Hepatitis E virus in patients with decompensated chronic liver disease: a prospective UK/French study

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SUMMARY

Background
In developed countries, hepatitis E is a porcine zoonosis caused by hepatitis E virus (HEV) genotype 3. In developing countries, hepatitis E is mainly caused by genotype 1, and causes increased mortality in patients with pre-existing chronic liver disease (CLD).

Aim
To determine the role of HEV in patients with decompensated CLD.

Methods
Prospective HEV testing of 343 patients with decompensated CLD at three UK centres and Toulouse France, with follow-up for 6 months or death. IgG seroprevalence was compared with 911 controls.

Results
11/343 patients (3.2%) had acute hepatitis E infection, and three died. There were no differences in mortality (27% vs. 26%, OR 1.1, 95% CI 0.28–4.1), age ($P = 0.9$), bilirubin ($P = 0.5$), alanine aminotransferase ($P = 0.06$) albumin ($P = 0.5$) or international normalised ratio ($P = 0.6$) in patients with and without hepatitis E infection. Five cases were polymerase chain reaction (PCR) positive (genotype 3). Hepatitis E was more common in Toulouse (7.9%) compared to the UK cohort (1.2%, $P = 0.003$). HEV IgG seroprevalence was higher in Toulouse (OR 17, 95% CI 9.2–30) and Truro (OR 2.5, 95% CI 1.4–4.6) than in Glasgow, but lower in cases, compared to controls (OR 0.59, 95% CI 0.41–0.86).

Conclusions
Hepatitis E occurs in a minority of patients with decompensated chronic liver disease. The mortality is no different to the mortality in patients without hepatitis E infection. The diagnosis can only be established by a combination of serology and PCR, the yield and utility of which vary by geographical location.

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INTRODUCTION

Hepatitis E is endemic in many developing countries where it causes a high disease burden in humans.[1] In these geographical locations, hepatitis E is caused by hepatitis E virus (HEV) genotypes 1 and 2, spread orofaecally by contaminated drinking water. Hepatitis E predominantly affects young adults with an acute self-limiting hepatitis, except in pregnant women in whom the mortality is approximately 20%.[2] Several studies have shown that patients with underlying chronic liver disease (CLD) who develop hepatitis E also have a high mortality.[3–5] This included a prospective study from India that showed a 12-month mortality of 70%, which was significantly higher than in patients with CLD who decompensated due to any other cause.[3]

Over the last few years, evidence has emerged that HEV is also endemic in industrialised nations.[6–12] In such settings, hepatitis E is caused by HEV genotypes 3 and 4,[13, 14] and is mainly a porcine zoonotic infection spread predominantly by consumption of food products contaminated by HEV.[6–12, 15, 16] Cases of hepatitis E are sporadic and more commonly affect older males.[2, 6] Most patients have an acute self-limiting hepatitis, but infections with HEV genotype 3 can become chronic in the immunosuppressed.[17–24] In developed countries, excess mortality in pregnant women infected with HEV genotype 3 has not been observed.[2, 6]

Small case series suggest that, as has been documented in developing countries with HEV genotype 1, patients in developed nations with acute hepatitis due to locally acquired HEV genotype 3 who have underlying CLD may also have an adverse prognosis.[25, 26] In addition, two studies have shown a strong direct relationship between pork consumption and deaths from CLD in developed countries.[27, 28] Taken together, these data suggest that HEV may have a role in causing decompensation and death in patients with underlying CLD. However, to date, no large-scale studies have been reported from industrialised countries on the role of HEV in decompensated CLD.

The aim of this study was to define the role of HEV in patients with decompensated CLD in two developed countries.

MATERIALS AND METHODS

Patients with decompensated CLD were prospectively recruited from three hospital sites in the UK over a 2-year period during 2012–2013 (Truro $n = 105$, Glasgow $n = 119$, Norwich $n = 20$). Additional patients were prospectively recruited from one centre in France over an 18-month period from August 2011 to January 2103 (Toulouse $n = 99$). All patients were treated as hospital in-patients. Exclusion criteria were age <18 years and unwillingness to give consent (UK centres only).

Decompensated CLD was defined as: clinical and/or radiological and/or histological evidence of CLD, presenting with jaundice, and/or encephalopathy, and/or ascites, and/or variceal haemorrhage. Each patient was tested for HEV and followed up for a minimum of 6 months or until death.

