

Figure. Phylogenetic tree of bovine kobuvirus (kobuvirus/bovine/Aba-Z20/2002/Hungary, in **boldface**) based on the 455-nt fragment of the kobuvirus 3D regions. The phylogenetic tree was constructed by using the neighbor-joining clustering method with distance calculation and the maximum composite likelihood correction for evolutionary rate with help of MEGA version 4.1 software (10). Bootstrap values (based on 1,000 replicates) are given for each node if >50%. Reference strains were obtained from GenBank. Scale bar indicates nucleotide substitutions per site.

### Gábor Reuter and László Egyed

Author affiliations: ÁNTSZ Regional Institute of State Public Health Service, Pécs, Hungary (G. Reuter); and Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary (L. Egyed)

DOI: 10.3201/eid1505.081427

### References

1. International Committee on Taxonomy of Viruses. Virus taxonomy 2008 [cited 2009 Feb 23]. Available from <http://www.ictvonline.org/virusTaxonomy.asp?version=2008>
2. Yamashita T, Kobayashi S, Sakae K, Nakata S, Chiba S, Ishihara Y, et al. Isolation of cytopathic small round viruses with BS-C-1 cells from patients with gastroenteritis. *J Infect Dis*. 1991;164:954–7.
3. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K. Isolation and characterization of a new species of kobuvirus associated with cattle. *J Gen Virol*. 2003;84:3069–77. DOI: 10.1099/vir.0.19266-0

4. Reuter G, Boldizsár Á, Kiss I, Pankovics P. Candidate new species of *Kobuvirus* in porcine hosts. *Emerg Infect Dis*. 2008;14:1968–70. DOI: 10.3201/eid1412.080797
5. Khamrin P, Maneekarn N, Peerakome S, Okitsu S, Mizuguchi M, Ushijima H. Bovine kobuviruses from cattle with diarrhea. *Emerg Infect Dis*. 2008;14:985–6.
6. Pham NT, Khamrin P, Nguyen TA, Kanti DS, Phan TG, Okitsu S, et al. Isolation and molecular characterization of Aichi viruses from fecal specimens collected in Japan, Bangladesh, Thailand, and Vietnam. *J Clin Microbiol*. 2007;45:2287–8. DOI: 10.1128/JCM.00525-07
7. Oh DY, Silva PA, Hauroeder B, Deidrich S, Cardoso DD, Schreier E. Molecular characterization of the first Aichi viruses isolated in Europe and in South America. *Arch Virol*. 2006;151:1199–206. DOI: 10.1007/s00705-005-0706-7
8. Ambert-Balay K, Lorrot M, Bon F, Giraudon H, Kaplon J, Wolfer M, et al. Prevalence and genetic diversity of Aichi virus strains in stool samples from community and hospitalized patients. *J Clin Microbiol*. 2008;46:1252–8. DOI: 10.1128/JCM.02140-07

9. Sdiri-Loulizi K, Gharbi-Khélifi H, de Rougemont A, Chouchane S, Sakly N, Ambert-Balay K, et al. Acute infantile gastroenteritis associated with human enteric viruses in Tunisia. *J Clin Microbiol*. 2008;46:1349–55. DOI: 10.1128/JCM.02438-07
10. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol*. 2007;24:1596–9. DOI: 10.1093/molbev/msm092

Address for correspondence: Gábor Reuter, Regional Laboratory of Virology, National Reference Laboratory of Gastroenteric Viruses, ÁNTSZ Regional Institute of State Public Health Service, Szabadság út 7, H-7623 Pécs, Hungary; email: reuter.gabor@baranya.antsz.hu

## Candidate Porcine *Kobuvirus*, China

**To the Editor:** The picornaviruses constitute a large, diverse family of positive-sense RNA viruses, which comprise 8 genera: *Enterovirus*, *Aphthovirus*, *Cardiovirus*, *Hepatovirus*, *Parechovirus*, *Erbovirus*, *Kobuvirus*, and *Teschovirus* (1). The genus *Kobuvirus* contains 2 known species: *Aichi virus*, which was identified in humans in 1989 and was found to be associated with human acute gastroenteritis (2), and *Bovine kobuvirus*, which was identified in 2003 in apparently healthy cattle (3). In our study, we identified a candidate novel strain of kobuvirus from porcine fecal specimens; this strain is markedly different from Aichi virus and bovine kobuvirus.

