APPENDIXES

LEGENDS TO APPENDIX FIGURES

Figure A1. Phylogenetic tree based on a 343-nucleotide partial sequence corresponding to nucleotides 5999-6342 of open reading frame 2 (ORF2) of the HEV genome (GenBank accession no. AF082843).

The legend of this figure is the same as that of Figure 1, except the tree was built using the Maximum Likelihood method based on the General Time Reversible model. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.6239)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 261 nucleotide sequences and there were a total of 343 positions in the final dataset.

Figure A2. Phylogenetic tree based on a 231-nucleotide partial sequence of open reading frame 2 (ORF) 2 of the HEV genome (a fragment from the 343 nucleotide-long sequence analyzed in Figure 1 that corresponds to nucleotides 6,063-6,293 of genome GenBank accession no. FJ705359).

The HEV sequences obtained in our laboratory are indicated by a black frame. The 10 sequences with the highest BLAST scores (excluding those from our laboratory or with a query coverage <98%) recovered from the NCBI GenBank nucleotide sequence database (indicated in boldface, underlined, labeled with BBH (for best BLAST hit), GenBank accession number, host, country and year of sample collection or sequence submission) with the near full-length genome obtained in this study (GenBank accession no. KJ701409) have been incorporated into the phylogeny reconstruction in addition to a comprehensive set of 245 full-length genome sequences that were downloaded from the Virus Pathogen Resource database (1) (labeled, if they are clustered with sequences from the present study, with genotype and subtype, GenBank accession number, host, country and year of sample collection or sequence submission). To allow for the better legibility of the tree, branches were collapsed when they did not directly link to sequences from the present study and when the bootstrap value at node was >90%. Nucleotide alignments were performed using the MUSCLE software (http://www.ebi.ac.uk/Tools/msa/muscle/). The tree was constructed using the MEGA 5 software (http://www.megasoftware.net/) and the neighbor-joining method. Branches with bootstrap values were obtained from 1,000 resamplings of the data, and values >50% are labeled on the tree. The scale bar indicates the number of nucleotide substitutions per site.

BBH, best BLAST hit; BOL, Bolivia; GER, Germany; FRA, France; Hu, Human; JAP, Japan; KYR, Kyrgyzstan; MON, Mongolia; NLD, the Netherlands; Sw, Swine; SWE, Sweden; URU, Uruguay; Wb, wild boar.

APPENDIX TABLES

Table A1. Main characteristics of the 8 chronically-HEV infected patients

Case no.	Age (years)	Gender	Date of presumed HEV infection	Time since kidney-transplantation (months)	HEV testing at diagnosis		
					HEV RNA	Anti-HEV IgM	Anti-HEV IgG
1	46	M	27-Apr-09	56	+	+	+
2	55	M	02-Apr-04	70	+	+	+
3	64	M	19-Jun-06	121	+	+	+
4	53	M	27-Jun-06	14	+	+	-
5	43	M	26-Sep-06	25	+	+	+
6	73	M	02-May-07	107	+	+	-
7	50	F	05-Nov-07	12	+	N.t.	N.t.
8	46	M	18-Aug-08	59	+	-	-

+, positive; -, negative; IgG, immunoglobulin G; IgM, immunoglobulin M; M, male; F, female; N.t., not tested

