# Mortality and Morbidity of Locally-Acquired Hepatitis E in the National Scottish Cohort: A Multicentre Retrospective Study

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**Summary**

Background: Hepatitis E virus (HEV) is the most common acute viral hepatitis in Scotland. Little is known about the burden of morbidity and mortality, which can be high in chronic liver disease or immunocompromised states.

Aims: The study aims to record the morbidity and mortality of HEV in Scotland.

Methods: Demographic, clinical and laboratory data were collected retrospectively from all cases of HEV reported to virology departments across 9 NHS health boards, between January 2013 and January 2018.

Results: 511 cases were included (Mean age 62, 64% male). 58 (11%) cases had pre-existing cirrhosis and 110 (21%) had diabetes. 303 patients required admission (59%), totalling 2747 inpatient-bed-days. 17 (3.3%) HEV related deaths were recorded. Factors that predicted mortality included haematological malignancy (OR 51.56, 95% CI 3.40-782.83, p=0.005), cirrhosis (OR 41.85, 95% CI 2.85-594.16, p= 0.006), higher serum bilirubin (OR 1.01, 95% CI 1.01-1.02, p=0.011) and chronic HEV infection (OR 0.02, 95% CI 0.02- 0.28, p=<0.001).

HEV infection affected 35 transplant patients of 106 total immunosuppressed patients (21%). Of these, 25 patients received Ribavirin therapy with a sustained virological remission of 76%. 35 (6.7%) patients developed acute or acute-on-chronic liver failure with 2 requiring transplant. 37 (7.2%) patients reported neurological complications with 10 developing neuralgic amyotrophy, 6 Guillain-Barré and 2 encephalitis. 44 (8.6%) patients developed acute kidney injury.

Conclusion: In Scotland, HEV causes a significant burden of inpatient admissions, organ failure and death. Cirrhosis and haematological malignancy are significant predictors of mortality. Neurological and renal complications occur in a significant minority.

**Introduction**

In the past decade, our understanding of the role that the *Hepatitis E* virus (HEV) plays in human disease has changed dramatically. First identified from outbreaks in lower-income countries, HEV genotypes 1 and 2 are obligate human pathogens, spread via faecal-oral transmission. They cause an acute hepatitis with no recorded cases of chronic infection.[1] Genotype (G) 1 and 2 are responsible for 28.7% of cases of acute liver failure (ALF) in India with a high mortality in pregnant women.[2] There are an estimated 20.1 million infections each year worldwide with 70,000 associated deaths.[3] In comparison, G3 and G4 are now understood to be responsible for autochthonous (locally acquired) infections throughout Europe, North America, China and higher-income countries.[4] The mode of infection is primarily zoonotic with pigs acting as the primary reservoir.[5] Infection can also occur through contaminated water courses and vegetable irrigation systems and shellfish.[6] 1% of Scottish shellfish at the point of sale were recently found to contain HEV RNA.[7]

At least 70% of HEV G3 infections are thought to be asymptomatic in humans.[8] Laboratory confirmed cases have increased in many European countries in recent years and represent the 'tip of an iceberg' of undetected infections in any given region.[9] There is evidence to show that, in contrast to being a self-limiting illness, G3 infection can carry significant morbidity in vulnerable patient groups. Though uncommon, cases of acute liver failure (ALF) due to HEV infection have been reported.[10] Patients with chronic liver disease (CLD) infected with HEV are considered at-risk of acute-on-chronic liver failure (ACLF) or decompensated CLD (dCLD).[11] Another potential at-risk group are immunocompromised patients, in particular transplant recipients and patients with haematological malignancy.[12,13] HEV infection also causes harm in extra hepatic organ systems and is particularly associated with neurological injury.[14] HEV associated kidney injury has been described with both membranoproliferative and membranous glomerulonephritis, being reported to improve with viral clearance.[15]

The rising numbers of confirmed cases of HEV infection throughout Europe and the surprisingly high numbers of blood donors found to have circulating viral RNA, suggest that HEV is very common in humans.[9] HEV is now the most common acute viral hepatitis in many western European countries (including Scotland) with IgG seroprevalence being 16.2% in southwest England and as high as 52% in southwest France.[16-18] To date, there are limited data on the clinical impact of autochthonous HEV infection. The aim of this study was to determine the natural history and burden of disease from locally acquired hepatitis E in Scotland, as well as factors which predict adverse outcome, including mortality.

## Methods

### *Population studied*

Data were collected from four reference virology laboratories; covering nine of the 14 regional NHS health-boards (NHSHB) in Scotland which cover an estimated 71.5 % of the Scottish population (Figure 1, see also supplementary data for health board populations).

### *Clinical definitions and Inclusion criteria*

*HEV cases definition*: This was defined as HEV RNA viraemia **and/or** anti-HEV IgM and IgG reactive with biochemical evidence of raised transaminases (ALT ≥2.5 ULN). Cases that had travelled to a G1&2 endemic region and/or confirmed cases of G1&2 were excluded as probable imported cases.

*HEV related death*: This was defined as any patient who had HEV as a documented cause of death in their clinical records **and/or** death certification. This included those attributed **EITHER** directly to HEV infection **OR** HEV related death in the context of co-morbidities or concurrent illness **OR** any death within 30 days of recorded HEV viraemia.