Using reverse transcription-PCR (RT-PCR) to characterize calcivirus in porcine fecal specimens with a primer pair of p289/VN3T20 designed for a 3-kb fragment of the virus, we observed an unexpected band on aga-

rose gel electrophoresis (4). After purification and sequencing, the 1,185-bp fragment was found to share 73% similarity with the 3D region of bovine kobuvirus. A pair of primers was then designed from this sequence and synthesized (forward: 5'-TGGAC GAC-CAGCTCTTCCTTAAACAC-3' and reverse: 5'-AGTGCAAGTGCAAGTCTGGGTTGCAGCCAACA-3'; 495 bp) to screen other porcine samples for the virus by PCR. Our samples were 322 fecal specimens collected during 2006–2007 from healthy piglets <15 days of age from 3 different farms and several sporadically distributed families that raised pigs in Lulong County, China. Of the 322 samples, 97 were positive. All products were sequenced, and 18 were chosen randomly for deposit in GenBank under accession nos. FJ459895–FJ459912.

To further characterize the virus, we designed primers corresponding to the viral protein (VP) 0 region of kobuvirus on the basis of conserved sequences deduced by comparing the sequences of bovine kobuvirus (GenBank accession no. NC\_004421) and Aichi virus (GenBank accession no. NC\_001918). An 823-bp fragment was examined and then submitted, together with the 1185-bp sequence, to GenBank under accession no. FJ493623. The obtained sequences were analyzed by using the DNASTAR software package ([www.dnastar.com](http://www.dnastar.com)) and were compared with other sequences in GenBank by using BLAST ([www.ncbi.nlm.nih.gov/blast/Blast.cgi](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi)).

The results demonstrate that the 3D partial region of the novel strain has nucleotide homology of 73% and 70% to that of bovine kobuvirus and Aichi virus, respectively. The sequence in the VP0 region was less conserved; nucleotide and amino acid homologies were 69% and 71%, respectively, to those of bovine kobuvirus and 66% and 69%, respectively, to those of Aichi virus.

Partial sequences of the 3D region have been used to deduce phylogenetic

relationships among picornaviruses; these sequences have been particularly useful for placing viruses within species or genera or for comparing viruses of different genera or families (5). We constructed phylogenetic trees by using MEGA software version 3.1 ([www.megasoftware.net](http://www.megasoftware.net)). The phylogenetic analysis showed a single genetic lineage for the novel virus, close to *Kobuvirus* but phylogenetically distinct from both bovine kobuvirus and Aichi virus, which sug-

gests that the porcine fecal specimen contained a candidate novel species of *Kobuvirus* (Figure).

The filtered fecal samples positive for the virus were inoculated onto RD cells. After 3 serial passages, no obvious cytopathic effect in RD cells was noted. The cells and supernatants in every passage were collected separately for RNA extraction. We then used semiquantitative RT-PCR with glyceraldehyde-3-phosphate dehydrogenase as an internal reference

