MERS-Related Betacoronavirus in Vespertilio superans Bats, China

To the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV), a novel lineage C betacoronavirus, was first described in September 2012, and by April 16, 2014, the virus had caused 238 infections and 92 deaths in humans worldwide (1). Antibodies against MERS-CoV in dromedary camels were recently reported (2), as was the full genome of MERS-CoV from dromedary camels (3). Finding the natural reservoir of MERS-CoV is fundamental to our ability to control transmission of this virus to humans (4).

We report a novel lineage C betacoronavirus identified from Vespertilio superans bats in China. The full-length genome of this betacoronavirus showed close genetic relationship with MERS-CoV. Together with other evidence of MERS-CoV–related viruses in bats (5–8), our findings suggest that bats might be the natural reservoirs of MERS–related CoVs.

In June 2013, we collected anal swab samples from 32 V. superans bats from southwestern China. A small proportion of each sample was pooled (without barcoding) and processed by using virus particle–based metagenomic technology Information (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) by using BLAST (http://blast.ncbi.nlm.nih.gov) with the nonredundant protein database: 72,084 of the 32 samples (~16%) were positive for the novel betacoronavirus, and the PCR amplicons shared >98% nt identity with each other. Using a set of overlapped nested PCR primers, we amplified the cDNA ends method, we determined the full-length genome of this bat CoV (BtCoV) HKU4-1, 70.1% of the 3′ poly (A) tails, and a G+C content of 43.1%. Pairwise genome sequence alignment, conducted by the EMBL-EBI Needle (http://www.ebi.ac.uk/Tools/psa/emboss_needle/) with default parameters, suggested that the genome sequence of BtVs-BetaCoV/SC2013, GenBank accession no. KJ473821).

The betacoronavirus strain had a genome length of 30,413 nt, excluding the 3′ poly (A) tails, and a G+C content of 43.1%. Pairwise genome sequence alignment, conducted by the EMBL-EBI Needle software (http://www.ebi.ac.uk/Tools/psa/emboss_needle/) with default parameters, suggested that the genome sequence of BtVs-BetaCoV/SC2013 showed 75.7% nt identity with that of human MERS-CoV (hCoV-MERS); this shared identity is higher than that for other lineage C betacoronaviruses (from bats and hedgehogs) with full genomes available. hCoV-MERS showed 69.9% nt identity with bat CoV (BtCoV) HKU4-1, 70.1%
Figure. Phylogenetic trees based on the deduced amino acid sequences of the partial RNA-dependent RNA polymerase (RdRp; an 816-nt sequence fragment corresponding to positions 14817–15632 in human Middle East respiratory syndrome coronavirus [hCoV-MERS; KF192507]) and complete spike (S) protein. The novel virus is shown in gray, and hCoV-MERS is shown in bold. The following coronaviruses were used (GenBank accession numbers are shown in parentheses): severe acute respiratory syndrome coronavirus (SARS-CoV; NC004718), Bat Rp-coronavirus/Shaanxi2011(JX993987), Bat Cp-coronavirus/Yunnan2011(JX993988), Bat coronavirus HKU9-1 (BtCoV-HKU9-1; EF065513), BtCoV-133/2005(NC008315), BtCoV-HKU4-1 (EF065505), BtCoV-HKU4-2 (EF065506), BtCoV-HKU4-3 (EF065507), BtCoV-HKU4-4 (EF065508), BtCoV-HKU5-1 (EF065509), BtCoV-HKU5-2 (EF065510), BtCoV-HKU5-3 (EF065511), BtCoV-HKU5-5 (EF065512), BtCoV-ITA26/384/2012 (KJ312399), BtCoV-KW2E-F82 (JX899382), BtCoV-KW2E-F93 (JX899383), BtCoV-KW2E-F53 (JX899384), BtCoV-V-724 (KC243390), BtCoV-V-691 (KC243391), BtCoV-UKR-G17 (KC243392), Human betacoronavirus 2c EMC/2012 (hCoV-MERS/EMC; JX869059), hCoV-OC43 (NC005147), hCoV-NL63 (NC005831), Betacoronavirus ErinaceusCoV/2012-174 (ErCoV-2012-174; KC545383), and ErCoV-2012-216 (KC545386). Scale bars indicate genetic distance estimated by using WAG+G model for the RdRp and WAG+G+F model for the S protein implemented in MEGA5 (http://www.megasoftware.net).
nt identity with BtCoV-HKU5-1, and 69.6% nt identity with hedgehog CoV EriCoV-2012–174.

