REVIEW

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Future MICROBIOLOGY

Hepatitis E: latest developments in knowledge

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Hepatitis E, caused by Hepatitis E virus (HEV), is a highly prevalent disease in developing countries. In developed nations, autochthonous HEV infections seem to be an emergent disease. Its clinical manifestations and epidemiology are well known for endemic countries. It has been confirmed that hepatitis E is a zoonosis and that parenteral transmission can also occur. The molecular mechanisms of HEV replication are not fully understood, mostly because there are no efficient cell culture systems. HEV can cause chronic hepatitis in organ transplant recipients and immunocompetent patients. Cases with fulminant hepatitis and other extrahepatic manifestations have also been reported. The diagnosis is based on serological studies and detection of HEV RNA in blood and feces. Treatment with ribavirin and/or pegylated-IFN-α have proven to be successful in some cases. The recently approved/marketed vaccine is a good option in order to prevent this infection.

First draft submitted: 20 January 2016; Accepted for publication: 30 March 2016;
Published online: 20 May 2016

KEYWORDS
• epidemiology • hepatitis E • hepatitis E virus • HEV • replication • treatment • vaccine

Hepatitis E virus (HEV) is the main cause of enterically transmitted hepatitis worldwide, which is responsible for >50% of acute viral hepatitis cases in endemic countries. It is estimated that approximately 2 billion individuals, representing a third of the world's population, live in hepatitis E-endemic areas and, therefore, are at risk of infection. According to WHO, 20 million cases of HEV infections are registered annually, of which >3 million are acute cases and approximately 56,600 result in death [1].

With the exception of China, where hepatitis E is a zoonosis, in developing countries with poor sanitation, this disease is transmitted through contaminated water and is associated with large epidemics [2,3]. Developed countries appear to be free of this infection, with the exception of cases that are sporadically described in individuals coming from endemic regions. For this reason, hepatitis E is considered one of many diseases linked to poverty in tropical and subtropical countries. The combined use of serological and molecular techniques has proved much higher incidence and prevalence rates than expected in developed countries, confirming the existence of a zoonotic reservoir among domestic animals. In this respect, the infection of pig livestock and its connection with cases in humans has been proven [4,5]. Moreover, cases of parenteral transmission have also been detected.

This review describes recent advances in knowledge regarding the virus and the disease, with transcendence from a clinical, epidemiologic and sanitary point of view.

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History of hepatitis E

The first epidemic attributed to HEV occurred during 1955 [6] in New Delhi (India) when approximately 30,000 individuals fell ill due to a supposed hepatitis A infection after consuming contaminated water. However, it was not until 1980 when it was observed that they did not react against HAV antigens [7], thus this epidemic was attributed to a new etiological agent.

In 1983, a volunteer was experimentally infected by ingesting a mixture of extracts obtained from an individual who suffered the disease in the 1955 epidemic in Kyrgyzstzan [8]. Viral particles, 27–34 nm in size, were observed in the feces.

This observation was confirmed in patients from distant geographical regions, all of them affected by hepatitis no-A no-B [9]. In 1989, the first cynomolgus macaque monkeys were effectively infected [10].

In 1990, HEV RNA was detected in clinical samples of affected individuals in Burma, Mexico, Somalia and Pakistan [11]. Experimental infection of pigs was also performed, achieving the excretion of the virus in feces [12]. This new agent was named hepatitis E virus. The letter 'E' makes reference to the enteric, endemic and epidemiologic characteristics of the disease.

In 1996, a strain different from those known at the time was isolated in an American individual with no prior history of trips overseas [13]. This fact, along with the emergence of a great number of hepatitis E cases in developed countries considered nonendemic, such as Holland [14], Japan [15] and Spain [16], suggested the existence of animal reservoirs. Consequently, many seroprevalence studies were performed in different animal species, for example, pigs [17–19], poultry [20] and rodents [21].

In 1997, the first HEV strain of porcine origin was isolated in the USA [22], with only a 74% of resemblance in the nucleotide sequence when compared with the classic strains isolated in Burma and Mexico, but with a resemblance of 90% when compared with the human isolates of the same region. Since then, there have been many cases of porcine strains isolated in industrialized countries, such as Spain, The Netherlands and the UK [23].

Viral particle

HEV is a nonenveloped virus with an icosahedral symmetry and size between 32 and 34 nm in diameter. The buoyant density of HEV is between 1.39 and 1.40 g/cm^3 in CsCl and its sedimentation coefficient is 183S [24]. HEV genome is between 6.6 and 7.3 kb long and comprises a single-stranded positive polarity RNA molecule showing three open reading frames (ORFs) [25]. The ORF1 is the longest reading frame at the 5′-end of the genome. The ORF1 encodes a nonstructural polyprotein including a methyltransferase, a Y-domain, a papain-like protease, a polyproline region (hypervariable region), a macro-domain, a helicase and an RNA-dependent RNA polymerase. ORF2 is located at the 3′-end of the genome and encodes the major capsid protein. ORF3 overlaps partially with ORF2. It encodes a phosphoprotein that modules cellular activities [26]. All ORFs are expressed during viral infection since antibodies against these regions have been detected in naturally infected humans and in experimentally infected monkeys [27]. Additionally, there are two untranslated regions in 3′- and 5′-terminal portions.

