

Review article: hepatitis E—a concise review of virology, epidemiology, clinical presentation and therapy

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Full Title

REVIEW ARTICLE: HEPATITIS E: A CONCISE REVIEW OF VIROLOGY, EPIDEMIOLOGY, CLINICAL PRESENTATION AND THERAPY.

Short Running Title

Hepatitis E

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SUMMARY

Background

Hepatitis E virus (HEV) is a leading cause of acute icteric hepatitis and acute liver failure in the developing world. During the last decade, there has been increasing recognition of autochthonous – or locally acquired- HEV infection in developed countries. Chronic HEV infection is now recognised, and in transplant recipients this may lead to cirrhosis and organ failure.

Aim

To detail current understanding of the molecular biology of HEV, diagnostic and therapeutic strategies and propose future directions for basic science and clinical research.

Methods

PubMed was searched for English language articles using the key words 'hepatitis E', 'viral hepatitis', 'autochthonous infection', 'antiviral therapy', 'liver transplantation', 'acute', 'chronic', 'HEV', 'genotype', 'transmission' 'food-borne', 'transfusion'. Additional relevant publications were identified from article reference lists.

Results

There has been increasing recognition of autochthonous HEV infection in Western countries, mainly associated with genotype 3. Chronic HEV infection has been recognised since 2008, and in transplant recipients this may lead to cirrhosis and organ failure. Modes of transmission include food-borne transmission, transfusion of blood products and solid organ transplantation. Ribavirin therapy is used to treat patients with chronic HEV infection, but new therapies are required as there have been reports of treatment failure with ribavirin.

Conclusions

Autochthonous HEV infection is a clinical issue with increasing burden. Future work should focus on increasing awareness of HEV infection in the developed world, emphasising the need for clinicians to have a low threshold for HEV testing, particularly in immunosuppressed patients. Patients at potential risk of chronic HEV infection must also be educated and given advice regarding prevention of infection.

INTRODUCTION

Hepatitis E (HEV) was initially identified in the late 1970s as an epidemic non-A non-B hepatitis that caused an infectious waterborne illness similar to hepatitis A¹. Now, HEV is a leading cause of icteric hepatitis and acute liver failure in the developing world. Worldwide, the estimated annual incidence of HEV infection is 20 million, resulting in around 56,600 deaths². Predominantly, infections are recognised as occurring in developing countries as large epidemics due to poor sanitation and are mainly associated with what are known as genotypes 1 and 2. In this instance, pregnant individuals are more susceptible to severe infection, and often develop a more aggressive clinical course associated with a poor outcome (3000 stillbirths annually are reportedly caused by HEV³). Over the course of the last decade, there has been increasing recognition of autochthonous (locally acquired) HEV infection in Western countries, mainly associated with genotype 3^{4, 5}. Worldwide **endemicity** for HEV (all genotypes) is detailed in Figure 1⁶. Furthermore, chronic HEV infection has been recognised since 2008⁷, and in transplant recipients this may lead to cirrhosis and organ failure⁸.

In this review we detail current understanding of the molecular biology of HEV, clinical relevance of genotypic HEV infection, diagnostic and therapeutic strategies and propose future directions for basic science and clinical research.

METHODS

A systematic search was performed using the PubMed electronic database for relevant English language abstracts and full text articles published between 1990 and January 2017. Manual review of the reference lists of selected full text articles was also undertaken to identify additional eligible articles. The search terms included: 'hepatitis E', 'viral hepatitis', 'autochthonous infection', 'antiviral

therapy', 'liver transplantation', 'acute', 'chronic', 'HEV', 'genotype', 'transmission' 'food borne', 'transfusion' and 'ribavirin'.

THE MOLECULAR BIOLOGY OF HEPATITIS E VIRUS

HEV is a small (27-34nm) single-stranded RNA virus⁹, approximately 7.2kb in length with 7 currently recognised genotypes¹⁰. It is known to be non-enveloped in bile and faeces, and is present in blood, coated in a lipid membrane. HEV consists of a 5' short non-coding region (NCR), ORF1 (which encodes the non-structural proteins), ORF3 (which encodes a small multifunctional protein), ORF2 (which encodes the capsid protein) and a 3'NCR followed by a polyadenosine tail approximately 150-200 bases long (Figure 2). A novel fourth open reading frame (ORF4) has been identified, situated within the ORF1 region of the HEV genome, encoding a 20kDa (139-158aa long) protein during endoplasmic reticulum stress which interacts with host and viral proteins to control the activity of the viral RNA dependent RNA polymerase¹¹. However, ORF4 is unique to, although universal amongst, genotype 1 HEV strains.

ORF1 and ORF2/3 are separated by a junction region (JR), whereas ORF2 and ORF3 overlap and are transcribed as a bicistronic subgenomic mRNA¹². The genome contains 2 cis-reactive elements (CREs), one in the JR and one overlapping the 3' end of ORF2 and the 3'NCR^{13, 14}. The sequence and stem loop structure of the CRE in the JR is essential for replication and may also serve as the promoter for the subgenomic region¹². The 3'NCR CRE localises the RNA-dependent RNA polymerase¹⁵. The 5' end of both the genomic and subgenomic RNA is capped¹⁶. A 76 nucleotide region in the 5'NCR is responsible for binding the ORF2 protein and is considered to play a role in viral assembly¹⁷. The coding region of ORF1 begins immediately after the 5'NCR and extends over 5082 nucleotides. ORF1 encodes a 1693 amino acid polyprotein with a molecular mass of approximately 186kDa and several putative functional domains (Figure 2) including; a methyltransferase (MT) domain for 5' capping, a Y domain of unknown function, a papain-like cysteine protease (PCP) domain, a proline rich region that contains a hypervariable region, a X domain of unknown function, a helicase (Hel) domain, and a RNA dependent RNA polymerase (RdRp)¹⁸. However, the protease activity of the PCP domain is a subject of some debate, with some reports suggesting that this domain may instead de-ubiquitinate proteins, preventing the prosomal degradation of protein required for viral replication¹⁹. The capsid protein of HEV is expressed by

ORF2. The ORF2 protein contains 4 domains; the N terminals, arginine rich, shell, middle and protruding domain. Neutralizing antibodies have been shown to bind to the P and M domains, suggesting that these play a role in cell binding and entry²⁰. Studies involving the expression of a truncated form of ORF2 by a baculovirus expression system in insect cells have produced HEV like particles²¹. Such virus like particles have been shown to bind to heparan sulfate proteoglycans (HSPGs) and HSPG expression on the cell surface is required for in vitro infection²². ORF3 encodes a small 114 amino acid protein approximately 13kDa in size. The ORF3 protein is believed to interact with several cell proteins to facilitate HEV replication, for example; interacts with hemopexin (affecting iron homeostasis), binds to SH3 domain containing proteins (which function in the signal transduction pathway and promote cell survival) and interacts with Tsg101 and α 1-microglobulin to facilitate the sorting of the endosomal sorting complexes required for transport (ESCRT)^{23, 24, 25}. However, ORF3 protein's key role is believed to be in viral assembly and egress, with phosphorylated ORF3 interacting with the capsid protein²⁶. Interestingly monoclonal antibodies to ORF3 were able to bind nascent virions but not faecal virions²⁷. Further studies indicated that virions circulating in human serum banded at a lower sucrose gradient in comparison to faecally derived virions and were not neutralized by the presence of antibodies in cell culture systems in the way faecally derived virions were suggesting that virions circulating in sera are protected by a membrane, containing, at least in part, ORF3²⁸.