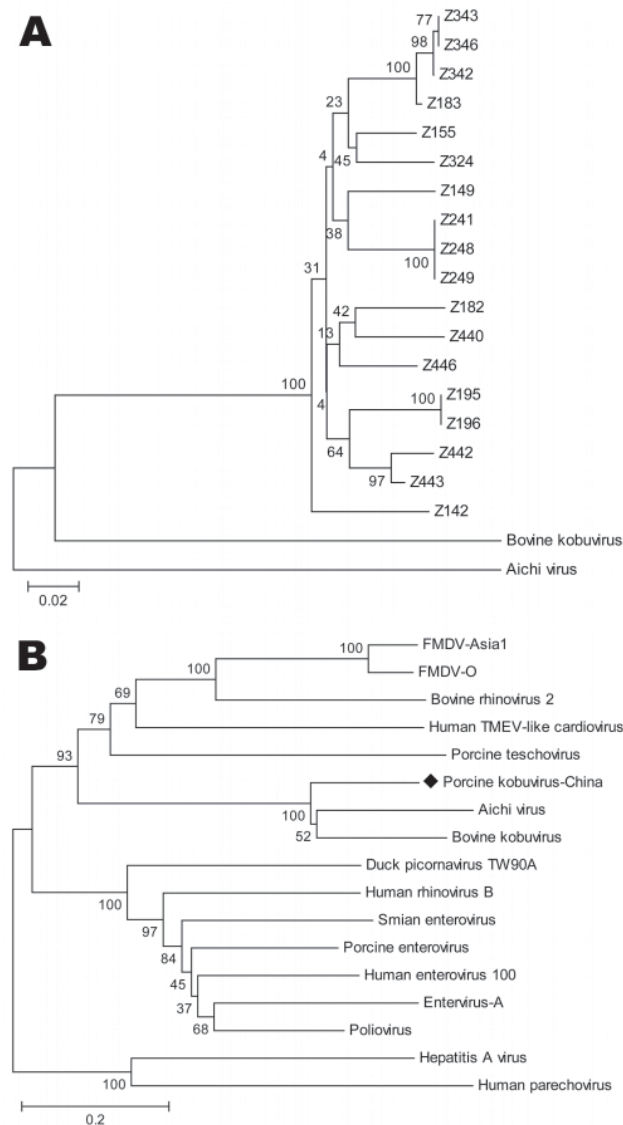


Figure. A) Phylogenetic tree of the partial sequences in the 3D region of the candidate novel virus, Aichi virus, and bovine kobuvirus. B) Relationships between the candidate novel virus and other picornaviruses based on nucleotide differences in the 3D region. FMDV, foot and mouth disease virus; TMEV, Theiler's murine encephalomyelitis virus. Scale bars indicate nucleotide substitutions per site.

to detect the virus and determine the amount of the viral RNA in the medium. Cells and supernatants of the 3 passages were virus positive, and the control inoculated with phosphate-buffered saline was virus negative. However, with each passage, the amount of viral RNA in the medium decreased. Whether the positive result was caused by residual viruses of the initial inoculation or by the decreased propagation of the virus in the cells is not clear. Further studies, such as continuous serial passages and neutralization assay, are needed to determine the final activity of the virus in RD cells, as well as in other cells such as Vero and HeLa, because several species of picornaviruses have been identified as causing persistent infections in these cells in vitro (6–10).

In conclusion, we report the genetic characterization and biological properties of a new agent in China. Of note, while we were preparing this article, a similar article from Hungary was published (5). After comparing our 1,185-bp sequence with the sequence from Hungary, we found that our sequence was 171 bp longer at the 3' end and 50 bp shorter at the 5' end and that the truncated sequence in the middle (same length) had a nucleotide homology of 92.1%. Phylogenetic analysis indicated that the 2 sequences may share the same origin (Figure, panel B). In addition, prevalence of our virus (30.12%) was higher than that of the virus from Hungary (13.3%). Further studies are needed to determine the complete genome and the relevance of the candidate porcine *Kobuvirus* as a causative agent of disease in pigs and a potential zoonotic agent.

This work was partly supported by "973" National Key Basic Research Program of China (grant no. 2007CB310500) and National High Technology Research and Development Program of China (grant no. 2006AA02A215).