*Chronic Hepatitis E:* Defined as HEV RNA viraemia for 3 months or greater[16]

*Cirrhosis*: Defined by **EITHER** biopsy proven cirrhosis **OR** Fibroscan® (Echosense) > 20 kpa or CT/MRI criteria (irregular liver contour± portal hypertension or evidence of previous admission with dCLD.[19]

*Immunosuppressed*: This was sub-categorised as: 1- Transplant recipient taking immunosuppressive therapy 2- Haematological malignancy 3- Disease modifying drugs for inflammatory conditions (including corticosteroids) 4- Cytotoxic chemotherapy for solid organ malignancy 5- HIV or other immune deficiency illness.

*Acute Liver failure (ALF)*: This was defined by severe liver injury (an acute abnormality of liver blood tests with subsequent jaundice and coagulopathy of liver aetiology), followed by the onset of hepatic encephalopathy within 12 weeks of the first symptoms in the absence of pre-existing liver disease.[20]

*Decompensated CLD (dCLD)*: This was defined as development of jaundice, ascites, variceal bleeding or mild hepatic encephalopathy (grade 1-2) in patients with chronic liver disease (with or without previously diagnosed cirrhosis) with NO extrahepatic organ failure.[21]

*Acute on Chronic Liver Failure (ACLF)*: This included any acute decompensation in patients with chronic liver disease (with or without previously diagnosed cirrhosis): with one or more extrahepatic organ failure(s), defined as:

- Liver failure: serum bilirubin > 177µmol/l (2.0mg/dl)
- Coagulation failure:- INR > 2.5 ± platelets $20x10^9$/l
- Renal failure: serum creatinine > 177µmol/l (2.0 mg/dl) or use of renal replacement therapy
- Circulatory failure: use of vasopressor to support blood pressure
- Respiratory failure: Pa02/FiO2 ratio >200 or SpO2/FiO2 <357
- Cerebral: grade 3-4 encephalopathy[21]

*Acute Kidney Injury (AKI)*: An eGFR decrease by ≥ 50% from baseline within seven days (RIFLE criteria).[22]

Proteinuria: Albumin/Creatinine ratio (ACR) of >3mg/mmol or Protein/Creatinine ratio of >15mg/mmol.

*HEV-associated neurological sequelae*: Any diagnosis of Guillain-Barré syndrome (GBS), neuralgic amyotrophy, meningo-encephalitis or reported neuralgia, myalgia, paraesthesia, weakness, meningitic headache or vertigo within 30 days of HEV diagnosis or viraemia.

*Lasting neurological disability*: The Guy's Neurological Disability Scale was used to define lasting disability at final follow up: 0= no complaints; 1=complaints but no restrictions; 2=restrictions but no help in daily life; 3= needs help in daily life but independent; 4=not independent, requires help regularly; 5=in need of continuous care.[23]

*Critical care*: Inclusion was based on the UK system of higher-level care including: Intensive care (ICU) for ventilated patients or two organ systems requiring invasive monitoring or support. High dependency unit (HDU) for single organ system requiring support

**Virological testing**

Over the timeframe studied, all laboratories HEV IgG and IgM serology used the RecomWell assay (Mikrogen, Germany). HEV PCR was tested on plasma with an in-house method designed in Glasgow using the Superscript III Platinum One Step Quantitative RT-PCR System (Invitrogen). All other Health boards used an easyMAG semi-automated nucleic extraction system (Biomerieux) followed by the ampliCube HEV 2.0 kit (Mikrogen Diagnostik) as previously described.[17] Seroconversion was not routinely assessed for.

**Data Acquisition**

This was a retrospective, five-year study with data collected from laboratory-confirmed HEV cases from each NHSHB from 1 January 2013 to 31st December 2017. Clinicians based at each NHSHB participated through a network set up through the Scottish Society of Gastroenterology (SSG).Cases were identified through local microbiology or virology databases. Detailed demographic information was acquired from electronic and paper records for each case (all datasets are included in the supplementary data). Virological data on genotype and sub-genotype was not routinely tested in Scotland during the study period. However, 34 cases were retrospectively tested for genotype in December 2019.

**Statistical analysis**

Logistic regression models were used to identify risk factors of different adverse outcomes. Exposure effects were expressed as odds ratios, linear regression coefficients and incidence (hazard) ratio with p-values and 95% confidence intervals. All statistical analyses were conducted using the R version 3.4.1. Missing data were assumed to be missing at random and handled by multiple imputation method using the mice package in R. We used the variable selection, which contains two steps. The first step involved performing stepwise model selection separately on each of the 100 imputed datasets, followed by the construction of a new supermodel that contains all variables that were present in at least half of the initial 100 models. R function step() was implemented for the stepwise method, which uses forward and backward selections together and repeatedly. Second, backward selection was applied to all variables present in the supermodel. Each variable was removed in turn and D1 statistic (similar to Wald test) was calculated by using the R function D1().If the p-value is larger than 0.05, the corresponding variable is removed, and the procedure is repeated on the smaller model. The procedure stops if all p-values are less or equal to 0.05. [24,25] **A Cox regression model was used to identify variables that predicted length of hospital stay.**

**Ethics**

Study registration and Caldicott approval for data-handling was granted and data were anonymised at NHSHB level. All data, including laboratory results were retrospective and originally recorded in the context of routine clinical care.

