Hepatitis E: a largely underestimated, emerging threat

ABSTRACT

Hepatitis E virus has two distinct clinical and epidemiological patterns based on the varying genotypes. Genotypes 3 and 4 cause widespread, sporadic infection in high-income countries and are emerging as the most common type of viral hepatitis in much of Europe. These infections carry significant morbidity and mortality in the growing numbers of immunosuppressed patients or in patients with established liver disease. Furthermore the growing extra-hepatic associations of the virus, including neurological and kidney injury, suggest that it may have been misnamed as a 'hepatitis' virus. This review explores current understanding of the epidemiology, virology and clinical presentations of hepatitis E infection and identifies vulnerable patient groups, who are at serious risk from infection. Guidance is offered regarding the diagnosis, treatment and prevention of this growing public health hazard.

Our knowledge of the hepatitis E virus and its role in human disease has changed dramatically since its discovery in 1983. Before its characterisation in 1991, an acute, non-A-C, viral hepatitis had been repeatedly described, not only as outbreaks in Asia but also as a sporadic phenomenon in high-income countries. The virus was finally sequenced as an RNA virus of the Hepeviridae family. Initial understanding of hepatitis E virus in human infection was based on genotypes 1 and 2, which solely infect humans via faecal-oral transition and cause a self-limiting hepatitis. It was observed in large outbreaks in developing countries with no evidence of chronic infection (Purcell and Emerson, 2008). Cases observed in high-income countries were seen in travellers returning from endemic regions. These early observations set the prevailing understanding of hepatitis E virus: it was not considered of relevance in high-income countries.

Genotypes 3 and 4 are now understood to be responsible for locally-acquired infections throughout Europe (including the UK), North America, China and other high-income countries (Kamar et al, 2014a). The mode of infection is primarily zoonotic from pigs, which act as a viral reservoir (Debing et al, 2016). Over the past decade in Europe, heightened surveillance and improved sensitivity of serological markers have demonstrated a ten-fold increase in cases of hepatitis E virus infection. Furthermore, increasing IgG seroprevalence and large increases of circulating viral RNA, detected in blood donors, within certain 'hot spots', have revealed hepatitis E virus infection to be increasingly common. Hepatitis E virus is now the most common acute viral hepatitis in many western European countries (Mansuy et al, 2016; Thom et al, 2016; Dalton et al, 2018). There is also growing evidence to suggest that, rather than being a self-limiting illness, hepatitis E virus infection can cause significant morbidity and mortality in vulnerable patient groups. Hepatitis E virus infection also appears to cause harm in extra-hepatic organ systems and is particularly associated with neurological (Dalton et al, 2016) and kidney injury (Del Bello et al, 2015). This review explores current understanding of hepatitis E virus infection, the morbidity and mortality associated with infection and the potential burden of disease.

Epidemiology

In the developing world, it is estimated that 20 million hepatitis E infections occur yearly, with 3 million symptomatic cases and 70 000 deaths a year. In countries where genotypes 1 and 2 are prevalent, the mode of infection is faecal-oral with outbreak studies implicating contaminated water supplies. There is no known animal vector. The disease is particularly problematic in pregnant women who develop higher viral loads and fulminant hepatitis. Case fatality in pregnancy can be as high as 25% (Hakim et al, 2017).

Hepatitis E virus genotypes 3 and 4 are endemic in some developing countries and most high-income countries (Madden et al, 2016). In Europe, an estimated 2 million locally-acquired infections occur yearly (Adlhoch et al, 2016). Domestic pigs are the primary host with 85% of UK pigs becoming infected and re-infected and excreting large quantities of the virus. Human infection can also occur through contaminated water courses and vegetable irrigation systems (Dalton et al, 2008). Hepatitis E virus RNA has been found in 1% of Scottish shellfish, thought to be from abattoir effluent and pasture run-off (O'Hara et al, 2018).

