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Hepatitis E: the current state of play

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SUMMARY

The hepatitis E virus (HEV) is a major cause of acute hepatitis globally. Genotypes 1 and 2 (G1 and G2) are obligate human pathogens transmitted faeco-orally, leading to epidemics in developing countries. In contrast, genotypes 3 and 4 (G3 and G4) have a wider host range, including humans, but are primarily porcine viruses and are transmitted from animals to humans as a food-borne zoonosis when meat from an infected animal is consumed. HEV is increasingly recognised as a problem in developed countries, including countries in Europe. G3 HEV is now the most common cause of acute viral hepatitis in the UK and cases continue to rise. The majority of these infections are acquired within the UK and thought to be from insufficiently cooked meat, predominantly processed pork meat. Previously thought to only cause self-limiting disease, HEV infection can persist in immunosuppressed patients, which may lead to chronic hepatitis and the rapid development of cirrhosis. Of particular interest to the transfusion community has been the possibility of transfusion-transmitted HEV, which has been reported from countries classically considered HEV-endemic but also non-endemic countries in Europe and Japan. This has prompted some countries to introduce screening for HEV in blood donations.

The hepatitis E virus (HEV) is a major cause of acute hepatitis globally. In endemic areas, the virus is transmitted faeco-orally, leading to sporadic cases punctuated by occasional epidemics when a drinking water source is faecally contaminated. By contrast, in industrialised countries, transmission occurs from animals to humans as a food-borne zoonosis when meat from an infected animal is consumed.

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The World Health Organization (WHO) estimate that 20 million HEV infections occur annually, with over 3 million symptomatic cases and in excess of 55 000 HEV-related deaths (WHO, 2016). These figures reflect the epidemiology in endemic regions such as Africa and Asia where large waterborne outbreaks occur and lead to the majority of symptomatic cases and mortality, but they do not account for HEV infections in industrialised countries. HEV is increasingly recognised as a problem throughout Europe, New Zealand, Japan and North America (Adlhoch *et al.*, 2016). It is now the most common cause of acute viral hepatitis in the UK and parts of Europe, and cases continue to rise. In 2015, 958 confirmed cases were recorded across England and Wales, continuing a pattern of a year-by-year increase in cases (Fig. 1, B. Said, personal communication, Public Health England (PHE)) (Ijaz *et al.*, 2014). Notably, the prevalence of HEV viraemia in blood donors in England during 2016/2017 is 1 in 2600, but marked fluctuations occur from month to month for reasons which currently remain obscure (NHSBT/PHE Epidemiology Unit, personal communication). While some of these cases in the UK are acquired HEV from endemic areas, the majority are acquired locally and thought to be from insufficiently cooked meat, predominantly processed pork meat (Said *et al.*, 2014).

Previously thought to only cause self-limiting disease, HEV infection can persist in immunosuppressed patients, which may lead to chronic hepatitis and the rapid development of cirrhosis in a proportion (Kamar *et al.*, 2008). However, HEV continues to be under-diagnosed globally because of limited access to diagnostic tests and lack of awareness amongst physicians in many countries. Of particular interest to the transfusion community has been the possibility of transfusion-transmitted HEV, which has been reported from countries classically considered HEV-endemic, but also non-endemic countries in Europe and Japan (Khuroo *et al.*, 2004; Nelson, 2014). This has prompted some countries to introduce screening for HEV in blood donations. Opinions are divided about the indication for, and cost-effectiveness of, providing HEV-screened blood for a virus that leads to a mild self-limiting infection in the immunocompetent but which, for a proportion of immunocompromised patients, can result in a clinically significant, perhaps even fatal, infection.

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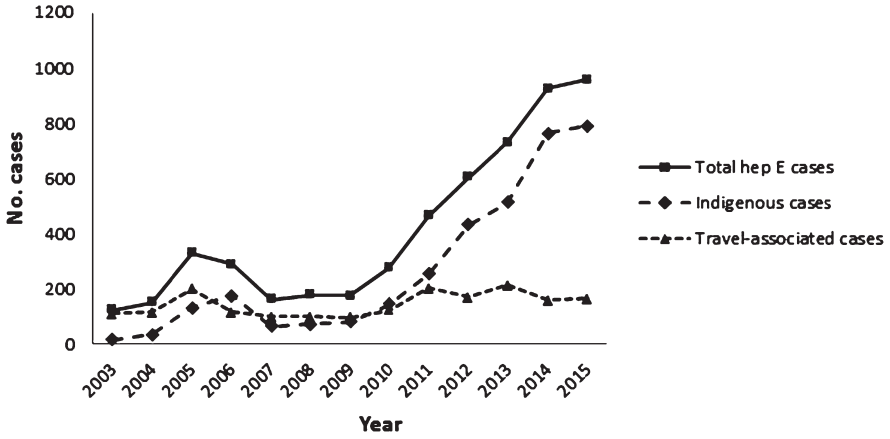

Fig. 1. Reference laboratory-confirmed HEV cases in England & Wales 2003–2015.