At presentation, 5 mL of blood was collected from each patient by vacutainer into SST-II Gold tubes (BD Biosciences, Oxford, UK). After inversion and clot activation at RT for 30 min, samples were spun at 1100 g for 10 min. Serum was separated and stored in 1 mL aliquots at −80 °C. The serum samples were tested for HEV IgM and IgG using the Wantai ELISA kit (Wantai Biologicals, Beijing, China). Samples that gave borderline values (O.D. 0.9–1.1), as defined by the manufacturer, were excluded from data analysis. Viral ribonucleic acid (RNA) was extracted using the Qiagen vRNA method (Qiagen, Surrey, UK) and HEV virus amplified using both validated quantitative and qualitative polymerase chain reaction (PCR) as described previously.[29, 30] In the UK, testing was performed at the Glasgow Caledonian University Laboratory for all the UK sites. In France, testing was done in the French National Reference Laboratory for HEV in Toulouse. Samples were coded before testing. Data collection was done prospectively and prior to testing.

A case of hepatitis E was defined as:

- Anti-HEV IgM positive and/or
- HEV PCR positive

In three of the four centres (Truro, Glasgow and Toulouse), the anti-HEV IgG seroprevalence of the patients with decompensated CLD was compared to a control population from the same geographical location. In the two UK centres, the control groups comprised of age/sex-matched patients admitted to the participating hospitals with non-liver related illnesses (and normal biochemical liver tests) including cardiac and respiratory patients (Truro $n = 300$, Glasgow $n = 99$). The Toulouse group consisted of 512 consenting voluntary blood donors from the Midi Pyrennes region. Data from Norwich were excluded from the seroprevalence aspect of this study, as small numbers precluded meaningful analysis.

Data were collated and analysed using IBM SPSS (IBM SPSS Statistics for Windows, Version 21.0, Released 2012; IBM Corp., Armonk, NY, USA). Case survival

curves were compared using Cox regression. Adjusted odds ratios were calculated from logistic regression analyses. This study was approved by the Scotland A Research Ethics Committee 10/MRE00/74. The UK cases and controls all gave informed consent. Consent was not obtained from the cases in Toulouse, as HEV testing is regarded as standard clinical care in patients with decompensated CLD.

RESULTS

Of the 345 patients recruited, two patients were excluded from further analysis due to missing data. The demographic, clinical and laboratory data of the patients are shown in Table 1. Eighty-eight patients (25%) died within 6 months of presentation (Table 1). On multivariate analysis, 180-day mortality was associated with increasing age, with poorer survival for patients aged 60–69 years ($P < 0.05$) and aged 70 years or older ($P < 0.001$).

Eleven of the 343 (3.2%) patients had evidence of acute hepatitis E infection at presentation (Table 2). None of the patients reported travelling to an HEV-endemic developing country in the previous 3 months. Other than the immune-dysregulation often associated with CLD, none of the patients were immunocompromised. The median age of the patients with hepatitis E was 54 years (range 34–75 years), 10 of whom were male ($P = 0.18$ compared to patients without hepatitis E). At presentation, there was no difference in age ($P = 0.9$), serum bilirubin ($P = 0.5$), alanine aminotransferase (ALT; $P = 0.06$) albumin ($P = 0.5$) or international normalised ratio ($P = 0.6$) in patients with decompensated CLD and hepatitis E infection compared to patients without hepatitis E infection. Only two patients (patients 1 and 7; Table 2) had an ALT at presentation >2000 IU/L. Five patients (including both patients with an ALT>2000 IU/L) had HEV RNA detectable in their serum; on sequencing, it was HEV genotype 3 in all cases.

Of the 11 cases of acute hepatitis E, three (27%) died from liver failure within 6 months of presentation. The mortality of cases of decompensated CLD without hepatitis E was 26%. There was no difference between the 6-month mortality of cases with or without acute hepatitis E (Odds ratio, OR 1.1, 95% CI 0.28–4.1). Cases of acute hepatitis E were not seen uniformly across all sites of the study. Only three cases (1.2%) were identified in the combined UK group of 244 patients. By contrast, there were eight cases (7.9%) among the 99 patients from Toulouse, which was significantly more than seen in the UK group of cases ($P = 0.003$). Within the Toulouse group, there was no difference in 6-month mortality in cases with hepatitis E, compared to those without evidence of hepatitis E infection (29% and 38% respectively, OR = 1.5, 95% CI 0.33–6.6). Two patients from Toulouse were treated with ribavirin, shortly after presentation (Table 2). One survived and one died.

