Autochthonous hepatitis E in Southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease

Harry R. Dalton[a], William Stableforth[a], Prem Thurairajah[c], Simon Hazeldine[d], Rene Remnarace[e], Warshow Usama[c], Liz Farrington[a], Noor Hamad[a], Cyril Sieberhagen[f], Vic Ellis[b], Jonathan Mitchell[c], S. Hyder Hussaini[a], Malcolm Banks[g], Samreen Ijaz[h] and Richard P. Bendall[b]

Aims **To report the natural history of autochthonous hepatitis E and hepatitis E virus (HEV) IgG seroprevalence in Southwest England.**

Methods **Patients with unexplained hepatitis were tested for hepatitis E and cases followed until recovery or death. Five hundred blood donors, 336 individuals over the age of 60 years and 126 patients with chronic liver disease were tested for HEV IgG.**

Results **Forty cases of autochthonous hepatitis E (genotype 3) were identified. Hepatitis E was anicteric in 25% of cases and usually caused a self-limiting hepatitis predominantly in elderly Caucasian males. Six of 40 had a significant complication and three patients died, two of who had previously undiagnosed cirrhosis. Hepatitis E shows a seasonal variation with peaks in the spring and summer and no cases in November and December. HEV IgG prevalence increases with age, is more common in men and is 16% in blood donors, 13% in patients with chronic liver disease and 25% in individuals over 60 years.**

Conclusion **Autochthonous hepatitis E is more common than previously recognized, and should be considered in the differential diagnosis in patients with hepatitis, whatever their age or travel history. It carries a significant morbidity and when seen in the context of chronic liver disease carries an adverse prognosis.** ***Eur J Gastroenterol Hepatol*** **20:784–790 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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[a]Cornwall Gastrointestinal Unit, [b]Clinical Microbiology, Royal Cornwall Hospital Trust, Truro, [c]Departments of Gastroenterology, Derriford Hospital Plymouth, [d]Royal Devon and Exeter Hospital, Exeter, [e]Torbay Hospital, Torbay, [f]North Devon District Hospital, Barnstaple, [g]Veterinary Laboratories Agency, New Haw, Addlestone, Surrey and [h]Virus Reference Department, Centre for Infections, Health Protection Agency, London, UK

Correspondence to Harry R. Dalton, Cornwall Gastrointestinal Unit, Royal Cornwall Hospital Trust, Truro, Cornwall TR1 3LJ, UK
Tel: +44 1872 252749; fax: +44 1872 252794;
e-mail: harry.dalton@rcht.cornwall.nhs.uk

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Introduction

Hepatitis E is endemic in parts of the developing world and occurs in large outbreaks in areas with poor water sanitation. It causes a brief icteric illness, similar to hepatitis A and most often affects young adults [1]. The prognosis of hepatitis E is generally good, except in pregnant women and in patients with underlying chronic liver disease where the mortality rates are 20% and up to 75%, respectively [1–3].

Hepatitis E in developed countries has previously been considered rare and usually seen in patients who have recently travelled to endemic areas [1]. However, over the past few years, a number of cases of autochthonous (locally acquired) hepatitis E have been reported in the UK [4–8], Japan [9], Holland [10], France [11], USA [12] and New Zealand [13]. Although the source of these infections is not known, zoonotic transmission from pigs has been suggested [6–12]. The emergence of autochthonous hepatitis E as a public health issue in developed countries was recognized in the UK in 2005 by the Health Protection Agency, who called for a period of enhanced surveillance [14].

We have previously reported 21 cases of autochthonous hepatitis E in Southwest England [8], and showed that it is caused by hepatitis E virus (HEV) genotype 3 and has a predilection for middle-aged/elderly Caucasian males in whom it causes a self-limiting hepatitis. The aim of this study is to extend these observations in light of the new cases we have documented following the period of enhanced surveillance. In particular, we report the natural history, seasonal clustering, complications and predictors of outcome of autochthonous hepatitis E. In addition, we present data on the HEV IgG seroprevalence in our community in blood donors, a group of individuals over

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the age of 60 years and a cohort of patients with chronic liver disease.

Methods

Identification of cases of hepatitis E

From 1999 to 2004 stored sera from patients presenting with unexplained hepatitis to the Royal Cornwall Hospital's 'jaundice hotline' service [15] were retrospectively tested for HEV by IgM, IgG and reverse transcriptase (RT)-PCR assays. From 2005 onwards, patients presenting with unexplained hepatitis to the five major hospitals in Cornwall and Devon were prospectively tested for HEV IgM, IgG and by RT-PCR. The cases of hepatitis E identified were asked a detailed travel and dietary questionnaire and were followed up until recovery or death.