Negative sense intermediate replicates are detected during replication and attachment receptors have been identified but overall, the viral life cycle of HEV has not been extensively studied^{29,30}. This is, in part, due to the slow progress in the development of reliable culture methods for HEV. Similarly, in vivo studies have been hampered due to the absence of a small animal model, until recently; with three studies achieving viral inoculation of human liver chimeric mice with HEV^{31, 32, 33}. Interestingly, these reports indicated greater success using genotype 1 strains as inoculants (in contrast to in vitro studies) and all achieved greater infection and viraemia rates in inoculated mice using faecally derived HEV virions, in comparison to serum or culture derived. Two studies examined the use of ribavirin in inoculated chimeric mice and demonstrated the treatment to be successful in reducing viraemia in therapeutics^{31, 33}. However, ribavirin induced anaemia was common in the treated mice (a side effect documented in humans)³¹.

HEV Phylogeny

All HEV strains belong to the family Hepeviridae, which has not been assigned to any order. The family Hepeviridae contains 2 genera; *Piscihepevirus* and *Orthohepevirus*. *Piscihepevirus* contains one species; *piscihepevirus A*, which contains all known cutthroat trout strains of hepatitis E. The *Orthohepevirus* genus contains 4 species; *orthohepevirus A*, *orthohepevirus B*, *orthohepevirus C* and *orthohepevirus D*. All avian strains are contained in the species *orthohepevirus B*. The strains isolated from humans and pigs are all assigned to *orthohepevirus A*, as are the strains which infect camels, deer, rabbits, mongooses and some rat strains. *Orthohepevirus C* contains all strains isolated from ferrets and some rat strains. *Orthohepevirus D* contains only strains isolated from bats³⁴

HEV has 7 known genotypes; genotypes 1-4 and 7 displaying human tropism³⁵. Whilst genotypes 1 and 2 infect only humans, genotype 3 and 4 strains have been isolated from various animals and genotype 7 strains have been isolated from camels³⁶. HEV appears to be unique amongst human hepatitis viruses, as recombination events appear to alter the replicative capacity, tissue specificity and pathogenicity of HEV³⁷.

Genotypes 1 and 2

Genotypes 1 and 2 are endemic in developing countries, where they cause water-borne outbreaks. These are obligate human pathogens, transmitted via the faeco-oral route and clinical presentation with genotype 1 or 2 infection is indistinguishable from any other cause of acute viral hepatitis.

Genotypes 3 and 4

The most common mode of HEV transmission in developed countries is believed to be food-borne zoonosis³⁸. Evidence of HEV as a zoonosis first came from the detection of HEV in pigs with a high homology to HEV strains found in humans³⁹. Since then many potential animal reservoirs for HEV have been identified⁴⁰. Only genotypes 3 and 4 of the species *orthohepevirus A*, and genotype 7, are recognised as zoonotic and circulate mainly in developed countries⁴⁰. Host species for genotypes 3 and 4 include pigs, deer, rabbits, mongoose, cattle, sheep and horses^(39, 41, 42; 43; 44; 45). Food-borne zoonosis of HEV has been documented in several case reports, and undercooked or raw pork has now been identified as a significant risk factor for human HEV infection. Transmission via

contaminated shellfish⁴⁶ and soft fruits⁴⁷ is also recognised as a potential source of food-borne transmission. Transmission via blood transfusion and transplantation has also been well documented.

Genotype 7

To date, only one incidence of genotype 7 infection in humans has been documented; this was a case of a liver transplant recipient in the United Arab Emirates who regularly consumed camel milk and meat⁴⁸. However, the incidence of camelid HEV in humans in countries with large camel populations deserves further attention.

Importantly, the virus can present as two quite distinct clinical conditions: large epidemics in endemic areas (genotype 1 in Africa and Asia, genotype 2 in Mexico and Africa: sporadic cases are recognised but less common than epidemics) and isolated cases amongst asymptomatic individuals in developed countries (genotype 3 and 4). HEV is a virus with 'two faces', behaving in a remarkable contrast between developing and developed countries and according to genotype⁴⁹ (Table 1).

GENOTYPE 1 AND 2 HEV INFECTION

Epidemiology – Figure 1

Genotype 1 and 2 cause a waterborne, epidemic hepatitis with the virus being transmitted via the faeco-oral route. Recent and novel work using human liver chimeric mice as a model of HEV infection demonstrated that HEV genotype 1 infection was established after intravenous injection of stool derived HEV virions, whereas intraperitoneal or intravenous injection of HEV-positive patient serum did not lead to active HEV infection³³. This finding suggests that at least for HEV genotype 1, stool derived HEV virions are more infectious than virions derived from serum. HEV genotypes 1 and 2 are endemic in developing countries; HEV genotype 1 is a common cause of acute hepatitis in Asia (in particular India), whereas genotype 2 is prevalent in Central America, Mexico and Africa. These genotypes are restricted to humans.

The World Health Organization estimates that there are 20 million HEV infections worldwide, leading to an estimated 3.3 million symptomatic cases of HEV⁵⁰, and 56,600 HEV related deaths². However, this estimate was based on data from only 9 out of 21 of the Global Disease Burden areas worldwide,

and it is likely that the true burden of disease is much higher. The majority of these infections are genotype 1 and 2, affecting patients in the developing world; imported cases are also observed in developed countries. Case fatality rates in epidemics range from 0.2 to 4%⁴⁹. However, mortality rates are significantly higher in particular populations; reportedly up to 20% of infected pregnant patients in endemic countries⁵¹.

Hepatitis E infection and Pregnancy

HEV infection during pregnancy (particularly during the third trimester) leads to a worsening in both maternal and foetal outcomes compared with other acute viral hepatitis, however the pathogenesis of HEV infection in pregnant females, and the resultant high mortality rate, is incompletely understood. The high mortality rate is likely to be a result of a number of complex and interacting factors, including viral, host, hormonal and immunological factors. Potential important contributing factors include high viral load⁵², dysregulation of the progesterone receptor signalling pathway and other hormonal changes in pregnancy⁵³. In pregnant women with genotype 1 HEV infection, a significantly higher viral load is seen in patients with acute liver failure compared with acute hepatitis, and higher viral loads were observed in patients with foetal death compared with patients without foetal death⁵². Defective monocyte-macrophage function occurs in pregnant patients with HEV-induced acute liver failure compared with HEV-induced acute liver injury, with reduced toll-like receptor 3 and toll-like receptor 7 expression and concomitant reduction in toll-like receptor downstream signalling⁵⁴. This suggests an inadequate trigger for the innate immune response contributes to the development and severity of HEV-induced acute liver failure in pregnancy. Pregnancy is associated with a high level of steroid hormones, which may promote viral replication and suppress CD4 cells⁵⁵. HEV-infected women with acute liver failure have lower CD4 counts and higher CD8 counts. Pregnant women with HEV acute liver failure have also been shown to have higher levels of oestrogen, progesterone and B-HCG compared with HEV negative patients or control healthy pregnant women⁵⁵. The role of herbal medicines has also been debated, with one group suggesting that HEV-infected pregnant women may be more likely to take herbal medicines, which could also contribute to the high mortality in certain geographical regions⁵⁶. In addition, in Central Asia and eastern Africa, high mortality rates have also been reported amongst HEV infected children aged under 2 years^{57,58}.

