

# Hepatitis E Virus Infection Among Solid Organ Transplant Recipients at a North American Transplant Center

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**Background.** Autochthonous hepatitis E virus (HEV) infection has been reported in over 200 solid organ transplant (SOT) recipients since 2006, yet little is known about the burden of HEV among SOT recipients in North America. We performed a retrospective, cross-sectional study to investigate the prevalence and risk factors associated with HEV infection among SOT recipients at our institution.

**Methods.** Children and adults (n = 311) who received allografts between 1988 and 2012 at the Johns Hopkins Hospital were assessed for evidence of HEV infection by testing posttransplantation serum samples for HEV antibody by enzyme immunoassay and HEV RNA by reverse transcription quantitative polymerase chain reaction. Individuals with evidence of posttransplant HEV infection (presence of anti-HEV immunoglobulin [Ig]M antibody, anti-HEV IgG seroconversion, or HEV RNA) were compared with individuals without evidence of infection and assessed for risk factors associated with infection.

**Results.** Twelve individuals (4%) developed posttransplant HEV infection. Posttransplant HEV infection was associated with an increased risk for graft rejection (odds ratio, 14.2; *P* = .03). No individuals developed chronic infection.

**Conclusions.** Solid organ transplant recipients in the United States are at risk for posttransplant HEV infection. Further studies are needed to characterize environmental risk factors and the risk of HEV infection after SOT in North America.

**Keywords.** hepatitis E virus; renal transplantation; solid organ transplantation; viral hepatitis.

Hepatitis E virus (HEV) is a leading global cause of acute viral hepatitis, with 20 million estimated infections and 70 000 deaths per year. Epidemic outbreaks due to genotypes 1 and 2 occur primarily in subtropical climates due to contaminated water and are associated with high mortality rates during pregnancy [1, 2]. In contrast, autochthonous HEV infections in industrialized countries are infrequently linked to waterborne outbreaks or poor sanitation, are often due to genotypes 3 and 4, and lead to serious illness among immunocompromised persons and individuals with chronic liver disease [2, 3]. Although humans are the only known hosts for HEV genotypes 1 and 2, genotype 3 and 4 infections are primarily zoonotic in origin, and transmitted through contact with infected

mammalian reservoirs (deer, swine) or through ingestion of solid organ meats and undercooked pork [4, 5]. Hepatitis E virus transmission via blood products, including packed red blood cells and platelets, has also been demonstrated [6–9] and can lead to acute hepatitis, fulminant hepatic failure, and chronic infection among immunosuppressed individuals [10].

In recent years, autochthonous HEV infection has emerged as an important cause of hepatitis among solid organ transplant (SOT) recipients, with over 200 cases reported to date. In 2008, Kamar et al [11] reported evidence of active HEV infection in 14 of 217 (6.5%) SOT recipients with idiopathic hepatitis. Progression to chronic HEV infection, defined as HEV viremia persisting for >6 months, occurred in 8 individuals (57%). Since that time, additional studies have demonstrated the morbidity associated with chronic HEV infection among SOT recipients, with up to 14% of such individuals progressing to cirrhosis [12]. Risk factors for chronic HEV infection among SOT recipients include immunosuppression with tacrolimus, leukopenia, lymphopenia, and thrombocytopenia [11, 12].

However, despite growing evidence for the global burden of HEV, data regarding infection among SOT recipients in North America remain sparse. Estimates of HEV seroprevalence in the US population range from 6% to 21%, suggesting a high degree of population exposure to HEV, yet fewer than 10 cases of HEV infection among transplant recipients have been reported by the

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Centers for Disease Control and Prevention (CDC) since 2005 [13–17]. The lack of an US Food and Drug Administration (FDA)-approved diagnostic test, in addition to a low index of clinical suspicion among healthcare providers, has likely led to an underestimate of the burden of HEV infection among SOT recipients in North America. Although multiple studies have examined the burden of HEV infection among SOT recipients in Europe [10, 18, 19], only a single study addressing the prevalence of HEV infection after solid organ transplantation has been performed in the United States to date [17, 20]. We conducted a retrospective, cross-sectional study to investigate the prevalence and characteristics associated with posttransplant HEV infection at our institution.

