

9. Halperin JJ. A tale of two spirochetes: Lyme disease and syphilis. *Neurol Clin.* 2010;28:277–91. <http://dx.doi.org/10.1016/j.ncl.2009.09.009>
10. Cadavid D, Barbour AG. Neuroborreliosis during relapsing fever: review of the clinical manifestations, pathology, and treatment of infections in humans and experimental animals. *Clin Infect Dis.* 1998;26:151–64. <http://dx.doi.org/10.1086/516276>

Address for correspondence: Luis Castilla-Guerra, Servicio de Medicina Interna, Hospital Universitario Virgen Macarena, 41009, Seville, Spain; email: castillafernandez@hotmail.com

New Hepatitis E Virus Genotype in Bactrian Camels, Xinjiang, China, 2013

Patrick C.Y. Woo,¹ Susanna K.P. Lau,¹ Jade L.L. Teng,¹ Kai-Yuan Cao, Ulrich Wernery, Tony Schountz, Tsz Ho Chiu, Alan K.L. Tsang, Po-Chun Wong, Emily Y.M. Wong, Kwok-Yung Yuen

Author affiliations: The University of Hong Kong, Hong Kong, China (P.C.Y. Woo, S.K.P. Lau, J.L.L. Teng, T.H. Chiu, A.K.L. Tsang, P.-C. Wong, E.Y.M. Wong, K.-Y. Yuen); Sun Yat-sen University, Guangzhou, China (K.-Y. Cao); Central Veterinary Research Laboratory, Dubai, United Arab Emirates (U. Wernery); Colorado State University, Fort Collins, Colorado, USA (T. Schountz)

DOI: <http://dx.doi.org/10.3201/eid2212.160979>

To the Editor: Hepatitis E virus (HEV) is a member of the family *Hepeviridae*, genus *Orthohepevirus*, which comprises 4 species, *Orthohepevirus A–D*. *Orthohepevirus A* contains 7 genotypes (HEV1–7) (1,2). HEV1 and HEV2 infect humans only; HEV3, HEV4, and HEV7 can infect humans and other mammals; and HEV5 and HEV6 have been detected in animals only.

Worldwide, HEV is the most common cause of acute viral hepatitis in humans. The disease is generally self-limiting, but high death rates have been observed among HEV-infected pregnant women. Chronic HEV infection is a problem in immunocompromised patients, such as solid organ transplant recipients (3). Human HEV3 and HEV4 infections have been associated with consumption of undercooked pork or game meat (4).

In 2014, we described the discovery of a novel genotype of HEV in dromedaries (*Camelus dromedarius* or 1-humped camels), suggesting another possible source of human HEV infection (5). This dromedary HEV was subsequently classified as a novel *Orthohepevirus A* genotype,

HEV7 (1,2). Recently, this HEV7 genotype was also isolated from a liver transplant recipient from the Middle East with chronic HEV infection (6). The patient regularly consumed dromedary camel meat and milk, implying camel-to-human transmission of the virus (6).

Like the dromedary, the Bactrian camel (*Camelus bactrianus* or 2-humped camels) is an Old World camelid species. Thus, we hypothesize that Bactrian camels may also be reservoirs of HEV. To test this hypothesis and increase our understanding of the epidemiology of HEV in camels, we performed a molecular epidemiology study using feces samples from camels in China.

During November 2012–May 2013, we collected and tested 1 feces sample each from 205 Bactrian camels on a farm in Xinjiang, China. We performed RNA extraction and reverse transcription PCR (RT-PCR) as previously described (7). We screened for HEV by PCR amplification of a 251-bp fragment of open-reading frame (ORF) 2, using primers 5'-GTTGTCTCAGCCAATGGCGA-3' and 5'-GTAGTTGGTCATACTCAGCAGC-3'. PCR was performed, using previously described conditions (7), with the annealing temperature set at 50°C. DNA sequencing and quantitative real-time RT-PCR were performed as previously described (7). Three samples were positive for HEV; we performed complete genome sequencing of these samples as described (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/22/12/16-0979-Techapp1.pdf>) (5,7). We also performed comparative genomic analysis as previously described (1,2,8). We constructed a phylogenetic tree using the maximum-likelihood method and MEGA7 (9); bootstrap values were calculated from 1,000 trees. The optimal substitution model for each ORF was selected by MEGA7 (Figure).