Clinical Features

The majority of acute HEV infections are asymptomatic; if present (in 20% of those with genotype 1 or 2 HEV infection)³, symptoms are often non-specific and include anorexia, nausea, fatigue, myalgia and jaundice. Laboratory tests show elevated serum bilirubin levels and a marked rise in liver enzymes. The mean incubation period is 40 days (range 15-60 days). Most acute infections resolve spontaneously, with symptoms disappearing within 4-6 weeks. However, acute HEV infection can cause acute liver failure, most commonly in pregnant females in the developing world as described above. Whereas acute HEV infection can cause severe acute liver injury in the Western world, it rarely causes acute liver failure. Genotype 1 and 2 infections have also been implicated in the development of acute on chronic liver failure/decompensated liver disease. In countries where HEV is endemic, the number of cases of acute on chronic liver failure secondary to HEV is variable (ranging from 4% to 75%) with a median short term mortality rate of 34% (range 0% - 100%)⁵⁹. One study from India reported a 70% 12-month mortality rate in patients with HEV infection superimposed upon chronic liver disease and interestingly, HEV infection was associated with a higher mortality rate than decompensation due to any other cause⁶⁰.

GENOTYPE 3 AND 4 INFECTION

Epidemiology – Figure 1

HEV genotypes 3 and 4 are recognised to infect both humans and animals, in contrast with genotype 1 and 2; pigs, deer and wild boar have all been identified as reservoirs of infection. Genotype 4 infection mainly occurs in South-East Asia^{39,41}. Genotype 3 HEV is the most prevalent genotype causing autochthonous (locally acquired) infection in developed countries⁵. Many seroprevalence studies have been undertaken in Europe, and the results have shown a high variability in seroprevalence rates. A recent meta-analysis identified 73 studies of HEV seroprevalence in Europe; estimates of seroprevalence ranged from 0.6% to 52.5%, with rates increasing with age but unrelated to gender⁶¹. In the United States, seroprevalence for anti-HEV is around 6%⁶², in the United Kingdom 3-16%⁶³ and in some regions of France up to 52%⁶⁴. In England, the number of confirmed (symptomatic) cases of non-travel associated HEV infection has steadily increased over the past 14 years, from 124 in 2003 to 958 in 2015⁶⁵. However, a study from South-East England suggested that there are 80,000-100,000 infections per year in England, the majority of which are asymptomatic⁶⁶.

Autochthonous (locally acquired) HEV infection is not a benign condition, with mortality rates up to 27% reported in patients with underlying chronic liver disease⁶⁷.

Interesting geographical variations in genotype 3 HEV infection have been observed in France where there is considerable variation in seroprevalence by region from 8-86%⁶⁸; very high seroprevalence occurs in the southwest, southeast and northeast of the country^{67,68,69}. The reason for this interesting observation is unclear, but contaminated foodstuffs in the food chain are likely to explain these geographical differences in part. There appears to be no correlation with potential transmission routes (e.g. location of pig farms). Although Scotland is a relatively low seroprevalence region, seroprevalence rates are also variable⁷⁰. This geographical variation in HEV infection is worthy of further consideration and investigation. As in France the main pig-rearing/farming region is located in the North East of Scotland, in contrast to the area of peak HEV seroprevalence^{70, 71}. In a study of patients with decompensated chronic liver disease from the United Kingdom and France, HEV was significantly more common in the French cohort compared with the United Kingdom cohort (7.9% versus 1.2% respectively; $p = 0.003$)⁶⁷. Potential explanations for this include the quantum of circulating HEV in these respective regions, and the exposure to contaminated foodstuffs.

Many countries have undertaken epidemiological studies of HEV seroprevalence in their respective blood donor populations (Table 2)⁷²⁻⁸¹. Most countries report increasing incidence of HEV infection due to increased awareness of HEV, increased testing for the virus and a true increase in the numbers of new infections. Data from the Netherlands and Scotland suggests that this increase in incidence appears to be in younger patients, in contrast with previous observations that autochthonous HEV infection predominantly affects older (>60 years) males^{70,82}. The results of a large survey of hepatitis E infection in French blood donors have recently been reported. Overall IgG seroprevalence was 22.4%, with an IgM seroprevalence of 1%⁶⁸. IgM seroprevalence was highest in those patients living in the south of France and in those patients who self-reported consumption of pork liver sausage, pate and wild boar meat. The presence of HEV RNA was not reported upon in this large study, and there was no information given to suggest that any recipients of products from donors who were IgM positive developed active HEV infection.

HEV genotype 4 is endemic in China, Japan and Indonesia, and recently cases have been reported in Western countries including Belgium, Germany and France^{83,84,85}. There was an outbreak of genotype 4 infection in Italy in 2011⁸⁶. This outbreak was not directly linked to travel or infection by imported foods, raising the possibility of newly imported strains. All patients affected in this outbreak were male (mean age 59 years), and fatigue was the most frequently reported symptom.

Importantly, the presence of HEV antibodies does not protect from further infection. A French group performed a longitudinal study of multi-transfused immunocompetent patients in France. In this study, one seropositive patient demonstrated an increase in IgG level and HEV RNA reappearance, suggesting that reinfection does occur. The rates of reinfection and association with HEV antibodies warrants further investigation⁸⁷.

Transmission: Foodborne

Genotype 3 and 4 infections are most commonly transmitted via contaminated foodstuffs. These foodstuffs include porcine liver and sausage products, shellfish, green vegetables and strawberries. In the United Kingdom, a questionnaire-based study identified that risks for autochthonous infection include consumption of processed pork products, including pork pies (OR 6.33), sausages (OR 7.59) and ham (OR 10.98)⁸⁸. In a study of blood donors exposed to HEV infection in Southern France, foodstuffs associated with positive antibodies against HEV on multivariate analysis were uncooked pork liver sausages, offal and mussels⁷⁴. To support this, HEV has been found to be highly prevalent amongst global pig populations. For example, in Germany, 33% of wild boar and 50% of domestic pigs are seropositive for anti-HEV IgG^{89,90}. HEV infection has been identified in more than 80% of some pig herds in the United States, Canada and in the United Kingdom (England)^{91,92}, although Scottish herds have a lower seroprevalence of around 62%⁹³. In a more recent report on English and Northern Irish pigs, 93% of slaughter age animals were seropositive⁹¹.

In the West of Scotland, 92% of tested wild caught mussels were PCR positive for HEV and consumption of undercooked/raw shellfish is another viable route of transmission⁴⁶. Likewise, numerous studies have indicated HEV contamination of soft fruits, likely via exposure to contaminated water. In Quebec, Canada swine HEV was detected in strawberries (1.6% of samples tested)⁹⁴. A separate study of the European berry fruit supply chain identified HEV in 2.6% of berries (raspberries)

at point of sale⁹⁵. A more recent study identified HEV in 5% of irrigation water samples from leafy green vegetable production chains⁹⁶.