Classification

Although a single serotype has been identified, a great genetic diversity between the different HEV isolates has been widely reported [28]. Recent studies have proposed several classifications of HEV in different genotypes and subtypes [29,30]. The International Committee of Taxonomic Virology, in its last meeting that took place in July 2014 in Montreal, Canada [31], classified HEV into the family *Hepeviridae* **(Table 1)**. This family is divided in two genera, *Orthohepevirus* (all mammalian and avian HEV isolates) and *Piscihepevirus* (cutthroat trout HEV). Species within the genus *Orthohepevirus* are designated *Orthohepevirus A* (isolates from human, pig, wild boar, deer, mongoose, rat, rabbit and camel), *Orthohepevirus B* (isolates from chicken), *Orthohepevirus C* (isolates from greater bandicoot, Asian musk shrew, ferret and mink) and *Orthohepevirus D* (bat isolates). Within species *Orthohepevirus A*, there are currently four genotypes described that infect humans (G1, G2, G3 and G4) and three genotypes (G5, G6 y G7) that affect wild boards and camels [32]. Two genotypes exist within the genus *Orthohepevirus C*, C1 has been detected in greater bandicoot and Asian musk shrew and genotype C2, detected in ferret and mink [33,34].

The cutthroat trout HEV isolate has been classified in the genus *Piscihepevirus A*. This isolate exhibits the characteristic three ORFs

Table 1. Classification of the family *Hepeviridae*.

Family	Genus	Species	Genotype	Source
Hepeviridae	*Orthohepevirus*	*Orthohepevirus A*	1	Human
			2	Human
			3	Human, swine, mongoose, rabbit, deer, rat
			4	Human, swine
			5	Wild board
			6	Wild board
			7	Camel
		Orthohepevirus B	–	Avian
		Orthohepevirus C	C1	Greater bandicoot, Asian musk shrew
			C2	Ferret, mink
		Orthohepevirus D	–	Bat
	Piscihepevirus	*Piscihepevirus A*	–	Cutthroat trout

genome arrangement seen among avian and mammalian members of the family *Hepeviridae*. Additionally, motifs characteristics for methyltransferase, papain-like protease, macro domain, helicase and replicase are found in the cutthroat trout virus ORF1. However, amino acid sequence similarities between cutthroat trout virus and others members of this family are only 26–27% (ORF1), 18–21% (ORF2) and 13–16% (ORF3). By contrast, these similarities are 42–49% (ORF1), 42–55% (ORF2) and 20–29% (ORF3) between avian, rat and mammalian *Hepeviridae* [31,35].

Cell culture models of HEV replication

The molecular mechanisms of HEV replication are not entirely understood, mostly because there are no efficient cell culture systems. The first cell culture model for G3 and G4 of HEV that showed optimal results was developed using an inoculum with high titer of virus from serum and feces, diffused efficiently in PLC/PRF/5 cells derived from human hepatocarcinoma and A549 cells obtained from lung cancer **(Table 2)** [36–38]. Although A549 cells are not hepatocytes, it was found that the virus was able to replicate in other tissues of infected pigs, such as kidney, liver, spleen, tonsils and colon [39].

Subsequently, in 2009, Tanaka *et al.* [38] designed a cell culture with G4 strains HE/JF5/15F isolated from the feces of a patient with fulminant hepatitis E, which grew more efficiently in PLC/PRF/5 and A549 cells than the strain A549 JE03-1760F [44]. Thus, this model has been proposed to study the factors related to fulminant hepatitis.

Another study [40] demonstrated that it was possible to grow the G3 KernowC1 strain, isolated from the feces of an infected patient, in human hepatoma cells HepG2/C3A. This strain contains a recombinant insert in the ORF1 region of the viral genome of 174 nucleotides of a human gene for a ribosomal protein, which apparently facilitated the adaptation to cell culture [45].

Moreover, several studies have focused on getting spread of HEV strains isolated from pigs in human cells, and vice versa, to obtain information about the zoonotic potential of the virus, the mechanisms that facilitate cross-species infection and help to elucidate the replicative cycle of the virus. Thus, one study showed that HEV G3 could infect LLC-PK1 pig cells more efficiently than human cells HepG2/C3A [40]. In that sense, it also described that porcine hepatitis E G4 strains could replicate in human A549 cells [46].

Additionally, in 2012, Rogée *et al.* [41] developed two cell culture models HepaRG from human hepatoma cells and PICM-19 cell line from pig embryonic stem cells.

Recently, a new *in vitro* cell culture model has been performed with HEV G3 and G4 strains (G3jp, G3US, G3sp and G4jp) isolated from the feces of naturally infected pigs [42], in PHCs cells (primary human hepatocytes).

• Life cycle of HEV

The mechanism by which the virus penetrates the cell depends largely on the type of viral structure; big differences exist between enveloped viruses and nonenveloped viruses, such

Table 2. Cell lines used for propagation of different hepatitis E virus strains.

Cell line	Origin	Strain (genotype, origin)	Ref.
PLC/PRF/5	Human liver hepatoma	JE03-1760F (G3, H)	[36–38]
A549	Human lung carcinoma	HE/JF5/15F (G4, H)	
HepG2/C3A	Human hepatocellular carcinoma	KernowC1 (G3, H) SAR55 (G1, H)	[40]
LLC-PK1	Swine kidney epithelial	KernowC1 (G3, H)	[40]
Hepa-RG	Human hepatocytes	GenBank no JN906976 (G3f, S)	[41]
PICM-19	Pig liver stem		
PHCs	Primary human hepatocytes	G3jp (G3, S), G3US (G3, S), G3sp (G3, S), G4jp (G4, S)	[42]
S10-3	Human hepatocellular carcinoma	SAR55 (G1, H)	[43]

H: Human; S: Swine.

as HEV. Furthermore, the site of replication of HEV RNA, is located in the cytoplasm, unlike DNA viruses that typically replicate in the cell nucleus **(Figure 1)** [47].