Testing for hepatitis E virus and case definition

Patients with unexplained hepatitis were tested for HEV IgM and IgG. All sera were stored at −70°C before testing. Serum anti-HEV IgM and IgG were measured using Genelabs Diagnostics HEV ELISA kits (Genelabs Diagnostics, Singapore). All reactive sera (test : cut-off ratio > 1) were tested for HEV RNA by RT-PCR using a previously documented technique [7]. Samples that proved to be HEV PCR positive underwent RNA sequence analysis [16] to determine genotype.

Cases were defined as follows:

1. Biochemical evidence of hepatitis (raised serum alanine aminotransaminase) and
2. Strong reactivity for anti-HEV IgM (test : cut-off ratio > 5), or
3. A rising titre of anti-HEV IgG, or
4. Detectable viraemia by RT-PCR.

Hepatitis E virus IgG serosurvey

Five hundred anonymized samples of serum were obtained from the regional blood transfusion service in Bristol. These samples originated from blood donors from the whole of the southwest region of Britain, including Cornwall and Devon. Three hundred and thirty-six individuals over the age of 60 years were also tested for HEV IgG. They were drawn from medical and surgical day cases and inpatients, and medical outpatients at the Royal Cornwall Hospital in Truro, UK, in the summer of 2006. Patients were excluded from the serosurvey study if they were jaundiced or had an abnormal serum alanine aminotransferase (ALT). In each individual a dietary history was taken to establish if they were vegetarian or not. One hundred and twenty-six patients with established chronic liver disease attending the hepatology clinic at the Royal Cornwall Hospital Truro were also tested for HEV IgG. Serum samples were stored at −70°C and tested for HEV IgG. HEV IgG was measured using the Wantai kit (Wantai, Beijing, China). This assay uses a recombinant antigen corresponding to the structural region of ORF-2 and has previously been used for serological surveys [13,17].

Ethics

This study was given ethical approval by the Cornwall and Isles of Scilly Ethics Committee.

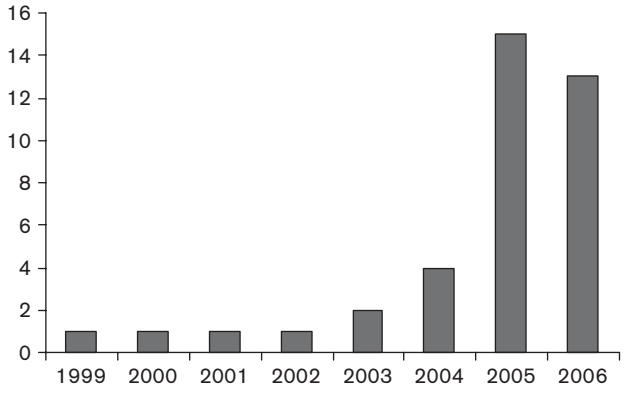
Results

Clinical and laboratory features of autochthonous hepatitis E

From 1999 to February 2007, 42 cases of hepatitis E were documented. Of these 42, 40 had autochthonous hepatitis E, as there was no history of travel to an area endemic for HEV. Two cases of 'imported' hepatitis E in travellers returning from areas where the disease is endemic (India and China) were found. These two cases will not be discussed further. During the final two years of the study (January 2005–December 2006), following the introduction of prospective testing, 28 cases of locally acquired hepatitis E were documented (Fig. 1). The results of virological testing are shown in Table 1. All PCR-positive cases were caused by HEV genotype 3.

Of the 40 cases, none had visited an area endemic for hepatitis E in the preceding 3 months, but five patients had travelled to nonendemic areas including Majorca ($n = 1$), Canary Islands ($n = 2$), Czech Republic ($n = 1$) and Australia ($n = 1$). Three of 40 cases were UK nationals visiting Cornwall and Devon (from Southampton $n = 1$, South Yorkshire $n = 1$, Cambridgeshire $n = 1$) at the time they developed the illness. The patients affected were all Caucasian and were predominantly elderly (median age 65 years, range: 35–86) males (M : F = 31 : 9). Four patients drank no alcohol at all; the median alcohol consumption was 5 units/week (range:

Fig. 1



Cases of autochthonous hepatitis E in Cornwall and Devon 1999–2006. The cases in the first 2 months of 2007 are not included.

