Hepatitis E: an *old* infection with *new* implications

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Introduction

The availability of safe blood and blood products is an important public health issue. Improvements in donor screening and testing, pathogen inactivation[1] and removal methods, the use of serological tests with greater diagnostic efficacy and the introduction of nucleic acid testing (NAT) have resulted in a substantial drop in transfusion-transmitted infections over the last two decades[2]. Nonetheless, blood supplies remain vulnerable to emerging and re-emerging infections. In recent years, numerous infectious agents found worldwide have been identified or reconsidered as potential threats to blood supplies[3-5].

Hepatitis E virus (HEV) has long been considered an enterically transmitted virus causing self-limiting acute viral hepatitis. The disease is endemic in many developing countries, but in recent years an increasing number of autochthonous and sporadic HEV infections have been described in developed countries[6]. This virus usually causes an acute self-limiting hepatitis, but in some cases fulminant hepatic failure resulting in morbidity and mortality may occur, especially in at-risk groups such as the elderly, pregnant women and patients with pre-existing liver disease or those who are immunocompromised. Furthermore, recent seroprevalence studies are questioning the concept of the low circulation of HEV in developed countries[7].

This narrative review aims at providing a comprehensive view of HEV and its possible "role" in transfusion medicine.

History

Many years before the discovery of HEV, epidemiological evidence had already given rise to the suspicion of the existence of a new enterically transmitted hepatitis virus. An enterically transmitted non-A non-B hepatitis (NANBH) virus was first suspected by Khuroo in 1980, during an outbreak of acute viral hepatitis in the Kashmir Valley, India[8]. A few months later, Wong *et al*.[9] reported the results of the retrospective serological testing of stored sera. These sera had been in store since a widespread epidemic of NANBH that broke out in New Delhi in 1955-1956 as a result of faecal contamination of drinking water. More than 29,000 people were infected, that is 2.3% of the population residing in the affected areas. The epidemic peaked after 2 weeks and declined in about 7 weeks. The incidence of HEV infection was highest in young adults. The peculiarities of this form of hepatitis were its brief prodromal period and the high frequency of fulminant hepatic failure in pregnant women with a high mortality rate[10]. The name "enterically transmitted NANBH virus" was coined[9].

Interestingly, HEV has a long military association being first discovered during the Soviet occupation of Afghanistan in the 1980s after an outbreak of unexplained hepatitis at a military camp[11].

Nearly a decade after the initial discovery of the new virus, Reyes *et al*. isolated a complementary DNA, representing a part of the genome of the virus responsible for enterically transmitted NANBH, from bile obtained from an experimentally-infected animal[12]. They also identified similar genomic sequences in clinical specimens obtained from several geographical regions at different time-points. The molecular cloning and sequencing of the entire genome of the virus soon followed, in 1991[13].

The virus and its geographic distribution

Taxonomy, morphology and genomic organisation

HEV belongs to the genus *Hepevirus*, as its sole member, in the *Hepeviridae* family[14]. This family contains mammalian HEV infecting human beings, domestic pigs, wild boar, deer, and rodents, but also avian HEV and cutthroat trout virus, which represent a potential separate genus[11].

HEV is a 7.2 kb single-stranded RNA non-enveloped virus which has a diameter between 27 and 34 nm[11,13]. The genome of the virus contains three open reading frames (ORF) (Figure 1). ORF1 encodes a protein containing functional motifs and domains common to other positive-stranded RNA viruses. ORF2 is

responsible for virion assembly, interaction with target cells and immunogenicity. ORF3 encodes a small protein of 114 amino acids involved in virion morphogenesis and release[11]. The genome of the HEV also includes a striking hypervariable region with multiple substitutions between isolates of the same virus but its function is currently unknown (Figure 1)[15].

Although a single serotype has been described, several authors have reported a great genetic diversity between the different HEV isolates. Recent studies have proposed several classifications of HEV into different genotypes and subtypes[17]. According to the most accepted classification there are four HEV genotypes, namely genotype 1, 2, 3 and 4 (Gt1, Gt2, Gt3 and Gt4, respectively) on the basis of nucleotide sequence homology, and 24 subtypes[17] with genomic sequence similarity greater than 90%[18].