## Results

Over the five-year period 758 cases of HEV were notified to Health Protection Scotland from all Scottish NHS health boards. The four participating microbiology laboratories reported 582 cases of HEV. 54 of these were excluded as they were anti-HEV IgG positive only. 12 cases were removed due travel to an HEV G1-2 endemic area within eight weeks, two of these were found to have G1 infection with recent travel to India. Five were removed as duplicate entries between health boards. This left a total of 511 cases of hepatitis E that fulfilled the case definition (figure 2). Of these, in 372 cases HEV RNA was detected in sera. 139 cases tested RNA negative but were anti-HEV IgM and IgG reactive. The characteristics of these cases are described in table 1. Genotype data was available for 32 of these cases, all of which were G3. Cases varied per year from a lowest of 79 in 2013 to a peak of 123 in 2016 (see supplementary data figure 1).

| | Total (%) |
|---|---|
| N | 511 |
| Male | 325 (63.6%) |
| Female | 186 (36.4%) |
| Median age: years (IQR) | 62 (18.5) |
| Median SIMD: 1 to 10; decile: 10= least deprived (IQR) | 6 (5) |
| Acute HEV | 478 (93.5%) |
| Chronic HEV | 33 (6.5%) |
| Cirrhosis | 58 (11.4%) |
| Total Immunosuppressed | 106 (20.7%) |
| • Liver transplant recipient | 10 (1.9%) |
| • Kidney Transplant recipient | 20 (3.9%) |
| • Other transplant recipient | 5 (0.98%) |
| • Haematological malignancy | 17 (3.3%) |
| • Other Immunosuppressed | 54 (10.8%) |
| Diabetic | 110 (21.5%) |
| Median peak ALT: iu/L (IQR) | 1079 (1488) |
| Median peak bilirubin: µmol/L (IQR) | 38 (85.5) |

Table 1: Case characteristics. SIMD: Scottish Index of Multiple deprivation ranks data zones from most deprived (ranked 1) to least deprived (ranked 6,976) using multiple variables.

**HEV related deaths**

A total of 17 HEV- associated deaths were recorded (total mortality 3.3%), 13 with concurrent liver disease and four with significant immunosuppression at the time of infection. Twelve were male and five female with a mean age of 61 years (range 44-85). Two cases had a first presentation of ACLF and died after admission to ICU with encephalopathy and multiple organ failure: both of these cases had underlying alcohol related chronic liver disease. Ten cases had an established diagnosis of cirrhosis with decompensation, two of these patients received ribavirin during their acute illness but died within 3 weeks of treatment.

Four patients who died were immunosuppressed: one renal transplant recipient and three with haematological malignancy (high grade lymphoma n=2; myeloma n=1). Three had chronic HEV viraemia, two of whom were treated with ribavirin but died before viral response could be assessed. (see table 2)

| Age Sex M/F | HEV | Cirrhosis Cause | Immuno-suppression | Cause of death and Comorbidities (In addition to HEV infection) | Peak ALT iu/L | Peak Bili µmol/L | Peak INR | AKI Stage | HEV Specific Treatment | Diagnosis-Death (Days) ∞ |
|---|---|---|---|---|---|---|---|---|---|---|
| 71 M | Acute | ARLD | No | Decompensated Alcohol related Cirrhosis Diabetes type 2 | 607 | 331 | 1.6 | 3 | None | 28 |
| 85 F | Acute | NAFLD | No | NAFLD Cirrhosis. ACLF | 1901 | 339 | 5.5 | 1 | None | 9 |
| 44 M | Acute | ARLD | No | Decompensated Alcohol related Cirrhosis | 85 | 659 | 2.1 | 2 | None | 28 |
| 48 M | Acute | ARLD | No | Alcohol related Liver Disease, ACLF, treated for alcoholic hepatitis (prednisolone) | 1611 | 562 | 1.9 | 0 | None | 42 |
| 59 F | Acute | NAFLD | No | Decompensated NAFLD cirrhosis. Diabetes type 2 | 874 | 602 | 2.3 | 3 | None | 40 |
| 60 F | Acute | ARLD | No | Alcohol related liver disease, cirrhosis, ACLF | 184 | 495 | 2.2 | 0 | None | 2 |
| 64 M | Acute | ARLD | No | ACLF* | 881 | 402 | 1.5 | 3 | None | 4 |
| 54 M | Acute | ARLD | No | Alcohol related liver disease, cirrhosis, ACLF (Started Ribavirin) | 1079 | 1075 | 3.4 | 3 | Ribavirin (5 Days before death) | 10 |
| 71 F | Acute | No | Cytotoxic Chemo-therapy | High Grade Lymphoma on Chemotherapy, Shingles. | 91 | 150 | 1.7 | 2 | None | 9 |
| 76 M | Acute | NAFLD | No | Decompensated NAFLD Cirrhosis, HCC | 96 | 543 | 2.8 | 0 | None | 5 |
| 54 M | Acute | ARLD | No | ACLF, ARLD | 1922 | 601 | 2.1 | 2 | Ribavirin (6 weeks before death) | 42 |
| 61 M | Acute | ARLD | No | Decompensated Alcohol related cirrhosis, Hepatitis C | 372 | 560 | 2.8 | 3 | Ribavirin for unclear | Unclear |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | treatment duration | |
| 61 M | Acute | ARLD | No | ACLF* | 2087 | 1035 | 3 | 3 | None | 12 |
| 62 F | Chronic | NAFLD | Chronic Lymphocytic Leukaemia | NAFLD cirrhosis, Diabetes type 2, Chronic Lymphocytic Leukaemia (Chronic HEV viraemia) | 110 | 32 | 1 | 0 | None | Unclear raised ALT for >6 months |
| 62 M | Chronic | No | Enteracept | High grade lymphoma, Stem cell transplant, Graft-versus-host disease | 260 | N/A | N/A | N/A | 12 weeks Ribavirin | 84 |
| 46 M | Chronic | No | Lenolidomide Dexamethasone | Multiple Myeloma | 1261 | 66 | N/A | 3 | Immuno-suppression reduced | 168 |
| 57 M | Chronic | Yes | Tacrolimus Myco-phenolate Prednisolone | Renal Transplant with relapsed Chronic HEV infection causing cirrhosis | 60 | 35 | 1.1 | >60 | Ribavirin 6 months, Tacrolimus reduced | 168 |