In view of the seroprevalence in animal populations described above, it seems logical that HEV can be transmitted to humans via the consumption of contaminated foodstuffs. Several studies have identified actual transmission of HEV to humans via the consumption of contaminated foodstuffs. One study investigated the role of figatellu (a traditional pig liver sausage eaten in France and commonly consumed raw): anti-HEV IgM or HEV RNA was positive in 7 out of 13 individuals who consumed raw figatellu, compared with 0 out of 5 individuals who did not eat figatellu⁹⁷. Genetic links were identified between HEV RNA sequences recovered from supermarket figatellu and sequences recovered from patients eating the same product, providing firmer evidence that human HEV infection is associated with figatellu consumption. More recently, Lee reported upon a 55 year old man who was found to be HEV RNA positive at 22 months after liver transplantation⁴⁸. The patient was Muslim, and therefore the potential route of food-borne transmission unclear. Phylogenetic analysis confirmed the patients HEV sequence to belong to camelid HEV; the patient owned a camel farm and subsequently confirmed regular consumption of camel meat and milk, making transmission via camel products the most likely source. In China, where mixed farming is common practice, a high prevalence of active HEV infection in cows was identified, and Huang demonstrated that HEV is excreted into milk that is produced by infected cows⁹⁸. Therefore, HEV-contaminated cows milk is another potential zoonotic source; gavage of infected milk to rhesus macaques resulted in active HEV infection as confirmed by HEV RNA in blood and faeces. However, in milk samples collected from dairy farms (i.e. not mixed farms) in Germany, no HEV RNA was detected⁹⁹. The exact contribution of zoonotic HEV infection via dairy milk, and the potential contamination via mixed farming remains to be established.

Transmission: via blood products

Transmission of genotype 3 and 4 HEV by transfusion of blood products (including red cells, platelets and even pathogen-inactivated [Intercept treated] fresh frozen plasma) that are HEV-infected has also been reported in many Western (and some Asian) countries^{66,100,101}. The incubation period for genotype 3 HEV infection in immunosuppressed patients with blood-borne HEV infection has been demonstrated to be 50-60 days, compared with less than 30 days for immunocompetent patients with genotype 1 infection¹⁰². Many countries have undertaken seroprevalence studies in their blood donor

populations

(Table

2)⁷²⁻⁸¹.

In Scotland, an increase in seroprevalence of genotype 3 infections has been observed; studies of the Scottish blood donor population in 2012 revealed 1 in 14,500 donors to be viraemic. More recent data, have shown donor viraemia in Scotland to have increased significantly, including in younger donors¹⁰³.

In southeast England, retrospective screening of 225,000 individual blood donations identified HEV RNA in 79 samples, equating with a prevalence of viraemia of 1 in 2848 donations⁶⁶. Of all RNA-positive samples undergoing genotyping, genotype 3 virus was identified in all cases. 79 donations from viraemic donors had been used to prepare 129 blood components, 62 of which had been transfused. Of the recipients of these components, 42% had evidence of infection, and 10 patients developed persistent infection. This study also suggested increased levels of circulating virus were associated with increased risk of infection.

A study of Catalonia (Spain) blood donors reported a prevalence of anti-HEV IgG of 19.96% (Wantai assay), with a HEV RNA positivity rate of 0.03%, or one per 3333 donations⁷². Study of the blood donor population in southwestern France found that anti-HEV IgG was detectable in 52.5% of blood donors, with seroprevalence increasing with age and associated with rural residence⁶⁴. Another group studied the presence of HEV RNA in manufacturing plasma pools from North America, Europe, the Middle East and Asia¹⁰⁴. Asian pools were most frequently positive for HEV RNA and had higher viral loads, and there was no evidence of HEV in pools tested from the Middle East, presumably relating to the low rates of pork consumption in this region.

Transmission: via solid organ transplantation

Transmission of HEV infection can occur via liver transplantation and transplantation of non-hepatic grafts. Schlosser described a case of a 73 year old man in whom HEV transmission occurred after transplantation of a HEV-infected liver from a donor with occult HEV infection¹⁰⁵. At the point of donor death, alanine aminotransferase was 4x upper limit of normal. The patient developed rapid graft cirrhosis and died from decompensated liver disease and septic shock. HEV was diagnosed at the time of hepatic decompensation; retrospective testing of a stored serum sample from 150 days post-transplant was also positive for HEV RNA. Pre-mortal blood and liver tissue from the donor confirmed

that the patients serum was HEV PCR negative, but HEV RNA was detected in high concentrations in the liver tissue of the donor. Sequence data from recipient serum and donor liver tissue were concordant, suggesting transmission of the virus via the transplanted liver. More recently, there has been a report of HEV transmission via renal grafts¹⁰⁶. The donor kidneys were transplanted into two separate recipients, and of note donor LFTs had been abnormal prior to transplantation. The first infected recipient presented at 9 months with deranged LFTS. Retrospective analysis detected negative HEV RNA until the day of transplantation, with positive RNA from the first month post transplant. The second recipient was identified after biomonitoring. Donor serum was positive for HEV RNA; genotype 3f was identified and this genotype was also identified in both recipients, providing evidence for transmission via non-hepatic solid organs.

Clinical Features

Immunocompetent individuals clear HEV promptly, usually within a few weeks. In these patients, HEV infection usually runs a mild course and is often asymptomatic. However, in the immunocompromised, HEV infection is often more difficult to clear, and about 60% of these patients go on to develop chronic HEV infection^{6,7}. Chronic HEV infection may lead to complications such as liver cirrhosis.

Clinical Features of Genotype 3 HEV infection

In 2008 it was established that genotype 3 HEV infection may progress to chronic infection in patients who are immunocompromised (e.g. patients with HIV, leukaemia, high dose steroid therapy) and solid organ transplant recipients^{6,107,108}. To date, chronic HEV infection has not been documented with genotype 1 or genotype 2 HEV infection. Chronic infection with genotype 4 HEV was reported in 2015, and there has only been one case reported in the literature to date¹⁰⁹.

Chronic HEV infection (genotype 3) is generally defined as persisting serum HEV RNA and elevated liver enzymes 6 months after the acute phase⁶, although some units use a 3 month cut-off to define chronicity¹¹⁰. In Toulouse, France between January 2004 and December 2009, 50 cases of HEV infection in solid organ transplant patients were identified: 32 kidney transplant recipients, 3 kidney-pancreas recipients and 15 liver transplant recipients. 45.7% of the kidney recipients in this cohort developed chronic HEV infection¹¹¹. In a retrospective study analysing stored plasma from 2,919 HIV-infected patients, 3 female patients were identified to have had HEV infection: 2 patients had acute HEV infection and 1 patient had chronic HEV infection for > 4 years (all infections genotype 3a)¹¹². In

addition to chronic HEV infection, reactivation of resolved infection has also been reported. One patient who had undergone stem cell transplant for leukaemia and was therefore immunosuppressed. Versluis described a patient who had initially cleared HEV infection, evidenced by 53 days with an undetectable HEV RNA¹¹³. At the time of allogeneic stem cell transplantation, HEV RNA was detectable, although viral load was low. Viral reactivation post stem cell transplant was based upon rising HEV RNA levels and identical HEV-ORF1b sequences. This patient later cleared infection with a reduction in immunosuppression and ribavirin therapy.

