# Toward Systematic Screening for Persistent Hepatitis E Virus Infections in Transplant Patients

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**Background.** Persistent hepatitis E virus genotype 3 (HEV G3) infections affect solid organ transplant (SOT) recipients and hematopoietic stem cell transplant (HSCT) recipients, but the burden in these cohorts in the United Kingdom is unknown. We established an audit to determine the point prevalence of HEV viremia in SOT and HSCT patients in the United Kingdom and compare different testing approaches to inform screening strategies. **Methods.** Between January 5, 2016, and September 21, 2016, 3044 patients undergoing therapeutic drug monitoring at a single transplant center were screened for HEV ribonucleic acid (RNA) in minipools. A total of 2822 patients who could be characterized included 2419 SOT patients, 144 HSCT patients and 259 patients with no available transplant history. HEV RNA-positive samples were characterized by serology and genomic phylogeny. HEV antigen (HEV-Ag) testing was performed on RNA-positive samples, 420 RNA-negative samples and 176 RNA-negative blood donor samples. **Results.** Nineteen of 2822 patients were viremic with G3 HEV giving a prevalence of 0.67%. The median alanine aminotransferase was significantly higher in the HEV viremic patients ($P < 0.0001$); however, 2 viremic patients had an alanine aminotransferase value within the normal range at the time of screening. The HEV-Ag assay identified 18/19 viremic patients and all those patients with proven viremia longer than 4 weeks. **Conclusions.** Transplant recipients in the United Kingdom are at a low but significant risk of HEV infection. HEV-Ag detection could be an alternative to RNA detection where the goal is to identify established persistent HEV infection, particularly where expertise, facilities, or cost prohibit RNA testing.

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Locally acquired hepatitis E virus (HEV) infection has become the commonest cause of acute viral hepatitis in the United Kingdom and many other European countries.[1,2] These infections, caused principally by genotype 3 (G3) HEV, are self-limiting in immunocompetent patients but can lead to persistent infections in immunocompromised patients, predominantly solid organ transplant (SOT) recipients and hematopoietic stem cell transplant (HSCT) recipients.[3,4]

HEV G3 may be acquired through diet, blood transfusion or organ transplantation. In the majority of patients the greatest risk is from diet, predominantly through the consumption of insufficiently cooked pork products.[5,6] However, in patients with high transfusion requirements, the risk from blood components (HEV unscreened red blood cells, fresh frozen plasma or platelets) may exceed the annual dietary risk, particularly in the immediate posttransplant period.[7]

The diagnosis of persistent HEV infection remains a major challenge because it is commonly asymptomatic usually with

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only a mild elevation in transaminases.[3] These infections are therefore often underrecognized clinically and raised transaminases may be mistakenly ascribed to other conditions including drug-induced liver injury and graft-versus-host disease (GvHD).[8-10] If there is a delayed or missed diagnosis of HEV infection, progressive liver damage may occur and evolve to cirrhosis in up to 15% of patients.[11] Retransplantation has been required in cases of persistent HEV infection in liver graft recipients.[11] Such complications can be prevented by prompt diagnosis to enable timely liver assessment and appropriate management which may include the modulation of immunosuppression and the addition of antiviral therapy, both intended to bring about termination of the infection.

The prevalence of persistent HEV infection in cohorts of SOT recipients in Western Europe varies between 0.7% and 1.5% in studies to date, but as high as 3.2% in one small study of lung transplant recipients.[12-16] However, although Public Health England (PHE) has observed a steady rise in the numbers of persistent HEV infections diagnosed each year across England and Wales (5 cases in 2012 rising to 25 cases in 2015), the prevalence of these infections in SOT and HSCT recipients in the United Kingdom is unknown.

A strategy of routinely screening at-risk populations could be considered though it remains unknown whether selective or nonselective screening strategies would be more effective pathways for identifying persistent HEV infections. A selective strategy could be based on known viremia prevalence rates within a population or defined by clinical parameters which may include deranged alanine aminotransferase (ALT) values, specific immunosuppression drug regimens and/or previous levels of exposure to blood components.[7]

Recently, a commercial HEV antigen (HEV-Ag) enzyme-linked immunosorbent assay (ELISA)-based assay which detects the product of open reading frame 2 has demonstrated high sensitivity in the detection of persistent HEV infections (n = 20), suggesting a possible role for screening.[17]

We established an audit to determine the point prevalence of HEV viremia in SOT and HSCT patients in a single transplant center in the United Kingdom and compared HEV ribonucleic acid (RNA) detection with HEV-Ag detection, HEV serology and ALT values to inform screening strategies.

## MATERIALS AND METHODS

### Audit Design

A prospective pseudo-anonymized prevalence audit of HEV viremia in patients undergoing routine therapeutic drug monitoring (TDM) for tacrolimus, cyclosporine, sirolimus, or everolimus. Patients were screened for HEV RNA in minipools, anti-HEV serology was performed on RNA-positive samples and HEV-Ag testing was performed on RNA-positive samples, 420 RNA-negative samples and 176 RNA-negative blood donor samples.

