## 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
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Primers	5' 10' 3'
Forward	
LPW28520	GTTGTCTCAGCCAATGGCGA
LPW28892	CGAAGGCTTACGAATGTTGC
LPW29061	ATCCGTTGGTCATTGAGA
LPW29066	ACTGTTGAGCTTACAGTTG
LPW29070	TGCATGGTGTTTGAGAATGA
LPW31223	CCGGCCCCTACAGTCTTTCATAT
LPW31225	CCGCTAATCCTGGTGCTATTA
LPW29072	TGCTGGACTTGACTAACTCA
LPW31228	GTATTGCCTCTGAACTTGT
LPW31226	GTACGAAGCTGTATGAAGCTGCTCA
LPW31432	GAAGGGTCTGAGGTCGATT
LPW31299	GGCTGTACTGTTGCTGTTCCT
LPW32175	CCATGTGTGGGAGTCCAA
Reverse	
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW28893	CTGAGAATCAACCCGGTCA
LPW29062	CAACTGTAAGCTCAACAGT
LPW29067	CGAGTGAGTGCAACAATAGCA
LPW29071	GCTGAGAATCAACCCGGTCA
LPW31231	CTTACCAGAACCAGGGACA
LPW31233	CAGAACCCTTTCAGAGACTCCTT
LPW28521	GTAGTTTGGTCATACTCAGCAGC
LPW29077	GCCCTGAGTGTAATTCTCTT
LPW31233	CAGAACCCTTTCAGAGACTCCTT

