SaBTO

Advisory Committee on the
Safety of Blood, Tissues and Organs

Hepatitis E Virus: Transfusion and Transplantation Risk Reduction Working Group Report

Table of Contents

Tables

Figures

1. Executive Summary

1.1. Background

A. In 2005, a study in England and Wales indicated that 25% of adults in the sixth and seventh decades of life have serological evidence of previous HEV infection.

B. The first UK transmission of HEV by a blood component was reported in 2006. It was identified by look back on the recipients of components taken from a blood donor who reported acute jaundice due to hepatitis E 24 days after blood donation.

C. In 2013 a study jointly funded by Public Health England and NHS Blood and Transplant confirmed a RNA prevalence of approximately 1 in 2850 within blood donors in the SE of England. Morbidity in recipients of components from HEV RNA positive donors was generally absent or mild, but viral clearance was delayed in those on immunosuppression.

1.2. Remit of HEV Working Group

A SaBTO Working Group was established in 2013 with the following remit:

A. To review the evidence base, including findings of the joint PHE/NHSBT study, of HEV prevalence in blood/organ donors and transmission through transfusion/transplantation.

B. To consider the impact of HEV on transfusion recipients and recipients of organs, tissues and cells.

C. To consider and identify any steps UK Blood Services and the transplant community should take to mitigate the risks associated with HEV infection in recipients of blood, organs, tissues and cells.

D. The remit included consideration of:

- Efficacy of current strategies in mitigating risk of transmission/long-term term liver disease;
- Steps being taken/under consideration in other countries
- Determining whether there are specific patient groups, if possible, who should be prevented from becoming infected
- Secondary transmission ie human to human through social or sexual contact
- The practicalities and impacts of mitigation steps on donors/recipients and on the blood/organ supply
- The cost-effectiveness of any proposed mitigation steps
- Further research or information required
- Disseminating the outcome of the review.

E. Out of scope were:

- Risk mitigation steps for the general population
- Antenatal screening

- HEV in fractionated plasma products
- Dietary advice to high risk patient groups.

1.3. Epidemiology and pathogenesis

A. The status of HEV infection in W Europe is evolving, and information on all factors which ultimately determine its impact on transfusion and transplant recipients is both limited and changing. Thus current observations and recommendations will require frequent review.

B. HEV is a zoonotic infection in the UK, most likely acquired through eating infected pork products. However, the HEV genotype in human UK cases does not match that of the UK pig population.

C. The UK incidence of clinical infection is increasing, with 600 indigenous clinical cases reported in 2012, 700 in 2013, and 800 in 2014. High rates are also reported in Netherlands and parts of France. However, this figure underestimates the true incidence, as infection is usually asymptomatic or mild. A study of UK blood donors revealed a viraemia rate of ~1 in 2850, equivalent to 2-3 donors/day across the UK.

D. Short-lived viraemia and complete viral clearance is the norm in immune competent individuals. However, long-term viral carriage may occur in immunosuppressed individuals, with progression to chronic liver disease; the frequency of this remains uncertain.

E. In patients with chronic liver disease, serious worsening of liver function has been described.

F. Clinical features other than hepatitis have been described, such as Guillain-Barré syndrome, but it is not clear whether these are more common with HEV than after other viral infections .

1.4. Transmission through blood, tissues, organs and cells

A. Transfusion transmission of HEV is well described in the literature, with the first reported UK case in 2006 from red cells, and 3 cases reported by the UK haemovigilance scheme Serious Hazards of Transfusion (SHOT) from 2011-14, all from fresh frozen plasma (FFP); no UK deaths were attributed to HEV. Most clinically apparent cases in the UK and other countries have been in organ or stem cell transplant recipients. Even in such recipients, acute severe hepatitis and death appear to be rare, but the precise frequency is unknown.

B. HEV may be transmitted by red cells, platelets and FFP, with a transmission rate of ~40% in the PHE/NHSBT study, still the only donor/recipient prospective study available at the time of this review. In this study, albeit with small numbers of recipients, transmission was more likely at higher viral loads and with exposure to larger volumes of plasma (25% transmission rate from red cells, 50% from FFP/platelets). Most recipient infections were asymptomatic or mild, with only one case of clinical hepatitis in an immunocompetent recipient. Spontaneous viral clearance with sero-conversion was the commonest outcome, but a small

number of immunosuppressed individuals required anti-viral therapy and/or reduction of immunosuppression to achieve clearance. Special consideration is therefore needed regarding risk mitigation in recipients of solid organ and stem cell transplants in view of the increased risk of chronicity. This is explored further in section 1.7 below.

1.5. Possible risk mitigation steps for the blood supply

A. No country has yet adopted universal blood donor screening, and Netherlands (where there is evidence of higher prevalence than recorded in the UK) has taken a decision not to implement screening at present. France screens donors for manufacture of solvent-detergent FFP, and discussions there and in Ireland are ongoing regarding broader donor screening.

B. There is no evidence which can be used to develop questions which could be added to the blood donor health check questionnaire to identify donors at particularly high risk of HEV viraemia.

There have been no reported cases of HEV in vegetarians, but the population rate of self-declared vegetarians (7%) is too low to use this characteristic to identify a low-risk donor population for high risk recipients.

Based on the known incubation period, the period following donation for which donors are asked to report new illness need not be extended beyond the current 2 weeks.

C. Should testing be proposed, HEV NAT testing, which detects viraemia and hence potentially infectious donors, is the testing strategy of choice. There are CE marked assays currently available and suitable for HEV NAT testing of blood donors. Testing by serology detects recent and past infections, and thus would exclude many safe donors; there is no added value in carrying out serology testing in addition to NAT.

Blood donor screening by NAT could safely be performed in pools of 16-24, which would be determined as part of validation. Reactive samples would then be confirmed by a validated different HEV RNA assay of equivalent sensitivity, along with assessment of viral load, genotype and serological status.

Universal donor screening would yield over 500 donors/year who would require deferral and retest before returning to donation. Look-back of previous recipients would also be required, with an indicative look-back period of 4 months.

Due to variable levels of viraemia which in some donors will be below the detection limits of the assay, routine screening by pooled testing would potentially miss 16-44 donations/year, a figure which will vary with the population incidence.

D. There is insufficient information at present to recommend creation of a panel of sero-positive immune donors to provide transfusions for high risk recipients. For example, there is

no definition of IgG or IgM levels to define immunity, nor adequate information on antibody decay.

E. It is uncertain at present what degree of protection would be provided by pathogen inactivation of platelets or single unit FFP, as not all available methods have been tested by HEV, and breakthrough transmissions have been seen with FFP treated by [redacted] method. Manufacturers should be asked to provide such data.

The solvent detergent method of inactivation for FFP is not in itself effective against HEV. Therefore, specifications are being set for plasma used for manufacture with regard to HEV testing. If these are implemented, solvent detergent treated FFP (SDFFP) is likely to carry a significantly reduced risk of HEV, and merits further investigation with the manufacturer. Its cost-effectiveness as the FFP product of choice for all or high-risk recipients is as yet uncertain.

There are no PI methods available for red cells.

1.6. Risks in specific groups of transfusion recipients

A. No cases arising from transfusion transmission have been reported in pregnant recipients. There is no evidence of risk in pregnancy related to the G3 HEV genotype found in the UK. There is a major risk in the third trimester of pregnancy from a G1 HEV genotype. It is important to prevent G1 infection at this stage of gestation but it is highly unlikely that a G1 infection would be acquired in the UK (from any route).

B. No cases from arising from transfusion transmission have been reported in neonates. There are few data regarding the risk to neonates from HEV infection. Awareness of HEV in the paediatric community is low and consideration should therefore be given as to how such awareness could be increased, along with inclusion of HEV in the investigation of the jaundiced neonate

C. There have been occasional case reports of HEV in HIV positive patients. No cases arising from transfusion transmission have been reported.

D. There are no case reports of HEV in patients who receive regular red cell transfusions for either haemoglobinopathies or chronic haematological disorders such as myelodysplasia. Such patients would be a relevant sentinel group for cross-sectional and longitudinal monitoring.

1.7. Risks in solid organ and stem cell transplants

A. Solid organ and stem cell recipients may become infected through the transplanted organ itself, by transfusion at the time of transplantation, or by diet (infected pork) in the months or years following the transplant, when still on immunosuppression.

B. It is not known whether solid organ or stem cell transplant recipients are at particular risk of HEV acquisition. A sub-set of infected transplant patients go on to delayed clearance or

chronic infection; some of those will develop chronic liver disease. However, there is considerable uncertainty as to the size of the risk.

C. In deceased organ donors, there are many causes for mildly abnormal liver tests during the final illness, and chronic infection with HEV is rare. Based on average annual figures for each type of donor, it is calculated that there would be 1 infected deceased organ donor/year, 1 infected living organ donor every 1-3 years, and 1 infected unrelated stem cell donor every 1-3 years.

D. Based on the number of reported cases in the population, the annual dietary risk for transplants is calculated to be 0.1-0.2% ie 1 in 500 transplant recipients will become infected annually through diet, with 99% remaining negative for evidence of current or past HEV infection after 5 years. However, this makes the assumption that their risk of acquiring the infection is identical to the general population; as stated above, whether immunosuppression causes increased risk of virus acquisition is not known at present. Therefore, more data are required regarding the background rate of HEV acquisition from diet in transplant recipients.

E. Blood component exposure is low in renal transplantation (mean 0.5 donor exposures/procedure), rising to 9/procedure for liver transplantation, and 68/procedure for multivisceral transplantation. Approximately 25% of these exposures are through FFP.

F. Donor exposure during allogeneic stem cell transplantation is higher than for solid organ transplants, due to multiple platelet transfusions. Recent data from a single stem cell transplant centre in England (courtesy of Dr Kate Pendry, NHSBT/Central Manchester Hospitals) show that both adult and paediatric recipients of allogeneic (donor) stem cell transplants receive a median of 19 donor exposures/procedure, of which 12 are from platelets. It should be noted that donor exposure via platelets will increase by approximately 75% over the next 2 years as the percentage of apheresis platelets falls from 80% to 40%. The impact of platelet additive solution for pooled platelets is unknown.

G. Based on transplant activity, donor exposure and transmissibility, the estimated risk from blood components is equivalent to 1 infection/1-2 years from blood components in liver transplant recipients. The corresponding figure for renal and multivisceral transplants is 1 infected recipient every 5 years and 8-10 years respectively. The estimated infection rate in allogeneic stem cell transplant recipients is approximately 4 per year.

H. There is considerable uncertainty regarding the frequency with which HEV becomes chronic in organ and stem cell transplant recipients, and the likelihood of chronicity leading to serious sequelae such as cirrhosis.

I. Possible options to provide risk mitigation for solid organ and stem cell transplant recipients were considered. Because the clinical burden of HEV infection in transplant recipients is not defined, a full cost-effectiveness analysis was not possible, but broad costs were estimated. Options considered were:

(1) selective screening of the blood supply to provide HEV negative components for solid organ and allogeneic stem cell transplant patients. It is estimated that 50,000 donations/year would need to be screened, at an indicative cost of [redacted]

(2) Provision of immune donors (rejected) (for reasons given in paragraph D above)

(3) Test all solid organ and allogeneic stem cell transplant recipients at pre-defined interval(s) eg annually and treat if positive. There are >40,000 individuals alive with a transplanted solid organ in situ, and the clinical effectiveness, feasibility, and cost-effectiveness of annual testing is uncertain; indicative costs are [redacted]

(4) A combination of (1) and (3); indicative costs [redacted]

(5) No prevention or patient monitoring, but a low index of suspicion for HEV testing of patients if liver enzymes become abnormal, with treatment when chronic infection identified. HEV infected transplant recipients can be treated by modification (usually reduction) of immune suppression and, if indicated, anti- viral agents such as ribavirin.

J. The requirement and optimal strategy for clinical risk mitigation in transplant patients is not yet clear and further information is needed (see section 1.10).

1.8. Banked tissues, gametes and embryos

A. The intrinsic risk from tissue transplants appears to be extremely low. Tissue transplants are not commonly accompanied by a need for transfusion, but even if transfusion occurs, most tissue recipients are not immunosuppressed and therefore not at high risk from serious clinical sequelae. The exceptions are recipients of pancreatic islets and hepatocytes, who receive immunosuppression and who are therefore at equivalent risk of clinical sequelae as solid organ transplant recipients.

B. Likewise the risk of both infection and clinical sequelae from donation of eggs, sperm or embryos is extremely low.

1.9. Recommendations

A. On the albeit limited evidence available, there is no pressing case for HEV RNA screening of the entire blood supply at this time. However, the pattern of HEV infection in the UK is evolving and this recommendation should be reviewed at the earliest opportunity when the findings become available from the additional work recommended below (see section 1.10) , or new evidence from other countries.

B. The requirement and optimal strategy for clinical risk mitigation in solid organ and stem cell transplant patients is not yet clear. While the additional evidence is bring gathered, UK Blood Services should without delay develop a costed operational plan for blood donor testing to provide HEV-tested components for solid organ and allogeneic stem cell transplant recipients.

C. Testing of deceased organ donors for HEV should be imited to those with an unexplained hepatitis where a viral cause is being considered on clinical or epidemiological grounds. Specific pre-donation screening is not indicated for deceased organ donors with normal liver function tests.

D. Organs from deceased HEV infected donors should be used only in exceptional cases and only after full discussion and treatment planning with an expert microbiologist and with suitably informed patient consent.

E. Living organ or stem cell donors with unexplained liver function tests should be investigated and HEV infection considered. Organs from infected donors should not be used until the donor has been consistently negative for HEV RNA with a documented acceptable level of detectable IgG.

F. HEV testing should be considered in any solid organ, stem cell transplant or chronic liver disease patient with unexplained changes in liver enzymes.

G. No specific steps are required to mitigate the HEV risk in recipients of banked tissues. However, any future recommendations for recipients of pancreatic islets or hepatocytes should follow that for solid organ recipients.

H. No specific steps are required to mitigate the HEV risk in recipients of donated gametes or embryos.

I. Awareness of HEV should be increased in clinical teams treating organ and stem cell transplant recipients, neonates, pregnant women and transfusion-dependent patients such as those with haemoglobinopathies.

J. The section below highlights the further information needed to make definitive recommendations. A costed plan of this further work should be produced without delay, showing time lines as to when each piece of information will become available.

1.10. Additional information needed

Obtaining the information below will need collaboration between UK Blood Services, Public Health England and equivalent organisations in the devolved administrations, and clinicians in the solid organ and stem cell transplant communities.

1. How the incidence of new HEV infections in UK blood donors varies over time.
2. The rate of HEV acquisition and its clinical sequelae in specific patient groups, including children, transfusion-dependent patients, and solid organ/stem cell transplant recipients
3. Work to investigate the prevalence of chronic HEV infection in the UK and understanding the determinants associated with viral persistence in the immunosuppressed population.

4. Effectiveness and cost-effectiveness of different methods of Pathogen Inactivation for FFP and platelets.
5. . A feasibility study of testing all transplanted patients (organ or stem cell) eg annually.

2. Background, Remit and Methodology

Hepatitis E virus (HEV) has been increasing in prevalence throughout the UK and mainland Europe. HEV comprises four genotypes of which two, genotypes 1 and 2 are human viruses and two, genotypes 3 and 4, are animal viruses which infect humans as a zoonosis.
In 2005, a study in England and Wales indicated that 25% of adults in the sixth and seventh decades of life have serological evidence of previous HEV infection [1].
Public Health England has maintained enhanced surveillance of HEV since 2003, undertaking epidemiological investigation of confirmed cases. In 2013, PHE received reports of 691 cases of acute hepatitis E, exceeding the reported cases of hepatitis A. PHE ascertained that 69% of these cases occurred in people who had not travelled outside of the UK, leading to the conclusion that infection had occurred within England and Wales. Questionnaire based studies undertaken in cases from England have indicated an association between the consumption of processed pork products and HEV infection [2]. The concept that food can act as a vehicle of HEV transmission is further supported by reports of HEV infection following the consumption of undercooked/raw pig, deer and boar meat [3,4,5,6].

However, despite a reported increase in incidence, in routine clinical practice post-transfusion HEV infection is rarely reported. The first UK report of the virus being transmitted through the transfusion of a blood component was received in 2006. The incident was identified by a look back on the recipients of components taken from a blood donor who reported acute jaundice due to HEV 24 days after blood donation. Since the 2006 case, there have been eight (two in 2013, five in 2012, and one in 2011) suspected transfusion-transmitted HEV cases notified to NHS Blood and Transplant (NHSBT) for investigation. Of cases investigated to date, 3 have been confirmed to have arisen from transfusion (all 3 from FFP), with the remaining cases concluded not to have arisen from transfusion. Two of these cases appear in annual reports of the UK haemovigilance scheme Serious Hazards of Transfusion, and the third will be included in the 2014 report, due to be published in July 2015.