RT-PCR for a 251-bp fragment in ORF2 of HEV was positive for 3 (1.5%) of the 205 fecal samples; virus loads were 1.6×10^3 , 2.1×10^3 , and 1.8×10^4 copies/mg, respectively. Whole-genome sequencing of the 3 Bactrian camel HEV (BcHEV) strains (GenBank accession nos. KX387865–7) showed genome sizes of 7,212–7,223 bp and a G + C content of 52.7%–53.1%. Overall, nucleotides in the BcHEV genome differed by >20% compared with those in all other HEVs. Genomes of the 3 BcHEV isolates contained 3 major ORFs; genome organization was typical of and characteristics were similar to those of HEVs from other *Orthohepevirus A* species. Phylogenetic trees constructed using ORF1, ORF2, ORF3, and concatenated ORF1/ORF2, excluding the hypervariable region, showed that these 3 BcHEV isolates clustered with the 2 dromedary camel HEV7 strains and the HEV7 strain from the liver-transplant recipient with chronic hepatitis (Figure; online Technical Appendix Figure 1)

¹These authors contributed equally to this article.

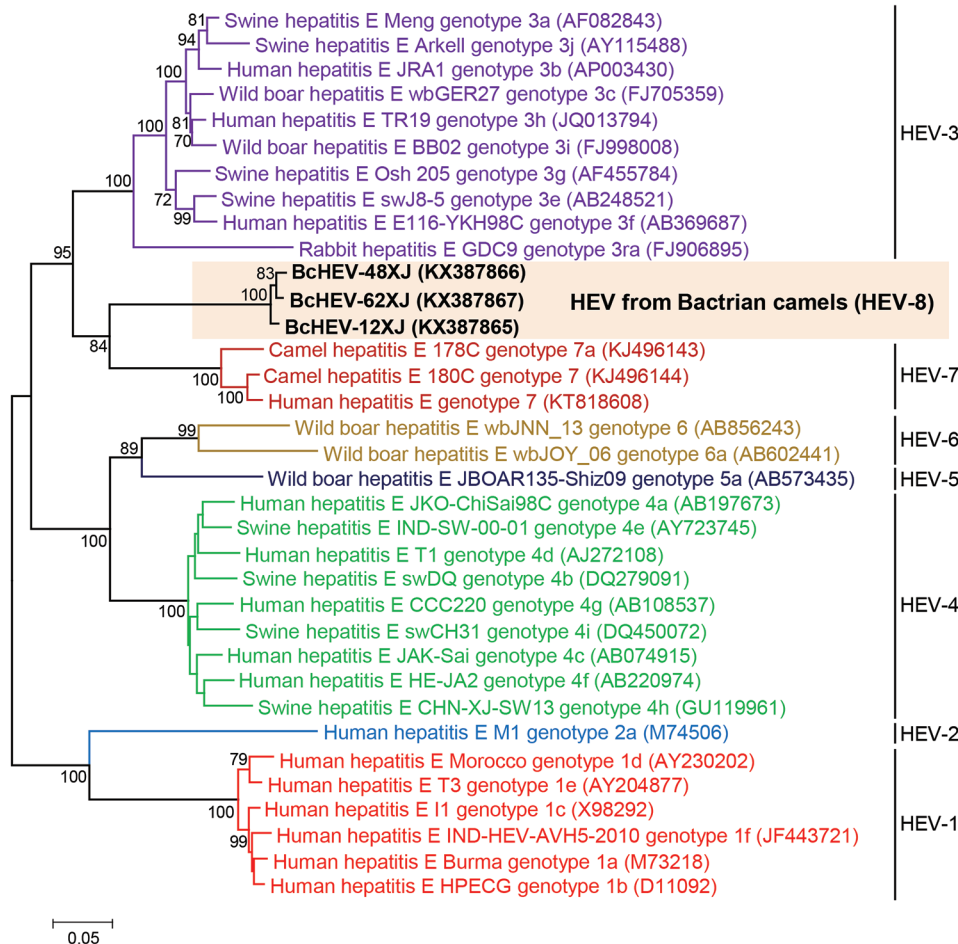


Figure. Phylogenetic analyses of the proteins of concatenated ORF1/ORF2, excluding the hypervariable region, of Bactrian camel hepatitis E virus (HEV) and other HEV genotypes (HEV1–HEV7) within the species *Orthohepevirus A* (family *Hepeviridae*). The tree was constructed using the maximum-likelihood method using the Jones–Taylor–Thornton substitution model with invariant sites and gamma distributed rate variation. The analysis included 2,282 amino acid positions (aa residues 1–706 and 789–2409, numbered with reference to GenBank sequence M73218). Bold indicates the 3 strains of BcHEV with complete genomes sequenced in this study. GenBank accession numbers are shown in parentheses. Scale bar indicates the estimated number of substitutions per 20 aa. ORF, open-reading frame.

