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R E V I E W

Transmission, diagnosis, and management of hepatitis E: an update

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Abstract: Hepatitis E virus (HEV) infection is an important public health concern in many developing countries, causing waterborne outbreaks as well as sporadic autochthonous hepatitis. HEV is mainly transmitted by the fecal–oral route in endemic areas through drinking of contaminated water. However, zoonotic transmission from animal reservoirs to humans has also been suggested. Three additional routes of HEV transmission have been proposed to occur: blood borne, human to human, and vertical transmission from mother to child. Acute HEV infection is usually diagnosed by detecting specific anti-HEV antibodies. However, the performance of the available assays in different settings is not optimal. Analysis of HEV ribonucleic acid in biologic specimens such as stools, serum, and liver biopsy by using nucleic acid amplification techniques is also employed. Nonetheless, additional consensus regarding the best technologies suitable for serosurveys and diagnosis of acute HEV infection is also needed. This review article summarizes the current status of HEV infection end epidemiology with particular emphasis in transmission, diagnosis, and clinical management.

Keywords: HEV, epidemiology, clinical features, routes of infection

Introduction

Hepatitis E virus (HEV) is the etiological agent of acute hepatitis E, an infection considered to be endemic in many developing countries in Africa and Asia.[1] HEV is transmitted primarily by the fecal–oral route and has been reported to occur as large waterborne epidemics and small outbreaks in developing areas.[2–4] However, over the last decade, it has occurred in several high income countries, in an increasing number of sporadic locally acquired cases in which it is often not possible to establish the route of acquisition of infection.[5,6] Besides humans, HEV strains have also been isolated from several other animal species including wild and domestic swine, deer, chicken, rat, ferret, and rabbit.[7–9] Hepatitis E is considered a zoonotic infection with pig and wild boar serving as the main reservoir for human infections. The global burden of HEV infection is thought to be due to sporadically transmitted hepatitis E cases rather than to cases due to hepatitis E epidemic. It has been estimated that two billion people, representing one third of the world's population, live in endemic areas for HEV and, therefore, are at risk of infection.[10] It is not clear whether HEV incidence rates in nonendemic areas are actually changing or if more cases are detected through increased surveillance.[11]

HEV typically causes an acute and self-limiting infection in immune-competent individuals, with low mortality rates in general, though fulminant hepatitis and high mortality are described in some settings. However, in the last few years, unknown

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aspects regarding HEV infection have been uncovered in nonendemic and industrialized countries, such as the possibility of the disease becoming chronic in transplant patients and the immunocompromised.[12] Chronic HEV infection in these individuals is indeed an emerging and significant clinical problem.

This review article summarizes recent advancements in the knowledge of the biology and epidemiology of HEV, with special emphasis on the transmission routes, diagnosis, and management of the infection.

Biology and molecular virology

HEV is currently classified as a member of the genus *Hepevirus* in the family Hepeviridae.[13,14] It is a small, nonenveloped, spherical particle of approximately 32–34 nm in diameter and has a single-stranded, positive sense ribonucleic acid (RNA) genome surrounded by an icosahedral capsid.[1]

HEV genome organization

The HEV genome is ~7,200 nucleotides (nt) in length, consisting of a short 5′ untranslated region (27–35 nt), three discontinuous and partially overlapping open reading frames (ORFs) 1, 2, and 3, and a short 3′ untranslated region (65–74 nt) that is terminated by a polyadenylated tract (Figure 1). The capped 5′ end, essential for viral infectivity, and the 3′ end of the viral genome are noncoding regions and cis-acting elements involved in the regulation of viral replication and translation.[15,16]

ORF1, the largest coding unit, encompassing approximately two thirds of the viral genome, is located at the 5′ end and is approximately 5,000 nt in length. This region is involved in viral replication and protein processing, and it encodes nonstructural proteins including putative methyltransferase, guanylyl transferase, papain-like cysteine protease, RNA helicase, and RNA-dependent RNA polymerase.[17,18] Also, some uncharacterized domains homologous to other animal and plant positive-strand RNA viruses have been identified in the ORF1.[16] The hypervariable region, a noncoding region within ORF1 that displays substantial genetic diversity, was recently proposed to modulate the efficiency of HEV replication.[19] Notably, the differences in the genome size among different HEV strains are confined mainly to this region.[20]

The viral ORF2 encodes the viral capsid protein of 660 amino acids that encapsidates the viral RNA genome.[16] Capsid is the only structural protein and was shown to assemble into a highly structured multimer (60 copies).[21,22]

ORF3 overlaps the other two ORFs and encodes a small phosphoprotein of 123 amino acids that may cooperate in replication and cytoskeleton synthesis,[23,24] and it is thought to interact with cellular mitogen-activated protein kinase phosphatase and other extracellular kinases, promoting cell survival through activation of intracellular signaling pathways.[25]

Genetic variability

Although a single serotype has been proposed,[26] extensive genomic diversity has been observed among HEV isolates.[23] Human infecting HEV sequences have been classified into four major genotypes (1–4) according to analysis of the complete genome sequence and/or variable partial HEV genomic regions within the ORF1 and ORF2.[27–29] However, the existence of a new HEV genotype infecting wild boar was recently been proposed.[30] This, together with the increasing number of HEV and HEV-like sequences published in the last few years, which increase the number of potential new genotypes or genetic groups, brought into question the current system of classification within the *Hepevirus* genus.[31]

