HOKKAIDO UNIVERSITY

| | |
|---|---|
| Title | Detection of Human Bocaviruses 1 to 4 from Nasopharyngeal Swab Samples Collected from Patients with Respiratory Tract Infections |
| Author(s) | Koseki, Naoko; Teramoto, Shinobu; Kaiho, Miki et al. |
| Citation | Journal of Clinical Microbiology, 50(6), 2118-2121<br>https://doi.org/10.1128/JCM.00098-12 |
| Issue Date | 2012-06 |
| Doc URL | https://hdl.handle.net/2115/50783 |
| Rights | © 2012 American Society for Microbiology |
| Type | journal article |
| File Information | JCM50-6_2118-2121.pdf |




Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

# Detection of human bocavirus 1-4 from nasopharyngeal swab samples collected from patients with respiratory tract infections

Naoko Koseki,[1†] Shinobu Teramoto,[1†] Miki Kaiho,[1] Rika Endo (Gomi),[2] Mikio Yoshioka,[3] Yutaka Takahashi,[3] Tsuguyo Nakayama,[4] Hiroyuki Sawada,[4] Mutsuko Konno,[5] Hiroshi Ushijima,[6] Hideaki Kikuta,[7] Tadashi Ariga,[1] and Nobuhisa Ishiguro[1*]

Department of Pediatrics[1], Department of Microbiology[2], Hokkaido University Graduate School of Medicine, Sapporo, Japan; Department of Pediatrics, KKR Sapporo Medical Center, Sapporo, Japan[3]; Department of Pediatrics, Hokkaido Social Insurance Hospital, Sapporo, Japan[4]; Department of Pediatrics, Sapporo Kosei General Hospital, Sapporo, Japan[5]; Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan[6]; Pediatric Clinic, Touei Hospital, Sapporo, Japan[7]

* Corresponding author. Mailing Address: Department of Pediatrics, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku,

19 Sapporo 060-8638, Japan. Phone: 81-11-706-5954. Fax: 81-11-706-7898.

20 E-mail: nishigur@med.hokudai.ac.jp

21 †N.K. and S.T. contributed equally to the research described in this paper.

22

23 ABSTRACT

24 Human bocavirus (HBoV) 1, 2, 3 and 4 were detected in 132 (15.5%), 5 (0.6%),

25 3 (0.4%) and 5 (0.6%) of 850 nasopharyngeal swab samples collected from

26 children with respiratory tract infections, respectively. Out of the 145

27 HBoV1-4-positive samples, 62 (42.8%) were co-detected with other

28 respiratory viruses. (47 words)

29

## TEXT

Human bocavirus (HBoV, lately denoted HBoV1), belonging to the family *Parvoviridae*, subfamily *Parvovirinae* and genus *Bocavirus*, was first identified by molecular screening of pooled human respiratory tract samples in 2005 (4). HBoV1 has been detected world-widely in 1.6% to 19% of patients with respiratory tract infections (4, 6, 8, 10, 23, 26, 27). Recently, serological and quantitative PCR analyses have provided compelling evidence for HBoV1 being an etiologic agent for respiratory tract infections (RTIs) (3, 8, 13, 21, 24, 30, 34). In 2009–2010, three additional species of human bocaviruses, HBoV2, HBoV3 and HBoV4, were discovered from fecal samples (5, 19, 20). In contrast to HBoV1, HBoV2-4 were predominantly detected in human stool samples and were therefore thought to be involved in enteropathogenesis (5, 18, 20). Only HBoV2 of HBoV2-4 has been detected in nasopharyngeal samples from children so far (16, 31). In this study, we examined the presence of HBoV2-4 as well as HBoV1 in nasopharyngeal swab samples (NPSs) from children with RTIs.