Serological results were available from 911 unmatched controls at three sites (Toulouse, Truro and Glasgow), 332 of who were HEV IgG seropositive, giving a

Table 1 | Demographic, laboratory and clinical data of patients with decompensated chronic liver disease ($n = 343$)

Age (years median, quartiles)	55 (46, 63)
Sex (M:F)	251:91 (1:n/k)
Acute HEV infections (*n*, %)	11 (3.2)
Bilirubin (μmol/L median, quartiles) (data missing for one case)	73 (36, 152)
ALT (IU/L median, quartiles) (data missing for three cases)	38 (25, 62)
ALKP (IU/L median, quartiles) (data missing for four cases)	182 (131, 316)
Albumin (g/L median, quartiles) (data missing for seven cases)	27 (23, 32)
INR (median, quartiles) (data missing for 102 cases)	1.5 (1.2, 1.8)
Cause of chronic liver disease (*n*, %)*	
Alcohol	274 (79.9)
HBV	10 (2.9)
HCV	36 (10.5)
NASH	21 (6.1)
PBC	2 (0.6)
Others	25 (7.3)
Child–Pugh Score (median, quartiles) (data missing for 26 cases)	10 (8, 11)
Death at 6 months, *n* (%)†	88 (25.7)

Bilirubin normal range 3–17 μmol/L. ALT (alanine aminotransferase) normal range <41 IU/L, ALKP (alkaline phosphatase) normal range 40–160 IU/L, albumin normal range 35–50 g/L, INR (international normalised ratio) range 0.8–1.2. n/k, not known.

* Note that some patients have more than one recorded cause of chronic liver disease.

† Excludes patients lost to follow-up within 6 months.

Table 2 | Demographic, laboratory and clinical data of the patients with acute hepatitis E infection and decompensated chronic liver disease

Case	1	2	3	4	5	6*	7	8	9	10	11†
Age (years)	60	34	60	36	50	39	53	54	75	59	61
Sex	M	M	M	F	M	M	M	M	M	M	M
HEV IgM	+	+	+	+	+	+	+	+	+	+	+
HEV IgG	+	+	+	+	+	+	+	–	+	+	–
HEV PCR	+	–	–	–	+	NA	+	–	+	–	+
Genotype	3				3		3		3		3
Bilirubin (μmol/L)	304	22	45	275	173	61	292	131	797	42	451
ALT (IU/L)	2419	10	70	129	128	129	2060	30	215	126	95
AST (IU/L)	NA	NA	NA	178	168	143	2091	121	214	76	152
ALKP (IU/L)	195	70	134	319	73	561	400	768	413	425	421
Albumin (g/L)	35	36	24	29	31	26	25	30	29	29	21
INR	1.5	0.9	1.6	2.1	NA	1.7	NA	1.6	2.3	1.2	NA
Cause of liver disease	NASH	Alcohol	Alcohol	Alcohol	Alcohol HBV	HCV NASH	Alcohol	Alcohol	Alcohol	Nash	Alcohol
Child–Pugh	8	5	NA	11	10	10	11	8	14	10	13
Death within 3 months	No	No	No	No	No	No	No	Yes	Yes	No	Yes

Cases 1 and 2 were from Truro and case 3 from Norwich, UK. The rest of the cases were from Toulouse, France. Bilirubin normal range 3–17 μmol/L. ALT (alanine aminotransferase) normal range <41 IU/L, ALKP (alkaline phosphatase) normal range 40–160 IU/L, albumin normal range 35–50 g/L, INR (international normalised ratio) range 0.8–1.2. NA, not available.

* Treated for 1 week with ribavirin, at the end of which time HEV became absent from serum.

† Treated with ribavirin, but made no response and died of liver failure 1 week into therapy. Of the three patients with HEV who died, one was too old to be considered a transplant candidate. Transplantation was contraindicated in the other two patients due to multifocal hepatocellular carcinoma and continued alcohol use/sepsis.

seroprevalence of 36.4%. At these sites, 64 of 321 patients with liver disease were seropositive (19.9%). Table 3 summarises the demographic details for cases and controls at the three centres, together with the anti-HEV IgG seroprevalence. The anti-HEV IgG seroprevalence increased with age in the control/patient groups, but was unrelated to sex (Table 4). The age-adjusted anti-HEV IgG seroprevalence was higher in Toulouse than in Glasgow (OR 17, 95% CI 9.2–30), and higher in Truro than in Glasgow (OR 2.5, 95% CI 1.4–4.6, Table 4). The anti-HEV IgG seroprevalence was significantly lower in cases, compared to controls (adjusted OR = 0.59, 95% CI 0.41–0.86, Table 4), and was not a predictor of 6-month mortality (Table 5). After adjusting for age, sex and geographical site, there was no difference in anti-HEV IgG seropositivity in patients with alcohol-related liver disease and those with liver disease of other aetiologies ($P = 0.13$).