In humans, at least 70% of hepatitis E virus genotype 3 infections are asymptomatic (Guillois et al, 2013). Laboratory confirmed cases represent the 'tip of the iceberg': a minority who develop symptoms or deranged liver blood tests and in whom hepatitis E virus is clinically

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Table 1. Global comparisons of hepatitis E virus IgG seroprevalence

Region	IgG seroprevalence
England	16.2%
Scotland	4.7%
France	22.4%
Germany	29.5%
Netherlands	27.0%
USA	9.0%
China	32.6%
India	13.7%
Japan	5.3%
South Africa	24.0%
Saudi Arabia	18.7%

From Guo et al (2010), Hartl et al (2016), Hakim et al (2017), Horvatits et al (2018)

suspected and tested for. The increase in cases reported to public health bodies across Europe is likely, in part, to represent greater clinical awareness and improved case ascertainment. Nevertheless, the number of cases has risen markedly across Europe. The sharpest rise appears to have occurred between 2006 and 2014 where France reported a rise in cases from 22 to 1813, Germany from <50 to 1266 and England and Wales from 279 to 925. This increase has continued with 5617 cases confirmed in the European Union in 2015, a 10-fold increase from 2005. The highest reporting countries were Germany, France and England. The vast majority of European hepatitis E virus infections were locally acquired genotype 3 (Adlhoch et al, 2016; European Centre for Disease Prevention and Control, 2017).

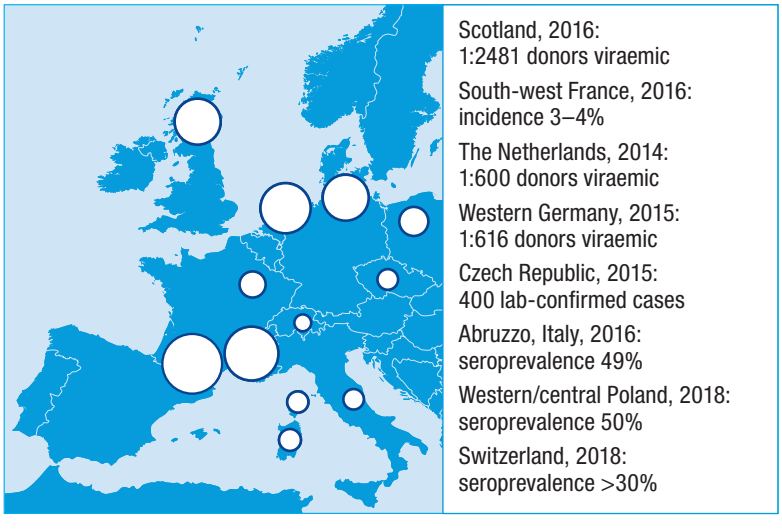

Figure 1. European Association for the Study of the Liver European 'hot spots' based on seroprevalence, circulating hepatitis E virus RNA or confirmed cases. From Dalton et al (2018).

The seroprevalence of patients with hepatitis E virus IgG antibodies shows what proportion of the population has had previous infection. Seroprevalence varies considerably from 4.7% in Scotland to 52% in southwest France, where it is hyper-endemic (*Table 1*). Scotland, with a historically low seroprevalence, reported a 15-fold increase in hepatitis E virus diagnoses from 13 to 206 between 2011 and 2016 and the number of viraemic blood donors increased from 1:14 500 in 2011 to 1:2481 in 2016. It is one of a number of identified European hot spots where there is a high incidence of circulating hepatitis E virus RNA and/or seroprevalence in blood donors (*Figure 1*).

Virology

Hepatitis E virus is a positive-sense, single-stranded RNA virus with a 7.2 kb genome. Like eukaryotic mRNA, the genome has a 5' 7-methylguanylate cap and a 3' polyA tail. It contains three or four open reading frames (ORFs) (*Figure 2*). ORF1 generates a functional protein involved mainly in genome replication, ORF2 encodes the viral capsid and ORF3 encodes a small protein involved in viral egress (Graff et al, 2006). The ORF2/ORF3 overlapping region is highly conserved between hepatitis E virus genotypes and thus is often the target region of pan-genotypic polymerase chain reaction tests. Hepatitis E virus genotype 1 strains contain an additional open reading frame within the ORF1 region (ORF4). While the exact function of G1 pORF4 is unknown, it enhances the activity of the viral RNA-dependent RNA polymerase (Nair et al, 2016). Short conserved sequences essential for viral replication, termed cis-reactive elements, have been identified in the 3' non-coding region and in the non-coding region between ORF1 and ORF3 (Yamada et al, 2009).

Virus particles have a 27–34 nm icosahedral capsid and, in bile and faeces, are non-enveloped. However, in blood the virus circulates in a host membrane-derived quasi-enveloped form (eHEV) (Purdy et al, 2017). This quasi-envelope protects the virus from antibody neutralization and eHEV is thought to play a major role in cell to cell spread. However, eHEV cell attachment is much less efficient than that of hepatitis E virus, which likely explains the reduced infectivity of serum-derived *vs* faecal-derived hepatitis E virus (Yin et al, 2016).