History of HEV

Prior to the 1980s, contaminated drinking water was thought to transmit only the one human hepatitis virus, type A. However, a large waterborne outbreak of acute non-A non-B hepatitis and jaundice in Kashmir, India in 1978 was suspected to be caused by a novel virus (Khuroo, 2011). This virus was identified during a subsequent outbreak in a soviet military camp in Afghanistan in the early 1980s. A member of the research team confirmed the presence of an infective agent in an unorthodox manner, by the ingestion of pooled faecal extract from the affected soldiers. This novel virus, later named HEV in 1983, was seen through electron microscopic examination of his stool after he fell ill with jaundice. HEV was subsequently shown to be circulating during an earlier outbreak in Delhi, India in the 1950s through retrospective serological testing of stored samples (Khuroo, 2011). Large epidemics of HEV affecting thousands of people have continued to occur periodically in the developing world, particularly in parts of Africa, Asia and Mexico (Teo, 2012).

In the late 1990s, the surprise finding of seroprevalence figures for antibody to HEV (anti-HEV) of between 1% and 4% in developed countries and the subsequent recognition of locally acquired infections in the United States and European countries began a new chapter in the HEV story (Thomas *et al.*, 1997). At around a similar time, novel viruses, designated swine HEV, were identified in pigs (Meng *et al.*, 1997). A further surprise followed in the late 2000s when HEV was recognised to lead to persistent infections in immunocompromised patients in France (Kamar *et al.*, 2008), Box 1.

Virology

HEV is a small, non-enveloped, positive-sense, single-stranded RNA virus. It is a member of the widespread *Hepeviridae* family within the genera *Orthohepevirus*, which includes all mammalian and avian HEV isolates under a newly proposed consensus classification (Smith *et al.*, 2014). There are four major genotypes of related viruses (HEV G1-4) that infect humans but only one recognised serotype. Genotypes 1 and 2 (G1 and G2) are obligate human pathogens and do not infect other hosts, whilst genotypes 3 and 4 (G3 and G4) have a wider host range including humans but are enzootic in pigs and are primarily porcine viruses (Table 1). The extent to which the other proposed genotypes 5, 6 and 7 can infect humans is largely unknown (Meng, 2013).

The ~7·2 kb genome contains three partially overlapping open reading frames (ORFs). ORF1 encodes a polyprotein with multiple putative functional domains vital for RNA replication (Cao & Meng, 2012). ORF3 codes for a small phosphoprotein vital for virion release, whilst ORF2 encodes the single capsid protein that carries the neutralising epitope(s) (Cao & Meng, 2012). This capsid protein is essential for virion assembly, host cell interaction and immunogenicity (Cao & Meng, 2012). The nucleocapsid structure is very tight, which helps to explain the considerable thermal stability of HEV, which is highly relevant for food-borne transmission and the relative inefficiency of generic pathogen inactivation methods when used against HEV within transfusion medicine (Hauser *et al.*, 2014; Owada *et al.*, 2014). The immunogenicity of the ORF2 product has been exploited to generate a vaccine by expressing high levels of the antigen *in vitro* (Zhu *et al.*, 2010).

Box 1. Key points outlining the current knowledge status of HEV

Four genotypes (HEV G1-4) cause disease in humans in two epidemiological patterns

HEV is predominantly either waterborne (G1 & G2) or food-borne (G3 & G4), but transmission by blood components is increasingly recognised

Severe disease occurs with G1, especially in pregnancy. Persistent infections are caused by G3 in immunocompromised patients

Ribavirin is effective for treatment to persistent HEV infection. Its role in the treatment of acute infection is unclear

Table 1. Comparison of the four main HEV genotypes that affect humans

	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Species infected	Humans only		Primarily pigs but many other mammals including humans	
Route of infection	Waterborne/faeco-oral		Food-borne	
	Direct person-to-person spread very limited		Direct person-to-person spread not proven	
Epidemiological pattern	Large epidemics with sporadic cases in between		Sporadic cases	
Clinical features	Acute hepatitis can be severe		Symptomatic hepatitis common in elderly males	
	Severe disease seen in pregnancy		Chronic in immunocompromised[1]	
Geographical distribution	Asia and Africa	Mexico and Africa	Worldwide	Asia, recent spread to Europe

[1]Only one chronic G4 HEV infection described in the literature.

Further detailed molecular understanding of the HEV lifecycle and pathogenesis has been hampered by the lack of an efficient cell culture system, but over recent years, several groups have successfully propagated HEV in a variety of cell lines from stool-derived virus (Okamoto, 2013). Plasma-derived virus has been more challenging to rescue in cell culture, and the reasons for this remain largely unexplained. This is perplexing in the context of the transmission of infection to patients by plasma transfusion. A key difference between stool-derived virus and plasma-derived virus is the presence of a lipid coat derived from host membranes surrounding HEV particles found in plasma (Feng & Lemon, 2014). This envelope of host membrane appears to protect the virus from neutralising antibodies (Feng & Lemon, 2014).

