Hepatitis E

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Hepatitis E virus (HEV) was discovered during the Soviet occupation of Afghanistan in the 1980s, after an outbreak of unexplained hepatitis at a military camp. A pooled faecal extract from affected soldiers was ingested by a member of the research team. He became sick, and the new virus (named HEV), was detected in his stool by electron microscopy. Subsequently, endemic HEV has been identified in many resource-poor countries. Globally, HEV is the most common cause of acute viral hepatitis. The virus was not initially thought to occur in developed countries, but recent reports have shown this notion to be mistaken. The aim of this Seminar is to describe recent discoveries regarding HEV, and how they have changed our understanding of its effect on human health worldwide.

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Virus biology and evolution

Hepatitis E virus (HEV) belongs to the genus *Hepevirus* in the *Hepeviridae* family. This family contains mammalian HEV infecting human beings, domestic pigs, wild boar, deer, and rodents,[1,2] but also avian HEV[3] and cut-throat trout virus,[4] representing a potential separate genus. The two latter groups share about half of the nucleotide sequence of mammalian HEV strains and have not been associated with cases in human beings.[3,4]

HEV is a small non-enveloped virus with a size of 27–34 nm, and has a positive-sense, single-stranded, 7·2 kb, RNA genome, which is capped at the 5′ termini and polyadenylated at the 3′ termini.[5,6] The HEV genome contains three open reading frames (ORF). ORF1 encodes a protein of 1693 aminoacids containing functional motifs and domains present in the non-structural proteins of other positive-stranded RNA viruses.[7] These functional domains include methyltransferase, protease, RNA helicase, and RNA-dependent RNA polymerase. ORF2 encodes the viral capsid protein of 660 aminoacids that is responsible for virion assembly,[8] interaction with target cells,[9] and immunogenicity.[10] ORF3, which overlaps ORF2, encodes a small protein of 114 aminoacids involved in virion morphogenesis and release.[11,12]

Structural analysis of virus-like particles obtained with truncated HEV capsid protein by cryoelectron microscopy and crystallography provide a basic understanding of the HEV capsid organisation.[13–16] Three domains have been defined: the shell domain (S; aminoacids 129–319), the middle domain (M; aminoacids 320–455), and protruding domain (P; aminoacids 456–606). These studies placed the neutralising epitope(s) in the P domain of ORF2. Sucrose-density gradient fractionation revealed that HEV particles in serum have lower density (1·15–1·16 g/mL) than do HEV particles in faeces (1·27–1·28 g/mL), reflecting the fact that serum HEV particles are associated with lipids.[17]

HEV has proven difficult to cultivate. An efficient culture system for HEV genotype 3 (HEV3) was established for the first time with PLC/PRF/5 hepatic carcinoma cell lines and A549 lung carcinoma cell lines and faecal samples from a patient with acute hepatitis in Japan containing a very high titre of HEV RNA.[18] These cell lines also permitted the propagation of HEV genotype 4 (HEV4) from a faecal extract[19] as well as HEV genotype 1 (HEV1), HEV3, and HEV4 from serum samples.[17] Recently, another strain of HEV3 from a chronically infected patient was adapted to grow in the HepG2/C3A hepatoma cell line.[20] Culture systems are essential for a better understanding of the biology of HEV and potential targets for the development of antiviral drugs.

Molecular characterisation of various HEV strains circulating among human beings and animals has led to the recognition of four major genotypes, representing a single serotype. HEV1 and HEV2 are restricted to human beings and transmitted via contaminated water in developing countries. HEV1 occurs mainly in Asia, and HEV2 in Africa and Mexico. HEV3 and HEV4 infect human beings, pigs, and other mammalian species and are responsible for sporadic cases of autochthonous hepatitis E in both developing and developed countries.[21] HEV3 has a worldwide distribution. By contrast, HEV4 mostly occurs in southeast Asia,[22] but has recently been isolated in European pigs.[23] Although HEV3 and HEV4 infections have been linked to the consumption of raw or undercooked pork or game meat,[24,25] the full range of species that are reservoirs for HEV is still unknown. On the basis of full-length genome-sequence analyses, HEV genotypes have recently been characterised in rats in Germany,[26] wild boars in Japan,[27] and farmed rabbits in China.[28] HEV1 can be classified into five subgenotypes, HEV2 into two, HEV3 into three, and HEV4 into seven.[22] Phylogenetic analyses show that HEV subgenotypes circulating in human beings and animals in the same area are closely related, supporting zoonotic transmission.[29] Studies of the evolutionary history and population dynamics of HEV show that HEV has evolved through a series of steps, in which the ancestors of HEV might have adapted to a succession of animal hosts leading to human beings.[30]

Search strategy and selection criteria

We searched Medline, Current Contents, and references from relevant articles published between January, 1986, and September, 2011; many articles were identified through searches of the extensive files of the authors. Search terms were "hepatitis E", "HEV transmission", "HEV genotypes", "HEV serology", and "the discovery of HEV".