• HEV binding to cell process

The specific life cycle of HEV is largely unknown due to the lack of an efficient cell culture system. In-depth knowledge of the capsid protein encoded by ORF2 has allowed the study of HEV interactions with target cells. It is believed that the C-terminal region of the ORF2 plays an important role in this stage of HEV life cycle [48].

This protein region first interacts with specific receptors on the cell membrane, including: heparan sulfate proteoglycans [49] and heat-shock protein cognate 70, facilitating virus binding to the hepatocyte.

A recent study [50] performed with HEV-like particles, showed how the entrance of HEV into Huh-7 hepatoma cells is associated with endocytosis-mediated GTPase Dynamin-2, playing an essential role in the cleavage of plasma membrane to form Clathrin-coated vesicles. Additionally, the process involves the cholesterol membrane, clathrin and actin (clathrin-mediated endocytosis), suggesting that macropinocytosis mechanisms are not involved in this process.

• Intracellular trafficking

HEV enters the cell through endosomes and replicates in the cytoplasm for subsequent release. This process is pH-independent, unlike other viruses that require acidic pH conditions [51,52].

The heat-shock 90 and Grp78 proteins bind to the cell membrane once the virus contacts the cell, participating in the intracellular plasma transportation of viral capsids in the early stages of infection with HEV [53,54].

• Replication

Once the RNA is released from the virion to the cell cytoplasm, translation of ORF1 nonstructural polyprotein occurs [55]. It is not yet confirmed if proteolytic processes occur or not. An RNA-dependent RNA polymerase synthesizes intermediate negative polarity copies from positive polarity RNA [56,57]. These serve as a template to synthesize new copies of viral RNA. The RNA-dependent RNA polymerase interacts with *cis*-reactive elements (CRE) in the 3′NCR of the genome of the HEV, which could have an important role in HEV replication.

Moreover, ORF2 and ORF3 proteins are translated in the cytoplasm. ORF2 proteins with new copies of viral RNA are assembled to form new virus particles. In this regard, it is thought that the C-terminal region of viral capsid is essential for encapsidation and particle stabilization [48], as suggested by a study of mutant strains of HEV for the region C-terminal 52 amino acids, in which premature signal STOP was artificially generated. This prevents the correct synthesis of this domain, which apparently protects the particle of C-terminal degradation.

A recent study [58] conducted a mutagenic analysis in the active site of 'X domain' suggests that X-domain residues intervene decisively in HEV replication. However, additional studies are required to confirm this relationship with the pathogenesis of the virus.

• Egression stage

New viral particles assembled in the cytoplasm are transported to the cell membrane; the ORF3 protein facilitates the process [59]. In the release process of new viral particles through the membrane of the infected cell, the action of PSAP (Pro, Ser, Ala and Pro) motif in ORF3 protein [60] is important. This protein is highly

conserved in all the HEV strains isolated from mammals and poultry.

Finally, the particles leave the cell and are released into the environment. In this last phase, it has been seen that the PSAP motif of the ORF3 protein interacts with the *Tsg101*, which is a cellular protein linked to the process of egress of other viruses, such as HIV [59,61]. The *Tsg101* is a required component for the transportation related to the mechanism of egress associated with multivesicular bodies. Thus, it is suggested that HEV uses multivesicular bodies pathway to exit the cell.

Epidemiology

Hepatitis E infection is an increasingly common cause of acute hepatitis, especially in developing countries, such as India or Bangladesh, where robust sanitation systems and hygiene are more required. HEV infection is found worldwide, with East and South Asia having the highest prevalence rates [62]. Different studies have shown that HEV has spread globally [63], but the genotypes found vary depending on the geographical region. G1 (Central and South Asia and North Africa) and G2 (West Africa and Mexico) are predominant in developing countries, whereas G3 is mostly found in the American continent and Europe, and G4 is most common in China **(Figure 2)**.

Regardless of the global distribution, the majority of the disease burden is in less developed countries. According to WHO, there are approximately 20 million hepatitis E infections every year, causing over 3 million acute cases of hepatitis and 56,600 hepatitis E-related deaths [1].

Types of presentation (sporadic vs epidemic)

Infection with HEV has two types of presentations, as endemic outbreaks or as sporadic events. Whereas sporadic hepatitis E occurs worldwide, outbreaks generally appear in developing countries and are mainly due to water sources that are contaminated with fecal matter [8]. These endemics become more frequent after natural disasters or in overcrowded refugee camps. Between one and 15% of the population is usually affected when an outbreak of hepatitis E occurs in a community [64]. HEV infection is considered endemic in India, Central Asia,