Epidemiology

Serological and molecular studies have shown that HEV is globally distributed (Figure 2)[11]. It is estimated that two billion people have been infected with HEV with 14 million symptomatic cases, and 300,000 deaths occurring annually around the world[19].

Each HEV genotype has a specific geographic distribution. Gt1 has been isolated from human cases of epidemic and sporadic hepatitis E in parts of Asia and Africa, where the disease is highly endemic, and also from travellers to these regions from low-endemic areas[11]. Gt2, first reported following an outbreak in Mexico, was subsequently described in patients in West Africa (Nigeria and Chad)[11]. Gt3, first identified in a few rare cases of locally acquired hepatitis E in the USA, was subsequently reported in several industrialised European countries[20] (the United Kingdom [UK], France, the Netherlands, Spain, Austria, Greece and Italy), Japan[21], New Zealand[22], China[23], and North America[24]. Gt4 has been found in sporadic cases of acute hepatitis in China, Taiwan, Japan, and Vietnam[11]. Gt1 and Gt2 infect only humans, whereas Gt3 and Gt4 also infect other animals, particularly pigs, and have different routes of transmission[25].

According to the prevalence rate of HEV antibody it is possible to differentiate endemic, hyper-endemic, and non-endemic countries for HEV[11]. Although the true burden of hepatitis E is unknown, as in most cases it is self-limiting and occurs in areas of the world where serology tests are not always available, its epidemiology is quite different in endemic and non-endemic countries.

In developing (endemic and hyper-endemic) countries, located in tropical and subtropical regions, hepatitis E has a common epidemiological feature, namely it occurs both sporadically and as an epidemic disease, affects a large part of the population, and is largely due to Gt1 (with Gt2 accounting for cases in Mexico and parts of Africa)[11,26]. Large outbreaks affecting thousands of people also occurred in China, India, Somalia, and Uganda[27]. The rates of anti-HEV antibody among adults in these areas range from 30% to 80%[28]. Although each HEV genotype is believed to have a specific geographic distribution (Figure 2), exceptions have been observed[11].

The recent discovery of locally-acquired cases in every developed (non-endemic) country where they were looked for (Europe, Oceania, Japan, and North America)[26] has substantially changed our understanding of HEV infections. Most of the infections are diagnosed in individuals who travel to endemic areas. However, sporadic hepatitis E associated with indigenous viral circulation has also been reported in developed countries[11,26]. Gt3 and Gt4 are responsible for autochthonous sporadic cases, mainly in non-endemic countries[20]. In non-endemic countries such as the USA, Japan, and other European countries, HEV seroprevalence rates were generally low and ranged between 0% and 7% but recent data show that they have increased up to four times[29,30]. Therefore, additional studies to clarify the real seroprevalence of HEV in the general population of these countries could be necessary[31,32].

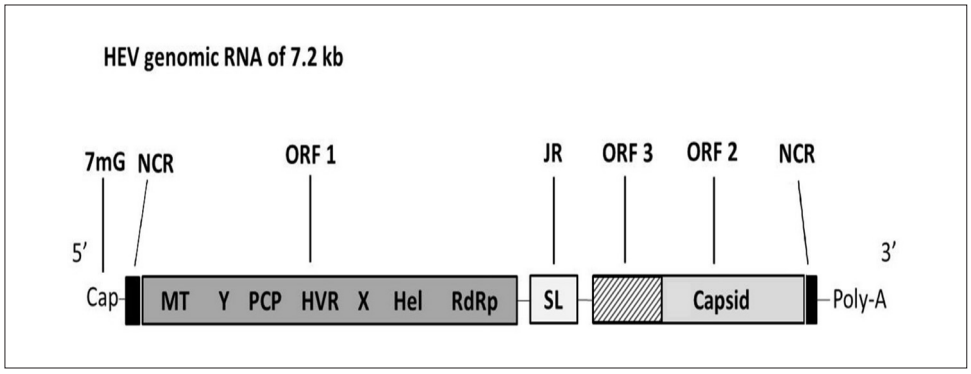

Figure 1 - A schematic representation of the genomic organisation of the hepatitis E virus (HEV). ORF: open reading frame; MT: methyltransferase; Y: Y domain; PCP: a papain-like cysteine protease; Hel: helicase; HVR: hypervariable region; X: macro-domain; RdRp: RNA-dependent RNA polymerase; JR: junction region; SL: stem-loop structure; NCR: non-coding region. Modified from Cao D *et al.,* 2010[16].

