Probable transmission of hepatitis E virus (HEV) via transfusion in the United States

John R. Ticehurst,[1,2,3] Nora Pisanic,[4] Michael S. Forman,[3] Carly Ordak,[4] Christopher D. Heaney,[4,1,5] Edgar Ong,[6] Jeffrey M. Linnen,[6] Paul M. Ness,[7,8] Nan Guo,[1] Hua Shan,[9] and Kenrad E. Nelson[1,2]

BACKGROUND: Hepatitis E virus (HEV) can inapparently infect blood donors. To assess transfusion transmission of HEV in the United States, which has not been documented, a donor-recipient repository was evaluated.

STUDY DESIGN AND METHODS: To identify donations that contained HEV RNA and were linked to patient-recipients with antibody evidence of HEV exposure, we assayed samples from the Retrovirus Epidemiology Donor Study (REDS) Allogeneic Donor and Recipient repository that represents 13,201 linked donations and 3384 transfused patients. Posttransfusion samples, determined to contain IgG anti-HEV by enzyme-linked immunosorbent assay, were reassayed along with corresponding pretransfusion samples for seroconversion (incident exposure) or at least fourfold IgG anti-HEV increase (reexposure). HEV-exposed patients were linked to donations in which HEV RNA was then detected by reverse-transcription quantitative polymerase chain reaction, confirmed by transcription-mediated amplification, and phylogenetically analyzed as subgenomic cDNA sequences.

RESULTS: Among all patients, 19 of 1036 (1.8%) who had IgG anti-HEV before transfusion were reexposed; 40 of 2348 (1.7%) without pretransfusion IgG anti-HEV seroconverted. These 59 patients were linked to 257 donations, 1 of which was positive by reverse-transcription quantitative polymerase chain reaction and transcription-mediated amplification. Plasma from this donation contained 5.5 log IU/mL of HEV RNA that grouped with HEV genotype 3, clade 3abchij. The patient-recipient of RBCs from this donation had a greater than eightfold IgG increase; however, clinical data are unavailable.

CONCLUSIONS: This is the first report of probable HEV transmission via transfusion in the United States, although it has been frequently observed in Europe and Japan. Additional data on the magnitude of the risk in the United States are needed.

Hepatitis E virus (HEV) is a global pathogen that, among humans, is represented by a single serotype with four genotypes.[1,2] The virus is

ABBREVIATIONS: ARC = American Red Cross; C_T = threshold cycle (of a RT-qPCR run); BioLINCC = Biologic Specimen and Data Repository Information Coordinating Center, NHLBI, NIH; ELISA = enzyme-linked immunosorbent assay; HEV = hepatitis E virus; JHBSPH = Johns Hopkins Bloomberg School of Public Health; NHANES = National Health and Nutritional Evaluation Survey (National Center for Health Statistics, CDC); NHLBI = National Heart, Lung, and Blood Institute, NIH; NIH = National Institutes of Health, US Department of Health and Human Services; NIH CC = NIH Clinical Center; ORF = open reading frame; PCR = polymerase chain reaction; RADAR = Retrovirus Epidemiology Donor Study (REDS) Allogeneic Donor and Recipient; RT-qPCR = reverse-transcription quantitative PCR; S/CO = sample-to-cutoff value; S/CO_{post} = posttransfusion S/CO; S/CO_{pre} = pretransfusion S/CO; TMA = transcription-mediated amplification; WHO = World Health Organization.

From the [1]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland; [2]Division of Infectious Diseases, Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, Maryland; [3]Division of Medical Microbiology, Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Maryland; [4]Department of Environmental Health and Engineering, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland; [5]Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland; [6]Hologic Inc, San Diego, California; [7]Division of Transfusion Medicine, Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, Maryland; [8]Division of Oncology, Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, Maryland; and the [9]Department of Transfusion Medicine, Stanford University, Palo Alto, California.

Address reprint requests to: in lieu of initial corresponding author Kenrad Nelson, John Ticehurst, Division of Medical Microbiology, Department of Pathology, Meyer B-130, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21287; e-mail: jticehur@jhmi.edu

This work was supported by grant R21 HL121740 from NHLBI, NIH. CDH was supported by E.W. "Al" Thrasher Award 10287 from the Thrasher Research Fund; grant 1316318 from NSF as part of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program; grant K01OH010193 from NIOSH, CDC; and grant R01ES026973 NIEHS, NIH.

Received for publication January 1, 2018; revision received September 30, 2018, and accepted October 2, 2018.

doi:10.1111/trf.15140

© 2019 AABB

TRANSFUSION 2019;59;1024–1034

commonly acquired by enteric transmission and, in developing countries, genotypes 1 and 2 can cause large waterborne epidemics associated with monsoon rain or humanitarian emergencies with contaminated supplies of drinking water. More recently, autochthonous HEV infections have been reported frequently among populations in industrialized countries. Such infections are associated with genotype 3 or 4, and usually occur as isolated cases or in small clusters. They commonly include asymptomatic infections of adults who acquire HEV from contaminated food, especially solid-organ meats from swine, wild boar, deer, or raw shellfish.

HEV transmission by transfusion has been reported since 2004 from Europe and Japan.[3–12] A study of 225,000 southeastern UK donors identified 79 (0.035%) with detectable HEV RNA.[8] Among 43 patients who were transfused with these donors' HEV RNA-containing products, 18 (42%) became infected. Chinese, European, and American investigators have detected HEV RNA in blood products, including pooled plasma, from otherwise acceptable donors.[6,13–17] In Japan, a total of 20 patients were reported to have acquired HEV by transfusion of blood products.[12] Consequently, blood centers in Hokkaido prefecture of northern Japan have routinely screened donors for HEV RNA during the past 10 years to prevent transmission by transfusion.[11,12]

Despite these international reports, limited data have been reported from blood centers in the United States. A study of 1939 donors at the National Institutes of Health Clinical Center (NIH CC, Bethesda, MD), who were sampled in 2006 and 2012, found 18.8% with IgG anti-HEV and 0.4% with IgM anti-HEV but none had detectable HEV RNA.[18] A study of 18,829 American Red Cross (ARC) donation samples, collected during 2013, identified 2 (0.01%) with HEV RNA, 7.7% with IgG anti-HEV, and 0.58% with IgM anti-HEV.[16] Another study of ARC donors, 5040 who were sampled in 2015, detected IgG anti-HEV among 11.4%; 0.18% had IgM anti-HEV detected by each of three assays, among which there was only 22% agreement.[19] HEV transmission in these US studies could not be assessed, however, because donations were not linked to blood product recipients.

To evaluate the risk of HEV transmission by transfusion in a US population, we tested samples from the Retrovirus Epidemiology Donor Study (REDS) Allogeneic Donor and Recipient (RADAR) repository. [20] This collection was organized between 2000 and 2003 by seven US blood centers. It links 13,201 donations, from 12,408 donors, with 3575 patients in eight California, Florida, Maryland, Michigan, Oklahoma, and Pennsylvania hospitals; these patients had cardiac, vascular, or orthopedic operations. The RADAR repository contains plasma samples from donors, and paired plasma samples that were collected from patients before or immediately after transfusion, and 6 to 12 months later.