DISCUSSION

The findings presented in the current study confirm that in south-west France and the UK, locally acquired acute HEV genotype 3 infection is present in a minority of patients with decompensated CLD, and some of these patients die. The mortality of patients with decompensated CLD and acute hepatitis E infection was 27%. This is not significantly different to patients without hepatitis E infection ($P = 0.9$), although the number of patients with hepatitis E was relatively low. These findings contrast to those found in some developing countries, including south-east Asia. In such settings HEV genotype 1 predominates, and patients with decompensated CLD and acute hepatitis E infection have a worse prognosis compared to patients who have decompensated from other causes.[3–5] In one study from Delhi, India, the 6-month mortality in patients with acute hepatitis E and decompensated CLD approached 70%.[3] The reason for the above difference in mortality in patients with CLD and hepatitis E possibly relates to viral factors, as HEV genotype 1 may be more pathogenic to humans compared to genotype 3.[31]

Hepatitis E infection appears to be an uncommon finding in patients with decompensated CLD in developed countries, and was documented in only 3.2% of cases we tested. This observation concurs with findings from a recent retrospective study from France, which

Table 3 | Demographic data and HEV IgG seroprevalence for cases and controls at three centres

	Glasgow	Truro	Toulouse	Total (three sites)
Controls				
Number of controls	99	300	512	911
Age (years, median, quartiles)	66 (51, 77)	65 (47, 77)	41 (31, 50)	48 (36, 64)
Sex (M:F)	29:70	134:166	329:183	492:419
IgG seropositive (*n*, %)	8 (8.1)	55 (18.3)	269 (52.5)	332 (36.4)
Cases				
Number of cases	119	105	99	323
Age (years, median, quartiles)	54 (43, 64.5)	54 (44, 61)	56 (49, 64)	54 (45, 62.5)
Sex (M:F)	90:28 (1 n/k)	71:34	74:25	235:87
IgG seropositive (*n*, %)	7 (5.9)	16 (15.2)	41 (41.4)	64 (19.8)

n/k, not known.

Table 4 | Odds ratios for IgG seropositivity, comparing cases (n = 323) and controls (n = 911) adjusted for age groups and study centres

Category	Adjusted odds ratio (95% CI)
Case/control	
Case	0.59 (0.41–0.86)
Age (years)	
≤39	Reference
40–49	1.2 (0.83–1.7)
50–59	1.9 (1.3–2.8)
60–69	2.2 (1.3–3.5)
≥70	2.4 (1.4–4.0)
Site	
Glasgow	Reference
Truro	2.5 (1.4–4.6)
Toulouse	17 (9.2–30)

Note that sex was not associated with HEV seropositivity in multivariate analysis.

Table 5 | Odds ratios for death within 6 months among decompensated chronic liver disease, by age groups at three centres

Category	Odds ratio (95% CI)
Age (years)	
≤39	Reference category
40–49	1.0 (0.38–2.7)
50–59	1.3 (0.54–3.4)
60–69	2.5 (1.0–6.1)
≥70	4.1 (1.5–11)

Note that sex, site and seropositivity were not associated with risk of death within 180 days.

showed that 3/84 (3.6%) of patients with severe alcoholic hepatitis had evidence of acute hepatitis E infection, one of whom died.[32] However, the incidence of hepatitis E infection in patients with decompensated CLD is likely to vary considerably by geographical location: hepatitis E infection was significantly more common in Toulouse than in the UK centres. This observation is probably a reflection of the differences in the quantum of circulating HEV in Toulouse and the UK, suggested by much higher anti-HEV IgG seroprevalence in Toulouse compared to the centres in the UK, documented in both the current and previous studies.[33–35]

The diagnosis of hepatitis E in patients with decompensated CLD is not straightforward. There were no differences in mode of clinical presentation or laboratory parameters in those with and without hepatitis E infection. Only 2 of the 11 patients with hepatitis E had an ALT >2000 IU/L at the time of hospital admission, which might have suggested the diagnosis to the attending clinician prior to the HEV results being available. A diagnosis of acute hepatitis E in patients with decompensated CLD can, therefore, only be made with appropriate virological testing. Our data suggest that this needs to be done with a combination of serology and PCR, as less than 50% had HEV RNA recoverable from the serum at presentation. This latter result suggests that some of our patients were infected with HEV several weeks prior to presentation, as HEV viraemia usually lasts for up to 1 month.[2, 6] This may also explain why only a small minority had very high ALT's when first seen.