(5,6). However, amino acid distances based on the concatenated ORF1/ORF2, excluding the hypervariable region of the 3 BcHEV isolates and the existing genotypes, ranged from 0.095 to 0.148, which was greater than the threshold (p -distance = 0.088) to demarcate intergenotype distance (1,2). Using this criterion, we propose that the 3 BcHEV isolates should constitute a new HEV genotype, HEV8.

A recent study in Dubai, United Arab Emirates, showed that HEV accounted for 40% of acute hepatitis cases (10). Even though HEV is an emerging pathogen in the Middle East, limited sequence data exist regarding the virus on the Arabian Peninsula. Recently, we discovered the HEV7 genotype in 1.5% of 203 feces samples from dromedaries in Dubai (5). In the current study, we detected a new HEV genotype in 1.5% of 205 Bactrian camels on a farm in Xinjiang. Comparative genomic and phylogenetic analyses showed that BcHEV represents a previously unrecognized HEV genotype. It has been shown that HEV7 from dromedaries can be transmitted to humans; thus, meat and milk from Bactrian camels might pose a similar risk to humans. The increasing

discoveries of camel viruses and of their transmission to humans highlight the need for caution when handling these mammals and processing food products derived from them.

This work was partly supported by the Theme-based Research Scheme (project no. T11/707/15), Research Grant Council Grant, University Grant Committee; the Hong Kong Special Administrative Region Health and Medical Research Fund; and the Strategic Research Theme Fund, The University of Hong Kong.

References

- Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, et al. Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol*. 2016;97:537–42. <http://dx.doi.org/10.1099/jgv.0.000393>
- Smith DB, Simmonds P, Jameel S, Emerson SU, Harrison TJ, Meng XJ, et al.; International Committee on Taxonomy of Viruses Hepeviridae Study Group. Consensus proposals for classification of the family Hepeviridae. *J Gen Virol*. 2014;95:2223–32. <http://dx.doi.org/10.1099/vir.0.068429-0>
- Kamar N, Selves J, Mansuy JM, Ouezanni L, Péron JM, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant

