Human Bocavirus as the Cause of a Life-Threatening Infection[▽]

Tina Uršič,^{1*} Andrej Steyer,¹ Silvester Kopriva,² Gorazd Kalan,² Uroš Krivec,³ and Miroslav Petrovec¹

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia¹;

Department of Pediatric Surgery and Intensive Care, University Medical Centre Ljubljana, Bohoričeva 20, 1000 Ljubljana,

Slovenia²; and Department of Pulmonology, University Children's Hospital, University Medical Centre Ljubljana,
Bohoričeva 20, 1000 Ljubljana, Slovenia³

Received 23 November 2010/Returned for modification 20 December 2010/Accepted 28 December 2010

Human bocavirus is a recently described respiratory pathogen. A case of a life-threatening human bocavirus infection of a previously healthy pediatric patient is described. An initial clinical presentation of acute bronchiolitis developed into an extremely severe course of disease characterized by pneumothorax, pneumomediastinum, and acute respiratory failure with pronounced air-leak syndrome.

CASE REPORT

In January 2010, a 20-month-old female patient was hospitalized due to acute respiratory infection with signs of respiratory distress. She was diagnosed as having acute bronchiolitis. The patient was prematurely born as a twin sister after 27 weeks of gestation, with a birth weight of 1,430 g, and her previous medical history was unremarkable.

She required additional oxygen on admission and was treated with a short-acting beta-2 agonist. Her medical condition failed to improve, so chest radiography was performed, and hyperinflation with an infiltrate in the left lower lung field was observed. Parenteral steroid and amoxicillin-clavulanic acid were commenced. In the next 24 h, her condition further deteriorated, despite treatment. Neck emphysema was observed, prior to imminent respiratory failure, and the girl was intubated and transferred to the intensive care unit (ICU). The child was sedated, relaxed, and mechanically ventilated (100% O₂). Laboratory tests revealed a leukocyte count of 22.0×10^9 /liter, a C-reactive protein level of 14 mg/liter, and a hemoglobin concentration of 9.4 g/dl. She received one dose of concentrated erythrocytes. Moderate hypotension was present, and circulatory stability was supported by intermittent intravenous saline boluses with continuous infusion of dopamine. Repeated chest radiography revealed pneumothorax of the left lung, which was immediately drained. Pneumothorax of the right lung occurred and was also drained. Bronchoscopy performed through an endotracheal tube showed edema and inflammation of the lower respiratory tract with a large amount of mucus. Several mechanical ventilation modes, including high-frequency oscillations, were tried over the next few hours without improvement in clinical or laboratory respiratory parameters. Carbon dioxide partial pressure (PaCO₂) increased and, 18 h after ICU admission, reached a maximum of 19.6 kPa, with excessive respiratory acidosis (pH 6.92). Assisted controlled ventilation with positive inspiratory pressure was effective in lowering the PaCO₂. Subcutaneous emphysema

occurred on her head, cheeks, neck, and chest, because of a pronounced air leak. Pneumoperitoneum was also observed on the radiograph. Her clinical condition substantially stabilized after surgical incision in the neck, and insertion of a new thoracic drain on the left side partially relieved pneumomediastinum. Air leakage decreased steadily, whereas subcutaneous emphysema persisted. Chest drains were removed on the seventh day in the ICU. She was extubated on the ninth day, and on the eleventh day she left the ICU. The girl then breathed independently, recovered from a mild withdrawal syndrome, and was neurologically intact on discharge from the hospital 4 days later. The child made a complete recovery.

A nasopharyngeal swab (at admission), tracheal aspirate (after intubation), and blood sample (at admission) were analyzed to assess the etiology of the respiratory disease. All samples were tested by real-time PCR for the presence of respiratory syncytial virus (RSV), human rhinoviruses (hRV), human metapneumovirus (hMPV), adenoviruses (Ad), influenza viruses A and B (INF A/B), human coronaviruses (hCoV; hCoV-OC43, hCoV-229E, hCoV-NL63, HKU11), parainfluenza viruses 1 to 3 (PIV 1 to 3), enteroviruses, and human bocavirus (HBoV). At admission to the ICU, blood was collected for hemoculture. Bronchoalveolar (BAL) fluid, tracheal aspirate, and thoracic drainage fluid were also collected, and appropriate cultures were set up for the detection of bacterial and fungal pathogens.