MATERIALS AND METHODS

Patient-recipient and donation samples

The RADAR repository[20] is maintained by the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC, NHLBI, NIH; see Web Resources). We initially obtained all 3384 posttransfusion samples that were available from the 3575 patient-recipients, and subsequently obtained selected pretransfusion and donation samples according to the testing algorithm below.

Reference materials

We conducted limited assessments of assay performance with two World Health Organization (WHO) reference materials and a characterized research-specimen. These WHO materials were: WHO Reference Reagent for HEV Antibody, reconstituted with water to 100 U/mL ([UK] National Institute for Biological Standards and Control code 95/584); and First WHO International Standard for Hepatitis E Virus RNA Nucleic Acid Amplification Techniques-Based Assays, reconstituted with water to 5.7 log IU/mL (Paul-Ehrlich-Institut code 6329/10). The latter contains genotype 3 strain HRC-HE104, the complete genomic sequence of which has GenBank accession AB630970.[21] The characterized research specimen was bile, containing approximately 9.8 log IU/mL of HEV subtype 2a, from a cynomolgus monkey that was experimentally infected with strain Mexico-14 in human feces; GenBank M74506 and KX578717 correspond to HEV in the fecal specimen.[22–24]

Testing algorithm for repository specimens

Our approach was intended to identify patients who had antibody evidence of HEV exposure during the pre- to posttransfusion sampling interval, and then HEV RNA–containing donations that were likely sources of such exposure (Fig. 1). We use the term *exposure,* rather than *infection* because the latter term might imply degrees of HEV replication and HEV-associated disease that could not be determined.

Detection and semiquantitation of IgG anti-HEV

We assayed patients' plasma specimens by using a commercially available enzyme-linked immunosorbent assay (ELISA) (Wantai HEV-IgG ELISA [WE-7296], Beijing Wantai Biological Pharmacy Enterprise), generally following manufacturer's instructions. Minor modifications included reassaying selected patients' specimens, which had yielded posttransfusion IgG anti-HEV sample-to-cutoff values (S/CO_{post}) > 6.0, diluted 1:4 or 1:8 in phosphate-buffered saline, pH 7.0, with 1% wt/vol bovine serum albumin. By assaying such two-fold dilutions of the WHO Reference Reagent for HEV Antibody, we determined analytic sensitivity of the IgG anti-HEV ELISA to be 1 U/mL according to preassay concentration, or

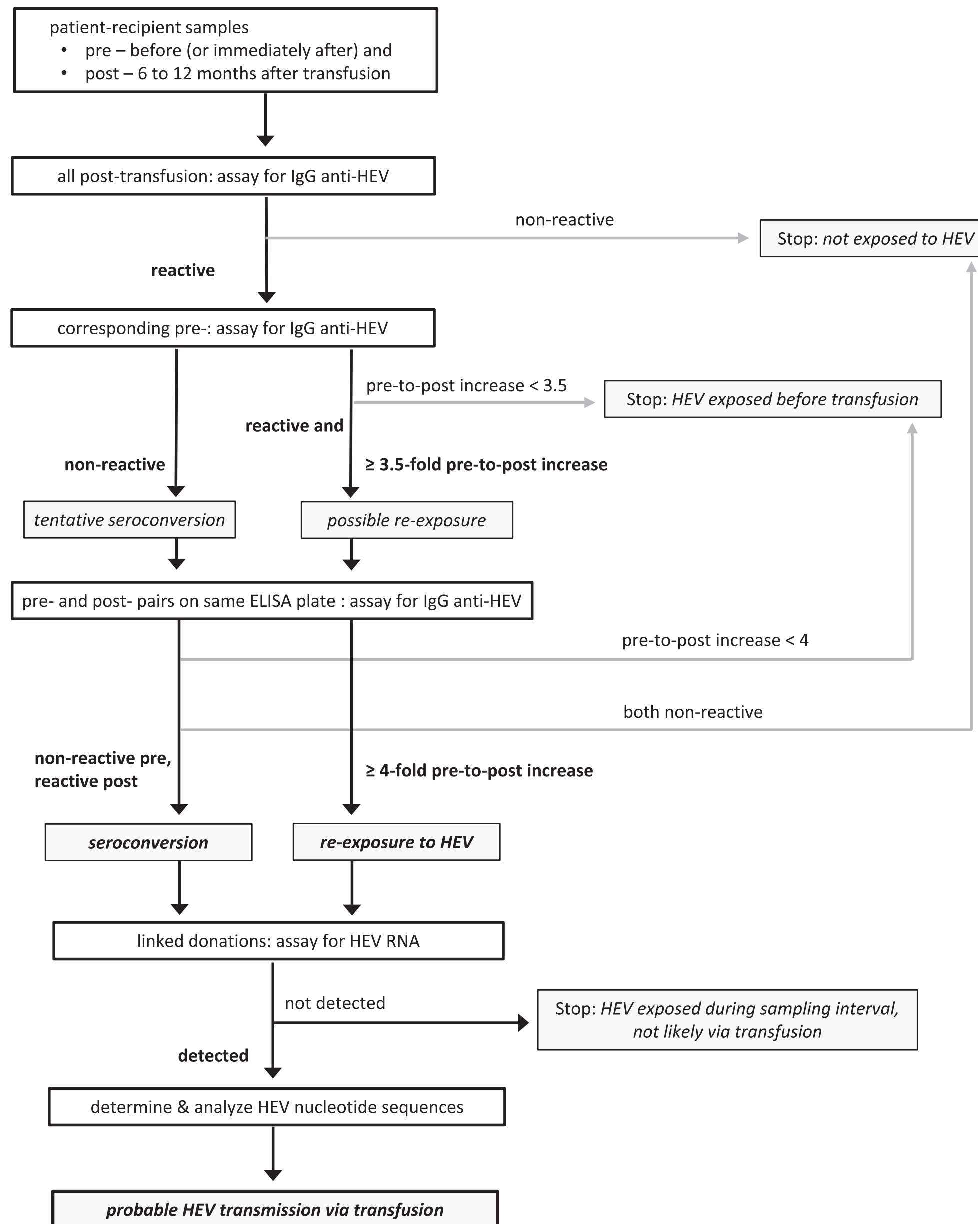


Fig. 1. Testing algorithm for patient-recipient and donation samples from the RADAR repository. We assayed posttransfusion specimens for IgG anti-HEV and, for those with reactive results, tested corresponding pretransfusion specimens in subsequent assay runs. Each specimen pair that yielded preliminary evidence of HEV exposure during the sampling interval, as manifested by seroconversion or by a 3.5-fold or greater increase in IgG anti-HEV S/CO value, was reassayed on a single ELISA plate. We then assayed for HEV RNA in donations that were linked to patients who had single-plate confirmed seroconversion or a fourfold or greater increase of IgG anti-HEV concentration. Finally, we determined and analyzed partial nucleotide sequences of any detected HEV RNA in donation samples.