Table 1 The virological findings in 40 cases of autochthonous hepatitis E

	HEV IgM	Initial HEV IgG	Convalescent HEV IgG	HEV RT-PCR
Positive	34	35	30	26
Negative	4	3	3	11[a]
Equivocal	2	0	0	0
Not tested	0	2	7	3

Figures represent totals from the cohort of 40 patients with autochthonous hepatitis E, with all patients fulfilling the case definition. Median (range) delay from onset of symptoms to hepatitis E virus (HEV) PCR sampling was 2 weeks (<1 to 3 weeks) for patients who were PCR positive, and 2 weeks (1–4 weeks) for patients who were PCR negative (P=NS).
[a]The serological results for the PCR negative were as follows: HEV IgM positive and rising HEV IgG n=7; HEV IgM positive and HEV IgG negative n=1; negative IgM and rising HEV IgG n=3.

Table 2 Symptoms at presentation in 40 cases of autochthonous hepatitis E

Symptom/frequency	Symptom/frequency
Jaundice n=30	Pruritis n=4
Anorexia n=15	Weight loss n=3
Malaise/lethargy n=15	Headaches n=3
Abdominal pain n=14	Back pain n=2
Nausea n=13	Arthralgia n=2
Fever/chills n=8	Rash n=1
Vomiting n=7[a]	Paraesthesiae n=1[b]
Myalgia n=5	No symptoms n=2

The total duration of symptoms was a median of 4 weeks (range: 2–18 weeks). Symptoms of dark urine and pale stools are included under 'jaundice'.
[a]Vomiting when present was often a dominant symptom and in one case resulted in a ruptured oesophagus (see main text).
[b]This patient developed a demyelinating polyradiculopathy precipitated by genotype 3 hepatitis E infection (see main text).

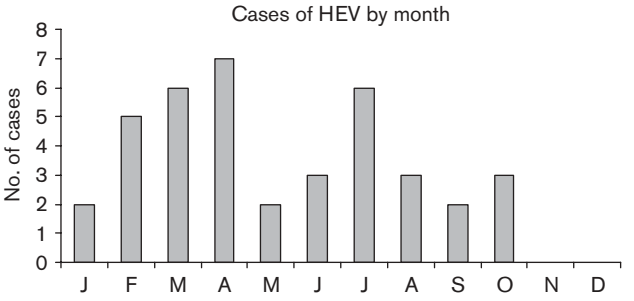
0–70 U/week). Thirty of 40 presented with jaundice, but 10 patients remained anicteric throughout their illness. Other symptoms were largely non-specific and are detailed in Table 2. Two patients had no symptoms at all, and were diagnosed by an incidental finding of a transaminitis on their liver blood tests taken for screening purposes (cardiac transplantation $n = 1$, hypercholesterolaemia $n = 1$). Ten of 40 were erroneously assumed to have drug-induced liver injury, prior to their HEV results being available. No cases were observed in the months of November and December, and the number of cases peaked in the spring, with a smaller peak in the summer (Fig. 2).

At presentation the median serum bilirubin was 105 μmol/l (range: 3–417 μmol/l) and ALT 1380 IU/l (range: 50–3346 IU/l). Thirty-four of 40 patients made a full clinical and biochemical recovery within 4–6 weeks. Six patients developed significant complications (three hepatic and three nonhepatic). Three survived but three died, two patients from liver failure.

Nonhepatic complications of hepatitis E

Vomiting was a symptom present in seven of 40 patients (Table 2), and in some this was a prominent and persistent problem. This was the case in a 75-year-old man who, 2 weeks into his illness, developed signs and symptoms of a spontaneous ruptured oesophagus (Borhaeve's syndrome). He was treated surgically and survived after a 2-month stay on the Intensive Care Unit.

Fig. 2



The seasonal variation of autochthonous hepatitis E. Months of the year are expressed on the x-axis, and have been abbreviated to their initial letter for the sake of clarity.

The second complication was seen in a 42-year-old man who presented with paraesthesiae and weakness in his lower limbs, and abnormal liver blood tests (ALT 632 IU/l, bilirubin 16 μmol/l). Nerve conduction studies confirmed the diagnosis of inflammatory polyradiculopathy that resolved after 3 months. Analysis of his serum showed him to be genotype 3 HEV PCR positive, his CSF was HEV PCR negative. He remained anicteric throughout his illness.