In organ transplant recipients, chronic HEV genotype 3 infection may lead to cirrhosis and liver failure within 1-2 years⁷. This may in turn lead to a requirement for re-transplantation. In transplant patients, anti-HEV IgM and IgG may be negative and therefore RNA testing by polymerase chain reaction (PCR) must be employed. Pas demonstrated that anti-HEV IgM could only be detected in 7/16 immunocompromised patients compared with 18/18 immunocompetent patients in the acute phase of infection, suggesting a delayed immune response and abnormal IgM antibody kinetics in the immunocompromised group¹¹⁴.

A UK/French study also looked at the role of HEV infection in patients with decompensated chronic liver disease⁶⁷. Acute HEV infection (genotype 3) was identified in a minority (3.2%) of patients with decompensated chronic liver disease, and there were no differences in mortality between patients with and without HEV infection. It is likely that HEV genotype 3 infection in patients with chronic liver disease confers an adverse prognosis (similar to that of other insults causing decompensation), but this effect is less than that seen with HEV genotype 1 infection as seen in a cohort of Indian patients⁶⁰. The role of the genotype of HEV in acute on chronic liver failure/decompensated chronic liver disease and its effect on patient outcome is therefore less certain, and requires further investigation.

EXTRAHEPATIC MANIFESTATIONS OF HEV

HEV infection may also present with extrahepatic manifestations. *In vitro* data has identified that HEV can replicate in non-liver cells including human intestine¹¹⁵.

Neurological manifestations have been reported in HEV genotypes 1 and 3 infection. HEV RNA has been found in the cerebrospinal fluid of patients with neurological symptoms during HEV infection¹¹⁶.

A Dutch study reported up to 5% of patients with Guillain-Barre syndrome had associated acute HEV infection¹¹⁷. Other reported neurological disorders include neuralgic amyotrophy, transverse myelitis and cranial nerve palsies. Other recognised extrahepatic manifestations of HEV infection include renal impairment with cryoglobulinaemia, pancreatitis and haematological abnormalities. HEV infection can cause severe kidney disease and should be considered in cases of unexplained glomerular disease. Arthritis and pancreatitis have also been reported.

DIAGNOSIS OF HEV INFECTION

Clinically, cases of HEV infection are indistinguishable from other causes of acute viral hepatitis. HEV infection can be diagnosed either indirectly by the demonstration of anti-HEV antibodies or directly by detecting HEV RNA using a quantitative reverse transcription polymerase chain reaction in serum, plasma or stool samples. During acute HEV infection, anti-HEV IgM becomes detectable in the days prior to the onset of symptomatic illness and becomes undetectable again at 4-6 months. Anti-HEV IgG becomes detectable soon after the presence of anti-HEV IgM, and persists for many years, even life-long in some patients. In 95% of patients, anti-HEV IgG is detectable at time of first clinical presentation¹¹⁸. Nucleic acid testing is essential to exclude HEV infection in the immunosuppressed population in view of the poor antibody response in such individuals.

There is currently no consensus across laboratories for HEV testing, and the sensitivity and specificity of HEV assays vary widely. This may at least in part account for the differences in reported rates of anti-HEV antibody in various populations. For example, within one country (UK), the prevalence of anti-HEV antibody in the blood donor population was 3.6% as detected by one assay, compared with 16.2% with the use of an alternative⁶³. In the recent meta-analysis of HEV seroprevalence in Europe by Hartl, seroprevalence again varied depending upon the assay used, with the Wantai assay reporting significantly higher seroprevalence rates across all cohorts tested⁶¹. As a result, it can be difficult and unreliable to compare data from different populations obtained by different laboratory methods. Several serological methods are available for diagnosis of HEV, including enzyme immunoassay and immunochromatography. Anti-HEV IgM can be difficult to detect, which may hinder the diagnosis of acute HEV infection, Abravanel reported upon the performance of a HEV IgM rapid test from Wantai in detecting anti-HEV IgM in both immunocompetent and immunocompromised

patients¹¹⁹. The rapid Wantai assay is a relatively new, immunochromatographic assay which can rapidly detect anti-HEV IgM. Abravanel identified that the sensitivity of this assay was higher in immunocompetent patients (sensitivity 97.7%; 95% CI 87.9-99.9%) compared with the immunocompromised (sensitivity 85%; 95% CI 70.2-94.3%).

In an attempt to harmonise HEV PCR techniques and standards, the World Health Organisation initiated the production of international standards for anti-HEV IgG and HEV RNA¹²⁰. This work involved 23 laboratories from 10 countries; in summary the World Health Organisation established a genotype 3a HEV strain as the International Standard strain for HEV RNA, with an assigned a unitage of 250,000 IU/mL. With regards to serology, there are no World Health Organisation reference materials available at present but this work is in progress.

Serum anti-HEV IgM and IgG may be negative in the presence of active HEV infection, and this may be as a result of the sensitivity of assays used, and/or the immunocompetence status of the patient. There have been reports of false positive results from anti-HEV IgM assays in cases of Epstein-Barr virus and cytomegalovirus infection¹²¹. Therefore, HEV RNA PCR is the favoured diagnostic test, particularly in the immunocompromised. If the patient has undergone liver biopsy for investigation of acute or chronic hepatitis, histology commonly demonstrates a non-specific hepatitis which may easily be attributed to an alternative cause, and the diagnosis must be confirmed with HEV Ag immunohistochemistry. However, the availability of this technique is limited.

DIAGNOSTIC MIMICRY

As HEV infection (both acute and chronic) is clinically indistinguishable from other causes of hepatitis, it is likely that HEV infection is under-diagnosed. The relevance of autochthonous HEV infection is only recently recognised, and it is likely that patients who have presented with severe acute liver injury/acute liver failure due to HEV in the past have been mislabelled as having an alternative diagnosis. As such, several centres have retrospectively analysed stored sera from patients with indeterminate acute liver failure (i.e. non A to E hepatitis, seronegative hepatitis) for presence of HEV. One German group retrospectively analysed stored sera from patients with 'indeterminate acute liver failure' for anti-HEV IgM, IgG and HEV RNA¹²². 10% of samples tested positive for HEV RNA and had clinical findings which would support the diagnosis of acute HEV infection. In Scotland, 80 patients with severe acute liver injury were tested for serological markers of HEV infection¹²³. 3 patients tested

positive for anti-HEV IgG, anti-HEV IgM and HEV RNA. 1 further patient tested anti-HEV IgG and IgM positive, but HEV RNA negative. The patient with negative HEV RNA testing had been initially diagnosed as having acute liver failure secondary to a paracetamol overdose. Of the other patients, one was initially diagnosed as having a drug induced liver injury, another had travel-acquired HEV infection and the remainder was found to have liver cirrhosis on further investigation, presenting with decompensated disease secondary to HEV. In addition, patients previously having been labelled as having drug induced liver injury are increasingly recognised to in fact have had acute HEV infection. In a United Kingdom study of patients with drug induced liver injury, on retrospective testing 13% were found to have autochthonous HEV infection¹²⁴. Smaller numbers have been reported from the United States- 3% of patients with suspected drug induced liver injury retrospectively tested positive for anti-HEV IgM¹²⁵. In patients undergoing allogeneic haematopoietic stem cell transplant, graft versus host disease may present with clinical features similar to HEV infection. In one study of stem cell transplant recipients, 2.4% of 328 patients developed HEV infection, in which the pattern of liver function test abnormality was indistinguishable from that of graft-versus-host disease¹¹³. This is clearly an important clinical distinction to make as the two conditions are treated entirely differently in terms of adjustments of immunosuppression.