## METHODS

### Study Subjects

The Johns Hopkins Hospital conducts approximately 300 SOTs (pediatric and adult) annually. Pre- and posttransplantation serum specimens are routinely collected for renal transplant histocompatibility testing and for other select SOT recipients, at the clinician's discretion; residual samples are stored at  $-30^{\circ}\text{C}$ . To identify study subjects, we reviewed pre- and posttransplantation serum specimens of SOT recipients from 1988 to 2010. Those with available stored sera at least 6 months after transplantation were included in the study. Individuals with no residual serum or no available serum drawn  $\geq 6$  months after SOT were excluded. The protocol was reviewed and approved by the Johns Hopkins Medicine Institutional Review Board.

### Study Design

Patient records were reviewed for baseline demographic characteristics (transplant type, age, gender), liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and bilirubin), and serum creatinine. Initial HEV testing (antibody and RNA detection as described below) was performed on sera collected  $\geq 6$  months posttransplantation. Among individuals with evidence of elevated aminotransferases (defined as  $>1.5\times$  upper limit of normal; ALT  $\leq 31$  U/L, AST  $\leq 34$  U/L), available specimens most closely associated with the period of abnormal aminotransferases were chosen for testing. Among participants who received plasmapheresis in the context of an ABO or human leukocyte antigen (HLA) antibody incompatibility, preplasmapheresis samples were selected.

Subjects with detectable anti-HEV immunoglobulin (IgM) in their posttransplantation serum were classified as having acute HEV infection after transplant. Subjects with evidence of anti-HEV IgG posttransplant were classified as having prior HEV infection. To investigate when infection occurred among anti-HEV IgG-positive subjects, pretransplantation sera were tested for anti-HEV IgM and IgG. Hepatitis E virus infection was considered to have occurred after transplantation if HEV antibodies were detectable after but not before transplant. Individuals with

**Table 1. Definitions of HEV Infection Status per Testing Protocol**

HEV Infection Status	Posttransplantation Specimen			Pretransplantation Specimen		
	PCR	IgG	IgM	PCR	IgG	IgM
Active Infection	POS	+/-	+/-	NT	NT	NT
Acute/Recent Infection <sup>a</sup> (IgM)	NEG	+/-	POS	NEG	NEG	NEG
Acute/Recent Infection <sup>a</sup> (Seroconversion)	NEG	POS	NEG	NEG	NEG	NEG
Past Infection	NEG	POS	NEG	+/-	POS	+/-

Abbreviations: HEV, hepatitis E virus; Ig, immunoglobulin; NEG, negative; NT, not tested; PCR, polymerase chain reaction; POS, positive.

<sup>a</sup> The term "acute/recent" here refers to HEV infection after transplantation.

evidence of anti-HEV IgG alone, both before and after transplantation, were not considered to have posttransplantation HEV infection (Table 1). Previously seropositive individuals with evidence of rising anti-HEV IgG ( $>4$ -fold) but negative anti-HEV IgM and HEV RNA were considered to have antibody evidence of HEV exposure during the interval between collection of the 2 specimens, but they were not considered to have active HEV infection or reinfection [21, 22].

Initial evaluation of posttransplant sera also included testing for HEV RNA by reverse transcription quantitative polymerase chain reaction (RT-qPCR) using the method described below. Individuals with detectable HEV RNA were classified as actively infected at the time of sample collection. In addition, HEV RNA testing of pretransplantation specimens was performed in all subjects with evidence of posttransplant HEV seroconversion to assess for prior viremia. Among individuals with evidence of HEV infection after transplantation (positive PCR, anti-HEV IgM, or anti-HEV IgG seroconversion), serial PCR of samples taken before (3 months) and after (3 and 6 months) the posttransplant specimen were also tested to document viremia and assess for evidence of chronic HEV infection.

### Serologic Testing for Hepatitis E Virus Antibodies

Anti-HEV IgG and anti-HEV IgM antibodies were detected using a commercially available enzyme immunoassay (Wantai Diagnostics, Beijing, China). Sera (10  $\mu\text{L}$ ) were added into each well of a microtiter plate coated with recombinant HEV ORF2 antigen (for detection of HEV IgG) or with anti-IgM (class capture assay for detection of HEV IgM). The remainder of the test was performed according to the manufacturer's instructions. Each plate contained 1 blank, 3 negative, and 2 positive controls (provided by the manufacturer). Absorbance values of negative controls were used to calculate signal-to-cutoff (S/CO) ratios. An S/CO ratio  $>1.1$  was considered reactive. All serum samples were tested in duplicate. Borderline specimens (S/CO ratio 0.8–1.1) were retested in duplicate to determine final serostatus. Among individuals with borderline pre- and posttransplant anti-HEV IgG, anti-HEV IgG seroconversion was defined as a 4-fold or greater rise in S/CO ratio posttransplant.