The most serious nonhepatic complication was seen in an 82-year-old man with a severe hepatitis (bilirubin 320 μmol/l, INR 1.3) requiring inpatient care. He died suddenly at day 3 from a ruptured common iliac aneurysm, which had previously been clinically silent.

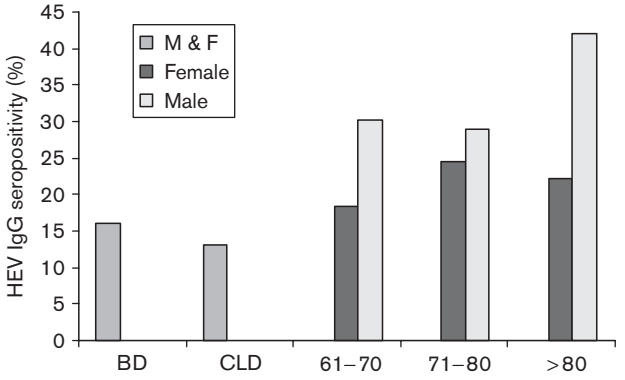
Hepatic complications of hepatitis E

Liver biopsies were performed in six of 40 patients. The histological features have been described in detail elsewhere [18]. In three male patients liver biopsy showed previously undiagnosed cirrhosis (ethanolic $n = 2$, idiopathic $n = 1$). The patient with idiopathic cirrhosis developed transient encephalopathy and recovered. The two patients with ethanolic cirrhosis (aged 59 and 76 years) both showed biochemical evidence of impaired synthetic function on presentation (see below), developed subacute liver failure and died at 4 and 5 months, respectively. The 59-year-old patient was referred for liver transplant assessment, but was declined.

Predictors of outcome

At presentation, four of 40 patients had an albumin of less than 34 g/l and an INR > 1.2. Three of these patients were found to have previously undiagnosed cirrhosis on

Fig. 3



Comparison of hepatitis E virus (HEV) IgG seropositivity rates in anonymous blood donors (BDs, $n=500$), chronic liver disease (CLD, $n=126$), and individuals over the age of 60 years without CLD and a normal alanine aminotransferase ($n=336$). Eighty out of 500 (16%) of the BDs were HEV IgG positive compared with 86 of 336 (25.5%) in the above 60 years cohort [$P=0.002$, odds ratio 0.6 (0.42–0.85), χ^2 test]. In the above 60 years group males had a significantly [$P=0.05$, odds ratio 1.63 (0.97–2.76), χ^2 test] higher HEV IgG seroprevalence (48 of 157, 30.5%) compared with females (38 of 179, 21.2%). Five of 336 were vegetarian, one of five tested positive for HEV IgG. Seventeen of 126 (13.4%) of the CLD group were HEV IgG positive. This is not significantly different from the BD group.

liver biopsy, two of who died from subacute liver failure, the other developed self-limiting encephalopathy (see above). The fourth patient had a severe hepatitis (bilirubin peaked at 417 μmol/l) but survived. He did not have a liver biopsy.

Lifestyle data

On lifestyle enquiry, 21 of the cases were retired and two unemployed. The remainder had various jobs, including one patient who worked as a butcher. None of the other patients' occupations brought them into contact with pigs, uncooked pig products or animal slurry. None were vegetarian and all patients were pork eaters.

IgG seroprevalence studies

Sixteen percent of blood donors, 13% of patients with chronic liver disease and 25.5% of the over 60 year olds were HEV IgG positive. HEV IgG seroprevalence increased with age and was more common in men (Fig. 3).

Discussion

This study describes a remarkable number of cases of autochthonous hepatitis E in southwest England, particularly in 2005–2006 following the Health Protection Agency's call for enhanced surveillance. In common with other studies, autochthonous hepatitis E in the developed world seems to have a predilection for middle aged and elderly men and is caused by HEV genotype 3 [6–13]. Hepatitis E usually causes a self-limiting hepatitis [4–13], but in our series six patients had a significant complication, two of who died from subacute liver failure. It is interesting to note that three of these six patients had previously undiagnosed cirrhosis. It thus seems likely that autochthonous genotype 3 hepatitis E in the developed world carries an adverse prognosis in patients with underlying chronic liver disease, as has been clearly demonstrated in genotype 1 'epidemic' hepatitis E in the Indian subcontinent [2,3]. The at-risk population is large, as only 13% of our patients with chronic liver disease have detectable anti-HEV antibodies. Very recent data from France have shown that in patients with chronic liver disease the mortality of autochthonous hepatitis E is approximately 70% [19].