While the cell receptor governing virus entry remains unidentified, initial attachment occurs via heparan sulfate proteoglycans. Entry is through clathrin-mediated endocytosis, requiring dynamin-2 (Holla et al, 2015) (*Figure 3*). Following entry, genome replication occurs in the cytoplasm, with the 5' cap recruiting the 40s ribosomal subunit to initiate translation of ORF1 (Perttilä et al, 2013). The RNA-dependent RNA polymerase domain of pORF1 then reverse transcribes the genomic RNA to generate the subgenomic RNAs and full length genomes (Nair et al, 2016). The methyltransferase domain of pORF1 facilitates the capping of full-length genomic and subgenomic RNAs at the 5' end. The pORF2 capsid proteins translated from the subgenomic RNA package the

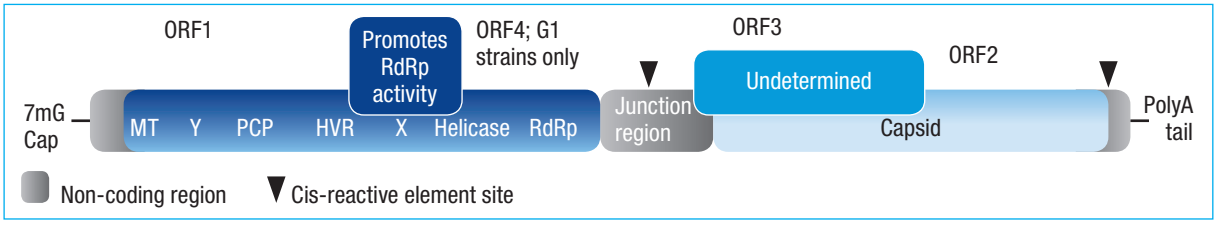

Figure 2. A schematic diagram of the open reading frame (ORF) positions within the HEV genome. HVR = hypervariable region; MT = methyltransferase; PCP = papain-like cysteine protease; RdRp = RNA-dependent RNA polymerase.

viral RNA and form virions. These are transported to the cell membrane, facilitated by pORF3 (Nagashima et al, 2011). It is probable that eHEV is released from the apical surface of hepatocytes but that the action of bile results in the loss of envelope from virus particles (Yin et al, 2016).

Thus hepatitis E virus RNA can be detected by polymerase chain reaction (conventional and quantitative) in both the blood and faeces of infected patients. However, the window of detectable virus is sometimes small and a negative polymerase chain reaction result should not immediately exclude a diagnosis of hepatitis E virus. In immunocompetent and symptomatic patients, primary infection elicits an IgM response and many diagnostic laboratories will accept a positive IgM result without a polymerase chain reaction-positive result as confirmation of an acute case of hepatitis E virus.

Clinical course, morbidity and mortality in healthy and vulnerable groups

Most clinically diagnosed hepatitis E virus infections will follow a self-limiting course of transaminitis, often with cholestasis and jaundice. The incubation period is 2–10 weeks. If taken at the time of acute infection peak serum alanine aminotransferase level is usually >1000 iu/litre. This rises within days of symptoms and normalises over 1–4 weeks, often followed by a cholestatic phase. Clinical jaundice occurs in roughly a quarter of infections. Genotype 3 infections seem to present more severely (and are therefore more commonly diagnosed) in older men and patients with diabetes (Wallace et al, 2017). A currently uncertain proportion of hepatitis E virus-infected patients will come to significant harm. Progression to acute liver failure is uncommon, but cases have been reported. A German single-centre study of 80 patients with acute liver failure found hepatitis E virus RNA in 10% of patients (Hartl et al, 2016). Excess mortality in pregnant women is not seen in the context of genotypes 3 and 4. In contrast, hepatitis E virus gt1 is responsible for 28.7% of cases of acute liver failure in India (Shalimar et al, 2017).

Patients with chronic liver disease infected with hepatitis E virus are at significant risk of acute-on-chronic liver failure or decompensated cirrhosis. Decompensated cirrhosis has many precipitant factors and carries a high mortality. A multi-centre Anglo-French study of 343 patients with decompensated chronic liver disease found that 3.2% had active hepatitis E virus infection. Mortality was 27% in this group (Blasco-Perrin et al, 2015). Importantly, cases often did not have a significantly raised alanine aminotransferase level to prompt testing for hepatitis E virus. This raises the possibility that morbidity and mortality in patients with chronic liver disease could be much higher than is currently assumed and gives weight to the notion that all decompensated patients without clear cause should be tested for hepatitis E virus (Dalton et al, 2018).