HEV REINFECTION

Re-infection with HEV is reported, and can be identified by a rapid increase in anti-HEV IgG levels, with HEV RNA becoming detectable. Abravanel followed a cohort of 263 solid organ transplant recipients for one year; in addition to three cases of de novo HEV infection, there were 3 cases of HEV reinfection¹²⁶. Patients who tested positive for anti-HEV IgG, with or without detection of anti-HEV IgM at transplantation, and tested positive for HEV RNA during follow up were considered to have become reinfected. Reinfection with HEV can lead to a chronic infection and further studies are required to evaluate the clinical importance of HEV reinfection in immunosuppressed patients.

Previously, reinfection or chronic infection was associated only with immunosuppressed patients and not the healthy donor population. However, Baylis recently reported HEV re-infection in a small percentage of plasma donors, as suggested by anti-HEV IgG with high avidity and high viral loads, in the absence of anti-HEV IgM¹²⁷. Schemmerer also reported reinfection in 8.8% of individual patient

courses¹²⁸; the preexisting anti-HEV IgG concentration was $<7 \text{ WU mL}^{-1}$, and one patient had a serologic profile indicating 4 consecutive reinfections in intervals of 1.2-3.4 years.

TREATMENT OF HEV INFECTION

Liver transplantation for fulminant hepatitis

It is rare for patients with HEV induced acute liver failure to require emergency liver transplantation. The United States Acute Liver Failure Study Group reported upon 681 patients with acute liver injury/failure who were tested for anti-HEV IgM and IgG levels¹²⁹; those with detectable IgM levels underwent HEV RNA testing. One patient who was initially found to have a positive anti-HEV IgM proceeded to emergency liver transplantation. However, in this case the diagnosis of HEV and its potential causative role in acute liver failure was not clear cut; repeat samples for anti-HEV IgM were negative, and although initial anti-HEV IgG was positive, repeat serum samples were negative. HEV RNA was never detected. Following further investigation, it was felt likely that the cause of the acute liver failure and requirement for transplantation was in fact inadvertent paracetamol overdose. There are no other reports of emergency liver transplantation for acute HEV infection in the literature.

Medical Therapy

Following the identification of chronic HEV infection, there were case reports of successful viral clearance following ribavirin and/or pegylated interferon treatment. Both ribavirin and pegylated interferon inhibit HEV replication *in vitro*. However, pegylated interferon is contraindicated in kidney transplant recipients due to an appreciable risk of acute rejection and ribavirin has subsequently become the first line medical treatment for both acute (if required) and chronic HEV infection (Table 3)^{111, 130-135}. Gerolami first reported upon the use of ribavirin in the treatment of acute HEV genotype 3 infection in 2011¹³⁶; the patient was immunocompetent and was treated for 3 weeks, with normalisation of alanine aminotransferase and a fall in detectable HEV RNA levels. Treatment is most commonly started if HEV RNA remains detectable at 3 months, however this remains an off licence use. Ribavirin has also been used to treat acute HEV genotype 1 infection: Pischke described treatment of a patient with acute HEV genotype 1e infection (acquired in Eritrea) with ribavirin for 6 weeks and the patient obtained SVR¹³³. The effect of ribavirin treatment on HEV genotype 1 infection has recently been studied using human liver chimeric mice. Ribavirin treatment led to a statistically

significant decrease in viraemia after 6 weeks of treatment, together with a sharp decline in ORF2 and ORF3 proteins as detected by immunofluorescence in the livers of treated animals compared with controls³³.

Worryingly, cases of ribavirin resistance and treatment failure have been reported, often related to a reduction in ribavirin dose because of side effects (e.g. anaemia). In one case series of patients undergoing treatment of HEV infection, ribavirin-induced anaemia necessitated dose reduction in 29%; the use of erythropoietin in 54% and blood transfusions in 12%¹³⁰. The G1634R mutation in the HEV ORF1 protein has also been associated with treatment failure; one study demonstrated that in patients with ribavirin treatment failure, all patients had this mutation¹³¹. The G1634R mutation increases the replicative capacity of HEV in the human liver, and thereby reduces the efficacy of ribavirin.

Recently, ribavirin has been reported as mutagenic for the HEV genome during treatment of chronic infection, with an increasing number of variants being identified and mutations in all open reading frames of the genome¹³⁷. In addition to the previously identified G1634R mutation, K1838N, D1384G, V1479I and Y1587F mutations are selected in non-responders to ribavirin therapy. In essence, ribavirin exerts mutagenic pressure on the viral genome and whilst this may result in viral clearance, it may also lead to the selection of resistant variants in those patients who do not respond.

Debing described a patient with chronic HEV infection, who experienced treatment failure with ribavirin, with a resistant phenotype¹³⁸. In this case, although HEV RNA was undetectable after 10 weeks of treatment, eight weeks after treatment cessation the patient had viral relapse. Ribavirin was subsequently restarted and at 58 weeks post re-introduction of ribavirin, HEV RNA was still detectable. Next generation sequencing was performed to identify mutations associated with ribavirin resistance: resistance was associated with Y1320H, K1383N and G1634R mutations in the viral polymerase, in addition to an insertion in the hypervariable region. Subsequent *in vitro* studies identified that Y1320H and G1634R mutations have replication-increasing roles, whereas the K1383N mutation suppressed viral replication and in fact increased the *in vitro* sensitivity to ribavirin. Further deep sequencing of hepatitis E genomes demonstrated that ribavirin is mutagenic to viral replication *in vitro* and *in vivo*.

Treatment of chronic HEV infection in transplant recipients (and other immunocompromised patients)

Solid organ transplant recipients represent a unique therapeutic challenge in the management of HEV infection. Commonly used immunosuppressive agents are now known to affect the *in vitro* replication of HEV. mTOR (mammalian target of rapamycin) inhibitors (e.g. everolimus) promote *in vitro* HEV replication via mTOR inhibition¹³⁹. The calcineurin inhibitors (tacrolimus, ciclosporin A) have also been shown to have a pro-proliferative effect, in contrast to mycophenolate mofetil which inhibits HEV replication *in vitro*¹⁴⁰.

In transplant recipients, the initial treatment approach should be to reduce immunosuppression if this is feasible. Reduction in immunosuppression by approximately 30% results in clearance of chronic HEV infection in around 30% of this patient cohort⁷. For patients who cannot reduce immunosuppression or who fail to clear the virus despite a reduction in immunosuppression, ribavirin monotherapy is the treatment of choice for the majority of patients (Table 3)^{111, 130-135}. Sustained viral response is the aim of therapy, defined as an undetectable serum HEV RNA level at least 6 months after treatment cessation. There is no definitive guidance as to the ideal treatment duration and dosage; there are reports of treatment courses lasting 1 month to 9 months, with the majority of units who have reported their experience favouring a 3-month course. Initial starting doses of ribavirin range from 600-1000mg. Factors associated with achieving a sustained viral response include a higher lymphocyte count when ribavirin therapy was initiated¹³⁰. Kamar reported that in 59 solid organ transplant recipients who were treated with a median of 9 months of ribavirin for chronic HEV infection, sustained viral response was obtained in 78%. Importantly, in those patients who had recurrence and completed a second, prolonged course of ribavirin, sustained viral response could be achieved in the majority of patients¹³⁰.