- recipients. *N Engl J Med*. 2008;358:811–7. <http://dx.doi.org/10.1056/NEJMoa0706992>
4. Debing Y, Moradpour D, Neyts J, Gouttenuire J. Update on hepatitis E virology: implications for clinical practice. *J Hepatol*. 2016;65:200–12. <http://dx.doi.org/10.1016/j.jhep.2016.02.045>
 5. Woo PC, Lau SK, Teng JL, Tsang AK, Joseph M, Wong EY, et al. New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis*. 2014;20:1044–8. <http://dx.doi.org/10.3201/eid2006.140140>
 6. Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150:355–7.e3. <http://dx.doi.org/10.1053/j.gastro.2015.10.048>
 7. Woo PC, Lau SK, Lam CS, Tsang AK, Hui SW, Fan RY, et al. Discovery of a novel bottlenose dolphin coronavirus reveals a distinct species of marine mammal coronavirus in *Gammacoronavirus*. *J Virol*. 2014;88:1318–31. <http://dx.doi.org/10.1128/JVI.02351-13>
 8. Smith DB, Purdy MA, Simmonds P. Genetic variability and the classification of hepatitis E virus. *J Virol*. 2013;87:4161–9. <http://dx.doi.org/10.1128/JVI.02762-12>
 9. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33:1870–4. <http://dx.doi.org/10.1093/molbev/msw054>
 10. Abro AH, Abdou AM, Saleh AA, Ustadi AM, Hussaini HS. Hepatitis E: a common cause of acute viral hepatitis. *J Pak Med Assoc*. 2009;59:92–4.

Address for correspondence: Patrick C.Y. Woo, State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong; email: pcywoo@hku.hk; Kwok-Yung Yuen, State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, The University of Hong Kong, University Pathology Bldg., Queen Mary Hospital, Hong Kong; email: kyyuen@hku.hk

Avian Influenza Virus H5 Strain with North American and Eurasian Lineage Genes in an Antarctic Penguin

Gonzalo P. Barriga, Dusan Boric-Bargetto, Marcelo Cortez-San Martin, Víctor Neira, Harm van Bakel, Michele Thompsom, Rodrigo Tapia, Daniela Toro-Ascuy, Lucila Moreno, Yesseny Vasquez, Michel Sallaberry, Fernando Torres-Pérez, Daniel González-Acuña, Rafael A. Medina

Author affiliations: Pontificia Universidad Católica de Chile, Santiago, Chile (G.P. Barriga, R.A. Medina); Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile (D. Boric-Bargetto, F. Torres-Pérez); Universidad de Santiago, Santiago

(M. Cortez-San Martin, D. Toro-Ascuy, Y. Vasquez); Universidad de Chile, Santiago (V. Neira, R. Tapia, M. Sallaberry); Icahn School of Medicine at Mount Sinai, New York, New York, USA (H. van Bakel, R.A. Medina); Universidad de Concepción, Concepción, Chile (M. Thompsom, L. Moreno, D. González-Acuña); Millennium Institute on Immunology and Immunotherapy, Santiago (R.A. Medina)

DOI: <http://dx.doi.org/10.3201/eid2212.161076>

To the Editor: Previous studies have reported avian influenza virus (AIV)–positive serum samples obtained from Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*), and gentoo (*Pygoscelis papua*) penguins (1–4). Only recently was an H1N2 subtype virus isolated from Adélie penguins in Antarctica (5). We performed AIV surveillance in the Antarctic Peninsula to identify the strains currently circulating in different penguins species in this area.

During 2015–2016, we sampled penguin colonies from 9 locations on the Antarctic Peninsula. We collected 138 blood samples from Adélie penguins at Ardley Island (62°13'S, 58°56'W), Arctowski Base (62°9'S, 58°28'W), and Bernardo O'Higgins Base (63°19'S, 57°53'W) and identified 5 serum samples positive for influenza. We also collected 513 cloacal swabs from Adélie, chinstrap (online Technical Appendix Figure 1, panel A; <http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf>), and gentoo penguins from Mikkelsen Harbor (63°54'S, 60°47'W), Dorian Bay and Port Lockroy (64°48'S, 63°30'W), Pleanau Island (65°06'S, 64°04'W), Brown Base (64°53'S, 62°52'W), Orne Harbor (64°37'S, 62°32'W), and Aitcho Island (62°23'S, 59°46'W) during January–March of 2 consecutive seasons (2015 and 2016; online Technical Appendix Figure 1, panel B; <http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf>). Quantitative reverse transcription PCR (RT-PCR) analysis of the matrix segment (6) identified 21 positive AIV samples from penguins (8 chinstrap, 13 gentoo) on Aitcho Island, demonstrating the presence of AIV in 2 additional penguin species in a new location in Antarctica.