Seroprevalence

Anti-HEV IgG seroprevalence data, reflecting prior HEV infection from various countries, has been difficult to compare historically because of the variable performance of different anti-HEV assays. By restricting comparisons to studies using the Wantai IgG enzyme-linked immunosorbent assay (ELISA) as a screening assay, there is a consistent wide variation of seroprevalence between countries (Table 2). This assay is regarded as one of the most sensitive diagnostic assays available. Although in the absence of a confirmatory algorithm, it is plausible to suggest that any single assay may overestimate the true seroprevalence, experience suggests that for the Wantai assay this is not significant. Areas of the highest seroprevalence are observed in geographical areas considered HEV-endemic, such as Nepal (47%) and Bangladesh (50%), yet the European countries of France (25–53%) and the Netherlands (21–27%) also record very high figures (Mansuy *et al.*, 2011; Slot *et al.*, 2013; Gallian *et al.*, 2014; Hogema *et al.*, 2014; Izopet *et al.*, 2015). The lowest seroprevalence figures are found in New Zealand (4%), Australia (6%), Canada (6%) and Scotland (5%) (Dalton *et al.*, 2007; Cleland *et al.*, 2013; Fearon *et al.*, 2014; Shrestha *et al.*, 2014). Such high figures in industrialised European countries appear surprising when relatively few clinically symptomatic cases are identified but can be understood in the context of blood donor studies where asymptomatic infections are the norm (Tedder *et al.*, 2016). A recent trend highlighted from studying seroprevalence data and the attack rates in blood donors, particularly from the UK and the Netherlands, has been a year-by-year rise in HEV cases over the last 5 years in Western Europe (Adlhoch *et al.*, 2016).

There is also considerable variation from region to region within certain industrialised countries, most marked in France (Mansuy *et al.*, 2011). The consumption of regional delicacies, such as the raw liver sausage figatellu and fitone, may partly explain these variations, but obvious regional dietary delicacies are not evident in other countries (Meng, 2013).

Epidemiology and transmission

In broad terms, transmission of HEV to humans can be considered to be either enteric or parenteral. Genotypes 1 and 2 are human-only pathogens acquired through the consumption of food or water contaminated with human faecal material shed from an acutely infected person. This can lead to large outbreaks in areas of poor sanitation, such as refugee camps. Genotypes 3 and 4 are zoonotic and are also acquired through the consumption of food containing the virus; although, in this case, it is through eating meat from an animal that is viraemic at the time of slaughter. Pork meat, particularly that which contains liver, is the predominant food source, but infection from venison and wild boar is also recognised (Meng, 2013; Said *et al.*, 2014). Interestingly, the majority of human infections in the UK are not acquired from UK pigs and are likely to arise from the importation of continental pig meat of animals viraemic at the time of slaughter (Grierson *et al.*, 2015). Other food products, including shellfish, soft fruits and vegetables, may be contaminated by pig effluent or irrigation water and lead to HEV infection, but there is currently only minimal evidence to suggest these are important transmission routes (Said *et al.*, 2009; Meng, 2013).

HEV strains related to G3 are found in a variety of other animals and hence may lead to human infection. Of these, rabbit strains appear the most likely to be a risk to human health because they are antigenically related and able to infect pigs experimentally (Lhomme *et al.*, 2013). A human infection from a rabbit-related strain has been described (Lhomme *et al.*, 2013). More diverse strains of HEV have been identified in chickens, rats, camels, ferrets, foxes and bats (Smith *et al.*, 2014).

Table 2. Prevalence of HEV viraemia in blood donors and IgG seroprevalences in both blood donors and the general population from selected countries where G3 is the dominant genotype. All studies used Wantai IgG assay (Fortress Diagnostics Ltd, Antrim, Northern Ireland, UK). All percentages rounded up to whole numbers

	Country	Blood donors HEV RNA positive	Anti-HEV IgG seroprevalence (blood donors)	Anti-HEV IgG seroprevalence (general population)	Year of sampling
Europe	Austria	1 : 8416	14%		2013/2014, Fischer *et al.* (2015)
	England	1 : 2848			2012/2013, Hewitt *et al.* (2014)
			12%		2010, Beale *et al.* (2011)
	France	1 : 2218	25%		2012/2013, Gallian *et al.* (2014)
	SW France			34%	2010/2011, Mansuy *et al.* (2011)
			53%		2003/2004, Mansuy *et al.* (2011)
	Germany	1 : 1240			2011, Vollmer *et al.* (2012)
		1 : 4525			2011, Baylis *et al.* (2012)
				30%	2010, Wenzel *et al.* (2013)
	Netherlands	1 : 762			2013/2014, Hogema *et al.* (2016)
		1 : 2671	27%		2011/2012, Slot *et al.* (2013)
			21%		2011, Hogema *et al.* (2014)
	Spain	1 : 3333	20%		2013, Sauleda *et al.* (2015)
	Sweden	1 : 7986			2011, Baylis *et al.* (2012)
North America	USA	1 : 9500			2013, Stramer *et al.* (2016)
			16%		2012, Xu *et al.* (2013)
		0 : 1939			2011, Baylis *et al.* (2012)
	Canada	0 : 5000	6%		2013, Fearon *et al.* (2014)

HEV transmission from blood components, tissues and organs

Where acutely infected viraemic individuals become blood donors, blood and its components will also infect those who receive such substances. Initially, transfusion transmission of HEV was described in HEV-endemic countries where G1 is dominant (Khuroo *et al.*, 2004). More recently, the transmission of G3 through transfusion has been reported in European countries, including France, the UK and Germany (Colson *et al.*, 2007; Hewitt *et al.*, 2014; Huzly *et al.*, 2014). Prevalence of G3 viraemia in donors has been considered to range from 1 : 762 in the Netherlands to 1 : 9500 in the United States (Stramer *et al.*, 2016; Hogema *et al.*, 2016).