**Jie-mei Yu, Miao Jin,  
Qing Zhang, Hui-ying Li,  
Dan-di Li, Zi-qian Xu,  
Jin-song Li, Shu-xian Cui,  
Su-hua Yang, Na Liu,  
Zhao-jun Duan**

Author affiliation: China Center for Disease Control, Beijing, People's Republic of China

DOI: 10.3201/eid1505.081518

#### References

1. Pringle CR. Virus taxonomy at the XIth International Congress of Virology, Sydney, Australia, 1999. *Arch Virol.* 1999;144:2065–70. DOI: 10.1007/s007050050728
2. Yamashita T, Sakae K, Tsuzuki H, Suzuki Y, Ishikawa N, Takeda N, et al. Complete nucleotide sequence and genetic organization of Aichi virus, a distinct member of the *Picornaviridae* associated with acute gastroenteritis in humans. *J Virol.* 1998;72:8408–12.
3. Yamashita T, Ito M, Kabashima Y, Tsuzuki H, Fujiura A, Sakae K. Isolation and characterization of a new species of kobuvirus associated with cattle. *J Gen Virol.* 2003;84:3069–77. DOI: 10.1099/vir.0.19266-0
4. Wang QH, Han MG, Cheetham S, Souza M, Funk JA, Saif LJ. Porcine noroviruses related to human noroviruses. *Emerg Infect Dis.* 2005;11:1874–81.
5. Oberste MS, Maher K, Pallansch MA. Genomic evidence that simian virus 2 and six other simian picornaviruses represent a new genus in *Picornaviridae*. *Virology.* 2003;314:283–93. DOI: 10.1016/S0042-6822(03)00420-3
6. de la Torre JC, Davila M, Sobrino F, Ortin J, Domingo E. Establishment of cell lines persistently infected with foot-and-mouth disease virus. *Virology.* 1985;145:24–35. DOI: 10.1016/0042-6822(85)90198-9
7. Gibson JP, Righthand VF. Persistence of echovirus 6 in cloned human cells. *J Virol.* 1985;54:219–23.
8. Matteucci D, Paglianti M, Giangregorio AM, Capobianchi MR, Dianzani F, Bendinelli M. Group B coxsackieviruses readily establish persistent infections in human lymphoid cell lines. *J Virol.* 1985;56:651–4.
9. Roos RP, Richards OC, Green J, Ehrenfeld E. Characterization of a cell culture persistently infected with the DA strain of Theiler's murine encephalomyelitis virus. *J Virol.* 1982;43:1118–22.
10. Vallbracht A, Gabriel P, Maier K, Hartmann F, Steinhardt HJ, Muller C, et al. Cell-mediated cytotoxicity in hepatitis A virus infection. *Hepatology.* 1986;6:1308–14.

Address for correspondence: Zhao-jun Duan, Department of Viral Diarrhea, National Institute for Viral Disease Control and Prevention, China Center for Disease Control, 100 Ying-Xin St, Xuan-Wu District, Beijing 100052, China; email: zhaojund@126.com

## Postoperative Panophthalmitis Caused by Whipple Disease

**To the Editor:** The clinical spectrum of Whipple disease has widely expanded since its etiologic agent, *Tropheryma whipplei*, was isolated in 2000 (1). Systematic 16S rDNA sequencing unexpectedly identified *T. whipplei* in patients for whom blood cultures were negative for endocarditis, spondylitis, and uveitis (2). Features common to these conditions and to Whipple disease include long-standing, unexplained arthralgia and deterioration of the patient's condition after treatment with immunosuppressive drugs (2). We report an unexpected case of postoperative panendophthalmitis identified by systematic 16S rDNA sequencing of a vitreous sample in a patient who had unexplained arthralgia and had been given topical corticosteroids after cataract surgery.

A 78-year-old woman in France underwent left eye phacoemulsification with intraocular lens implantation in May 2005 and retinal surgery followed by local corticoid application in April 2006. She had experienced cortisone-resistant polyarthralgia for 2 years before the first surgery. In July 2006, she showed decreased vi-