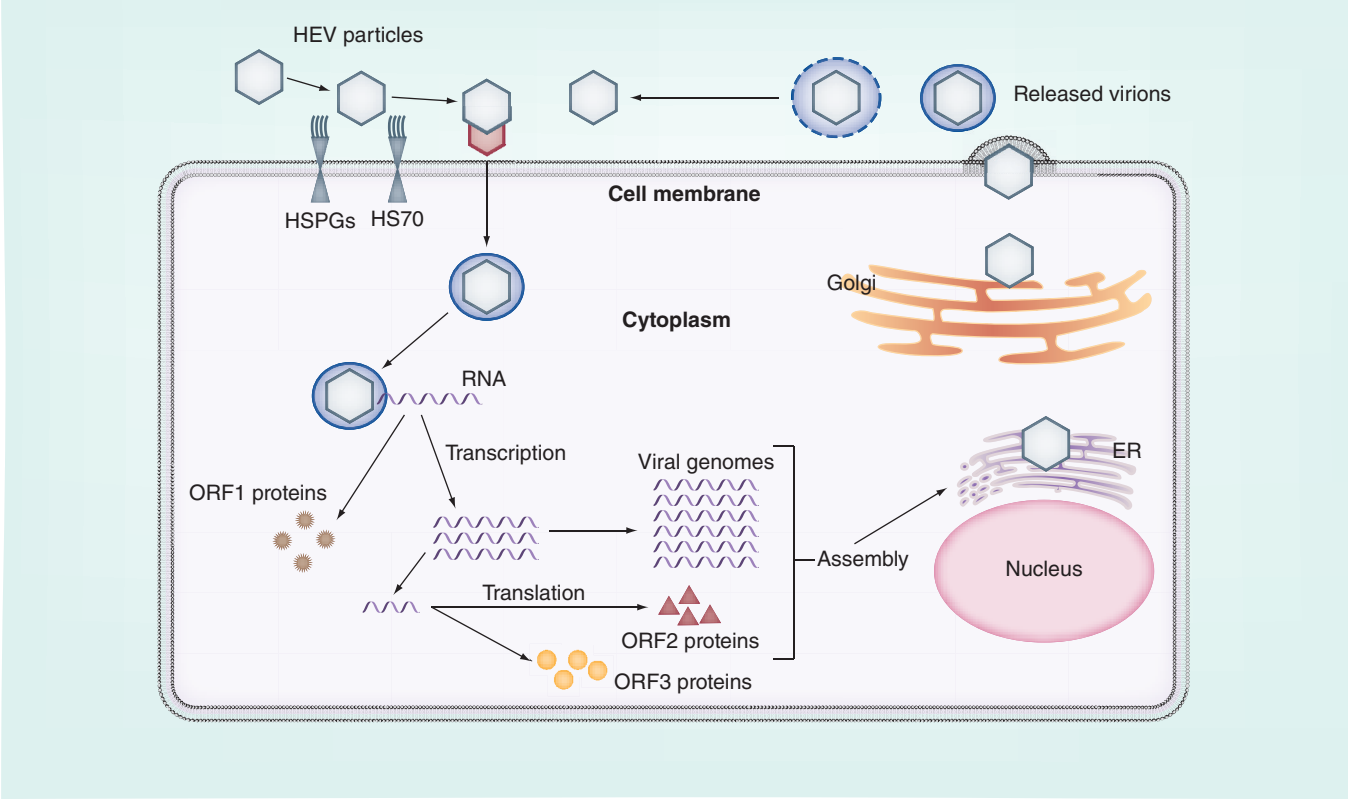

Figure 1. Hepatitis E virus replicative cycle.