This study extends our previous observations that autochthonous hepatitis E in developed regions occurs mainly in older male Caucasian patients. This observation was also noted in a study recently reported from southwest France [20]. The reason for this finding is uncertain. It could be related to increased exposure to HEV in men, as our data show that HEV IgG seroprevalence is higher in men. In our population autochthonous hepatitis E shows a seasonal variation, with a peak in the spring, a smaller peak in the summer and no cases in November and December. This is the first report in the literature of a seasonal variation of hepatitis E. The reason for this observation is also uncertain, but is likely to be related to the mode of transmission.

The source of autochthonous genotype 3 hepatitis E infection in developed countries is not known. It is thought that it is a zoonosis [21], and there is considerable evidence that pigs may be the reservoir, as genotype 3 HEV has been documented in pig herds throughout the world [22–24]. Anti-HEV IgG seroprevalences in pig-handlers and veterinarians caring for pigs are high [25]. HEV genomic sequences from pigs and humans are very closely related [6–12]. Porcine HEV is transmissible to nonhuman primates and human HEV is transmissible to pigs [26]. HEV has been demonstrated in retail pork meat products in Japan [27], the Netherlands [28] and USA [29], and in the latter study HEV recovered from pigs liver obtained from retail outlets was shown to be viable and infectious. Finally, HEV seems to be resistant to cooking in temperatures up to 60°C [30]. It is interesting to note that all our PCR-positive cases were HEV genotype 3 and all were pork eaters. This latter point is, however, less compelling evidence in support of the zoonotic theory than might at first be thought, as our seroprevalence data show that only 1.5% of Cornish residents over the age of 60 years are vegetarian.

The seroprevalence data presented in our study deserves further discussion. HEV IgG seroprevalence in develop-

ing countries is high, reflecting the endemic nature of the disease in this setting. For example, the HEV IgG seropositivity in adults in urban Delhi, India is 35.6% [31]. There have been a number of studies of HEV IgG seroprevalence in developed countries, reporting divergent results. The majority of studies report seroprevalence rates of 4–6% [13,32,33]. However, there have been a number of studies reporting much higher figures, including 17–21% in US blood donors [25,34] and 14% in adults in certain parts of Japan [35]. These divergent results may represent true geographical variations in the burden of hepatitis E infection. These data are, however, derived from diverse study populations with differing ages, and HEV IgG seroprevalence increases with age [25]. Moreover, differing HEV IgG assays were used with different target antigens. Thus, the wide range of results from developed countries reported in the literature could, at least in part, be methodological in origin.

We chose to use the Wantai HEV IgG kit to assess HEV seroprevalence because animal studies suggest that this assay has better sensitivity compared with other commercially available kits [36] and we were able to verify the manufacturer's claims that it was able to detect lower concentrations of reference sera (data not shown). We found a seroprevalence rate of 16% in blood donors, which is similar to that in US blood donors [25] but quite different to the only previous study from the UK by Bernal *et al.* [37], which reported a seroprevalence rate of 3.8% in individuals born in the UK and 8.8% in individuals born outside the UK. The disparity between our results and that of Bernal *et al.* could have a number of explanations. The populations studied and the HEV IgG assays were different. It could also be that in the intervening 10 years between the studies that there has been a true increase in the seroprevalence of HEV. Whatever the explanation, an HEV IgG seroprevalence rate of 16% in blood donors reported in this study seems plausible, given the number of cases of autochthonous hepatitis E documented in our community over the last few years. If our seroprevalence result is accurate, it indicates that unrecognized infection has hitherto been common place.

We cannot be certain about the incidence of hepatitis E in our population. Our data show that hepatitis E can occur either without any, or with nonspecific symptoms and that 25% are anicteric. Such patients may well not seek medical advice and if they do they may well not be tested for HEV. In light of our findings of a 16% HEV IgG seroprevalence in blood donors, it is possible that the majority of patients with hepatitis E have a clinically silent infection. Furthermore, one of the diagnostic criteria we used for hepatitis E infection was the presence of anti-HEV IgM tested by the Genelabs kit. It has been shown that this assay has poor sensitivity, giving false-negative results in up to 45%, particularly if there is delay in sampling after the onset of icterus [38]. This may have led us to miss a number of cases of hepatitis E. This effect was, however, mitigated to some extent by testing putative cases for acute and convalescent anti-HEV IgG, which has a sensitivity and specificity of approximately 90% [38]. It is thus likely that there were a considerable number of cases in our population during the study period of whom we were unaware.