Other antiviral drugs have been investigated in the treatment of HEV in view of potential treatment failure with ribavirin. The role of sofosbuvir, a directly acting antiviral which is the oral prodrug of a nucleotide hepatitis C virus-RNA-dependent polymerase inhibitor, has been studied. Dao Thi demonstrated that sofosbuvir efficiently inhibited HEV genotype 3 replication *in vitro*¹⁴¹. Furthermore, this group were able to demonstrate an additive effect when combined with ribavirin. The authors of this paper did recognise that the anti-HEV property of sofosbuvir is less marked than its anti-hepatitis C property, and clinical studies were required to confirm the efficacy of sofosbuvir in treating human HEV infection, particularly in those who have failed to clear HEV with ribavirin therapy alone.

Following this, Donnelly described a patient with chronic hepatitis C and HEV infection post-transplant, who was treated with sofosbuvir and daclatasvir (predominantly for hepatitis C infection and in view of previous ribavirin intolerance) and observed the effects of this treatment on HEV RNA and HEV-specific T-cell responses¹⁴². Despite the previous report of the inhibition of HEV replication *in vitro* with sofosbuvir, in this human study, no effect was seen on either HEV RNA levels or HEV-specific T-cell responses. Brown later characterised host T-cell responses against HEV and similarly demonstrated that in organ transplant recipients, anti-HEV T-cell responses were reduced in breadth and magnitude¹⁴³. Another group studied the effect of sofosbuvir combined with ribavirin against HEV genotype 3 infection in a human patient: sofosbuvir at a standard dose had some antiviral activity against HEV (as evidenced by a decline in HEV RNA levels) but was not potent enough to induce viral clearance¹⁴⁴. The authors suggest that higher doses of sofosbuvir may be required to completely suppress viral replication; this would be an expensive approach to HEV treatment, and the potential side effects with this dose of therapy are unknown.

An American study analysed the prevalence and clinical consequences of HEV infection in patients who had previously undergone liver transplantation for chronic hepatitis C infection¹⁴⁵. 42% of patients had detectable anti-HEV IgG at some point from baseline (pre-transplantation) until the end of the five year follow up period; 5 patients were anti-HEV IgM positive pre-transplant, one patient demonstrated IgM seroconversion post-transplant and eight patients had IgG seroconversion post-transplant. Of those patients seroconverting post-transplant, eight had been treated for hepatitis C recurrence before or at the time of seroconversion. The authors felt that post-transplant treatment of hepatitis C recurrence with ribavirin/PEG-IFN may have afforded a degree of protection against HEV, and warned that with increasing use of new directly acting antiviral agents, the prevalence of chronic HEV infection in this population may in fact begin to increase.

PREVENTION OF HEV INFECTION

GENOTYPE 1 and 2 INFECTION

HEV is becoming a real global public health problem, and a focus on prevention of infection must be considered. Globally, basic sanitation must remain the first line of defence against HEV infection. However, it is recognised that during outbreaks, basic sanitation and simple health interventions do

not adequately prevent additional infections. Therefore, a vaccine against HEV is highly desirable, particularly for residents living in highly endemic areas and for those at high risk of developing complications e.g. the immunosuppressed. As all HEV genotypes belong to the same serotype, it is thought that one HEV vaccine should provide protection against all HEV genotypes. Due to difficulties in culturing HEV, it has not been feasible to produce enough virus for vaccine production for either live attenuated or inactivated vaccine against this virus¹⁴⁶. Vaccine development therefore relies on preparation of recombinant HEV antigens or DNA. At least 11 experimental vaccines have been evaluated in non-human primates¹⁴⁶. Two recombinant HEV vaccines (developed from genotype 1) have been shown to have short term efficacy in humans^{147, 148}. A genotype I HEV recombinant protein (rHEV) vaccine had been trialled in volunteers from the Nepalese army, however this vaccine has been removed from the development pipeline^{146, 147}.

The long term efficacy of the licensed Xiamen Innovax Biotech anti-HEV vaccine (Hecolin) has recently been studied in adult patients in China¹⁴⁸. Patients were randomly assigned to receive either three doses of the HEV vaccine, or a hepatitis B vaccine. In the HEV vaccine group, 0.3 cases per 10,000 person-years were identified, compared with 2.1 cases per 10,000 person-years in the hepatitis B vaccine (control) group, affording a vaccine efficacy of 86.6%. On follow up, the HEV vaccine induced antibodies against HEV and provided protection against HEV for up to 4.5 years, and importantly, no safety concerns relating to the use of this vaccine were reported. Although there were some clinical issues relating to this study, for example the potential for missed cases of HEV despite vaccination due to the lack of regular follow up assessments, the promise of a safe and effective anti-HEV vaccine seems achievable in the near future. The WHO SAGE working group on Hepatitis E has identified and recognised the need for reviewing the existing data on the safety, efficacy and cost-effectiveness of the licensed hepatitis E vaccine and identifying the potential indications and uses for the hepatitis E vaccine in the context of other hepatitis E preventative strategies¹⁴⁹.

GENOTYPE 3 and 4 INFECTION

In the Western world, as transmission is predominantly via undercooked foodstuffs there must be education on adequate cooking to minimise risk of transmission via the food chain: for example, the risk of HEV transmission via foodstuffs is significantly reduced by cooking meat for 1 minute at 70°C¹⁵⁰. With regards to prevention of transmission via contaminated blood products, there is no

evidence at present to support the need for HEV negative blood components for pregnant women. At present in England, and more recently in Scotland, NHS Blood and Transplant recommend that HEV negative blood should be used in patients who have undergone allogeneic stem cell transplant or solid organ transplant¹⁵¹. The UK Advisory Committee on the Safety of Blood, Tissues and Organs make a number of additional recommendations regarding the use of HEV-screened blood components- Table 4¹⁵².

NEW APPROACHES TO TREATMENT, FUTURE WORK AND RECOMMENDATIONS

Firstly, standardisation of diagnostic assays is key to ensure as many cases of HEV infection are detected as possible. Work is currently underway to develop World Health Organisation reference materials for HEV serology, which will be available in due course as a worldwide resource. Dedicated studies are required to clarify the optimal dose and treatment duration of ribavirin therapy. With regards to the development of new treatment strategies, targeting viral polymerase may provide a new approach to therapy, and the recent availability of cell culture models/systems will allow new opportunities for the study of HEV biology and the development of targeted therapeutic and/or prophylactic strategies. Deep sequencing technology may prove invaluable in identifying patients at risk of treatment failure with ribavirin; its use may become important in a 'personalised medicine' approach to the treatment of chronic HEV infection. Future work should focus on increasing awareness of HEV infection in the developed world, emphasising the need for clinicians to have a low threshold for HEV testing, particularly in immunosuppressed patients. Patients at potential risk of chronic HEV infection must also be educated and made aware of modes of transmission of infection and given advice regarding prevention of infection e.g. routine advice should be given to stem cell and solid organ transplant recipients regarding risk of eating under or poorly cooked pork or pork products. Other attempts to reduce the risk of transmission of infection and infection in high risk patients could include the global use of HEV negative blood in immunosuppressed patients, including the organ and stem cell transplant population. Future clinical trials could include trials of alternative immunosuppression regimens in the transplant population: as described above the role of certain therapies in the development of HEV infection is now recognised, and trials of alternative treatment options for those patients who have failed ribavirin therapy, and/or have the presence of the G1634R mutation are required. Clinicians must be vigilant to the possibility of HEV infection, particularly in

elderly men, solid organ transplant recipients and the immunosuppressed, and understand how to manage the patient with HEV infection – see table 5.