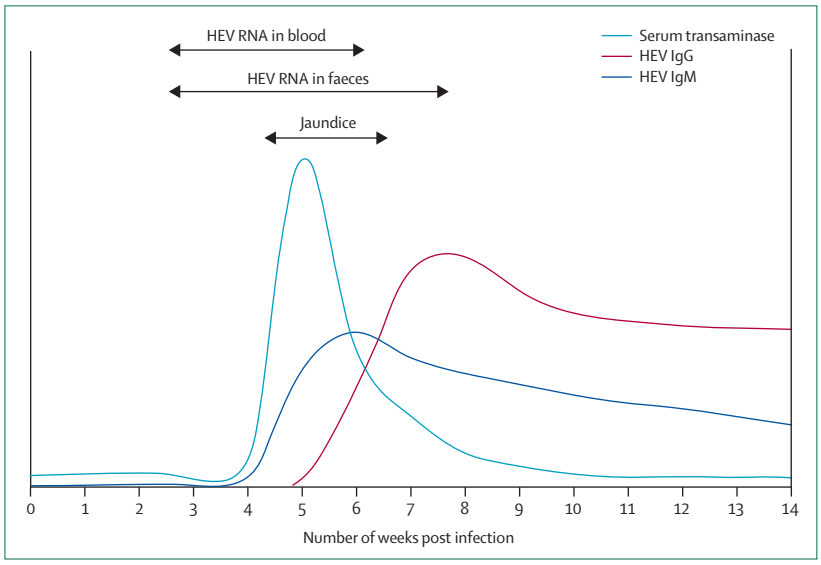

***Figure 1*: Schematic representation of HEV infection, showing virus detection at different sites and serological response**
Reproduced from Dalton and colleagues,[21] by permission of Elsevier.

Few data are available regarding differences in pathogenicity between HEV genotypes. In a Japanese study,[31] patients with HEV4 had a significantly higher peak alanine transaminase level than did patients with HEV3. Further studies are needed to clarify whether genotype is associated with the severity and outcome of HEV infection.

Laboratory diagnosis of HEV infection

HEV can be diagnosed either directly by detecting its nucleic acids or indirectly by detecting an immune response in the host. The initial diagnosis is commonly made indirectly with serological techniques, because of the ease of these techniques and the short duration of viraemia.

Serology

After an incubation period of 2–6 weeks, the immune response to HEV follows the usual pattern: an initial short-lived IgM response followed by more durable IgG antibodies (figure 1).[32] As the response matures the low avidity antibodies are replaced by antibodies with higher avidity.[33] Although four genotypes of HEV are recognised, they elicit very similar antibody responses and appear to represent a single serotype.[34,35]

The diagnosis of acute hepatitis E usually depends on the detection of specific IgM antibodies directed against a range of recombinant viral antigens by enzyme immunoassay or rapid immunochromatographic kits.[36] Comparative studies[29,37] show that these tests differ substantially in their accuracy and users should ensure that a test is used that has been validated in their population. Confirmation of acute cases detected in this way is either by molecular techniques, detecting rising reactivity in a specific IgG assay, or positivity in immunoblot IgM assays.[38]

The determination of immunity or previous exposure to HEV by detection of IgG antibodies is more problematic. Available enzyme immunoassays use different antigens and vary in their effectiveness.[39,40] Additionally, most assays have been validated with sera from patients with recent hepatitis E so their suitability for other purposes such as detecting immunity or previous infection is not known. A concentration of anti-HEV antibody that reliably prevents infection has not been defined, but a vaccine study[41] suggests antibody concentrations of 20 Walter Reed units/mL (2·5 WHO units/mL) are protective. The cutoff for some commercial assays is close to this figure,[33,42] so they might not reliably detect protective concentrations of anti-HEV IgG. Similarly, in some cases, anti-HEV IgG is undetectable or disappears rapidly in some assays,[33,43] which make them unsuitable for detecting previous infection. This variability might also account for the differing estimates of HEV seroprevalence in some populations. The use of more sensitive IgG assays has led to a three-times or four-times increase in estimates of seroprevalence,[39,44] thus direct comparisons of seroprevalence (and by inference, rates of HEV infection) between populations can really only be made if the same assay has been used. IgG immunoblot assays have been used to confirm the results of anti-HEV IgG testing, but this technique is unreliable because a study[38] in the Netherlands calculated the specificity of IgG immunoblot to be only 66%.

Molecular techniques

The detection of HEV RNA in samples is an important method for the diagnosis, confirmation, and monitoring of HEV infections. However, a recent investigation into nucleic-acid-based assays highlighted the variability in the accuracy of these methods between different laboratories.[45] This study formed part of initial investigations into establishing a WHO international standard and clearly showed the need for producing suitable control material with which to standardise detection assays and to accurately quantify HEV RNA.

In patients with an acute HEV infection, peak viraemia occurs during the incubation period and early phase of disease (figure 1). After exposure, viral RNA can be detected just before the onset of clinical symptoms in both blood and stool samples. HEV RNA does not persist for long, becoming undetectable in blood about 3 weeks after the onset of symptoms. The virus is shed in stool for a further 2 weeks.[46,47] The window of detectable RNA is therefore narrow and if patients present late in their illness, an undetectable HEV RNA result does not exclude recent infection.