The sensitivity and specificity of this enzyme immunoassay has been previously reported to be 97% and >99%, respectively, for the anti-HEV IgG assay and 75%–99% and >99%, respectively, for the anti-HEV IgM assay, and were additionally confirmed at our institution using the World Health Organization (WHO) 95/584 HEV antibody reference reagent (anti-HEV IgG and IgM positive) and the WHO nucleic acid standard 6329/10 (anti-HEV antibody negative) [23–28].

#### Hepatitis E Virus RNA Detection

One-step RT-qPCR targeting highly conserved ORF2/3 sequences [29] was performed to detect HEV RNA. RNA was extracted using an automated M48 nucleic acid extraction platform (QIAGEN, Valencia, CA) from a 200  $\mu$ L aliquot of serum sample and eluted in 100  $\mu$ L. Five microliters of each sample was subsequently tested for HEV RNA by RT-qPCR (Applied Biosystems 7500) in a total reaction volume of 25  $\mu$ L. Reverse transcription was performed using the QIAGEN RT-qPCR kit at 50°C for 30 minutes, followed by denaturation at 95°C for 15 minutes. Complimentary DNA amplification was subsequently performed for 45 cycles at 95°C for 15 seconds and 60°C for 1 minute. Bile from a monkey infected with HEV genotype 2 served as a positive extraction and PCR control, and HEV RNA from individuals infected with HEV genotype 3 were used as PCR-positive controls.

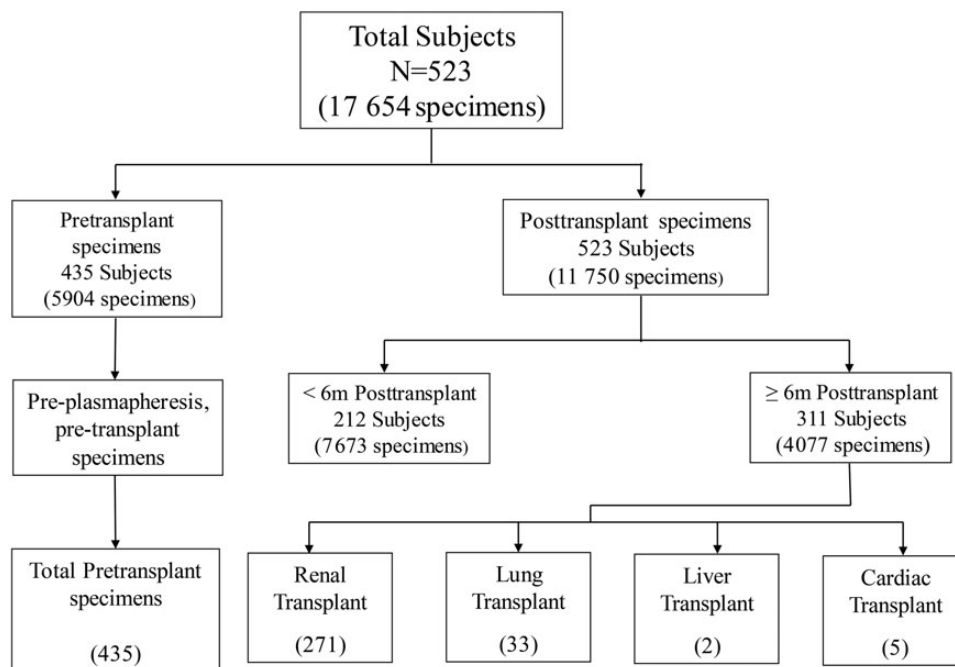
#### Statistical Analysis

Subjects were classified as either recently infected or noninfected after transplantation. Characteristics including ALT, AST,

and creatinine were compared between the 2 groups, and Shapiro-Wilk testing performed to assess for the presence of nonparametric data. The Wilcoxon rank-sum test was applied using Stata statistical software (version 12.1; StataCorp) to assess for associations between posttransplant HEV infection and the continuous variables above.