Table 2. Cases of HEV related Mortality. Footnotes: ALT = alanine aminotransferase (normal range 10-40iu/L); bili = serum bilirubin (Upper Limit of Normal = <40iu/L: ); INR = International Normalised Ratio; AKI = acute kidney injury; NAFLD = non-alcoholic fatty liver disease; ARLD = Alcohol related liver disease; ACLF = acute and chronic liver failure; HCV = hepatitis C virus; HCC = hepatocellular carcinoma; N/A = not available; Y=yes; N=no

Stages of AKI (RIFLE criteria, based on eGFR alone)

1. Risk: Decrease of eGFR by 25%
2. Injury: Decrease of eGFR by 50%
3. Failure: Decrease of eGFR by 75%

*first presentation of chronic liver disease

∞time to death from initial diagnosis of HEV

Using multivariate analysis, factors that significantly predicted mortality (table 3) were haematological malignancy (OR 51.56, 95% CI 3.40-782.83, p=<0.05), cirrhosis (OR 41.85, 95% CI 2.85-594.16, p= 0.006), chronic HEV infection (OR 0.02, 95% CI 0.02- 0.28, p=<0.001), hepatic encephalopathy (OR 38.86, 95% CI 1.13, 75.94 p= <0.01), higher peak serum bilirubin (OR 1.01, 95% CI 1.01-1.02, p=0.011) and higher INR (OR 1.34, 95% CI 1.044- 1.706, P=0.02) . Age, gender, diabetes, index of deprivation, immunosuppression, higher peak ALT, HEV PCR+ status or treatment centre did not predict mortality.

| | **Proportion of HEV related death** | **Multivariate Analysis** | | | **Univariate Analysis** | | |
|---|---|---|---|---|---|---|---|
| | | **Odds ratio** | **P-value** | **95% confidence interval** | **Odds Ratio** | **P-value** | **95% Confidence interval** |
| Age | Continuous | 1.04 | 0.54 | (0.928, 1.154) | 1.004 | 0.819 | (0.97, 1.04) |
| Gender | Female (5/188) | - | - | - | | | |
| | Male (12/323) | 1.44 | 0.77 | (0.12, 17.36) | 1.412 | 0.523 | (0.488, 4.083) |
| SIMD | Continuous | 0.74 | 0.24 | (0.44, 1.229) | 1.051 | 0.576 | (0.883 1.252) |
| HEV Acute* | No (4/33) | - | - | - | | | |
| | Yes (13/478) | 0.02 | 0.001 | (0.002, 0.28) | 0.202 | 0.008 | (0.06, 0.66) |
| Haematological Malignancies* | No (14/494) | - | - | - | | | |
| | Yes (3/17) | 52.98 | 0.005 | (3.39, 782.83) | 7.347 | 0.004 | (1.89, 28.59) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cirrhosis* | No (3/453) | - | - | - | | | |
| | Yes (14/58) | 41.17 | 0.006 | (2.85, 594.16) | 54.316 | <0.001 | (13.21, 223.32) |
| Immunosuppression | No (12/405) | - | - | - | | | |
| | Yes (6/106) | 2872.31 | 0.069 | (0.46, 558501.343) | 2.157 | 0.14 | (0.777, 5.992) |
| Transplant recipient | No (15/477) | - | - | - | | | |
| | Yes (2/34) | 0.06 | 0.30 | (0.00, 12.10) | 1.911 | 0.404 | (0.417, 8.753) |
| Diabetes | No (13/401) | - | - | - | | | |
| | Yes (4/110) | 0.57 | 0.66 | (0.05, 6.50) | 1.122 | 0.843 | (0.357, 3.524) |
| Peak Bili | Continuous | 1.01 | 0.011 | (1, 1.02) | 1.010 | <0.001 | (1.007, 1.013) |
| Highest INR** | Continuous | 1.34 | 0.021 | (1.044, 1.706) | 1.338 | 0.029 | (1.030, 1.739) |
| Peak ALT | Continuous | 1.00 | 0.18 | (1.00, 1.00) | 0.999 | 0.046 | (0.999, 1.000) |
| Hepatic Encephalopathy* | No (7/492) | - | - | - | | | |
| | Yes (10/19) | 9.206 | 0.038 | (1.13, 75.07) | 76.984 | <0.001 | (23.84, 248.61) |