Should clinicians routinely test patients with decompensated CLD for hepatitis E? Some clinicians might argue that it is not worth the bother and expense: HEV is an uncommon infection in such patients; the mortality is no worse than in those not infected; and there are no interventions that have been shown to improve outcome. We would argue that such patients should be routinely tested, as this will help our understanding of the emerging clinical

phenotype of hepatitis E infection at both patient and population level. In addition, a small case series suggests that patients with acute HEV and decompensated CLD may benefit from early intervention with anti-viral therapy.[36–38] Whether such intervention improves outcome remains to be established. We acknowledge that the decision whether to routinely test patients with decompensated CLD will be influenced by local economic and virological factors. For example, our data suggest that the clinical utility and yield of testing is much higher in Toulouse (8/99 cases, 7.9%) compared to Glasgow (0/119 cases, 0%).

Two previous population studies have shown that there is a correlation between national per capita consumption of pork and deaths from CLD in developed countries.[27, 28] The strength of this association is similar to that seen with alcohol. These observations have never been explained. One hypothesis is that this association might be accounted for by HEV, as this virus been found in the human food chain in pork products in many developed countries[15, 16, 39–42] and could cause excess mortality in patients with CLD via previously unrecognised infection. The data presented in the current article would argue against this hypothesis. However, our data do not completely negate this as a possible explanation. The reason for this is that the data showing the association between pork consumption is old, and dates from the 1960s and 1970s[27] and 1990–2000.[28] Seroprevalence data from Denmark[43] and the UK[44] show that during the above time-frames HEV was far more common than is currently the case. In addition, pork consumption has decreased in many countries in recent years.[45] Further studies are required to determine the reason for the previously observed relationship between pork consumption and liver deaths.

The seroprevalence data deserve brief comment. A previous study from south-west England showed that there was no difference in anti-HEV IgG seroprevalence in patients with *compensated* CLD and controls, but patients with compensated *alcoholic* liver disease had a significantly lower seroprevalence.[34] The reason for this observation is unknown, but one explanation is that hepatitis E might carry an excess mortality in patients with established alcoholic liver disease. This explanation would seem to fit well with another study from the UK which showed that individuals who drink excessive amounts of alcohol are more likely to develop clinically recognisable disease when exposed to HEV.[46] However, the seroprevalence data reported in the current study showed lower anti-HEV IgG seroprevalence in patients with decompensated CLD compared to controls. In addition, IgG seropositivity was unrelated to alcohol and did not predict mortality.

The strengths of the current study are its prospective nature and that it was performed in two countries, with markedly differing incidences of hepatitis E. This latter point gives the study more depth and improves the generalisability of the findings. In addition, we employed a serological assay which is specific and has a high (95–98%), validated sensitivity,[47] and a PCR technique which performs well against WHO standards.[48] The serological assay we employed is used by the HEV National References Centres in a number of European countries, including France and the UK.[49] The weaknesses of the study are that in one of the UK centres, recruitment was poor. In addition, the French controls used in the seroprevalence part of the study were an unmatched 'convenience' sample of blood donors. The seroprevalence data should be considered with this in mind.

In conclusion, locally acquired hepatitis E occurs in a minority of patients with decompensated CLD in the UK and south-west France. The mortality is 27%, which is no different to the mortality in patients without hepatitis E infection (26%). The diagnosis can only be established by a combination of serology and PCR, the yield and utility of which vary considerably by geographical location.

AUTHORSHIP

Guarantor of the article: H. R. Dalton.

Author contributions: HBP, RGM, AS, JGH, LV, KL, NDJ, CM, BS, HH, MP, EF collected the data and reviewed the drafts. CC, JP, JMM, LS, JI did the laboratory work and reviewed the drafts. OB did the statistical analysis. OB, LS, JMP and HRD co-designed and co-instigated the study and wrote the drafts.

All authors approved the final version of the manuscript.

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