A total of 850 NPSs were collected from 757 children (436 males and 321

48 females) aged 0 - 136 months (average age, 17.9 months) with RTIs at four
49 hospitals (KKR Sapporo Medical Center, Hokkaido Social Insurance Hospital,
50 Sapporo Kosei General Hospital and Nemuro City Hospital) in Hokkaido,
51 Japan during the period from June 2005 to August 2011 after obtaining
52 informed consent from the children's parents. DNA was extracted from 200
53 µl of NPSs by using the method according to Chomczynski's protocol (11).
54 Elution volume of the extractions was 90 µl. Pan-bocavirus nested PCR
55 targeting the VP1 region was used for detection of HBoVs as described by
56 Kapoor et al. (19). Both sense and antisense strands of the second PCR
57 products were sequenced directly by using a BigDye terminator cycle
58 sequencing ready reaction kit (Perkin-Elmer Applied Biosystems, Tokyo,
59 Japan) with an ABI Prism 310 genetic analyzer (Perkin-Elmer Applied
60 Biosystems). The nucleotide sequences of the second PCR products were
61 assembled by using CLUSTAL W software. Phylogenetic trees were
62 generated by the neighbor-joining method with the MEGA program (22).
63 Using DNA extracted from fecal specimens collected in Vietnam as templates
64 of the pan-bocavirus nested PCR, the first-round PCR products of HBoV1
65 and HBoV2 were cloned into the vector pT7Blue (Novagene, Inc., Madison,

66 WI, U.S.A.). The sequences were deposited at GenBank (accession numbers
67 JQ734543 and JQ734544). The corresponding regions of HBoV3
68 (EU918736) and HBoV4 (FJ973561) were synthesized and cloned into
69 pUC57 by Genscript (Piscataway, NJ, U.S.A.). These four vectors were used
70 as positive controls for the nested PCR. DNA solutions containing 5, 25,
71 125, 625 and 3125 copies of these vectors per μl were prepared to determine
72 the sensitivities of the nested PCR. All of the specimens were also assayed
73 for the presence of thirteen other respiratory viruses: human respiratory
74 syncytial virus (hRSV), human metapneumovirus (hMPV), human
75 rhinovirus (HRV), parainfluenza viruses (PIV 1-3), influenza A and B viruses
76 (FluA and FluB), human enterovirus (HEV), human coronaviruses (HCoV),
77 adenoviruses (AdV), KI polyomavirus (KIPyV) and WU polyomavirus
78 (WUPyV). The PCR and RT-PCR protocols used for detecting these thirteen
79 viruses were the same as those previously described (1, 2, 12, 15, 17, 25, 28,
80 29, 32).
81
82 Of the 850 NPSs tested, 145 (17.1%) had confirmed positive results by
83 sequencing. By determining the phylogenetic relationships among various

84 HBoV strains, the final prevalences of HBoV1, 2, 3 and 4 in the NPSs were
85 132/850 (15.5%), 5/850 (0.6%), 3/850 (0.4%) and 5/850 (0.6%), respectively.
86 The five HBoV2 strains belong to the HBoV2A clade described by Kapoor et
87 al. (19). Two of the HBoV1-positive samples were collected from two
88 children, from whom HBoV1 had been detected two months before. The
89 other HBoV1-4-positive samples were collected from different children. The
90 amplicon sizes were 525 bp (nucleotides 3353 to 3877 in accession no.
91 NC_007455.1) for HBoV1 and HBoV2 and 528 bp (nucleotides 3320 to 3847
92 in accession no. NC_012564.1) for HBoV3 and HBoV4. The sequences were
93 deposited at GenBank (accession numbers JQ346532 to JQ346676).
94 According to calculations by amplification of serial limiting dilutions of
95 cloned HBoV1-4 DNA, the copy numbers of 67 (50.8%) of the 132
96 HBoV1-positive NPS samples were estimated to be more than $5.5 \times 10^4$
97 copies / ml, and those of the remaining 65 HBoV1-positive NPS samples were
98 estimated to be between $1.2 \times 10^4$ and $5.5 \times 10^4$ copies / ml. The copy
99 numbers of the HBoV2, 3 and 4-positive NPS samples were estimated to be
100 between $2.3 \times 10^3$ and $1.2 \times 10^4$ copies / ml. At least 10-fold differences in
101 sensitivities of the pan-bocavirus PCR for HBoV1-4 have already been