Nucleic-acid-based methods of detection are crucial in the diagnosis of patients with persistent HEV infections. These infections occur in immunosuppressed individuals in whom antibodies to HEV might

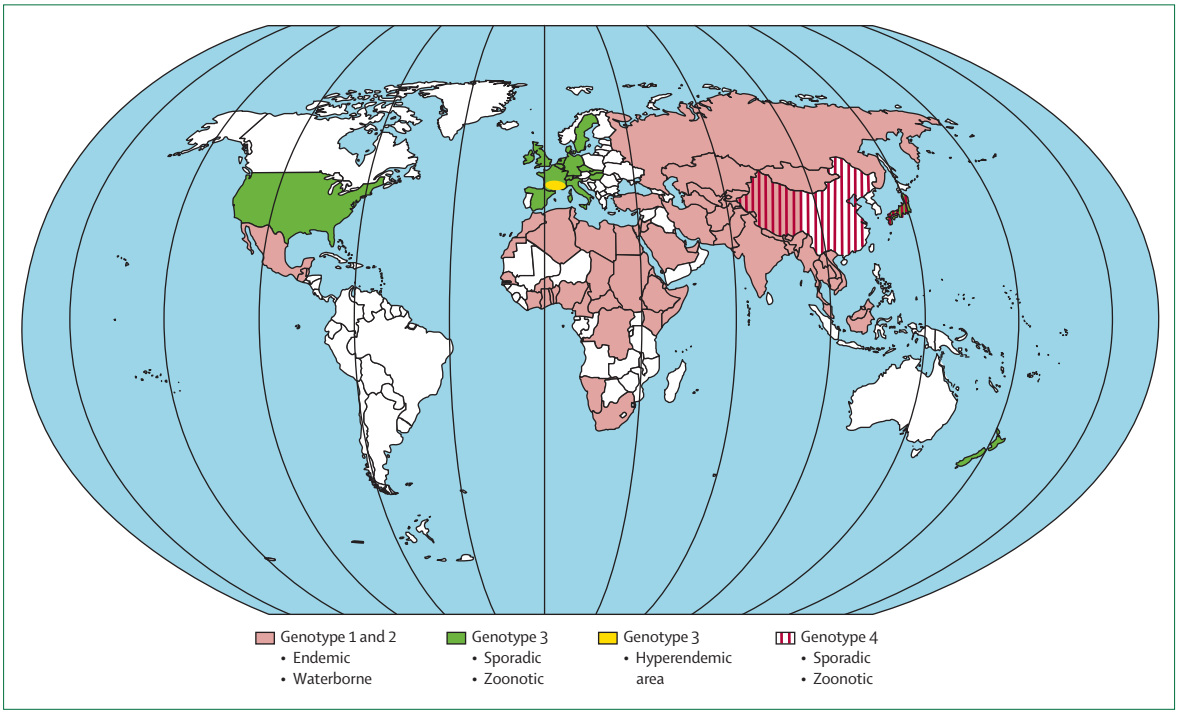

***Figure 2*: Worldwide distribution of clinical cases of HEV infection**
Note, that in several countries, including in South America, there have been occasional reports of HEV3 infection. Countries left blank are those with insufficient data.

be absent and are therefore unreliable markers for infection.[48] Emerging data on the use of antiviral treatment in patients with chronic hepatitis E has highlighted the role for HEV RNA detection and quantification in monitoring response to therapy.[49]

Cellular immunity

T-cell responses to previous HEV infection could prove to be a useful test for confirming positive serology and for understanding the mechanisms of chronic infection. Although some techniques[50] have only detected a short-lived response, others have detected a memory T-cell response to HEV antigens.[51]

Acute HEV infection

HEV in developing countries

Epidemics of hepatitis E occur periodically throughout the developing world, and are mainly caused by HEV1 in Asia and HEV2 in Africa and Mexico (figure 2). The first, retrospectively identified outbreak of hepatitis E caused 29 300 cases in India from 1955–56.[52] Other large outbreaks affecting thousands of people have occurred in China,[53] India,[54] Somalia,[55] and Uganda.[56] In addition to epidemic infection, sporadic cases of HEV occur throughout endemic regions. Infection is mainly transmitted via faecally-contaminated water (figure 3). Person-to-person spread is uncommon, but a recent study from Uganda suggests that household factors are important in outbreaks.[57] Mortality rates in epidemics range from 0·2% to 4·0%. For unknown reasons, mortality is higher in infants under 2 years of age[56,58] and it reaches 10% to 25% in pregnant women. Maternal mortality occurs largely in the third trimester, and is caused by fulminant hepatic failure and obstetric complications such as eclampsia or haemorrhage.[59]

Water-borne epidemics of hepatitis E mainly affect young adults, the clinical attack rate being highest among 15–35 year-olds.[60] Men are clinically infected two to five times more than women in most outbreaks.[53,61] However, no sex difference exists in exposure to HEV,[56,62] implying that more men develop symptomatic hepatitis. Asymptomatic infections have been estimated to exceed the number of symptomatic cases by two to four times in waterborne outbreaks[62–64] and sporadic cases.[65]

China is generally judged to be an HEV-endemic area. Most infections are due to HEV1 and HEV4, though HEV3 has recently been isolated.[66] The extended waterborne outbreak of 1989 in Xinjiang Province, northwestern China[53] resulted in 120 000 cases. The outbreak was caused by HEV1 and affected mainly young adults. Since then, hepatitis E in China has occurred mainly as sporadic cases and occasional food-borne outbreaks. The predominant circulating genotype is HEV4, with only occasional HEV1 cases. Sporadic HEV4 cases are more common in elderly men.[67] This pattern of infection is similar to that seen in Europe with HEV3. The reason for the shift towards HEV4 as the predominant genotype in China is unclear, but it might reflect