### Patient Samples

Between January and September 2016, samples from 3044 transplant recipients undergoing TDM for immunosuppressive drugs were identified in the Queen Elizabeth Hospital biochemistry laboratory in Birmingham. Duplicate samples were removed using an automated tool within the database and a second check was performed by imputing the date of birth.

Residual plasma was separated, aliquoted and stored at −20°C before shipping to the Blood Borne Virus Unit, PHE, Colindale. All patient-identifiable information was retained at Queen Elizabeth Hospital, sample tubes were labelled with a unique research code which was used for result reporting and data analysis.

For the HEV-Ag assay evaluation a random selection of 420 of the screened transplant recipients were tested alongside samples from the 19 HEV viremic patients and 176 anonymized blood donors. Anonymized blood donor and convalescent blood donor samples from a previous HEV donor-transmission study were donated by National Health Service Blood and Transplant (NHSBT).[18]

### Patient Characterization

Demographic data (age and sex), transplant history, immunosuppressive drug regimen and biochemical parameters (ALT and bilirubin) were triangulated from the TelePath Laboratory Information System and an in-house system called Prescribing Information and Communication System. Test results and the prescription details were recorded for the date of the plasma sample.

### Virological Testing

Three thousand forty samples from individual patients were tested for HEV RNA in minipools of 16 samples of 100 μL each. Four additional samples were tested individually due to either low volume (n = 1) or detached tube labels (n = 3).

HEV RNA from 1.2 mL of each minipool was extracted on the QiaSymphony (Qiagen, Crawley, UK; virus-specific cell-free protocol), detected and quantified using an in-house and validated quantitative HEV PCR (expressed in international units per mL; IU/mL) as previously described (limit of detection 22 IU/mL).[19] Reactive pools were resolved to individual samples by extraction of 200 μL of each primary sample on the MagNA Pure 96 (Roche Diagnostics Ltd. Burgess Hill, UK; virus-specific cell-free protocol). HEV antibody detection was performed on RNA-positive individual samples using the Wantai IgM and IgG detection assays (Fortress Diagnostics, Antrim, Northern Ireland, UK). Samples underwent sequence and phylogenetic analyses across part of the open reading frame 2 of HEV as previously described and were ascribed genotype and subtype.[20]

### HEV Antigen Testing

HEV-Ag testing was performed using a commercial ELISA (HEV-Ag ELISA; Fortress Diagnostics) according to the manufacturer's recommendations. We considered any samples with a S/CO ratio greater than 1.0 on initial testing as reactive and any >1.0 on repeat testing as repeat reactive.

To confirm specificity of a sample found reactive in the HEV-Ag assay, reactivity was subjected to neutralisation by a pool of high-titre convalescent plasma. In brief, preincubation of the test sample with the neutralising reagent was carried out for 1 hour at room temperature before subjecting the mixture to analysis in the assay.[21]

### Audit Ethics and Management of HEV-Infected Patients

Independent advice was sought from the London Bridge Ethics Committee; the Chair advised that this study was

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considered to be an audit using residual patient samples and therefore did not require approval through the centralised National Health Service research ethics committee process. The protocol was approved by University Hospital Birmingham Clinical Audit Department in line with the advisory letter from the chairman of the UK Advisory Committee on the Safety of Blood, Tissues, and Organs to all clinicians in England and Wales looking after SOT and HSCT recipients. This letter indicated the requirement to consider investigating such patients for HEV infection as part of their clinical care.

The audit steering committee reviewed progress monthly and the audit lead clinician communicated positive HEV RNA results to the relevant clinical team. As part of the ongoing clinical management of the patient, a confirmatory sample was taken, a hepatology assessment was arranged and expert clinical advice was offered.

### Data Analysis

Of the individual patients tested, 2822 out of 3044 had sufficient clinical and/or demographic data for further analysis. For statistical analysis, all patients with a liver transplant were grouped together including kidney/liver and lung/liver dual transplants. Heart and lung transplants were grouped including heart/kidney dual transplants. Patient characteristics were compared for patients who were infected and those not infected. Continuous variables were compared using the Wilcoxon 2-sample test and categorical variables compared using Fisher's exact test. Confidence intervals (CIs) for measures of prevalence were calculated using the Wilson method. Missing data are summarized and inequality symbols were removed for the purposes of data analysis (eg, a value of <5 ng/mL for tacrolimus was set to 5 ng/mL).

## RESULTS

Patients lived in England or Wales, 96% of whom lived within 100 miles of central Birmingham. A minimum dataset was available for 2822 patients who were included in the statistical analysis, consisting of 2419 SOT patients (1181 kidney, 869 liver, 229 heart, 110 lung, 21 kidney/liver, 6 heart/lung, 2 heart/kidney and 1 lung/liver), 144 allograft HSCT patients and 259 patients with no available transplant history. The majority of SOT patients were greater than 6 months from the transplant date at the time of screening for HEV RNA, whilst the majority of the HSCT patients were within 6 months of the transplant date (Figure 1). Seven hundred thirteen patients were prescribed cyclosporine, 2066 tacrolimus, 42 sirolimus, and 1 everolimus.