In 2013 a study, jointly funded by Public Health England and NHSBT, was undertaken to establish:
(1) the prevalence of HEV Ribonucleic Acid (RNA) in blood donors
(2) the transmission rate of HEV by a range of blood components and
(3) the outcome in recipients of receiving HEV containing components.
The study findings confirmed a HEV RNA prevalence in NHSBT blood donors of approximately 1 in 2850. Blood components manufactured from HEV RNA positive donations were tracked and where possible recipients were tested for HEV demonstrating a

transmission rate of approximately 40%. Transfusion-transmitted infections were found to rarely cause acute morbidity but in some immunosuppressed patients the virus became persistent and took some time to clear.

The working group have reviewed and evaluated the evidence base for HEV transmission through blood, cells, tissues and organs, including the findings of the joint PHE/NHSBT study of HEV prevalence in blood/organ donors and evidence of transmission through transfusion/transplantation. In so doing, information has been gathered on steps being taken or considered to reduce the risk of HEV transmission in other countries.

The group have considered the impact of HEV on transfusion recipients and recipients of organs, tissues and cells and have investigated the risks of secondary transmission. The group sought to identify any steps (e.g. donor selection; pathogen inactivation) that the UK Blood Services and the transplant community might take to mitigate the risks associated with HEV infection in recipients of blood, organs, tissues and cells and considered the benefits, practicalities, costs and disadvantages of implementing such steps. The group aimed to determine whether there are specific patient groups who should be protected from HEV infection and to determine the efficacy of different strategies in mitigating both the risk of HEV transmission, and long term liver disease. The impact of the group's recommendations for donors and recipients, and on UK blood services' operational blood supply, UK organ donation activity and the supply of tissues and cells has been considered to ensure that they are appropriate, feasible and deliverable.
The group agreed that the scope of the review would be as follows:

- Consideration of HEV screening for blood, tissue, organ and cell donors
- Consideration of potential steps to be taken to reduce the risks of transmission of HEV and its clinical sequelae
- Consideration of the cost effectiveness of any proposed risk mitigation strategies
- Review of actions undertaken internationally to mitigate risk of HEV transmission

The following were agreed as out of scope (though it was recognised that fractionated plasma products are regulated by the MHRA and therefore the group's recommendations related to blood components may be reviewed for relevance by the MHRA.)

- Risk mitigation steps for the general population
- Antenatal Screening
- HEV in fractionated plasma products
- Steps to reduce dietary risks

3. Population Epidemiology, Natural History and Pathogenicity of HEV

Hepatitis E virus (HEV) is increasingly described as having 'two faces', the outbreaks of HEV genotype 1 seen in developing countries that often results in significant morbidity and mortality and the usually asymptomatic cases of HEV genotype 3 reported in the developed world [7]. Differences in transmission routes, disease pattern and outcome are broadly dictated by viral genotype, geography and socio-economic status. These aspects are discussed in this document but with a focus on HEV genotype 3 infections that occur in the UK.

3.1. Epidemiology

HEV is hyper-endemic through much of the developing world where sanitation and food/water hygiene may be poor. Infections in the developing world are usually linked to genotype 1 (South Asia, Middle East and Africa) and genotype 2 viruses (Mexico and Africa). In these countries the virus results in sporadic cases of hepatitis but also in large water-borne outbreaks associated with faecal contamination of water. The virus remains a major public health concern in these regions with approximately 50% of acute viral hepatitis cases being due to HEV.

Cases in the developed world are mainly sporadic and linked to genotype 3 (Europe, North America and Japan) and genotype 4 viruses (South East Asia). In these regions the virus transmits via a zoonosis with animals acting as a reservoir for infection in humans. The pig remains the best studied animal and high HEV antibody prevalence rates have been described in pigs worldwide. The concept of a zoonosis is further supported by the close sequence homology observed between the HEV genotype 3 and 4 viruses found in humans and animals. There are now also good data supporting food as a vehicle for transmission with infections in industrialised countries linked to the consumption of undercooked/raw pig, deer and wild boar meat.

3.2. Course of infection and tissue distribution

The incubation period ranges from 15-60 days (average 40 days). In an acute HEV infection, peak viraemia occurs during the incubation period and early phase of disease. Viral RNA can be detected just before the onset of clinical symptoms in both blood and stool samples. HEV RNA does not persist in immunocompetent individuals becoming undetectable in blood about 3 weeks after the onset of symptoms. Some reports suggest that the virus is shed in the stool for a further 2 weeks. HEV IgM is detected during the acute phase of the illness and can persist for 4 to 5 months. HEV IgG appears shortly after IgM and levels rise rapidly.

Estimates of the duration of the IgG response and immunity to subsequent infection vary, but antibody has been detected up to 12 years after infection.

3.3. Pathogenesis, clinical features and sequelae

The majority of infections regardless of HEV genotype are asymptomatic. In symptomatic cases the disease is usually mild and symptoms may be non-specific, such as fatigue, loss of appetite, abdominal pain, fever and nausea. Symptomatic acute illness may include typical hepatitis symptoms such as jaundice, dark urine and pale stools. Differences in disease patterns and outcome have been noted in relation to HEV genotype.

Through hyper-endemic regions where genotypes 1 and 2 are found, clinical attack rates are highest amongst young adults aged between 15-35 years old. Case-fatality rates in these regions range from 0.2% to 4% but rise dramatically to between 10-25% in pregnant women, especially during the third trimester.

The demography of cases from the developed world linked to genotypes 3 and 4 infections is striking with the majority occurring in older males. Poor outcome in relation to pregnancy does not appear to be a feature of genotype 3 and 4 infections. However, the development of chronic HEV infection is increasingly recognised in immunosuppressed individuals including children [8,9,10]. All cases except one have been linked to genotype 3 infections; one recent paediatric case has been shown to be associated with a genotype 4 infection. It remains unclear whether HEV genotypes 1 and 2 are associated with chronicity.

Current knowledge suggests chronic infection in up to 60% of solid organ recipients who are HEV viraemic, but is based on small case series. Reports of chronic infection have also appeared in relation to stem cell transplant recipients, patients with other haematological disorders and HIV-infected persons. These cases are in the main asymptomatic with only mild liver enzyme derangement although the long-term prognosis for individuals with chronic hepatitis E is poor. Chronic hepatitis E infection can result in rapidly progressive liver fibrosis and cirrhosis with death due to decompensated liver disease. In addition, acute HEV infection in patients with pre-existing liver disease has been associated with a poor outcome. A 70% mortality rate linked to HEV infections has been reported in patients with underlying chronic liver disease.

3.4. Extra-hepatic manifestations

A number of extra-hepatic manifestations, clinical features other than hepatitis, linked to acute and chronic hepatitis E have been reported. These include a range of neuropathologies, thrombocytopenia, glomerulonephritis, acute pancreatitis, and acute thyroiditis. In a recent retrospective review of 106 hepatitis E cases from South West

England, eight (7.5%) presented with neurological syndromes, which included brachial neuritis, Guillain-Barré syndrome, peripheral neuropathy, neuromyopathy and vestibular neuritis. Patients with neurological syndromes were younger and had a more modest transaminitis compared to cases without neurological symptoms. Twelve patients (11.3%) presented with thrombocytopenia, fourteen (13.2%) with lymphocytosis and eight (7.5%) with a lymphopenia. Seventeen of 65 patients had a monoclonal gammopathy of uncertain significance (MGUS). Two cases developed haematological malignancies 36 and 18 months after presenting with acute HEV infection. Additional studies are required to understand the role of HEV in contributing to diseases other than hepatitis [11,12,13,14,15].

3.5. Management and treatment

In the majority of hepatitis E cases no treatment will be required as these infections will clear uneventfully. Individuals with persistent HEV infection may require intervention. Data from the transplant setting have shown that a reduction in immunosuppression levels (in particular drugs that target T cells) led to viral clearance in 30% of cases. Clearance in this setting is usually associated with sero-conversion and frequently with a transaminitis. However, reduction in immunosuppression levels needs to be balanced with the risk of graft loss in transplant patients. Antiviral treatment or changes in immunosuppression regimens should be considered for patients in whom reduction of immunosuppression has either not been possible or ineffective in achieving viral clearance.

Antiviral treatment has been used successfully to treat chronic HEV infections. Treatment regimens vary and include interferon-α and ribavirin as monotherapy or in combination. Ribavirin monotherapy is becoming the drug of choice with viral clearance usually achieved within a few weeks. However, caution is needed as interferon therapy is contraindicated in kidney transplant patients due to increased risk of acute rejection. In addition, to avoid ribavirin-induced haemolytic anaemia, the dose should be adjusted according to renal function.

3.6. Prevention

Prevention in endemic regions is best achieved by reducing faecal-oral transmission through the provision of clean drinking water and good sanitary infrastructure. In the developed world, ensuring meat products are thoroughly cooked and appropriately handled will be good measures for reducing transmission. Hepatitis E is a notifiable infection and as such all new cases should be reported to the relevant public health team for follow-up. Public Health England have published guidance for follow-up of affected individuals and wider public health actions. However, there is no data to suggest that there is frequent transmission of HEV from person-to person, where infection occurs in families it is thought that this is due to a common

food source rather than person-to-person transmission. Usually only patients with significant symptoms will be identified. In most cases the only public health action will be maintaining a heightened awareness for any associated cases particularly in those individuals who would normally be considered to spread faecal-oral infections such as young children, food handlers, those with poor personal hygiene and front-line health and social care workers.

A Hepatitis E vaccine [redacted] has been licensed for use in China in those aged between 16 and 65 years. The vaccine, also known as HEV 239, is a 26 KDa protein encoded by ORF2 of HEV genotype 1. In a recent phase 3 trial of the vaccine using a 3 dose schedule found it gave very good protection (protective efficacy rate of 100% (95% CI 72.1-100.0). Hecolin® was well tolerated with local reactions at the injection site being the main adverse event associated with its use. The data also showed that despite being based on genotype 1 virus the vaccine provided protection against genotype 4 infections. Its efficacy against genotype 3 is not known. However, there is limited or no data available on the safety and immunogenicity of the vaccine amongst children, pregnant women and in specific groups such as individuals with chronic liver disease and immunocompromised patients. The long-term efficacy of the vaccine, duration of protection and the need for a booster dose has not been determined. Currently there are no vaccines licensed for use in Europe.

3.7. HEV in the UK

Public Health England has had a programme of enhanced surveillance for hepatitis E running since 2003. The data shows that whilst cases are observed from travellers returning from HEV hyper-endemic areas, the majority of HEV cases are acquired indigenously (Figure 1). The data collected over a ten year period also shows the virus to be dynamic in our population. Travel associated cases have remained steady and are mainly associated with genotype 1 infections. In contrast, major fluctuations have been noted in indigenously acquired cases with a dramatic year on year increase in case numbers since 2010. What influences these changes is unclear but the increase in case numbers observed since 2010 suggest that the risk of acquiring HEV has changed and that we are currently in a period of heightened activity for acquiring the virus. Molecular characterisation demonstrated indigenous infections to be due to genotype 3 viruses and for these to form two distinct phylogenetic groups. Of interest, the recent rise in indigenous cases has been associated with the emergence of a novel HEV G3 phylotype not commonly circulating prior to 2010.

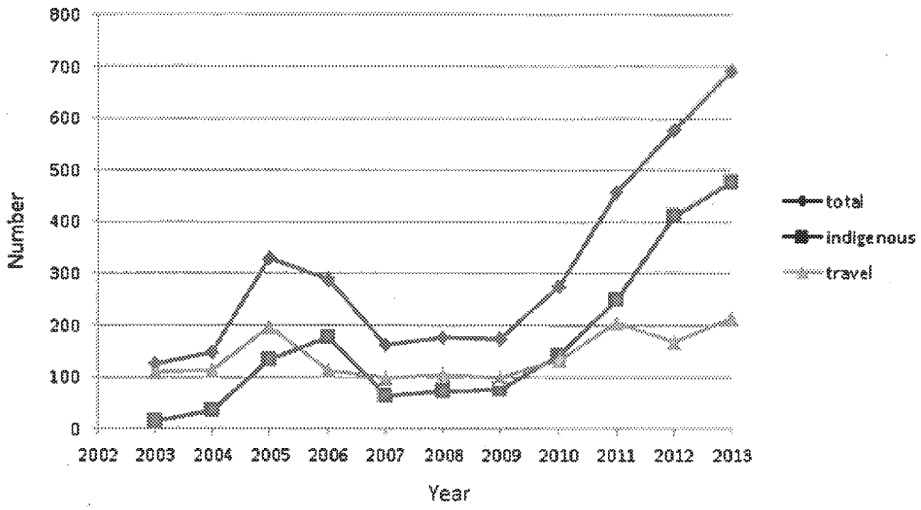

Figure 1: Total, indigenous and travel-associated cases of acute hepatitis E diagnosed between 2003–2013 in England and Wales

Sero-epidemiological studies have also been carried out in the general population in England indicating HEV seroprevalence to be high at approximately 13%. The seroprevalence rates were found to increase with age and in a cohort from 2004, it peaked at approximately 25% in those aged 50 years and over. Additional data modelling suggested that there is an annual attack rate for the indigenous virus of between 0.1-0.2% and that around 60 000 infections of HEV occur yearly in England.

The reported incidence of Hepatitis E (HEV) infection in Scotland has increased dramatically in recent years. Between 2000 and 2011, the number of laboratory confirmed cases of HEV varied from 3 to 13 per year. Since 2011 there has been a substantial increase in laboratory reports of HEV. In 2011 Health Protection Scotland received 13 reports, increasing to 78 in 2012 and 95 in 2013, and a provisional total of 160 in 2014. In 2014, 60% of HEV reports were from males, with 73% of the cases in males reported from those aged 50 years and over (personal communication Alison Smith-Palmer Health Protection Scotland).

Hepatitis E is not a notifiable infection in Northern Ireland. Currently local laboratories test on request but there is no standardised policy, and this may lead to an underestimation of the number of infections. Between 2000 and 2013 3 cases were reported to the NI Public Health Agency whereas in 2014 9 cases were reported (personal communication Philip Veal, Public Health Agency, Northern Ireland).

As a result of increased awareness and testing, persistent HEV infections are being increasingly recognised in the immunosuppressed population in the UK. These have been described in HIV-infected patients but primarily in haemato-oncology patients and solid organ transplant recipients. The overall UK seroprevalence and complication rate in this cohort has not been clearly established.

3.8. HEV epizoology in England and Wales

A case controlled, questionnaire based study undertaken by PHE in 2012 indicated the consumption of pork based products to be associated with cases of hepatitis E from England and Wales. In order to better understand HEV infections in the UK pig population, a joint PHE-DeFRA-FSA investigation was undertaken studying pigs at time of slaughter. The study reported a high HEV seroprevalence rate of 93% with the detection of IgM antibody in half of the seropositive animals indicating many pigs to have had recent infection and some 6% to be undergoing a current infection and to be viraemic (HEV RNA in their plasma) at the time of slaughter. Molecular characterisation of identified viruses showed that the UK pig could be the likely source of a small proportion of the infections currently being diagnosed in the England but that two thirds of the viruses that infect patients are not found in the UK pig. The precise source of these infections remains to be confirmed.

3.9. Epidemiology in UK blood donors

In recent years the blood and transplant community have become more aware of the potential impact of transmission of hepatitis E to immunosuppressed individuals through blood transfusion and organ transplant. Hepatitis E is not routinely included in the screening of blood donations although in the last year there has been much debate as to whether this should be implemented in Europe.

A study published by Beale in 2011 [16] looked at the rate of HEV RNA and seroprevalence in unselected UK blood donors (n=262) and with a history of jaundice unrelated to hepatitis B (n=333). Seroprevalence for anti-HEV IgG was 12% in unselected donors and 8% in those with a history of jaundice. Two samples in each group were IgM positive but HEV RNA negative. These results suggested that there was likely HEV infection turning over in the blood donor panel which warranted further investigation. A more recent study [17] looked at a greater number of donations and the presence of active infection. Donations from blood donors from England living in the south of the country were screened between October 2012 and 2013 for the presence of HEV RNA. Of 225,000 donations tested 79 were found to be positive for HEV RNA, genotype 3, a rate of 1/2848 or 0.04%. A recent study carried out in the Scottish National Blood Transfusion Service looked for HEV seroprevalence in a collection of 1559 anonymised samples collected between 2004 and 2008 and an additional

528 samples collected in 2012. The samples from 2012 were included for comparison to ensure that long-term storage did not affect on the test results. In addition 43,560 anonymised samples in minipools were tested for the presence of HEV RNA. HEV RNA was detected in 1/14,520 or 0.007% of donations, with anti-HEV IgG seroprevalence of 4.7%, lower rates than detected in England. As this was retrospective testing there was no follow-up of those samples found to be HEV RNA positive.