According to the currently accepted system of classification, the four major HEV genotypes are further subclassified
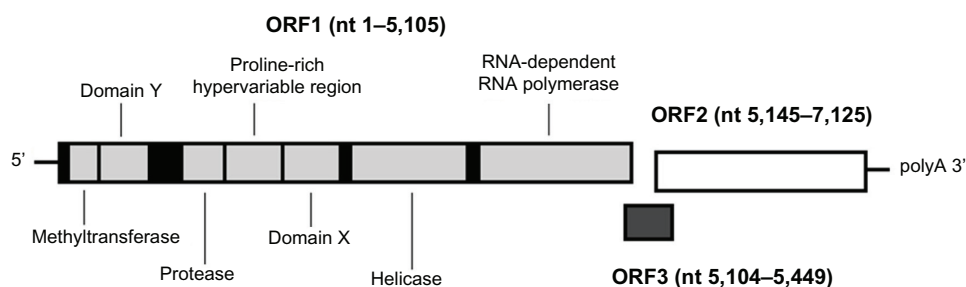
Figure 1 Organization of the hepatitis E virus genome.
Notes: Scheme showing the organization of the three viral open reading frames (ORFs); ORF1 encodes a nonstructural polyprotein comprising a methyltransferase, Domain Y (nonfunction assigned), papain-like protease, proline-rich hypervariable region (HVR, in text), Domain X (nonfunction assigned), RNA helicase, and an RNA-dependent RNA polymerase; ORF2 encodes the capsid protein and ORF3 encodes a small phosphoprotein; nucleotide positions are relative to the genotype 1 Burmese SAR-55 isolate.
Abbreviations: RNA, ribonucleic acid; nt, nucleotides.

into subtypes, defined on the basis of five different phylogenetic reconstructions: 5′ ORF1, 3′ ORF1, 5′ ORF2, 3′ ORF2, and complete genome.[28]

HEV genotype 1 sequences are divided into five subtypes, 1a–e, and genotype 2 into two subtypes, 2a and 2b.[28] Genotype 1 is responsible for most endemic and epidemic cases of HEV infection in Asia and has been also detected in small outbreaks from Cuba and sporadic cases from Venezuela and Uruguay, respectively[1,32,33] (Figure 2). Genotype 2 is prevalent in Mexico (probably subtype 2a, based on the characterization of a single strain) and Africa (subtype 2b).[1] By contrast, genotype 3 is widely distributed, and sequences of this genotype are extremely diverse,[23] comprising ten (3a–j) subtypes. Genotype 4 sequences, even though they display high heterogeneity (subtypes 4a–g), are geographically restricted to Asia and Central Europe (Figure 2).[28,34] Recently, this subtype-based classification has also been challenged by Smith et al, in which an exhaustive molecular and phylogenetic analysis revealed several inconsistencies with the currently defined subtype designations.[35] This observation was also supported by other similar studies.[31]

HEV epidemiology

HEV is considered hyperendemic in many developing countries such as India, Bangladesh, and the People's Republic of China, where major waterborne outbreaks occur, generally associated to isolates of genotypes 1 and 2. In turn, the case of Mexico, a country which is usually qualified also as highly endemic, is questionable; hepatitis E outbreaks have never been reported again in the last two decades. Genotype 2 appears to be anthroponotic since it has been isolated exclusively from human cases while genotype 1 infects mainly humans but also has been detected in pigs.[28,36,37]

In developed and nonendemic countries, occurrence of HEV infection has usually been associated with travelers returning from endemic countries.[38] Recently, accumulating lines of evidence indicate that sporadic and locally acquired cases of genotype 3 and 4 HEV infection also occurs among individuals from industrialized countries who have no history of travel to endemic areas.[1,7,39–41] In fact, in the last decade, the number of autochthonous cases of genotype 3 and 4 infection reported in developed areas has increased dramatically, and evidence for animal reservoirs and zoonotic transmission has been brought to light. HEV strains of these zoonotic genotypes have been found in humans and other animal reservoirs including swine, wild boar, and deer.[9,42–45] Most episodes of zoonotic transmission associated to genotypes 3 and 4 are foodborne and have been linked to ingestion of raw or undercooked meat from wild and domestic pigs and deer.[46,47] Table 1 summarizes epidemiological features of the four recognized human HEV genotypes.

Recent advancements in the understanding of global epidemiology of HEV infection reveals a picture quite more complex than initially assumed. Nevertheless, distinct epidemiological patterns are clearly distinguished in endemic regions compared to nonendemic areas.

Endemic countries were shown to have an overall HEV prevalence of 25% of all non-A, non-B acute hepatitis cases,[48] and while the anti-HEV immunoglobulin (Ig)G prevalence among healthy blood donors may be as high as 45% in some hyperendemic countries, reports from industrialized countries, although highly variable from study to study, show prevalence ranging from 1% to 4%.[49]
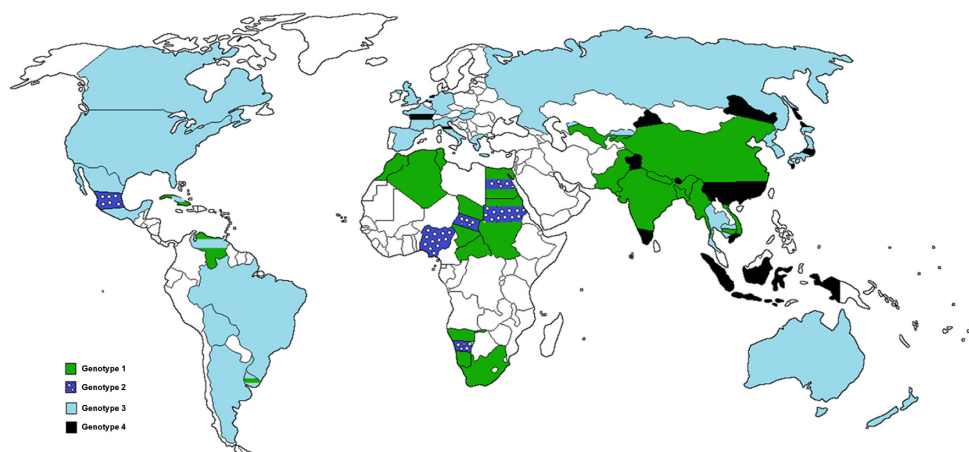
Figure 2 Geographical distribution of hepatitis E virus genotypes.
Notes: Genotypes 1 and 2 consist of epidemic strains reported in Asia and several Latin American countries and Africa and Mexico, respectively; genotypes 3 and 4 comprise of zoonotic strains and have been isolated from sporadic cases of acute human hepatitis E viral infection and from several animal reservoirs, particularly domestic and wild pigs and deer; genotype 3 is distributed worldwide in human and pigs while genotype 4 was reported in East Asia and Central Europe.