0.091 U/mL in ELISA (Table S1, available as supporting information in the online version of this paper).

When a posttransfusion specimen was nonreactive for IgG anti-HEV, the corresponding pretransfusion sample was not tested, and the patient interpreted as not HEV exposed before or during the sampling interval. When a posttransfusion specimen was reactive, we tested the corresponding pretransfusion sample and compared results with those obtained earlier for posttransfusion specimens. We defined patients as having tentatively seroconverted when pretransfusion results were nonreactive or equivocal (S/CO_{pre} <1.1), and possibly increased IgG anti-HEV concentration when S/CO_{post} was at least 3.5-fold higher than S/CO_{pre} of 1 or greater.

We then reassayed, on the same ELISA plate, each specimen pair that yielded such preliminary evidence of sampling interval exposure. Same-plate IgG anti-HEV

results were used to identify 1) incident exposure, or seroconversion, defined as pretransfusion nonreactive (S/CO_{pre} <1) and posttransfusion reactive; 2) reexposure, as both specimens reactive and posttransfusion concentration at least fourfold higher than before transfusion (S/CO_{post} after fourfold dilution ≥ S/CO_{pre}); and 3) past exposure, as both specimens reactive and S/CO_{post} after fourfold dilution less than or equal to S/CO_{pre} or, without dilution, S/CO_{post} less than 6.0 and less than four times S/CO_{pre}. Our reexposure criterion was based on a linear and approximately 1:1 correlation between WHO U/mL and IgG anti-HEV S/CO ranging from 0.25 to 6.0 (Table S1).

Detection and quantitation of HEV RNA

We tested donation samples, identified as linked to patients who had serologic evidence of HEV exposure during the sampling interval, by using assays that are based on polymerase chain reaction (PCR) or transcription-mediated amplification (TMA).

To generate templates for reverse-transcription quantitative PCR (RT-qPCR) and for sequence analysis (below), we added approximately 4.7 log pfu of coliphage MS2[25] and 8 μg of yeast transfer RNA to 200 μL of plasma or reference material. We then purified RNA and DNA by using MagNA Pure LC Total Nucleic Acid Isolation-High Performance kits with the MagNA Pure LC 2.0 instrument, Roche Diagnostics), eluting into 100 μL of proprietary (Roche) buffer.

We detected and quantified HEV RNA with a RT-qPCR assay that our Johns Hopkins Bloomberg School of Public Health (JHBSPH) laboratory implemented for environmental and plasma samples[26,27] and then adapted to increase sensitivity and throughput. We mixed 5 μL of purified nucleic acids into a 20-μL reaction with a master mix product (VeriQuest Probe One-Step qRT-PCR Master Mix, Affymetrix/USB) and oligonucleotide sets for amplifying a highly conserved segment of the HEV genome[28] (primers, 500 nM; probe, 250 nM) and for MS2[25] (primers, 250 nM; probe, 125 nM; for sequences, see Table S2, available as supporting information in the online version of this paper). RNAs were reverse transcribed and then amplified in a qPCR system (Applied Biosystems StepOnePlus Real-Time PCR system, Thermo Fisher Scientific) by incubating at 50°C for 15 minutes and 95°C for 10 minutes; and then 45 cycles of 95°C for 15 seconds, 55°C for 20 seconds, and 60°C for 20 seconds. The quantitation standard was cloned HEV cDNA, assayed as a 10-fold dilution series of concentrations between 0.5 log and 5.5 log copies/reaction. Samples that yielded an HEV threshold cycle (C_T) value of 38.0 or less were considered to be positive. Analytic sensitivity was 2.5 log IU/mL of plasma, or 0.5 log IU/reaction; 0.5 $\log_{10}$ IU corresponded to 1.5 $\log_{10}$ copies of cloned HEV cDNA.

To confirm selected RT-qPCR results, donation specimens were tested with a TMA-based assay (Procleix HEV, Hologic; and Grifols Diagnostic Solutions) that has a 95% detection probability of 0.90 log IU/mL.[16] This assay requires 0.7 mL of specimen for singulate testing; because the volume of many RADAR samples is extremely limited, selected samples were diluted as much as eightfold (i.e., 0.1 mL of sample with 0.7 mL of proprietary buffer).

Determination and analysis of HEV cDNA nucleotide sequences

We synthesized and then amplified HEV cDNA via nested PCR with primers that represent segments of HEV open reading frame (ORF) 1 and ORF2[29–31] (Table S2; Ticehurst and Forman, unpublished data). Sanger sequence reads were generated from nested PCR products by using a genetic analyzer (3500 Genetic Analyzer, Applied Biosystems) and then base-called, trimmed to amplicon-length without primers, and assembled by computer software (Aligner version 8.0.1, CodonCode; and BioEdit version 7.2.5 [for availability, see Web Resources]) to yield sequences for phylogenetic analysis.

We constructed maximum-likelihood phylogenetic trees via PhyML[32] at the Web site of Le Laboratoire d'Informatique, de Robotique et de Microélectronique de Montpellier (Université Montpellier; see Web Resources) with this laboratory's default parameters; we did not choose optional Gblocks curation.[33] Before submitting for analysis, we used BioEdit version 7.2.5 to trim reference-sequences to RADAR HEV cDNA length, and to align all sequences via ClustalW[34] (included with BioEdit). We initially constructed a partial-ORF1 tree by using 158 unique HEV reference sequences[35] and the approximate likelihood ratio test for branch assessment.[33] For clarity in this presentation, we remade the partial-ORF1 tree with 32 taxa, 27 of which represent consensus reference strains for the four human HEV genotypes and a genotype 5 representative as outgroup,[36] plus three other well-characterized human strains.[35] For this tree, we statistically assessed branches by bootstrapping with 100 resamplings, outgroup-rendered the tree with computer software (TreeView version 1.6.6; for availability, see Web Resources), and annotated it by using presentation software (PowerPoint 2016, Microsoft). We similarly generated trees from partial ORF2 sequences (not shown). We also compared nucleotide sequences representing our JHBSPH laboratory's HEV strains to determine if those representing the RADAR donation are unique; that is, not the result of contamination.

RESULTS

IgG anti-HEV in single and paired specimens from RADAR patient-recipients

Among all 3384 patients, 1036 (30.6%) had detectable IgG anti-HEV before transfusion (Table 1). Based on changes in IgG anti-HEV reactivity at 6 to 12 months after transfusion,

TABLE 1. Evidence of HEV exposure among 3384 RADAR patients, based on comparison of pre- and posttransfusion results for IgG anti-HEV

After transfusion		Before transfusion		Pre- to posttransfusion		
IgG anti-HEV result	No.	IgG anti-HEV result	No.	Change	No.	Interpretation
Reactive	1076	Nonreactive	40	Seroconversion	40	Incident exposure
		Reactive	1036	≥ Fourfold increase*	19	Reexposure
				< Fourfold increase	1017	Past exposure (without reexposure)
Nonreactive	2308	Not tested (presumed nonreactive)	2308	None determined	2308	No exposure
Total	3384					

* A four-fold or greater increase, S/CO value of 1:4 diluted posttransfusion sample greater than or equal to S/CO value of undiluted pretransfusion sample (see Materials and Methods and Table S1).