Table 3: Multivariate, logistic regression results for all the potential associated factors HEV related mortality.
* Risk factors selected by stepwise model selection ** Risk factors selected after stepwise model selection (including highest INR as a significant factor)

***Hospital admissions and higher-level care***

Three hundred and three patients were admitted to hospital (59.3%). The median length of admission was 5 days with a range of 1-208 (IQR:7): the high outlier being a patient with HEV encephalitis, confirmed with HEV RNA on lumbar puncture, who required prolonged rehabilitation. Ten patients were admitted to Intensive Care and a separate 13 patients were admitted to a high dependency unit. Reasons for critical care admission included ALF (2), deteriorating chronic HEV (3), ACLF/dCLD (13), dCLD + Variceal haemorrhage (2), GBS (2) and encephalitis (1). The cumulative inpatient bed days was 2747 over the five-year period.

Patients with a lower serum albumin, higher peak ALT, liver failure and neurological sequelae were all significantly more likely to be admitted to hospital (see table 4). Predictors of critical care admission (ICU or HDU) that were statistically significant were being a transplant recipient (OR 16.44, 95% CI 3.39-79.04, p=0.000) and all-cause liver failure (ALF/ACLF and dCLD) (OR 52.46, 95% CI 11.82-230.44, p=0.000). Associations with length of hospital stay were examined as a surrogate marker of disease severity. Factors that predicted a longer hospital stay included lower serum albumin, higher peak ALT, lasting neurological disability at final follow up and critical care admission (see table 4).

| Morbidity/ Complication | Associated factor | OR | P | 95% CI |
|---|---|---|---|---|
| Admissions to Hospital | Neurological manifestations | 4.21 | <0.001 | (1.52, 11.66) |
| | Higher peak ALT | 1.001 | <0.001 | (1.000, 1.001) |
| | Liver failure (ALF/ACLF/dCLD) | 14.78 | 0.02 | (1.40,155.72) |
| Admission to critical care | Transplant recipient | 16.44 | <0.001 | (3.39, 79.04) |
| | Liver failure (ALF/ACLF/dCLD) | 52.46 | <0.001 | (11.82,230.44) |

| | | | | |
|---|---|---|---|---|
| Longer duration of Hospital stay* | Admitted to critical care | 0.39 | <0.001 | (0.22, 0.69) |
| | Lower serum albumin | 1.1 | <0.001 | (1.07, 1.13) |
| | Higher peak ALT | 1.0001 | 0.01 | (1.00, 1.0002) |
| All cause Liver Failure ALF/ACLF/dCLD | Cirrhosis | 194.09 | <0.001 | (17.694, 2128.93) |
| Acute kidney Injury | Peak bilirubin | 1.004 | 0.005 | (1.001, 1.007) |
| | Cirrhosis | 8.41 | 0.001 | (2.457, 28.809) |
| Neurological Sequelae | Male sex | 0.50 | 0.05 | (0.25,1.01) |
| Neurological Functional Disability at final follow up | Age | 0.90 | 0.01 | (0.90, 0.99) |

Table 4: Factors with a significant association with HEV morbidity using multivariate logistic regression. *Using Cox regression.

Footnotes: ALT = alanine aminotransferase, ALF = acute liver failure, ACLF = acute and chronic liver failure, dCLD = decompensated chronic liver disease

***ACLF, Decompensated cirrhosis and ALF***

Thirty-three patients were documented as having ACLF or decompensated cirrhosis. Median peak ALT was 581.5iu/L with a range of 81-1901 and eight cases had a peak ALT <200iu/L. Morbidity included variceal haemorrhage (n=4), bacterial peritonitis (n=8) and encephalopathy (n=20). Twelve of these 33 patients died making the mortality in this group 36.4%. IU/L.

Within the 58 cases with confirmed or clinically suspected cirrhosis 30 were recorded as having ACLF/dCLD with 28 following a compensated, self-limiting acute hepatitis. The ACLF/dCLD group had a blood test profile in keeping with liver failure with a mean bilirubin and mean INR of 194 and 2.2 respectively, compared to 69 and 1.3 in the compensated group. ALT did not significantly differ between decompensated/ACLF (581iu/l) and compensated patients (689iu/l). A far higher proportion of the dCLD/ACLF group were of a background ARLD cirrhosis (55% vs 15%) and a higher proportion of the compensated cirrhotic cases were of NAFLD aetiology (36% vs 14%).

Four ACLF patients with acute HEV infection were treated with ribavirin, 3 patients died, two during a rapid deterioration before completing a course of Ribavirin and one after successfully clearing the virus on repeat PCR but deteriorating over with dCLD over six weeks (see table 2). 1 Case survived with viral clearance, a 69 year old with ARLD and cirrhosis, presenting with a peak ALT of 1314iu/L and a peak bilirubin of 682µg/l. Before serology, this patient was treated with steroids for alcoholic hepatitis. One patient who was on the transplant waiting list for NAFLD cirrhosis proceeded to liver transplant in the context of HEV-associated ACLF.