Table 1 Epidemiological and clinical features of the four human hepatitis E virus genotypes

Genotype	1	2	3	4
Host	Human; also isolated from pigs	Human	Human, swine, and other mammalian species	
Route of transmission	Waterborne (fecal contaminated water); human to human; maternofetal; probably zoonotic (genotype 1)		Zoonotic: mainly via consumption of raw or undercooked contaminated animal meat; environmental (shellfish); to date environmental shellfish has been reported exclusively for genotype 3; blood transfusion	
Geographical distribution	Asia and Latin America (Cuba, Venezuela, and Uruguay)	Mexico and several African countries	Worldwide	East Asia and Central Europe
Epidemiological features	Endemic in Asia and Cuba; causes outbreaks and sporadic cases in developing countries; more common in young people and travelers	Endemic in Mexico? Causes outbreaks and sporadic cases in developing countries; more common in young people	Causes autochthonous sporadic cases in developed and nonendemic areas; highest attack rate in middle-aged and elderly men; higher overall mortality rate compared to genotype 1	
Clinical presentation in humans	Mostly asymptomatic infections; acute self-limited hepatitis; high mortality in pregnancy and in patients with underlying chronic liver disease	Mostly asymptomatic infections; moderately severe acute self-limited hepatitis	Moderately severe acute self-limited hepatitis; asymptomatic; infection may lead to chronicity in solid organ transplant recipients (to date, only reported for genotype 3) and immunocompromised patients	

Additional dramatic differences were observed in the size and frequency of outbreaks, overall attack rates, and duration of viremia.[49] These issues are extensively reviewed by Kumar et al.[9]

Clinical features

Historically, data on clinical manifestations of hepatitis E are available from basically two sources of evidence: 1) reports of HEV infection outbreaks and sporadic disease from highly endemic areas and 2) case reports and case series from developed nonendemic countries. However, the recent observation that HEV infection may progress to chronicity in transplant and immunocompromised patients has increased the complexity of the disease and has rendered it to be a public health problem of major concern.[15] Clinical outcomes associated with HEV infection from the two sources are reviewed here. In addition, recent advancements in the comprehension of the chronic course of HEV infection and the identification of its distinct clinical features are also discussed.

HEV infection in highly endemic regions

In moderate and high endemic areas, HEV infection is the most common cause of acute viral hepatitis.[12,50] The infection varies in severity, ranging from subclinical to fulminant hepatitis. Most acute HEV hepatitis cases have a clinically silent course,

and patients clear the virus rapidly.[51,52] Symptomatic disease is reported in about 20% of patients and is observed most frequently in youths and adults (14–40 years old).[50] Symptoms associated with HEV infection are moderately severe and generally self-limiting, with case fatality rates of 0.5%–4%. In symptomatic cases, the incubation period ranges from 2 to 8 weeks (mean 40 days), and initial symptoms are mainly unspecific. Typically, these include flu-like myalgia, arthralgia, anorexia, hepatomegaly, fever, weakness, and vomiting, but also patients may present more specific signs such as jaundice, itching, pale stools, and darkened urine. Additionally, clinical symptoms are usually accompanied by increased levels of liver enzymes, especially bilirubin, aspartate aminotransferase, and alanine aminotransferase.[50–52] However, HEV infection can lead to more severe liver disease in pregnant women or patients with underlying chronic liver diseases, sometimes progressing to fulminant hepatic failure (FHF).[53]

Of major concern, is the relationship between HEV genotype 1 and 2 infections and pregnancy. In pregnant patients, acute liver disease can lead to FHF, and increased mortality (up to 30%) is observed in endemic regions.[9] This link was first reported during several outbreaks in India but has also been found in sporadic cases in highly prevalent areas.[54] HEV infection during pregnancy is also associated with abortion, prematurity, low birth weight, and an increased risk

of perinatal mortality.[55] Unfortunately, despite the suggested roles and association of immunological and/or hormonal factors, it is not currently understood why pregnancy results in severe hepatitis E manifestation.[56,57]

HEV infection in low endemic areas

The differences in clinical and epidemiological features of acute HEV infection between patients living in nonendemic countries and highly endemic areas are noticeable.[58] The highest attack rates in middle and elderly males, highest overall mortality rates, and greater frequency of unspecific symptoms are clearly distinguishable. Additionally, severe clinical manifestations during pregnancy have only been occasionally reported for HEV genotype 3.[59]

Chronic HEV infection

Though HEV infection was conventionally believed to be a self-limited infection with spontaneous clearance in most of the patients, in recent years episodes of persistent viral shedding have been recognized in low endemic countries and in sporadic cases from endemic regions.[60–62]

The ability of HEV to progress to chronic state is of major concern in organ transplant recipients who receive immunosuppressive medication to prevent rejection. This group is thought to be the main population at risk for chronic hepatitis E, which is well documented among patients with liver and kidney transplants.[63–65] Recently, it was also suggested that HEV chronic infection is an important cause of abnormalities in liver enzyme tests observed after lung and heart transplantation, sometimes leading to rapid liver fibrosis.[66] Furthermore, HEV chronic infection has been shown to occur in patients with other conditions associated with immunosuppression, such as HIV infection and lymphoma and leukemia.[59,61] However, the establishment of persistent viral infection seems to be much less common in this group than in those patients with solid organ transplantation.

Therefore, chronic HEV infection in immunocompromised individuals is an emerging and significant clinical problem. Recently, González Tallón et al reported the first case of a healthy, immunocompetent man who developed an episode of acute HEV hepatitis, which progressed to chronic liver disease within a 1-year period.[67]

Nonhepatic manifestations of HEV infection

There is some evidence that both acute and chronic infections are associated with extrahepatic manifestations, particularly acute pancreatitis and neurological disorders including encephalitis, polyradiculopathy, Guillain–Barré syndrome, bilateral brachial neuritis, and proximal myopathy.[68,69] These issues are extensively discussed in greater detail in other reviews.[12,70]

HEV transmission

Drinking fecal contaminated water in highly-endemic regions with poor sanitation practices (waterborne transmission) and the consumption of raw or undercooked meat of HEV-infected animals (zoonotic, foodborne transmission), especially in industrialized countries, account for most HEV transmissions, resulting in an important public health problem.