Transmission is described for red blood cells, platelet preparations, pooled granulocytes and fresh frozen plasma. The risk of transmission is influenced by the presence of antibodies and viral load in the donor and also the volume of plasma transfused in the final blood component (Hewitt *et al.*, 2014). Overall, the risk of a viraemic donation leading to infection in the recipient is estimated to be 40–50% (Hewitt *et al.*, 2014). Extrapolating figures from a transmission study in the UK, the lowest total virus inoculum of G3 known to have led to infection in the recipient is 2×10^4 international units (IU) (Hewitt *et al.*, 2014).

To date, there has been only one reported transmission of HEV from solid organ transplantation; a liver transplant containing HEV led to cirrhosis in the recipient (Schlosser *et al.*, 2012). The donor was aviraemic at the time of donation. There are no reported cases of stem cell donation leading to infection in the recipient, but a potential stem cell donor was identified as having HEV viraemia prior to donation (Koenecke *et al.*, 2014).

Balance of dietary risk and transfusion risk in a given individual

A paradox arises in that at the same time the virus is affecting the population from which blood donors are drawn, patients themselves are also subjected to the same dietary exposure. Therefore, for any duration of time, the potential exposure to transfusion-transmitted infection in a given patient has to be set against the dietary acquisition over the same period. In principle, given that the duration of viraemia is around 8 weeks, and it is known that approximately half of the components drawn from a viraemic donor are likely to lead to infection, the ratio between diet and transfusion risk is fixed. Thus, approximately 13 donor exposures are required to provide the same risk of infection as 1 year of dietary exposure on a population basis (Fig. 2). Clearly, if an individual patient elects not to consume those foods that are likely to carry an HEV risk, this equation no longer holds.

Other routes of transmission

The consistent finding of high anti-HEV seroprevalence in people with occupational exposure to pigs, including veterinarians, pig handlers, forestry workers, slaughterhouse workers and butchers, is suggestive that direct animal contact may lead to HEV infection (Chaussade *et al.*, 2013).

Human-to-human transmission of G3 is extremely rare but occurs in outbreaks of G1 HEV, leading to prolonged epidemics

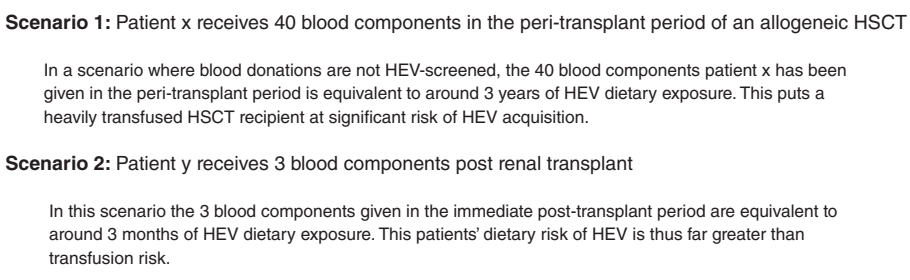

Fig. 2. Worked examples of how dietary exposure and transfusion differ in different patients.

(Mansuy *et al.*, 2009; Teshale *et al.*, 2010). There is no direct evidence of sexual transmission. Mother-to-child transmission occurs commonly with G1 HEV infection in pregnancy, with reported ranges of 33–100%, particularly if HEV is acquired in the third trimester, but it is not known whether transmission occurs *in utero*, through labour, breastfeeding or close contact post-delivery (Krain *et al.*, 2014). HEV RNA has been found in breast milk, suggesting this as a possible route of infection (Rivero-Juarez *et al.*, 2016).

CLINICAL ASPECTS

Acute hepatitis E

Many HEV infections will be completely asymptomatic, more so in the case of the zoonotic G3 and G4 infections than in G1. Where symptoms arise, they do so with a prodrome of non-specific malaise late in the incubation period followed by the onset of acute jaundice. This clinical symptomatology is indistinguishable from other causes of acute viral hepatitis, including hepatitis A. Acute G1 infections in the context of chronic liver disease can lead to decompensation with excess mortality (Kumar Acharya *et al.*, 2007). Pregnant women in their third trimester are also at risk of severe acute infections (Sultana & Humayun, 2014). By contrast, in developed countries, G3 infections do not express this increased pathogenicity in pregnant women (Tabatabai *et al.*, 2014; Blasco-Perrin *et al.*, 2015). The overall mortality from G1 infections ranges from 0·5 to 3·0% but is significantly lower with G3 infections.

Extra-hepatic manifestations of HEV infection

Infection has been associated with a wide range of extra-hepatic disease processes, including neurological, immune complex and haematological complications.

Neurological symptoms

Neurological symptoms have been observed during G1 and in up to 5% of both acute and chronic G3 cases (Kamar *et al.*, 2011a). Symptoms may be more commonly seen in younger males and with only modestly deranged liver function tests, which can mislead clinicians (Kamar *et al.*, 2011a). Manifestations include Guillain-Barré syndrome, neuralgic amyotrophy, acute transverse myelitis and acute meningoencephalitis. Bilateral neuralgic amyotrophy is suggested to be a specific variant clinical phenotype associated with HEV (van der Eijk *et al.*, 2014).