Another at-risk group is immunocompromised patients. This includes individuals taking immunosuppressive therapy for autoimmune disease, cancer and following organ transplantation, as well as patients immunocompromised with haematological disorders or HIV. A Scottish study of 283 clinical cases found 15.5% were immunosuppressed (Wallace et al, 2018). Between 50% and 66% of immunosuppressed transplant recipients develop a chronic hepatitis on exposure to hepatitis E virus, defined as the presence of hepatitis E virus RNA for >3 months. Of these, 10% developed a rapidly progressive cirrhosis (Behrendt et al, 2014). In one case series of 85 patients with solid organ transplant, only one third of patients were symptomatic, with fatigue as the main symptom. Patients with chronic hepatitis E virus may only have moderately raised alanine aminotransferase levels and the threshold for testing should be low (Kamar et al, 2014b).

Hidden hepatitis E virus: extrahepatic morbidity

An association between hepatitis E virus genotype 3 and a number of neurological injuries has recently been

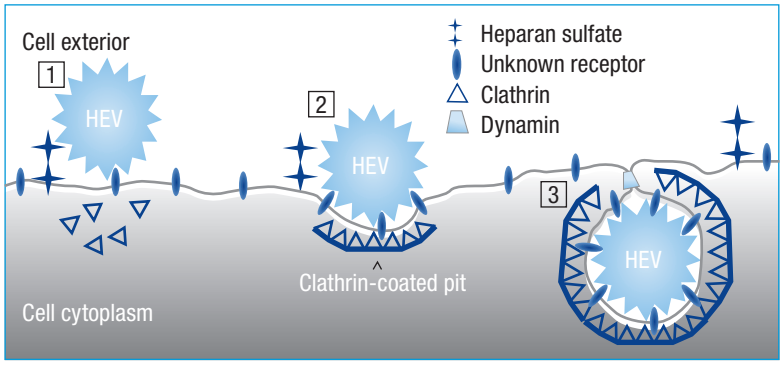

Figure 3. A schematic diagram of the proposed mechanism of hepatitis E virus (HEV) entry into cells. 1. HEV first attaches to heparan sulfate on the cell and binds via an unknown receptor. 2. The cytoplasmic tail of the virus-bound receptors bind clathrin via adapter proteins, forming a clathrin-coated pit. 3. The continued binding of clathrin causes curvature of the virus-bound area of the membrane, resulting in membrane invagination and the formation of a virus-containing vesicle, which is ultimately scissioned by dynamin.

Table 2. Patients who should be tested for hepatitis E virus

Acute hepatitis E	■ Biochemical evidence of hepatitis ■ Decompensated chronic liver disease† ■ Suspected drug-induced liver injury† ■ Guillain–Barré syndrome* ■ Neuralgic amyotrophy*
Chronic hepatitis E (immunosuppressed only)	■ Persistently raised alanine aminotransferase level

** Irrespective of alanine aminotransferase result; †With any abnormality of alanine aminotransferase*

identified. Case and cohort studies from Europe have found hepatitis E virus infection in the context of neuralgic amyotrophy, Guillain–Barré syndrome, encephalitis, Bell's palsy, mononeuritis multiplex, peripheral neuropathy and vestibular neuritis. Of 450 patients with non-traumatic neurological injury, 2.4% had underlying hepatitis E virus infection (Dalton et al, 2017). A review of 283 patients with laboratory-confirmed hepatitis E found 30 (10.6%) developed neuralgic amyotrophy, Guillain–Barré syndrome or other neurological complaints (Wallace et al, 2018).

Neuralgic amyotrophy, Guillain–Barré syndrome and encephalitis have a causal relationship with hepatitis E virus infection. A multicentre cohort study found evidence of hepatitis E virus infection in 10.6% of patients with neuralgic amyotrophy. Hepatitis E virus infection has a distinct phenotype and cases were significantly more likely to develop bilateral involvement, more extensive nerve damage to, and nerve damage outside, the brachial plexus. These patients often had little evidence of hepatitis with normal or modestly raised transaminase levels (van Eijk et al, 2017). Three case-control studies from the Netherlands, Bangladesh and Japan found concurrent hepatitis E virus infection to be present in 5–11% of patients with Guillain–Barré syndrome, significantly higher than controls (Dalton et al, 2017). There are several case reports of hepatitis E virus-infected patients developing encephalitis (Wallace et al, 2018). In such cases hepatitis E virus RNA can be found in the CSF suggesting that hepatitis E virus may be neurotropic in humans. The causal role of hepatitis E virus in neurological injury is supported by in-vitro studies. For example, Zhou et al (2017) demonstrated that human neural cell lines are highly susceptible to hepatitis E virus infection in vitro, and hepatitis E virus RNA was detected in the brain tissue of peripherally inoculated mice and donkeys (Drave et al, 2016).