Conditional logistic regression was performed to investigate the relationship between posttransplant infection and previously reported risk factors for ongoing HEV infection. Individuals with evidence of posttransplant infection were matched to controls by transplant type and year of transplantation in a 1:3 ratio, and a  $\chi^2$  test was used to assess for potential confounders such as low white blood cell count (defined as  $<4.5 \times 10^3/\mu$ L), platelet count ( $<140 \times 10^3/\mu$ L), graft rejection, and immunosuppressive regimen. In cases for which fewer than 5 events were noted, the Fisher's exact test was used in place of the  $\chi^2$  test. Univariate and multivariate logistic regression with robust variance was subsequently used to estimate odds ratios to determine the association between risk factors and the risk of posttransplant HEV infection.

Akaike information criteria was used to assess goodness of fit, with nonconvergent covariates subsequently eliminated from the multivariate model. Given the number of covariates ultimately included (4), Bonferroni correction was not used in the multivariate model. Odds ratios comparing the group with posttransplant HEV infection to the negative control were calculated, with corresponding *P* values and 95% confidence intervals reported accordingly ( $P > .05 =$  nonsignificant).



**Figure 1.** Specimen testing for evidence of hepatitis E virus infection.

**Table 2. Baseline Characteristics at Time of Posttransplant Sample Collection<sup>a</sup>**

Characteristic	Posttransplant HEV Infection (N = 12) n (%) or Median (IQR)	No Posttransplant HEV Infection (N = 299) n (%) or Median (IQR)	P Value
<b>Age at transplant in years</b>			
Median (range)	39 (30–52)	48 (37–58)	.12
Female Sex, No. (%)	7 (58)	166 (56)	.85
<b>Organ Transplant Type</b>			
Kidney, No. (%)	10 (83.3)	261 (87.3)	.69
Lung, No. (%)	1 (8.3)	32 (10.7)	.79
Heart, No. (%)	0 (0)	5 (1.7)	.82
Liver, No. (%)	1 (8.3)	1 (0.3)	.08
<b>Serum aminotransferases</b>			
ALT, median (range)	43 U/L (14–60)	24 U/L (14–47)	.58
AST, median (range)	28.5 U/L (14–47)	23 U/L (16–36)	.64
Bilirubin, median (range)	0.4 U/L (0.2–1.3)	0.4 U/L (0.1–23.0)	.74
Creatinine, median (IQR)	1.75 mg/dL (1.1–2.1)	1.6 mg/dL (1.3–2.2)	.88

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HEV, hepatitis E virus; IQR, interquartile range.

<sup>a</sup> Wilcoxon rank-sum test was done for nonparametric distribution, and  $\chi^2$  and Fisher's exact tests were used for categorical variables.