### Viremic Patients

Nineteen minipools containing HEV RNA were resolved to identify 19 viremic patients, giving an overall RNA prevalence of 1 in 149 (0.67%; 95% CI, 0.43-1.05%). Individual viremia levels ranged from 352 IU/mL to $9.09 \times 10^6$ IU/mL. Phylogenetic analysis demonstrated all samples to harbor HEV G3 viruses of which 6 (31.6%) were group 1 (subtypes efg) and 13 (68.4%) were group 2 (subtypes abchij). Three of the viremic patients (15.8%) were allogeneic HSCT recipients with a median duration of time since transplant of 11.3 months (interquartile range [IQR], 2.3-23.0). Sixteen (84.2%) were SOT recipients (6 kidney, 9 liver, 1 heart) with a median duration of time since transplant of 88.4 months (IQR, 19.3-122.6) (Table 1).

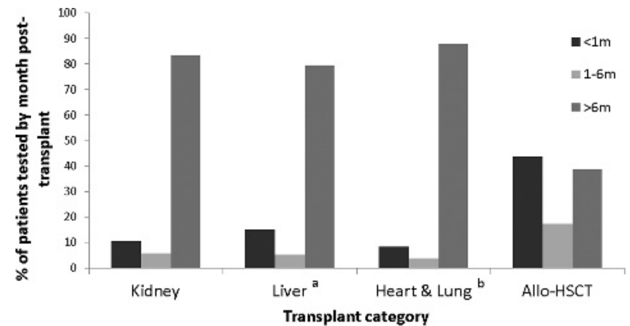


**FIGURE 1.** Bar chart representing the proportion of patients within one month, between one and six months and greater than six months from transplantation in those screened for HEV RNA. [a] includes liver/kidney (n=21) and lung/liver (n=1); [b] includes heart/kidney (n=2).

Full clinical details were available on 16 of the viremic patients. The diagnosis of HEV infection was only considered clinically in 1. Four had rises in ALT which were thought not to be clinically significant. The working diagnoses in the remainder were GvHD (n = 2), graft rejection (n = 2), autoimmune hepatitis (n = 2), statin-induced liver injury (n = 1), Epstein-Barr virus–associated hepatitis (n = 1), alcohol excess (n = 1), recurrent primary biliary cirrhosis (n = 1), and recurrent primary sclerosing cholangitis (n = 1).

A follow-up sample taken at a median of 9 weeks after the initial diagnosis of HEV infection (range, 1.6-22.1 weeks) as part of the routine clinical care of the viremic patient was available for 15 individuals, all but one of whom remained viremic. The follow-up sample for the patient who cleared HEV viremia was taken 22.1 weeks after the initial sample (patient 6, Table 1). Two further patients who were viremic at initial follow-up cleared their virus within 3 months of the initial screening test (patients 7 and 9, Table 1).

At the time of writing during the follow-up period, 12 patients had evidence of established persistent infection; viremia longer than 12 weeks (n = 11) or an unchanged viral load over a period of at least 8 weeks (n = 1). Four patients had insufficient follow-up to comment.

### Predictive Factors for HEV Viremia

The characteristics of HEV viremic patients were compared with uninfected aviremic patients. At the single time point of random screening, the HEV viremic patients had statistically significantly higher ALT ($P < 0.0001$), bilirubin ($P = 0.01$), tacrolimus levels ($P = 0.002$), and cyclosporine levels ($P = 0.02$), with the caveat of relatively small numbers (Table 2). The median ALT was 156 IU/L (IQR, 57-298) in HEV viremic patients compared to a median ALT of 19 IU/L (IQR, 13-30) in HEV RNA-negative patients (Figure 2). A sub analysis of only liver transplant patients found a similar difference with a median ALT of 127 IU/L (IQR, 57-298) in HEV viremic patients compared to a median ALT of 24 IU/L (IQR, 15-62) in HEV RNA-negative patients ($P = 0.005$) (Table 3). Comparison of transplant types did not identify any particular SOT category as being a risk factor for being HEV viremic, however, when SOT patients were compared with HSCT patients, there was borderline evidence ($P = 0.09$) that HSCT patients were more likely to be viremic. Sex, drug administered (cyclosporine or tacrolimus), age, and log (time since transplant) were not