Two patients were referred to the Scottish Liver transplant Unit with ALF in the context of acute hepatitis E. Both patients developed encephalopathy with no history of cirrhosis. Peak Bilirubin 472µmol/l and 499µmol/l: Peak INR 2 AND 2.9. One patient recovered with supportive care alone and the other required liver transplantation.

***Acute Kidney Injury (AKI)***

Forty-four patients had a documented AKI. Twenty of these were in the context of decompensated cirrhosis or ACLF. However twenty-four patients had AKI with acute hepatitis E with no evidence of synthetic liver dysfunction to indicate ACLF or hepatorenal syndrome (HRS) as the cause. These 24 patients all saw a resolution of AKI as their transaminases settled. Seven of these patients had new or significantly worsening proteinuria (>100% higher than baseline measurement), two of which were chronic HEV patients who developed urinary high protein:creatinine ratio (PCR) values of 190mg/mmol and 70mg/mmol, both of which resolved with HEV viraemic remission. Of note, one acute case had raised free light chains for the duration of their HEV viremia. Six cases with proteinuria were tested for glomerulonephritis autoantibodies, all being negative and no patient had a renal biopsy. Four patients had normal urinary protein and the remaining 15 had no urinary protein recorded. Nine cases were on nephrotoxic

medication (including tacrolimus), one patient had a concurrent coliform grown in urine culture and one case was documented with dehydration indicating alternative aetiologies for their AKI.

Statistically significant predictors of AKI included higher serum peak bilirubin, (OR 1.004 P=0.005, 95% CI 1.001-1.007), and cirrhosis (OR 8.414, 95%CI 2.457-28.809, p=0.001) (see table 4). There was also a significant association between AKI and bilateral neuralgic amyotrophy (OR 12.55 95% CI 1.80-88.23, p=0.01).

***Immunosuppressed patients***

One hundred and six (20.7%) patients diagnosed with HEV infection were immunosuppressed (figure 3) at the time of infection: 30 with solid organ transplants; five stem cell transplant recipients; 3 with HIV; 51 receiving cytotoxic chemotherapy for cancer or disease-modifying drugs for inflammatory conditions and 17 with haematological malignancy. Seventy-three (68.9%) of the cases behaved as an acute hepatitis with resolution of viraemia and raised transaminases. Median, peak ALT in this group was (987iu/L) with a range of (43-6900). Forty-four (41.5%) patients achieved spontaneous viral clearance without reduction in immunosuppressive therapy. Eleven patients had their immunosuppression stopped and 18 had it reduced.

The remaining 33 cases developed chronic hepatitis E with a sustained viraemia of >3 months. This included 25 solid organ transplant patients, 6 patients with haematological malignancy (including two with stem cell transplant) and two immunocompetent patients. Median, peak ALT in this group was (172iu/L) with a range of (32-445iu/L). Five achieved viral clearance with a reduction in immunosuppression and two died without receiving ribavirin (see figure 3).

**Anti-viral therapy**

Thirty patients were treated with ribavirin: 25 with chronic HEV and five for acute infection and ACLF. 19 chronic cases achieved SVR with 4 chronic patients having a later relapse (SVR 76%). Two chronically infected patients died despite ribavirin therapy as shown in figure 3. Of the five acute cases treated with ribavirin, three died before viral response could be measured (SVR 40%).

**Immunocompetent cases with prolonged viraemia**

HEV infection was documented in pregnancy in one (0.19%) case of the 511 studied. This was a previously fit, immunocompetent pregnant woman with persistently deranged LFTs and a peak ALT of 111iu/L. The diagnosis was delayed, but retrospective sampling showed that she was viraemic from repeated samples for 5 months before achieving spontaneous viral clearance. A second, apparently immunocompetent case was reported in a 65-year-old female with untreated sarcoidosis and persistently deranged LFTs for one year. Peak ALT was 171iu/L. Viraemia persisted for >3 months and resolved with raised transaminases after a 3-week course of ribavirin (300mg bd).

**HEV-associated neurological Sequelae**

A total of 37 (7.2%) patients reported overt neurological complications, all in the context of acute infection in immunocompetent individuals (mean age = 59). . These comprised: neuralgic amyotrophy (n=10), Guillain-Barré syndrome (n=6), and encephalitis (n=2). Some cases had more than one neurological sequelae. There were 20 additional cases with other neurological symptoms not falling into the above criteria. These included neuralgia, parasthesia, meningitic headache prompting lumbar puncture, vertigo and Bell's palsy (n=20). 20 patients reported a longstanding disability at final follow up, ranging from loss of function that did not interfere with daily activities through one case of HEV encephalitis requiring long term rehabilitation and care. All six GBS patients reported longstanding disability at final follow up. The median, peak ALT for all neurological cases was 855iu/L with a range of 147-4829, however median peak ALT was lower in GBS patients (379iu/L).

HEV-associated neurological injury was more common in females with 19/188 effected compared with 18/323 males (OR 0.5, 95% CI 0.25-1, p= 0.05), but no other factors were identified as predictors of neurological illness. No significant predictors of neurological Injury were identified, though younger patients were more likely to report residual neurological functional disability at final follow up (OR 0.90, 95% CI 0.90, 0.99, p=0.01) (see table 4).

**Uncomplicated versus Complicated**

Of the 511 cases of HEV 379 (74.2%) reported an uncomplicated, acute hepatitis with complete recovery. The remaining 132 cases (25.8%) had recorded mortality or morbidity the breakdown of which is summarised in figure 4.