Figure 2 - Worldwide distribution of hepatitis E virus infection per genotype (Gt). Modified from Kamar N *et al*., 2012[11].

Route of transmission

The route of transmission of HEV is still, even today, one of the most controversial aspects of hepatitis E as there is a remarkable difference from one geographic area to another. In developing countries, the faecal-oral route was fully confirmed several years ago. On the other hand, the route of transmission remains unclear in infections occurring in industrialised countries[11].

The faecal-oral route is predominant in endemic countries, where hygienic conditions make the contamination of drinking water or food[20,33] the main cause of the transmission of Gt1 and Gt2 HEV to human beings[12,20].

In industrialised countries, hepatitis E is regarded as a zoonotic disease and the pig its main reservoir. A high prevalence of HEV antibodies was observed in pig farmers, slaughterhouse workers, veterinarians, and farm labourers. Food-borne disease, transmitted through the consumption of contaminated animal meat (undercooked pig liver), has been confirmed[34-36] and in Western Europe the food chain is the main source of infection[37].

Person-to-person transmission is uncommon (0.7-2.2%)[38,39], although evidence of domestic HEV transmission during a large outbreak in Uganda was recently reported[40] and dissemination of the virus by close contact with infected patients was also documented by Khuroo *et al*[41].

Nosocomial and parenteral transmission in haemophiliacs[42] and in haemodialysis patients[43] has also been reported[41]. Transplacental vertical transmission of HEV was described in the third trimester of pregnancy[34,41] and, mainly in endemic countries, there is a high rate of perinatal mortality[44].

Although sexual transmission is not frequent, homosexual males show a higher prevalence of HEV antibodies (20%) than the general population[45] and the sexual transmission of HEV is still under debate.

Cases have also been described in recipients of a variety of transplants, including kidney, liver, heart, bone marrow, and lung. In solid organ recipients the prevalence of immunoglobulin G (IgG) anti-HEV is 11.6% and that of genomic viral RNA is 2%[46-48]. However, especially in industrialised countries, the mode of transmission in most of the patients with hepatitis E cannot be determined, thus suggesting the existence of still unknown routes of transmission.

Transfusion-transmitted hepatitis E virus: fear-mongering or reality?

The history of transfusion-transmitted HEV can be traced back to 2000 when the first two cases of transfusion-transmitted hepatitis E were retrospectively reported in India although the authors were not able to unequivocally demonstrate the association of blood transfusion with hepatitis E infection through molecular diagnostic tools[49]. Indeed, the first demonstration of the transmission of hepatitis E through transfusion therapy in a developed country (Hokkaido, Japan) dates back to 2002 when Matsubayashi *et al*. showed an identical sequence of HEV (Gt4) RNA genome in

both the donor of a whole-blood fresh-frozen plasma unit and in the patient who had received the unit during open-heart surgery[50]. In 2004, the second case of transfusion-transmitted Gt4 hepatitis E, again in Hokkaido, was associated, through sequence analysis, with a zoonotic food-borne infection in a male patient who developed acute hepatitis after a platelet transfusion[51]. In this country, since 2005 in-house HEV RNA testing in mini-pools of 20 specimens has been implemented in addition to blood donor screening for elevated alanine transaminase (ALT) levels. Despite this, seven HEV-positive blood products were transfused and two cases of post-transfusion hepatitis E occurred before March 2006, when HEV NAT started to be systematically used only for qualified donors (i.e. donors who passed the serological tests for hepatitis B virus, hepatitis C virus, and human immunodeficiency virus 1⁄2 and also had ALT levels below 60 IU⁄L), thus preventing the further release of HEV-positive blood products[52].

In different parts of Japan, two additional patients developed post-transfusion hepatitis E and the plasma fractionator of the Japanese Red Cross detected HEV RNA in three units of source plasma[52-54]. In 2006 and 2007, two post-transfusion HEV infections were reported in European countries: the UK[55]and France[56].

The transfusion of infected blood products was, therefore, included in the possible routes of transmission of HEV infection but the question posed by the risk of HEV parenteral transmission to transfusion safety is still largely unanswered[57] although the above case reports have paved the way to a growing debate in the transfusion medicine community.