102 reported (19). HBoV1 was detected solely in 76 (57.6%) of the 132
103 HBoV1-positive samples, and one or more other viruses were co-detected in
104 56 (42.6%) of the 132 HBoV1-positive samples. hMPV (20 samples), hRSV
105 (15 samples) and HRV (7 samples) were the most frequently detected viruses.
106 HBoV2 was detected solely in 3 (60.0%) of the 5 HBoV2-positive samples,
107 and another virus was co-detected in 2 (40.0%) of the 5 HBoV2-positive
108 samples. HBoV3 was co-detected in 3 (100.0%) of all of the 3 hRSV-positive
109 samples. HBoV4 was detected solely in 4 (80.0%) of the 5 HBoV4-positive
110 samples, and another virus was co-detected in 1 (20.0%) of the 5
111 HBoV4-positive samples (Table 1).
112
113 The ages of patients with HBoV1-positive samples ranged from 1 month to 6
114 years and 9 months (average age, 18.2 months), and the ages of patients with
115 HBoV2-4-positive samples ranged from 1 month to 3 years and 6 months
116 (average age, 17.1 months). HBoV1 genomes were detected in every month
117 with peaks in May (18 cases) and June (17 cases). All of the 13
118 HBoV2-4-positive samples were collected in fall or winter except for one in
119 April (Supplemental table). Fifty (37.9%) of the 132 HBoV1-positive

patients were admitted to hospital for 3 to 11 days, but none of the 13 HBoV2-4-positive patients were admitted to hospital. The clinical diagnoses of the HBoV-positive patients are summarized in Table 2. Thirty-four HBoV single-positive patients (28 with HBoV1, 2 with HBoV2 and 4 with HBoV4) were diagnosed as having bronchitis. Twenty HBoV1 single-positive patients were diagnosed as having wheezy bronchitis. Twenty-one HBoV single-positive patients (20 of HBoV1 and 1 of HBoV2) were diagnosed as having pneumonia. Although HBoV2-4 were thought to be involved in enteropathogenesis, a gastrointestinal symptom (diarrhea) was observed in only one of the four HBoV4-positive patients and was not observed in any of the HBoV2-3-positive patients. It is known that low-load HBoV1 DNA is long-lasting in the mucosa (7), and quantification of HBoV DNA by real-time PCR is therefore necessary to estimate the time of infection to the present symptoms. In this study, detection of HBoV DNA was done by semi-quantitative nested PCR instead of real-time PCR. Scince “semi-quantification” according to the first and second PCR-round sensitivities is limited, the seasonal distributions of HBoVs and the associations of clinical diagnoses with the presence of HBoVs described

138 above might not be accurate.

139

140 In our study, we identified HBoV2, HBoV3 and HBoV4, as well as HBoV1, in
141 NPSs collected from patients with RTIs. The detection rate of HBoV1
142 (15.5%) in NPSs was within the range of previously reported rates (1.6-19%)
143 (4, 6, 8, 10, 23, 26, 27). In contrast, the detection rates of HBoV2 (0.6%),
144 HBoV3 (0.4%) and HBoV4 (0.6%) in NPSs were much lower than that of
145 HBoV1 (15.5%). The hospital admission rates of HBoV2-4-positive patients
146 (0%) were lower than that of HBoV1-positive patients (37.9%). These facts
147 suggest that the roles of HBoV2-4 in RTIs might be limited in comparison
148 with the role of HBoV1. Differences in the duration of persistence of HBoVs
149 in the nasopharynx might also have caused the lower prevalences of
150 HBoV2-4 than that of HBoV1. Previously reported detection rates of
151 HBoV2 in NPSs were 4.3% in China (31) and 2.3% in South Korea (16),
152 which were higher than the rate in our study. HBoV2 was not detected
153 among 6,524 respiratory samples in the United Kingdom and Thailand (9).
154 The majority of respiratory samples in that study (6,138 of 6,524) were not
155 obtained from individual persons but from pooled stock, which might have