Occasionally, the exact mode of HEV transmission remains controversial, and sources of viral infection are often not well identified, particularly in sporadic cases of acute hepatitis E. In this setting, the infection may be also related to the level of population immunity, sanitary conditions, living conditions, and others factors.[71] Recent investigations reinforce the notion of the existence of three additional routes of HEV transmission: parenteral (blood borne transmission),[72] human to human,[73] and vertical transmission from mother to child (perinatal transmission).[74] Even though these routes of transmission are thought to be less frequent, the existence of growing lines of evidence in this matter encourages prophylactic measures that contribute to the prevention of HEV infection.

Waterborne transmission

The earliest well-documented report of the disease was of a large epidemic of waterborne hepatitis occurring in New Delhi, India in 1955.[75] Even though it was initially believed to be related to hepatitis A virus, subsequent testing of stored sera from this epidemic and another outbreak during 1978 to 1979 in Kashmir, India failed to demonstrate serological markers for hepatitis A and B viruses.[54] HEV is mainly an enterically-transmitted pathogen and has been reported to occur as large waterborne epidemics and small outbreaks. In addition, in developing regions, it is occasionally associated with hepatitis A virus outbreaks in the form of dual infection, which increases the complexity of the HEV epidemiology and makes it more difficult to understand.[32] These waterborne outbreaks, have been exclusively associated with genotype 1 and 2 strains.[76–79] Nonetheless, environmental presence of HEV has also been demonstrated both in industrialized and developing countries, and raw sewage and seawater have been shown to contain infectious HEV strains that are

closely related to the strains circulating in humans and other animals.[80–85] Many reports have described genotype 3 as the main type that circulates in several environmental conditions, playing a potential role in the spread of HEV infection among humans.[82–84] Although it has been suggested that the HEV virion is stable enough to be transmitted from environmental sources, additional studies should be performed to evaluate the ability to transmit and cause infection in cases where the virus contamination levels are low.[85]

Zoonotic and foodborne transmission

Since HEV is unique among the known hepatitis viruses, with several susceptible animal species capable of functioning as reservoir of the disease (swine, wild boar, and deer),[86] the zoonotic transmission caused by genotype 3 and 4 HEV strains has been uncovered.[5,6,36,42,43,87,88]

HEV remains infectious at up to 60°C, suggesting the possibility of HEV transmission by consumption of raw and slightly steamed contaminated food.[85] Indeed, the foodborne transmission by consumption of raw or undercooked meat of wild (deer and boar) and domestic (pig and probably rabbit) animals contaminated with HEV has been clearly demonstrated, and compelling evidence exists.[7,89] More recently, fully infective HEV virions were detected, isolated, and characterized from pork liver sausage produced in France, suggesting that the consumption of these products may be a risk factor for HEV infection in humans, and for which measures to eliminate the source of viral origin must be warranted.[47]

Zoonotic transmission through direct contacts with infected animals has also been reported. Farmers, veterinarians, and workers attending animals comprise the highly at-risk, exposed group for HEV infection.[90–92] Shellfish consumption has also been suggested as a potential source of HEV infection to human and other animal reservoirs. Said et al, identified HEV-contaminated shellfish as the almost exclusive risk factor involved in an outbreak occurring aboard a cruise ship in European waters.[93] Afterwards, several HEV strains were detected in commercial mussels of different European countries.[94,95]

In summary, zoonotic risk for HEV transmission is currently well known and broadly studied, with an ever-expanding host range and newly identified animal reservoir species, revealing a health problem of major concern.

Vertical transmission

In endemic areas, HEV is the most common cause of hepatitis during pregnancy, but the cause of increased severity of the virus is not known.[9] Notably, to date, the high mortality in pregnant woman has been associated with genotype 1 HEV infections only, and there have been no reports of HEV genotype 3 or 4 infections.[96,97] However, HEV genotype 2 infection was also associated with acute liver failure in a pregnant woman during an outbreak in Namibia.[79]

HEV infection is commonly transmitted from mother to child via the intrauterine and perinatal routes, and in fetuses and neonates it causes severe liver disease with high rates of mortality.[55,74,98–100] The clinical course in vertically transmitted HEV infection in survivor neonates is self-limiting with short lasting viremia.[74]

In developing countries, HEV may be responsible for 2,400–3,000 stillbirths each year, with many additional fetal deaths linked to antenatal maternal mortality; it remains unclear if the increase of stillbirths is attributable to vertically transmitted infection or if it is the result of maternal complications of hepatitis E.[96] Other studies suggest that the severity of HEV infection in mother and baby may be related to each other, and fetal disease influenced the course of maternal HEV infection.[101,102]

Furthermore, there are no reliable data on whether HEV can be transmitted through breast milk, and there is no information about the influence of asymptomatic infection in pregnancy.[96] In this direction, serologic surveillance and monitoring the entire pregnancy period and neonatal outcomes may be necessary to understand these issues.

Additional investigations of the mechanisms of HEV pathogenesis in pregnant women are needed to understand the role of transplacental transmission in fetal loss and stillbirth.

Person-to-person transmission

The role of person-to-person transmission in HEV infection is still controversial and is thought to not contribute significantly to morbidity in epidemics.[71,103] However, in developing countries, poor hand hygiene and common drinking, hand washing, and eating vessels may offer repeated opportunities for cross-contamination of water and food with the virus, increasing the human-to-human transfer during an HEV outbreak.[104]

Many lines of evidence have indicated that person-to-person transmission is uncommon, especially in sporadic cases of hepatitis E.[103,105] However, in endemic areas, even though most outbreaks of HEV infection have displayed a short course and are linked to a common drinking water source, some multimodal and prolonged outbreaks have also occurred that could be the result of continued person-to-person transmission.[106]

Indeed, some studies have suggested that person-to-person transmission is possible in HEV epidemics.[103,107] Recently, the first direct evidence of this mode of transmission was reported from an HEV outbreak in northern Uganda.[71] The lack of a clear continuing common source of infection necessary to sustain the epidemic for many months, the high estimated secondary attack rate, and the absence of HEV detection in zoonotic sources provided supporting data to the hypothesis. Nevertheless, additional studies are required to better understand the probable mechanisms and vehicles of transmission.[71]