Non-neurological

Glomerulonephritis, polyarthritis and haematological complications, such as autoimmune haemolytic anaemia, severe thrombocytopenia, macrophage activation syndrome and cryoglobulinaemia, have all been associated with acute HEV infection (Bazerbachi *et al.*, 2016). Acute pancreatitis, including necrotising forms, is also described in G1 infections within 1–3 weeks of hepatitis onset (Haffar *et al.*, 2015).

Persistent hepatitis E infections

Persistent HEV infections were first described 8 years ago in solid organ transplant (SOT) recipients, and since the first reports, they have been increasingly recognised (Kamar *et al.*, 2008). They are also reported in other immunosuppressed patients, including haematopoietic stem cell transplantation (HSCT) recipients, those co-infected with human immunodeficiency virus (HIV), those receiving immunosuppressive therapy for chronic inflammatory diseases and patients undergoing chemotherapy (Gauss *et al.*, 2012; van der Eijk *et al.*, 2014; Debes *et al.*, 2016). Almost exclusively caused by G3 (G4 and G7 camelid HEV-persistent infections are described in case reports), infections are frequently pauci-symptomatic, and the transaminitis is usually mild (Geng *et al.*, 2014; Lee *et al.*, 2015). If the infection persists for a long time, chronic hepatitis, fibrosis and rapidly progressive cirrhosis may follow (Kamar *et al.*, 2008).

Currently, persistent HEV infection is defined as 'ongoing viraemia in excess of 3 months duration' (Kamar *et al.*, 2013). It is likely in an immunosuppressed patient that the detection of viraemia at a steady level over a period of a month or more is a more timely working definition of persistence. However, this definition may not hold true in patients with fluctuating levels of immunosuppression, particularly HSCT recipients.

Solid organ transplant patients

An estimated 60% of SOT patients who acquire HEV infection will develop persistent infection, and 10%–15% of these

patients may develop cirrhosis (Kamar *et al.*, 2008). The level and the duration of iatrogenic immunosuppression mean that this patient group is at high risk for the development of cirrhosis, which can occur within 2 years (Kamar *et al.*, 2008).

Risk factors for the development of a persistent infection include a shorter time from the transplant to diagnosis, a recent episode of acute rejection and low CD4 and CD8 T cell counts (Kamar *et al.*, 2016). The use of the calcineurin inhibitor immunosuppressant tacrolimus, as opposed to ciclosporin A, is another independent risk factor for persistent infection in this cohort (Kamar *et al.*, 2011b).

There has been significant interest in which immunosuppressants pose the greatest risk of chronic HEV infection. *In vitro* studies demonstrate increased HEV replication in cell culture in the presence of both the mammalian target of rapamycin (mTOR) inhibitors and calcineurin inhibitors (tacrolimus and ciclosporin), but mycophenolic acid has an inhibitory effect in these models (Wang *et al.*, 2013; Zhou *et al.*, 2014). The analysis of small numbers of cases suggests that mycophenolate mofetil is associated with the spontaneous clearance of HEV (Pischke *et al.*, 2012). In contrast, once persistent HEV is established, the response to antiviral treatment does not appear to be influenced by the specific drug regime but by the net level of immunosuppression (Kamar *et al.*, 2015).

Haematopoietic stem cell transplant patients

Less is known about the overall risk and natural history of persistent HEV in HSCT patients than SOT patients. The severity of immunosuppression, time of acquisition in reference to the transplant and comorbidities, including graft versus host disease (GvHD), all affect the chance of HEV clearance. A retrospective study of 328 alloHSCT patients in the Netherlands found a prevalence of HEV viraemia of 2·4% (8/328), of whom 63% developed persistent infection (Versluis *et al.*, 2013).

Hepatitis E can be misdiagnosed in this cohort because of overlapping clinical presentation with hepatic GvHD or drug-induced liver injury (DILI) (Bettinger *et al.*, 2015). Increasing immunosuppression to alleviate GvHD will potentiate viral replication; thus, isolated hepatic GvHD in the absence of other peripheral signs of GvHD should be investigated to exclude persistent HEV.

HIV-infected patients

There are several case reports of persistent HEV infection in the context of HIV, although it remains rare in this cohort and is usually observed only with advanced HIV with CD4 counts below 200 mm^3 (Debes *et al.*, 2016). Several studies across Europe have systematically tested for HEV in HIV cohorts and found six cases of active viraemia amongst over 3000 patients tested. Of these six infections, two patients had preserved CD4 counts, but follow-up was insufficient to confirm persistent infection (Debes *et al.*, 2016). Patients who do develop persistent infection are at risk of an immune reconstitution hepatitis as the CD4 count rises in the context of antiretroviral treatment. No cases of persistent HEV have been described in G1-endemic areas with high rates of HIV infection, including Ghana and Cameroon (Feldt *et al.*, 2013).

Patients on other immunosuppressive regimens

The extent to which immunosuppressed patients beyond transplantation and advanced HIV can support persistent HEV is not known. Cases of persistent HEV leading to rapidly progressive liver fibrosis have been reported in patients treated with rituximab-based chemotherapy for underlying haematological malignancies (Gauss *et al.*, 2012). In addition, cases of persistent infection are emerging in patients with less well-defined immunosuppression (Grewal *et al.*, 2014).