Unfortunately, retrospective studies in transfusion recipients have been inconsistent. Studies from non-endemic areas have shown no clear association of HEV seroprevalence with previous transfusion[58], while retrospective studies from endemic areas suggested the possibility of transmission through blood transfusion and also highlighted a significantly higher prevalence of markers of acute HEV in transfusion recipients[49,59].

Interestingly, multi-transfused patients have a significantly higher incidence of markers of HEV infection (i.e. IgG/IgM anti-HEV and HEV-RNA) than those who have received fewer blood transfusions and five patients who developed post-transfusion hepatitis E had haematological diseases[52,55,57,60-62].

Many studies show that any blood product, including red blood cells[55,57,60,61], platelets[51,61], and fresh-frozen plasma[50,62,63] can transmit HEV but the viral load required to induce transfusion-transmitted hepatitis E in recipients is unclear[64,65].

The presence of HEV-RNA has been reported in both mini- and large-plasma pools[63,66], including those for fractionation[67]. In fact, HEV RNA was detected in four out of 41 plasma pools for fractionation obtained from Europe and North America[67]. Notwithstanding these findings, different seroprevalence rates of HEV in patients with haemophilia were reported[42,68] and it is still unclear whether HEV may be transmitted to recipients of plasma-derived medicinal products. In fact, several factors may play a significant role in preventing its transmission, such as the dilution of any initial viral load, the presence of neutralising antibodies in the plasma pools, and the size of the plasma pools[69-71]. Interestingly, a recent work provides, for the first time, indirect evidence of HEV transmission through solvent/detergent plasma in two patients with thrombotic thrombocytopenic purpura[72].

Furthermore, the high seroprevalence of infection in asymptomatic individuals raises the potential risk of HEV transmission through blood transfusion[57]. Hepatitis E should therefore be considered as a risk to transfusion safety, especially in high-risk recipients (pregnant females, patients with pre-existing chronic liver disease, and immunocompromised patients), for two reasons. Firstly, the HEV-positive donor may have asymptomatic viraemia with normal aspartate transaminase and ALT and[57,73,74], secondly, the time of seroconversion is not clearly defined[75].

Viraemia in individuals infected with HEV is usually of short duration, but there are reported instances of protracted viraemia, such as after acute HEV hepatitis in children[76]. A brief incubation period can be followed by a symptomatic phase although the infection in the recipient is generally asymptomatic apart from mild jaundice and elevated ALT.

However, a thorough evaluation of the incidence of transfusion-associated hepatitis E is lacking probably also because it has been recorded only in several case reports from Europe and Japan showing the transmission of HEV through blood products donated by HEV-infected donors[49-53,55,56,60,62]; for the same reason its natural clinical course is not well known but in high-risk recipients it is thought to be associated with considerable morbidity and mortality[57].

Seroprevalence of hepatitis E virus in blood donors worldwide

Hepatitis E is an enterically transmitted disease (water-borne), which can cause large epidemics due to Gt1 or (to a lesser extent) to Gt2 in developing countries. In industrialised countries, the autochthonous hepatitis E cases are caused by Gt3 (mainly) or Gt4 of zoonotic origin, which can occasionally be transmitted also via the parenteral route. Therefore, the original mode of (enteral) transmission is no longer thought to be the only route of HEV transmission[11]. Besides this paradigm shift, HEV is also considered a re-emerging infectious

disease. The rapidly growing number of seroprevalence studies published over the past 20 years shows the ever-increasing interest in HEV among the transfusion medicine community.

However, to establish whether HEV may be a risk to transfusion safety and, more in general, a problem for public health, the first step is to assess its real seroprevalence in blood donors and the general population. In addition, as the prevalence rates of HEV vary greatly not only over time in the same country[29,30], but also from one country to another and from one geographical area to another[57], extensive seroprevalence studies should be carried out in each single country to assess how widespread HEV is at a local level.