Provisional data from 283 Scottish hepatitis E virus cases showed that 28 (9.8%) had a documented acute kidney injury, mostly in the context of hepatitis E virus-associated acute-on-chronic liver failure (Wallace et al, 2018). Cases of glomerulonephritis (membranoproliferative and membranous) have been identified in both the immunocompetent and immunosuppressed with renal function improving upon clearance of the virus (Del Bello et al, 2015).

Hepatitis E virus: who should we test?

Detection of hepatitis E virus infection relies on changing the commonly held belief that hepatitis E virus is rare or limited to endemic areas in Africa and Asia. Within the UK, despite the wide availability of hepatitis E virus assays, hepatitis E virus may still be seen as an 'exotic' test requiring specialist request, rather than part of the initial liver screen. This practice needs updating given that hepatitis E virus is the most common cause of acute viral hepatitis in many countries including the UK. European guidelines (Dalton et al, 2018) recommend that any patient with an unexplained, raised alanine aminotransferase level should have hepatitis E virus testing as a first-line investigation (*Table 2*). This is particularly the case with drug-induced liver injury which is a common misdiagnosis of hepatitis E virus infection. Acute hepatitis E virus has historically accounted for some diagnoses of suspected drug-induced liver injury and no diagnosis of drug-induced liver injury is secure without first excluding hepatitis E virus (Dalton et al, 2018).

Adopting the above approach will mean an enormous increase in individuals who are currently tested for hepatitis E virus. In one study using receiver-operator curve analysis, Wallace et al (2017) found that hepatitis E virus testing could be focussed to patients with an alanine aminotransferase level >300 iu/litre. However, this threshold does not apply in cases of neurological injury, decompensated cirrhosis or the immunosuppressed. Immunosuppressed patients with a sustained rise in alanine aminotransferase levels should always have hepatitis E virus RNA tested as they may not produce antibodies (*Figure 4*).

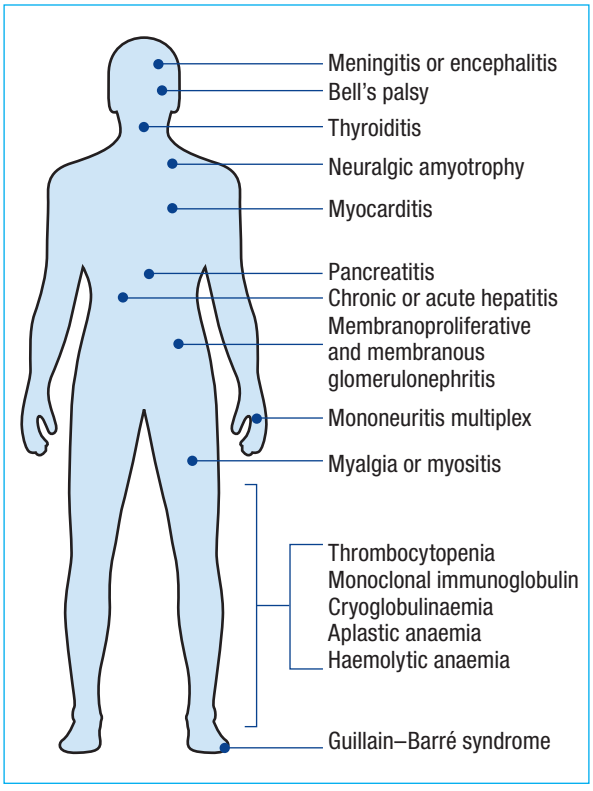

Figure 4. Extrahepatic manifestations of hepatitis E virus.

Blood donors

As hepatitis E virus infection is so commonly and mostly asymptomatic, it is no great surprise that hepatitis E virus has found its way into the human blood supply. However, the high numbers of viraemic donors have caused consternation in the blood transfusion community. Transfusion-transmitted infection with hepatitis E virus, sometimes with adverse outcomes, has been documented in many countries, including the UK (Hewitt et al, 2014). As a result, routine blood donor screening for hepatitis E virus has started in the UK (2016–17), Ireland (2016), Netherlands (2017) and Switzerland (2018). Several other European countries have decided to implement donor screening or are actively considering it. It is likely that blood donor screening will have a modest effect on the prevention of hepatitis E virus as most infections are dietary in origin. However, a cost–benefit analysis from the Netherlands showed donor screening for hepatitis E virus compared favourably with other screening measures already in place including HIV, hepatitis C virus and hepatitis B virus testing (de Vos et al, 2017).