3.10. Population and blood donor studies in other countries

Data from the Netherlands [18] reported a viraemia rate of 1:1761 (0.06%) in 35,220 plasma donations tested (Table 1). It has also been shown that anti-HEV IgG sero-prevalence has declined over time in donors aged 18-64 from 46.6% in 1988 to 20.9% in 2011 in The Netherlands. However, an increase in seroprevalence has been observed in younger donors aged 18-21 from 4% in 2000 to 13% in 2011, suggesting that prevalence and acquisition of HEV has changed over time. The circulating virus is a genotype 3 virus and, as in the UK, is thought to be associated with consumption of contaminated meat products.

Country	Time period	Number screened	HEV RNA positive	Method	Rate	Ref
France	Nov. 2012-Dec. 2013	53,234	24	Pool	1/2218	Gallian et al., 2014 [19]
The Netherlands	2013	35,220	20	Pool	1/1761	Hogema et al., 2014 [20]
The Netherlands	2011-2012	45,415	17	Pool	1/2671	Slot et al., 2013 [21]
Germany	July-Sept 2011	16,125	13	Individual	1/1240	Vollmer et al., 2014 [22]
Scotland	2004-2008	43,560	3	Pool	1/14,520	Cleland et al., 2013 [23]
England	2012-2013	225,000	79	Pool	1/2848	Hewitt et al., 2014 [17]

Table 1 Hepatitis E rates in blood donors

A recent study in Germany [24] found relatively low rates of past infection in donors; anti-HEV IgG of 6.8% in 1019 donors tested. Seroconversion was observed in 7/69 donors within a 2 year period, an incidence of 0.35% per year. HEV RNA was detected in 0.08% of donations (1 in 1,250) with a report of transfusion transmitted infection (TTI) from two donations from a single donor via apheresis platelets. One patient was immunosuppressed and developed chronic infection. Data from South West France have shown over half of all blood donors (52.5%) have been shown to be HEV IgG positive, probably reflecting the local diet.

Although the level of HEV infection and seroconversion is high in blood donors a large proportion of the general population and blood recipients will also have immunity [25].

A nationwide survey of 12,600 blood donors in Japan found 3.4% to be HEV IgG positive with prevalence varying by geography. There was a relationship between elevated alanine aminotransferase and positive anti-HEV IgG. The predominant genotypes were both 3 and 4 with 4.1 % of samples were HEV RNA positive [26].

Work has been carried out in the USA to investigate the anti-HEV IgG seroprevalence. Of 916 donations investigated in 2006, prevalence was 21.8% compared with 1023 donations in 2012 when seroprevalence was 16%. None of the donations were positive for HEV RNA although 0.4% were IgM positive. In addition 362 recipients were followed up by testing pre and post-donation samples but no TTIs were observed. The authors note that no TTIs were observed despite the relatively high seroprevalence rate [28].

3.11. Conclusion

HEV is a zoonotic infection in the UK, most likely acquired through eating infected pork products. The UK prevalence of clinical infection is increasing, with 500 indigenous cases reported in 2013, and high rates also reported in Netherlands and parts of France. However, this figures underestimates the true prevalence, as infection is usually asymptomatic or mild. A UK study of blood donors revealed a viraemia rate of ~1 in 2850, equivalent to 2 donors/day. Short-lived viraemia and complete viral clearance is the norm in immune competent individuals, in whom severe acute hepatitis and chronic liver disease are rare. However, long-term viral carriage may occur in immunosuppressed individuals, with progression to chronic liver diease; the frequency of this remains uncertain. Worsening of chronic liver disease may also occur. Extra-hepatic manifestations have also been described.

4. Transmission Through Blood, Tissues and Organs, and Possible Mitigation Steps

4.1. Transmission through blood components

The first case of confirmed transfusion-transmitted hepatitis E infection was reported by [27] in Japan. The recipient had received 23 components following heart surgery in 2002 and was readmitted 46 days post transfusion with hepatitis. The donor was asymptomatic at the time of donation and donated Fresh Frozen Plasma (FFP) which was found to be the source of infection, with molecular typing showing identical genotype 4 virus in donor and recipient.

Subsequently the first British TTI was reported [28] following a transfusion in 2004. The donor was asymptomatic but reported flu-like symptoms 14 days post-donation and jaundice a further 10 days later; there was no history of travel. The donation had been made into red cells and a platelet pool. The patient who received the red cells had B-cell lymphoma and developed HEV which persisted for several weeks; the same virus (genotype 3) was identified in the donor and recipient.

Further transmissions in the UK occurred in 2011, 2012 and a single case in 2014; no transmissions were reported in 2013. All 3 transmissions were from FFP. The transmission in 2011 was identified in an adult stem cell recipient who had received blood components from 34 donors, two of whom were found to be viraemic at the time of donation following analysis of archive samples. One of these donors had donated FFP; donor and recipient were found to have the same genotype 3 virus. Unfortunately the recipient died from other causes. The 2012 recipient was receiving immunosuppressive therapies and had experienced 129 donor exposures. One donor had evidence of HEV RNA at the time of donation, the infection cleared and the donor seroconverted 5 months later. This donor was identified as the source of infection having donated FFP to the recipient. The 2014 patient had underlying chronic liver disease and was treated with FFP (details will appear in the 2014 SHOT report due to be published in July 2015). Two other cases are under investigation where donors reported post-donation illness and were investigated by their GPs. Both were found to be HEV RNA positive and lookback investigations on the recipients are ongoing. Now that investigations for possible hepatitis include HEV testing, more such reports are likely.

There have also been case reports of TTIs in Germany and indirect evidence of transmission from solvent-detergent treated fresh frozen plasma plasma (SDFPP) in Canada [29,30]. In the Canadian study, patients with Thrombotic Thrombocytopaenic Purpura were receiving up to

40L of plasma as either SDFFP or cryosupernatant plasma. Patients were followed up at baseline, 1 and 6 months and tests for anti-HEV antibodies and where seroconversion had been observed HEV RNA. Seroconversion was observed at one month in 2/17 patients treated with SDFFP.

The recent Hewitt study [17] actively followed up recipients of blood donations given in the South East of England. Of 60 patients receiving blood components 17 were unable to be followed up for various reasons. In the 43 recipients with outcomes 18 had evidence of a HEV TTI. Six recipients had serological evidence of infection and 12 had proven viraemia on at least one point following transfusion. TTIs were more often associated with higher viral load in the donor and absence of antibody. In the recipients, 4/16 receiving red cells developed infection, 4/10 receiving pooled platelets, 5/14 receiving apheresis platelets and both pooled granulocytes (n=1) and FFP (n=2) resulted in a TTI. Although numbers are small it does appear that those components containing large volumes of plasma from an infected donor may be expected to transmit HEV RNA. Analysis of outcome in the infected recipients indicated that level of immunosuppression was linked to the duration of infection and median weeks to seroconversion (Table 2). Prolonged viraemia with delayed development of the antibody response was observed in those recipients with moderate and severe immunosuppression levels. Intervention either through the reduction of immunosuppression, or through antiviral treatment led to viral clearance in three recipients.

Based on this study, it would be expected that there would be 400-500 transfusion transmissions annually in UK, yet only occasional cases are reported. This illustrates the asymptomatic or mild nature of HEV infection in most recipients, even those ill enough to require transfusion.

Inferred immun-suppression	Number of recipients	Median weeks*			Proportion (%) who developed		
		to RNA detection	to seroconvert	duration infection	anti-HEV	clearance	clinical hepatitis
None or mild	8	5	7	10	8 (100%)	8 (100%)	1 (12.5%)
Moderate	6	8	11	18	5/6 (83%)**	3/4 (75%)**	0
Severe	4	9	37.5	30	2/3 (66%)**	2/3 (66%)**	0

Table 2: Outcome in 18 recipients infected by transfusion of a blood component from a viraemic donor, ranked by immunosuppression.

4.2. International situation

No country has yet introduced routine blood donor screening. The Netherlands has been the lead country in terms of epidemiological studies, with 3.5% of donors sero-positive and 1 in 3000 RNA positive (Hans Zaaijer, presentation to HEV group). The Dutch authorities considered the issue of blood donor screening, but concluded that in view of the high population prevalence, the added risk from transfusion did not warrant screening.

France has introduced NAT for donors contributing to pools of SDFFP. Universal or selective screening of blood donors is under consideration. Other countries in Europe plus Canada are carrying out epidemiological studies, but have not taken any decision regarding blood donor screening. USA and Australia are maintaining a watching brief.

4.3. Transmission through stem cells, tissues and organs

One case has been ascribed to a liver transplant from an HEV positive donor [31]. There have been no other cases ascribed to stem cell, tissue or organ transplants themselves, although a stem cell donor was reported to have been undergoing acute HEV infection at the time of transplant [32].

4.4. Donor selection and post-donation information

Donor selection criteria are fundamental to blood and tissue donation processes and support decision-making in organ donation. Information is collected about the donor's general health, recent illness and any risk behaviours that may put them at increased risk of infectious diseases. The majority of the blood donor selection criteria also apply to both live and deceased tissue donors; however, donor selection for stem cell and organ donation tends to be on a case by case basis and certainly for organ donation there are very few conditions which would result in a donor not being accepted. Every time an individual attends to donate blood they are given information about blood donation and then asked to complete and sign a donor health check form (DHC) which asks a number of questions about general health, sexual behaviour, travel and any known exposure to infectious diseases. The DHC is used in conjunction with the donor selection guidelines to ensure both the safety of the donor, and of the blood supply.

It is known that the majority of people with HEV may not be aware of their illness at the time of donation. In a recent English study it was observed that approximately a third of donors experienced some illness which was probably related to their infection [17]. Some donors may have relatively minor symptoms which they did not disclose - it is known that donors do not always fully disclose illness, particularly if they think it is not relevant.

It would be difficult to introduce any donor selection criteria for a food-mediated infection; currently there are no deferral criteria in the UK that are related to diet. It seems unlikely that there are any donor selection criteria that would mitigate against HEV genotype 3. There have been no reported cases of HEV in vegetarians, but the population rate of self-declared vegetarians (7%) is too low to use this characteristic to identify a low-risk donor population for high risk recipients. Some donors may be more at risk of acquiring hepatitis E due to the nature of their work i.e. working in animal husbandry or welfare, but rates of viraemia or sero-positivity are not known in the populations.

Other genotypes of hepatitis E infection such as those viruses found in Africa and SE Asia are mitigated against by other travel deferrals. Donors are asked about any travel overseas either since the last donation or for new donors in the last 12 months [33]. Most of those countries with endemic HEV are in areas where donors would already be deferred for 6 months due to the malarial risk deferral. It may be possible to target particular donor groups to identify those donors with possibly protective HEV IgG antibodies i.e. known that older males are more likely to have evidence of HEV IgG. HEV has been described in men who have sex with men (MSM) [34,35] the risk from such individuals as blood donors is already covered by the 12 month deferral period.

Post-donation information

There are specific questions on the DHC which ask all donors about illness in the last 2 weeks, known contact with an infectious individual in the last 4 weeks and any history of jaundice. However, these questions depend on the donor having symptoms. It is possible that viraemic donors will develop symptoms some days after donation and therefore it is important that all donors are aware, and reminded, that they should report any illness (other than a cold) that develops within two weeks of donation. Hepatitis E symptoms may develop more than two weeks post donation so the group considered extension of the period of post-donation reporting. However, any extension of the reporting time for post-donation infections would be likely to result in a large increase in donor reports but unlikely to yield many hepatitis E infections.

Recommendation: the period following donation for which donors should report symptoms need not be extended beyond the current 2 weeks.

4.5. Donation screening

Assays available

There are two primary screening targets for HEV, HEV nucleic acid (RNA) and IgG antibody to HEV (anti-HEV). HEV RNA is the first target to appear, followed some time later by the

appearance of anti-HEV. However in the context of donation safety, screening for the presence of HEV RNA is required to identify potentially infectious donations. The presence of antibody provides evidence of infection including acute, resolving or resolved, but on its own antibody screening cannot identify all viraemic, and thus potentially infectious, donations. In practice most antibody positive donors will not be viraemic, they will have undergone infection in the past and very few will be undergoing an acute infection and be viraemic. To maximise product safety donations must be screened for HEV RNA if there is a desire to remove viraemic components from the inventory.

Currently there are only two main global suppliers of CE marked molecular donation screening assays, [redacted] (Appendix 1), and both of these suppliers have a suitable HEV RNA assay on their automated platform. Assay evaluation and validation would need to be performed prior to any implementation. [redacted]

[redacted]

[redacted]

Individual donations or pooled screening

Currently there are no specific published data on the minimum infectious dose that would be transmitted by transfusion, the only data available are from the inoculation of Macaques, demonstrating clinical disease, viraemia and seroconversion in those inoculated with higher doses (10^4-10^6 infectious doses), with just viraemia and seroconversion in those with lower doses. However, although based upon small numbers, one of the outcomes of the NHSBT study was to identify the lowest level of viraemia associated with transmission, 409 IU/ml, in

a dose of apheresis platelets. Although only a single datum, this figure does provide the only estimate currently available upon which further calculations can be made.

Options for HEV RNA screening include both individual donation (ID) and pooled, with a range of possible pool sizes. Although ID screening is the most sensitive approach, pooled screening has operational and financial advantages when screening large numbers of donations and has been successfully used by a number of countries for routine molecular screening of donations for HBV, HCV and HIV for some years, and in a range of pool sizes. Pooled screening is current practice for the molecular screening of blood donations within the UK Transfusion Services

There are a number of factors that need to be considered when deciding whether any HEV RNA screening should be ID or pooled: the range of viral loads expected in viraemic donors; the sensitivity of the assays available; the lowest level of viraemia that would be expected to transmit; the quantity of virus likely to be present in the products provided to recipients and the susceptibility of recipients to infection. Whilst all of these factors have a degree of interaction in determining whether any virus present in a donation would be detected, and if not detected whether it would transmit, the prime consideration is whether the assay is sensitive enough to support pooled screening given the range of viral loads expected in HEV viraemic donors. Looking at the NHSBT study outcomes, the only currently published study assessing HEV viraemia in screening pools, viral loads in pick-ups ranged from 50 - 2.37 $x10^6$ IU/ml, using an in-house HEV RNA assay (PHE) with a 60% detection limit of 22 IU/ml. If screening pools of 24 donations, individual donations with HEV RNA levels less than 528 IU/ml would theoretically not be detectable. However, although the median viral load in the viraemic donors was determined to be 3900 IU/ml, viral loads as low as 50 IU/ml were identified. The lowest level associated with transmission in the NHSBT/PHE study was 409 IU/ml in a seronegative donor. This was in a dose of apheresis platelets transfused into an immunocompetent individual.

Further analysis of the recipient outcomes from the NHSBT/PHE study have determined that the minimum infectious dose in a blood product that could result in transmission is estimated to be $2x10^4$ IU. Different products have different residual plasma volumes and consequently contain different overall quantities of virus. Taking $2x10^4$ IU as the minimum total viral input in each product type that would lead to HEV infection in the recipient, the minimum viral load in any donation that would be expected to lead to HEV infection in the recipient, based upon final product type, can then be estimated (Table 3). However it must be stressed that these figures are based on very limited data and therefore need to be judged with caution. In

addition the effect of any other products given at the same time needs to be considered, although currently any such effect can only be surmised.

		Platelets				
	Red cells	**Apheresis**	**Pooled[1]**	**PAS/ plasma (30-40% plasma)**	**FFP (not MB treated)**	**Cryo[2] (pool)**
Residual plasma volume in product[3] (ml)	7.6 - 17.4ml, depending on pack type	180	290	105	275	43 (172/215)
Viral load in donor plasma required to result in infection (IU/ml)	1.15 – 2.63 x10^4	111	69	190	73	465 (116/93)

Table 3 Estimation of infectivity per product type based on residual plasma volume

[1] Pools of 4 but the majority of the plasma from 1 donor
[2] Pooled figures in brackets; pools of 4 for untreated and 5 for MB treated plasma
[3] Mean actual values for NHSBT products obtained from routine Quality Monitoring data

Pooled screening may therefore be considered a viable option if the expected viraemia is high enough to still be detectable when diluted in a pool. Table 4 presents the manufacturer's claimed sensitivity for their respective assays, with the impact of different pool sizes included. On the basis of these data together with data from the NHSBT/PHE study, both assays could be considered to be suitable for use up to and including a pool size of 16 donations.