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**TABLE 1.**

**Patient characteristics of HEV viremic patients**

| Pt No. | Transplant | Time since transplant (yrs) | Age (yrs) | Sex | Immune suppression | Bilirubin µmol/L | ALT IU/L | Serology | | HEV viral load IU/ml | HEV-Ag S/CO |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | IgM S/CO | IgG S/CO | | |
| 1 | Liver | 0.17 | 27 | F | Azathioprine, prednisolone, tacrolimus | 16 | 15 | 0.01 NEG | −0.01 NEG | 8.57E + 02 | 8.36 POS |
| 2 | Liver | 5.49 | 65 | F | Tacrolimus | 6 | 47 | 13.44 POS | 23.38 POS | 9.97E + 05 | 19.08 POS |
| 3 | Liver | 9.13 | 69 | M | Tacrolimus | 8 | 57 | 9.37 POS | 6.61 POS | 1.86E + 05 | 18.16 POS |
| 4 | Liver | 16.89 | 55 | F | Tacrolimus | 11 | 78 | 13.52 POS | 19.25 POS | 1.30E + 05 | 18.19 POS |
| 5 | Liver | 1.22 | 52 | M | Prednisolone, sirolimus | 8 | 127 | 11.11 POS | 23.45 POS | 8.38E + 05 | 17.00 POS |
| 6 | Liver | 2.06 | 21 | M | Mycophenolate mofetil, tacrolimus | 11 | 229 | 6.60 POS | 24.60 POS | 3.58E + 04 | 17.93 POS |
| 7 | Liver | 0.01 | 43 | M | Basiliximab, prednisolone, tacrolimus | 38 | 298 | 2.01 POS | −0.17 NEG | 8.58E + 03 | 16.94 POS |
| 8 | Liver | 14.15 | 57 | F | Prednisolone, tacrolimus | 361 | 602 | 10.95 POS | 15.32 POS | 2.27E + 04 | 17.09 POS |
| 9 | Liver | 1.61 | 61 | M | Tacrolimus | 10 | 794 | 14.24 POS | 19.46 POS | 4.32E + 04 | 6.51 POS |
| 10 | Kidney | 10.22 | 58 | M | Tacrolimus | 129 | 93 | 9.64 POS | 24.74 POS | 7.56E + 04 | 18.57 POS |
| 11 | Kidney | 8.69 | 66 | F | Mycophenolate mofetil, prednisolone, tacrolimus | 5 | 156 | 7.38 POS | 23.80 POS | 3.48E + 05 | 18.45 POS |
| 12 | Kidney | 7.70 | 24 | M | Prednisolone, tacrolimus | 9 | 272 | 13.51 POS | 23.35 POS | 3.08E + 05 | 18.65 POS |
| 13 | Kidney | 7.37 | 52 | M | Tacrolimus | — | — | 11.91 POS | 24.84 POS | 3.56E + 05 | 17.72 POS |
| 14 | Kidney | 12.61 | 56 | F | Tacrolimus | — | — | 15.20 POS | 26.27 POS | 1.16E + 06 | 17.94 POS |
| 15 | Kidney | — | 61 | M | Cyclosporine | 25 | 415 | 12.30 POS | 0.16 NEG | 9.09E + 06 | 17.87 POS |
| 16 | Heart | 2.58 | 67 | M | Mycophenolate mofetil, prednisolone, tacrolimus | 9 | 53 | 4.44 POS | 2.59 POS | 7.52E + 05 | 18.03 POS |
| 17 | Allo-HSCT | 1.92 | 36 | F | Cyclosporine | 10 | 161 | 13.22 POS | 18.37 POS | 7.50E + 05 | 17.76 POS |
| 18 | Allo-HSCT | 0.19 | 44 | F | Cyclosporine | 14 | 17 | 3.26 POS | 17.45 POS | 3.52E + 02 | 0.26 ND |
| 19 | Allo-HSCT | 0.94 | 34 | M | Cyclosporine | 24 | 392 | 0.00 NEG | 0.00 NEG | 1.10E + 06 | 18.34 POS |

Patient characteristics of 19 HEV viremic patients identified by HEV RNA screening.
ND, not detected; F, female; M, male; NEG, negative; POS, positive.

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**TABLE 2.**

**Comparison of selected demographic, clinical, and biochemical parameters of HEV viremic patients and HEV RNA-negative patients**

| | HEV viremic patients (n = 19) | HEV RNA-negative patients (n = 2803) | *P* |
|---|---|---|---|
| Transplant type | | | |
| SOT | 16 (84%) | 2403 (94%) | |
| Kidney | 6 | 1175 | |
| Liver[a] | 9 | 882 | 0.09 |
| Heart/lung[b] | 1 | 346 | |
| HSCT | 3 (16%) | 141 (6%) | |
| Allo-HSCT | 3 | 141 | |
| Not reported | 0 | 259 | |
| Sex | | | |
| Male | 11 (58%) | 1662 (59%) | >0.99 |
| Female | 8 (42%) | 1141 (41%) | |
| Drug administered | | | |
| Tacrolimus | 14 (74%) | 2052 (73%) | |
| Cyclosporine | 4 (21%) | 709 (25%) | 0.3 |
| Other | 1 (5%) | 42 (2%) | |
| Age, y | | | |
| Median (IQR) | 55 (36-61) | 54 (42-63) | 0.6 |
| Number included | 19 | 2803 | |
| Log (time since transplant), y | | | |
| Median (IQR) | 3.8 (2.7-4.7) | 3.8 (2.4-4.8) | 0.9 |
| Number included | 18 | 2586 | |
| ALT, IU/L | | | |
| Median (IQR) | 156 (57-298) | 19 (13–30) | <0.0001 |
| Log (ALT), median (IQR) | 5.0 (4.0-5.7) | 2.9 (2.6-3.4) | <0.0001 |
| Number included | 17 | 2746 | |
| Bilirubin, µmol/L | | | |
| Median (IQR) | 11 (9-24) | 8 (6-13) | 0.01 |
| Log (bilirubin), median (IQR) | 2.4 (2.2-3.2) | 2.1 (1.8-2.6) | 0.01 |
| Number included | 17 | 2803 | |
| Tacrolimus level, µg/L | | | |
| Median (IQR) | 8.4 (7-8.9) | 5.8 (4.4-7.7) | 0.002 |
| Number included | 14 | 2052 | |
| Cyclosporine level, µg/L | | | |
| Median (IQR) | 166.5 (118.5-434.5) | 73 (43-119) | 0.02 |
| Number included | 4 | 709 | |