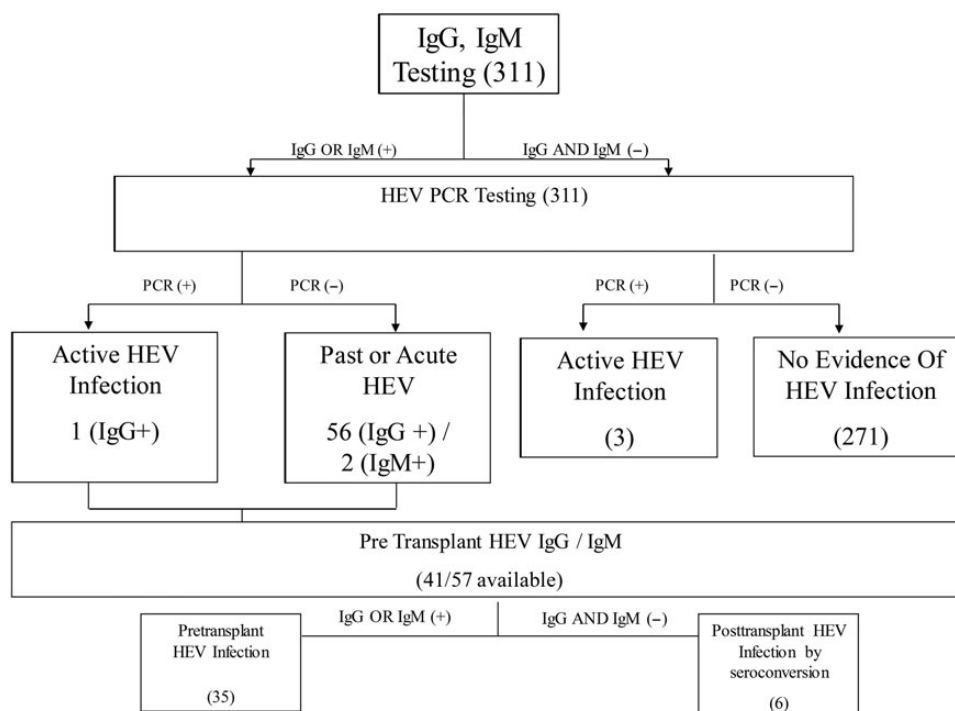
## RESULTS

### Baseline Characteristics

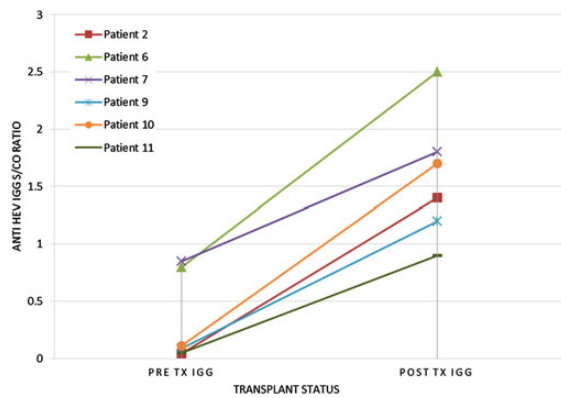
We reviewed data from 523 SOT recipients, from whom 17 654 serum samples were collected; 311 subjects (173 females, 138

males) met eligibility criteria and were tested for evidence of posttransplantation anti-HEV IgG, IgM, and HEV RNA. Subjects included 271 kidney, 33 lung, 5 cardiac, and 2 liver transplant recipients (Figure 1). Seventy of 311 individuals (23%) demonstrated evidence of increased ALT or AST >1.5 upper limit of the normal range after transplantation. Median age at time of transplant was 47.5 years (range, 2–80 years), with 17 patients under 18 years of age (1 liver, 14 kidney, 2 cardiac). There was no difference in gender, age, or transplant type between posttransplant HEV-infected individuals and noninfected individuals (Table 2). Fifty-seven individuals (18%) tested positive for anti-HEV IgG antibody suggestive of previous infection. Twelve (4%) individuals demonstrated evidence of HEV infection after transplantation per initial criteria (Figure 2). Among those, 6 (2%) individuals showed evidence of posttransplant anti-HEV IgG seroconversion (pretransplant sample was negative and posttransplant sample positive for HEV IgG) and 6 (2%) showed evidence of active HEV infection after transplantation (posttransplant samples from 2 individuals were positive for anti-HEV IgM, and samples from 4 individuals were positive for HEV RNA) (Figure 2).

Anti-HEV IgG (+) individuals demonstrated higher proportions of elevated ALT (32%) and AST (28%) compared with anti-HEV IgG (–) individuals (18% and 10% with  $P = .07$  and  $P = .04$ , respectively).



**Figure 2.** Results of posttransplant hepatitis E virus (HEV) infection testing among solid organ transplant recipients. Abbreviations: Ig, immunoglobulin; PCR, polymerase chain reaction.



**Figure 3.** Signal-to-cutoff (S/CO) ratio for anti-hepatitis E virus (HEV) immunoglobulin (IgG) among individuals with evidence of posttransplant seroconversion. PRE TX IGG, pretransplantation IGG; POST TX IGG, posttransplantation IGG.

Among individuals with evidence of anti-HEV IgG seroconversion (6), pre- and posttransplantation S/CO values were quantitatively assessed, and a 4-fold rise was noted in 4 of 6 individuals (Figure 3). Three additional cases demonstrated qualitative borderline seroconversions with minimal increases in S/CO ratio (<2-fold increase) and were subsequently excluded from further analysis. One additional case demonstrated a weakly positive pretransplantation anti-HEV IgG S/CO ratio (1.07) with an almost 4-fold increase 14 months after transplantation (S/CO ratio, 4.20), suggestive of interval re-exposure, but did not meet criterion for posttransplant seroconversion. HEV RT-qPCR was performed on all pretransplant samples, and did not identify additional cases of HEV viremia.

Characteristics of posttransplant HEV-infected individuals are shown in Table 3. Although kidney transplant recipients (KTR) accounted for the largest number of posttransplant-infected individuals (N = 10), 1 lung transplant and 1 liver transplant recipient also showed evidence of posttransplant HEV infection. There were no differences in median AST,

ALT, serum creatinine, or bilirubin noted between the HEV-infected and uninfected groups (Table 2).