reduced the sensitivity of their PCR assay. These different results can be explained partially by the difference in PCR primers or by the regional difference. On the other hand, HBoV2 (3 samples) and HBoV4 (4 samples) were detected without co-detection of other respiratory viruses in a few NPSs, suggesting that HBoV2 and 4 might play some roles in RTIs in children. To clarify the clinical impact of HBoV2-4 in RTIs, quantitative PCR study in various age groups and various clinical groups including healthy controls is needed. Adenoviruses 40 and 41 cause gastroenteritis, but respiratory symptoms are not frequent (21% of cases) (33). The unique physicochemical properties of adenovirus 41 partially explain its enteric tropism (14). Structural studies of HBoV1-4 might be also helpful to clarify the roles of HBoVs in RTI and gastroenteritis.

## ACKNOWLEDGEMENTS

This research was supported in part by a Grant-in-Aid for Scientific Research (C), 2010 (22591174), from the Ministry of Education, Science, Sports and Culture of Japan and by Global COE Program “Establishment of International Collaboration Centers for Zoonosis Control”, MEXT, Japan.

174 All of the necessary ethics approval for this study was obtained from the
175 Institutional Review Board of Hokkaido University Hospital for Clinical
176 Research. We thank Kunio Ozutusmi of Nemuro City Hospital for providing
177 nasopharyngeal swab samples. We thank Stewart Chisholm for
178 proofreading the manuscript.

179

180 **REFERENCES**

181 1. **Aguilar, J. C., M. P. Perez-Brena, M. L. Garcia, N. Cruz, D. D. Erdman,**
182 **and J. E. Echevarria.** 2000. Detection and identification of human
183 parainfluenza viruses 1, 2, 3, and 4 in clinical samples of pediatric
184 patients by multiplex reverse transcription-PCR. J Clin Microbiol
185 **38:**1191-1195.
186 2. **Allander, T., K. Andreasson, S. Gupta, A. Bjerkner, G. Bogdanovic, M.**
187 **A. Persson, T. Dalianis, T. Ramqvist, and B. Andersson.** 2007.
188 Identification of a third human polyomavirus. J Virol **81:**4130-4136.
189 3. **Allander, T., T. Jartti, S. Gupta, H. G. Niesters, P. Lehtinen, R.**
190 **Osterback, T. Vuorinen, M. Waris, A. Bjerkner, A. Tiveljung-Lindell, B.**
191 **G. van den Hoogen, T. Hyypia, and O. Ruuskanen.** 2007. Human

bocavirus and acute wheezing in children. Clin Infect Dis **44:**904-910.

4. **Allander, T., M. T. Tammi, M. Eriksson, A. Bjerkner, A. Tiveljung-Lindell, and B. Andersson.** 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A **102:**12891-12896.

5. **Arthur, J. L., G. D. Higgins, G. P. Davidson, R. C. Givney, and R. M. Ratcliff.** 2009. A novel bocavirus associated with acute gastroenteritis in Australian children. PLoS Pathog **5:**e1000391.

6. **Bastien, N., N. Chui, J. L. Robinson, B. E. Lee, K. Dust, L. Hart, and Y. Li.** 2007. Detection of human bocavirus in Canadian children in a 1-year study. J Clin Microbiol **45:**610-613.

7. **Blessing, K., F. Neske, U. Herre, H. W. Kreth, and B. Weissbrich.** 2009. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. Pediatr Infect Dis J **28:**1018-1019.