59 patients (1.7%) were determined to have seroconverted or been reexposed after the pretransfusion specimen was collected. Incident exposures occurred in 40 of 2348 patients (1.7% of 2308 + 40; Table 1) who had not been HEV exposed before transfusion. The 19 reexposures represent 1.8% of the 1036 previously exposed patients.

Detection and analysis of HEV RNA

The 59 RADAR patients who had evidence of HEV exposure were linked to 257 donations from 257 donors, all of which were assayed for HEV RNA by RT-qPCR. Fifteen of 257 (5.8%) were positive: one yielded a C_T of 26.9, the 14 other C_T values ranged between 33.9 and 37.5. Seventeen of these 257 donations, including 14 RT-qPCR positives and 1 that yielded an invalid result (MS2 internal control not detected), were also assayed by using the Procleix HEV assay. One (0.008% of 13,201 linked donations) RT-qPCR-positive was confirmed, that for which C_T was 26.9. We also RT-qPCR assayed remaining plasma (50 μL before transfusion, 200 μL after transfusion) from the recipient of the HEV RNA–containing donation; neither had detectable HEV RNA.

By RT-qPCR, the HEV RNA-confirmed specimen contained 5.5 $\log_{10}$ IU per mL of plasma. This RNA phylogenetically represents HEV genotype 3, clade 3abchij,[37] based on HEV ORF1 (Fig. 2) and ORF2 (data not shown) nucleotide sequences that are also distinct from those of all other strains in our JHBSPH laboratory. Analogous subgenomic sequences of coamplified cDNAs that represent the First WHO International Standard for HEV RNA (genotype 3, grouping with clade 3abchij[37]) are identical to those in GenBank and are represented in Fig. 2. Sequences representing HEV subtype 2a strain Mexico-14 in monkey bile, determined from separately amplified cDNA, are 99.7-100% identical to those in GenBank. (See Web Resources for new accession numbers.)

Characteristics of the donation with detectable HEV RNA and selected patient-recipients

The HEV RNA–containing donation was from an individual who made a single donation that was documented in the RADAR archive. This donation was transfused as RBCs to one patient who received three other RBC units, each from one donor. This patient's IgG anti-HEV concentration increased more than eightfold after transfusion; that is, the S/CO of a 1:8 diluted posttransfusion sample was greater than the S/CO of a neat pretransfusion sample (Table 2). As noted above, HEV RNA was not detected in either of this patient's specimens.

Nine of the other 58 IgG anti-HEV seroconversions and reexposures were linked to an HEV RNA–negative donation that was also linked to a second recipient. In two such instances, both recipients seroconverted. Otherwise, the second recipient did not have evidence of exposure during the sampling interval: both specimens were reactive for IgG anti-HEV without a pre- to posttransfusion increase, or both were nonreactive (data not shown).

It is not known if any donor or patient developed symptoms or signs of HEV-associated disease or if patients had foodborne or other types of exposure to HEV because repository data do not contain such information about RADAR subjects. Certain demographic characteristics of RADAR donors and patient-recipients are available; BioLINCC and NHLBI do not allow such characteristics in publications, however, because of privacy concerns.

DISCUSSION

To our knowledge, our report provides the first documentation of probable HEV transmission via transfusion in the United States, from an HEV RNA–containing donation to a patient who had antibody evidence of HEV exposure. Our data are suggestive of reexposure because the patient had IgG anti-HEV that increased in concentration after transfusion; IgG anti-HEV evidence of HEV reexposure has been reported.[38] Because the RADAR database does not include subjects' clinical data, we cannot determine if this patient-recipient developed any HEV-associated illness. Pathogenic association with either clade 3abchij, with which the RADAR-donation HEV RNA phylogenetically grouped (Fig. 2), or clade

3efg, was not identified by an analysis of genotype 3 infections in the United Kingdom and Western Europe during 2003 to 2015.[37]

To detect HEV transmission that was temporally associated with transfusion, our strategy was to evaluate all possible incident and secondary exposures by assaying linked

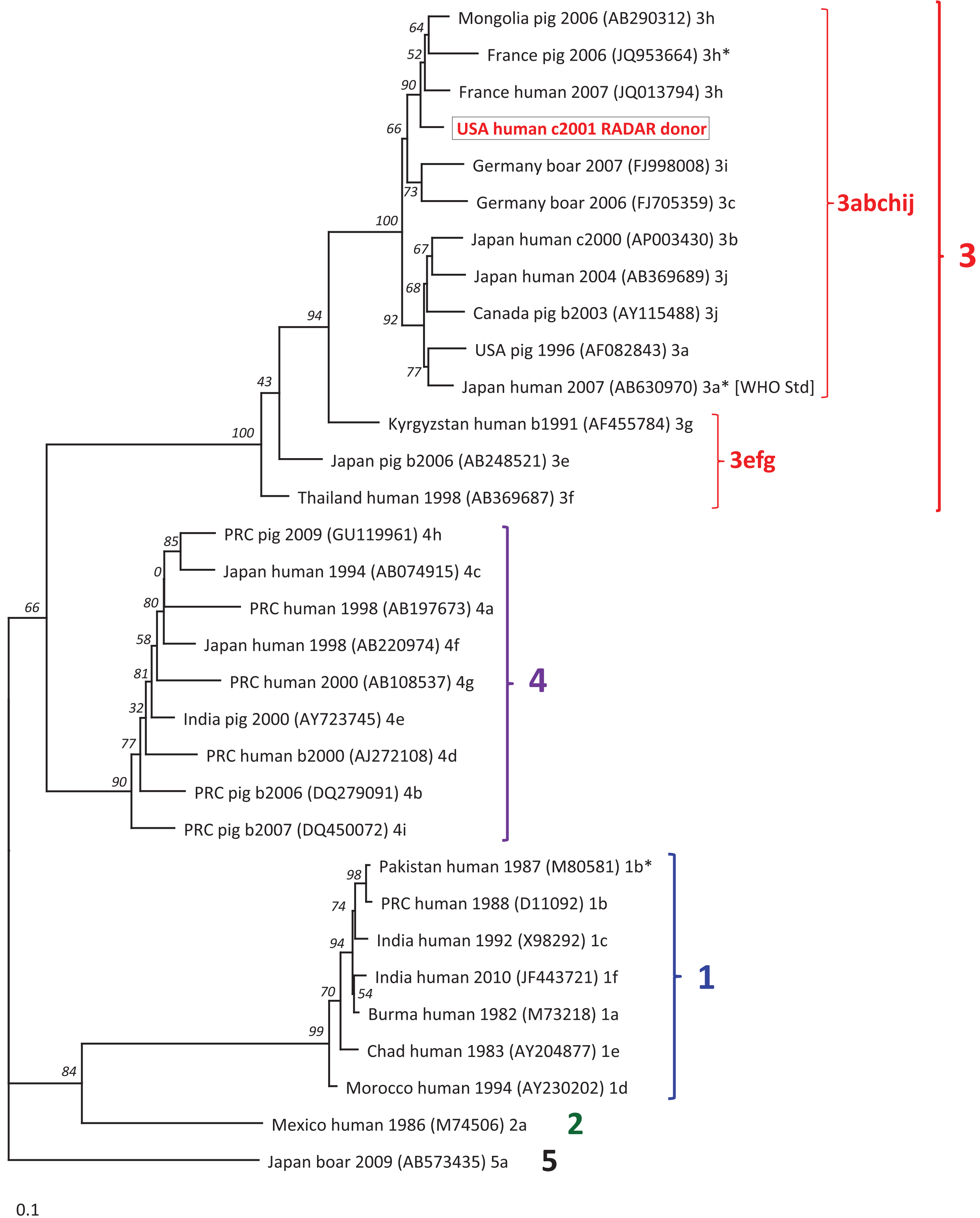


Fig. 2. Legend on next page.