Actually, in the last two decades, many studies have examined the prevalence of HEV in several countries, but most of them dealt with the prevalence of HEV in adults or in selected groups of subjects or small communities in a vast territory (Appendix 1)[22,29,30,58,66,67,74,77-129]. These methodological choices and the lack of sensitivity/specificity of the detection methods are real flaws as it was often impossible to determine the true incidence of HEV and, consequently, the real frequency of transmitted HEV infection might have been underestimated[57]. Naturally, the same applies to the seroprevalence in blood donors.

In this review article, we have analysed several studies published between 1994 and 2014. The data reported on blood donor populations substantially confirm the great difference between non-developed countries (endemic and hyper-endemic) and developed countries (non-endemic) and seem to reflect the prevalence found in the respective general populations.

The seroprevalence of HEV ranges from 0.26%[116] to 52.5%[30] for IgG (including those studies only using the generic term "HEV antibodies"), from 0.4%[58] to 5.9%[112] for IgM, and from 0%[58] to 14.6%[83] for HEV-RNA (when reported).

Data from studies analysing the seroprevalence in donors with elevated ALT showed higher rates of IgG in this cohort of subjects (range, 3.2%[101] to 7.5%[87]) in comparison to those without elevated ALT. In addition, the prevalence of IgG anti-HEV was significantly higher in rural areas (41.7%[95]) than in urban areas (22.7%[96]) of China, in eastern Japan (5.6%) than in western Japan (1.8%) ($p<0.001$)[100], and varied greatly between different states/regions of the USA (range, 1.2%[90] to 21.3%[91]) and Europe (range, 0.26%[116] to 52.5%[30]) (Appendix 1).

Published data on the estimated incidence of viraemic donations are scarce[57,130]. In Europe, in the western part of which a high incidence and prevalence of HEV has been reported, a rough estimate of the incidence of viraemic donations ranges from 1:1,000 to 1:15,000[131] (Mayr W, personal communication, Workshop on "Qualification of new blood donors before donation: Pros & Cons", Rome, Italy, February 3rd, 2014) .

In some countries the seroprevalence of anti-HEV IgG has been stable over time, being about 5% from 2007 to 2012 in Japan[99-102], about 3% from 2000 to 2012, from 1999 to 2010, and from 1994 to 2012 in Brazil[84-87], Spain[126,128], and Italy[118-121], respectively, and about 18% from 1997 to 2013 in the USA[58,89-91]. On the other hand, in other countries IgG seroprevalence has increased over time (Germany: from 5.5%[111] in 2010 to 15.5%[114] in 2013; Greece: from 0.26%[116] in 1998 to 9.43%[117] in 2013; France [Midi-Pyrénées region]: from 16.6%[29] in 2008 to 52.5%[30] in 2011).

In about two-thirds of the studies the population analysed was exclusively composed of healthy blood donors, while about one-third evaluated selected groups of the population (patients with hepatitis, workers at zoonotic risk, rural populations, etc.) and reported a higher seroprevalence[30,77,79,81,82,84,86,87,89,91,104,107,114-116,119,123,127,128] (see Appendix 1). It is, however, unclear how much the differences represent true changes in prevalence or just changes in methodology as there is no recognised standard for these assays.

Laboratory diagnosis of hepatitis E infection

HEV can be diagnosed either directly by detecting its nucleic acids or more frequently indirectly, due to the relative short duration of viraemia, by detecting the immune response in the host through serological techniques (Figure 3).

Serological techniques

As in other forms of viral hepatitis, after an incubation of 2 to 8 weeks, viraemia arises followed by IgM and IgG antibodies and HEV is also present in stools (Figure 3)[11,132]. Recovery is characterised by viral clearance, an increase in IgG titres, and a decrease in IgM levels[133]. The duration of viral shedding is variable, as is the presence of antibodies. IgM antibodies remain detectable for 3 to 12 months, whereas IgG antibodies persist for years, if not for life[28]. The four HEV genotypes elicit very similar antibody responses, as if they were a single serotype[11,134].

The serological diagnosis of acute hepatitis E is usually performed through enzyme immunoassays or rapid immunochromatographic kits, which detect specific IgM antibodies directed against a range of recombinant viral antigens[11,135]. The detection of IgG antibodies is more problematic as the available enzyme immunoassays use different antigens and have variable effectiveness[11,28,136]. Moreover, as most assays have been validated with sera from patients with recent hepatitis E their suitability for other purposes, such as detecting immunity or previous infection, is not known[11].