Although uncommon, the intrafamilial spread of sporadic HEV infection has been suggested as a likely mode of transmission by several lines of evidence.[73,105]

Parenteral transmission

In industrialized countries, despite that only in a few cases of sporadic HEV infections where the source of infection could be certainly identified, most cases are probably linked to foodborne transmission through consumption of raw meat of reservoir animals. This mode of transmission, in which most of the infections will be asymptomatic, may lead to the spread of HEV infection by subclinically infected individuals. Indeed, several investigations have reported remarkably high HEV seroprevalence rates (5%–50%) among healthy individuals, suggesting a widespread circulation of HEV strains.[108,109] This issue is of great significance to transfusion transmissibility of HEV because potentially infected donors may never have shown clinical signs. Thus, considering that HEV cannot be fully inactivated in blood-derived products, this virus has recently emerged as a transfusion-transmitted pathogen of concern and has led to a resurgence of interest to researchers in this field.

Although the parenteral route of transmission is not thought to be a frequent occurrence, and its clinical consequences have not been systematically evaluated, the potential risk of transmission of HEV by transfusion has been suggested by several studies.[2,110–112] Additionally, comparative molecular analysis of HEV sequences from blood donor and recipient have been performed, and direct evidence for parenteral transmission (HEV genotypes 1 and 3) has been reported.[72,113]

Certainly, in the last few years, the knowledge regarding HEV epidemiology, its biology, and its modes of transmission, especially in nonendemic regions, has been remarkably increased. Thus, in the near future, it will be necessary to establish standardized guidelines and policies for, when possible, routine HEV RNA screening of blood banks and organ donors. Indeed, recently, a novel approach for routine detection of HEV infection in German blood donors has been performed.[114] However, especially in developing countries, it still remains very expensive to efficiently perform nucleic acid amplification techniques (NATs)-based screening programs aimed to prevent HEV transmission by blood transfusion. Although currently available evidence indicates that transfusion-transmitted HEV infection indeed occurs and is more frequent than initially recognized, cost-effective analysis should be conducted in each case by local public health policy makers.[115] In spite of this, and considering that fatal HEV outcomes such as FHF or chronicity may be triggered in recipients who are immunosuppressed, pregnant, or have chronic liver disease, special measures and considerations must be taken into account in these high-risk patients.

Diagnosis of HEV

Acute HEV infection is usually diagnosed by detecting specific anti-HEV antibodies (IgM and IgG). However, the performance of the available assays in different settings is not optimal, and highly variable values are observed depending on the commercial assay employed.[116,117] Analysis of HEV RNA in biologic specimens such as stools, serum, and liver biopsy using NATs is also used for diagnosis. Even though in light of recent advancements in the understanding of HEV epidemiology and viral pathogenesis several diagnostic flow charts have been proposed,[118] the standardization of diagnostic criteria still remains critical. Additional consensus regarding the best technologies suitable for serosurveys and diagnosis of acute HEV infection is also needed. Besides this, the diagnosis of HEV infection in immunocompromised patients and solid organ transplant recipients in whom seroconversion to anti-HEV antibodies is delayed or may not occur at all is of particular concern. In this review, we summarize the most common available diagnostic tools currently employed for the identification of acute HEV infection, discussing their performance in different settings on the basis of recent findings.

Serologic tests

Little was known concerning the immunological aspects of the HEV infection until the last few years, when a pattern of antibody response to HEV has been identified and understood. Anti-HEV IgM appears during acute stage of the infection and is detectable 4 days after the onset of jaundice and persists for up to 5 months.[119] However, strongly positive reactions are rare after 3 months.[59] Overall, 90% of patients coursing an acute hepatitis E infection have detectable anti-

HEV IgM within 2 weeks of illness onset[119] while anti-HEV IgG antibodies are detectable shortly after the appearance of anti-HEV IgM. Therefore, the fact that both classes of antibodies probably develop simultaneously in acute infection makes it difficult to establish an accurate serological diagnosis of acute HEV infection. Moreover, while the anti-HEV IgM antibodies rapidly decline within a few months, IgG persists for longer, a variable period within 1 to 14 years postinfection.[120]

Currently, commercially available immunoassays for the detection of specific anti-HEV antibodies during acute infection differ dramatically in their sensitivity and specificity, which may partly explain the discrepancies in the HEV seroprevalence observed in different studies.[121]

Traditional immunoassays for IgM have a sensitivity ranging from 90% to 97%, with false positive results of up to 2.5%,[122,123] and several of them have been shown to produce false negative results in patients infected with genotype 1 strains.[124] In fact, many diagnostic methodologies are based on HEV genotype-specific antigens, thus limiting the detection of all HEV genotypes.

Intrinsic genetic variability of HEV leads to significant modifications of antigenic sites that have important implications in the development of reliable immunoassays. Despite this variability, the four HEV genotypes share cross-reactive domains within capsid protein (ORF2), which include a minimum neutralizing domain comprising amino acids 458–607.[49,125] ORF2 and ORF3 antigens or immunodominant peptides were widely used in commercial HEV serological tests for the detection of IgM, IgG, and IgA antibodies against HEV.[126,127]

Recent developments have focused on the construction and improvement of recombinant ORF2-derived antigens expressed either in baculovirus or *Escherichia coli*, which include the minimum neutralizing domain.[16] In particular, the approaches that use virus-like particles (VLPs) technologies in the eukaryotic-expressing system, such as baculovirus, seem to be the more appropriate methods for constructing HEV antigens for diagnostic devices.[118]

Even though all the ORF2-based immunoassays have indeed improved sensitivity, the specificity still needs to be increased, especially in low endemic regions where the occurrence of IgM false positive cases are more frequent.[128]