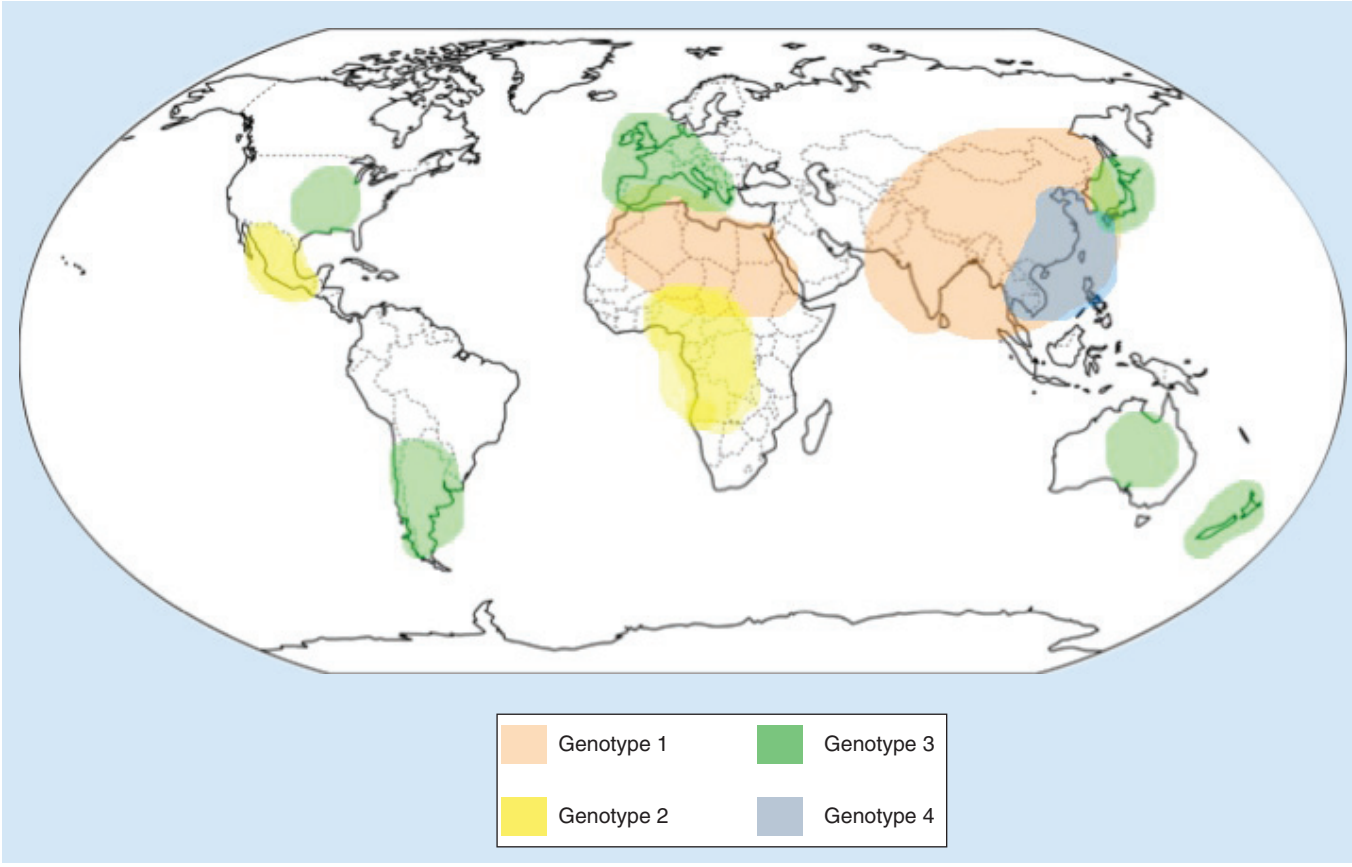

Figure 2. Geographic distribution of the hepatitis E virus genotypes affecting humans.

Africa and Mexico. India is one of the countries where HEV is responsible for a high percentage of acute viral hepatitis cases, accounting for approximately 40% according to CDC estimates. In fact, the first identified epidemic outbreak of HEV took place in New Delhi in 1955 [65]. The most recent epidemic took place in 2014, in Biratnagar, eastern Nepal, due to a contaminated water supply [66]. Epidemic outbreaks registered in each continent from 1955 to the present day are described in **Table 3**.

Historically, HEV infections in industrialized countries were connected to patients that had traveled to endemic areas [78–80], but recent studies found locally acquired HEV infections in these nonendemic countries that prove this theory wrong. G3 and G4 are responsible for autochthonous cases in nonendemic countries, such as the USA, Japan or European countries [81]. In these countries, the most probable transmission route for these sporadic cases is the contact with animals that act as reservoirs. This could include both, cattle handlers and veterinarians who work with pigs, and people who ingest raw or undercooked meat from these infected animals [82].

Transmission routes

As with genotype distribution, there is also a remarkable difference in transmission routes between geographical areas **(Figure 3)**.

The most predominant transmission route in developing countries is the fecal–oral route; these countries have deficient hygienic conditions, which increase the contamination of food and, especially, drinking water [81]. Generally, contamination occurs after torrential rains, floods or natural disasters, when consumption water is mixed with animal and human sewage [83]. This is the main transmission route for G1 and G2, which can only be acquired person-to-person.

Other potential human-to-human transmission routes include sexual transmission and *in utero* vertical transmission. *In utero* transmission has been described in several studies [84,85]. Mother-to-child transmission rates vary between 23.3 and 50% depending on the study [85], and

Table 3. Major outbreaks and number of cases of hepatitis E.

Continent	Country	Year(s)	Cases	Ref.
Africa	Algeria	1979–1980	715	[67]
	Botswana	1985	273	[68]
	Central African Republic	2002	20	[67]
		2004–2005	411	[67]
	Chad	1983–1984	38	[68]
		2004	1442	[68]
	Cote d'Ivoire	1983–1984	623	[68]
	Djibouti	1992–1993	43	[67]
	Eritrea	1988–1989	>750	[67]
	Ethiopia	1988–1989	423	[68]
		2014–2015	1117	[69]
	Kenya	1991	1765	[67]
		2012	223	[70]
	Morocco	1994	>75	[67]
	Namibia	1983	201	[67]
		1995–1996	>600	[68]
	Somalia	1988–1989	11,413	[68]
	Sudan	1988	>55	[67]
		2004	3753	[68]
		2004	2621	[68]
		2013	5080	[71]
	Uganda	2008	9500	[72]
America	Costa Rica	1976	5	[73]
	Mexico	1986	223	[68]
Asia	China	1982	45	[74]
		1983	520	[74]
		1985	9	[74]
		1986–1988	119,280	[68]
	India	1955–1956	29,300	[75]
		1978–1979	20,000	[68]
		1979–1980	6000	[68]
		1981–1982	26,000	[76]
		1984	3005	[76]
		1987	2215	[68]
		1988	17	[68]
		1988–1989	53	[68]
		1989–1990	3000	[68]
		1991–1992	79,091	[68]
		1992	2427	[68]
		1992–1993	3682	[68]
		1995	21	[68]
		1998	NA	[68]
		2002	185	[76]
		2003	>123	[76]
		2004	1338	[76]
		2005	1611	[76]
		2005–2006	3170	[76]
		2007	400	[76]
		2008	23,915	[76]
		2010	161	[76]

Table 3. Major outbreaks and number of cases of hepatitis E (cont.).

Continent	Country	Year(s)	Cases	Ref.
Asia (cont.).	Indonesia	1998	>600	[68]
	Kyrgyzstan	1955	NA	[68]
		1987–1989	>500	[76]
	Myanmar	1982	399	[68]
		1999	111	[68]
	Nepal	1981	4337	[68]
	Pakistan	1986	85	[68]
		1987	133	[68]
		1998	109	[68]
		2000–2001	18	[68]
	Turkmenistan	1985	16,175	[68]
	Vietnam	1994	150	[77]

are most frequent in the third trimester of pregnancy. In one of these studies, premature births and increased prenatal mortality were observed in a group of infected pregnant women [86]. Homosexual males show a higher prevalence of HEV antibodies (20%) than the general population, thus it has been suggested that sexual intercourse is a viable transmission route, however, further data are required [87].