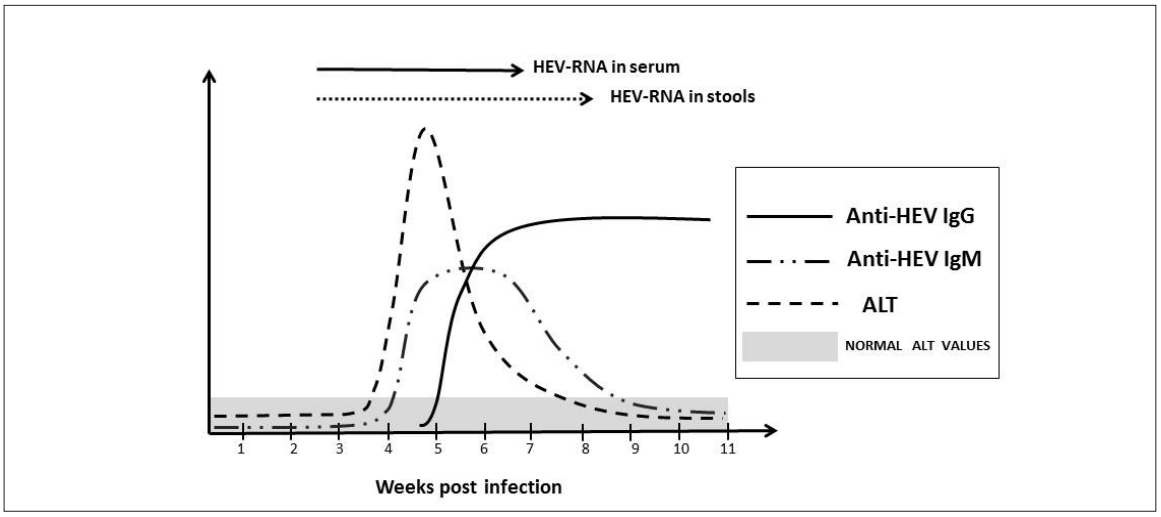

Figure 3 - Schematic representation of the course of acute HEV infection.
HEV RNA becomes detectable in stools and serum during the incubation period, with the subsequent appearance of IgM and IgG HEV antibodies. The level of IgM antibodies peaks early and then these antibodies become undetectable during recovery, whereas the level of IgG antibodies keeps on increasing and can persist in the long term. Clinical symptoms (fatigue, nausea, and jaundice) begin shortly after the increase of serum alanine aminotransferase (ALT) levels. HEV RNA disappears from the serum with recovery, whereas the virus usually remains detectable longer in stools (arrows). Modified from Hoofnagle JH, 2012[28].

Finally, although specific assays for IgG and IgM HEV antibodies are available commercially their sensitivity and specificity vary greatly. The use of more sensitive IgG assays has led to a three to four-fold increase in estimates of HEV seroprevalence and, by inference, rates of infection[28]. As different antibody testing assays produce different seroprevalence data, only by using the same assays can the rates of HEV antibodies in the various populations be compared[28,37,136].

Molecular techniques

The detection of HEV RNA has a key role in the diagnosis (especially in immunosuppressed patients without HEV antibodies and with persistent infection[137]), confirmation, and monitoring of HEV infection as well as determining the response to antiviral therapy in patients with chronic hepatitis[138]. However, the variability of assay sensitivity highlights the need for the standardisation of HEV RNA assays as well[139].

In patients with an acute HEV infection, viral RNA can be detected in both blood and stool samples just before the onset of clinical symptoms. It does not persist for long and becomes undetectable in blood about 3 weeks after the onset of symptoms while it is shed in stools for a further 2 weeks[11,28]. The period in which RNA can be detected is usually short. The asymptomatic window of infectivity in (the great part of) asymptomatic subjects coincides with the 4 to 6 weeks of the viraemic phase[140] while in (the small portion of) symptomatic cases it can last 3 to 4 weeks (Figure 3). The very high rate of asymptomatic infections complicates the detection of potentially infected blood donors and also explains the lack of comprehensive data on donors presenting with asymptomatic viraemia at the time of donation.