8. **Brieu, N., G. Guyon, M. Rodiere, M. Segondy, and V. Foulongne.** 2008. Human bocavirus infection in children with respiratory tract disease. Pediatr Infect Dis J **27:**969-973.

9. **Chieochansin, T., A. Kapoor, E. Delwart, Y. Poovorawan, and P. Simmonds.** 2009. Absence of detectable replication of human bocavirus species 2 in respiratory tract. Emerg Infect Dis **15:**1503-1505.

10. **Choi, E. H., H. J. Lee, S. J. Kim, B. W. Eun, N. H. Kim, J. A. Lee, J. H. Lee, E. K. Song, S. H. Kim, J. Y. Park, and J. Y. Sung.** 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. Clin Infect Dis **43:**585-592.

11. **Chomczynski, P.** 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques **15:**532-534, 536-537.

12. **Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, H. Ishiko, M. Hara, Y. Takahashi, and K. Kobayashi.** 2004. Human metapneumovirus infection in Japanese children. J Clin Microbiol **42:**126-132.

13. **Endo, R., N. Ishiguro, H. Kikuta, S. Teramoto, R. Shirkoohi, X. Ma, T. Ebihara, H. Ishiko, and T. Ariga.** 2007. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. J Clin Microbiol

228 **45:**3218-3223.

229 14. **Favier, A. L., W. P. Burmeister, and J. Chroboczek.** 2004. Unique
230 physicochemical properties of human enteric Ad41 responsible for its
231 survival and replication in the gastrointestinal tract. Virology
232 **322:**93-104.

233 15. **Gaynor, A. M., M. D. Nissen, D. M. Whiley, I. M. Mackay, S. B.**
234 **Lambert, G. Wu, D. C. Brennan, G. A. Storch, T. P. Sloots, and D.**
235 **Wang.** 2007. Identification of a novel polyomavirus from patients with
236 acute respiratory tract infections. PLoS Pathog **3:**e64.

237 16. **Han, T. H., J. Y. Chung, and E. S. Hwang.** 2009. Human bocavirus 2 in
238 children, South Korea. Emerg Infect Dis **15:**1698-1700.

239 17. **Ishiko, H., Y. Shimada, M. Yonaha, O. Hashimoto, A. Hayashi, K.**
240 **Sakae, and N. Takeda.** 2002. Molecular diagnosis of human
241 enteroviruses by phylogeny-based classification by use of the VP4
242 sequence. J Infect Dis **185:**744-754.

243 18. **Kantola, K., M. Sadeghi, J. Antikainen, J. Kirveskari, E. Delwart, K.**
244 **Hedman, and M. Soderlund-Venermo.** 2010. Real-time quantitative
245 PCR detection of four human bocaviruses. J Clin Microbiol

48:4044-4050.

19. **Kapoor, A., P. Simmonds, E. Slikas, L. Li, L. Bodhidatta, O. Sethabutr, H. Triki, O. Bahri, B. S. Oderinde, M. M. Baba, D. N. Bukbuk, J. Besser, J. Bartkus, and E. Delwart.** 2010. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. J Infect Dis **201:**1633-1643.

20. **Kapoor, A., E. Slikas, P. Simmonds, T. Chieochansin, A. Naeem, S. Shaukat, M. M. Alam, S. Sharif, M. Angez, S. Zaidi, and E. Delwart.** 2009. A newly identified bocavirus species in human stool. J Infect Dis **199:**196-200.

21. **Karalar, L., J. Lindner, S. Schimanski, M. Kertai, H. Segerer, and S. Modrow.** 2009. Prevalence and clinical aspects of human bocavirus infection in children. Clin Microbiol Infect.

22. **Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei.** 2001. MEGA2: molecular evolutionary genetics analysis software. Bioinformatics **17:**1244-1245.