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FIGURES

Figure 1. Worldwide endemicity for HEV infection

Figure 2. Organisation of the HEV genome.

A schematic diagram of the genomic and subgenomic organisation of the HEV genome. The open reading frames are shown as boxes and labelled. The non-coding features are labeled and the putative domains of ORF1 are also shown. **In genotype 1 strains, a putative ORF4 has been identified and falls within the ORF1 coding region.** Modified from Cao & Meng 2012. CRE, cis-reactive element; Hel, Helicase; HVR, hypervariable region; JR junction region; MT, methyltransferase; NCR, non-coding region; PCP, papain-like cysteine protease; RdRp, RNA dependent RNA polymerase; SL, stem-loop structure.

TABLES

Table 1. Characteristics of HEV infection according to genotype.

Table 2. HEV seroprevalence in screened blood donor populations

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| Table 5. Clinicians guide to testing for and treatment of hepatitis E

Table 1. Characteristics of HEV infection according to genotype.

CHARACTERISTIC	GENOTYPE 1+2	GENOTYPE 3+4
Species specificity	Restricted to humans	Zoonotic
Geography	Developing world	Developing and developed world
Pattern of spread	Epidemic and sporadic	Sporadic
Mode of transmission	Faecal-oral spread (contaminated water)	Contaminated food products (e.g. pork) Blood products Solid organ transplantation
Age distribution	More common amongst adolescents and young adults	More common amongst older adults
Sex distribution	Affects males and females equally	More common in males
Chronic infection	Not recognised	Now recognised, common in immunosuppressed patients
Therapy	None	Ribavirin, peginterferon
Mortality	High amongst pregnant women	Higher amongst older adults

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Table 2. HEV seroprevalence in screened blood donor populations

COUNTRY OF SCREENED BLOOD DONORS	ANTI-HEV IgG SEROPREVALENCE	HEV RNA POSITIVITY RATE
Spain, 2014 ⁷²	19.96% (Wantai assay) 10.72% (Mikrogen assay)	1 per 3333 donations
US, 2015 ⁷³	9.5%	1 per 9500 donations
Southern France, 2011 ⁷⁴	39.1% (Wantai assay)	1 case detected out of 591 tested
Denmark, 2016 ⁷⁵	<i>Data not available</i>	1 per 2330 donations
Brazil, 2015 ⁷⁶	10%	0%
Austria, 2014 ⁷⁷	13.55% (Wantai assay)	1 per 8416 donations
Sweden, 2012 ⁷⁸	NA	1 per 7986 donations
Germany, 2012 ⁷⁹	5.94% (Mikrogen assay)	1 per 1240 donations
England, 2010 ⁶³	16.2% (Wantai assay) 3.6% (MP assay)	<i>Data not available</i>
England, 2014 ⁶⁶	29% (Wantai assay)	1 per 2848 donations
Holland, 2013 ⁸⁰	27% (Wantai assay)	1 per 2671 donations
Scotland, 2013 ⁸¹	Wantai	1 per 14520 donations

Table 3. Treatment of chronic HEV infection with ribavirin in immunosuppressed patients

STUDY	POPULATION	TREATMENT REGIMEN	OUTCOME
Kamar N et al 2010 ¹¹¹	Renal transplant recipients, France	Dose: median 800mg per day Duration: 3 months	SVR 67%
Kamar N et al 2014 ¹³⁰	Solid organ (all) transplant recipients with genotype 3 infection, France	Dose: median 600mg per day Duration: median 3 months	SVR in 78%
Debing Y et al 2014 ¹³¹	Solid organ (all) transplant recipients with genotype 3 infection, Germany	Dose: initial daily dose 600-1000mg Duration: not specified	Treatment successful in 87%
Mallet V et al 2010 ¹³²	Kidney-pancreas transplant and idiopathic immunodeficiency	Dose: 400-600mg per day Duration: 3 months	RNA negative at 2 and 3 months post treatment cessation
Pischke S et al 2013 ¹³³	Solid organ (all) transplant recipients	Dose: initial daily dose 600-1000mg Duration: 5 months	SVR in 81%
Tavitian S et al 2015 ¹³⁴	Haematological malignancy	Dose: median 800mg per day Duration: median 3 months	RNA undetectable after 30 days in all treated patients
Galante A et al 2015 ¹³⁵	Orthotopic liver transplant recipients	Dose: initial daily dose 400-800mg Duration: 3 months	SVR in 75%

SVR = sustained virological response

Table 4. Recommendations on the use of HEV-screened blood components¹⁵²

PATIENT GROUP	RECOMMENDATION REGARDING BLOOD COMPONENTS
<i>SOLID ORGAN TRANSPLANTATION(SOT)</i>	
All	HEV-screened components should be given to all SOT recipients taking immunosuppressant medication
Potential SOT recipients	From 3 months prior to date of elective SOT potential recipients should only receive screened components. Patients likely to be transplanted within 3 months and currently not receiving immunosuppression should be given HEV screened components.
Any patient receiving immunosuppression before SOT	Should receive screened components only
Extra corporeal procedures	Screened components should be used for extra corporeal circulatory support for patients undergoing SOT, and for SOT patients receiving immunosuppression
<i>HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)</i>	
Allogeneic HSCT	Screened components should be given to potential allogeneic HSCT recipients from 3 months prior to date of planned HSCT until 6 months following HSCT, or for as long as patient is immunosuppressed
Autologous HSCT	No convincing evidence at present to support recipients receiving screened components.

Table 5. Clinicians guide to testing for and treatment of hepatitis E

Question?	Take home message
Which patients should be tested for HEV?	<ul style="list-style-type: none"> • ALT \geq 300 IU/L (all patients) • ALT/ALP ratio \geq 2 (all patients) • Suspected drug induced liver injury • Severe acute liver injury (all patients)
How should we test for HEV?	<ul style="list-style-type: none"> • Anti-HEV IgM should be the initial serological test of choice • If IgM positive, HEV RNA by PCR in serum or stool should be used to confirm active infection • HEV RNA by PCR should be the initial test of choice in immunosuppressed patients
How long should I treat HEV infection with ribavirin for?	<ul style="list-style-type: none"> • For chronic HEV infection, the initial course of ribavirin therapy should be for 3 months • If the patient is immunosuppressed e.g. is a solid organ transplant recipient, consider a trial of a reduction in immunosuppression in the first instance
What should I do in the case of ribavirin non-response?	<ul style="list-style-type: none"> • Extend course of ribavirin therapy for a further 3 months • If still no response, continue ribavirin for further 6 months • Consider trial of pegylated interferon for 3 months (not in renal transplant recipients)

- Not endemic
- Endemic (HEV infection accounting for <25% of non-A non-B hepatitis)
- Highly endemic (HEV infection accounting for $\geq 25\%$ of non-A non-B hepatitis or waterborne outbreaks)