Categorical values were compared using Fisher exact test and continuous variables are compared using Wilcoxon 2-sample test.
[a] Includes kidney/liver (n = 21) and lung/liver dual transplants (n = 1).
[b] Includes heart/kidney dual transplants (n = 2).

statistically significant univariable risk factors for HEV viremia in this audit.

## Predictive Value of a Raised Serum ALT Value for HEV Viremia

Of the HEV viremic patients with an available ALT result (n = 17), 15 (88.2%) had an abnormal ALT value at the time of screening (>41 IU/L) compared with only 452 (16%) of the HEV RNA-negative patients. The positive predictive value (PPV) of an abnormal ALT result (>41 IU/L) as a surrogate for HEV infection in this cohort was 3.2% and did not rise significantly by raising the ALT threshold (PPV, 3.7%/sensitivity 70.6% for ALT >57 IU/L; PPV, 5.2%/sensitivity 47.1% for ALT >156 IU/L; PPV, 3.8%/sensitivity 23.5% for ALT >298 IU/L). No correlation was observed between ALT value and plasma HEV viral load (correlation coefficient, 0.11; *P* = 0.7).

Both of the 2 patients with a normal ALT (patients 1 and 18, Table 1) at the time point of screening subsequently developed an abnormal ALT result during follow-up with a rising viral load (352 rising to $3.0 \times 10^5$ IU/mL, $8.57 \times 10^2$ rising to $7.6 \times 10^4$ IU/mL, respectively), suggesting that the screening test was during early infection.

## HEV Markers in Viremic Patients

Most patients, 15 (78.9%) of 19 patients, were seropositive for IgM and IgG anti-HEV, 2 (10.5%) were seropositive for IgM anti-HEV only and 2 (10.5%) were seronegative (patients 1 and 19, Table 1). Of the 2 patients who were seronegative at the time of screening, one was an allogeneic HSCT recipient (HEV RNA $1.10 \times 10^6$ IU/mL) and one was a liver transplant recipient (HEV RNA $8.57 \times 10^2$ IU/mL). Both patients subsequently seroconverted for IgM and IgG antibody but had remained seronegative for at least 4 months and 2 months, respectively, from the time of first testing.

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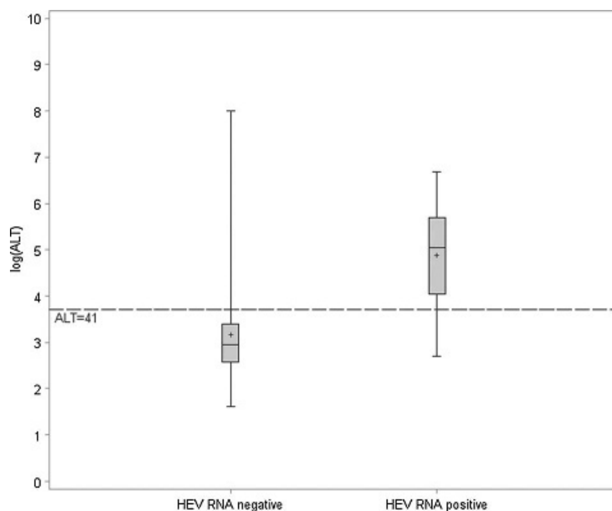


**FIGURE 2.** Box plot of the distribution of ALT values at the time of screening for HEV viraemic and aviraemic patients. Median ALT values were significantly higher in the HEV viraemic patients (ALT 156 IU/L) compared to the aviraemic patients (ALT 19 IU/L) (P<0.0001). The hatched line represents the laboratory upper limit of normal for ALT (41 IU/L). The boxes are defined by the first and third quartiles and the band represents the median value. + = mean value.