**Discussion**

This study represents the majority of Scottish health boards, making up roughly 71% of the nation's population.[26] Clinical data could not be collected from a minority of health boards including NHS Forth Valley, Lanarkshire, Highland, Western Isles and Dumfries and Galloway. Nevertheless, the data is broadly applicable to the whole of Scotland and covers the majority of all confirmed HEV cases in Scotland over the five-year timeframe. For comparison 853 cases were reported to HPS during the study time-period. However this includes probable cases and imported cases excluded from this study as well as (presumably) asymptomatic viraemic blood donors, picked by routine screening by the Scottish national blood transfusion service and reported to HPS. The total number of symptomatic cases diagnosed within all NHS health boards was 758.

This study represents roughly 67% of the total Scottish symptomatic caseload,[27] covering a timeframe where reported cases and circulating virus increased dramatically in Scotland. This is of particular concern as it is likely that the population will continue to become increasingly vulnerable to the complications of HEV infection, with liver disease increasing[28] and the number of patients receiving immunosuppression also rising.[29]

Between 2004-2008 the HEV IgG seroprevalence in Scotland was comparatively low at 4.7%.[30] However HPS reported a 15-fold increase in HEV diagnoses from 13 to 206 cases between 2011 and 2016. In addition, Thom et al. demonstrated that the number of viraemic blood donors (all G3) in Scotland increased from 1:14,500 in 2011 to 1:2481 in 2016, suggesting an increase in exposure to HEV in a largely HEV-naïve population.[17] Genotyping data in the current study, although limited in number, showed the patients included were uniformly infected with HEV G3. These findings are congruent with studies from many other European countries where the vast majority of infections with HEV are locally acquired G3.[31] So that autochthonous HEV infection was represented, we actively excluded patients with a recent travel history to Africa or Asia, which are areas endemic for HEV G1 and2. At the time of writing, a more detailed study of Scottish HEV phylogeny is currently underway could be important as a study of Genotype 3 cases in Belgium showed that there was a significantly lower rate of hospital admissions from HEV 3c subtype infections when compared to subtype HEV 3f.[32]

The five-year timeframe represents a period where clinicians became increasingly aware of HEV. However, it is likely that a significant caseload of HEV in Scotland was missed in sub-clinical cases or lack of HEV testing. A high proportion of HEV cases were diabetic (21.5%), immunosuppressed (20.7%), cirrhotic (11.4%) and there was a male to female ratio of 13:8 with a median age higher than the forecasted median age for Scotland (41.9 years).[26] These findings are in keeping with previous observations in UK in patients with HEV G3, and are unlikely to represent increased risk of infection in these high risk groups but rather a higher chance of developing a clinically detectable infection in these cohorts.[17,33] Despite the high proportion of cases, older age and diabetes were not multivariate predictors of HEV associated mortality.

As the number of reported cases represents a fraction of total HEV infections in the population, the true denominator and thus the true mortality and morbidity as a percentage are unclear. This is especially true in decompensated cirrhosis where patients may not have the biomass of hepatocytes necessary to generate an ALT rise and prompt testing. A point made all the more pertinent as previous studies have shown that 43% of patients with ACLF have no identifiable trigger.[34] The median peak ALT in the cirrhotic group was 581.5iu/L with several cases

demonstrating no significant ALT rise. Neurological injury is another cohort that often do not always present clinically as a hepatitis and the median, peak ALT for this cohort was 855iu/L. Decompensated chronic liver disease and neurological cases of HEV infection, without significant ALT rise have also been identified in the South of England.[31] This produces a clear selection bias as only the most clinically severe manifestations of HEV are included.

Despite the unknown denominator of undiagnosed HEV cases, the cumulative inpatient bed days, critical care admissions and patients left with disability represent a significant burden to the Scottish healthcare system. Based on an average inpatient admission cost of £3037, an average bed-day cost of £234/day for NHS Scotland with £717/day for HDU and £2085/day for ICU, HEV admissions alone have cost Scottish hospitals a conservative estimate of between £750,000 and £1,050,000 over the five year time period.[35,36] This does not include the cost of outpatient follow up, liver transplant, long term disability. These rough estimations of clinical cost could be used to influence the decision on the cost effectiveness of routine testing for HEV in blood products, which is under debate in many European countries.

The data show that a significant minority of the HEV infections come to harm with 25.8% of reported HEV cases dying, requiring critical care, developing liver or kidney failure, associated neurological morbidity or chronic hepatitis E. In many cases, it was a combination of these. Our study demonstrates how HEV can be fatal in vulnerable groups. Mortality was 20% in patients with chronic liver disease (12/58) and 36.4% (12/33) in cases with dCLD or ACLF. This compares with the CANONINC study that identified 28-day mortality of 33% of all cases of ACLF.[34] Similarly, a multi-centre Anglo-French study of 343 patients with decompensated CLD found 3.2% to have active HEV infection. Mortality was 27% in this group.[11] Cirrhosis was significantly associated with mortality (p=0.006) and peak bilirubin rather than ALT was the laboratory test significantly associated with mortality (p=0.011) with INR as a useful marker of liver failure. Rather than suggest a novel scoring system to forecast the severity a HEV infection, the authors suggests that these findings support the CLIFF-C ACLF scoring system and recommends this for acute HEV infection. In the Scottish ACLF cases, ribavirin seems only to have been given to the sickest patients and therefore its efficacy in this context is unclear.