23. **Ma, X., R. Endo, N. Ishiguro, T. Ebihara, H. Ishiko, T. Ariga, and H. Kikuta.** 2006. Detection of human bocavirus in Japanese children with

lower respiratory tract infections. J Clin Microbiol **44:**1132-1134.

24. **Martin, E. T., M. P. Fairchok, J. Kuypers, A. Magaret, D. M. Zerr, A. Wald, and J. A. Englund.** 2010. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis **201:**1625-1632.

25. **Miura-Ochiai, R., Y. Shimada, T. Konno, S. Yamazaki, K. Aoki, S. Ohno, E. Suzuki, and H. Ishiko.** 2007. Quantitative detection and rapid identification of human adenoviruses. J Clin Microbiol **45:**958-967.

26. **Monteny, M., H. G. Niesters, H. A. Moll, and M. Y. Berger.** 2007. Human bocavirus in febrile children, The Netherlands. Emerg Infect Dis **13:**180-182.

27. **Neske, F., K. Blessing, F. Tollmann, J. Schubert, A. Rethwilm, H. W. Kreth, and B. Weissbrich.** 2007. Real-time PCR for diagnosis of human bocavirus infections and phylogenetic analysis. J Clin Microbiol **45:**2116-2122.

28. **Offringa, D. P., V. Tyson-Medlock, Z. Ye, and R. A. Levandowski.** 2000. A comprehensive systematic approach to identification of influenza A

virus genotype using RT-PCR and RFLP. J Virol Methods **88:**15-24.

29. **Osiowy, C.** 1998. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription-PCR assay. J Clin Microbiol **36:**3149-3154.

30. **Soderlund-Venermo, M., A. Lahtinen, T. Jartti, L. Hedman, K. Kemppainen, P. Lehtinen, T. Allander, O. Ruuskanen, and K. Hedman.** 2009. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis **15:**1423-1430.

31. **Song, J. R., Y. Jin, Z. P. Xie, H. C. Gao, N. G. Xiao, W. X. Chen, Z. Q. Xu, K. L. Yan, Y. Zhao, Y. D. Hou, and Z. J. Duan.** 2010. Novel human bocavirus in children with acute respiratory tract infection. Emerg Infect Dis **16:**324-327.

32. **Stephensen, C. B., D. B. Casebolt, and N. N. Gangopadhyay.** 1999. Phylogenetic analysis of a highly conserved region of the polymerase gene from 11 coronaviruses and development of a consensus polymerase chain reaction assay. Virus Res **60:**181-189.

33. **Uhnoo, I., G. Wadell, L. Svensson, and M. E. Johansson.** 1984. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. J Clin Microbiol **20:**365-372.

34. **Wang, K., W. Wang, H. Yan, P. Ren, J. Zhang, J. Shen, and V. Deubel.** 2010. Correlation between bocavirus infection and humoral response, and co-infection with other respiratory viruses in children with acute respiratory infection. J Clin Virol **47:**148-155.

## FIGURE LEGEND:

Figure 1. Phylogenetic analysis of partial VP1 genes of human bocaviruses amplified by nested PCR. Trees were constructed by the neighbor-joining method with the MEGA program; bootstrap values >= 70% are shown.