Compared with those lineage C betacoronaviruses, which had an 816-bp partial RdRp sequence fragment available, BtVs-BetaCoV/SC2013 shared 96.7% aa identity with hCoV-MERS. Pipistrellus BtCoVs found in Europe (BtCoV-8-724, BtCoV-8-691, BtCoV-UKR-G17) shared 98.2% aa identity with hCoV-MERS, and Eptesicus BtCoV found in Italy (BtCoV-ITA26/384/2012) and other lineage C betacoronaviruses shared 96.3% aa and <95% aa identity, respectively, with hCoV-MERS.

To clarify the evolutionary relationship between BtVs-BetaCoV/SC2013 and other lineage C betacoronaviruses, we performed phylogenetic analyses based on the deduced RdRp and the spike, envelope, membrane, and nucleocapsid proteins by using MEGA5 (http://www.megasoftware.net) (Figure; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/7/14-0318-Techapp1.pdf). For RdRp and the envelope, membrane, and nucleocapsid proteins, BtVs-BetaCoV/SC2013 always clustered with hCoV-MERS with short branch lengths, reflecting their high sequence similarities.

In the spike protein phylogenetic tree, BtVs-BetaCoV/SC2013 clustered with a clade defined by BtCoV-HKU5, with which it shares 74.8% aa identity. The spike proteins of hCoV-MERS form a sister clade of the clade defined by HKU5 BtCoVs and BtVs-betaCoV/SC2013, and the spike proteins share 69.0% aa identity with BtVs-betaCoV/SC2013. Spike proteins of BtVs-BetaCoV/SC2013, HKU5 BtCoVs, HKU4 BtCoVs, and hCoV-MERS, rather than EriCoV-2012–174, EriCoV-2012–216, and BtCoV-KW2E-F93, form a super clade. Spike protein is the critical factor for receptor recognition, binding, and cellular entry of CoVs in different host species (10), which may explain why the spike proteins in our study were relatively conserved within the same host species.

We identified a novel lineage C betacoronavirus from a V. superans bat and determined its full-length genome sequence. This novel betacoronavirus represents one of the most MERS-like CoVs that have been identified in bats as of the end of March 2014. The full-length genome sequence of the novel virus showed a closer genetic relationship with hCoV-MERS and camel MERS-CoV than with any other fully sequenced lineage C betacoronaviruses previously identified in bats or hedgehogs. Further studies of CoVs from more bat species worldwide may therefore help provide additional clues to the origins of pathogenic hCoV-MERS.

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Li Yang,1 Zhiqiang Wu,1 Xianwen Ren,1 Fan Yang,1 Junpeng Zhang, Guimei He, Jie Dong, Lilian Sun, Yafang Zhu, Shuyi Zhang, and Qi Jin

Author affiliations: Ministry of Health Key Laboratory of Systems Biology of Pathogens, Beijing, China (L. Yang, Z. Wu, X. Ren, F. Yang, J. Dong, L. Sun, Y. Zhu, Q. Jin); Institute of Pathogen Biology, Beijing (L. Yang, Z. Wu, X. Ren, F. Yang, J. Dong, L. Sun, Z. Zhu, Q. Jin); and East China Normal University, Shanghai, China (J. Zhang, G. He, S. Zhang)

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Address for correspondence: Qi Jin, Ministry of Health Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 6, Rongjing East St, BDA, Beijing 100176, China; email: zdsys@vip.sina.com

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Technical Appendix

Technical Appendix Figure. Phylogenetic trees based on the deduced amino acid sequences of the complete envelope (E), membrane (M), and nucleocapsid (N) proteins. The novel virus is shown in red, and human Middle East respiratory syndrome coronaviruses (MERS-CoVs) in blue. SARS, severe acute respiratory syndrome; BtCoV, bat CoV; HCoV, human CoV; EriCoV, Erinaceus CoV. Scale bar indicates genetic distance estimated by using the WAG+G model implemented in MEGA5 (www.megasoftware.net).