TABLE 2. IgG anti-HEV S/CO values for recipient of HEV RNA-containing RBCs, demonstrating increased concentration of IgG anti-HEV after transfusion*

	Pretransfusion			Posttransfusion		
ELISA plates	Neat	1:4	1:8	Neat	1:4	1:8
Separate (initial-testing runs)	8.91			17.11		
Same (reassay run)	10.16	3.63	1.29	16.15	14.93	14.12

* Neat, assayed without dilution; 1:4 and 1:8, assayed after respectively diluting four- and eightfold in phosphate-buffered saline, pH 7.0, with 1% wt/vol bovine serum albumin.

donations for HEV RNA (Fig. 1). By testing paired recipient specimens for IgG anti-HEV, we identified patients who had antibody evidence of exposure during the pre- to posttransfusion interval, thereby reducing the number of donations to assay for HEV RNA. The 59 identified HEV exposures are based on same-plate ELISA testing that reproduced earlier results from separate runs in which the identity of specimen pairs was blinded.

One cannot unambiguously conclude that a blood product is the source of HEV transmission unless the donor is determined to circulate infectious HEV, which most likely would require inoculation of a susceptible primate. While more definitive evidence of transfusion transmission would include a donation and linked recipient with identical or nearly identical HEV RNAs, it is extremely unlikely that RADAR posttransfusion samples, like others collected from immunocompetent patients at least 6 months after exposure,[12,39–41] would contain HEV RNA. We also cannot rule out a temporal association, without transmission, between the HEV RNA–containing donation and linked patient-recipient: better evidence would include detectable or increased anti-HEV in posttransfusion specimens collected sooner than those in the RADAR repository.

Transfusion transmission accounts for a minority of all HEV infections except possibly those among highly transfusion-dependent patients. Based on an estimated 0.2% annual HEV incidence in the United Kingdom, investigators there estimated that the ratio of foodborne to transfusion-acquired HEV was approximately 13:1.[42] This ratio may be higher in the United States because, among the 59 incident exposures and reexposures that we identified, only one could be associated with an HEV RNA–containing donation.

We may have underestimated transfusion transmission risk, however, because RADAR patients also received 11,141 blood components from donors who were not enrolled in the study and therefore could not be linked to HEV-exposed patient-recipients and screened for HEV RNA.[20] In addition, RADAR patients who died less than 6 months after transfusion were not studied because posttransfusion specimens could not be collected. Patients who contributed paired specimens were generally immunocompetent, but others who might have been at higher risk of HEV infection (e.g., organ transplant recipients) were not included.[20] We also may have underdetected HEV RNA–containing donations because the confirmatory assay for HEV RNA was considerably more sensitive than that we used for initial HEV RNA detection.

We encountered other limitations that are worth noting. First, our RT-qPCR assay yielded positive results that failed confirmation via the more sensitive TMA-based assay, and we generated HEV sequences only from the TMA-confirmed donation and two HEV RNA reference materials. While it is very unlikely that these false positives resulted from cross contamination, several pertinent samples yielded human DNA after nested PCR with primers for HEV ORF1 or ORF2 (data not shown). Computer-assisted searches did not reveal high identity between GenBank human sequences and our RT-qPCR oligonucleotides for HEV ORF3 and coliphage MS2. Other groups have noted failure to reproduce initial HEV RNA detection[16] or have successfully coamplified HEV and MS2 cDNAs.[43] Second, we attempted to generate IgM anti-HEV data with a commercial μ-capture

FIG. 2 Phylogenetic tree of a 530-nucleotide segment of HEV ORF1 from 31 reference taxa and a RADAR donation. This tree is a rectangular phylogram with an HEV genotype 5 outgroup. Bootstrap values, as a percentage of 100 resamplings, are indicated by italicized numerals near branch points. Reference sequences[35,36] are designated by country (PRC, People's Republic of China); host; collection year (b, before the earlier of GenBank deposition or publication; c, circa, the midpoint in a range of possible years); GenBank accession number, in parentheses; and clade assignment by Smith et al.[36,37] or with asterisk, Vina-Rodriguez et al.;[35] [WHO Std], First WHO International Standard for Hepatitis E Virus RNA Nucleic Acid Amplification Techniques-Based Assays. All recognized human subtypes of genotypes 1, 2, 3, and 4 are represented except 3d, for which only ORF2 sequences have been reported,[36] and 3ra that primarily represents rabbits and for which there is one reported human-strain sequence that includes the pertinent ORF1 segment.[57,58] Boxed "USA human c2001 RADAR donor" designates sequence from this study. Largest numerals and brackets indicate genotypes; numeral 3 followed by letters indicate proposed monophyletic groups.[37] Bar indicates genetic distance. [Color figure can be viewed at wileyonlinelibrary.com]

ELISA and an analogous approach to that for detecting IgG anti-HEV. The overall frequency of IgM anti-HEV reactivity was higher than in other studies, and most reactive results were implausible or uninterpretable; some such reactivity may have been caused by nonspecific binding between captured IgM or other specimen material and reagent HEV ORF2 protein (data not shown). Furthermore, sample-collection timing made it impossible to determine if any patient developed IgM anti-HEV soon after transfusion and then "seroreverted" to undetectable when the posttransfusion specimen was collected. While posttransfusion IgM and IgG anti-HEV might be considered as more definitive evidence for exposure, assays for IgM antiviral antibodies are typically configured to yield predominantly nonreactive results by 6 months into convalescence. Third, although interrun repeatability was a requirement for incident- or reexposure categorization, we cannot exclude the possibility of false-positive IgG anti-HEV results because we did not independently verify reactivity (e.g., via western blot immunoassay) and our 31% frequency of IgG anti-HEV reactivity is high. Noting that RADAR patient-recipients were older (91% and 74% at least 50 and 60 years old, respectively) and predominantly male (54%),[20] our IgG anti-HEV frequency is consistent with several of those reported for older US subpopulations[18,19,44,45] (see discussion below about prevalence).