Automatic nucleic acid extraction was carried out using the total nucleic acid isolation kit on a MagNA pure compact instrument (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. Nucleic acid (5 µl; automatic extraction from 190 µl of sample) was used in an internally controlled real-time quantitative PCR (qPCR) (15). Real-time qPCR was performed with the platinum quantitative PCR SuperMix-UDG kit (Invitrogen, Carlsbad, CA), using 88 bp of the HBoV NS1 gene as the target (15). HBoV DNA was the only pathogen detected. The highest viral loads were detected in tracheal aspirate (2.1×10^{10} copies/ml), and then in the nasopharyngeal swab (8.6×10^9 copies/ml) and the plasma sample (1.8×10^6 copies/ml). HBoV viral particles were visualized using electron microscopy (EM) in a nasopharyngeal swab sample and by immunoelectron microscopy (IEM) using an autologous plasma sample from the patient (Fig. 1).

* Corresponding author. Mailing address: Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia. Phone: 386 1 543 74 58. Fax: 386 1 543 74 01. E-mail: tina.ursic@mf.uni-lj.si.

[▽] Published ahead of print on 12 January 2011.

FIG. 1. EM image of HBoV in a direct preparation from a nasopharyngeal swab suspension (A) and HBoV in the patient's nasopharyngeal swab suspension mixed with the patient's plasma (ratio 1:1) (B), marked with protein A gold (15 nm). Both EM preparations were negatively stained with 2% phosphotungstic acid (PTA; pH 4.5) and examined with a transmission electron microscope (JEM 1200 EX II; Jeol, Japan) at a magnification of $\times 200,000$.

Tracheal aspirates and blood were taken from the patient every two days. The viral load decreased slowly, and before she left the hospital, the HBoV load was 9.9×10^4 copies/ml in a nasopharyngeal swab and 3.6×10^4 copies/ml in the plasma. Hemoculture, bronchoalveolar fluid, tracheal aspirate, BAL fluid, and thoracic drainage fluid cultures remained negative for pathogenic bacteria and fungi during hospitalization.

A 1,136-bp-long region of the VP1/VP2 gene of the HBoV genome was amplified by using in-house-developed PCR and Promega PCR MasterMix (Promega, Madison, WI). The PCR products were purified and subsequently sequenced by using BigDye terminator chemistry on an ABI Prism 310 genetic analyzer (Applied Biosystems, CA). Four forward and four reverse sequencing primers were newly synthesized and employed to achieve the appropriate sequence coverage. The sequence obtained showed 99% identity with an HBoV ST2 isolate (accession no. DQ000496) and was deposited in GenBank (accession no. GU989638).

A nasopharyngeal swab and blood sample were taken on a follow-up visit 1 month after hospital discharge. Both samples were tested for HBoV, RSV, hRV, hMPV, Ad, INF A/B, hCoV (hCoV-OC43, hCoV-229E, hCoV-NL63, HKU11), PIV 1 to 3, and enteroviruses. The nasopharyngeal swab was negative for all viruses, including HBoV. In contrast, the plasma sample was positive for HBoV, with a viral load of 2.1×10^3 copies/ml (Fig. 2).

The thorough virological follow-up was designed as a study after HBoV was found as a sole pathogen in a severe medical condition. The study was reviewed and approved by the National Medical Ethics Committee, and written informed consent was obtained from the patient's parents.

HBoV was discovered in 2005 in Swedish children with acute respiratory infections, and the virus was subsequently detected in children with respiratory infections all over the world (2, 3, 4, 9, 13, 16, 20, 22). Many studies have been published investigating the role of the virus in respiratory tract illness (6). Some raise the possibility that HBoV is associated with severe respiratory disease (7, 8). HBoV is frequently found in hospi-

talized children under 2 years of age with bronchiolitis, asthma exacerbation, and wheezing (1, 11, 12). Most patients also presented with symptoms of cough, fever, rhinorrhea, dyspnea, and hypoxia. Some authors suggest that clinically relevant disease is associated with the detection of high HBoV DNA viral load (1, 7). True pathogenicity of HBoV as a sole pathogen in respiratory tract infections has been largely obscured by the fact that HBoV is frequently found simultaneously with other respiratory viruses (16). While infections caused by HBoV are frequent, severe cases are rare in immunocompetent (17, 19, 21) and immunocompromised (10, 14, 18) patients. Until now, visualization of HBoV virions by EM was described only twice, in patients with high viral loads (5, 7). To the best of our knowledge, there is no documented case of life-threatening HBoV infection related to acute respiratory failure and air-leak syndrome in immunocompetent patients.

The twin brother of the case patient reported here was hospitalized 1 week previously, because of acute bronchiolitis, and required additional oxygen for 2 days. His hospital stay was uneventful. A nasopharyngeal swab taken on admission tested negative for RSV, hMPV, Ad, INF A/B, and PIV 1 to 3 with a direct immunofluorescence test. Retrospective PCR analyses of the same sample showed that he was positive for HBoV

FIG. 2. HBoV DNA viral loads in respiratory and plasma samples of patient during hospitalization and on the first follow-up visit are shown.