The definition of a case of hepatitis E that we chose to use is similar to that used in a number of other recent studies [7–13]. Initially, we chose a cut-off for the ALT of >500 IU/l as part of the case definition. During the course of our study it became apparent that this cut-off was arbitrary and unhelpful as five of our cases (three of whom were PCR positive) had an ALT at presentation of less than 500 IU/l and in three of the cases the ALT never went above this level throughout the course of their illness (data not shown). Indeed, it has previously been documented that PCR-positive hepatitis E infection can occur with completely normal liver blood tests [13]. Four of our cases were PCR negative. All these cases were either HEV IgM positive and/or had a rising titre of HEV IgG. These PCR-negative cases illustrate that the duration of viraemia during the course of an infection may be brief, and show that PCR negativity, even at the time of icterus, does not exclude the diagnosis.

Do the cases of autochthonous hepatitis E reported in this study represent a local outbreak in southwest England, or are our findings representative of a more widespread problem? Considerable evidence exists that genotype 3 hepatitis E is a problem both throughout England and Wales and in other developed countries. Three of our patients were from other areas of the UK and were visiting Devon and Cornwall on holiday and became ill a few days after arriving, suggesting that they brought the infection with them. There are reports of hepatitis E from other areas of the UK [5,7]. Over the last few years, the number of documented cases of locally acquired hepatitis E in England and Wales has increased rapidly, and these cases are from a wide geographical area ([7], Ijaz, unpublished observations). Autochthonous genotype 3 hepatitis E has been described in a number of other developed countries including Japan, The Netherlands, France, USA, New Zealand and Spain [9–13,39]. Thus, it is likely that our observations are representative of a more widespread burden of hepatitis E throughout the developed world, rather than a local outbreak in southwest England.

In conclusion, we have documented 40 cases of autochthonous hepatitis E in Cornwall and Devon UK, and this infection thus seems to be much more common than previously recognized. All the PCR-positive cases

were caused by HEV genotype 3. Hepatitis E appears to have a predilection for middle-aged/elderly Caucasian males. It usually causes a self-limiting hepatitis and 25% of cases are anicteric. Significant complications occur in a minority and, in patients with underlying chronic liver disease, hepatitis E carries a significant mortality. Autochthonous hepatitis E has a seasonal variation in incidence, being uncommon in November and December with peaks in the spring and summer. The seroprevalence of HEV IgG increases with age, is higher in men and is 16% in blood donors from southwest England. This result seems plausible given the number of cases of hepatitis E we have seen in our population, and indicates that unrecognized and/or asymptomatic infection has hitherto been common. We recommend that all patients with unexplained hepatitis, whatever their age or travel history, are tested for HEV. Hepatitis E is a public health issue in the UK.

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H.R.D. instigated the study, wrote the paper and is the guarantor. W.S. recruited patients for the serosurvey, identified the cases and reviewed the drafts. P.T. identified the cases of hepatitis E in Plymouth, provided follow-up data and reviewed the drafts. S.H. identified the cases of hepatitis E in Exeter, provided follow-up data and reviewed the drafts. R.R. identified the cases of hepatitis E in Torbay, provided follow-up data and reviewed the drafts. U.W. identified the cases of hepatitis E in Plymouth, provided follow-up data and reviewed the drafts. L.F. recruited the patients with chronic liver disease and reviewed the drafts. N.H. recruited the patients for the serosurvey and reviewed the drafts. C.S. identified the cases of hepatitis E in Barnstaple, provided follow-up data and reviewed the drafts. V.E. performed the IgG studies and reviewed the drafts. J.M. identified the cases of hepatitis E in Plymouth and Truro, provided follow-up data and reviewed the drafts. S.H.H. helped establish the jaundice hotline in Truro, identified a number of cases, helped with serology study and reviewed the drafts. M.B. helped with the molecular characterization of HEV and reviewed the drafts. S.I. performed the HEV serology, HEV RT-PCR and molecular characterization and reviewed the drafts. R.B. coinstigated the study, performed the IgG studies and reviewed the drafts of the study.

Conflict of interest: none declared.

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