In industrialized countries, hepatitis E is most commonly identified as a zoonotic disease, with swine being the main reservoir. In these countries, the infection can be acquired in one of two different ways: direct contact with infected animals (veterinarians and cattle handlers) or the ingestion of foods that have been in contact with infected animals or feces. Pig farmers, veterinarians, slaughterhouse workers and farmers have shown a high prevalence of HEV antibodies [88]. Infection through the consumption of undercooked or raw animal meat is also common in Europe and the USA, so much so that some epidemiological studies have identified the consumption of game and swine meat as a risk factor for the infection with HEV [89,90]. Bearing in mind that animal sewage can also contaminate water in developed countries, it is not surprising that the consumption of raw seafood could also lead to HEV infection. This was the case of a hepatitis E outbreak that took place in 2008 during a cruise [91]. The concept of hepatitis E as a food-borne disease is particularly strong in western Europe, where the food chain is the main source of infection [92].

Awareness of transfusion-transmitted HEV is increasing exponentially as more cases are retrospectively identified in both industrialized and developing countries. The first proven hepatitis E transmission through transfusion therapy in a developed country took place in Hokkaido (Japan) in 2002, when a group of researchers showed an identical sequence of G4 HEV RNA genome in a blood donor and a patient who had received his plasma unit during open-heart surgery [93]. After several other cases with the same characteristics were identified, in-house HEV RNA testing has been implemented in Japan in addition to blood donor screening for elevated ALT levels. Europe's first cases of post-transfusion HEV infections were reported in the UK [94] and France [95] during 2006 and 2007, respectively. Many studies show that any blood product (red blood cells, platelets, fresh frozen plasma, etc.) can transmit HEV, however, the viral load required to induce symptoms is unclear [96].

Clinical manifestations

HEV infection may cause a wide range of clinical presentations from subclinical or asymptomatic forms to fulminant liver failure [97,98]. However, acute hepatitis is the most common presentation in both industrialized and developing countries, although the mortality rate is much higher in the latter. Chronic hepatitis has also recently been described but only in infection with HEV G3.

Common features are:

- The incubation period is approximately 40 days, ranging from 2 to 10 weeks;
- Viremia, being transitory, occurs primarily during the preicteric phase and disappears with the development of clinical symptoms, except in cases of chronic hepatitis E;

- Fecal excretion of the virus begins around 5 days prior to jaundice and diminishes 2 or 3 weeks later, at the onset of jaundice [83].

• Acute hepatitis E

The illness is usually self limiting, lasting <6–7 weeks and is almost identical to other acute viral hepatitis, such as hepatitis A or B. Jaundice is present in approximately 40% of patients [99] and is the most common clinical manifestation of the infection. It may last for 2–4 weeks in most cases, or longer if cholestasis is prolonged. Flu-like myalgia, asthenia, fever, nausea, vomiting, joint and abdominal pain are other common symptoms [100].

The prevalence of the disease in industrialized countries is much higher among the middle aged and elderly. Patients are mostly males, with the median age being 65 years [101]. The reason why symptomatic infection is more common in this population still remains unknown, but it may have a relationship with the transmission route. HEV G3 infections are considered a zoonotic disease, although most patients have no contact with pigs, other animals or inadequate handling of foods. Certain nonconfirmed environmental factors, such as recreational waters, may play a role. On the other hand, in endemic areas, the large majority of patients are young adults from 10 to 40 years. The reason for that remains unclear, although it may be related to major exposure to contaminated water [100]. The clinical features in endemic areas are indistinguishable from those in industrialized countries.

During pregnancy, the number of cases of severe disease and mortality caused by G1 or G2 is very high, approximately 15–20%. Complications in the mother, such as eclampsia, hemorrhage and fulminant hepatic failure, can occur at a high rate. Abortion, premature delivery, death of the mother and fetus or of a live-born baby after birth have also been reported [102].

In industrialized countries, sporadic acute hepatitis E is commonly misdiagnosed as autoimmune hepatitis or drug-induced liver injury, with the HEV infection being diagnosed later, during retrospective serological testing [103]. As liver histology is not required for most patients, there are few studies regarding to support this. Acute cholangitis and polymorph inflammation, severe intralobular necrosis and lobular disarray with reticulin framework distortion have been