### Risk Factors for Hepatitis E Virus Infection Among Solid Organ Transplant Recipients

Univariate analysis of subjects with evidence of posttransplant HEV infection demonstrated a nonadjusted association between the risk of posttransplant HEV infection and low white blood cell count (odds ratio [OR], 5.6;  $P = .05$ ; 95% confidence interval [CI], 1.0–31), as well as graft rejection (OR, 6.9;  $P = .02$ ; 95% CI, 1.3–37.5). Multivariate conditional logistic regression also demonstrated an association between risk of posttransplant HEV infection and graft rejection (OR, 14.2;  $P = .03$ ; 95% CI, 1.26–160), but no association with leukopenia, tacrolimus exposure, or thrombocytopenia (Tables 4 and 5). Variables from the univariate analysis (prednisone, mycophenolate mofetil, lymphopenia, and cyclosporine), which failed to converge upon multivariate analysis due to sample size and the skewed distribution of covariates, were ultimately excluded.

### Chronic Hepatitis E Virus Infection

To assess for cases of chronic HEV infection, sera from 3 months prior, as well as 3 and 6 months after transplant, were tested for HEV RNA as described above (N = 12). No HEV RNA was detected in any of these samples, suggesting that no individuals developed chronic HEV infection during the study period.

### DISCUSSION

Globally, autochthonous HEV infection has emerged as an important cause of morbidity among SOT recipients. Multiple studies from France, the Netherlands, and Germany suggest that 6%–8% of all idiopathic liver dysfunction among SOT recipients may be due to HEV infection, with up to 60% of these progressing to chronic hepatitis [11, 18, 19, 30]. In the Americas, evidence of posttransplant HEV infection was demonstrated among 3% of KTR in a recent Brazilian cohort, and in 6% of

**Table 3. Individuals With Evidence of Posttransplantation HEV Infection**

Patient No.	Organ Transplanted	Age	Gender	AST (U/L)	ALT (U/L)	Graft Rejection	Immunosuppression	HEV Diagnosis Method
1.	Kidney	44	F	40	72	No	MMF, prednisone, tacrolimus	(+) PCR
2.	Kidney	51	F	52	44	Yes	MMF, prednisone, tacrolimus	(+) IgG seroconversion
3.	Kidney	30	M	41	94	Yes	MMF, prednisone, tacrolimus	(+) PCR
4.	Kidney	58	F	26	48	Yes	MMF, prednisone, tacrolimus	(+) PCR
5.	Kidney	17	M	24	8	Yes	MMF, prednisone, tacrolimus	(+) PCR
6.	Kidney	30	M	13	9	Yes	MMF, prednisone, tacrolimus	(+) IgG seroconversion
7.	Kidney	64	F	10	18	No	MMF, prednisone, cyclosporine	(+) IgG seroconversion
8.	Kidney	29	F	12	14	Yes	MMF, prednisone, tacrolimus	(+) IgM
9.	Kidney	38	F	12	11	Yes	MMF, sirolimus	(+) IgG seroconversion
10.	Kidney	39	M	31	42	Yes	MMF, prednisone, tacrolimus	(+) IgM
11.	Lung	23	M	57	65	Yes	MMF, prednisone, cyclosporine	(+) IgG seroconversion
12.	Liver	52	M	141	55	Yes	MMF, prednisone, tacrolimus	(+) IgG seroconversion

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HEV, hepatitis E virus; Ig, immunoglobulin; MMF, mycophenolate mofetil; PCR, polymerase chain reaction.

**Table 4. Risk Factors Associated With Posttransplantation HEV-Infected Individuals<sup>a</sup>**

Exposure	Posttransplant HEV Infection (n = 12)	No Posttransplant HEV Infection (n = 36)	P Value
Tacrolimus, No. (%)	9 (75)	33 (91)	.16
Mycophenolate mofetil, No. (%)	11 (92)	29 (81)	.5
Cyclosporine, No. (%)	2 (17)	0 (0)	.06
Prednisone, No. (%)	12 (100)	32 (89)	.54
Triple Therapy, No. (%)	11 (92)	29 (81)	.51
Leukopenia (<4.5 K/mm <sup>3</sup> ), No. (%)	5 (42)	3 (9)	.02
Thrombocytopenia (>140 K/mm <sup>3</sup> ), No. (%)	2 (18)	1 (3)	.15
Lymphopenia (<1100/mm <sup>3</sup> ), No. (%)	6 (50)	13 (36)	.58
Graft rejection, No. (%)	9 (75)	10 (28)	.005

Abbreviations: HEV, hepatitis E virus.