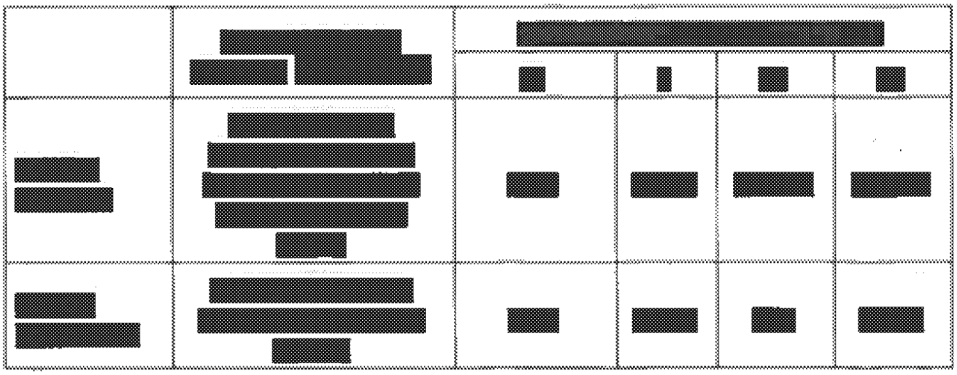

Table 4 Estimated assay sensitivity for different pool sizes

[1] using the WHO International Std for HEV

Confirmation

It is best practice to independently confirm screening reactivity. Whether screening in pools or ID, all screen reactives should be confirmed by a sensitive and independent HEV RNA assay, the viral load determined and genotype ascribed. Reactive pools would automatically be resolved to the individual donation by the screening laboratory. The individual donations would then be referred to the appropriate reference laboratory for confirmation using an alternative, validated, quantitative HEV RNA assay. Additionally, the serological status of all HEV RNA positive donors would be determined to build up a body of data on the biology of HEV infection in UK donors. Sequencing would also be required. Full molecular and serological follow-up of all confirmed HEV RNA positive donors would be required to enable re-instatement to the donor panel once viraemia has cleared.

4.6. Donor management and deferral

Currently any donor reporting a known history of HEV is deferred for 12 months from the time of recovery [36]. If HEV screening were introduced in the UK blood services then arrangements would need to be in place for the management of donors including post-donation information and discussion. During the recent study carried out between NHSBT and PHE, all donors who tested positive were sent a letter and an information leaflet about hepatitis E, the donors had already received information about the additional screening test at the donation session. All positive donors were advised that the infection was notifiable and hence the local public health team would be informed. Donors were asked to ring the clinical team to discuss their results. During this post-test discussion the nature of the infection was explained, the donor was asked about any travel or unusual foods and any symptoms. The majority of donors were asymptomatic but were warned that symptoms may develop. Consent was sought to send a letter to the donor's GP for information. A similar process would need to be put in place if HEV screening became part of mandatory testing.

Currently a 12 month deferral is in place from the time of recovery for those individuals with a confirmed hepatitis E infection. However, if screening were in place it may be possible to reduce this deferral by several months. Donors would be retested before being returned to the panel to ensure that HEV RNA had cleared and seroconversion had taken place with the presence of IgG. It is expected that the number of donors with an acute HEV infection would be considerably greater than those whose serology is positive for hepatitis B/C, HIV and HTLV in an average year.

Lookback investigations

When new viral infections are detected in regular donors it is good practice to review the previous donation for presence of virus which may have been missed due to a window-

period infection. Usually archives are retrieved and retested using individual nucleic acid testing. If a previous donation is found to be positive then a lookback investigation on previous recipients will be carried out. In brief the fate of all components manufactured from the previous donation will be identified and where units have been transfused the consultant haematologist will be contacted, informed of the issue and asked for help in identifying the recipient. The recipient will then be followed up by either the hospital consultant, their GP or in some cases by the blood services. The recipient will be advised of the situation, and where appropriate testing will be offered.

Until more is known about the natural history of HEV it is likely that a previous donation within the last 4 months will require follow-up but this would normally be decided on a case-by case basis.

4.7. Residual risk if screening were introduced

Estimation of residual risk for HEV can be performed, but with some degree of uncertainty. The key data needed for residual risk estimation for any transmissible infectious agent are incidence and window period. Published HEV incidence data are limited to the study of German blood donors [37], which determined a seroprevalence of 6.8% and annual incidence of 0.35%.

The NHSBT/PHE study identified 79 HEV viraemic individuals. As HEV, in such healthy individuals, is an acute infection it could be argued that this figure can be used to generate an incidence figure. However a percentage of these also had concomitant antibody detected, a further small number also had evidence of HEV infection in the stored archive sample from their previous donation. Removal of these donors to leave just those with only HEV RNA present in the pick-up donation provides a figure, 56 donations (donors), from which a tentative annual incidence, based upon the testing of 225,000 donations could be determined.

For the purposes of estimating HEV residual risk for this assessment the following values were used:

1. the length of the HEV window period is uncertain, but HEV RNA would be expected to be seen within 2-4 weeks of exposure; an infectious window period being from 1-2 weeks
2. the annual incidence of HEV in blood donors, based upon the total number of RNA positive donors identified, with or without serological evidence of infection was determined to be 0.035%.

3. the annual incidence of HEV in blood donors, based upon the number of RNA positive donors identified who have no serological evidence of infection and whose archive sample also had no serological or molecular evidence of infection, was determined to be 0.024%.

The residual risk estimation, the annual risk of failing to detect HEV viraemic donations across all of the UK Blood Services, was performed by the NHSBT/PHE Epidemiology Unit using the above figures . These figures were generated from the outcomes of screening in pools of 24 donations, although the maximum pool sizes validated for the CE marked commercial assays are 6 and 16 and can therefore be considered to be 'worst case'

- Using an annual incidence of 0.035% the number of donations that may not be detected in one year is estimated to range from 22 – 44
- Using an annual incidence of 0.024% the number of donations that may not be detected in one year is estimated to range from 16 - 31,

The full figures are presented in Appendix 2.

Existing screened and banked donations

In the past, the implementation of an additional screening test to the UK blood services has not required the retrospective screening of all existing banked products. If screening were to be implemented there would therefore be a period when untested products would be in inventory until they could be replaced. However unless universal screening is recommended the HEV RNA status of screened and banked products is unlikely to be an issue.

4.8. Creation of a panel of immune donors

Background

The use of bespoke panels to reduce the risk of pathogen transmission to vulnerable recipients is well established. With hepatitis E virus infections in immunosuppressed patients linked to the development of chronic hepatitis and a poor hepatic outcome, the need to define strategies to reduce the risk of transmitting HEV through blood/blood components to at risk recipients is clear. With a view to explore the role of a bespoke panel of known HEV immune donors in addressing this concern, we examine what is known about HEV seroepidemiology in blood donors and discuss what is understood about HEV antibody dynamics.

Defining HEV seroepidemiology in blood donors

A study on 3000 donors from England, sampled in 2010, demonstrated an overall anti-HEV prevalence rate of 7.5% (personal communication, NTMRL, NHSBT unpublished data). Additional breakdown of the data indicated a cohort effect with antibody prevalence rates increasing with age peaking at approximately 35% in those aged 60 years and over (fig 2). A slight predominance of males was also observed. Studies undertaken in the general population have also shown that the risk of acquiring HEV has fluctuated over time [38,39]. What causes these changes in prevalence is unclear but it is likely that the seroepidemiological profiles identified in donors will be fluid and continue to change.

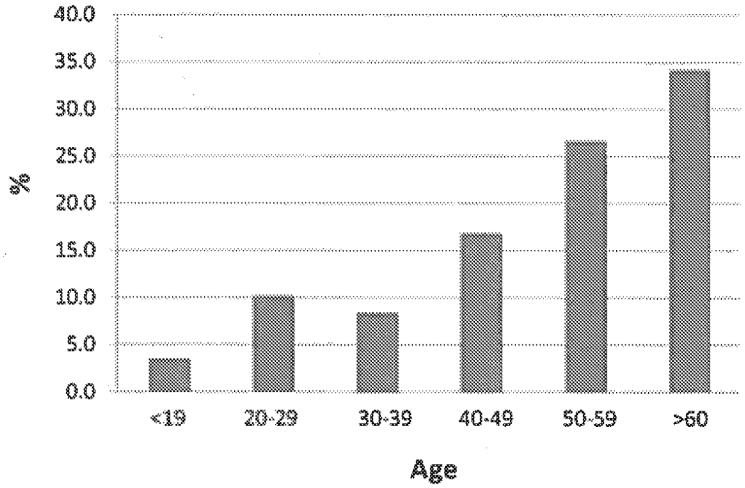

Figure 2: HEV IgG prevalence in blood donors shown by age

Understanding the dynamics of HEV antibody

Analysis of HEV IgG antibody levels in the 3000 donors from England showed 60% to have IgG S/CO levels <10 (figure 3). Additional break down of the data did not show a relationship between IgG antibody levels and age.

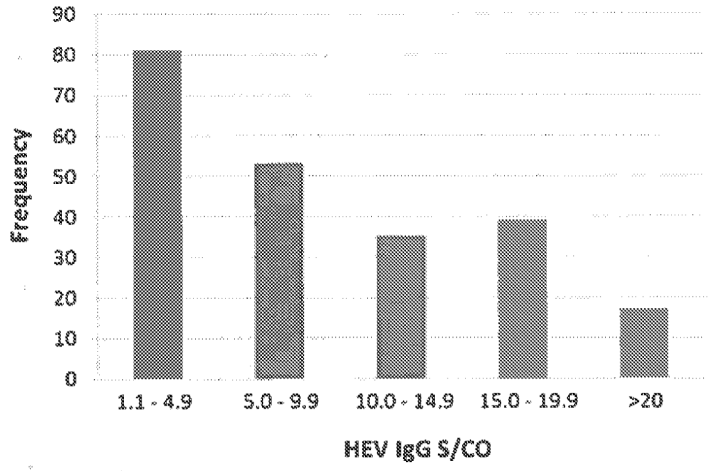

Figure 3: Breakdown of antibody levels in 3000 blood donors

There remains much debate on the persistence of HEV antibody following recovery. Understanding antibody dynamics has been hampered by the unreliability and lack of sensitivity of commercially available HEV IgG detection assays, further exemplified by the poor concordance between many of these tests. Whilst improved assays are now increasingly available, data on the long term persistence of HEV antibody over time is lacking.

Longitudinal follow up of acutely-infected blood donors identified through a recent study has started and antibody levels on sequential samples taken between 6 to 18 months after infection are currently available on 58 donors. In the majority of donors, HEV antibody levels have remained steady and high. However, a drop in antibody levels was noted in 12 (21%) donors, with the majority (58%) showing reductions to S/CO <10. We accept that the numbers involved are small and that the period of follow up is short. Clearly more long term data is needed to define the decay of HEV antibody in this group. Nonetheless, the observation of rapid antibody decay even in the minority of donors does raise interesting questions. What level of HEV antibody is protective? Is there a gradient in antibody titre, with ensuing complete and partial protection? Would the eventual decline in antibody levels mean that donors could become susceptible to re-infection? If so, what would be the risk from a donor undergoing re-infection?

The recent NHSBT/PHE study on HEV and blood safety involving some 225,000 samples identified 79 viraemic blood donors [17]. Retrospective testing of archival samples available prior to the donor being identified as being viraemic, showed all samples to be completely

unreactive for anti-HEV. These data confirm that all 79 donors found to be viraemic were likely the result of primary infections. These occurred in an estimated 190,000 sero negative donors. Given that the modal age for repeat donors lies between 40 and 45 and the seroprevalence at this age is around 16%, we could have anticipated pro rata around 15 viraemic donors in the estimated 34,000 previously infected and now seropositive donor but in fact found none. This is a highly significant difference indicating that seropositivity protects against viraemia but does not exclude the occurrence of reinfections in those whose antibody titres has waned or declined; such infections in the UK would seem to be rare. However data from a group of closely monitored individuals in China report re-infections in 17% of diagnosed cases [40]. These cases were associated with a less severe hepatitis with a boost in IgG levels. Whether the re-infections were due to viruses of the same or different genotype to the original infection remains unknown. Re-infections in the transplant setting linked to lower HEV antibody levels have also been reported [41]. HEV RNA has been detected in solid organ recipients who had demonstrable HEV IgG prior to transplant; numbers involved were very low, and titres <7 WHO units/ml did not seem to be sufficient to prevent or control viraemia, with one patient out of 3 progressing to chronicity. It is worth noting that descriptions of HEV re-infections remain rare, but there have not been large systematic studies looking at this.

Considerations for creating a bespoke panel of HEV immune donors

Head to head comparisons with the introduction of NAT screening shows that the creation of a bespoke panel of immune donors does have many advantages, in particular with respect to donor selection, donor deferral and donor follow up (Table 5). However, there remain many issues that warrant further discussion and understanding.

Information on the persistence of HEV antibody and protective levels are lacking. Modelling of HEV antibody data from population based surveys indicates that a S/CO ratio of ≥5.0 is likely to be related to recovery antibody [33]. Used as a proposed cut off, only 144 (4.8%) of the analysed 3000 donors from the 2010 study would be eligible for inclusion in the panel. These figures may suggest that assembling a panel of immune donors to meet demand, in particular when considering the use of platelets, may not be possible. This could be further complicated as considerations for ABO matching will mean that the panel will need to be representative. An understanding of the predicted haematological support that will be needed for identified vulnerable populations would be an essential starting point. This will determine how many donors will be required and the level of antibody screening that will be needed in order to assemble the panel.

There are currently no platforms available that support HEV antibody testing. Logistics as well as costs for the implementation of liquid handlers, consumables and staff would need to be investigated. Protocols for maintaining and monitoring the panel will need to be established. One possible suggestion is that the panel is maintained with antibody testing at a year to ensure that levels remain at a S/CO ratio of ≥5.0. A second possibility is that a new panel is constructed very year. Perhaps most critical are considerations needed for the initial assembly of the panel. Whilst screening for HEV IgG will identify those donors with antibody, additional HEV RNA testing will have to be undertaken in this group in the first instance. During an acute infection HEV IgG will be detectable during the viraemic phase. The levels of IgG will rise as HEV RNA clears. However to screen and therefore base inclusion criteria solely on the presence of HEV IgG will not be acceptable.

	NAT screening	**Panel of HEV immune donors**
Donor Selection	-----	-+
Donor Follow Up	++++	----
Donor Deferral (Could be decreased from 12/12)	++++	----
Ease of adding to current tests	Requires new UK wide tender process	Would require new microplate and implementation of liquid handlers
No. required to meet demand	+ 2999 of 3000 are negative Potential for selected screening to supply all platelets, RBC's and FFP to high risk recipients	+++ IgM Neg, IgG ≥5: 144 out of 3000 donors will fit this criteria
Estimated residual risk	16-44/year	

Table 5: Head to head comparison of the introduction of NAT screening vs the creation of a bespoke panel of immune donors

4.9. Pathogen inactivation (PI)

Red cells: There are no licenced methods for PI of red cells.

FFP: There are 3 licenced methods for PI of FFP.

4.9.1 Methylene blue

This is a phenothiazine-based photosensitizer process with affinity to guanosine-cytosine pairs. It is said to inactivate all enveloped viruses and some non-enveloped like Parvovirus B14 for Plasma. [redacted]

[redacted]

4.9.2 Solvent detergent

This is medicinal product licensed by the MHRA. This requires pooling of 2000-3000 donations and is available commercially as Octaplas[redacted]

[redacted]

4.9.3 Amotosalen/UV treated

The Cerus Intercept System uses amotosalen HCl (a photoactive compound) for both platelets & plasma and long-wavelength ultraviolet (UVA) illumination to photochemically treat components.

The Intercept Platelet and Plasma systems use amotosalen (also known as S59). Whereas the Intercept Red Cells system uses S303 (still in trials, not licensed).

[redacted]

4.9.4 Platelets

There are 2 licensed methods, although routine use is limited.

1. Amotosalen/UV - the same method as for FFP.
2. Riboflavin activated with light.

The Mirasol Pathogen Reduction Technology (PRT) System renders a broad range of disease-causing viruses, bacteria and parasites less pathogenic, and inactivates residual white blood cells found in blood components. It uses a combination of riboflavin (vitamin B2), a non-toxic, naturally occurring compound, and a specific spectrum of ultraviolet (UV) light to inactivate viruses, bacteria, parasites and white blood cells that may be present in collected blood products.

[redacted]

4.10. Conclusions

There is no evidence which can be used to develop questions which could be added to the donor health check questionnaire to identify donors at particularly high (or low) risk of HEV carriage for selective testing.

HEV NAT testing, which detects viraemia and hence potentially infectious donors, is the testing strategy of choice. There are CE marked assays available suitable for HEV NAT testing of blood donors. Serology detects recent and past infections, and thus would exclude many safe donors; there is no added value in carrying out serology testing in addition to NAT. Blood donor screening by NAT could safely be performed in pools of 16-24, which would be determined as part of validation. Reactive samples would be confirmed by a different HEV RNA assay, along with assessment of viral load and genotype. Universal donation screening would yield over 500 donors/year for deferral and retest before returning to donation. Lookback of previous recipients would also be required, with an indicative lookback period of 4 months. Routine screening would potentially miss 16-44 donations/year, a figure which will vary with the population incidence.

There is insufficient information to recommend creation of a panel of immune donors for transfusion to high risk recipients. There is no definition on IgG or IgM levels to define immunity, nor adequate information on antibody decay.

Transfusion transmission of HEV is well described in the literature but clinical disease is uncommon, with the first reported UK case in 2006 from red cells, and 3 cases in the UK from 2011-14, all from FFP; no deaths were attributed to HEV. Most UK and literature cases

have been in organ or stem cell transplant recipients. Acute severe hepatitis is rare and death very rare.