Previous case series have shown that between 50-66% of immunosuppressed transplant recipients develop chronic hepatitis on exposure to HEV G3. Of these, 10% develop rapidly progressive cirrhosis.[12,13] Of the total immunosuppressed caseload of 106 patients, mortality was recorded at 4 (3.8%). However, three deaths were in the context of haematological malignancy and one in a kidney transplant recipient. Total haematological malignancies counted at 18 making mortality in this cohort 16.6%. Haematological malignancy was a strong independent predictor of mortality (p=0.005). This supports the work of von Felden et al. who's recent multi-centre European study found a mortality of 16% among viraemic HEV patients with haematological malignancy. In the current study, ribavirin SVR rate in the 25 chronic HEV was 76% with four patients having a relapse and two patients dying during their first course of ribavirin. Of the remaining three chronic cases, two died without receiving ribavirin, both being viraemic within 30 days of death, and one achieved SVR with a reduction of immunosuppression. Numbers are too small to demonstrate significance, but von Felden et al found that mortality was lower with ribavirin treatment compared to reducing immunosuppression.[35] The two individual cases of persisting HEV viraemia in the immunocompetent also raises concerns about who indeed is vulnerable to complicated infection, particularly as one patient was pregnant. Whilst the data does not go into the detail necessary to draw conclusions about individual cases, it does highlight a new research question on screening pregnant patients for chronic HEV.

There is already a demonstrable causal relationship between neuralgic amyotrophy (NA), Guillain-Barré syndrome (GBS) and encephalitis with HEV infection, often with little evidence of an associated hepatitis.[14] In this national Scottish cohort, a large number of cases with harmful neurological sequelae were identified. In particular eight cases of bilateral NA, five cases of GBS and two with encephalitis. Disability was often longstanding and most cases having a modest peak ALT below 400iu/L. Hepatitis E has been found in situ and in animal models to be neurotropic and whilst there may be an element of immune activation HEV could be causing direct nerve damage through infection.[38] The viral or host factors that leave a patient vulnerable to nerve damage are unclear and the role of ribavirin therapy in such cases remains to be established[39] One interesting observation was the association between bilateral NA and a reduction in EGFR (Log HR 2.53, 95% CI 0.59, 4.48, p=0.01). This represents three cases, only one of which had evidence of ACLF and possible hepatorenal syndrome. Whilst our data does not offer in-depth clinical information about the aetiology of each patient's renal failure, for 24 cases without liver decompensation, 15 had no alternative

aetiology in their clinical history. Furthermore the significant, transient proteinuria suggests a proportion of patients had an intrinsic renal injury related to HEV viraemia.

With regards to protecting vulnerable groups, Scotland routinely screens donated blood for HEV to try and reduce blood transmission. However sporadic cases from environmental exposure appear to have significantly increased, with an associated rise in seroprevalence in the past five years.[17] Only a major public health initiative to identify and control contaminated food and water would reduce the significant exposure to the population of Scotland. Identification of vulnerable groups and a low threshold for testing can direct early treatment early ribavirin therapy should be considered in patients with chronic HEV, Neurological injury and perhaps ALF/ACLF. Finally, there remains no consensus regarding the role of ribavirin treatment in hepatitis E viraemic ACLF.

The study is limited by its retrospective nature. In common with all retrospective studies, data collection was not complete in all cases, and testing thresholds for HEV may well have varied between centres and over time. The low proportion of genotype data is also a weakness of the study: During the timeframe of this study, RNA sequencing and genotyping were not routinely performed. This study was designed to assess clinical outcomes and therefore detailed virological or phylogenetic data analysis was considered beyond the scope of the study aims. Whilst it is not possible to retrospectively test sera from the patients included in this study, a further study to analyse the prevailing clades in Scotland is currently underway. The limitation in our knowledge of HEV genotype is partially, addressed by the knowledge that the predominant circulating genotype in Scotland during the course of the study was G3,[17] and by excluding patients who had recently travelled to HEV G1 and G1 endemic areas.

To our knowledge, this is the first systematic, multicentre review of mortality, compound morbidity and burden to healthcare for HEV in a G3/G4 endemic region.[40] Our study serves best as an assessment of the burden of disease on secondary care, although even in this setting some cases would have been missed. The data available is less suitable for identification of epidemiological trends or estimate what proportion of all HEV infections develop complications. However, used in conjunction with our current understanding of the emerging HEV epidemiology in Scotland, this study is well-timed to provide an accurate assessment of the burden of disease from HEV and provides clear evidence supporting increased vigilance in clinicians for possible HEV in vulnerable groups.

**Conclusion**

Hepatitis E virus infections in Scotland are characterised by a wide spectrum of clinical phenotype, from asymptomatic circulating virus detected in blood donors through to multiple organ failure and death. This study offers an overview of the scale of this public health hazard. It confirms that HEV poses a significant risk of morbidity and mortality to vulnerable groups and causes further harm through the extra-hepatic manifestations of neurological and renal injury. This burden of disease should justify increased efforts and resources to prevent public exposure to circulating HEV, early identification and treatment of vulnerable groups and research focus on improved treatment.

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