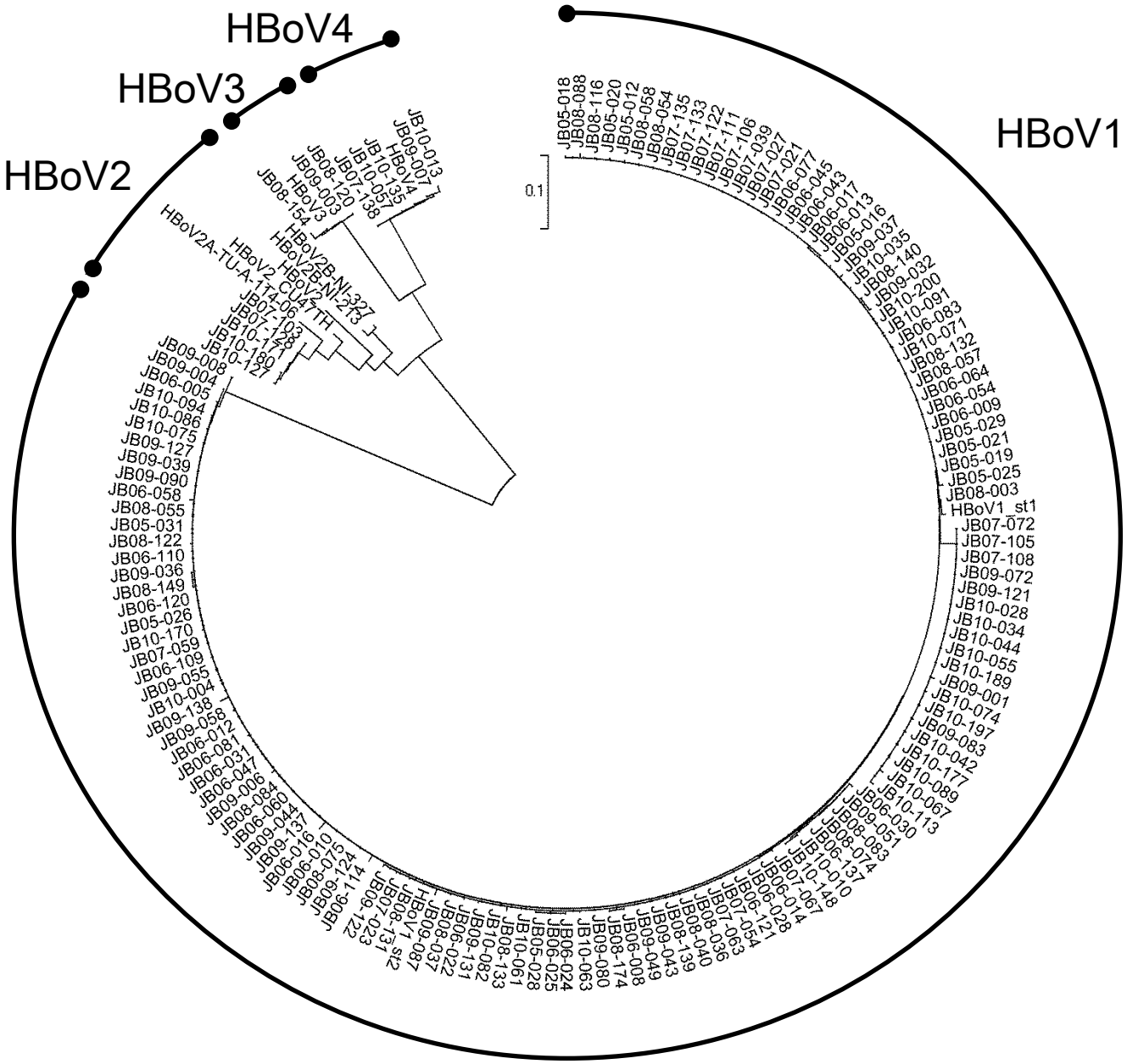

HBoV4
HBoV3
HBoV2
HBoV1
0.1
HBoV1_st1
HBoV2A-TU-A-114-06
HBoV2B-NI-327
HBoV2B-NI-2243
HBoV2-CC41TH
HBoV3
HBoV4
JB10-013
JB09-007
JB10-135
JB07-138
JB08-120
JB09-003
JB08-154
JB07-103
JB10-148
JB10-180
JB10-121
JB09-008
JB09-004
JB06-005
JB10-094
JB10-086
JB10-075
JB09-127
JB09-039
JB09-090
JB06-058
JB08-055
JB05-031
JB08-122
JB06-110
JB09-036
JB08-149
JB06-120
JB05-026
JB10-170
JB07-059
JB06-109
JB09-055
JB10-004
JB09-138
JB09-058
JB06-012
JB06-081
JB06-031
JB06-047
JB09-006
JB08-084
JB06-060
JB09-044
JB09-137
JB06-016
JB06-010
JB08-075
JB09-124
JB06-114
JB09-123
JB08-023
JB08-131
JB09-087
JB08-037
JB06-022
JB09-131
JB09-082
JB10-133
JB10-061
JB05-028
JB06-024
JB10-063
JB09-080
JB08-174
JB06-008
JB09-049
JB09-043
JB08-139
JB08-040
JB08-036
JB07-063
JB07-054
JB06-121
JB06-028
JB07-014
JB07-067
JB10-148
JB10-010
JB06-137
JB08-074
JB09-083
JB09-051
JB06-030
JB10-113
JB10-067
JB10-089
JB10-177
JB10-042
JB09-083
JB10-197
JB10-074
JB09-001
JB10-189
JB10-055
JB10-044
JB10-034
JB10-028
JB09-121
JB09-072
JB07-108
JB07-105
JB07-072
JB08-003
JB05-025
JB05-019
JB05-021
JB05-029
JB06-009
JB06-054
JB06-064
JB08-057
JB08-132
JB10-071
JB06-083
JB10-091
JB10-200
JB09-032
JB08-140
JB10-035
JB09-037
JB06-016
JB06-013
JB06-017
JB06-043
JB06-045
JB06-077
JB07-021
JB07-027
JB07-039
JB07-106
JB07-111
JB07-122
JB07-133
JB07-135
JB08-054
JB08-058
JB05-012
JB05-020
JB08-116
JB08-088
JB05-018

FIG 1

Table 1. Summary of HBoV1-4 detection in nasopharyngeal swab samples from patients with RTIs