The only other HEV study of US recipients and linked donors, by Xu et al.,[18] investigated 362 patients near or in Washington, D.C. (NIH CC, Suburban Hospital, and Children's National Medical Center), starting in 2001. Two patient-recipients, including one who received an HEV RNA–containing and a "high-titer" anti-HEV product shortly before death, became reactive for IgG anti-HEV, but the authors concluded that neither patient had a transfusion-associated exposure to HEV. Another publication reported the results of retrospectively assaying cryopreserved specimens that were collected during the 1960s, prior to routine donor screening for viral markers, from 66 NIH CC cardiac surgery patients who developed post-transfusion hepatitis: 4 (6%), 20 (31%), and 1 (2%), respectively, were infected with hepatitis B virus, hepatitis C virus, and HEV.[46] It is highly likely that many transfusion transmissions of HEV have gone unrecognized in the United States. Linking donations to American recipients has been difficult because most blood product collection, processing, and distribution are centralized, and the products are often transfused after a substantial interval. Also, foodborne transmission of HEV genotype 3 is likely to be more common than infection from a transfusion in the United States, a likelihood with which our data are consistent. In addition, the lack of FDA-licensed assays for detecting serologic or virologic evidence of HEV infection, as well as US clinicians' unfamiliarity with hepatitis E and extrahepatic manifestations of HEV infection,[47] are important barriers to diagnosis.

However, large population-based surveys have documented high anti-HEV prevalence in the general US population. A study by Kuniholm et al.,[44] of 18,695 individuals from the Third National Health and Nutrition Examination Survey (NHANES) that represents the 1988–1994 US population, found an IgG anti-HEV prevalence of 21% by using an assay that was developed at NIH.[48] Another study, using an ELISA that has been reportedly[49] less analytically sensitive than the Wantai HEV-IgG ELISA (that we used) and the NIH-developed assay, determined a decline in IgG anti-HEV prevalence from that in the 1998–1994 NHANES population (10% weighted, 17% unweighted) to that in the 2005–2006 NHANES population (6%, weighted or unweighted).[50] Regardless of diminishing IgG anti-HEV prevalence, which others have recognized,[18,51,52] these reports have provided persuasive evidence that HEV infections, which often are subclinical, are common in the United States.

Population-based studies have consistently detected increasing IgG anti-HEV seroprevalence with age, and other studies have reported high anti-HEV frequency among older Americans, especially men, who were sampled at about the same time as RADAR patient-recipients. The above-cited study that demonstrated declining NHANES anti-HEV prevalence[50] detected, among subjects who were US born and at least 50 years old, 25% unweighted IgG anti-HEV reactivity in the 1988–1994 NHANES subpopulation and 11% in the 2005–2006 subpopulation. Among 1988–1994 NHANES US-born males studied by Kuniholm et al.,[44] approximately 31% of those who were 50 to 59 years old and approximately 39% of those 60 years old or older had IgG anti-HEV. A 2002 publication[45] reported using the same NIH-developed ELISA as Kuniholm et al.,[44] and detecting IgG anti-HEV among 27% of 120 blood donors who were 50 years old or older. Among 574 blood donors greater than 45 years old who were sampled during 2006 at the NIH CC, 30% had IgG anti-HEV detected by a Wantai ELISA[18] that was likely to be similar to the ELISA we used. In a study of more recently collected (during 2015) ARC samples, which included approximately 1600 from donors 50 years old or older and testing with a Wantai IgG anti-HEV ELISA, reactive frequencies ranged from approximately 16% for 50- to 55-year-old donors to approximately 44% for those between 80 and 93 years old.[19] Incident infections are also likely to be frequent in older men[53]; although the frequency of incident HEV exposures among RADAR patient-recipients was higher than that reported for general populations,[1,53] our data may reflect higher incidence in an older and predominantly male RADAR population.[20]

During recent years, there has been increasing recognition of the risk of transmitting HEV by transfusion outside of the United States. While infections with HEV genotype 3 are common among adults and frequently asymptomatic in the United States and Europe,[1,2] a substantial portion of patients in industrialized countries who need transfusions are immunocompromised. Several European countries and Japan's Hokkaido prefecture have considered or adopted selective

screening of blood products for transfusion into high-risk patients or routine screening of all donors.[11,12,54] The United Kingdom has elected to screen all donors for HEV RNA because a high proportion of transfusion recipients, those who are immunocompromised, may be at increased risk of more severe HEV[54]; chronic progressive hepatitis E has been reported among immunocompromised patients, especially those with solid-organ transplants.[12]

A recent publication from the Netherlands concluded that screening of blood donors for HEV could have a reasonable cost–benefit ratio.[55] Among US donations, reported HEV RNA detection frequencies (76, 102, and 23 per million, respectively, during 2000–2003 [this study], 2013,[16] and 2015[17]) are similar to those for hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type 1 RNA (76, 200, and 28 per million, respectively, during 2011–2012[56]), for which testing is currently performed; the latter frequencies are 41 to 240 times higher than corresponding infection frequencies (1, 0.83, and 0.67 per million[16]). It is not known if US infection and illness frequencies for HEV are comparable, for example, to those reported for the southeastern United Kingdom, where 18 of 43 recipients of HEV RNA–containing blood products became infected, among whom 5 had elevated serum concentrations of alanine aminotransferase, including 1 with clinically apparent hepatitis, and 10 developed prolonged or persistent infection.[8] Health economic analysis, similar to that performed for the Netherlands,[55] could be important for the United States; however, the data on HEV transmission in the United States are too scarce to do such an analysis at present.

In conclusion, we detected one case of likely transfusion transmission of HEV among a population of 3384 transfused patients in the United States. These recipients were exposed to approximately 25,000 blood components, among which 13,800 were from linked donations.[20] We were able to identify this case even though the RADAR population was much smaller than the UK linked study population.[8] Our study's source donor, who likely transmitted HEV, had an HEV RNA plasma concentration of 5.5 log IU/mL. This level of HEV RNA was consistently associated with HEV transmission from donors in the large UK study[8] and is much greater than those of the two HEV RNA positive donors in the ARC study[16] or the three in a recent study of US plasma donors.[17] To our knowledge, our report documents for the first time that the risk of transfusion-transmitted HEV probably exists in the United States. Further quantifying this risk, and potentially developing a strategy to prevent HEV transfusion transmission to US patients at high risk of complicated infections, should be priorities.

ACKNOWLEDGMENTS

We thank Robin Cory, Tim Shin, Anh Hoang, and Graham Anderson for their technical assistance. We also acknowledge BioLINCC and NHLBI staff for considering and fulfilling our requests.

CONFLICT OF INTEREST

JRT provides professional services, as a part-time contractor, to CSL Plasma Inc. EO and JML were Hologic Inc employees when the reported findings were generated. The other authors have disclosed no conflicts of interest.

WEB RESOURCES

We obtained RADAR samples from BioLINCC (biolincc.nhlbi.nih.gov/studies/radar). BioEdit software is available from author Tom Hall at www.mbio.ncsu.edu/bioedit/page2.html. We generated phylogenetic trees at the Phylogeny Analysis page (phylogeny.lirmm.fr/phylo_cgi/phylogeny.cgi) of Le Laboratoire d'Informatique, de Robotique et de Microélectronique de Montpellier, Université Montpellier, Montpellier, France.[33] Each of these sites was accessed January 12, 2019. TreeView was obtained from author Rod Page at taxonomy.zoology.gla.ac.uk/rod/treeview.html, a site that was no longer accessible on January 12, 2019, when it was available at treeview.software.informer.com/download.