### HEV-Ag Sensitivity and Specificity in Transplant Patients

In comparison to serology, 18 (94.7%) of the 19 samples from viremic patients were reactive in the HEV-Ag assay (S/CO range, 8.35-19.08). The patient, whose sample tested negative in the HEV-Ag assay, had a viral load of 352 IU/mL. Follow-up testing 10 weeks later demonstrated a rising viral load ($3.00 \times 10^5$ IU/mL) and a reactive HEV-Ag result (S/CO, 19.38). When testing was restricted to patients with established persistent infection (viral load stable for period of >4 weeks) sensitivity was 100% (14/14). A correlation was seen between log HEV RNA level (IU/mL) and HEV-Ag $OD_{450/630}$ S/CO ratio (correlation coefficient 0.7388, data not shown). Specificity was determined by performing HEV-Ag testing on plasma from anonymized blood donors and aviremic immunocompromised patients (Table 4). Thirteen were reactive (S/CO >1.0) of which 10 samples (2.4%) were repeatedly reactive. In contrast, none of the 176 plasmas from anonymized blood donors were reactive. Using a novel HEV-Ag neutralisation step, we were able to confirm that the HEV-Ag reactivity in the RNA-positive samples was specific, but for 9 of the 10 RNA-negative/HEV-Ag–reactive samples, the reactivity was nonspecific, confirming false-positive results.[21] Thus, the overall specificity in our immunocompromised cohort was 97.85% (95% CI, 95.96-99.01).

The S/CO ratios of the RNA-positive/antigen reactive samples were significantly higher than the RNA-negative/antigen reactive samples (median, 17.93; IQR, 17.0-18.3 vs median, 2.737; IQR, 1.48-4.47; *P* = <0.0001) (Figure 3).

**TABLE 3.**

**Comparison of ALT values at the random time-point of screening for HEV viremic patients and HEV RNA-negative patients by transplant group**

| | HEV viremic patients | HEV RNA-negative patients |
|---|---|---|
| | Median ALT, IU/L | Median ALT, IU/L |
| Kidney | 214 (124.5-343.5), n = 4[a] | 16 (12-22), n = 1120[b] |
| Liver[c] | 127 (57-298), n = 9 | 24 (15-62), n = 882 |
| Heart/lung[d] | 53 (—), n = 1 | 18 (14-26), n = 346 |
| Allo-HSCT | 161 (17-392), n = 3 | 29 (18-50), n = 141 |

[a] Data missing for 2 patients.
[b] Data missing for 55 patients.
[c] Includes kidney/liver (n = 21) and lung/liver dual transplants (n = 1).
[d] Includes heart/kidney dual transplants (n = 2).

**TABLE 4.**

**Determination of specificity of HEV-Ag assay by testing HEV RNA-negative anonymized blood donors and transplant recipients**

| | Anonymized blood donors | Immunosuppressed patients | *P* |
|---|---|---|---|
| Repeat reactive samples | 0 (0%) | 10 (2.4%) | 0.038 |
| Nonreactive samples | 176 (100%) | 410 (97.6%)[a] | |

Values were compared using Fisher exact test.
[a] Includes 3 samples which were reactive on initial testing (S/CO >1.0) but not reactive on repeat testing.

### Selective Screening Strategies

We considered 2 possible strategies for screening patients: testing all patients for HEV RNA; testing all patients for HEV-Ag; testing patients with an abnormal ALT for HEV RNA or testing patients with abnormal ALT for HEV-Ag (summarized in Figure 4). ALT data were missing for 2 viremic patients; however, if testing was restricted to those with an abnormal ALT, it would reduce the overall cohort size requiring testing to 467 (16.5%) and would have identified at least 15 (88.2% of HEV viremic patients with known ALT) using either HEV RNA or HEV-Ag detection. If screening were delayed to a fixed time-point posttransplant such as at 6 months then the proportion of patients with an abnormal ALT would be lower still (Figure 5). For example, in the cohort of patients greater than 6 months from transplant 4.1% kidney, 20.5% liver, 6.6% heart/lung, and 32.9% of HSCT patients had an ALT above the upper limit of normal (ULN). This would reduce the cost of screening significantly.

## DISCUSSION

Persistent G3 HEV infections are an important cause of chronic hepatitis among SOT recipients because of the risk of cirrhosis in up to 15% of patients.[3,11] We sought to establish the prevalence of HEV infection in an unselected cohort of SOT and HSCT patients to assess the need for systematic screening within the UK and investigate possible strategies including the role of HEV-Ag testing for this purpose.

This is the largest audit to screen for HEV viremia in a transplant cohort and the first in the United Kingdom. We identified a low but significant HEV RNA prevalence of 1 (0.67%) in 149, similar to studies in other European countries.[12-16] During the period of sampling, the prevalence of HEV viremia among blood donors in England was 1:1875 (0.05%) and in effect reflects the incidence rate for HEV infection in the English population (national screening of donors February to September 2016, England. NHSBT/PHE Epidemiology Unit, pers. comm. Dec 2016). The 12-fold higher prevalence rate in the transplant patients represents a