In a recent study aimed to evaluate the performance of currently available commercial kits for the detection of specific anti-HEV IgG/M, 300 serum samples from patients from nonendemic areas stored at the Center for Disease Control of Taiwan were tested.[118] Six immunoassays based on

different immunodominant determinants were included: the EIAgen HEV IgG/M (Adaltis, Bologna, Italy) included the HEV ORF2 (amino acids 619–660) and ORF3 (amino acids 101–123), derived from the Burmese strain (genotype 1) and the Mexican genotype 2 prototype strain, respectively; the recomWell HEV IgG/M (Mikrogen, Neuried, Germany) used purified recombinant HEV-ORF2 virus antigens derived from genotype 1 and 3 strains; the MP Biomedicals HEV IgM ELISA 3.0 (Santa Ana, CA, USA) detected antibodies targeting recombinant peptides coded by ORF2 and the complete recombinant protein specified by ORF3 from genotypes 1 and 3; and the rest were in-house hepatitis E VLP IgG/M assays, which used highly purified empty VLPs of HEV as an antigen expressed by a recombinant baculovirus, and self-assembled with an ORF2-coded recombinant protein consisting of amino acid residues 112–660 of the Burmese strain. This study showed that recomWell IgM, which had the highest sensitivity (93.3%), exhibited the best overall performance for diagnosing and screening of acute HEV infection in Taiwan. However, the hepatitis E VLP IgM had the highest specificity (95.6%) and positive predictive value (40%) in spite of its relatively low sensitivity rate (66%). Beyond evident differences in the sensitivity and specificity among assays, all anti-HEV IgM detection systems in this study were better than anti-HEV IgG assays for the purpose of screening. Additionally, it was possible to raise the sensitivity up to 100% and improve specificity as much as possible by combining IgM and IgG assays from the same brand or two different IgM detection systems. Thus, at least in nonendemic areas, these approaches seem to have reasonable robustness for use as screening tools for acute HEV infection in immunocompetent patients.

In addition to the inconsistent performances of sensitivity and specificity among different available immunoassays,[124,125] false reactivity for anti HEV IgM with other hepatotropic viruses such as Epstein–Barr virus (EBV) and cytomegalovirus (CMV) has been reported. This is clinically problematic and relevant since these viruses form the differential diagnosis for acute hepatitis once hepatitis A virus infection is excluded.[129] The cross-serological reactivity of HEV with other viruses known to cause hepatitis was further investigated.[130] In this retrospective study of HEV serology testing, 1,423 samples were analyzed with a commercially available kit. Of them, 1.9% were unequivocally IgM-positive and 9.3% were IgG-positive. Overall, the degree of EBV and CMV IgM cross-reactivity to anti-HEV IgM was strikingly high, with 33.3% and 24.2% of samples that were HEV IgM-positive also showing reactivity to EBV

and CMV, respectively. Additionally, in only 13.3% of the samples, anti-HEV IgM serology correlated to HEV polymerase chain reaction (PCR) positivity. These results are concerning and highlight the unreliability of anti-HEV IgM testing given high false positive reaction rates with EBV and CMV IgM and the extremely low level of correlation with PCR-confirmed HEV infections.[130]

The diagnosis of acute HEV infection in patients with conditions associated with immunosuppression, such as HIV infection and lymphoma and leukemia as well as solid organ transplant recipients, is of particular interest and to which much research has been focused. In these patients, seroconversion to anti-HEV antibodies during infection is delayed or may not occur at all, and thus while using serological tests for screening, this condition must be considered. Abravanel et al[131] evaluated the performance of the EIAgen HEV IgG and IgM kits (Adaltis) and anti-HEV IgM and IgG ELISA 3.0 Wantai kits (Wantai Biological Pharmacy Enterprise, Beijing, People's Republic of China), the two main anti-HEV IgM and IgG assays employed in Europe for diagnosing acute HEV infections in immunosuppressed patients. In the study, 40 samples from 35 solid organ transplant recipients and five hematological patients were analyzed for anti-HEV antibodies, with two additional panels consisting of 44 acute HEV-infected immunocompetent patients and 233 HEV-negative blood donors used for comparison. Both anti-HEV IgM tests showed good specificity and sensitivity (85%–87.5% and 85%, respectively) in these immunocompromised patients; although as expected, they were markedly lower than that observed for the immunocompetent HEV-infected group.

By contrast, the assays had failed in detecting specific IgG antibodies in the acute phase, with only half of the patients testing positive for HEV. Thus, the performance of these commercially available tests indicate that in this setting, the anti-HEV IgM assays could be used as first line tools for the routine diagnosis of acute HEV infection, even in immuno-compromised patients. However, according to this and several others studies,[65,109] the molecular detection of HEV RNA is essential for the diagnosis of acute hepatitis E in anti-HEV IgM-negative immunocompromised patients. In this setting, HEV RNA detection and quantification may also have a critical role in monitoring the response to antiviral therapy.[64]

RNA-based detection tests

The accumulating information concerning the highly variable performance of the currently available serological tests in endemic and nonendemic regions, together with the false negative results and cross-reactivity with other hepatotropic viruses reported in different clinical settings, raises the question as to whether HEV RNA testing should be conducted in all patients suspected of HEV infection.

However, the sensitivity of molecular tests for the detection of HEV RNA is highly dependent on patients' early presentation and timely collection of serum or stool samples as well as on appropriate transport and processing. Therefore, undetectable viral RNA does not rule out HEV infection.[72] Even though HEV RNA may be detected at the onset of illness and up to 6 and 4 weeks in stool and serum, respectively, the viral RNA levels can be low and thus reduce the capture window for HEV.[132]

Nonetheless, detecting viral RNA in biologic samples is the gold standard for the confirmation of acute HEV hepatitis since NATs can accurately identify active infection and help confirm serological findings.[133] Unfortunately, NAT-based detection is an expensive approach that might not be available for diagnostic laboratories, and additionally it demands highly specialized techniques and trained personnel.