Treatment of hepatitis E virus

Treatment is not usually required in immunocompetent patients with acute hepatitis E virus infection. In patients with acute-on-chronic liver failure and extrahepatic injury a small number of cases have been treated with antiviral therapy, with variable results (Dalton et al, 2018). In chronic cases, 30% sustained viral remission is achieved by reducing or stopping immunomodulatory medication, although a reduction of immunosuppression is not possible in some groups including renal and heart transplant recipients, because of the risk of organ rejection (Kamar et al, 2014b).

Case reports and case series in patients who have had solid organ transplant show ribavirin monotherapy to be effective at achieving sustained viral remission (defined as undetectable hepatitis E virus RNA after 3 months of treatment) and reducing the risk of progressive liver disease (Kamar et al, 2014b). A study of 59 transplant patients with chronic hepatitis E virus infection treated with ribavirin showed the sustained viral remission to be 78%. Those who relapsed were retreated with ribavirin for a 6-month period and achieved sustained viral remission, giving an overall sustained viral remission of about 90%. The optimal duration for ribavirin therapy is unclear (Behrendt et al, 2014). Patients who fail to respond or who are intolerant to ribavirin are problematic. Some such individuals have been treated successfully with pegylated interferon, but this is contraindicated in most transplant recipients because of the risk of organ rejection. The optimal therapy in such patients is currently unknown. In the 30% of patients with chronic infection who develop cirrhosis, partial reversal of cirrhosis has been observed when sustained viral remission was achieved (Kamar et al, 2014b).

Therefore, reducing or stopping immunosuppression is recommended for patients with chronic hepatitis E virus infection. Those who fail to clear the virus should be started on ribavirin for 3 months. Those who relapse after ribavirin is discontinued should then have 6 months of ribavirin. There are no placebo-controlled trials to support this treatment strategy (Dalton et al, 2018).

KEY POINTS

- Hepatitis E is a commonly occurring, locally acquired porcine zoonotic infection in high-income countries with evidence of increased viral circulation in some countries.
- Liver failure is rare in healthy individuals, but mortality is high in patients with underlying liver disease.
- Immunosuppressed patients are at risk of developing chronic hepatitis and subsequent cirrhosis.
- Hepatitis E virus is associated with extrahepatic manifestations including neurological and acute kidney injury.
- Hepatitis E virus testing should be part of the first-line diagnostic protocol in patients with a raised alanine aminotransferase level, including patients with suspected decompensated liver disease and the immunosuppressed. Patients with Guillain–Barré syndrome, neuralgic amyotrophy or encephalitis should be tested for hepatitis E virus even if there is no biochemical evidence of hepatitis.
- Ribavirin therapy and reduction of immunosuppression achieves viral clearance in most chronically infected patients.

Prevention of hepatitis E virus

There is a vaccine for hepatitis E virus which is currently only licensed for use in China. This provides 97% efficacy in preventing clinical hepatitis from hepatitis E virus infection but does not prevent sub-clinical infections (Zhu et al, 2010). Until an effective vaccine is widely available, reducing hepatitis E virus infection focuses on food selection, food preparation and hepatitis E virus-free blood products. Pork consumption is a significant risk factor for hepatitis E virus infection. The virus also occurs in shellfish and infected vegetables. In-vitro studies have shown that hepatitis E virus infectivity is eliminated when heated to 70°C for more than 2 minutes (Johne et al, 2016). Patients with immunosuppression or chronic liver disease should be advised to avoid undercooked pork and shellfish.

Conclusions

Locally acquired hepatitis E virus infection is a common cause of viral hepatitis in high-income countries. However, there is still a prevailing understanding that hepatitis E virus infection is a self-limiting, benign occurrence with complications confined to isolated case reports. There is growing evidence to the contrary. A large European multicentre study is currently underway to determine clinical outcomes of hepatitis E virus. The results are awaited with interest, as they will inform our understanding of the burden of disease. Following the provisional results from the Scottish study presented above, hepatitis E virus may well be identified as a more important public health issue than previously thought. **BJHM**

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