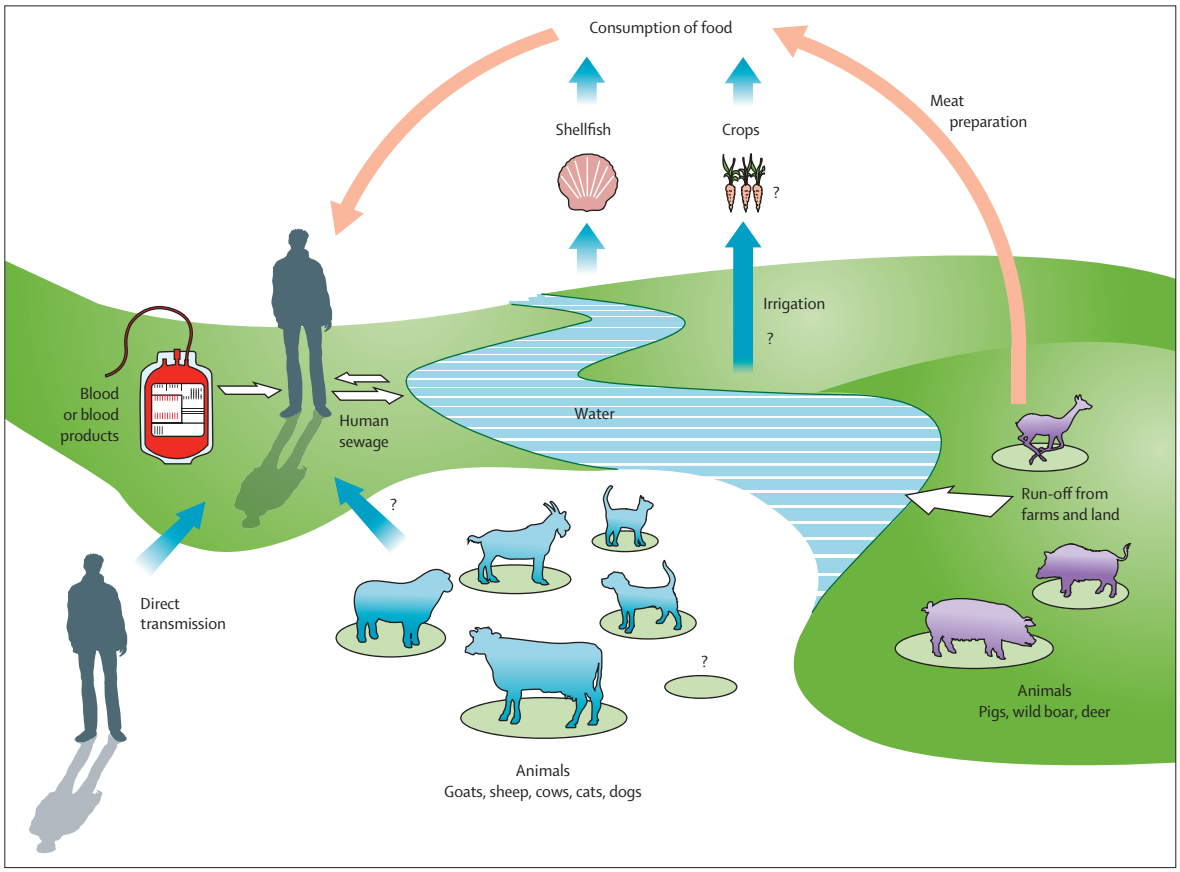

***Figure 3*: Source and route of HEV1–4 infection**
HEV1 and HEV2 are waterborne only, with possible human-to-human transmission, including vertical transmission.

improvements in water supply and the sanitary infrastructure in China over the past few decades, allowing zoonotically transmitted HEV4 to predominate in the human population.

Hepatitis E has been methodically studied in the area around Dongtai City in eastern China, the site of an HEV vaccine study.[68] Community-based studies of a population of about 500 000 before, during, and after the vaccine trial have produced important insights into HEV epidemiology. HEV (mostly HEV4) is the most common and clinically severe cause of acute viral hepatitis in this setting. Clinically apparent infection has an incidence of 0·028% and is more common in elderly men and patients with chronic hepatitis B infection. 17% of these cases are actually reinfections. Whether these cases are due to viruses of the same or different genotypes to the original infection is not known. Reinfection usually results in a less severe hepatitis and is more common in young women. The incidence of anti-HEV IgG seroconversion is 1·76%, suggesting that about 98% of infections are asymptomatic. These data might be relevant to HEV in developed countries, since HEV3 and HEV4 have a similar epidemiology.[31]

HEV in developed countries

The discovery of locally acquired cases in developed countries has substantially changed our understanding of HEV infections. In recent years, autochthonous HEV3 infections have been reported in Europe,[69–71] New Zealand,[72] and North America.[73] Both HEV3 and HEV4 are present in Japan (figure 2).[31,74] Autochthonous hepatitis E infection has been detected in every developed country in which it has been sought, with the possible exception of Finland.[75] As in China, acute hepatitis E is more common than hepatitis A in France, UK, and Japan.[74,76] In southwest France, HEV is hyperendemic.[44] Curiously, very few cases of hepatitis E have been reported from the USA, despite a documented seroprevalence of 21%[77] and an estimated annual incidence of 0·7%.[78] These findings probably reflect the fact that in the USA no diagnostic tests for HEV are currently licensed for use in human beings.

Estimating the burden of HEV infection in a population is difficult. Several studies report anti-HEV seroprevalence rates of less than 5% in developed countries.[79,80] The true figure could be much higher since these studies used assays with poor sensitivity. In the Toulouse region of southwest France, for instance, the seroprevalence in blood donors was initially thought to be 16%[81] but rose to

52%[44] when a more sensitive assay was used. The increase is unlikely to be due to false-positive results, as only 2% of infants from the same region were seropositive in the sensitive assay. Furthermore, the annual incidence of locally acquired HEV3 infection in transplant patients in Toulouse (assessed with molecular techniques) is 3·2%[82] and hepatitis E is common in this area.[69] A recent study[83] of UK blood donors estimated the annual incidence to be 0·2%, which equates to 120 000 infections per year and results in a seroprevalence of 11%. These findings, taken with the equivalent data from rural China[84] (annual incidence 4·3%, seroprevalence 43%), suggest that the high seroprevalence is a more accurate reflection of the cumulative incidence of infection in these areas.