Table A2. PCR primers used in the present study

Name	Orientation	Nucleotide positions amplified [a]	Sequence length (nt)	HEV genes	Sequences (indicated in 5'-3' orientation)	Reference
F25	ESP	7-251	245	ORF1	GGTCGAYGCCATGGAGGCCC	(2)
R5500	EAP				GVGGGGCGCTGGGACTGGTCAS	
HEVORF1con	ESP	54-471	418	ORF1	CTGGCATYACTACTGCYATTGAGC	(3)
HEVORF1con	EAP				CCATCRARRCAGTAAGTGCGGTC	
Mars1	ESP	369-1009	641	ORF1	AGAGAYGTYCAGCGCTGGTA	Present study
Mars2	AP				RAGCATGAGCCGRTCCCA	
None	ESP	933-2102	1170	ORF1	ACAGAGGTGTATGTTAGATCCATATTTGGC	(4)
None	AP				GGGGAGAAGTCGCTAGAGAAACCTGATGT	
MJ-B	ESP	2085-2351	267	ORF1	GCATRTTTGGGAGTCKGCTAATCC	(5)
MJ-B	EAP				GCATGGCAGAGSCCGCCGCCGGGGC	
MJ-B	IAP				ACCTTAGMGCCGTCMGGGTAGGT	
MJ-B	ESP	2148-2779	632	ORF1	GCATRTTTGGGAGTCKGCTAATCC	(5)
MJ-C	EAP				AGGGTGCCGGGCTCGCCGGA	
Mars3	ESP	2705-3847	1143	ORF1	GCAGCATATCGAGAGACCTG	Present study
Mars4	AP				GTGAGCTCTTGTGGCATGTA	
Mars5	ESP	3756-4473	718	ORF1	AATGCRTCKAAYCCYGGC	Present study
Mars6	AP				GCACARAGGTYTTRCTCCA	
Mars7	AP				CAAACTCAGAAAARTCRTTCTCAAT	
Mars8	AP				GCATSCCACACTCCTCCAT	
Mars9	AP				CRCCGGARTGYTTCTTCC	
MJ-B	ISP	3848-4579	732	ORF1	ACWTTKTATACCCGTACTTGGTC	(5)
MJ-C	IAP				TCACCGGAGTGYTTCTTCCAGAA	
None	SP	4559-5169	611	ORF1-ORF3	ACAGAGGTGTATGTTAGATCCATATTTGGC	(4)
None	AP				GGGGAGAAGTCGCTAGAGAAACCTGATGT	
F25	ESP	5152-5601	450	ORF3-ORF2	GGTCGAYGCCATGGAGGCCC	(2)
R5470	IAP				GGGACTGGTCRGCCAAG	
Mars10	SP	5213-5983	771	ORF3-ORF2	CATGCGCCCTAGGGYTGT	Present study
Mars11	AP				AGCCATGTATGCARAGCATWAC	
HevMrsFwd1	ESP	5711-6475	765	ORF2	AATTATGCYCAGTAYCGRGTT	(6)
HevMrsFwd2	ISP				GTWATGCTYTGCATACATGGCT	
HevMrsRev2	IAP				AGCCGACGAAATCAATTCTGTC	
HevMrsRev1	EAP				CCCTTRTCYTGCTGRGCATTCTC	
None	SP	6372-7125	754	ORF2	ACAGAGGTGTATGTTAGATCCATATTTGGC	(4)
None	AP				GGGGAGAAGTCGCTAGAGAAACCTGATGT	

[a] A, antisens; E, external; I, internal; P, primer; S, sens

Reference List

1. **Pickett, B. E., E. L. Sadat, Y. Zhang, J. M. Noronha, R. B. Squires, V. Hunt, M. Liu, S. Kumar, S. Zaremba, Z. Gu, L. Zhou, C. N. Larson, J. Dietrich, E. B. Klem, and R. H. Scheuermann**. 2012. ViPR: an open bioinformatics database and analysis resource for virology research. Nucleic Acids Res. **40**:D593-D598.
2. **Peralta, B., E. Mateu, M. Casas, D. N. de, M. Martin, and S. Pina**. 2009. Genetic characterization of the complete coding regions of genotype 3 hepatitis E virus isolated from Spanish swine herds. Virus Res. **139**:111-116.
3. **Erker, J. C., S. M. Desai, and I. K. Mushahwar**. 1999. Rapid detection of Hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers. J Virol.Methods **81**:109-113. doi:S0166-0934(99)00052-X [pii].
4. **Legrand-Abravanel, F., J. M. Mansuy, M. Dubois, N. Kamar, J. M. Peron, L. Rostaing, and J. Izopet**. 2009. Hepatitis E virus genotype 3 diversity, France. Emerg.Infect.Dis. **15**:110-114.
5. **Zhai, L., X. Dai, and J. Meng**. 2006. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. Virus Res. **120**:57-69.
6. **Kaba, M., B. Davoust, J. L. Marie, M. Barthet, M. Henry, C. Tamalet, D. Raoult, and P. Colson**. 2009. Frequent transmission of hepatitis E virus among piglets in farms in Southern France. J Med.Virol. **81**:1750-1759.