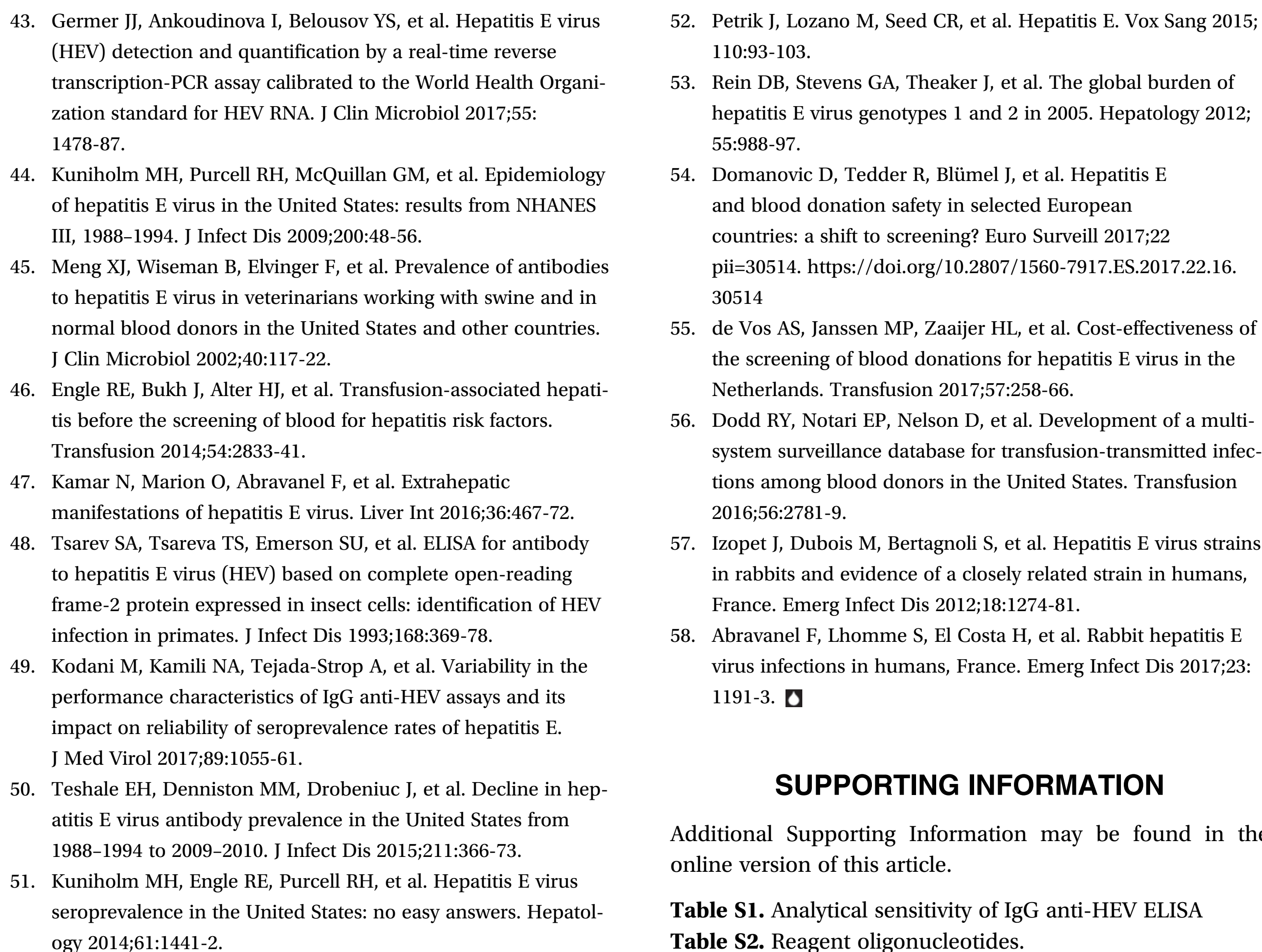
Nucleotide sequences, described above and representing segments of ORF1 and ORF2 of HEV RNA in RADAR-donation plasma, have been deposited to GenBank with accession numbers MK385653 and MK385654. Nucleotide sequences that represent segments of ORF1, the region in which ORFs 1-3 overlap, and ORF2 of HEV in cynomolgus monkey bile have accession numbers MK385655-MK385657.

REFERENCES

1. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. N Engl J Med 2012;367:1237-44.
2. Nelson KE, Heaney CD, Kmush BL. The epidemiology and prevention of hepatitis E virus infection. Curr Epidemiol Rep 2017; 4:186-98.
3. Ankcorn MJ, Tedder RS. Hepatitis E: the current state of play. Transfus Med 2017;27:84-95.
4. Boxall E, Herborn A, Kochethu G, et al. Transfusion-transmitted hepatitis E in a "nonhyperendemic" country. Transfus Med 2006;16:79-83.
5. Colson P, Coze C, Gallian P, et al. Transfusion-associated hepatitis E, France. Emerg Infect Dis 2007;13:648-9.
6. Haïm-Boukobza S, Ferey MP, Vétillard AL, et al. Transfusion-transmitted hepatitis E in a misleading context of autoimmunity and drug-induced toxicity. J Hepatol 2012;57:1374-8.
7. Huzly D, Umhau M, Bettinger D, et al. Transfusion-transmitted hepatitis E in Germany, 2013. Euro Surveill 2014;19 pii=20812. https://doi.org/10.2807/1560-7917.ES2014.19.21.20812
8. Hewitt PE, Ijaz S, Brailsford SR, et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet 2014;384:1766-73.
9. Mitsui T, Tsukamoto Y, Yamazaki C, et al. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan:

evidence for infection with genotype 3 HEV by blood transfusion. J Med Virol 2004;74:563-72.

