Supplementary material

Supplementary clinical information

Infectious disease panel: There was no evidence of serological IgM antibodies for cytomegalovirus, Epstein-Barr, measles, varicella, rubella, toxoplasmosis or hepatitis A viruses. However, IgG antibodies were found for rubella, Epstein-Barr, toxoplasmosis and hepatitis A viruses. Using RT-PCR, he was found to be negative for dengue, chikungunya and measles viruses. In addition, his bile culture was positive for non-specific gram-positive bacilli. Lastly, the patient's serum tested positive by polymerase chain reaction (RT-PCR) for Zika virus. Similarly, tests confirmed ZIKV in bile and gallbladder tissue *via* RT-PCR, with a 100% homology to a ZIKV sequence (see below).

Methods

Patient consent: Patient provided written consent for this case report

Genetic UGT1A1: Genomic DNA was isolated using QIAmp® DNA Blood mini kit (Qiagen, Hilden, Germany) and a 404 bp fragment was amplified by PCR with specific primers (UGT1A1F: 5' gaggttctggaagtactttgc 3' and UGT1A1R: 5' ccaagcatgctcagccag 3') designed for the promoter region of UGT1A1 gene from genomic DNA. Genotyping was performed by sequencing in 3500 ABI Applied Biossystems (Applied Biossystems, USA). Genotypes were assigned as Gilbert's syndrome if homozygous for the (TA)7TAA-allele (UGT1A1*28polymorphism), homozygous for the (TA)8TAA-allele or being (TA)7TAA-(TA)8TAA.

Antibody studies and cultures: Serum samples were tested at the reference laboratory by means of antibody-capture enzyme immunoassay for IgA, IgM, and IgG isotypes for pathogens described in *case report* section. Blood, urine, stool, bile and peritoneal fluid anaerobic and aerobic cultures were performed at a reference center.

Histological analysis of ZIKV: Gallbladder was collected for microscopic examination, and fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5μm and stained with hematoxylin and eosin for light-microscopy.

Immunohistochemistry staining for ZIKV was performed with either pan flavivirus 4G2 antibody (Millipore, Billerica, MA) or monoclonal anti-ZIKV kindly received from Dr. Sherif R. Zaki (Infectious Disease Pathology Branch, Centers for Disease Control and Prevention) as a primary antibody, however the results were negative. Inappropriate tissue storage at room temperature followed by freezing of formaldehyde-fixed material could partially explain these results.

Electron Microscopy: For transmission electron microscopy, small fragments of gallbladder were fixed in 2% glutaraldehyde in 0.15 M phosphate buffer, followed by post-fixation in 1% OsO_4, and block staining in 1% aqueous uranyl acetate overnight. The specimens were then embedded in an epoxy resin. Ultrathin sections were obtained with a Reichert ultra tome and double-stained by uranyl acetate and lead citrate. Micrographs were obtained with a JeolJEM 1010 electron microscope.

Detection of Zika virus (ZIKV) RNA: Bile specimen was diluted (1:10) in 1X phosphate-buffered saline and filtered using 0.2 µm filter. Total RNA purification from serum or filtered-diluted bile (300 µL) was performed using TRIzol LS kit (Ambion - Life Technologies, CA, USA). Chloroform was added to the homogenate, and the aqueous phase was collected after centrifugation followed by isopropanol precipitation with glycogen. After centrifugation, the pellet was washed with ice-cold ethanol, dried and eluted with RNase free water. Double stranded cDNA library was prepared using the SuperScript VILO Master Mix (Life Technologies, CA, USA). Real-time PCR assay for detection of the NS5 region (191bp) was performed with specific primers using the LightCycler 480 SYBR Green I Master (Roche, Mannheim, Germany).(1) In addition, next generation sequencing (NGS) was performed on RNA extracted from Vero cell supernatant following passage of infected tissue on the cell line. NGS was also performed on RNA extracted from the patient's gallbladder. After RNA purifications, sequence-independent single primer amplification and next generation sequencing (Illumina MiSeq, paired end 2x300 base pairs) were performed as described previously.(2) Read mapping and assembly were performed using CLC command-line algorithm. To identify ZIKV specific

sequences, all reads were mapped to a reference ZIKV genome center (accession number KU681082.3).

ZIKV sequencing: RT-PCR for ZIKV confirmed viral presence in bile and gallbladder tissue. Samples from cultured supernatant generated 284 reads mapped across the genome, with 279 reads forming a 327-base pair contig (average coverage of 196.89 across the contig). The nucleotide positions corresponded to 942 to 1268, with a 100% matching to the ZIKV genome KU681082.3 reference. RNA extracted from the gallbladder recovered two ZIKV specific reads when mapped against the KU681082.3 genome. Sequences will be deposited in the GenBank.

Supplementary references

1. Balm MN, Lee CK, Lee HK, Chiu L, Koay ES, Tang JW. A diagnostic polymerase chain reaction assay for Zika virus. J Med Virol 2012;84:1501-1505.
2. Moser LA, Ramirez-Carvajal L, Puri V, Pauszek SJ, Matthews K, Dilley KA, Mullan C, et al. A Universal Next-Generation Sequencing Protocol To Generate Noninfectious Barcoded cDNA Libraries from High-Containment RNA Viruses. mSystems 2016;1.