DNA by real-time qPCR and negative for all other viruses tested. The viral load in the nasopharyngeal sample was lower (3.7×10^7 copies/ml) than the viral load in the nasopharyngeal sample of our case patient. The case patient was hospitalized with similar symptoms 4 days after her twin brother was released from the hospital, but she took a much more severe clinical course. It is most likely that our case patient was infected by her twin brother, because neither child visits a day care center.

In this report, we present a case of a 20-month-old female patient with HBoV infection associated with acute bronchiolitis, complicated by pneumothorax, pneumomediastinum, interstitial emphysema, and acute respiratory failure. No other virus or bacterium was detected in the respiratory and blood samples of our case, which indicates that HBoV is likely to be a true respiratory pathogen that could cause severe and even life-threatening disease. We confirmed the abundant presence of the virus in respiratory tract specimens, by visualizing the viral particles with EM. We also indirectly confirmed an immune response against the virus in patient plasma by aggregation of viral particles with IEM, which further supports a causal role for HBoV.

However, the prolonged presence of viral DNA in the blood of the patient 50 days after contracting the disease warrants further studies to evaluate the appropriate methods for laboratory diagnosis of acute HBoV infections.

Nucleotide sequence accession number. The new sequence described here was deposited in GenBank under accession no. GU989638.

REFERENCES

1. Allander, T., et al. 2007. Human bocavirus and acute wheezing in children. *Clin. Infect. Dis.* **44**:904–910.
2. Allander, T., et al. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc. Natl. Acad. Sci. U. S. A.* **102**:12891–12896.
3. Bastien, N., K. Brandt, K. Dust, D. Ward, and Y. Li. 2006. Human bocavirus infection, Canada. *Emerg. Infect. Dis.* **12**:848–850.
4. Bonzel, L., et al. 2008. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr. Infect. Dis. J.* **27**:589–594.
5. Brieu, N., B. Gay, M. Segondy, and V. Foulongne. 2007. Electron microscopy observation of human bocavirus (HBoV) in nasopharyngeal samples from HBoV-infected children. *J. Clin. Microbiol.* **45**:3419–3420.
6. Chow, B. D., and F. P. Esper. 2009. The human bocaviruses: a review and discussion of their role in infection. *Clin. Lab. Med.* **29**:695–713.
7. Christensen, A., S. A. Nordbo, S. Krokstad, A. G. Rognlien, and H. Dollner. 2010. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J. Clin. Virol.* **49**:158–162.
8. Don, M., et al. 2010. Serologically verified human bocavirus pneumonia in children. *Pediatr. Pulmonol.* **45**:120–126.
9. Fry, A. M., et al. 2007. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J. Infect. Dis.* **195**:1038–1045.
10. Garbino, J., et al. 2008. Respiratory viruses in HIV-infected patients with suspected respiratory opportunistic infection. *AIDS* **22**:701–705.
11. Gendrel, D., et al. 2007. Human bocavirus in children with acute asthma. *Clin. Infect. Dis.* **45**:404–405.
12. Jacques, J., et al. 2008. Human bocavirus quantitative DNA detection in French children hospitalized for acute bronchiolitis. *J. Clin. Virol.* **43**:142–147.
13. Kaplan, N. M., et al. 2006. Human bocavirus infection among children, Jordan. *Emerg. Infect. Dis.* **12**:1418–1420.
14. Koskenvuo, M., et al. 2008. Human bocavirus in children with acute lymphoblastic leukemia. *Eur. J. Pediatr.* **167**:1011–1015.
15. Lu, X., et al. 2006. Real-time PCR assays for detection of bocavirus in human specimens. *J. Clin. Microbiol.* **44**:3231–3235.
16. Manning, A., et al. 2006. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J. Infect. Dis.* **194**:1283–1290.
17. Oikawa, J., et al. 2009. Human bocavirus DNA detected in a boy with plastic bronchitis. *Pediatr. Infect. Dis. J.* **28**:1035–1036.
18. Schenk, T., et al. 2007. Disseminated bocavirus infection after stem cell transplant. *Emerg. Infect. Dis.* **13**:1425–1427.
19. Simon, A., et al. 2007. Detection of bocavirus DNA in nasopharyngeal aspirates of a child with bronchiolitis. *J. Infect.* **54**:e125–e127.
20. Smuts, H., and D. Hardie. 2006. Human bocavirus in hospitalized children, South Africa. *Emerg. Infect. Dis.* **12**:1457–1458.
21. Terrosi, C., M. Fabbiani, C. Cellesi, and M. G. Cusi. 2007. Human bocavirus detection in an atopic child affected by pneumonia associated with wheezing. *J. Clin. Virol.* **40**:43–45.
22. Weissbrich, B., et al. 2006. Frequent detection of bocavirus DNA in German children with respiratory tract infections. *BMC Infect. Dis.* **6**:109.