<sup>a</sup> Based on  $\chi^2$  test for binary outcomes. Fisher's exact test was used in cases of fewer than 5 events.

Hepatitis C Virus (HCV) infected liver transplant recipients in a recent U. S. study. Yet, despite increasing recognition of the role of HEV infection among SOT recipients, little is known about the risk of autochthonous HEV infection in the United States [17, 31]. Between 2005 and 2011, the CDC reported 123 cases of suspected HEV infections in the United States, with fewer than 10 cases among SOT recipients [13]. Although this low prevalence may reflect the epidemiology of HEV infection in North America, it may also underestimate the true burden of disease, given the significant obstacles to testing, including the lack of an FDA-approved diagnostic test and a limited clinical awareness among practitioners [32].

Few studies to date have investigated the incidence and risk factors for HEV infection after SOT in North America. We examined 311 SOT recipients from our institution and found evidence of posttransplant HEV infection in 12 individuals (4%). Hepatitis E virus-infected individuals did not differ from non-HEV-infected individuals in regards to their maintenance immunosuppressive regimen, leukopenia, absolute lymphocyte count, or total platelet count but were at increased risk for graft rejection.

Evidence of previous HEV infection (anti-HEV IgG seropositivity) was detected in 18% of study subjects, consistent with

previously reported data from the US National Health and Nutrition Examination Survey (NHANES) 1988–1994 cohort [14]. Our seroprevalence was comparable to previously reported prevalence in England (15%), Germany (16%), and the Netherlands (21%), but was markedly lower than southwest France (53%) [18, 33–35]. Recent seroprevalence data from a single year in the United States (NHANES 2009–2010) was reported to be 6.0%, suggestive of a potential cohort effect and sporadic exposure risk over time to HEV within the US population [36]. Although the ingestion of pig liver sausage has been clearly identified as a source of HEV infection in southern France, the etiology of HEV exposure within the United States remains unclear [14, 37]. It is possible that exposure to animal hosts, poorly cooked organ meats and sausage, contaminated vegetables, or infected blood products and/or donor organs may be sources of infection that warrant further investigation.

Posttransplant HEV infection was strongly associated with graft rejection in our study (OR, 14.2;  $P = .03$ ). Although the majority of these cases were noted among renal transplant recipients, who comprised the vast majority of our cohort, graft rejection was also noted among the single HEV-infected lung and liver transplant recipients in our cohort as well. Hepatitis E virus infection has been associated with a number of extrahepatic manifestations, including posttransplant membranoproliferative glomerulonephritis, IgA nephropathy, and cryoglobulinemia, and has been suggested as a potential cause of graft hepatitis in liver transplant recipients; however, to our knowledge, this is the first study in which posttransplant HEV infection has been associated with increased risk of graft rejection, particularly among KTR [38–41].

In contrast to previous reports, we did not identify cases of chronic HEV infection, either among individuals with serologic or RT-qPCR evidence of posttransplant HEV infection, despite the presence of previously identified risk factors such as exposure to tacrolimus (9 of 12), leukopenia (5 of 12), and lymphopenia (6 of 12). Although the reason for this lack of chronic infections remains unclear, prior studies have demonstrated clearance of HEV viremia in 30% of chronic cases after reduction of immunosuppression [42]. Among the 4 individuals in our cohort with HEV viremia, we noted reductions in immunosuppression among three following their positive sample, as well as multiple administrations of intravenous Ig (IVIg) in the fourth individual, due to

**Table 5. Relative Risk of Posttransplant HEV Infection With Selected Risk Factors by Univariate and Multivariate Analysis**

Exposure	Unadjusted OR <sup>a</sup>	95% CI	P Value	Adjusted OR	95% CI	P Value
Tacrolimus	0.3	0.4–1.7	.16	0.4	.11–1.6	.20
Leukopenia (<4.5 × 10 <sup>3</sup> /mm <sup>3</sup> )	5.6	1.0–31	.05	3.2	.4–63	.30
Thrombocytopenia (>140 K/mm <sup>3</sup> )	5.3	0.4–60	0.18	7.7	.2–287	.27
Graft rejection	6.9	1.3–37.5	.02	14.2	1.26–160	.03

Abbreviations: CI, confidence interval; HEV, hepatitis E virus; OR, odds ratio.