HEV is transmitted by red cells, platelets and FFP, with a transmission rate of ~40% in the only UK prospective study and transmission more likely at higher viral loads and with exposure to larger volumes of plasma (25% transmission rate from red cells, 50% from FFP/platelets). In this study, most recipient infections were asymptomatic or mild, with 1 case of clinical hepatitis in an immunocompetent recipient. Viral clearance with sero-conversion was the commonest outcome, but a small number of immunosuppressed individuals required anti-viral therapy and/or reduction of immunosuppression.

On this albeit limited evidence, there is no pressing case for routine HEV screening or other steps affecting the entire blood supply at this time. No country has yet adopted universal blood donor screening, nor a selective screening strategy based on patient risk. Such options are under consideration only in France. However, the incidence of HEV should be monitored and this recommendation reviewed in the light of new evidence.

Special consideration is needed regarding risk mitigation in recipients of solid organ and stem cell transplants in view of the increased risk of chronicity. This is explored further in Section 5. HEV is reported in such patients from either diet or transfusion, with one case ascribed to a liver transplant from a HEV positive donor. There have been no other cases ascribed to stem cell, tissue or organ transplants, although a stem cell donor was reported to have been undergoing acute HEV infection at the time of transplant.

It is uncertain at present what degree of protection would be provided by PI of platelets or single unit FFP, as not all available methods have been tested by HEV, and breakthrough transmissions have been seen with the Intercept method. The solvent detergent method of inactivation for FFP is not in itself effective against HEV. Therefore specifications are being set for plasma pools for manufacture. If these are met, SDFFP is likely to be HEV safe. There are no PI methods available for red cells.

5. Consideration of Risks in Specific Groups of Transfusion Recipients

5.1. Pregnant women

In relation to pregnancy, it is necessary to consider the HEV genotypes differently. It is recognised that there are geographical differences in virus genotypes. The most common infection in the UK, annually running at somewhere between 100,000 and 150,000 infections in England and Wales is a zoonosis of genotype three (G3) acquired through the dietary consumption of contaminated porcine flesh. These infections are not known to be associated with severe disease in pregnancy.

During epidemics of G1, there is a significant risk of mortality from HEV infection in the third trimester of pregnancy. This has been seen in ongoing outbreaks in Africa and during epidemics in India where the mortality rate from liver failure is 15-20%. The neonatal outcome is also compromised [45]. The additional risk factors are low socioeconomic status, co-infection, poor sanitation and nutritional status [46]. There is also evidence that there may be an underlying genetic susceptibility. It is suggested that the adverse outcome in HEV infected pregnant women might be due to the presence of certain cytokines gene polymorphism [47]. The report of a case of HEV related liver failure in a Pakistani woman who had been resident in Portugal who probably acquired the infection during a visit to Pakistan, supports the suggestion of a genetic risk [48]. The underlying mechanism of liver failure in pregnancy is unknown [49].

It is known that the main pregnancy risk relates to the third trimester only although a potential risk to the foetus if there is an infection earlier in the pregnancy has not been excluded. There are a few well recognised viruses that have a teratogenic effect but the hepatitis group is not associated with this risk.

5.2. Neonates and infants

There are limited published studies and case reports of HEV genotype 3 in pregnancy and in the neonatal period. Few of the studies provide information specifying viral genotype, with diagnosis being made by measurement of anti-HEV-IgM/IgG titres. Most of these studies have been published in the past 5 years and are from resource-poor settings.

The prevalence of HEV in a small cohort of asymptomatic pregnant women in France yielded an overall HEV prevalence of 7.74%, which was deemed low. Genotype was not specified [50]. A case report describes autochthonous HEV genotype 3 infection in a 41 year

old pregnant woman living in South Eastern France (a non-endemic country). The acute hepatitis had a spontaneous good outcome for the mother and the child. It is suggested that in non-endemic areas where hepatitis E infections are emerging, unexplained cytolysis, whatever its level, in a pregnant woman could be investigated for HEV, using biological molecular and serology tools [51].

In another case report from Germany, an autochthonous HEV subgenotype 3c infection is described in a 27 year old pregnant woman and was the first documented case of a hepatitis E infection during pregnancy in Germany. The patient presented in week 26 of gestation with acute hepatitis and elevated transaminases. During follow-up, she tested positive for anti-HEV antibodies. HEV viral load during the acute hepatitis was 2.3×10^6 copies/ml serum, however viraemia declined and cleared rapidly. Sequence analysis revealed a HEV subgenotype 3c closely related to European isolates. The patient had not travelled outside Germany, had regular contact to animals, but the source of infection remained unclear. The newborn was delivered in week 40 of gestation in good health, HEV was not transmitted and liver enzymes were normal. The authors concluded that hepatitis E should be considered in differential diagnosis in patients with acute hepatitis especially during pregnancy, even without travel history to countries with high endemicity [52].

The role of the placenta in Mother to Infant HEV transmission

In a study to investigate if HEV replication occurs in the placenta of infected mothers, viral RNA was extracted from blood and placenta of 68 acute viral hepatitis (AVH) and 22 acute liver failure (ALF) pregnant patients. Replicative HEV RNA was detectable only in the placenta in ALF and AVH cases and not in blood samples. Positive staining of placental tissue sections with HEV antibody against the viral structural protein ORF3 was observed. HEV replication in placenta also correlated with foetal and maternal mortality in ALF patients. HEV replication thus occurs in human placenta and that placenta may be a site of extrahepatic replication of HEV in humans [53].

Archived sera dating from 1993 from Portuguese mothers with no history of travelling to HEV endemic countries, and their newborns, were tested for anti-HEV IgG antibodies to genotype 3 HEV. Four of the 12 maternal sera were positive for IgG anti-HEV, indicating exposure to this virus. Their newborns were also positive, showing higher anti-HEV IgG levels. These findings demonstrate an efficient transplacental transport of anti-HEV IgG, and also that there was circulation of autochthonous HEV in Portugal in the early 1990s [54].

Anecdotally, in the UK foetal medicine and neonatal clinical settings, hepatitis E virus is infrequently considered as a pathogen. HEV is infrequently tested for, and infrequently identified in association with jaundice and hepatitis (whereas hepatitis A, B and C and CMV are frequently tested for). In standard neonatal guidelines for investigating early and late-onset jaundice, testing for HEV is mostly absent.

The lack of UK data and studies of HEV in UK pregnant women and neonates, especially those who are symptomatic of liver disease, represents an absence of evidence versus evidence that HEV is not pathogenic in this group. It is difficult to quantify the risk of HEV to mother, foetus and neonate from genotype 3 infection as there is insufficient published information.

5.3. HIV-infected patients

The reported incidence of HEV infection in patients with HIV is low ranging from 0% to 0.9% [55,56,57,58] Of the 14 PCR-proven cases, ten were acute infections and four patients had chronic infections, two of whom had cirrhosis. All infections were linked to HEV genotype 3. Of note, patients who developed chronic infection had low CD4 counts despite their HIV infection being under control. There have been no reports of HEV infection linked to transfusion transmission in this population.

5.4. Transfusion-dependent patients

There have been no reports of transfusion associated HEV in transfusion dependent patients. However, in a survey of regularly transfused sickle cell (n= 437) and thalassaemia (n= 323) patients in London, donor exposure reached 27-42/year and 26-65/year respectively; moreover, in sickle cell patients treated by automated exchange transfusion, this rises to 50-110/year (Sara Trompeter, NHSBT audit data). Assuming a transmission rate of 25% from red cells, it would be expected that there would be 1-2 new infections/year in each of the sickle and thalassaemia populations. Such patients would therefore be a useful sentinel group in which to examine HEV sero-prevalence as an indicator of transfusion risk (see section 8).

5.5. Conclusions

There is no evidence of risk in pregnancy related to G3 HEV genotype. There is a major risk in the third trimester of pregnancy from a G1 HEV genotype. It is important to prevent G1 infection at this gestation but it is highly unlikely that a G1 infection would be acquired in the UK (from any route). No cases from transfusion have been reported in pregnant recipients.
There are few data regarding the risk to neonates from HEV infection. Awareness of HEV in the paediatric community is low and consideration should therefore be given as to how this

could be increased, along with inclusion of HEV in the investigation of the jaundiced neonate. No cases from transfusion have been reported in neonates.

6. Risks and Mitigation Options In solid Organ and Stem Cell Transplant Recipients

These patients are considered separately because:

(1) they are treated with long-term immunosuppression and appear to be at particular risk of HEV chronicity and progression to chronic liver disease

(2) they potentially have multiple sources of infection: the graft itself, transfusion around the time of the transplant or later, diet as an on-going source indefinitely, and reactivation of past infection(s).

6.1. Epidemiology and clinical impact

There are case reports and small series which have linked transmission of HEV by blood transfusion to organ transplant recipients. Initial reports of autochthonous acute HEV infections were in the setting of solid organ and haematopoietic stem cell transplants and there is an increasing number of reports of chronic HEV infection in immunosuppressed patients, including solid organ recipients. While markers of previous HEV infection are frequent among candidates for transplantation, active ongoing infection is less common [59]. However, seroconversion after transplantation does occur: one study from France estimated an incidence of seroconversion of 2.83 cases per 10 person years in liver transplant recipients [60]. Another study of 283 solid organ recipients followed for one year after transplantation found 38% had evidence of anti-HEV IgG at the time of transplantation with similar titres at one year; there were three de novo infections and three re-infections [41].

In one centre (Groningen), 34 of 1129 patients suspected of possible HEV infection were positive for HEV RNA: only 7 of these were immunocompetent [61].

Hepatitis E virus (HEV) infection is increasingly recognised as a cause of morbidity and occasional mortality in immunosuppressed patients. In transplanted individuals, HEV infection is due to G3 or 4. There are increasing reports and series of both acute and chronic HEV infections in immunosuppressed individuals but there have, as yet, been few large scale studies using robust approaches to diagnose infections.

The incidence of HEV G3 infection after organ transplantation has been estimated at 3·2 cases per 100 person-years in southwest France [26]. Consumption of game meat and pork products is associated with HEV infection after transplantation [62]. Despite a high seroprevalence of HEV in organ donors in the Toulouse area of southwest France, no cases of HEV transmission via a graft have been documented.

Most organ transplant patients have no symptoms when infected with HEV and very few present with jaundice. Liver abnormalities detected by blood tests are usually very modest (typically serum alanine aminotransferase is around 300 IU/L), anti-HEV IgG and IgM might be negative and seroconversion might never occur after infection [563]. Therefore, use of molecular techniques to confirm the diagnosis and assess the response to therapy is important. Patients present with usually a mild hepatitis, both biochemically (with elevated serum aminotransferases) and histologically, although fulminant cases may occur in immunosuppressed patients. Acute HEV infection proceeds to chronic infection in 50-60% [64] and in solid organ recipients, there is an increased rate of progression to advanced fibrosis and cirrhosis within 3-5 years [65]. Extra-hepatic manifestations of HEV may occur.

Kamar [66] followed up 217 solid organ transplant recipients (SOTR) and described 14 cases (3 liver, 3 kidney and pancreas, 9 kidney) of acute HEV , who presented with abnormal amino transferases with or without clinical symptoms. Eight patients developed chronic infection, with elevated liver enzyme levels, positive HEV RNA in plasma and histological evidence of hepatitis. When comparing those who resolved infection and those who did not, there were no differences in the level of serum transferase, immunosuppressive regimen or induction, nor demographics. Those who acquired infection in the earlier post-transplant period, whilst they had lower lymphocyte and platelet counts, were more likely to develop chronic infection. Histologically, Metavir activity and fibrosis score progressed from 1 to 2.2 and 1.2 to 1.5, respectively, from the acute to the chronic phase. Resolution of infection (seroconversions, clearance of HEV viraemia and normalisation of liver enzymes) occurred within 1 to 3 months from diagnosis of acute infection in 43% of patients.

Like solid organ recipients, patients having allogeneic stem cell transplants (allo-SCT) also appear to be at increased risk of chronicity with 5 of 6 patients developing chronic HEV infection in one study from the Netherlands [63] . Fibrosis of the liver was documented on histology in 2 of these patients (Table 6). Other than the UK study, there are no data on possible transmission of HEV infection via blood products or the transplant itself in these patients. In the absence of such data it would be difficult to establish algorithms for testing donor or recipient. Versluis' paper [67] highlights the higher detection of viraemia in transplant recipients by RT-PCR rather than ALT based algorithms.

The infection is mild but chronicity has been observed in SOT, and anti-viral agents have enabled clearance in some cases. This provides a basis for consideration of diagnostic HEV testing if deranged LFTs are seen post allogeneic-stem cell transplant in both seropositive

and negative recipients with reduction in immunosuppression and and antiviral treatment considered if infected.

Versluis 2013 **328 patients alloSCT** [67]	**Reizebos-Brilman 2013** **1129 hospitalised patients** [63]
Retrospective. Bloods for HEV RNA and serology undertaken if ALT abnormal. Additionally, bloods at time points for CMV for RT-PCR. Median f/u 40.9 months.	Prospective, in pts with unexplained hepatitis, abnormal ALT led to RT-PCR
44% sib 42% MUD 14% cord. Ac GVH 40%, cGVH 37%	34 infected (3%), of which 18 had solid organ transplant (SOT).
8 (2.4%) confirmed HEV infection. 7/8 (88%) detected by cross sectional RT-PCR testing, 1/8 by ALT abnormality and subsequent testing. 4 alive, 4 died with active HEV and hepatitis. (6/8 patients developed chronic disease (2 died before time-frame for chronicity was reached) Cause of death unrelated. 6/8 recd blood products. Blood products not tested for HEV transmission risk. 7/8 patients on immunosuppression.	SOT pts are more likely to get chronic infection. Blood products not tested.
Median time to infection 4.6 months, median time to clear infection 6.3 months.	Median 6 yrs (3 mo-12 years)post transplant to infection.

Table 6 HEV Infection in Solid Organ and Stem Cell Transplant Recipients

6.2. The effect of immunosuppression

In vitro studies suggest that different immunosuppressive agents affect HEV replication in different ways: for example, inhibitors of mammalian target of rapamycin (mTORi) (sirolimus and everolimus) and calcineurin inhibitors (CNI) (cyclosporin and tacrolimus), support HEV replication, whereas mycophenolate inhibits replication; corticosteroids have no effect [68]; whether these effects translate into in vivo effects is unclear.

6.3. Risk from deceased and living organ donors

Deceased donors

The selection criteria for organ donors are very different from those in place for blood and tissue donors. The person giving consent for the organ donation will be asked a number of questions about the donor's health and lifestyle. However, it is very unusual that an organ would not be offered because of this information; there are a very small number of absolute contraindications to organ donation but these are not related to donor behaviours. The age of the donor may increase the likelihood that the donor is HEV IgG positive but as with blood and tissue donors it would not be possible to select 'low-risk' donors on donor risk information alone.

At present, there are no robust data on the prevalence of HEV infection in deceased solid organ donors. At an annual UK target of 1439 deceased organ donors, if the rate of infection in organ donors were the same as in blood donors, it is estimated that there would be 1 infected donor/year (defined as HEV RNA positive). However, extrapolation from blood donors may give misleading conclusions as the demographics of the two populations show significant differences. There has been one case report of HEV transmission via liver transplantation from a deceased organ donor [31] no cases have been attributed to donor-derived transmission via other organs. In terms of screening of deceased organ donors, HEV IgG screening is not informative in terms of infectivity risk and HEV RNA results may not be available until after donation has occurred.

Living organ donors

The current annual UK target is 1143 living donors, the vast majority of which are kidney donors, although donation of a liver lobe from a living donor is possible. Again, current estimates would suggest that an HEV RNA positive living donor would be detected every 1-2 years. Screening for HEV would be possible in the work-up of living organ donors but since the risk from kidney transplantation is unknown, its requirement is unclear at present.

6.4. Risk from stem cell donors

There have been no confirmed transmissions attributed to the stem cell donor, although one donor with an acute infection has been reported. There are 1615 allogeneic stem cell transplants annually in the UK. If the rate of infection were to be similar to blood donors, there would be 1 HEV infected stem cell donor every 5-10 years.

6.5. Risk from diet

It is estimated that there are currently 60,000 infections from diet annually in England. This provides an annual dietary risk of 0.1-0.2% ie 1 in 500 transplant recipients will become

infected annually through diet. Put another way, after 5 years, 99% of transplant recipients will not have acquired HEV from their diet. However, this makes the assumption that their risk of infection is identical to other people. This may not be the case, given the over-representation of transplant recipients in case series of HEV infections in hospital populations. If the risk of acquiring HEV from diet is substantially increased in organ recipients, the number of patients infected will rise concomitantly. Therefore, more data are required regarding the background rate of HEV acquisition from diet in transplant recipients.

6.6. Risk from blood components

Blood component exposure is low in renal transplantation, where most procedures require no blood at all (mean 0.5 donor exposures/procedure). Donor exposure rises to 9/procedure for liver transplantation, with heart, lung and pancreas transplant being intermediate. The highest of all is multivisceral transplantation (68/procedure).

