

# Complete Genomes of Three Human Bocavirus Strains from Children with Gastroenteritis and Respiratory Tract Illnesses in Jiangsu, China

Xiaochun Wang,<sup>a</sup> Xiaodan Zhang,<sup>a</sup> Hua Tian,<sup>b</sup> Feng Ling,<sup>a</sup> Min Yu,<sup>a</sup> Guangming Sun,<sup>c</sup> Shixing Yang,<sup>a</sup> Quan Shen,<sup>a</sup> Yan Wang,<sup>a</sup> Shihe Shao,<sup>a</sup> and Wen Zhang<sup>a</sup>

Department of Microbiology, School of Medical Science and Laboratory Medicine, Jiangsu University, Zhenjiang, People's Republic of China<sup>a</sup>; Jiangsu Provincial Center for Disease Prevention and Control, Nanjing, People's Republic of China<sup>b</sup>; and the Fourth Affiliated Hospital of Jiangsu University, Zhenjiang, People's Republic of China<sup>c</sup>

**Human bocavirus (HBoV) is a newly discovered parvovirus associated with acute respiratory tract illness (ARTI) and gastrointestinal illness. No previous reports indicated the presence of HBoV infection in Jiangsu Province, China. Here we report three complete genomic sequences of HBoV strains from children with gastroenteritis and respiratory tract illnesses in Jiangsu, China. Phylogenetic analysis indicated that the three HBoV strains in the present study belong to the HBoV1 lineage, where jz-42 clustered separately, forming a single branch, while zj-68 and zj-92 existed in two separate branches, clustering with several other Chinese HBoV1 strains.**

Human bocavirus (HBoV) is a single-stranded DNA virus with a genome size of approximately 5.3 kb, which has three open reading frames (ORF) encoding two nonstructural proteins, NS1 and NP1, and the two structural proteins VP1 and VP2 (1). A number of epidemiological studies indicate that HBoV has spread worldwide and has been discovered in respiratory samples from children with respiratory tract illnesses and in stool samples from children with gastroenteritis (4, 8, 9). HBoV has four different genotypes, HBoV 1 to 4, according to phylogenetic analysis, among which HBoV1 was first described in children with respiratory infections (1) and HBoV2, HBoV3, and HBoV4 were discovered subsequently, sharing a mean similarity of 80% with HBoV1 (2, 7). Recently, new bocavirus species were isolated from gorilla and swine and were most closely related to HBoV (3, 6). In this study, two cases of gastroenteritis and one case of respiratory tract illness in three children <6 years old in Jiangsu, China, were determined to be positive for HBoV.

In order to further characterize the HBoV strains in the three cases of gastroenteritis or respiratory tract illnesses, we determined the complete genomes of the three HBoV strains from the three positive samples, using the method described in a previous report (5). The viral genomic DNA was extracted from the suspension of stool samples or nasopharyngeal secretion using the MiniBEST viral RNA/DNA extraction kit (TaKaRa, Japan) in accordance with the manufacturer's protocol. PCR-amplified DNA fragments of the expected sizes were sequenced in a DNA analyzer (Applied Biosystems 3730 DNA Analyzer; Invitrogen) and assembled using CodonCode Aligner software.

The complete genome of the HBoV strain from the case of respiratory tract illness is named jz-42 and comprises 5,301 nucleotides (nt). The NS1 gene begins at nt 254 and ends at nt 2173. The NP1 gene begins at nt 2411 and ends at nt 3070. The VP1 gene begins at nt 3057 and ends at nt 5072. VP2 begins at nt 3444 and ends at nt 5072. The two HBoV strains from the two cases of gastrointestinal illness are named zj-68 and zj-92. The complete genome of zj-68 has the same genomic length and structure as jz-42, though the two genomes have a sequence divergence of 23 nt. The genome of zj-92 comprises 5,300 nt. The NS1 gene begins at nt 253 and ends at nt 2172. The NP1 gene begins at nt 2411 and ends at nt 3069. The VP1 gene begins at nt 3056 and ends at nt 5071.

VP2 begins at nt 3443 and ends at nt 5071. Phylogenetic analysis over the complete genome sequences of the three HBoV strains in the present study and all HBoV strains with complete genomes available in GenBank indicated that the three HBoV strains in the current study belong to HBoV1. The phylogenetic tree shows that the three HBoV strains in this study lie in the same subgroup in HBoV1 lineage, where jz-42 clustered separately, forming a single branch, zj-68 clustered closely with two other Chinese HBoV1 strains, DQ988934 and GQ455988, forming a separate branch, and zj-92 clustered with other five HBoV1 strains in another subgroup.

**Nucleotide sequence accession number.** The virus genome sequence was deposited in GenBank under accession no. JX887480 to JX887482.

## ACKNOWLEDGMENTS

This work was partly supported by the Ph.D. Programs foundation of the Ministry of Education of China no. 20113227120008, the Natural Science Foundation of Jiangsu Province no. BK2012289, the Special Fund for Preventive Medicine of Jiangsu Province no. Y2012067, and the Professional Research Foundation for Advanced Talents of Jiangsu University under grant no.12JDGXX.

## REFERENCES

1. 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.
2. Arthur JL, Higgins GD, Davidson GP, Givney RC, Ratcliff RM. 2009. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathog.* 5:e1000391. doi:10.1371/journal.ppat.1000391.
3. Cheng WX, et al. 2010. Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PLoS One.* 5:e13583. doi: 10.1371/journal.pone.0013583.

Received 24 September 2012 Accepted 24 September 2012

Address correspondence to Wen Zhang, z2016wen@yahoo.com.

Xiaochun Wang and Xiaodan Zhang contributed equally to this work.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.02618-12

4. **Cashman O, O'Shea H.** 2012. Detection of human bocaviruses 1, 2 and 3 in Irish children presenting with gastroenteritis. *Arch. Virol.* **157**: 1767–1773.
5. **Chieochansin T, et al.** 2007. Complete coding sequences and phylogenetic analysis of Human Bocavirus (HBoV). *Virus Res.* **129**:54–57.
6. **Kapoor A, et al.** 2010. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J. Infect. Dis.* **201**:1633–1643.
7. **Kapoor A, et al.** 2009. A newly identified bocavirus species in human stool. *J. Infect. Dis.* **199**:196–200.
8. **Koseki N, et al.** 2012. Detection of human bocaviruses 1 to 4 from nasopharyngeal swab samples collected from patients with respiratory tract infections. *J. Clin. Microbiol.* **50**:2118–2121.
9. **Xu L, et al.** 2012. Surveillance and genome analysis of human bocavirus in patients with respiratory infection in Guangzhou, China. *PLoS One* **7**:e44876. doi:10.1371/journal.pone.0044876.