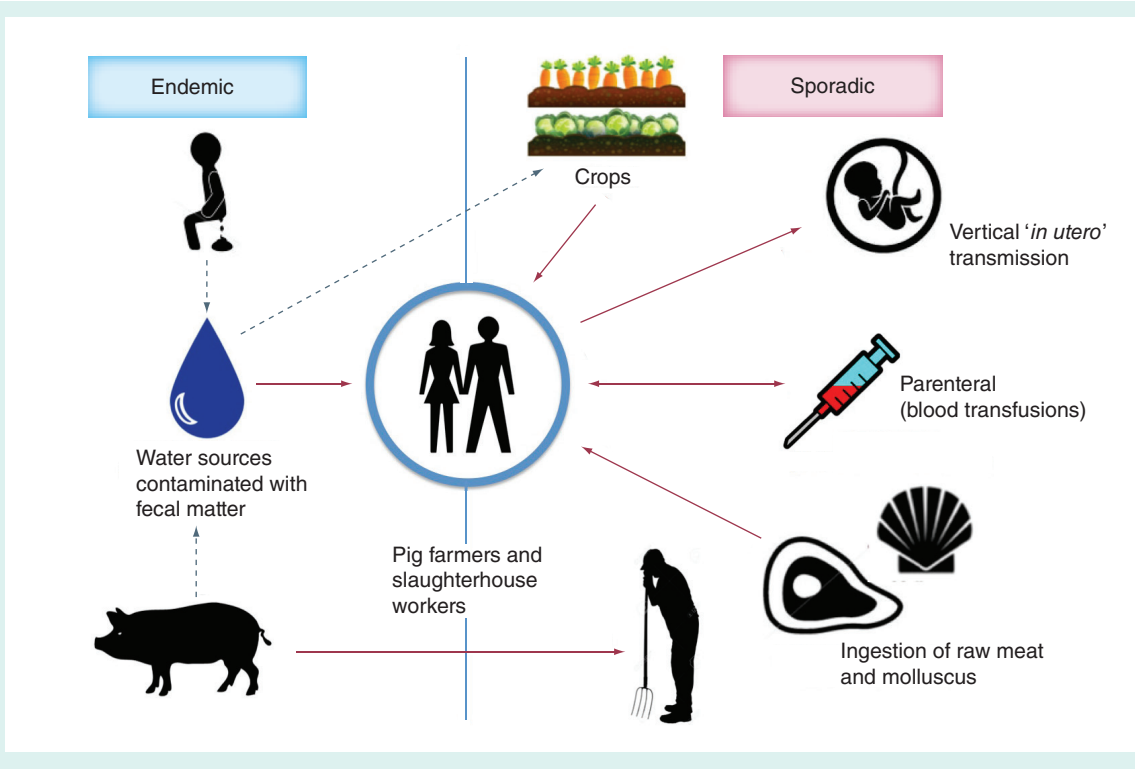

Figure 3. Transmission routes of hepatitis E virus.

detected. Portal tracts are expanded by a severe mixed polymorph and lymphocytic inflammatory infiltrate but no characteristic lesions have been observed [103]. Polymorphs concentrated at the interface and periphery of the liver, with lymphocytes, including aggregates, concentrated centrally were detected in three patients with autochthonous hepatitis E [104]. These findings may be useful when differentiating autochthonous hepatitis E from other causes of hepatitis (autoimmune hepatitis). However, these data are based on a small number of cases and further research is required.

• Chronic hepatitis E

The principal characteristic of chronic HEV infection is the presence of HEV RNA and/or IgM anti-HEV in blood and/or stools for >6 months in association with increased liver enzyme levels. However, if the virus has not been cleared in 3 months (HEV RNA persistence in blood by PCR) no spontaneous clearance is observed thereafter without treatment, suggesting that chronic hepatitis E can be diagnosed as soon as 3 months after infection [105]. HEV infection most frequently evolves into chronic liver disease in immunocompromised patients (patients with solid organ transplants, lymphoma, HIV, hematological patients receiving chemotherapy, primary immunodeficiencies and those under treatment with corticosteroids and immunosuppressive agents, such as rheumatology patients) [106]. Currently, only two cases of chronic hepatitis E in immunocompetent individuals have been reported [107,108]. Chronic hepatitis E has also been reported in children after liver or bone marrow transplantation [109].

Usually, there are no characteristic clinical features related to chronic hepatitis E as most patients only present a slight increase in liver enzyme levels and generally remain asymptomatic. Moreover, IgG and IgM anti-HEV in blood might not be detected due to the immunosuppression, leaving HEV RNA detection the only technique available to confirm the diagnosis of chronic hepatitis E.

To date, chronic hepatitis E has been observed almost exclusively in locally acquired cases of patients infected with G3. No chronic disease has ever been related to G1 and G2 [110] and concerning to G4 there is only one report in China in a patient with acute lymphoblastic leukemia [111].

• Chronic hepatitis E in transplant patients

Kamar *et al.* in France [112] first described chronic courses of hepatitis E in solid organ transplant patients and later on in different populations of immunosuppressed patients. With 60% of transplant patients being unable to eliminate HEV, and its corresponding evolution to liver fibrosis, cirrhosis and death, chronicity rates are elevated [113]. Estimates show that 10% of transplant patients suffering hepatitis E develop cirrhosis several years after primary infection [114] and, therefore, antiviral treatment must be started as soon as possible. This progressive evolution to cirrhosis has also been confirmed in liver-transplanted children infected with HEV [115].

The incidence of new HEV infection in liver transplant patients is 4.8 cases per year and HEV RNA is present in 0.9–3.2% of transplanted patients [106]. Immunosuppression degree, leukocyte count, T-cell composition and tacrolimus use are strongly related to the inability to clear HEV after acute infections. HEV reactivation, however rare, may occur, as it has been communicated in two allogenic stem cell transplant patients [116,117].

However scarce, the data available on liver pathology show progressive fibrosis and portal hepatitis with lymphocytic infiltration and piecemeal necrosis with progression to cirrhosis in a short period of time [111,118].

Extrahepatic manifestations of acute and chronic HEV infections are also described in transplant patients (see the 'Extrahepatic manifestations' section).

In addition to the well-known modes of transmission of HEV, other routes, such as transfusion of blood products, nosocomial infection or most rarely the graft must be considered in the transplant patients. In one case, an HEV infected liver has been transplanted to an IgG anti-HEV-negative patient. The transmission was confirmed by phylogenetic analysis [119]. Screening of blood or organ donors have not yet been recommended but this risk for patients in the transplant setting may be underestimated as supported by a recent study [120].

• Chronic hepatitis E in HIV patients

Chronic hepatitis E has been described in HIV-infected patients mainly in those treated with antiretroviral drugs [121,122]. The seroprevalence of IgG anti-HEV can be as high as 10.4% in some European countries [123], but according

to some authors it may not be so common and only observed in individuals with strong immune impairments. Low CD4 counts may be considered a risk factor for progression to chronicity since many authors have confirmed that all HIV-infected patients who developed chronic infection had low CD4 counts [124,125]. Since HEV infection may be fulminant in the presence of underlying liver disease or may lead to chronic infection [126], testing for detection of HEV RNA in blood should be considered essential for diagnosis and treatment.

