

Supplemental Digital Content

Materials and Methods

Genotyping and phylogenetic analyses of patient-derived hepatitis E virus genomes

First-round RT-PCRs for ORF1 and ORF2 were performed as a touchdown protocol with reverse transcription at 50°C for 30 min and subsequent PCR at 94°C for 5 min, 10 cycles with 94°C for 30 sec, a 1°C touchdown decrease of the annealing temperature to 50°C, and extension at 72°C for 45 sec, followed by 35 cycles at an annealing temperature of 52°C. Nested and seminested PCRs were performed at 94°C for 10 min then 40 cycles of 94°C for 30 sec, 52°C for 30 sec and 72°C for 45 sec. For the RT-PCRs the One Step RT-PCR Kit (Qiagen, Hilden, Germany) was used with 5 µl RNA, 0.2 mL dNTP, 400 nM (each) sense and antisense primer (supplementary Table S1, supplemental digital content), nested or seminested PCRs were performed with HotStarTaq Master Mix (Qiagen) using 1 µl of the first-round PCR product and 400 nM (each) sense and antisense primer. PCR products were gel purified with the MinElute Gel Extraction Kit (Qiagen) and sequenced with BigDyeTerminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Bundesstaat, USA) with both forward and reverse primer using the 3100 Sequencer (ABI, Waltham, MA, USA).

Table S1: Primers used for HEV-specific RT-PCR and sequencing

Primer	Sequence (5'-3') ^a	Targeted Region	Position ^b
HEV-F38	GAGGCYATGGTSGAGAARG	RdRp, ORF1	4084-4102
HEV-R39	GCCATGTTCCAGACRGTCTTCC		4622-4601
HEV-F37	GGTCCGYGCTATTGARAARG		4277-4297
HEV-R27	TCRCCAGAGTGYTTCTTCC		4583-4565
HEV-F30	CCGACAGAATTGATTTTCGTCGG	ORF2	6296-6317
HEV-R31	GTCTTGGARTACTGCTGR		6750-6733
HEV-F32	GTCTCAGCCAATGGCGAGCCRAC		6350-6372
HEV-F137	TCTAATGGCCTGGACTGTACTG	HVR, ORF1	1201-1222
HEV-R124	TGGACCGAYGAGGCYCGCTGCAT		3176-3154
HEV-R25	GGTTACGYTCCCAGGCRTC		2743-2725
HEV-F158	TTYTCYCCTGGGCAYMTYTGGGA		2071-2093
HEV-R159	TTAACCARCCARTCACARTCYGAYTCAAA		2441-2414
HEV-F160	ACYTGGTCHACATCTGGYTTYTC		2137-2156
HEV-F161	TTYTCCCCYCCTGAGGCGGC		2202-2223
HEV-R162	TACACCTTRGCSCCRTCRGGRTA		2399-2377

^a R is G/A, Y is C/T, S is G/C, W is A/T, M is A/C, K is G/T, H is A/C/T, N is A/T/C/G, and I is inosine.

^b Nucleotide positions are relative to species *Orthohepevirus A* reference strain Burma (GenBank acc. Nr.: M73218)