltrate in the right upper lobe. Chest computed tomography confirmed this finding and showed lymphadenopathy in the right hilus and mediastinum.

Notably, the tuberculin skin test result was negative at screening before she began the TNF- α treatment, but was now positive (20 mm), suggesting mycobacterial infection. Auramine and Ziehl-Neelsen staining of sputum and bronchoalveolar liquids showed no acid-fast bacilli, and *M. tuberculosis* infection was not confirmed by PCR or culture. Eventually, a mediastinal lymph node biopsy

was taken by endoscopic ultrasound guidance. Granulomatous inflammation and acid-fast bacilli were seen by microscopy. Corresponding cultures yielded a strain identified as *M. haemophilum* at the Netherlands National Institute for Public Health and the Environment (RIVM) by using the Inno-LiPA Mycobacteria v2 reverse line blot assay (Innogenetics, Ghent, Belgium). Strain identity was confirmed by sequencing of the complete 16S rDNA gene, which was identical to that of *M. haemophilum* available in the GenBank sequence database (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov; accession no. X88923).

The RIVM performed drug susceptibility testing by using a modified agar dilution method (5). Middlebrook 7H10 media were enriched with 10% sheep blood hemolyzed by 1:1 dilution with water and subsequent freezing–thawing. Historic drug susceptibility data was reviewed (Table). Initially, adalimumab was discontinued, and our patient was treated with isoniazid, ethambutol, rifampin, and pyrazinimide because *M. tuberculosis* infection was suspected. After identification of *M. haemophilum*, our patient was treated with rifampin and azithromycin. A total treatment duration of 10 months resulted in complete resolution of the

Table. Antimicrobial drug susceptibility test results for *Mycobacterium haemophilum* isolate from rheumatoid arthritis patient and other *M. haemophilum* isolates*

Antimicrobial drug	Case report		RIVM historic data (n = 49)	
	Classification	MIC, mg/L	% Susceptible	% Resistant
Isoniazid	Resistant	10	0	100
Rifampin	Susceptible	0.2	4	96
Ethambutol	Resistant	20	0	100
Streptomycin	Susceptible	<1.0	35	65
Cycloserine	Susceptible	50	78	22
Prothionamide	Susceptible	<1.0	61	39
Amikacin	Resistant	10	29	71
Ciprofloxacin	Resistant	4.0	88	12
Clofazimine	Susceptible	<0.5	92	8
Clarithromycin	Susceptible	<2.0	94	6
Rifabutin	Susceptible	<0.2	96	4

*All isolates submitted to the Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment (RIVM), the Netherlands, January 2000–January 2007. Before January 2004, strains tested were identified by 16S rDNA gene sequencing; after January 2004, strains were identified by the Inno-LiPA assay (Innogenetics, Ghent, Belgium).