Clinical features

After a short prodromal phase, the most common symptom of hepatitis E is jaundice, which can be accompanied by asthenia, fever, malaise, arthralgia, vomiting, and abdominal pain[28,141]. In symptomatic patients, the rate of mortality ranges from 0 to 10%[38] (some studies report 0.2 to 4%)[25,31]; it is higher in infants under 2 years of age for unknown reasons, and ranges from 10 to 25% in pregnant women. Maternal mortality occurs mainly in the third trimester caused by fulminant hepatic failure and obstetric complications[11,25,28,31].

Acute hepatitis E

Acute HEV infection is usually a self-limiting illness lasting less than 6-7 weeks. As already said, the clinical pictures range from subclinical or asymptomatic forms to fulminant hepatic failure. In industrialised countries the most common clinical presentation is acute hepatitis but sporadic cases are frequently misdiagnosed as drug-induced liver injury or autoimmune hepatitis, and HEV infection is frequently detected only with retrospective serological testing[142]. Alcohol consumption is a risk factor because it favours overt disease and is related to its severity.

Chronic hepatitis E

Chronic infection is defined by the persistence of (Gt3)[11] HEV RNA and/or HEV IgM antibodies in serum or stools for more than 6 months in association with increased liver enzyme levels. HEV infection can progress

to chronic liver disease mainly in immunocompromised patients[134] but also in immunocompetent individuals[143]. Most of the patients are asymptomatic. The chronicity rate is very high in transplant recipients (more than 50%), with a rapid progression to liver fibrosis[144]. Interestingly, a low risk of HEV reactivation was reported after allogeneic stem cell transplantation[145] and no reactivation after kidney transplantation[146].

A very recent study of Gt3 isolate from a patient with a chronic infection revealed a recombinant viral-host genome that was infectious to swine, deer and human hepatocytes *in vitro*[147]. This cross-species adaptation of zoonotic HEV strains and their pathogenicity in humans could also possibly explain why chronic HEV infection is unique to Gt3.

Extra-hepatic manifestations

In the last few years, several HEV-associated neurological syndromes have been described, the majority of which in the Indian subcontinent and related especially to Gt1 HEV. These complications, which appear to be viral load-dependent, include Guillain-Barré syndrome[148], Bell's palsy[149], neuralgic amyotrophy[150] and acute meningo-encephalitis[151]. Recently, neurological complications were also reported to have occurred in patients with acute and chronic infections due to Gt3 HEV[152].

Pancreatitis is also frequently reported in the second or third week after the onset of jaundice but most patients recover spontaneously[153]. Other complications include rash and arthralgia[154], thrombocytopenia associated with an immune mechanism[155], haemolysis and other immunological manifestations such as membranoproliferative and membranous glomerulonephritis[156], and Schonlein-Henoch purpura[157].

Treatment and preventive strategies

The treatment of HEV infection is supportive and aimed at dealing with symptoms or complications, which are not frequent as the disease is usually self-limited and without consequences. Hospitalisation is indicated only for patients unable to maintain oral intake and with hepatic complications. Ribavirin or pegylated α-interferon monotherapy is an effective treatment for most patients with severe or chronic HEV infection[138,158,159]. Reduction of immunosuppression and administration of antiviral medicines can be considered in immunocompromised patients[134].

Antiviral treatment of HEV infections has only been reported in patients infected with Gt3 HEV. In patients infected with Gt1 and Gt2 HEV or in patients with underlying chronic liver disease the best treatment option has not yet been defined[159].

Reduction of exposure to the virus and vaccination are the available preventive strategies for HEV infection.

Improving sanitary facilities and providing clean drinking water play key roles in developing countries. In developed countries, prevention is more complex because the several possible routes of transmission are not yet fully understood. Unfortunately, passive immunisation with IgG does not seem to be effective in preventing hepatitis E[41]; at present, it seems that HEV prevention through vaccination could be a realistic possibility although recombinant vaccines are not yet widely available. There are currently two recombinant vaccines: one tested in Nepalese military[160] and the other one (HEV 239) tested in the Chinese adult population[161]. In 2007, the Nepalese recombinant Gt1 HEV vaccine was tested in a phase II controlled trial and showed 95.5% efficacy in preventing infection and clinical disease; its safety and efficacy in women was not established. Three years later, the HEV 239 vaccine was registered in China and is now marketed in that country alone.