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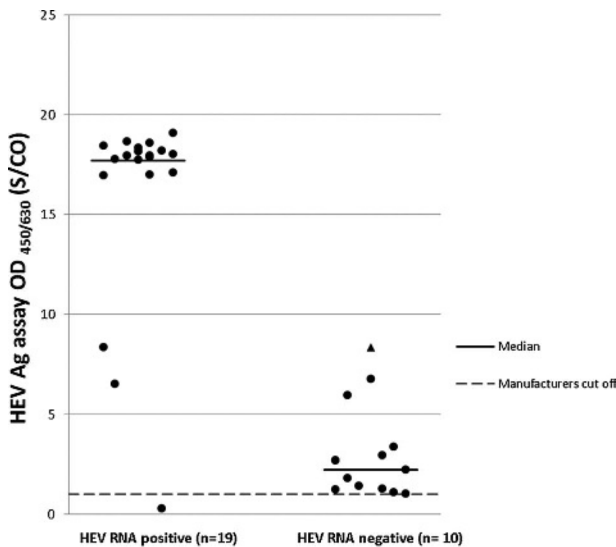


**FIGURE 3.** Comparison of HEV-Ag assay reactivity in HEV viremic and RNA-negative samples testing repeat reactive in the assay (n=10) at the time of sampling. Displayed values are normalised OD450/630 ratios (S/CO). There is a statistically significant difference in S/CO between the 2 groups;median 17.93 [IQR17.0-18.3] vs median 2.737 [IQR 1.48-4.47] ($P < 0.0001$). ▲ = sample harbouring neutralizable HEV-Ag (see results and discussion).

cumulative prevalence of infection as a consequence of the failure to clear infection during iatrogenic immunosuppression.

This audit represents a single transplant center experience in Birmingham where 96% of tested patients resided within a 100 mile radius. Whilst HEV exposure has been shown to vary geographically in some regions such as southern France, ongoing seroprevalence studies in English blood donors do not support great heterogeneity across England (Steve Dicks, pers. comm., NHSBT, March 2017).[22] Therefore, our data are likely to be representative of other transplant cohorts across the United Kingdom.

Higher tacrolimus and cyclosporine levels were found in the infected patients which would suggest that lower levels are associated with spontaneous clearance of viremia. However, no association was found between different SOT categories even though they have different levels of immune suppression. Our data suggested the possibility that HSCT patients, having a higher exposure to blood products, are more at risk of HEV infection (3/149, 2%). A larger population size may have reached statistical significance, but our selection criteria of patient identification through those undergoing TDM will have biased our findings to more heavily immunosuppressed HSCT patients by selecting those closer to transplantation or with concomitant GvHD. However any increased risk for this group is likely to diminish in the United Kingdom after the introduction of universal HEV screening of blood donations.

Crucially, 18 of the 19 HEV infections were previously undiagnosed clearly showing that the burden of HEV infection in immunosuppressed patients is under appreciated and relying on clinical suspicion to consider the diagnosis is insufficient. In 4 patients, the derangement of liver enzymes was not considered significant for further evaluation and alternative diagnoses were suggested in at least 11 patients, including many diagnoses where increasing immunosuppression would be a potential intervention. Such treatment would have the potential to further amplify viral replication and exacerbate HEV disease.

At the time of writing, 12 patients had virological evidence of established persistent infection leaving them at risk of chronic liver disease. As most of these infections were not recognized clinically, we considered strategies for the identification of these infections which would enable timely management to prevent complications such as cirrhosis. Given the recent findings by Behrendt et al[17] who demonstrated high sensitivity of the HEV-Ag assay for the detection of persistent infections we assessed the role of HEV-Ag detection for screening purposes. In this study, we found that the HEV-Ag assay had a sensitivity of 94.7% for viremia at the single random time-point of screening and 100% for identification of patients with proven viremia of greater than 4 weeks duration. Similar to Behrendt et al,[17] we found those patients with established infection had very high S/CO ratios (>16.0). The 2 patients with intermediate S/CO ratios of 8.36 and 6.51 (patient 1 and 9 respectively, Table 1) were considered to be in the early phase of infection in the case of patient 1 or in the process of spontaneous clearance in the case of patient 9 (data not shown). We also found the assay to be highly specific (97.85%) in immunosuppressed patients. However, in the context of a viremia prevalence rate found in this study, the ratio of false positives to true positives will be in the region of 3:1, giving a PPV of a reactive HEV-Ag result of approximately 25%. The addition of a novel neutralization step, described in detail elsewhere, can identify nonspecific reactivity in the HEV-Ag assay and may enable more widespread testing in laboratories unable to perform HEV RNA testing.[21]

The use of serology to diagnose chronic HEV infection in immunocompromised patients has been reported to be insufficient due to a delayed serological response.[23,24] In this audit, 17 (89.5%) of the 19 viremic patients had a detectable serological response (plasma IgM and IgG reactive or IgM reactive only) at the time of screening. This was higher than

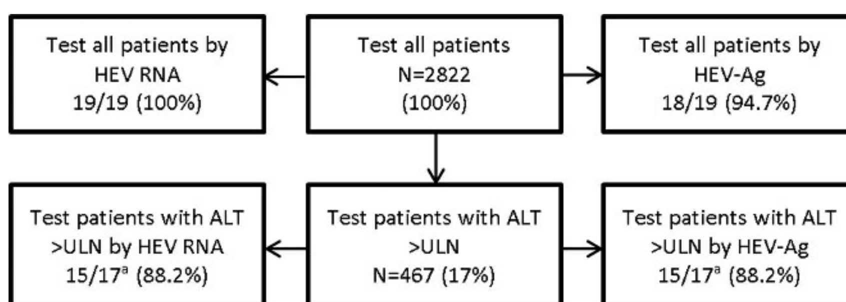


**FIGURE 4.** Consideration of possible screening strategies for identifying persistent HEV in a transplant cohort.The transplant cohort considered included heart, lung, kidney and stem cell recipients. [a] ALT data missing for two viraemic patients. ULN, Upper limit of normal.