Using multisegment RT-PCR performed with influenza-specific universal primers, we amplified all 8 virus segments from a chinstrap penguin specimen, which yielded cDNA products suitable for next-generation sequencing with a HiSeq 2500 System (Illumina, San Diego, CA, USA). This virus was subtyped as an H5N5 and named A/chinstrap_penguin/Antarctica/B04/2015 (H5N5). Analysis of its cleavage site confirmed this was a typical low pathogenicity AIV (LPAIV) containing cleavage motif PQRETRGLF (7).

To trace the origin of this H5N5 virus, we performed phylogenetic analyses of its hemagglutinin and neuraminidase genes (Figure, panels A, B; online Technical Appendix Figures 2, 3, <http://wwwnc.cdc.gov/EID/article/22/12/16-1076-Techapp1.pdf>). The hemagglutinin gene was placed

New Hepatitis E Virus Genotype in Bactrian Camels, Xinjiang, China, 2013

Technical Appendix

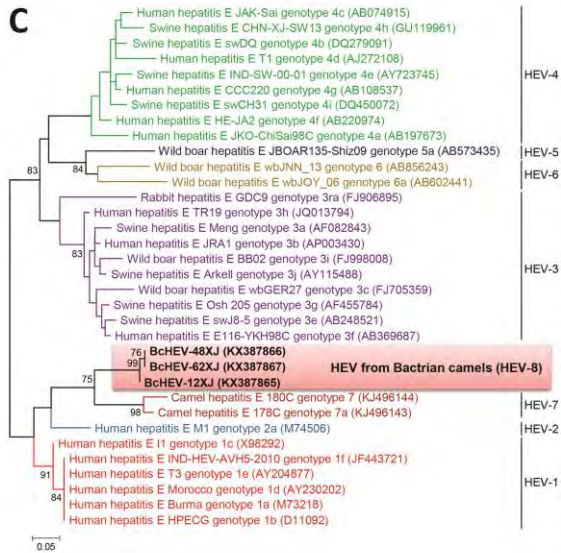
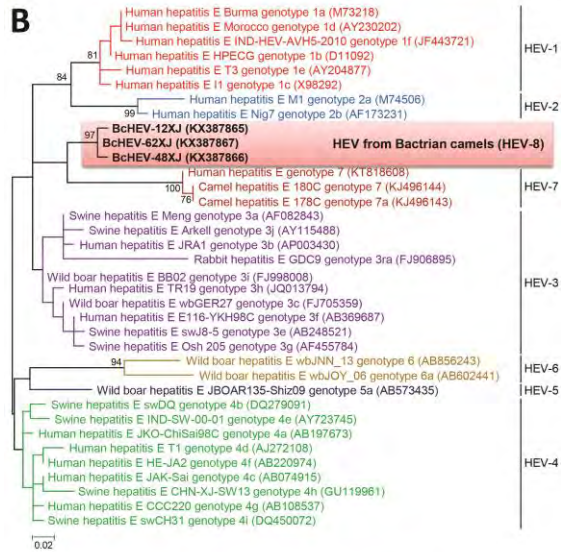
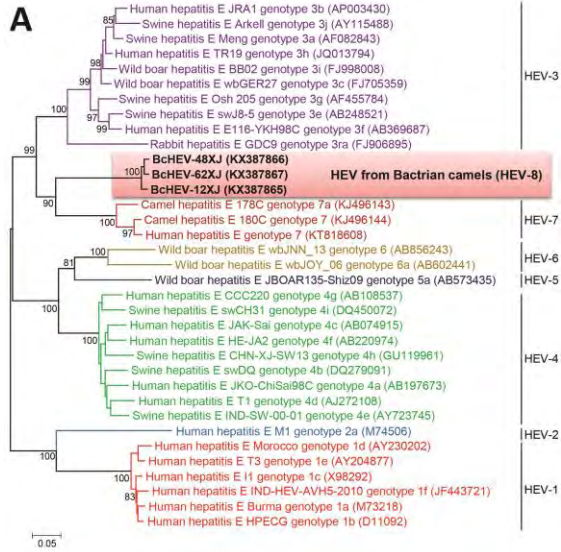
Complete Genome Sequencing