Based on data from the Hewitt study [17] for donor incidence and infectivity, it is estimated that for liver transplant recipients, the upper estimate of infection from blood components at the time of transplant is 0.14%, equivalent to 1 liver transplant recipient/year. Because multivisceral transplants are not commonly performed, the corresponding figure is 1 infected recipient every 6 to 7 years, with the number in heart and lung transplants being intermediate. Where there is considerable uncertainty is the frequency with which HEV becomes chronic in such recipients, and the likelihood of chronicity leading to serious sequelae such as cirrhosis.

Recipients of allogeneic (donor) stem cell transplants receive immunosuppression to prevent graft-versus-host disease from the transplant. Recent data from a single centre in England (courtesy of Dr Kate Pendry, NHSBT/Central Manchester Hospitals) show that both adult and paediatric recipients of allogeneic (donor) stem cell transplants receive a median of 19 donor exposures/procedure, of which 12 are from platelets. It should be noted that donor exposure via platelets will increase by approximately 75% over the next 2 years as the percentage of apheresis platelets falls from 80% to 40%. In contrast, recipients of autologous (patient's own) stem cells have a median of 5 donor exposures/procedure, and do not receve specific immunosuppression. .

Reduced intensity conditioning regimens have reduced the need for blood products; however patients are rendered immunosuppressed for longer periods.

6.7. Diagnosis and treatment

Prophylactic anti-viral therapy to cover a transplant procedure is not recommended. There is broad consensus that treatment should be considered for organ transplant recipients with HEV infection, balancing the risks and benefits of treatment:

- The diagnosis of ongoing HEV infection should be established by using a validated RNA-based test
- in some patients, no treatment is indicated
- review immunosuppression and minimise the total immunosuppressive burden only if clinically appropriate
    a. consider switching from inhibitors of mammalian target of rapamycin (mTORi) (sirolimus and everolimus) and calcineurin inhibitors (CNI) (cyclosporin and tacrolimus) to other medications such as mycophenolate and/or steroids
    b. monitor liver tests and HEV RNA levels closely
- if there is continuing infection, consider drug therapy with ribavirin for 3 months and then review response (note: ribavirin is not licenced for use in this indication).

Gamma interferon (IFN) has a significant morbidity and complication rate in immunosuppressed patients and can precipitate allograft rejection, so transplant physicians would not be keen to advocate IFN as a first line of treatment.

6.8. Risk mitigation options

Based on the considerations above, there are several options to mitigate risk for transplant recipients.

Option 1. Provide NAT negative blood components for organ and stem cell transplant recipients.

It is clinically acceptable to provide screened blood components to recipients at particularly high risk. UK Blood Services have experience with selective testing through many years of supplying CMV sero-negative components for high risk recipients. Provision of NAT negative components for all solid organ and stem cell transplant recipients would therefore be feasible as the vast majority of donors will be safe. It is estimated that testing 50,000-100,000 donors/year may be sufficient to meet demand. However, confirmation of these numbers will depend on gathering detailed prospective data on blood component usage in stem cell transplant recipients. This would generate a managable 15-40 HEV NAT positive donors/year for confirmatory testing and follow up.

Option 2. Provide blood components from immune donors.

This option was rejected as discussed in section 4.8.

Option 3. Test all transplant recipients at pre-defined intervals eg annually and treat if positive.

European organisations, who have seen increasingly high proportions of liver disease in organ transplant patients with chronic HEV, have suggested baseline HEV Ab and RNA testing, followed by 6 monthly and yearly monitoring.

There are no firm data at this point to support such a strategy. The risk from diet may be as low as 1% over 5 years, but this needs to be confirmed. Moreover, it is not clear whether the risk of serious clinical sequelae is similar in recipients of different types of transplant or in patients receiving different types of immune suppression. For example, patients receiving liver transplants may be at particularly high risk. There are currently 43,300 people alive in the UK with a functioning organ transplant, of whom 8,300 have transplanted livers (Rachel Johnson, NHSBT data). The 1 year survival of patients undergoing stem cell transplant is 62% for matched unrelated donors and 71% for related donors (BSBMT data). The approximate number of people alive after 5 years who have had an unrelated allogeneic stem cell transplant is 594.

The need for and feasability of testing all or selected transplant recipients at regular intervals whilst on immunosuppression or for life is not clear, and it would be premature to recommend such a strategy at this time. More information is needed from cross-sectional and longitudnal studies to understand the feasability of and yield from such an exercise.

Option 4. A combination of 1 and 3

Option 5. No prevention, but a low index of suspicion for HEV testing if abnormal liver enzymes arise in a transplant recipient. Positive patients would be treated as above. This recommendation can be promulgated immediately while additional information is gathered.

6.9. Cost effectiveness considerations

It follows from the above uncertainties that a full cost-effectiveness analysis is not possible at this time, as key inputs are not available regarding the frequency with which HEV transmission occurs in transplant recipients, the source of infection, and the rate of serious clinical sequelae in the highest risk populations. Hence the costs saved by their avoidance cannot be calculated. The paragraphs below are intended to give some only some **broad**

estimates of costs of different options. These would need confirmation and refinement before precise cost-effectiveness calculations could be performed.

Option 1: Provision of HEV NAT negative blood components for solid organ and stem cell transplant patients

Detailed costings of HEV blood donor screening would require discussion with manufacturers in the context of a tender exercise, and prices are highly volume dependent. [redacted] This does not include costs of confirmatory testing, staff costs nor costs of donor follow up and reinstatement testing.

Option 2 (Provision of Immune donors) was rejected on clinical grounds.

Option 3: Test all transplant recipients eg annually and treat if positive.

For this option, built around early detection and treatment, the major costs are:

(1) patient testing, either by NAT or serology. If annual testing of 100% of living transplant recipients were possible, something in the order of 50,000 tests/year would be needed, [redacted]

Option 4: A combination of Options (1) and (3)

[redacted]

Option 5: No prevention, but a low index of suspicion for HEV testing, treating infected patients.

Costs saved.

Options (1) and (3) might each prevent/detect 1 infection/year in liver transplant recipients, fewer in other types of solid organ recipients, and perhaps 4 infections/year in stem cell transplant recipients. Only a proportion of these infections will become chronic and cause

any clinical sequelae. Because the frequency of minor and severe clinical sequelae remain uncertain, the treatment costs saved by any of the risk mitigations are as yet unknown.

6.10. Conclusions

Solid organ transplant recipients appear to be at particular risk of clinical sequelae of HEV acquisition. A sub-set of infected patients go on to delayed clearance or chronic infection; some of those will develop chronic liver disease. However, there is considerable uncertainty as to the size of the risk.

Stem cell transplant recipients also appear to be at increased risk of chronic infection with an increased risk of chronic liver disease.

There are many causes for mildly abnormal liver tests in deceased donors and chronic infection is rare, so it is recommended that testing of deceased donors is limited to those with an unexplained hepatitis where a viral cause is being considered on clinical or epidemiological grounds. It is expected that this testing would have been part of the diagnostic work up for the donor, rather than a specific pre-donation screening.

Living organ donors with unexplained hepatitis should be investigated and HEV infection considered. Living donor organs should not be used until the donor has been consistently negative for HEV RNA with a documented acceptable level of detectable IgG.

Organ donors with known HEV infection, rarely, a donor may present with known HEV infection. It is recommended that organs from HEV infected donors are used only in exceptional cases and should be used after full discussion and treatment planning with an expert microbiologist and with suitably informed patient consent.

A study of HEV prevalence and clinical consequences of HEV infection associated with solid organ and stem cell transplantation should be undertaken before definitive recommendations can be made (see section 8).

HEV infected transplant recipients can be treated by modification of immune suppression and, if indicated, ribavirin.

The costs of selective blood donor screening and transplant recipient monitoring each appear to be [redacted] Savings in treatment costs are uncertain as the

frequency of serious sequelae is not known. Therefore a full cost-effectiveness calculation cannot be made at this time. Further information is needed (see section 8).

It is premature to recommend either provision of HEV negative blood components or routine monitoring of solid organ or stem cell transplant patients for HEV infection. Further information is needed on the degree of mitigation of complications that either of these options would provide. Where a transplant recipient has unexplained abnormal liver function tests, then evidence of HEV infection should be sought, testing for HEV RNA in plasma.

7. Transmission Through Tissues, Gametes and Embryos

7.1. Banked tissues

This section considers the factors determining the risk of HEV transmission by tissue transplantation from untested donors, particularly the effect of processing steps and storage conditions. The scope of this section is restricted to the tissue grafts donated for clinical use in the UK. Deceased tissues donors can donate skin, musculoskeletal tissues, cardiovascular and ocular tissues, pancreatic islets and hepatocytes for transplantation. Amnion and femoral heads are donated by living donors. Pancreatic islets and hepatocytes are normally donated by organ donors and will be considered in the section on organ transplantation.

Donor Selection

Selection of deceased tissue donors is carried out by asking the next of kin a standard set of questions relating to the general health and behaviours of the donor. The person assisting in the donor selection process may not have known the donor well. The information provided by the next of kin is supplemented by information provided by the general practitioner however, of course, only illnesses that were sufficiently serious or long lasting will have resulted in a GP visit therefore mild illness may be missed in the selection.

The selection criteria and post-donation information applied to blood donation also apply to live tissue donors. Live tissue donors reporting recent illness are excluded from donating tissues. Live tissue donors reporting post donation information would be managed in the same way as blood donors and donated tissues would not be issued for clinical use.

Testing

Blood samples from living and deceased tissue donors are currently tested for a range of mandatory tests namely HIV, HCV, HBV, HTLV and treponemal antibodies and additional tests where appropriate, following requirements of EU Tissues and Cells Directive. HEV testing could be added, subject to validation of assays for testing with deceased donor samples.

Tissue grafts can be processed and stored as non-viable or viable (cryopreserved) grafts. Bone, tendon, decelluarised dermis, irradiated skin, amnion and sclera are non viable grafts, Heart valves, pericardium, arteries, skin, osteochondral, meniscus, cornea are viable grafts. In addition pancreatic islets are minimally processed, fresh, viable grafts infused without storage. Hepatocytes may be transplanted without storage or cryopreserved for future use.

Femoral head donated by surgical donors is stored without additional processing or can be irradiated. Amnion donated at the time of delivery by live donors is processed and stored. The tissues grafts donated by deceased donors are processed and stored with the exception of pancreatic islets.

Infectivity of allografts

It is not known if the HEV load in unprocessed tissue is sufficient to infect the recipients. Donations are not pooled which is a general risk reduction measure to minimise risk of infection transmission. One tissue donor can donate one or more grafts. Some grafts e.g. bone and amnion can be transplanted to a number of recipients. One tissue donor therefore has the potential to infect one or more recipients. However, processing steps and storage conditions may minimise infectivity of tissue grafts.

Effect of processing, preservation and Storage

There are no specific studies in literature examining the effect of tissue processing steps on HEV. Experimental studies conducted to determine food safety have demonstrated that HEV can be inactivated above 71^{0}C for 20 minutes or by boiling for 5 minutes [69]. The heat sensitivity of HEV is dependent on the heating conditions [70]. HEV can survive frozen storage in dry ice (-80^{0} C) or vapour nitrogen (below -135^{0} C), but HEV is rapidly degraded by the freezing and thawing process [71]. As there is no tissue culture system for HEV, the ability to measure pathogen viability and sterility assurance level is affected. Viruses irradiated in a liquid medium are more sensitive than either dried or frozen samples. Fully wet virus is most sensitive with resistance increasing with dehydration. Gamma irradiation alone does not appear to be particularly effective for inactivating small, non enveloped viruses. In the absence of data for a virus family, it is advisable that higher radiation doses may need to be used

Most non viable tissue grafts are processed to deplete donor cell, blood and/or bone marrow contents. The processing steps may include physical processes, such as high pressure water jet, centrifugation or increased temperature up to 60°C, and chemical processes, including water washing or washing with solvents or detergents, depending on local protocols. Some types of non-viable tissue graft, such as bone, may also be terminally sterilised with gamma irradiation at an absorbed dose of 25-40kGy.

Viable grafts are either processed using decontamination with a cocktail of antibiotics, followed either by cryopreservation by impregnation with a cryoprotectant and controlled freezing to <-135°C, or stored at normothermic temperatures for up to 28 days .

Decellularised grafts are terminally sterilised and stored at room temperature. Non-viable allografts are either freeze dried, or immersed in solvent, and stored at ambient temperature or frozen and stored in a freezer.

Viable grafts are cryopreserved and stored at <-135°C in the vapour phase of liquid nitrogen or using ultra low temperature freezers. The processing and storage conditions may significantly reduce the viral load, but there is no evidence that any of these inactivate the virus.

Recipients

Most of the tissue allograft recipients are immunocompetent and do not require immunosuppression. For example, tendon and meniscus recipients are normally healthy individuals undergoing surgery following sports injuries. The clinical consequence of HEV infection in most tissue allograft recipients is not a serious concern. Some hepatocyte recipients can be neonates and children. Hepatocytes and pancreatic islets recipients receive immunosuppression. The guidance for organ donors and organ transplant recipients is applicable to hepatocytes and pancreatic islets.

There have, as yet, been no reports of HEV being transmitted by tissue allografting. However where a potential tissue donor is known to be actively infected with HEV it is not advised that donation and transplantation of tissue should take place. In the case of living tissue donors, tissue should not be donated until the donor has been microbiologically cleared to donate.

7.2. Gametes (eggs and sperm)

7.2.1 What is the chance of sexual transmission of HEV via semen?

HEV comprises a family of at least four major genotypes, each with its own epidemiology, global distribution and pathogenicity in humans. In the UK genotype one (G1), an endemic human infection in developing countries, is acquired through foreign travel and accounts for a minority of cases of hepatitis E and a very small fraction of HEV infections over all. The main genotype in the UK (G3) is not thought to have a significant person-to-person transmission. None of the different genotypes have been shown to be sexually transmitted. Whilst this does not preclude transmission by this route, the risk must be considered to be

very small. There may be an increased risk in MSM, this is mostly likely due to transmission via anal intercourse in relation to G1 [72]. Based on this information, it is concluded that there is unlikely to be a significant infective viral load in semen.

Processing of semen for donation

Semen collected for donation other than to a sexual partner is processed before use. The sperm are separated from the seminal plasma then re-suspended in sterile media. The sample is then placed into sealed vials or straws and cryopreserved in liquid nitrogen or vapour phase nitrogen. For use, the volume of media containing the sperm that is used is about 0.3ml. Following freezing and thaw, it is likely that the HEV will survive but also possible that its infectivity will be reduced.

Feasibility of screening sperm donors

Donors are screened for other viruses (HBV, HCV and HIV) prior to donation and the sample is usually quarantined for 6 months so that the donor can be retested before the sample is used. It would thus be possible to include HEV NAT screening in this procedure if it were considered to be required.

7.2.2 Risk of HEV transmission via egg donation

The risk from egg donation is different from sperm because eggs are collected via a surgical procedure as part of the IVF process. Although there may be some blood contamination, this is removed immediately after the egg is identified. The egg is a single cell with surrounding cumulus cells that form a complex about 2mm diameter. Whilst there is a potential risk that there may be HEV present in this complex if the donor were viraemic, the risk of there being an infective viral load in the egg must be very small. Thus risk of passing HEV to the egg recipient is equally small.

Processing of eggs for donation

Egg donation involves the IVF process during which the egg is mixed with sperm. If HEV is present with either the egg or sperm, then there is a theoretical risk to the embryo. There is no evidence of teratogenicity in those with the infection who conceive naturally although it is noted that the embryo in the laboratory has none of the protective immune factors present during natural conception. IVF is currently carried out for couples where either one or both couples carry HBV, HCV or HIV. There is no evidence that this has a pre-implantation developmental effect on the child.

Feasibility of screening egg donors

Most eggs are donated to another woman for treatment without being cryopreserved although the success rates of egg cryopreservation have improved and this could be a routine option in the future. This would facilitate screening if it were considered to be necessary.

7.3. Embryos

The situation for embryo donation is different from separate egg and sperm donation because the embryos are usually created and cryopreserved by a couple for their own use and then later donated. Results of screening at that later date may not reflect the viral situation at the time that embryos were created and cryopreserved. Given the information above about the risk of sperm and egg donation, it can be concluded that the risk from embryos is equally small.

7.3.1 Risk after conception if HEV is transmitted by gamete donation

Despite the very small risks identified above, if pregnancy does follow gamete donation and HEV transmission occurs, the disease is only likely to be limited to the first trimester.

7.4. Conclusions

No specific steps are required to mitigate the HEV risk in recipients of banked tissues. The risk from tissue transplants themselves appears to be extremely low. Tissue transplants are not commonly accompanied by a need for transfusion, but even if transfusion occurs, most tissue recipients are not immunosuppressed and therefore not at high risk from serious clinical sequelae. The recommendations for organ donors and organ transplant recipients is applicable to hepatocytes and pancreatic islets transplantation.