In the last few years, several NAT assays have been reported for the detection and identification of HEV RNA in serum and stool samples, including reverse transcription followed by PCR (RT-PCR), real-time RT-PCR, and reverse transcription loop-mediated isothermal amplification. These NATs include assays designed and optimized to broadly detect the four human HEV genotypes.[134–136]

However, methods of HEV nucleic acid detection have not been well standardized, and extreme variability has been observed in the performance of in-house tests.[137] In 2009, the Paul-Ehrlich-Institut (Langen, Hesse, Germany) initiated the development of an international standard (IS) for HEV RNA for use in NAT assays, with the approval of World Health Organization (WHO) Expert Committee on Biological Standardization. The initial study that investigated the performance of several in-house-developed NAT assays used in the detection of HEV infection was completed.[137] In their work, Baylis et al included four viruses belonging to genotypes 3 (subtypes 3a, 3b, and 3f) and 4 (subtype 4c) that underwent blinded independent testing in several laboratories. Even though it was concluded that any of these strains could be used for the development of an IS, genotype 3 strains seemed to be the best candidates since they have the widest distribution worldwide, and genotype 3 is identified in chronic infections. On the other hand, wide variations in assay sensitivity (100- to 1,000-fold) were observed.[137] Recently, two virus strains (of subtypes 3a and 3b) included in the panel, together with a Japanese national standard, were selected by the WHO as potential IS candidates and were further

investigated.[138] This international collaborative study, aimed to demonstrate the suitability of these IS candidates, evaluate their potency, and assign an internationally agreed upon unit, established a genotype 3a HEV strain as the IS for HEV RNA for use in NAT-based assays, with an assigned unit of 250,000 IU/mL.[138] Furthermore, this WHO IS was employed to validate two new commercially available HEV RNA assays and to evaluate their suitability in detecting and quantifying 3c, 3e, and 3f.[139] These strains were chosen since they are the most prevalent among genotype 3 isolates in industrialized countries. Both methodologies, Hepatitis@ceeram Tools kit by Ceeram (La Chapelle sur Erdre, France) and the RealStar HEV RT-PCR kit, version 1.0, by Altona Diagnostics (Hamburg, Germany) showed good analytical sensitivity with high reproducibility for detecting genotype 3 HEV RNA.

On the other hand, again, the extremely high variability observed in sensitivity among the assays highlights the need for the standardization of genotype-specific NAT assays and the development of methodologies capable of accurately detecting all HEV genotypes. To this end, much more study involving other HEV strains is required.

Management of HEV infection

Acute HEV is a self-limiting illness in immunocompetent patients. Some patients might require treatment of symptoms, but almost all are able to clear the HEV infection spontaneously. However, in patients with poor prognostic factors such as immunosuppressed status or underlying liver disease, HEV infection can develop to fulminant hepatitis or acute or chronic liver failure. In this setting, a short course of ribavirin has been shown to produce complete recovery and avoid the need for liver transplantation in case reports and small series.[70,140,141] However, many more investigative and prospective studies are needed to evaluate if ribavirin treatment can indeed prevent progression to liver failure in patients with severe acute hepatitis E. Currently, liver transplantation is the only validated treatment option for patients with FHF.[140,141]

Of major concern is the ability of HEV, especially genotype 1 strains, to cause fulminant hepatitis in pregnant women with high mortality rates. To date no data are available on the role of treatment in pregnant women with acute hepatitis E, non-pregnant patients with acute liver failure, or during neonatal HEV infection. These cases, which mainly occur in areas where HEV is highly endemic, differ from patients with chronic HEV infection in that the window of time available for the treatment to be effective may be much shorter.[70]

As mentioned, the chronic course of HEV genotype 3 infection has been described in solid organ transplant recipient patients with hematological disease and with HIV-positive patients who had low CD4 counts.[70,140–142] Chronic HEV infection can result in progressive liver fibrosis, cirrhosis, and subsequent liver failure, which occasionally requires liver transplantation.[70,140,143,144] Therefore, therapeutic interventions should be considered for immunocompromised patients with HEV infection.[70,140,141] The treatment goal for chronic HEV infection is the complete removal of the virus. Even though various treatment options have been attempted, and several methods were described, systematic guidelines have not yet been reported.[140,141,145]

Decreasing the doses of immunosuppressive drugs that are aimed at T cells (mainly calcineurin inhibitors) has been proposed as the first-line therapeutic approach in transplant patients. This leads to spontaneous HEV clearance in 30% of patients but also to rejection under certain circumstances.[141,145]

Currently, it is widely agreed that antiviral therapy should be considered for patients who fail to eliminate the virus from their system after reduction in immunosuppressive drugs or for those who cannot tolerate a reduced dose of immunosuppressive drugs.[146,147] In these cases, antiviral therapy for chronic infection consists of monotherapy with interferon-alpha or ribavirin or a combination of these medications, with a recommended dosage period of usually 2–3 months.[70,140,141,148] However, both of these medications induce some severe adverse effects. In transplant recipients, interferon-alpha has been shown to increase the risk of rejection, and severe hemolytic anemia was observed in patients undergoing ribavirin treatment.[70,140,146,149]

PEGylated interferon-alpha 2a (PEG-IFNa2a) has been used as monotherapy for liver transplant recipients with chronic HEV. In a small case series, three patients who received 3 months of therapy with PEG-IFNa2a were included, and while two had a virological response at 24 and 20 weeks after completion of treatment, respectively, one experienced acute rejection after the completion of the treatment.[65,150] A group from the Netherlands, has recently reported the findings and clinical outcomes of treatment with PEG-IFNa2b of two liver transplant recipients with chronic HEV. One patient treated for 1 year had normalization of liver enzymes tests and a complete virological response at week 20. On the contrary, no evidence of a virological response was detected in the second patient, and PEG-IFNa2b treatment was stopped at week 16; however, after the immunosuppression dose was decreased, there was a normalization of liver enzymes, and HEV RNA became undetectable in serum.[65,151] Treatment with interferon, however, has been considered as a possible risk for inducing acute rejection in liver transplant recipients and is particu-

larly contraindicated by clinicians in kidney, lung, and heart transplant recipients patients.[65,150]

Several studies have been carried out with the aim to evaluate the efficacy of treatment with ribavirin under a monotherapy approach. A study carried out in France evaluated this drug in six kidney transplant patients who were HEV RNA-positive. After 6 months of treatment, four patients had a sustained virological response, and two patients presented a viral relapse after the completion of their ribavirin treatment.[65,152]

