Supplementary material

Supplementary Table 1 Primers used for amplification of a part of the *ORF2* region of hepatitis E virus RNA from strains isolated from liver tissues of wild boars

Primer	Sequence (5' to 3')	Position according to the Burmese strain	Reference
F2	GCTCACGTCATCTGTCGCTGCTGG	5572-5595	2
R2	GGGCTGAACCAAAATCCTGACATC	5837-5859	
3156-EF	Aaytatgcmcagtaccgggttg	5687-5708	1
3157-ER	Cccttatcctgctgagcattctc	6395-6417	
3158-EF	Gtyatgytyygcatacatggct	5972-5993	1
3159-IRS	Agccgacgaaatyaattctgtc	6298-6319	
ORF 2-s1	Gacagaattratttcgtcggctgg	6298-6321	1
ORF 2-a 1	Cttgttcrtgytggttrtcataatc	6470-6494	

HEV-RNA detection in wild boars' liver tissue samples

From 2010-2014, liver tissue samples from 40 wild boars (*Sus scrofa*) originating from Northern and Western Greece were submitted to the Laboratory of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly and were stored at -80°C for HEVRNA examination. Viral RNA was isolated from 50 mg of liver tissue samples using Trizol reagent protocol (Thermofisher Scientific). A primer pair targeting at a 197-nucleotide part of *ORF2* gene (40) was used for amplifying HEV-RNA. An OneStep RT-PCR protocol (Qiagen, Germany) was performed in a final volume of 50 μL, according to the manufacturer's instruction. Following RT-PCR, 10 μL of amplification products were analyzed by electrophoresis in a 2% agarose gel stained with 0.5 mg/mL ethidium bromide. Product sizes were determined with reference to 100 bp DNA ladder. Amplicons were sequenced bidirectional by BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

HEV detection in wild boars is part of the European Union Seventh Framework Programme (2007–2013), a large collaboration project under grant agreement no. 222633 (Novel Technologies for Surveillance of Emerging and Re-emerging Infections of Wildlife—WildTech). All samples used in this study represent material collected by partners and other organizations for other purposes than this project as specified in deliverable D4.5/5.5 titled "Guidelines for ethical sample collection" submitted to the European Commission (26 February 2010, Dissemination Level: PP, restricted to other program participants, including Commission Services). The wild boar liver tissue samples were collected opportunistically (no active capture, killing, and sampling of wild animals specifically for this study were performed) from animal hunter-harvested by members of hunting federations. Thus, special approval was not necessary, and steps to ameliorate suffering were not applicable to this study. Research on animals as defined in the EU Ethics for Researchers document (European Commission, 2007, Ethics for Researchers—Facilitating Research Excellence in FP7, Luxembourg: Office for Official Publications of the European Communities, ISBN 978-92-79-05474-7) is not applicable to this study.

HEV genotyping and phylogenic analysis

HEV-RNA from patients and wild boars was used for partially amplification of the ORF2 gene using four overlapping primer pairs (Supplementary Table 1) [1,2]. An OneStep RT-PCR protocol provided by the manufacturer (Qiagen, Germany), was performed for each primer pair. RT-PCR products of expected sizes were analyzed by a 2% agarose gel electrophoresis and underwent bidirectional sequencing using the fluorescent BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

Evolutionary analyses were conducted in MEGA X [3] among 37 HEV strains including those described in this study. Twenty-seven HEV sequences of genotypes 1-4 were also retrieved from the GenBank database. Nucleotide sequences were aligned by CLUSTAL W and evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei substitution model [4]. One thousand bootstrap re-samplings were performed to calculate confident value of internal nodes.

Autoantibody testing in human serum samples

Antinuclear antibodies (ANA), smooth muscle antibodies (SMA), antibodies against liver kidney microsomal (anti-LKM), and against liver cytosol type-1 (anti-LC1) were initially detected by indirect immunofluorescence on 5-μm fresh frozen sections of in-house rodent kidney, liver, and stomach tissue substrates. Anti-LKM1, anti-LKM3 anti-LC1, and antibodies against soluble liver antigen/liver pancreas (anti-SLA/LP) were also evaluated by immunoblotting using rat liver microsomal or cytosolic extracts. Commercially available enzyme-linked immunosorbent assay kits using recombinant SLA/LP/tRNP (Ser) Sec (Inova Diagnostics, San Diego, CA, USA), cytochrome P450 2D6 (INOVA) and formiminotransferase cyclodeaminase (Euroimmun Medizinische Labordiagnostika, Lubeck, Germany) were also used for anti-SLA/LP, anti-LKM and anti-LC1 determination respectively, according to the manufacturer's instructions.

References to Supplementary material

1. van der Poel WH, Verschoor F, van der Heide R, et al. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands. *Emerg Infect Dis* 2001;**7**:970-976.
2. Oliveira-Filho EF, Bank-Wolf BR, Thiel HJ, König M. Phylogenetic analysis of hepatitis E virus in domestic swine and wild boar in Germany. *Vet Microbiol* 2014;**174**:233-238.
3. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;**35**:1547-1549.
4. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;**10**:512-526.