Acute HEV3 and HEV4 infection in developed countries is usually a self-limiting illness that lasts 4–6 weeks.[69–72] Typically, patients present with an alanine transaminase concentration of around 1500 IU/L, but the range is wide.[70] In some cases, the increase in alanine transaminase concentrations is more modest, and occasionally it is completely normal at the time of viraemia.[72] 30 (75%) of 40 patients in one hospital-based series of patients with HEV presented with jaundice.[70] Other symptoms are non-specific (malaise, anorexia, nausea, abdominal pain, fever, arthralgia), and indistinguishable from those of other viral infections.[70] As in China, most HEV3 infections in developed countries seem to be asymptomatic (figure 4). For instance, during an outbreak of HEV3 on a cruise ship 67% of cases were either asymptomatic or had symptoms unrelated to HEV infection.[86] The high pregnancy-associated mortality in HEV1 has not been reported with HEV3 or HEV4. These genotypes can, however, have serious consequences. Studies have shown overall mortality rates of up to 10%, which could reflect case selection and high rates of comorbidity in symptomatic cases of HEV infection.[70]

The demography of locally acquired HEV in developed countries is remarkably consistent. By contrast with HEV1 and HEV2 infections in the developing world, symptomatic HEV infection is much more common in middle-aged and elderly men.[69–72] Few data exists to explain this pattern of infection. Host factors must explain why these populations are more likely to develop overt hepatitis, because seroprevalence data suggests that exposure is unrelated to age or sex.[87] Although HEV3 infection is thought to be zoonotic, identification of a definite food source or history of significant animal contact in individual cases is unusual (figure 3).[88] Two studies[86,89] have suggested that clinically overt disease is more common in people who consume excessive amounts of alcohol. This finding could be because these individuals are at greater risk of hepatic steatosis or hepatic fibrosis, which could result in a more severe host response to HEV infection.[89]

Symptomatic infection may also be misdiagnosed. For example, HEV can be mistaken for drug-induced liver injury. A UK study showed that six (13%) of 47 patients with "criterion-referenced" drug-induced liver injury had been incorrectly diagnosed, and in fact had acute HEV3 infection.[90] These findings have been confirmed by a US study that showed nine (3%) of 318 cases of suspected drug-induced liver injury were due to HEV3 infection.[91]

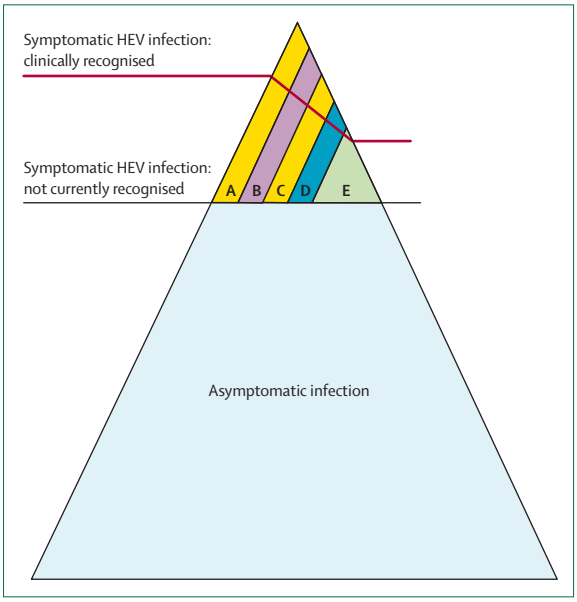

***Figure 4*: Symptomatic, unrecognised, and asymptomatic infections with HEV3 and HEV4**
Most infection with HEV3 and HEV4 is either asymptomatic or unrecognised. Current recognised clinical manifestations of HEV3 and HEV4 include: (A) acute icteric hepatitis (more common in elderly males and individuals who drink >22 units of alcohol per week) with a high mortality in patients with chronic liver disease. (B) Chronic infection (HEV3 only). It is usually asymptomatic, and anicteric, and seen in the immunosuppressed, including transplant recipients. (C) Drug-induced injury, often icteric. A few of such patients are misdiagnosed and have HEV3 infection. (D) Neurological injury, usually anicteric. HEV3 is neuropathogenic, but the incidence and scope of associated neurological injury are unknown. (E) Miscellaneous clinical syndromes (eg, HEV3 has been associated with glomerulonephritis).[85]

Acute HEV in the context of pre-existing chronic liver disease

Most deaths from HEV3 infection are caused by acute or subacute liver failure in patients with pre-existing liver disease.[92,93] Studies from the Indian subcontinent show a 12-month mortality of up to 70% in patients with HEV1 and chronic liver disease.[94] The burden of HEV infection (in terms of mortality and morbidity) in patients in the developed world with underlying chronic liver disease is unknown, since these patients are not routinely tested for HEV infection.

Two studies[95,96] have shown an association between pork consumption and mortality from chronic liver disease in developed countries. Alcohol consumption, pork consumption, and hepatitis B seroprevalence have been reported to be independent risk factors for death from chronic liver disease.[96] HEV3 has been detected in pork destined for human consumption in several countries,[25,44] which could explain this association. An

Anglo-French study is underway to determine the extent to which HEV infection accounts for hepatic decompensation in chronic liver disease.