<sup>a</sup> Due to small sample size and skewed distribution, a number of covariates did not converge in the multivariable analysis and were excluded.

rejection. It is possible that such changes in immunosuppression may have contributed to clearance of HEV viremia in these cases.

### Limitations

Our study had a number of limitations. Due to its retrospective design, detailed data on exposures and potential routes of HEV transmission were not available. In addition, as our study was limited to a single center, we did not capture potential regional differences in HEV exposure and risk of infection.

A disproportionate number of KTR were represented within our cohort. Among these, a high proportion of KTR were HLA-mismatched, highly sensitized individuals at increased risk for graft rejection and required higher levels of immunosuppression. As such, it is possible that the association we observed between HEV infection and graft rejection was particularly exaggerated in this population, although this association was also observed among other transplant types in our study.

Transmission of HEV infection through contaminated blood products has been previously described and as such, it is possible that a number of infected individuals within our cohort may have been exposed to HEV via contaminated blood products [7, 43]. However, we were unable to test blood products received by individuals for the presence of HEV, given the retrospective nature of our study as well as the lack of blood donor screening for HEV.

In regards to our HEV seroconversions, we considered the possibility that anti-HEV IgG may have been transmitted through IVIG administration after transplantation and contributed to a number of seroconversions we observed. To address this concern, we reviewed the records of the 6 individuals diagnosed by seroconversion, and we noted that only 2 received IVIG within 2 months before initial specimen testing (Cases 11 and 12). Of these 2 cases, 1 individual underwent plasmapheresis immediately after IVIG administration (making IVIG an unlikely cause of seroconversion), resulting in 1 individual within our cohort who may have seroconverted secondary to recent IVIG administration.

To evaluate the likelihood of anti-HEV IgG seroconversion secondary to recent IVIG administration in this individual, we reviewed records of all anti-HEV IgG seronegative subjects (ie, no evidence of seroconversion) for evidence of prior IVIG exposure. We found that 61% (162 of 254) of seronegative individuals previously received IVIG with no evidence of anti-HEV IgG seroconversion with our assay. Of these, 18% (29 of 162) received IVIG within the 8 weeks preceding specimen collection but yet remained anti-HEV IgG negative. Therefore, we concluded that recent administration of IVIG was unlikely to be a significant source for anti-HEV IgG seroconversion in the individual above.

Although it is likely that the majority of HEV infections we observed were due to genotype 3 virus, we were unsuccessful in our attempts to genotype the PCR-positive isolates in our

cohort. We attribute this to the (1) relatively lower levels of viremia we noted among our isolates as well as the (2) decreased sensitivity of the genotyping assay for HEV compared with RT-qPCR. We were also surprised to not observe any PCR (+) chronic infections. Circulating HEV RNA may have been reduced to undetectable levels as a result of plasma dilution in the setting of IVIG administration and plasmapheresis for ABO- or HLA-incompatible living donor transplant. This may have contributed to the absence of HEV RNA in the serial samples we tested. Finally, the use of rituximab in over one third of our cohort of KTR may have contributed to the low prevalence of anti-HEV IgM and led to an underestimate of recent posttransplant infections in our study compared with others.

### CONCLUSIONS

In summary, these data represent the largest investigation, to our knowledge, of posttransplant HEV infection among SOT recipients in North America. We observed evidence of posttransplant HEV infection in a relatively high proportion of individuals (4%), with detectable HEV RNA in 1% of cases. Posttransplant HEV infection was associated with an increased incidence of graft rejection exclusively among renal transplant recipients (OR, 14.2;  $P = .03$ ; 95% CI, 1.26–160), but was not associated with immunosuppressive regimen, leukocyte count, lymphopenia, or thrombocytopenia.

Hepatitis E virus-infected individuals did not differ significantly from noninfected individuals in terms of age, bilirubin, AST, ALT, or creatinine levels. Among 12 individuals with evidence of posttransplant infection, we observed no cases of chronic hepatitis, in contrast to previous studies of HEV-infected SOT recipients. A further multicenter, prospective study is needed to supplement the findings of this retrospective, single-center study and to better understand HEV infection among SOT recipients in North America.

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