LPW31433	GCATGTGCACGAGAAGATT
LPW32181	GCATAATTGGACGCCTCAG
LPW31433	GCATGTGCACGAGAAGATT

HEV-3	Human hepatitis E JRA1 genotype 3b (AP003430)     Swine hepatitis E Arkell genotype 3j (AY115488)     Human hepatitis E Meng genotype 3 (AF062843)     Human hepatitis E TR19 genotype 3h (JQ01794)     Wild boar hepatitis E Mod genotype 3c (F70559)     Wild boar hepatitis E Mod R27 genotype 3c (F70559)     Swine hepatitis E Obd Penotype 3g (AF458784)     Swine hepatitis E CloC9 genotype 3f (AB369687)     Human hepatitis E CDC9 genotype 3f (AB369685)	
-8)	HEV from Bactrian camels (HI	BCHEV-48XJ (KX387866) BCHEV-62XJ (KX387867) BCHEV-12X1 (KX387865)
HEV-7	pe 7a (KJ496143) /pe 7 (KJ496144)	Camel hepatitis E 178C genoty
HEV-6	(KT818608) V_13 genotype 6 (AB856243)	97- Human hepatitis E genotype 7 Wild boar hepatitis E wbJNN Wild boar hepatitis E wbJNN
HEV-5	VICY_06 genotype 5a (AB502441) 2135-Shiz09 genotype 5a (AB573435) ype 4g (AB108537) ype 4i (DQ450072) type 4c (AB074915)	Wild boar hepatitis E JBOAR Wild boar hepatitis E JBOAR Human hepatitis E SwCH31 genoty Human hepatitis E JAK-Sai genoty
HEV-4	ype 4f (AB220974) 3 genotype 4h (GU119961) e 4b (DQ279091) 3C genotype 4a (AB197673) 4d (AJ272108)	100 Human hepatitis E HE-JA2 genoty Swine hepatitis E CHN-XJ-SW1 Swine hepatitis E swDQ genotype Human hepatitis E JKO-ChiSai98
HEV-2	genotype 2e (AY/23/45) enotype 2a (M74506) genotype 1d (AY230202) be 1e (AY204877)	Human hepatitis E M generative E MD-SW-00-01 Human hepatitis E M generative E Morocco generat
HEV-1	a rc (X96292) AVH5-2010 genotype 1f (JF443721) totype 1a (M73218) anotype 1b (D11092)	Human hepatitis E IND-HEV- 83 – Human hepatitis E IND-HEV- 9 – Human hepatitis E Burma gen 9 – Human hepatitis E HPECG ge
HEV-1	e 1a (M73218) ype 1d (AY230202) H5-2010 genotype 1f (JF443721) = 1b (D11092) e (AY204877)	Human hepatitis E Burma genotyp Human hepatitis E Morocco genoty Human hepatitis E IND-HEV-AV Human hepatitis E HPECG genotype Human hepatitis E T3 genotype 16
HEV-2	(98292) I genotype 2a (M74506)	Human hepatitis E I1 genotype 1c (X
/-8)	HEV from Bactrian camels (H	97 BcHEV-12XJ (KX387865) BcHEV-62XJ (KX387867)
	notype 7 (KT818608)	BcHEV-48XJ (KX387866) Human hepatitis E ger
HEV-7	50C genotype 7 (KJ496143) 843) 15488) 103430) 269 genotype 3ra (FJ906895)	Tel Camel hepatitis E 1 File Camel hepatitis E 1 Swine hepatitis E Meng genotype 3a (AF0622 Swine hepatitis E Arkell genotype 3 (AP1 Human hepatitis E JRA1 genotype 3b (AP0 Rabbit hepatitis E CC
HEV-3	8) 3794) (FJ705359) 3 3f (AB399687) B248521) AF455784) 4 (AB152, a genetic 6 (AB55243)	Ild boar hepatitis E BB02 genotype 3( (F.J98800) Human hepatitis E TR19 genotype 3h (JQ013 Wild boar hepatitis E WoGER27 genotype 3c Human hepatitis E E 116-YKH98C genotype Swine hepatitis E wale 5 genotype 3 ( Al Swine hepatitis E Csh 205 genotype 3 (
HEV-6 HEV-5	E wbJQY_06 genotype 6a (A8602441) 9 genotype 5a (A8573435) 1) 47723745) B 197673)	Wild boar hepatitis E Wild boar hepatitis E JBOAR135-Shiz05 Wine hepatitis E IND-SW-00-01 genotype 4b (D0279097 Swine hepatitis E IND-SW-00-01 genotype 4e (A nan hepatitis E IND-SW-00-01 genotype 4e (A
HEV-4	8) 74) 115) 4h (GU119961) 537) 72)	<ul> <li>Human hepatilis E T1 genotype 4d (AJ27210 uman hepatilis E HE-JA2 genotype 4f (AB22097) uman hepatilis E JAK-Sai genotype 4d (AB074 — Swine hepatilis E CM-XJ-SW13 genotype uman hepatilis E CC220 genotype 4g (AB1888 wine hepatilis E swCH31 genotype 4l (DQ45007)</li> </ul>
HEV-4	otype 4c (AB074915) V13 genotype 4h (GU119961) e 4b (DQ270901) type 4d (AJ272108) 1 genotype 4e (AY723745) vpe 4d (AB18537)	Human hepatitis E JAK-Sai genc Swine hepatitis E CHN-XJ-SV Human hepatitis E T1 geno Human hepatitis E T1 geno Swine hepatitis E T1 geno
HEV-5	ype 4i (DQ450072) e 4f (AB220974) 3C genotype 4a (AB197673) R135-Shiz09 genotype 5a (AB573435)	Swine hepatitis E swCH31 genoty     Human hepatitis E HE-JA2 genotype     Human hepatitis E JKO-ChiSai96     Wild boar hepatitis E JBOAF
HEV-6	_13 genotype 6 (AB505243) (0Y_06 genotype 6a (AB602441) ype 3ra (FJ906895) Q013794) a (AF082843) (AP003430)	A Wild boar hepatitis E would Wild boar hepatitis E would Rabbit hepatitis E GDC9 genoty Human hepatitis E TR19 genotype 3h Swine hepatitis E Meng genotype 3a Human hepatitis E JRA1 genotype 3b
HEV-3	pp 3i (FJ998008) (AY115488) genotype 3c (FJ705359) 3g (AF455784) 3e (AB248521) anotype 3f (AB369687)	<sup>83</sup> Wild boar hepatitis E BB02 genoty Swine hepatitis E Arkell genotype 3( Wild boar hepatitis E swideS genotype Swine hepatitis E Sols 205 genotype Swine hepatitis E Sols 205 genotype Human hepatitis E E116-YK1980 ge 75 BcHEV-48XJ (KX387866)
-8)	HEV from Bactrian camels (HE	75 BcHEV-62XJ (KX387867) BcHEV-12XJ (KX387865)
HEV-7	enotype 7 (KJ496144) totype 7a (KJ496143)	Camel hepatitis E 180C g 98 Camel hepatitis E 178C gen
HEV-2	kotype 11 (JF443721) (7) 230202) 278	Human hepatitis E I1 genotype 1c (X98292) Human hepatitis E I1 genotype 1c (X98292) Human hepatitis E IND-HEV-AVH5-2010 gen Human hepatitis E Morocco genotype 1d (AY Human hepatitis E Morocco genotype 1d (AY
	3e (AB248521) enotype 31 (AB369687) HEV from Bactrian camels (HB enotype 7 (KJ496144) otype 7a (KJ496143) 4506) uotype 11 (JF443721) 7) 230202) 216) (092)	Swine hepatitis E sw0-8-5 genotype Human hepatitis E E116-VK1982C (KX387866) 99 BcHEV-482X (KX387866) 99 Camel hepatitis E 180C g 99 Camel hepatitis E 180C g 90 Camel hepatitis E 180C g 90 Camel hepatitis E 180C g 90 Camel hepatitis E 180C g 91 Camel hepatitis E 180C g 92 Camel hepatitis E 180C g 93 Camel hepatitis E 180C g 94 Camel hepatitis E 180C g 94 Camel hepatitis E 180C g 94 Camel hepatitis E 180 Cg 94 Camel hepatitis E 180 Cg 95 Camel hepatitis E 180 Cg 94 Camel hepatitis E 180 Cg 95 Camel hepatitis E 180 Cg 96 Camel hepatitis E 180 Cg 96 Camel hepatitis E 180 Cg 96 Camel hepatitis E 180 Cg 97 Camel hepatitis E 180 Cg 97 Camel hepatitis E 180 Cg 98 Camel hepatitis E 180 Cg 99 Camel hepatitis E 180 Cg 90 Cg 90 Camel hepatitis E 180 Cg 90

**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.