Studies following the outcome of 23 cases of acute HEV in patients with inflammatory arthritidies on a variety of immunosuppressants did not identify any chronic cases, suggesting that the risk is not high in this cohort (Bauer *et al.*, 2015). However, in this study, the majority of patients' immunosuppression was manipulated in light of HEV infection, thus making it difficult to determine the natural history of HEV infection in such patients (Bauer *et al.*, 2015). Nevertheless, it is known that the inadvertent introduction of an anti-TNF agent during an acute HEV infection can exacerbate the clinical presentation and tip the patient into a persistent infection (Behrendt *et al.*, 2015).

Diagnosis

An imputable diagnosis of acute hepatitis E in jaundiced, immunocompetent patients is through the detection of HEV RNA in plasma with coincident IgM anti-HEV and IgG seroconversion. In the absence of RNA testing, a diagnosis can usually be made through the interpretation of titres or the reactivity of anti-HEV IgG and IgM results. Several commercial HEV-specific ELISAs, originally designed to detect G1 and G2 HEV antibodies by using recombinant ORF 2/3 antigens, are equally good at detecting antibodies to other genotypes (Bendall *et al.*, 2010). Newer assays have been compared and show good sensitivity and specificity (Pas *et al.*, 2013).

RNA testing is especially useful when serology is difficult to interpret, for example, in the detection of an isolated HEV IgM reactive result. RNA amplification is also vital for genotyping for epidemiological purposes. The failure to develop anti-HEV IgG antibodies in an immunocompetent patient who is anti-HEV IgM sero-reactive confirms that the IgM reactivity was non-specific.

In contrast, the diagnosis of HEV in many persistently infected hosts relies solely on HEV RNA because of a delayed or non-existent serological response (Pas *et al.*, 2013). RNA assays are limited in availability, usually restricted to reference laboratories and are inherently expensive. As a result, there has been some interest in the utility of diagnostic assays which detect plasma HEV antigen. These relatively simple and inexpensive tests are less sensitive than RNA testing and so are

unlikely to be recommended for the diagnosis of acute HEV. As the viral load in persistent infection is high, it is possible that such assays may find a niche in identifying persistent infections and perhaps in the monitoring of response to treatment with antivirals (Behrendt *et al.*, 2016), Box 2.

Box 2.The key questions for future research

Issue	Question
Source of infection	• How can vulnerable groups be best protected from dietary exposure to HEV?
Pathogenesis	• Why does G1 lead to severe disease in pregnancy? • How does G3 lead to persistent infections in immunocompromised patients? Which are the at-risk immunocompromised groups?
Diagnosis	• What is the best screening strategy for persistent HEV in immunocompromised patients?
Blood safety	• What will be the impact of providing HEV-screened blood components? • Can generic pathogen-inactivation methods be used to reduce HEV transmission via blood components?
Prevention	• Is the current vaccine cross-protective of all genotypes? • Will there be a role for the current vaccine in protecting vulnerable populations?

TREATMENT AND PREVENTION

Management of acute HEV infection

Acute HEV infections are, for the most part, self-limiting with supportive care and no specific treatment required. There are no randomised controlled trials to guide treatment decisions, but ribavirin has been used successfully in small numbers of severe acute cases of both G3 and G1 (Gerolami *et al.*, 2011; Péron *et al.*, 2016). It may be a useful adjunct in the context of acute HEV superimposed on chronic liver disease or HIV (Goyal *et al.*, 2012; Robbins *et al.*, 2014).

Because of concerns of teratogenicity, ribavirin is contraindicated in pregnancy, but in cases of life-threatening infection, a clinician may be left with a complex dilemma given that the preliminary findings from the ribavirin pregnancy registry have not detected a signal indicating human teratogenicity (Roberts *et al.*, 2010). If liver failure ensues, then liver transplantation may be necessary.

Management of persistent HEV infection

It is considered best practice to attempt HEV clearance in patients presenting with persistent HEV in order to prevent liver disease and rarer neurological and renal complications that can develop during the course of persistent infection. Some patients will tolerate persistent infection with minimal disease progression; however, currently, there are no reliable tools to predict those patients who will progress to severe liver disease.

Clearance can be achieved by reducing iatrogenic immunosuppression or by the use of antivirals. Up to one third of SOT patients will clear HEV following reduction of immunosuppression, and this should be considered first in those whose graft will tolerate this (Kamar *et al.*, 2010). Drugs that specifically target T cells should be tapered preferentially as it is known that HEV-specific T-cell responses are decreased in SOT patients with persistent HEV.