Unanswered questions and future perspectives in Transfusion Medicine

Hepatitis E has been known for some time but only now is it showing its true face. Although our understanding of HEV has changed enormously over the past 10 years there are still many unanswered questions regarding the biology of this virus and the clinical course of the infection it causes, such as the replicative cycle, the cell-surface receptors, tissue and species specificity, the reason why only Gt3 has been associated with chronic infection, the unexplained high severity in pregnancy, and the current clinical impact of the various genotypes (Gt1, Gt2 *vs* Gt3, Gt4)[11].

Moreover, we need to identify other geographical areas of hyperendemicity also in developed countries and obtain accurate estimates of incidence and prevalence of HEV infection in the general population as well as in blood donors in order to estimate the incidence of viraemic donations with the greatest precision possible. However, a more thorough knowledge of the real epidemiology of HEV also requires more sensitive and more specific serological tests for IgM and total HEV antibodies as well as more effort to standardise both serological[162,163] and molecular tests[139].

As far as preventive strategies are concerned, effort by manufacturers is essential for the production of vaccines, which could play a crucial role in the control and elimination of the disease in endemic areas.

Last, but not least, although the clinical impact of transfusion-transmitted HEV is unclear, the transmission of HEV by transfusion does occur and causes clinical hepatitis in recipients[57,131]. Patients who receive many blood products (e.g. immunosuppressed patients or subjects with chronic liver disease) have a significantly higher probability of coming into contact with the virus and of developing acute or chronic hepatitis than

those who have received fewer blood transfusions[57]. In addition, it is also clear that any blood product can transmit HEV[57] and, interestingly, approximately 10% of the plasma pools for fractionation contain HEV RNA[67,164]. In this regards, a recent proposal to amend the European pharmacopoeia monograph 1646 -human plasma (pooled and treated for virus inactivation)- would see the introduction of HEV NAT in January 2015[165].

However, the viral load required to induce transfusion-transmitted hepatitis E in recipients is unclear and further investigation is required to clarify this issue[64,65]. Theoretically, the prevention of transmission through blood products is feasible by screening donated blood[32,57]. Unfortunately, at the moment, the lack of HEV screening of blood donors jeopardises the real assessment of the frequency of transfusion-transmitted HEV infection in developed countries, as well as the real estimate of seroprevalence of the virus in various geographical areas[57].

The solutions to all the above, still unresolved issues are necessary to set the framework for cost-benefit analyses before the possible implementation of a programme of systematic HEV screening of blood donors in areas with high HEV seroprevalence.

Conclusions

Three milestones about the possible risk of transfusion-transmitted hepatitis E have been reached, namely: (i) HEV transmission through blood product transfusion and post-transfusion hepatitis E have been clearly documented although the viral load required to induce transfusion-transmitted hepatitis E in recipients is unclear[64,65]; (ii) probably many cases of post-transfusion hepatitis E are unrecognised; and (iii) HEV IgM antibodies and HEV RNA are frequently detected in blood donors in several countries[166].

The ever-increasing number of papers on HEV published annually in peer-reviewed journals is the proof of the growing attention of the scientific community, which is striving to provide exhaustive answers to the open questions on this virus. The transfusion medicine community eagerly shares this effort since it is more than aware that more data are needed to propose recommendations on the management of this *old* infection with possible *new* implications for blood supply safety.

New answers will hopefully come from future and ongoing national HEV studies such as the look-back study on HEV-RNA positive donations, which is being carried out in the UK and the results of which are expected to be published soon (Mayr W, personal communication, Workshop on "Qualification of new blood donors before donation: Pros&Cons", Rome, Italy, February 3rd, 2014) or by exploiting available donor-recipient repositories such as the Retrovirus Epidemiology Donor Study Allogeneic Donor and Recipient (RADAR) repository[166], which is an invaluable resource for studies of infectious agents and their transmissibility through transfusion and can really provide transfusion medicine specialists, whose tasks also include the prevention and management of possible adverse effects of blood product transfusion, with a useful "prospective perspective on the past"[167].

Keywords: hepatitis E virus, HEV prevalence, HEV genotype, transfusion-transmitted hepatitis, blood donor.

Authorship contributions

All Authors made substantial intellectual contributions to the preparation of this article.

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