A prospective study done in Germany evaluated 33 solid organ transplant recipients with posttransplant HEV infection during the period from 2008 to 2012. Fifteen out of 33 patients (45%) had elevated alanine aminotransferase levels and active HEV infection (detectable RNA) for more than 2 months (prolonged HEV viremia), which was cleared in three of them after a reduction of immunosuppression. On the other hand, nine of eleven patients who received ribavirin became HEV RNA-negative after 3 to 6 weeks, and none of these patients had a recurrent HEV infection after the end of the treatment. The administered dose of ribavirin was 600 to 1,000 mg daily.[150]

Another interesting study showed that in a group of ten HEV RNA-positive patients of a cohort of 468 adult lung transplant recipients, eight patients developed a chronic HEV infection for more than 6 months. Thereafter, two of these patients were treated with ribavirin (400 mg twice daily) for 4 months and experienced a clearance of HEV RNA after 2 months as well as normalization of aminotransferases levels.[149]

The mechanism by which ribavirin suppresses HEV replication is not fully understood and requires further investigation.[141] For kidney, lung, and heart transplant recipients with chronic HEV infections, in which treatment with interferon is contraindicated because of the high risk of rejection, ribavirin could be an option as the first line of treatment since it seems to be well tolerated, safe, and capable of inducing a sustained virological response.[152,153] However, the optimal dosage and duration of the treatment still need to be determined and standardized. In Figure 3, an algorithm of management of HEV infection is proposed.

Appropriate prophylactic strategies and measures must be exercised in order to avoid HEV infection, particularly in high-risk groups such as pregnant women, immunosuppressed individuals, and solid organ transplant recipients. To date, two types of recombinant HEV vaccine have been developed. The first, developed by GlaxoSmithKline (Brentford, UK) and the Walter Reed Army Institute of Research (Washington, DC, USA), was tested in Nepalese males (n=1,900) with high safety and efficacy levels after three doses. However, this vaccine has
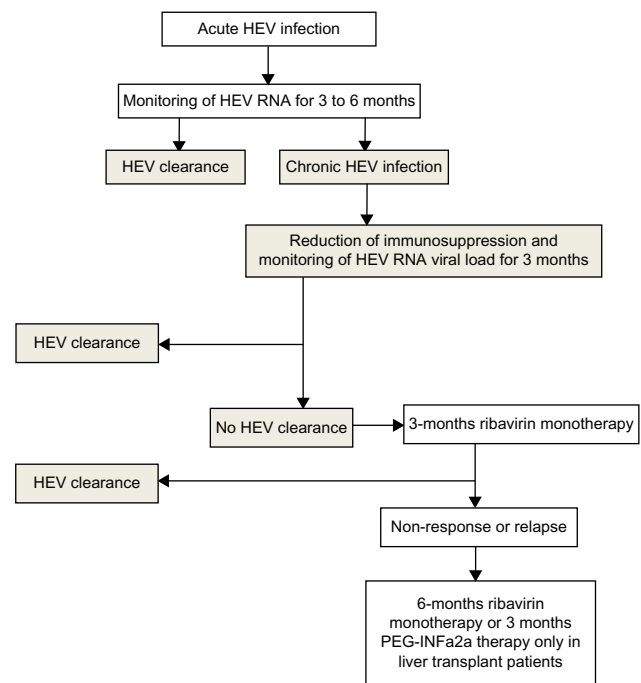
Figure 3 Proposed algorithm for the management of hepatitis E viral infection after solid organ transplantation.
Note: © Copyright 2012 The American Society of Transplantation and the American Society of Transplant Surgeons. Adapted with permission from Kamar N, Legrand-Abravanel F, Izopet J, Rostaing L. Hepatitis E virus: what transplant physicians should know. Am J Transplant. 2012;12(9):2281–2287.[155]
Abbreviations: HEV, hepatitis E virus; PEG-INFa2a, pegylated interferon-alpha 2a; RNA, ribonucleic acid.

not been further developed.[154] The second vaccine was tested in more than 112,000 Chinese participants, irrespective of HEV titers, and demonstrated a very high efficacy of around 94%–100% (95% confidence interval; 72.1–100) with a good safety profile. This vaccine, known as HEV 239, was licensed in the People's Republic of China in December 2011 and is approved for high-risk groups and will soon be available to other countries highly endemic for HEV.[52] However, both vaccines are genotype 1-based, and though they would be very useful in pregnant women and travelers to endemic regions, their efficacy in preventing HEV infection in nonendemic areas (where other genotypes predominate) is a matter of debate and needs to be investigated. Future developments including other genotypes, particularly genotype 3, will enable chronic HEV infection prevention and could be considered as an immunoprophylaxis measure in pretransplant recipients.[70]

Concluding remarks

HEV is the leading cause of non-A, non-B enterically-transmitted acute viral hepatitis in endemic regions and is considered a major global health problem that causes significant morbidity worldwide. Zoonotic transmission seems to be a major cause of HEV infections in industrialized countries, in the form of sporadic autochthonous cases. Prevention of foodborne HEV

transmission should rely on avoiding consumption of raw or undercooked animal meat and the revision of the minimum safety industry standards required for the production of ready-to-eat products of animal source. In addition, the currently increasing global burden of epidemic and sporadic forms of hepatitis E, the high mortality among pregnant women, the severity of autochthonous hepatitis E, and the ability of the virus to induce chronic infections highlights the need to give special attention to persons at higher risk of severe illness by giving priority to the prevention of infection in this vulnerable group during HEV outbreaks. Serological and NAT-based HEV tests should therefore be included in the evaluations of all patients with increased levels of liver transaminases. In this sense, it is particularly relevant that the test for HEV RNA be carried out in immunocompromised patients, such as HIV-infected individuals, solid organ transplant recipients, and people with hematological diseases. Patients with chronic hepatitis E should be considered for treatment with ribavirin since prolonged viremia has been associated with the development of liver cirrhosis and severe hepatic failure. The threat caused by the widespread prevalence of HEV infection reinforces the need to perform broad epidemiological studies in endemic and nonendemic regions and to conduct clinical trials of currently developing vaccines. Additional research to find the most highly effective and protective candidates for HEV vaccines, which include the immunodominant epitopes of the four human genotypes, should be pursued.

Acknowledgments

Disclosure

JA is a Level 3 Researcher at Sistema Nacional de Investigadores-ANII. The authors report no other conflicts of interest in this work.

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