If this strategy is contraindicated or unsuccessful, then ribavirin should be considered. Ribavirin has become the treatment of choice for persistent HEV because of its high efficacy, relatively good safety profile and low cost; however, no clinical trial data are available to support its use (Kamar *et al.*, 2014). The best available evidence comes from a multicentre retrospective study of 59 SOT patients, which found that 78% of patients achieved a sustained virological response (SVR), defined as an undetectable serum HEV RNA level, at least 6 months after cessation of ribavirin therapy following treatment for a median of 3 months (range: 1–18) (Kamar *et al.*, 2014). Optimal duration and dosing of ribavirin is undefined but should be tailored to individual patients by monitoring HEV RNA in both plasma and stool. Therapy should only be discontinued once clearance from both plasma and stool has been achieved. Persistence of RNA in the stool indicates that the infection has not been cleared and is predictive of relapse if ribavirin is stopped (Abravanel *et al.*, 2015). The monitoring of viral kinetics in plasma in the first week of treatment may also help predict which patients will achieve an SVR after 3 months of treatment with ribavirin. One small study found an 88% positive predictive value for an SVR after 3 months treatment with ribavirin if a patient achieved a reduction of plasma HEV RNA of 0·5 log or greater by day 7 (Kamar *et al.*, 2015).

Not all patients will tolerate ribavirin because of frequently occurring side effects, particularly anaemia, which can be significant but rarely life threatening. In the inaugural paper on the use of ribavirin in persistent HEV infection, 29% of patients required a dose reduction of ribavirin; 54% required supportive erythropoietin; and 12% required blood transfusion (Kamar *et al.*, 2014).

Both HIV-infected patients and HSCT recipients present unique treatment decisions, and there is sparse evidence on which to base the decisions. HIV-infected patients have cleared persistent HEV with immune reconstitution following the introduction of antiretroviral therapy, and this should be considered first (Andersson *et al.*, 2013). However, others have been successfully treated with interferon with/without ribavirin (Dalton *et al.*, 2011; Neukam *et al.*, 2013).

Similarly, haemato-oncology or HSCT patients may spontaneously clear HEV as immunosuppression varies. However, this is highly unpredictable, and treatment for HEV is often given to

these patients to enable them to complete the therapeutic schedule for their primary haematological disorder. Ribavirin is effective in this cohort, but decisions on the timing of treatment are more complex in the context of a recent HSCT and fragile bone marrow (Tavitian *et al.*, 2015).

Relapses

Relapses occur when infection rebounds following cessation of ribavirin therapy and generally may be predicted by detectable HEV RNA in the stool of patients displaying plasma clearance (Abravanel *et al.*, 2015). The majority of patients who relapse will clear HEV with a further 6 months of ribavirin if this can be tolerated (Kamar *et al.*, 2015). Whether these relapses constitute ongoing biliary excretion of the hepatic-derived virus in the absence of plasma spill over or an as-yet unidentified extra-hepatic site of replication is unresolved. Animal models in pigs and rabbits suggest that there may be important extra-hepatic sites of HEV replication (Williams *et al.*, 2001).

Antiviral resistance

A minority of patients are unable to clear HEV infection with ribavirin alone. Studies in these treatment failures have revealed that under the drug pressure of ribavirin, a number of mutations emerge in the viral polymerase region, which may contribute to drug resistance (G1634R, K1383N, D1384G, V1479I, Y1587F and Y1320H) (Debing *et al.*, 2016; Lhomme *et al.*, 2016; Todt *et al.*, 2016). However, the association is not straightforward as G1634R variants have been detected before therapy in patients achieving an SVR as well as those not achieving an SVR (Lhomme *et al.*, 2016). Furthermore, *in vitro* studies demonstrate that many of these mutations lead to increased viral replicative capacity rather than drug resistance *per se*, yet others appear to sensitise the virus to ribavirin, so there is still much to learn regarding the clinical relevance of these mutations (Debing *et al.*, 2016).

Very few alternative antiviral strategies currently exist; pegylated interferon α (PEG-IFN-α) has been used with some success as monotherapy and in combination with ribavirin, with which it shows synergy *in vitro* (Debing *et al.*, 2014; Peters van Ton *et al.*, 2015). Therefore, it remains an option for some liver transplant recipients and perhaps HSCT recipients, but interferon has no role in the treatment of HEV in heart, lung and renal transplant patients because of the high risk of acute rejection. Sofosbuvir has no clinical data to support its use, but this NS5B polymerase inhibitor, which was developed for use against hepatitis C virus (HCV), also inhibits HEV *in vitro*, suggesting it may be a promising drug given its favourable safety profile in transplant patients (Dao Thi *et al.*, 2015).

How can we mitigate the risk of HEV in developed countries?

Strategies to protect the most vulnerable immunosuppressed patients from HEV infection should take into account the likelihood of acquisition from the two main sources in developed countries and also need to be proportionate to the risk. The risk of HEV acquisition from diet and blood will vary as patients progress through their treatment pathway (Fig. 2).

As blood donors cannot be identified pre-donation because of the pauci-symptomatic nature of the infection and lack of objective criteria upon which to predict infected donors, a screening strategy is necessary if one intends to reduce the risk from blood transfusion (Tedder *et al.*, 2016). Screening would necessitate the detection of HEV RNA as the vast majority of viraemic donors will not have detectable antibodies at the time of donation (Hewitt *et al.*, 2014). Trial screening of individual donations started in 2005 in Hokkaido, Japan. More recently, in response to the perceived risk of HEV infection, Ireland and the UK have introduced pooled screening of blood donations for all patients (Ireland) or for select immunocompromised groups (UK). A recent full meeting of SaBTO at the end of November 2016 examined the cost effectiveness of the selective screening policy in place in the UK blood services. On the basis that it remained appropriate to provide screened blood for immunosuppressed recipients and that it was felt that the hidden costs of selective screening were excessive, universal testing of all donations was determined as the most cost-effective option. The decision to implement universal testing of all donations has been agreed by the UK blood services, and the implementation of this in England in the first half of 2017 has been planned.