There is no evidence of HEV transmission as a result of donation of gametes and the theoretical risk is extremely low. There is therefore no requirement to take steps to mitigate the risk of HEV through donation of eggs, sperm or embryos.

8. Recommendations

A. On the albeit limited evidence available, there is no pressing case for HEV RNA screening of the entire blood supply at this time. However, the pattern of HEV infection in the UK is evolving and this recommendation should be reviewed at the earliest opportunity when the findings become available from the additional work recommended below (see section 9) , or other new evidence from other countries.

B. The requirement and optimal strategy for clinical risk mitigation in solid organ and stem cell transplant patients is not yet clear. While the additional evidence is bring gathered, UK Blood Services should without delay develop a costed operational plan for blood donor testing to provide HEV-tested components for solid organ and allogeneic stem cell transplant recipients.

C. Testing of deceased organ donors for HEV should be imited to those with an unexplained hepatitis where a viral cause is being considered on clinical or epidemiological grounds. Specific pre-donation screening is not indicated for deceased organ donors with normal liver function tests.

D. Organs from deceased HEV infected donors should be used only in exceptional cases and only after full discussion and treatment planning with an expert microbiologist and with suitably informed patient consent.

E. Living organ or stem cell donors with unexplained liver function tests should be investigated and HEV infection considered. Organs from infected donors should not be used until the donor has been consistently negative for HEV RNA with a documented acceptable level of detectable IgG.

F. HEV testing should be considered in any solid organ or stem cell transplant patient with unexplained changes in liver enzymes.

G. No specific steps are required to mitigate the HEV risk in recipients of banked tissues. However, any future recommendations for recipients of pancreatic islets or hepatocytes should follow that for solid organ recipients.

H. No specific steps are required to mitigate the HEV risk in recipients of donated gametes or embryos.

I. Awareness of HEV should be increased in clinical teams treating organ and stem cell transplant recipients, neonates, pregnant women and transfusion-dependent patients such as those with haemoglobinopathies.

J. The section below highlights the further information needed to make definitive recommendations. A costed plan of this further work should be produced without delay, showing time lines as to when each piece of information will become available.

9. Further work needed

HEV in Europe is evolving, and the information on which to make decisions at this time is limited. Further studies to provide data critical to decision making are needed in the following areas.

1. **How the incidence of new HEV infections in blood donors varies over time.**

It is clear that the incidence of HEV infections is not constant in the UK population. What influences fluctuation in the risk of acquiring HEV remains unclear nor is the range of these excursions known. We suggest a rolling programme of work that monitors changes in the dynamics of HEV infection in blood donors at a national level. These investigations could be undertaken as:

a) HEV antibody studies to determine changes in HEV seroprevalence rates in blood donors over time

or

b) Determine attack rates by measuring HEV seroconversion in a cohort of regular donors

These data will inform on changes over time in the risk of HEV acquisition from blood/blood components. The frequency of the survey should be decided with statistical input.

2. **The rate of HEV acquisition and its clinical sequelae in specific patient groups**:

   a. **Transfusion-dependent patients.** Is there evidence that transfusion dependent patient groups who receive multiple blood components over extended time periods have a higher HEV seroprevalence rate than the rest of the population eg sickle cell/thalassemia patients?

   b. **Organ and stem cell transplant recipients.** A recent study has demonstrated that immunosuppressed transplant patients receiving HEV-containing products are at increased risk for the development of persistent infections. There are however no coherent data on HEV antibody prevalence rates in individuals on transplant waiting lists. Studies to determine HEV antibody status in these individuals would provide baseline information on how many are likely to be susceptible to infection but also provide opportunity for longitudinal studies to be undertaken in seropositive patients. These

investigations would ascertain whether the pre-existence of circulating antibody protects against HEV infection post-transplant or whether antibody disappears following initiation of immunosuppression regimens leaving recipients susceptible to infection.

c. **Children**. Data on HEV infections in children are lacking. Surveillance studies indicate seroprevalence rates to be low in children, an observation which in itself raises interesting questions about susceptibility. However, recent case reports of chronic HEV infections in children plus descriptions of HEV antibody detection in transplant recipients indicate that more work and a better understanding is needed in this population. Proposed studies could include:
    i. General population seroprevalence studies in children (<20 years).
    ii. Understanding persistent infections in children who receive solid organ transplant (SOT), and those who are immunosuppressed after treatment for haematological malignancies and lymphomas (HOnc), including those who have received stem cell transplants. These studies could be linked and developed along 3b and 4a.

d. **Sero-prevalence in deceased tissue donors**. These studies would provide an estimate of the likely HEV prevalence in organ donors, but avoid the problems associated with post hoc testing of organ donors for research purposes. Linked with Study B above, this will indicate how many organ transplant recipients are being exposed to HEV through their transplants.

3. Programme of work investigating the **prevalence of chronic HEV infection in the UK** and understanding the **determinants associated with viral persistence in the immunosuppressed** population.
    e. There is currently insufficient information for building strategies for monitoring patients at higher risk of persistent HEV infection post transfusion. A study is therefore needed that will define the clinical indicators of persistent HEV infection in two immunosuppressed patient groups, those who have received a solid organ transplant (SOT), and those who are immunosuppressed after treatment for haematological malignancies and lymphomas (HOnc), including those who have received stem cell transplants. The study will also inform on the prevalence and outcome of persistent hepatitis E and its relation to immunosuppression regimens. Since such patients will both be exposed by

transfusion (and potentially transplantation itself) and through dietary exposure during their life as a transplant recipient, so both cross-sectional and extended longitudinal studies will be necessary.

f. Functional assays for T-cell responsiveness to recombinant genotype 1 and 3 virus like particles will be mapped for recovered cases of hepatitis E and for patients who are persistently infected and those undergoing intervention for viral clearance. This is a necessary strategy as the vaccine is based on G1 but the principal challenge in the UK patient is G3. Study patients undergoing intervention may include both those in whom immune manipulation is attempted and those receiving specific antiviral therapy (ribavirin).

4. **Effectiveness of Pathogen Inactivation of blood components.** Reports of 'breakthrough' HEV infections despite Incercept treatment may suggest there is no merit in recommending PI. However, observations that the use of Mirasol leads to a reduction in HEV viral load are encouraging. A recent UK study looking at transmission from HEV containing blood products showed that donations with a lower viral load were less likely to be associated with a transmission event. Manufacturers wishing to supply PI systems for blood components in the UK should therefore be required to demonstrate the capacity of their system to inactivate HEV.

5. **A feasability study of testing all transplanted patients (organ or stem cell) eg annually.**

10. Appendices

Appendix 1

Commercial screening assays available

[redacted]

[redacted]

Appendix 2 HEV residual risk estimates

Estimated HEV risk **Katy Davison - NHSBT/PHE Epi Unit. Nov 2014**

Risk = proportion of donations that are infectious among those that are screened negative

Assumption for UK risk – incidence in SE England donors is equal to UK donors

		HEV incident infections - serostatus					
		Seronegative but HEV RNA positive donors			All HEV RNA positive donors irrespective of serostatus		
Time/place	**2012/13 NHSBT donors in SE England**						
Testing data[1]	Infectious WP in days	7	10.5	14	7	10.5	14
	Infectious WP in years	0.019	0.029	0.038	0.019	0.029	0.038
Period specific donor data[2]							
	number of donors with incident infections	56	56	56	79	79	79
	number of donations tested tests	225,000	225,000	225,000	225,000	225,000	225,000
	number of person years at risk (donations x avg IDI of 0.7 years)	157,500	157,500	157,500	157,500	157,500	157,500
	incidence per 100,000 person-years in all donors	35.56	35.56	35.56	50.16	50.16	50.16
	LOWER 95% CI incidence	26.86	26.86	26.86	39.71	39.71	39.71
	UPPER 95% CI incidence	46.17	46.17	46.17	62.51	62.51	62.51
During the first year of testing, window period risk per million							
	WP Risk (Incidence x WP) x 1,000,000	**6.81**	**10.22**	**13.63**	**9.61**	**14.42**	**19.23**
	Range - lower value (using lower value of 95% CI incidence)	**5.15**	**7.72**	**10.30**	**7.61**	**11.42**	**15.22**
	Range - upper value (using lower value of 95% CI incidence)	**8.85**	**13.27**	**17.70**	**11.98**	**17.97**	**23.96**
During the first year of testing, window period risk as 1 infectious donation in x million donations							
	1/(WP Risk x 1,000,000)	0.015	0.010	0.007	0.010	0.007	0.005
Based on 2.3 million donations made in UK, it is estimated that in 1 year HEV testing will not identify x donations		**16**	**24**	**31**	**22**	**33**	**44**
	Range - lower value (using lower value of 95% CI incidence)	**12**	**18**	**24**	**18**	**26**	**35**
	Range - upper value (using lower value of 95% CI incidence)	**20**	**31**	**41**	**28**	**41**	**55**

[1] source A Kitchen, personal communication, 2014.

[2] source Hewitt [17]

Appendix 3 Statistical Risk of HEV Infection in Solid Organ and Stem Cell Recipients

This note considers the risk of HEV infection in solid organ and stem cell transplant recipients. Given the available data, we estimate the probability of immunosuppressed individuals being infected with HEV, broken down by transplant type.

The PHE/NHSBT study in 2013 found a HEV RNA prevalence in blood donors of 1 in 2850 (0.04%) so we assume this as the rate of potentially infective blood components. HEV is transmitted at a rate of approximately 40%, with transmission more likely at higher viral loads and with exposure to larger volumes of plasma. Transmission rates do appear to vary by blood component as shown by the recipient outcomes from the PHE/NHSBT study provided in Table 1. However, these are based on small numbers so we use the 40% average as the transmission rate. Given these assumptions, we might expect there to be approximately 920 potentially infective issues of blood components, and 368 infections from exposures a year as shown in Table 2.

Table 1 – Association between transfused blood components and HEV transmission, Hewitt [17]

Component	Number of recipients	Infected Recipients (%)	Uninfected Recipients (%)
Red Blood Cells	16	4 (25%)	12 (75%)
Pooled Platelets	10	4 (40%)	6 (60%)
Apheresis platelets	14	7 (50%)	7 (50%)
FFP	2	2 (100%)	0 (0%)
Pooled Granulocytes	1	1 (100%)	0 (0%)
TOTAL	**43**	**18**	**25**

Table 2 – Number of Infective Blood Components

	Blood Component[1]			
	RBC	**Platelets**	**FFP**	**Total**
2013 UK Issues[2]	2,043,000	312,000	266,000	2,621,000
Potentially (RNA+) Infective Issues (0.04%)	717	110	94	920
Transmission rate	40%	40%	40%	
Predicted infections from exposure	287	44	37	**368**

[1] No account made for wastage
[2] From 2013 Annual SHOT report

The likelihood of an immune suppressed individual being infected with HEV following transplantation will be based on interaction of a number of factors: the level of viral load and antibody in the donation; the level of viraemia that would be expected to transmit; the quantity of products provided to the recipients, and the susceptibility of recipients to infection.

There are different transplant types for each organ, with each procedure requiring a varying number of units to be transfused. For the purposes of these calculations an "average" has been used. Table 3 provides estimates of the average number of donor exposures with each transplant based on information provided by Chairs of Organ Advisory Committees and Central Manchester hospitals for allogeneic stem cells.

Table 3 – Average donor exposure for recipients of transplants, information provided by Chairs of Organ Advisory Groups and Central Manchester Hospitals

Transplant	Average Donor Exposure with Transplant				Total Exposures	Transplant Activity 2013-14 UK	Total Units (Average Units x Activity)
	RBC	FFP	Platelets	Cryo			
Liver	6	2	2	0	10.0	900	8,100
Kidney	0.5	0	0	0	0.5	3,055	1,528
Lung	4	2	2	0	8.0	210	1,470
Heart	3	2	0	0	5.0	197	985
Pancreas	1.84	0.30	0.05	0	2.2	261	564
Intestinal	2	3	0	0	5.0	26	130
Multivisceral	40	20	6	4	70.0	13	884
Heart/Lung	6	4	2	0	12.0	8	92
Kidney & Pancreas	2.34	0.30	0.05	0	2.7	188	500
Kidney & Heart	3.5	2	0	0	5.5	1	6
Kidney & Liver	6.5	2	2	0	10.5	12	114
Allogeneic Stem Cell	7	0	12	0	19	1,615	20,188
						6,486	**34,560**

Sero-epidemiological studies have been carried out in the general population in England indicating HEV seroprevalence to be high at approximately 13%. The seroprevalence rates have been found to increase with age, peaking at approximately 25% in those aged 50 years and over. However, for the purposes of these calculations a single assumption of 13% is used.

There remains much debate on the persistence of HEV antibody following recovery and data is lacking on decay of HEV antibody and what level of HEV antibody is protective. It is also not clear whether the pre-existence of circulating antibody protects against HEV infection post-transplant or whether antibody disappears following initiation of immunosuppression regimens leaving recipients susceptible to infection.

For these reasons two scenarios of susceptibility are considered;

a) *A lower estimate:* There is 100% susceptibility to an infective issue for any immunosuppressed individual who has not had HEV prior to the transplant. Conversely, an individual is assumed to clear the virus if they have previously had HEV.

b) *An upper estimate:* There is 100% susceptibility to an infective issue regardless of whether an individual has had HEV prior to the transplant.

Using the assumptions outline above, the estimated probability of post-transfusion HEV infection is shown in Table 4.

Table 4 – Probability of immunosuppressed individual being infected with HEV

Transplant	Probability of Clinically significant HEV Infection		Transplant Activity 2013-14 UK	Number of years before HEV infection via transfusion	Number of transplants functioning at 31 March 2014	Yearly HEV infections via diet for all living transplant recipients
	Lower Estimate	Upper Estimate				
Liver	0.122%	0.140%	900	1-2	8,300	16-17
Kidney	0.006%	0.007%	3,055	4-5	31,000	62-63
Lung	0.098%	0.112%	210	4-5	3,600	7-8
Heart	0.061%	0.070%	197	7-8		
Pancreas	0.027%	0.031%	261	12-14	1,800	3-4
Intestinal	0.061%	0.070%	26	54-62	100	0-0.5
Multivisceral	0.851%	0.978%	13	7-9		
Heart/Lung	0.147%	0.168%	8	74-85		
Kidney & Pancreas	0.033%	0.038%	188	14-16		
Kidney & Heart	0.067%	0.077%	1	1300-1500		
Kidney & Liver	0.128%	0.147%	12	56-65		
Allogeneic Stem Cell	0.232%	0.267%	1,615	0.25-0.5		

It is estimated that for liver transplant recipients, the upper estimate of infection from blood components at the time of transplant is 0.14%, equivalent to 1 liver transplant recipient every 1 to 2 years. Because multivisceral transplants are not commonly performed, the corresponding figure is 1 infected recipient every 7-9 years, with the number in kidney and lung transplants being intermediate. The estimate of infection from blood components for

allogeneic stem cell recipients is 0.267%, but due to the large number of transplants performed this could result in 4 infections per year.

It is estimated that 60,000 infections of HEV occur yearly in England and an annual dietary risk of 0.1% - 0.2%. However, there is an upward trend in infections so an attack rate of **0.2% or 1 in 500 per year** is assumed as the dietary risk. This provides a comparison in Table 4 of HEV infection via diet to that via transfusion. For example, for liver transplant recipients we might expect 1 person a year to have HEV infection via blood components, but 16 infections to occur through diet in all liver transplant recipients.

11. References

1. Ijaz S, Vyse AJ, Morgan D, Pebody RG, Tedder RS, Brown D. Indigenous hepatitis E virus infection in England: more common than it seems. J Clin Virol. 2009; 44:272-6

2. Said B, Ijaz S, Chand MA, Kafatos G, Tedder R, Morgan D. Hepatitis E virus in England and Wales: indigenous infection is associated with the consumption of processed pork products. Epidemiol Infect. 2014; 142(7): 1467-75

3. Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Kurata Y, Ishida M, Sakamoto S, Takeda N, Miyamura T. Hepatitis E virus transmission from wild boar meat. EID 2005;11:1958-1960.

4. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet 2003; 362: 371–373.

5. Takahashi K, Kitajima N, Abe N, Mishiro S. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 2004; 330: 501–505.

6. Colson P, et al. 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. J. Infect. Dis. 202:825–834.doi:10.1086/655898.

7. Teshale EH, Hu DJ, Holmberg SD. The two faces of hepatitis E virus. Clin Infect Dis. 2010 Aug 1;51(3):328-34.

8. Geng Y, Zhang H, Huang W, J Harrison T, Geng K, Li Z, Wang Y. Persistent hepatitis e virus genotype 4 infection in a child with acute lymphoblastic leukemia. Hepat Mon. 2014 Jan 23;14(1):e15618.

9. Halac U, Beland K, Lapierre P, Patey N, Ward P, Brassard J et al. Chronic hepatitis E infection in children with liver transplantation. *Gut* 2012; 61: 597-603.