Three complete genomes of Bactrian camel HEV (BcHEV) strains, including BcHEV-12XJ, BcHEV-48XJ and BcHEV-62XJ, were amplified and sequenced using the RNA extracted from the original specimens as templates. The RNA was converted to cDNA by a combined random-priming and oligo(dT) priming strategy. The cDNA was amplified by primers designed by multiple alignments of the genomes of other HEVs with complete genomes available. Additional primers were designed from the results of the first and subsequent rounds of sequencing (Table). The 5' ends of the viral genomes were confirmed by rapid amplification of cDNA ends using the 5'/3' RACE kit (Roche, Germany). Sequences were assembled and manually edited to produce final sequences of the viral genomes.

Technical Appendix Table. Primers for amplification of the three BcHEV genomes

Primers	5' to 3'
Forward	
LPW28520	GTTGTCTCAGCCAATGGCGA
LPW28892	CGAAGGCTTACGAATGTTGC
LPW29061	ATCCGTTGGTCATTGAGA
LPW29066	ACTGTTGAGCTTACAGTTG
LPW29070	TGCATGGTGTGTTGAGAATGA
LPW31223	CCGGCCCTACAGTCTTTCATAT
LPW31225	CCGCTAATCCTGGTGCTATTA
LPW29072	TGCTGGACTTGACTAACTCA
LPW31228	GTATTGCCTCTGAACTTGT
LPW31226	GTACGAAGCTGTATGAAGCTGCTCA
LPW31432	GAAGGGTCTGAGGTCGATT
LPW31299	GGCTGTAAGTGTGCTGTTCTT
LPW32175	CCATGTGTGGGAGTCCAA
Reverse	
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW28893	CTGAGAATCAACCCGGTCA
LPW29062	CAACTGTAAGCTCAACAGT
LPW29067	CGAGTGAGTGCAACAATAGCA
LPW29071	GCTGAGAATCAACCCGGTCA
LPW31231	CTTACCAGAACCAGGGACA
LPW31233	CAGAACCCTTTCAGAGACTCCTT
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW29077	GCCCTGAGTGTAAATCTCTT
LPW31233	CAGAACCCTTTCAGAGACTCCTT

LPW31433	GCATGTGCACGAGAAGATT
LPW32181	GCATAATTGGACGCCTCAG
LPW31433	GCATGTGCACGAGAAGATT



Technical Appendix Figure. Phylogenetic analyses of A) ORF1, B) ORF2, and C) ORF3 and other genotypes of HEVs (HEV-1 to HEV-7) within the species *Orthohepevirus A*. The trees were constructed using maximum likelihood method and the optimal substitution models of JTT+G+I+F, JTT+G+I and JTT+G were used for ORF1, ORF2 and ORF3, respectively. Amino acid residues 1–1743, 1–660 and 10–123 in ORF1, ORF2 and ORF3, numbered with reference to GenBank sequence M73218, were included in the analyses. For ORF1 and ORF3, the scale bars indicate the estimated number of substitutions per 20 aa. For ORF2, the scale bar indicates the estimated number of substitutions per 50 aa. The three strains of DcHEV with complete genomes sequenced in this study are in bold.