The efficacy of employing generic pathogen inactivation methods for HEV is uncertain at present. Not all methods have been tested against HEV, but plasma treated with commercial systems, such as Intercept™ that relies on amotosalen (a photoactive compound) and long-wavelength ultraviolet (UVA) illumination as photochemical treatment, remains able to transmit HEV (Hauser *et al.*, 2014). Alternative systems such as the Mirasol® pathogen reduction technology utilising riboflavin (vitamin B2) with ultraviolet light shows only a limited reduction of infectious HEV titre (Owada *et al.*, 2014). Furthermore, HEV is a non-enveloped virus, making it less susceptible to solvent/detergent inactivation methods (Andonov *et al.*, 2014).

Reducing or preventing dietary exposure requires the education of immunocompromised patients on avoidance of risky foodstuff and/or safe cooking of meat products. Notably, HEV has a high thermal stability, requiring high cooking temperatures of over 70 °C for inactivation, enabling HEV to transmit in all but the most fastidiously cooked food stuff (Feagins *et al.*, 2008). The fact that pigs do not suffer ill effects from the virus also means it is not a disease of economic importance as far as the agricultural economy is concerned. There is no impetus to prevent viraemic pigs entering the food chain nor at this time is there any attempt to control the infection in pigs.

Even if blood screening and effective health education are implemented in a country, infections will continue to occur, and so, prompt recognition and treatment of HEV infection is a vital aspect of risk mitigation. A more cost-effective and catch-all strategy may be to initiate routine screening of immunosuppressed patients at a set time interval to capture both dietary-

and blood-acquired HEV infections. However, there is currently no consensus over who should be screened, the timing or the frequency of screening.

Immunisation for HEV

Vaccines against HEV are not yet available routinely outside of China. The only currently licenced vaccine (HEV239, Hecolin®) is a recombinant peptide of a region of HEV ORF2 (aa 368–aa 606) from a G1 strain of HEV (Zhu *et al.*, 2010). In healthy Chinese adults 16–65 years old, it is highly efficacious as part of a three-dose schedule, with a durable antibody response in 87% for at least 4·5 years (Zhu *et al.*, 2010; Zhang *et al.*, 2015).

There are many gaps in knowledge over the safety and efficacy of the vaccine in children and pregnant women, patients with chronic liver disease and immunocompromised patients. Encouragingly, no adverse effects were seen in the 37 pregnant women who received the vaccine in the original trial (Wu *et al.*, 2012). There is also limited evidence of genotype cross-protection; the vaccine trial was in an area where G4 and G1 co-exist, but it is not known whether it is equally efficacious against other genotypes.

The WHO has not recommended its integration into routine immunisation programmes but do suggest that it could be considered to curtail outbreaks and possibly to mitigate hepatitis E in high-risk groups, including pregnant women. This is likely to be the main role for the vaccine at this point in time. Reactive vaccination, however, will only be a viable option if a shortened schedule is proven to be effective; a two-dose schedule is promising (Zhu *et al.*, 2010).

A major challenge facing the international health community with future HEV outbreaks will be the logistics of mobilising teams to acquire and administer the vaccine. While the current manufacturer of the vaccine lacks the resources to complete registration outside China, it may be many years before this vaccine is utilised and the most vulnerable patients can benefit.

CONCLUSIONS

HEV is an infection with two contrasting aspects. In developing countries, genotypes 1 and 2 present a real danger in areas of poor sanitation with limited access to clean water, where HEV can lead to large epidemics, with a particular predominance of severe presentations of acute hepatitis in pregnancy. In more industrialised countries, genotypes 3 and 4 are acquired through pork and game products and usually lead to asymptomatic or pauci-symptomatic infections. There is a current upsurge of acute hepatitis E cases reported across many countries in Europe. However, it is unclear which precise factors are leading to this rise in cases and whether this will be sustained.

The recognition of transfusion-transmitted HEV and the development of persistent infections in immunocompromised patients associated with fibrosis and cirrhosis has prompted HEV screening of blood donations in some European countries. Whilst persistent infection is unlikely to be a common event, cases go undiagnosed or are misdiagnosed as DILI or GvHD. The introduction of HEV-screened blood in some countries will have a minimal impact on the overall burden of HEV as dietary-acquired HEV constitutes the majority of infections. However, food-borne HEV is considered difficult to prevent on a population level as it would require changes in animal husbandry in continental Europe or a societal change in relation to the importation and consumption of pork and pork products. On an individual level, prevention is best achieved by fastidious cooking of pork and game food stuff.

Globally, the biggest future challenge will be the way to protect vulnerable patients from HEV epidemics. The only licenced vaccine is not yet available outside China, but there is a growing body of evidence that the protective effects of immunisation are long lasting and may be cross-protective for all genotypes. Further challenges may lie ahead as HEV continues to surprise and challenge the scientific and medical community.

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CONFLICT OF INTEREST

The authors have no competing interests.

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