10. Halac U, Beland K, Lapierre P, Patey N, Ward P, Brassard J et al. Cirrhosis due to chronic hepatitis E in a child post-bone marrow transplant. *J Pediatr* 2012; 160: 871-4.

11. Dalton H.R.; Kamar N.; Izopet J.Hepatitis E in developed countries: Current status and future perspectives Future Microbiology, December 2014, vol./is. 9/12(1361-372)

12. Woolson K.L.; Vine L.; Beynon L.; McElhinney L.; Hunter J.G.; Madden R.G.; Glasgow T.; Palmer J.; McLean B.N.; Bendall R.P.; Warshow U.; Dalton H.R. Neurological manifestations of HEV genotype 3, Journal of Hepatology, April 2014, vol./is. 60/1

13. Madden R.G.; Van Den Berg B.; Van Eijk J.J.J.; Van Der Eijk A.A.; Hunter J.G.;Tio-Gillen A.P.; Reimerink J.; Bendall R.P.; Pas S.D.; Ellis V.; Van Alfen N.; Beynon L.;Southwell L.; McLean B.; Van Engelen B.G.M.; Jacobs B.C.; Dalton H.R.
Post-infectious peripheral nervous system disorders and hepatitis e virus Journal of Hepatology, April 2014, vol./is. 60/1

14. van Eijk, Jeroen J J; Madden, Richie G; van der Eijk, Annemiek A; Hunter, Jeremy G; Reimerink, Johan H J; Bendall, Richard P; Pas, Suzan D; Ellis, Vic; van Alfen, Nens; Beynon, Laura; Southwell, Lucy; McLean, Brendan; Jacobs, Bart C; van Engelen, Baziel G M; Dalton, Harry R. Neuralgic amyotrophy and hepatitis E virus infection.
Neurology, 11 February 2014, vol./is. 82/6 (498-503)

15. van den Berg, Bianca; van der Eijk, Annemiek A; Pas, Suzan D; Hunter, Jeremy G;

Madden, Richie G; Tio-Gillen, Anne P; Dalton, Harry R; Jacobs, Bart C
Guillain-Barre syndrome associated with preceding hepatitis E virus infection.
Neurology, 11 February 2014, vol./is. 82/6 (491-497)

16. Beale MA, Tettmar K, Szypulska R, Tedder RS, Ijaz S. Is there evidence of recent hepatitis E virus infection in English and North Welsh blood donors?Vox Sang. 2011;100:340-2.

17. Hewitt PE, Ijaz S, Brailsford SR, Brett R, Dicks S, Haywood B, Kennedy IT, Kitchen A, Patel P, Poh J, Russell K, Tettmar KI, Tossell J, Ushiro-Lumb I, Tedder RS. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet. 2014 Jul 26. pii: S0140-6736(14)61034-5. doi: 10.1016/S0140-6736(14)61034-5. [Epub ahead of print]

18. Hogema BM, Molier M, Slot E, Zaaijer HLPast and present of hepatitis E in the Netherlands. Transfusion. 2014. doi: 10.1111/trf.12733. [Epub ahead of print].

19. Gallian P, Lhomme S, Piquet Y, Sauné K, Abravanel F, Assal A, Tiberghien P, Izopet J. Hepatitis E virus infections in blood donors, France.
Emerg. Infect. Dis. 2014 Nov;20 (11): 1914-7

20. Hogema BM, Molier M, Slot E, Zaaijer HLPast and present of hepatitis E in the Netherlands. Transfusion. 2014. doi: 10.1111/trf.12733. [Epub ahead of print] .

21. Slot E, Hogema BM, Riezebos-Brilman A, Kok TM, Molier M, Zaaijer HL

Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. Euro Surveill. 2013 Aug 1;18(31).pii:20550.

22. Vollmer T, Knabbe C, Dreier J

J Clin Microbiol. 2014 Jun;52(6):2150-6. doi: 10.1128/JCM.03578-13. Epub 2014 Apr 16.

Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors.

23. Cleland A, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L, Petrik J
Vox. Sang. 2013 Nov;1054(4):283-9. Hepatitis E virus in Scottish blood donors.

24. Juhl D, Baylis SA, Blümel J, Görg S, Hennig H. Seroprevalence and incidence of hepatitis E virus infection in German blood donors. Transfusion. 2014 Jan;54(1):49-56

25. Mansuy JM, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V, Rech H, Destruel F, Kamar N, Dalton HR, Izopet J. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis. 2011;17: 2309-12.

26. Takeda H, Matsubayashi K, Sakata H, Sato S, Kato T, Hino S, Tadokoro K, Ikeda H. A nationwide survey for for prevalence of hepatitis E virus antibody in qualified blood donors in Japan. Vox Sang. 2010; 99(4):307-13.

27. Matsubayashi, K., Nagaoka, Y., Sakata, H., Sato, S., Fukai, K., Kato, T., Takahashi, K., Mishiro, S., Imai, M., Takeda, N. & Ikeda, H. (2004) Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. Transfusion, 44 (6), 934-940.

28. Boxall, E., Herborn, A. *; Kochethu, G. ++; Pratt, G. ++; Adams, D. [S]; Ijaz, S. [P]; Teo, C.-G. [P]. Transfusion-transmitted hepatitis E in a nonhyperrendemic country. [Article] Source Transfusion Medicine. 16(2):79-83, April 2006.

29. Huzly D, Umhau M, Bettinger D, Cathomen T, Emmerich F, Hasselblatt P, Hengel H, Herzog R, Kappert O, Maassen S, Schorb E, Schulz-Huotari C, Thimme R, Unmüssig R, Wenzel JJ,

Panning M. Transfusion-transmitted hepatitis E in Germany, 2013.Euro Surveill. 2014 May 29;19(21). pii: 20812. PMID: 24906377

30. Andonov A, Rock G, Lin L, Borlang J, Hooper J, Grudeski E, Wu J; Members of the Canadian Apheresis Group (CAG). Serological and molecular evidence of a plausible transmission of hepatitis E virus through pooled plasma.Vox Sang. 2014 May 15. doi: 10.1111/vox.12156. [Epub ahead of print] PMID: 24830322

31. B. Schlosser, A. Stein, R. Neuhaus, S. Pahl, B. Ramez, D.H. Krüger, T. Berg, J. Hofmann. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. Journal of Hepatology 2012 vol. 56 p500–502

32. C Koenecke, S Pischke, G Beutel, U Ritter, A Ganser, H Wedemeyer, M Eder. Hepatitis E virus infection in a hematopoietic stem cell donor Bone Marrow Transplantation (2014) 49

33. http://www.transfusionguidelines.org.uk/dsg/wb

34. Brendan A.I. Payne,Manjul Medhi, Samreen Ijaz,Manoj Valappil, Emma J. Savage, O. Noel Gill,Richard Tedder, Ulrich Schwab. Hepatitis E Virus Seroprevalence among Men Who Have Sex with Men, United Kingdom. Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 19, No. 2, February 2013 (333-335)

35. Michelle K. Yong A,C, Emma K. PaigeA, David AndersonB,D and Jennifer F. HoyA,C,E Hepatitis E in Australian HIV-infected patients: an under-recognised pathogen? Sexual Health, 2014, 11, 375–378.

36. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/328634/01_info-note-hepatitis-e.pdf

37. Juhl D, Bayliss SA, Blumel J, Gorg S, Hennig H. Serpoprevalence and incidence of hepatitis E virus infection in German blood donors. Transfusion. 2014; 54:49-56

38. Ijaz S, Vyse AJ, Morgan D, Pebody RG, Tedder RS, Brown D. Indigenous hepatitis E virus infection in England: More common than it seems. J Clin Virol 2009 44: 272-276.

39. Ijaz S, Said B, Boxall E, Smit E, Morgan D, Tedder RS. Indigenous Hepatitis E in England and Wales From 2003 to 2012: Evidence of an Emerging Novel Phylotype of Viruses. J Infect Dis. 2014 209(8):1212-8

40. Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wany H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010 376:895-902.

41. Abravanel F, Lhomme S, Chapuy-Regaud S, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Kamar N, Izopet J. Hepatitis E virus reinfections in solid-organ-transplant recipients can evolve into chronic infections. J Infect Dis. 2014 209(12):1900-6.

42. Bertollini, J. Goss,N. Curling, J.
Production of Plasma Proteins for Therapeutic Use. 2013. Wiley & Sons

43. Hauser, I. Roque-Afonso, A. Beylouné, A. Simonet, M. Deau, B.
Hepatitis E transmission by transfusion of Intercept Blood System treated plasma. lood 2014. 123:796-797

44. Owada,T. Kaneko,M. Matsumoto,C. Sobata, R. Igarashi, M. Suzuki,K. Matsubayashi,K. Mio,K. Uchida,S. Satake,M. Tadokoro, K.
Establishment of culture systems for Genotypes 3 and 4 hepatitis E virus (HEV) obtained from human blood and application of

HEV inactivation using a pathogen reduction technology system.
Transfusion 2014. 54(11):2820-7

45. Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. 1981.Incidence and severity of viral hepatitis in pregnancy. Am J Med 70:252–255.

46. Nargis Begum, Salam Gyaneshwori Devi, Syed A. Husain, Ashok Kumar & P. Kar. Seroprevalence of subclinical HEV infection in pregnant women from north India: A hospital based study 2009 Indian J Med Res 130, 709-713

47. Salam Gyaneshwori Devi,Ashok Kumar Premashis Kar, Syed Akhtar Husain, Shashi Sharma. Association of Pregnancy Outcome With Cytokine Gene Polymorphisms in HEV Infection During Pregnancy 2014 Journal of Medical Virology 86:1366–1376

48. Mónica Velosaa,b, António Figueiredo, Helena Glória, Ana Morbey, Elia Mateus, Zélia Nevesd, Ana Araújo, Ana Carvalho,Judite Oliveira, Eduardo Barroso. 2013 Fulminant hepatitis E in a pregnant woman GE J Port Gastrenterol. 20(5):210-214

49. Udayakumar Navaneethan, Mayar Al Mohajer, Mohamed T Shata. Hepatitis E and Pregnancy- Understanding the pathogenesis. 2008. Liver Int. 28(9): 1190–1199.

50. Renou C, Gobert V, Locher C, Moumen A, Timbely O, Savary J, Roque-Afonso AM; Association Nationale des Hépato-Gastroentérologues des Hôpitaux Généraux (ANGH). Prospective study of Hepatitis E Virus infection among pregnant women in France. Virol J. 2014 Apr 9;11:68.

51. Anty R, Ollier L, Péron JM, Nicand E, Cannavo I, Bongain A, Giordanengo V,Tran A. First case report of an acute genotype 3 hepatitis E infected pregnant woman living in South-Eastern France. J Clin Virol. 2012 May;54(1):76-8.

52. Tabatabai J, Wenzel JJ, Soboletzki M, Flux C, Navid MH, Schnitzler P. First case report of an acute hepatitis E subgenotype 3c infection during pregnancy in Germany. J Clin Virol. 2014 Sep;61(1):170-2.

53. Bose PD, Das BC, Hazam RK, Kumar A, Medhi S, Kar P. Evidence of extrahepatic replication of hepatitis E virus in human placenta. J Gen Virol. 2014 Jun;95(Pt 6):1266-71.

54. Mesquita JR, Conceição-Neto N, Valente-Gomes G, Gonçalves G, Nascimento MS. Antibodies to hepatitis E in Portuguese mothers and their newborns. J Med Virol. 2013 Aug;85(8):1377-8

55. Kenfak-Foguena A, Schoni-Aff olter F, Burgisser P, et al, and theData Center of the Swiss HIV Cohort Study, Lausanne, Switzerland.
Hepatitis E Virus seroprevalence and chronic infections in patients with HIV, Switzerland. *Emerg Infect Dis* 2011; 17: 1074–78.

56. Renou C, Lafeuillade A, Cadranel JF, et al, and the ANGH.
Hepatitis E virus in HIV-infected patients. *AIDS* 2010;
24: 1493–99.

57. Keane F, Gompels M, Bendall R, et al. Hepatitis E virus coinfection in patients with HIV infection. *HIV Med* 2012; 13: 83–88.

58. Kaba M, Richet H, Ravaux I, et al. Hepatitis E virus infection in patients infected with the human immunodefi ciency virus.
J Med Virol 2011; 83: 1704–16.
114 Thoden J, Venhoff N, Miehle N, et al.

59. Sherman KE, Terrault N, Barin B, Rouster SD, Shata MT. Hepatitis E infection in HIV-infected liver and kidney transplant candidates. J Viral Hepat. 2014 21(8) :e74-7.

60. Buffaz C, Scholtes C, Dron AG, Chevallier-Queyron P, Ritter J, André P, Ramière C. Eur J Clin Microbiol Infect Dis. 2014 Jun;33(6):1037-43. doi: 10.1007/s10096-013-2042-2. Epub 2014 Jan 21.

Hepatitis E in liver transplant recipients in the Rhône-Alpes region in France.

61. Riezebos-Brilman A, Verschuuren EA, van Son WJ, van Imhoff GW, Brügemann J, Blokzijl H, Niesters HG. J Clin Virol. 2013 Nov;58(3):509-14. doi: 10.1016/j.jcv.2013.08.022. Epub 2013 Sep 4.

The clinical course of hepatitis E virus infection in patients of a tertiary Dutch hospital over a 5-year period.

62. Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, Muscari F, Sallusto F, Rostaing L, Izopet J. J Infect Dis. 2010 Sep 15;202(6):835-44. doi: 10.1086/655899.

Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France.

63. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants.Gastroenterology 2011;140:1481-1489

64. Fujiwara S, Yokokawa Y, Morino K, Hayasaka K, Kawabata M, Shimizu T. J Viral Hepat. 2014 Feb;21(2):78-89. doi: 10.1111/jvh.12156. Epub 2013 Aug 12. Chronic hepatitis E: a review of the literature.

65. Unzueta A, Rakela J. Liver Transplantation 2014;20(1):15-24. Hepatitis E infection in liver transplant recipients

66. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med 2008;358:811-817

67. Versluis J, Pas SD, Agteresch HJ, de Man RA, Maaskant J, Schipper ME, Osterhaus AD, Cornelissen JJ, van der Eijk AA. Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation. Blood. 2013 Aug 8;122(6):1079-86. doi: 10.1182/blood-2013-03-492363. Epub 2013 Jun 21.

68. Zhou X, Wang Y, Metselaar HJ, Janssen HL, Peppelenbosch MP, Pan Q. Rapamycin and everolimus facilitate hepatitis E virus replication: revealing a basal defense mechanism of P13K-PKB-mTOR pathway . J Hepatol. 2014 Oct;(4):746-54.

69. Barnaud E, Rogée S, Garry P, Rose N, Pavio N. Thermal inactivation of infectious hepatitis E virus in experimentally contaminated food. Appl Environ Microbiol. 2012 Aug;78(15):5153-9

70. Yunoki M, Yamamoto S, Tanaka H, Nishigaki H, Tanaka Y, Nishida A, Adan-Kubo J, Tsujikawa M, Hattori S, Urayama T, Yoshikawa M, Yamamoto I, Hagiwara K, Ikuta K. Extent of hepatitis E virus elimination is affected by stabilizers present in plasma products and pore size of nanofilters. Vox Sang. 2008 Aug;95(2):94-100.

71. WHO Global Alert Response Hepatitis E http://www.who.int/csr/disease/hepatitis/whocdscsredc200112/en/index2.html accessed 19/08/2014

72. Lewis HC. Wichmann O Duizer E 2010 Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review Epidemiol. Infect. 138, 145–166.

Additional Reading.

A rare case of transfusion-transmitted hepatitis E from the blood of a donor infected with the hepatitis E virus genotype 3 indigenous to Japan: Viral dynamics from onset to recovery. Matsui T, Kang JH, Matsubayashi K, Yamazaki H, Nagai K, Sakata H, Tsuji K, Maguchi H. Hepatol Res. 2014 Jul 17. doi: 10.1111/hepr.12390. [Epub ahead of print] PMID: 25041213

An assessment of hepatitis E virus (HEV) in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence for HEV transmission to 362 prospectively followed recipients. Xu C, Wang RY, Schechterly CA, Ge S, Shih JW, Xia NS, Luban NL, Alter HJ. Transfusion. 2013 Oct;53(10 Pt 2):2505-11. doi: 10.1111/trf.12326. Epub 2013 Jul 7. PMID: 23829163

Autochthonous hepatitis e virus infections: a new transfusion-associated risk? Dreier J, Juhl D.
Transfus Med Hemother. 2014 Feb;41(1):29-39. doi: 10.1159/000357098. Epub 2013 Dec 30. Review. PMID: 24659945

Past and present of hepatitis E in the Netherlands. Hogema BM, Molier M, Slot E, Zaaijer HL.
Transfusion. 2014 May 29. doi: 10.1111/trf.12733. [Epub ahead of print] PMID: 24889277