Chronic HEV infection

HEV3 causes chronic infection, defined by persisting HEV RNA in serum or stools for 6 months or more, in immunosuppressed patients.[97,98] Most cases have occurred in solid-organ-transplant recipients.[97–101] Additionally, a few cases have been reported in individuals with HIV[48,102,103] and patients with haematological disorders receiving chemotherapy.[104–106] Chronic infection with HEV1 or HEV2 has not been reported.

Solid-organ-transplant recipients

The incidence of HEV3 infection after organ transplantation is 3·2 cases per 100 person-years in southwest France.[82] Consumption of game meat, pork products, and mussels is associated with HEV infection after transplantation.[107] 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.[82] However, in Germany, a case of occult HEV infection, transmitted via liver allograft resulted in rapidly progressive cirrhosis and death of the recipient.[108] Transmission of HEV by blood transfusion has not been reported in organ-transplant recipients,[82] nor has HEV reactivation in anti-HEV IgG seropositive organ-transplant patients at transplantation or in post-transplant patients.[82] HEV reactivation has been reported in one patient with acute lymphoblastic leukaemia after allogenic stem-cell transplantation.[109]

The clinical features of chronic HEV infection are often unremarkable. Most organ-transplant patients have no symptoms when infected with HEV (table 1), and very few present with jaundice,[101] Liver abnormalities detected by blood tests are usually very modest (typically alanine transaminase is around 300 IU/L), anti-HEV IgG and IgM might be negative, and seroconversion might never occur after infection.[101] Therefore, use of molecular techniques to confirm the diagnosis and assess the response to therapy is important.

About 60% of organ-transplant recipients infected with HEV fail to clear the virus, and develop chronic hepatitis.[101] Sequential liver biopsies of organ-transplant patients with chronic HEV infection show rapid progression of liver fibrosis[110] and 10% of patients progress to cirrhosis.[101] No correlation between serum HEV RNA concentration and liver fibrosis progression has been reported in this setting.[107] Death occurs in a few such patients due to decompensated chronic liver disease.[98] Recurrence of chronic HEV infection has been reported after a second liver transplantation. This patient was infected by HEV 2 years after the first liver transplantation. He developed chronic hepatitis E and cirrhosis that required a retransplantation 5 years later. At the time of retransplantation, he was still viraemic and infected his second graft.[99]

Many factors are associated with failure of immunosuppressed transplant recipients to clear HEV after acute infection.[97,101] These factors include the degree of immunosuppression, the time between the last episode of acute rejection and HEV infection, time since transplantation, low leucocyte count, low total-lymphocyte count, and low T-cell count. Multivariate analysis showed that the use of tacrolimus (rather than ciclosporin) and thrombocytopenia were the only two predictive factors for chronic hepatitis E in organ-transplant recipients.[101]

HIV co-infection

The incidence of HEV infection in patients with HIV is low, ranging from 0% to 0·9%[103,111–113] Only 14 PCR-proven cases (all HEV3) have been documented worldwide.[48,102,103,111,113–115] Of these patients, ten had acute hepatitis, and four patients had chronic HEV infection,[48,102,103,113] one of whom had histologically proven cirrhosis.[48] Of note, patients who developed chronic infection had low CD4 counts. A case-control study from the UK suggests sexual transmission is not an important route of infection.[112] Co-infection with HIV and HEV1, HEV2, or HEV4 has not yet been reported and warrants further study.

	Immunocompetent	Immunosuppressed
Presentation	Often symptomatic	Rarely symptomatic
ALT at diagnosis	≈1000–3000 IU/L	≈300 IU/L
HEV genotype	Genotype 1, 2, 3, or 4	Only genotype 3 HEV infection has been reported in this population
HEV diagnostics	Increase in IgG and IgM PCR is positive in 75%	Serological testing is unreliable, and seroconversion might never occur The diagnosis should be established by PCR
Outcome	Resolving hepatitis	Chronic infection occurs in 60% of patients, and 10% develop cirrhosis
Treatment	Ribavirin has been used in very few patients presenting with severe acute hepatitis	Interferon-α and ribavirin are effective treatments for treating chronic HEV infection in this population; a 3-month course of ribavirin therapy is recommended

ALT=alanine transaminase. HEV=hepatitis E virus.

Table 1: **Hepatitis E virus infection in immunocompetent and immunosuppressed patients**

Extra-hepatic manifestations of HEV

Neurological complications

In the past 10 years HEV-associated neurological syndromes have been described in developing countries. These reports include Guillain-Barré syndrome,[116] Bell's palsy,[117] neuralgic amyotrophy,[118] acute transverse myelitis,[119] and acute menginoencephalitis.[120] Few of these studies used molecular techniques to confirm the diagnosis or genotype. Since these cases mostly originate from the Indian subcontinent, HEV1 is probably the causative agent.