| | No. (%) of detection | Single detection | Codetection with one or more viruses | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | hMPV | hRSV | HRV | KIPyV | WUPyV | FluB | PIV1 | PIV3 | AdV | HCoV | two or more viruses |
| HBoV1 | 132/850 (15.5%) | 76 | 15 | 12 | 6 | 2 | 4 | 2 | 2 | 1 | 1 | 1 | 10 * |
| HBoV2 | 5/850 (0.6%) | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| HBoV3 | 3/850 (0.4%) | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| HBoV4 | 5/850 (0.6%) | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 145/850 (17.1%) | 83 | 17 | 15 | 6 | 2 | 5 | 2 | 2 | 1 | 1 | 1 | 0 |

*Among the 132 HBoV1-positive samples, 10 were detected simultaneously with two or more viruses (3 with hMPV and KIPyV, 3 with hRSV and HRV, 1 with WUPyV and HRV, 1 with hMPV and PIV3, 1 with KIPyV and WUPyV, and 1 with hMPV, KIPyV and WUPyV).

Table 2. Summary of HBoV1-4 detection in nasopharyngeal swab samples and clinical diagnoses

| HBoVs | single or codetection | bronchitis | wheezy bronchitis | pneumoniae | asthma attack | acute pharyngitis | croup syndrome | (Total) |
|---|---|---|---|---|---|---|---|---|
| HBoV1 | single detection | 28 | 20 | 20 | 5 | 2 | 1 | 76 |
| | co-detection with one or more viruses | 19 | 19 | 12 | 4 | 1 | 1 | 56 |
| HBoV2 | single detection | 2 | 0 | 1 | 0 | 0 | 0 | 3 |
| | co-detection with another virus | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| HBoV3 | single detection | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | co-detection with another virus | 2 | 0 | 1 | 0 | 0 | 0 | 3 |
| HBoV4 | single detection | 4 | 0 | 0 | 0 | 0 | 0 | 4 |
| | co-detection with another virus | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | 58 | 39 | 34 | 9 | 3 | 2 | 145 |