10. Matsubayashi K, Nagaoka Y, Sakata H, et al. Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion 2004;44:934-40.
11. Matsubayashi K, Kang JH, Sakata H, et al. A case of transfusion-transmitted hepatitis E caused by blood from a donor infected with hepatitis E virus via zoonotic food-borne route. Transfusion 2008;48:1368-75.
12. Satake M, Matsubayashi K, Hoshi Y, et al. Unique clinical courses of transfusion-transmitted hepatitis E in patients with immunosuppression. Transfusion 2017;57:280-8.
13. Wang M, He M, Wu B, et al. The association of elevated alanine aminotransferase levels with hepatitis E virus infections among blood donors in China. Transfusion 2017;57:273-9.
14. Slot E, Hogema BM, Riezebos-Brilman A, et al. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. Euro Surveill 2013;18 pii=20550. https://doi.org/10.2807/1560-7917.ES2013.18.31.20550.
15. Baylis SA, Corman VM, Ong E, et al. Hepatitis E viral loads in plasma pools for fractionation. Transfusion 2016;56:2532-7.
16. Stramer SL, Moritz ED, Foster GA, et al. Hepatitis E virus: seroprevalence and frequency of viral RNA detection among US blood donors. Transfusion 2016;56:481-8.
17. Roth NJ, Schäfer W, Alexander R, et al. Low hepatitis E virus RNA prevalence in a large-scale survey of United States source plasma donors. Transfusion 2017;57:2958-64.
18. Xu C, Wang RY, Schechterly CA, et al. An assessment of hepatitis E virus (HEV) in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence for HEV transmission to 362 prospectively followed recipients. Transfusion 2013;53(10 part 2):2505-11.
19. Zafrullah M, Zhang X, Tran C, et al. Disparities in detection of antibodies against hepatitis E virus in US blood donor samples using commercial assays. Transfusion 2018;58:1254-63.
20. Kleinman SH, Glynn SA, Higgins MJ, et al. The RADAR repository: a resource for studies of infectious agents and their transmissibility by transfusion. Transfusion 2005;45:1073-83.
21. Baylis SA, Hanschmann KM, Blümel J, et al. Standardization of hepatitis E virus (HEV) nucleic acid amplification technique-based assays: an initial study to evaluate a panel of HEV strains and investigate laboratory performance. J Clin Microbiol 2011;49:1234-9.
22. Ticehurst J, Rhodes LL Jr, Krawczynski K, et al. Infection of owl monkeys (*Aotus trivirgatus*) and cynomolgus monkeys (*Macaca fascicularis*) with hepatitis E virus from Mexico. J Infect Dis 1992;165:835-45.
23. Huang C-C, Nguyen D, Fernandez J, et al. Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). Virology 1992;191:550-8.
24. Kaiser M, Kamili S, Hayden T, et al. Genome sequence of a genotype 2 hepatitis E virus World Health Organization reference strain. Genome Announc 2017;5:e01664-16.
25. Rolfe KJ, Parmar S, Mururi D, et al. An internally controlled, one-step, real-time RT-PCR assay for norovirus detection and genogrouping. J Clin Virol 2007;39:318-21.
26. Gentry-Shields J, Myers K, Pisanic N, et al. Hepatitis E virus and coliphages in waters proximal to swine concentrated animal feeding operations. Sci Total Environ 2015;505:487-93.
27. Sue PK, Pisanic N, Heaney CD, et al. Hepatitis E virus infection among solid organ transplant recipients at a North American transplant center. Open Forum Infect Dis 2016;3 ofw006. https://doi.org/10.1093/ofid/ofw006
28. Jothikumar N, Cromeans TL, Robertson BH, et al. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods 2006;131:65-71.
29. Dong C, Meng J, Dai X, et al. Restricted enzooticity of hepatitis E virus genotypes 1 to 4 in the United States. J Clin Microbiol 2011;49:4164-72.
30. Wang Y, Ling R, Erker JC, et al. A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. J Gen Virol 1999;80:169-77.
31. Zhai L, Dai X, Meng J. Hepatitis E virus genotyping based on full-length genome and partial genomic regions. Virus Res 2006;120:57-69.
32. Guindon S, Dufayard JF, Lefort V, et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 2010;59:307-21.
33. Dereeper A, Guignon V, Blanc G, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 2008;36(Suppl 2):W465-9.
34. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
35. Vina-Rodriguez A, Schlosser J, Becher D, et al. Hepatitis E virus genotype 3 diversity: phylogenetic analysis and presence of subtype 3b in wild boar in Europe. Viruses 2015;7:2704-26.
36. Smith DB, Simmonds P, Izopet J, et al. Proposed reference sequences for hepatitis E virus subtypes. J Gen Virol 2016;97:537-42.
37. Smith DB, Ijaz S, Tedder RS, et al. Variability and pathogenicity of hepatitis E virus genotype 3 variants. J Gen Virol 2015;96(Pt 11):3255-64.
38. Baylis SA, Crossan C, Corman VM, et al. Unusual serological response to hepatitis E virus in plasma donors consistent with re-infection. Vox Sang 2015;109:406-9.
39. Clayson ET, Myint KSA, Snitbhan R, et al. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. J Infect Dis 1995;172:927-33.
40. Aggarwal R, Kini D, Sofat S, et al. Duration of viraemia and faecal viral excretion in acute hepatitis E. Lancet 2000;356:1081-2.
41. Chandra NS, Sharma A, Malhotra B, et al. Dynamics of HEV viremia, fecal shedding and its relationship with transaminases and antibody response in patients with sporadic acute hepatitis E. Virol J 2010;7:213.
42. Tedder RS, Ijaz S, Kitchen A, et al. Hepatitis E risks: pigs or blood-that is the question. Transfusion 2017;57:267-72.



43. Germer JJ, Ankoudinova I, Belousov YS, et al. Hepatitis E virus (HEV) detection and quantification by a real-time reverse transcription-PCR assay calibrated to the World Health Organization standard for HEV RNA. J Clin Microbiol 2017;55: 1478-87.
44. Kuniholm MH, Purcell RH, McQuillan GM, et al. Epidemiology of hepatitis E virus in the United States: results from NHANES III, 1988–1994. J Infect Dis 2009;200:48-56.
45. Meng XJ, Wiseman B, Elvinger F, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol 2002;40:117-22.
46. Engle RE, Bukh J, Alter HJ, et al. Transfusion-associated hepatitis before the screening of blood for hepatitis risk factors. Transfusion 2014;54:2833-41.
47. Kamar N, Marion O, Abravanel F, et al. Extrahepatic manifestations of hepatitis E virus. Liver Int 2016;36:467-72.
48. Tsarev SA, Tsareva TS, Emerson SU, et al. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. J Infect Dis 1993;168:369-78.
49. Kodani M, Kamili NA, Tejada-Strop A, et al. Variability in the performance characteristics of IgG anti-HEV assays and its impact on reliability of seroprevalence rates of hepatitis E. J Med Virol 2017;89:1055-61.
50. Teshale EH, Denniston MM, Drobeniuc J, et al. Decline in hepatitis E virus antibody prevalence in the United States from 1988–1994 to 2009–2010. J Infect Dis 2015;211:366-73.
51. Kuniholm MH, Engle RE, Purcell RH, et al. Hepatitis E virus seroprevalence in the United States: no easy answers. Hepatology 2014;61:1441-2.
52. Petrik J, Lozano M, Seed CR, et al. Hepatitis E. Vox Sang 2015; 110:93-103.
53. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. Hepatology 2012; 55:988-97.
54. Domanovic D, Tedder R, Blümel J, et al. Hepatitis E and blood donation safety in selected European countries: a shift to screening? Euro Surveill 2017;22 pii=30514. https://doi.org/10.2807/1560-7917.ES.2017.22.16.30514
55. de Vos AS, Janssen MP, Zaaijer HL, et al. Cost-effectiveness of the screening of blood donations for hepatitis E virus in the Netherlands. Transfusion 2017;57:258-66.
56. Dodd RY, Notari EP, Nelson D, et al. Development of a multi-system surveillance database for transfusion-transmitted infections among blood donors in the United States. Transfusion 2016;56:2781-9.
57. Izopet J, Dubois M, Bertagnoli S, et al. Hepatitis E virus strains in rabbits and evidence of a closely related strain in humans, France. Emerg Infect Dis 2012;18:1274-81.
58. Abravanel F, Lhomme S, El Costa H, et al. Rabbit hepatitis E virus infections in humans, France. Emerg Infect Dis 2017;23: 1191-3.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Analytical sensitivity of IgG anti-HEV ELISA
Table S2. Reagent oligonucleotides.