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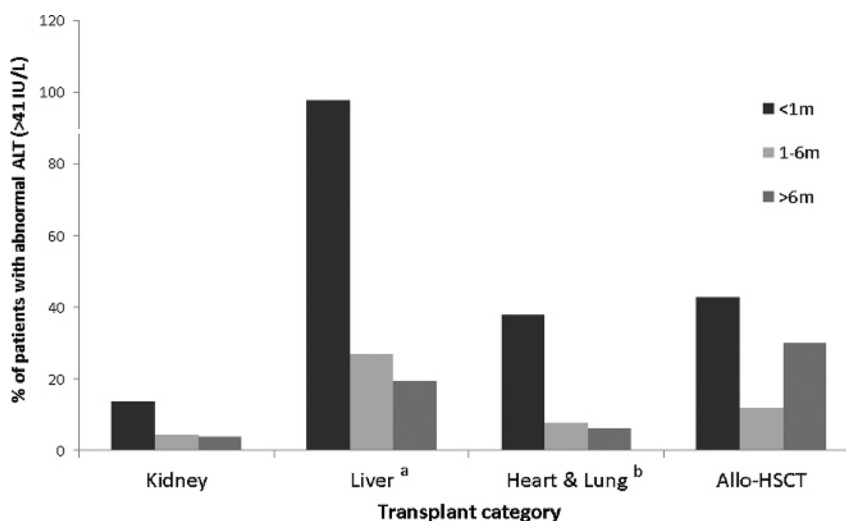


**FIGURE 5.** Bar chart representing percentage of patients with an abnormal ALT at timepoint of screening in relation to time since transplantation. [a] includes liver/kidney (n=21) and lung/liver (n=1); [b] includes heart/kidney (n=2).

expected but due to the lack of stored samples for retrospective testing, we are unable to comment on whether these serological responses were significantly delayed in individual patients. At PHE, we are also aware of patients with persistent HEV infection (n = 10) who have not seroconverted for anti-HEV despite viremia and serological follow up of at least 16 weeks (range 16-72 wks) (data unpublished). Therefore we would not advocate for serology alone to be used in this cohort to identify patients more likely to harbour persistent infection.

We specifically screened patients in an unbiased fashion disregarding ALT results to assess whether we would identify more HEV infections in this manner. By doing this, we identified 2 HEV viremic patients who had normal ALT results; however, both were likely to be in the early phase of infection as both returned elevated ALT levels when retested 29 to 30 weeks later with rising HEV RNA levels. If HEV screening was only performed on patients with an abnormal ALT, it would have reduced the numbers of patients screened by over sixfold; from 2822 to 467 but at the expense of a reduced sensitivity (87.5%) through failing to identify 2 infections. Raising the ALT threshold any higher than the ULN only reduced sensitivity and did not increase the PPV of the patient being HEV viremic.

It is likely that the most pragmatic and therefore cost-effective method of identifying persistent infection would be to test those patients with an abnormal ALT. Conventionally this would be by PCR but could also be by the less expensive HEV-Ag assay which in our study would have identified at least 15 of the 17 viremic patients who had an available ALT result. Whether consideration should be given to the introduction of a routine time-based screening policy in the absence of transaminitis is a matter for future discussion.

The strengths of this audit were the unbiased nature of testing which enabled us to compare different strategies for building testing algorithms, the size of the population and the wide range of different transplant populations screened. A major limitation was the single timepoint of testing at varying lengths of time posttransplant which rendered it difficult to ascribe specific infection risks such as risk from blood components around transplant or accumulated dietary risk over time. The lack of stored samples prevented us in determining the duration of infection before screening which would have informed the data on ALT levels.

We conclude that transplant recipients in the UK are at a low but significant risk of HEV infection. The majority of these infections go unrecognized despite increased awareness among the scientific and medical community. Although HEV screened blood components are now universally screened for HEV RNA within the United Kingdom, the dietary risk is now far greater than any residual transfusion risk in most patients and the rising risk of HEV acquisition from diet seen in Western Europe means that persistent infections will continue to occur.[25] Our data raise the question of whether structured systematic screening of transplant recipients for HEV infection by RNA testing or other virus-specific methods should be considered. We have demonstrated that HEV-Ag detection could be an alternative to RNA detection where the goal is to identify established HEV infection, particularly where expertise, facilities, or cost prohibit RNA testing. This audit should help inform health economic analyses and screening policies for HEV in immunosuppressed cohorts.

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