Recently, neurological complications were described in seven (6%) of 126 patients with acute and chronic HEV3 infection.[121] These complications included inflammatory polyradiculopathy, Guillain-Barré syndrome, bilateral

brachial neuritis, encephalitis, and ataxia and proximal myopathy. HEV RNA was detected in the CSF of all four patients with chronic HEV infection. Neurological symptoms completely resolved or significantly improved in patients who achieved viral clearance.[121] Clonal HEV sequences in the serum and CSF in one patient showed quasispecies compartmentalisation, suggesting that neurological symptoms could be linked to the emergence of neurotropic variants.[122] Two further cases of HEV-associated Guillain-Barré syndrome and one case of meningoencephalitis have been described in Belgium[123] and France.[124] One patient had antiganglioside GM1 antibodies (a feature of Guillain-Barré syndrome), and responded well to treatment with intravenous immunoglobulin.[123]

Other complications associated with HEV

Case reports of HEV-related membranoproliferative glomerulonephritis and membranous glomerulonephritis have been reported in France in patients with chronic HEV3 infection,[85] and in India in patients with acute HEV1 infection.[125] Acute pancreatitis[126] and severe thrombocytopenia[127] have also been reported during acute HEV infection. Further studies are needed to establish a causal relation and pathophysiological mechanisms.

Treatment of HEV infection

Acute HEV

Most cases of acute HEV infection are self-limiting and require no treatment. However, patients with or without pre-existing chronic liver disease with acute severe HEV3 infection, have been treated successfully with ribavirin monotherapy.[128,129] In developing countries, effective treatment of pregnant women with HEV1 infection is needed, but a treatment has yet to be established. Although ribavirin therapy is contraindicated in pregnancy due to teratogenicity, the risks of untreated HEV to the mother and fetus are high, and trials of antiviral therapy might be worthwhile.

Chronic HEV

In transplant recipients with chronic HEV infection, viral clearance is desirable. The first step is to reduce the immunosuppressive therapy (especially drugs that target T cells), if possible. Reduction of immunosuppression results in viral clearance in 30% of patients.[49,110] Antiviral therapy should be considered for patients for whom immunosuppressive therapy cannot be reduced and for those who fail to achieve viral clearance after reducing immunosuppression. Various treatment regimes have been used, including interferon-α and ribavirin as monotherapy or in combination.[49,130–132] Interferon-α increases the risk of acute rejection in kidney-transplant recipients and must be used with caution. HEV seems to be sensitive to ribavirin monotherapy, with viral clearance usually achieved within a few weeks. This treatment is now the antiviral agent of choice. Ribavirin should be prescribed for a duration of 3 months by physicians who are familiar with its use and side-effects. To avoid ribavirin-induced haemolytic anaemia, the dose should be adjusted according to renal function.

In other immunosuppressed patients, treatment options are less well established. Ribavirin and interferon-α have been successfully used alone and in combination in a few cases.[106,132–134]

HEV and safety of blood products

The high rate of asymptomatic HEV infections worldwide has raised concern of infection via blood donation. Post-transfusion hepatitis E has been reported in many countries.[135–137] A study of blood donors in London, UK, showed 11% of donor sera to be HEV IgG reactive and 0·7% IgM reactive.[83] 0·7% of plasma mini-pools from English donors contained HEV RNA.[138] Similar findings have been reported in China and a global investigation into plasma fractionation pools reported that 10% of pools tested were HEV-RNA positive.[45,139] The implication is that transmission of HEV associated with transfusion must be occurring, but is currently undiagnosed because donated blood is not tested for HEV and most resulting infections are asymptomatic. This fact might be of minor concern to immunocompetent recipients, but it is a clinically significant risk in immunosuppressed patients[48,97] and in those with chronic liver disease. Currently, 75% of blood or blood components used in the UK are given to immunosuppressed patients. Clearly, the issue of HEV and blood safety warrants further study.

Panel: **Is HEV vaccination warranted in Nepal? A case scenario**

- Every rainy season (end of May to mid September) the streets of Kathmandu are often flooded with polluted rainwater. Because of the rudimentary sewerage system, the flood water is contaminated with HEV, and so every summer the likelihood of an outbreak of HEV is high. When a summer outbreak of HEV occurs in the Kathmandu valley (population 1·5 million), more than 100 pregnant women affected by the virus have life-threatening complications. The burden of HEV infection in the rest of Nepal (population 30 million) is not known but could be associated with over 1000 maternal deaths per year (Buddha Basnyat, Oxford University Clinical Research Unit-PAHS, Kathmandu, Nepal, personal communication).
- The incidence of HEV has been well documented and is high in male Nepali army recruits,[41] but poorly documented in the general population, including pregnant women. Accurate incidence data for HEV in the general population (including pregnant women) will be very difficult to obtain, due to the fragmented health-care infrastructure and the fact that many Nepalis do not have access to or seek medical care, no matter how sick they are.
- Nepal is one of the poorest countries in the world. The gross domestic product per head is US$1200 (rank 204 of 226 countries) and 78% of the population live on less than $2 per day. The Nepalese Government has few funds to pay for studies to determine HEV disease burden or an HEV vaccination programme. The cost of the vaccine is unknown.
- With no accurate data for the burden of HEV disease in pregnant Nepalese women, WHO, GAVI Alliance, or the Gates Foundation are unlikely to be forthcoming with funding. How can Nepal address their problem with HEV?

HEV prevention

HEV infections could be prevented in two ways: reducing exposure to the virus and inducing immunity through vaccination. The key prevention strategy for HEV in developing countries is reducing exposure by improving the sanitary infrastructure, and providing clean drinking water. In developed countries, prevention is more complex because several possible routes of infection exist, which are not fully understood. Approaches might include ensuring that meat products are thoroughly cooked, advising that appropriate measures are taken when handling uncooked meat, and ensuring the proper disposal of pig faeces. Prevention of transmission through blood products is theoretically possible by screening donated blood, but its cost-effectiveness has yet to be established.

	Research questions
Epidemiology	
HEV3 is hyperendemic in southwest France	Are there any other areas of hyperendemnicity in developed countries?
HEV3 and HEV4 infection is more commonly seen in elderly men	Why?
Many seroprevalence studies have used assays of poor sensitivity and might have underestimated the seroprevalence	What are the accurate seroprevalence figures in differing geographical locations using accurate, validated assays?
In zoonotically transmitted HEV predominant areas, IgG anti-HEV levels of seropositive patients among general population is very low	What is the herd-immunity status in areas with high disease burden where HEV1 is endemic?
Accurate estimates of incidence are scarce	How does the incidence of infection vary in differing geographical areas?
Incidence of asymptomatic infection is poorly documented	How common is asymptomatic infection, and what factors predispose to this type of infection?
Transmission	
HEV occurs in the environment (eg, watercourses)	How widespread is HEV in the environment, and how long does it survive?
Several animals are reservoirs of infection	What other animals are reservoirs?
HEV3 and HEV4 can be transmitted by consumption of infected meat.	What other routes of infection are important?
HEV can be transmitted by transfusion	What is the risk of transfusion, and how does this vary from country to country?
Reinfection has been documented in developing countries	How commonly does this occur, and what are the clinical and epidemiological consequences?
Chronic hepatitis	
Only HEV3 has been associated with chronic infection	Can HEV1, HEV2, and HEV4 cause chronic infection?
HEV causes chronic infection in transplant recipients and in patients with HIV and haematological malignancy	Does chronic infection occur in individuals with more subtle defects of humoral and cellular immunity?
Treatment and prevention	
Ribavirin is an effective treatment for chronic HEV infection	What is its mechanism of action?
HEV 239 vaccine is well tolerated in healthy adults	Is it safe in pregnant women, children, elderly people over the age of 65 years and in patients with chronic liver disease?
HEV 239 vaccine is highly efficacious against hepatitis E	How long will the protection last? What is the protective antibody concentration? Does it also prevent subclinical HEV infection?
Other	
HEV is neuropathogenic	How common is neurological injury in HEV infection?
Few data are available on T-cell function	How do T cells interact with HEV?

HEV=hepatitis E virus. HEV1=hepatitis E virus genotype 1. HEV2=hepatitis E virus genotype 2. HEV3=hepatitis E virus genotype 3. HEV4=hepatitis E virus genotype 4.

Table 2: **Future research questions**

HEV prevention through vaccination is now a realistic possibility. Two candidate hepatitis E vaccines have been investigated in clinical trials. The first is a 56 kDa protein encoded by ORF2 of HEV1, expressed in insect cells. In a phase 2 trial in Nepal, male army recruits with undetectable anti-HEV were randomised to receive either three doses of 20 μg of 56 kDa vaccine (898 participants) or placebo (896 participants) at 0, 1, and 6 months, and were followed up for an average of 804 days.[41] The vaccine was well-tolerated and highly immunogenic, with 95·5% (95% CI 85·6–98·6) efficacy against hepatitis E. The vaccine's safety and efficacy in women has not been established.

The second vaccine, HEV 239, is a 26 KDa protein encoded by ORF2 of HEV1.[140] The vaccine is expressed in *Escherichia coli* and occurs as virus-like particles, 23 nm in diameter. In a phase 2 study of the vaccine in seronegative adults, the vaccine was safe and immunogenic and conferred protection against HEV infection, with an efficacy of 83%.[141] In a phase 3 trial in 11 townships in eastern China, participants were randomly assigned to receive either three intramuscular injections of HEV 239 at 0, 1, and 6 months (56 302 participants) or hepatitis B vaccine as a placebo (56 302 participants) and were followed up for occurrence of acute hepatitis to month 19.[68] The vaccine was well tolerated and protected against hepatitis E, with an efficacy of 100% (95% CI 72·1–100·0).

HEV239 vaccine has recently been licensed for use in China. It is uncertain if and when it will become available in other countries. When available, HEV vaccination would be of most use in developing countries where the virus is endemic. However, how such a vaccination programme will be funded is not clear, because some of the countries in greatest need are among the poorest in the world (panel). In developed countries vaccination might be useful in high-risk groups such as immunosuppressed patients and those with chronic liver disease, in addition to individuals intending to travel to endemic areas. Porcine vaccination might also be worthwhile if it is cost effective.

Future research

Our understanding of HEV has changed enormously over the past 30 years, from a waterborne infection causing outbreaks of acute hepatitis in developing countries to an infection of global distribution causing a range of hepatic and extra-hepatic illness. This development has been largely driven by the use of molecular techniques. However, many unanswered questions regarding HEV still remain (table 2). Before these questions can be addressed with any degree of certainty, properly standardised serological and molecular assays

must be adopted to accurately identify current and past infections. In some areas, such as the USA, the absence of any licensed assays severely limits the ability of clinicians to diagnose hepatitis E.

Clinicians should appreciate that HEV infection has a worldwide distribution. As well as classic acute hepatitis E, HEV causes chronic infection in immunocompromised patients and significant extra-hepatic complications. Unless HEV is specifically sought, the diagnosis will be missed because the clinical presentations overlap with many other disorders. We anticipate that as research progresses the range of diseases associated with HEV will widen.

Contributors

All authors contributed equally to the preparation of this Seminar.

Conflicts of interest

HRD has received travel and accommodation costs and consultancy fees from GlaxoSmithKlein and Wantai. All other authors declare no conflicts of interest.

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