• HEV infection in pre-existing chronic liver disease

Acute HEV infection coexisting with previous chronic liver disease with diverse origins has been widely reported. Initial symptoms are similar to those of acute hepatitis but soon develop complications due to the decompensation of chronic liver disease, appearance of ascites and hepatic encephalopathy. Alcohol, being a relevant risk factor, favors the onset of clinical symptoms and determines severity of the pathology. Patients with hepatic steatosis or hepatic fibrosis caused by alcohol consumption have been found to have a more severe host response to HEV infection [127]. In the absence of liver transplant, a negative outcome is highly probable, approaching a mortality rate of 70% in individuals infected with HEV G1 [128]. The liver histology of hepatitis E is nonconclusive for patients with underlying cirrhosis seeing as it can be mistaken for alcoholic hepatitis [129]. Reports show that, in some cases, a short treatment with ribavirin has avoided the need for liver transplants [129].

• Extrahepatic manifestations

Several nonhepatic diseases have been described in relation to HEV based on laboratory diagnosis of hepatitis E in addition to presence of symptoms in other organ systems apart from the liver. Neurological manifestations are the most frequent complications that have been described (5.5% of patients with acute and chronic HEV infection). Said manifestations include Guillain–Barré syndrome, Bell's palsy, meningoencefalitis and inflammatory polyradiculopathy, among others [130]. On the other hand, a large case–control study confirmed that 5% of patients with Guillain–Barré syndrome had acute hepatitis previously [131]. The cerebrospinal fluid of patients with neurological disorders has been analyzed for HEV RNA. HEV sequences in these individuals presented quasispecies, which suggests that neurotropic variants and extrahepatic replication can exist [113].

Several cases of pancreatitis have been reported. The symptom usually developed in the second or third week after the onset of jaundice and disappeared spontaneously in some cases [132]. Interestingly, acute pancreatitis has only been reported from endemic countries related to G1. On the other hand, hematological manifestations, such as thrombocytopenia and hemolytic anemia, have been communicated in endemic and nonendemic countries and it is associated to immune-mediated injury [133]. Henoch–Schonlein purpura in a child [134] has also been reported.

Kidney dysfunction has been communicated less frequently. In solid organ transplant patients, a decrease in glomerular filtration rate is observed in infections with G3, and some cases of membranous glomerulonephritis and cryoglobulinemia have also been reported [135].

Diagnosis

The tests carried out in the laboratory in order to detect HEV infection include molecular techniques and/or serological tests to detect specific humoral response in the host and IgM and IgG anti-HEV. HEV RNA levels in both serum and feces are transient. The virus can be detected in feces 1 week before the onset of the clinical signs and persists for 2 weeks, although, in some cases, the virus has been detected in feces up to 52 days after the appearance of the first symptoms. In blood, viremia is present during the incubation period and in the early symptomatic phase and became undetectable within 21 days of symptom onset.

• Detection of HEV RNA

There are some in-house real-time PCR assays to quantify HEV RNA in fecal and serum samples. The advantages of these techniques include a high sensitivity (ten molecules DNAc/PCR) and a high specificity. On the downside, their performance may vary between tests and laboratories. Due to this, and the short time of viremia, undetectable HEV RNA does not exclude HEV infection. However, detection of HEV RNA by PCR plays a critical role in the diagnosing of HEV infection, as well as the monitoring of antiviral therapy [136] in immunosuppressed patients in which diagnosis of acute or chronic

hepatitis E is of some concern since seroconversion to detectable levels of anti-HEV antibodies is delayed or not present at all in these patients. Recently, commercially available tests for quantification of HEV RNA have appeared but these have not yet been approved by the US FDA.

• Serologic assays

Most laboratories are choosing the method of serologic diagnosis for HEV infection, using ELISA **(Figure 4)**. The diagnosis of acute infection is based on the presence of IgM anti-HEV antibodies that can be detected during the acute phase of the illness and are present for up to 4–5 months. IgG anti-HEV are detected just after the raising of IgM anti-HEV, increasing from the acute phase until the convalescent phase. IgG anti-HEV have been detected up to 14 years after the acute phase [137]. Therefore, increased IgM anti-HEV levels represent acute infection, whereas IgG anti-HEV levels indicate previous contact with HEV. Recombinant proteins or synthetic peptides corresponding to immunodominant epitopes from ORF2 and/or ORF3 belonging to Burma and/or Mexico strains are being used as antigens in these assays. *In vitro* studies showed that all four HEV genotypes can be included in a single serotype. The performance characteristics of the commercially available ELISA tests are considered suboptimal since several studies have demonstrated that the results are frequently discordant. The sensitivity ranged from 17 to 100% and some of them did not detect IgM anti-HEV antibodies in patients infected with G2–G4 [100]. By contrast, specificity needs to be increased since high false-positive rates with IgM anti-EBV and anti-CMV have been recorded [138]. In addition, IgG immunoblot assays frequently used as a confirming test have been shown to be unreliable [139].

More research must be done for improving the laboratory diagnosis of HEV to provide more reliable and reproducible tests either molecular or serological assays especially in low prevalence areas.

Treatment

Currently, there is no specific antiviral drug for HEV infection since it was not considered necessary until recently when chronic cases were reported. This fact promoted the treatment studies for isolated cases in which drugs, such as ribavirin and/or pegylated IFN-α (peginterferon), were used empirically [140]. Peginterferon is an immunostimulator that is being used in hepatitis B and C treatments. In transplant patients, its use can cause allogeneic immunity, which could lead to rejection of the transplanted organ,

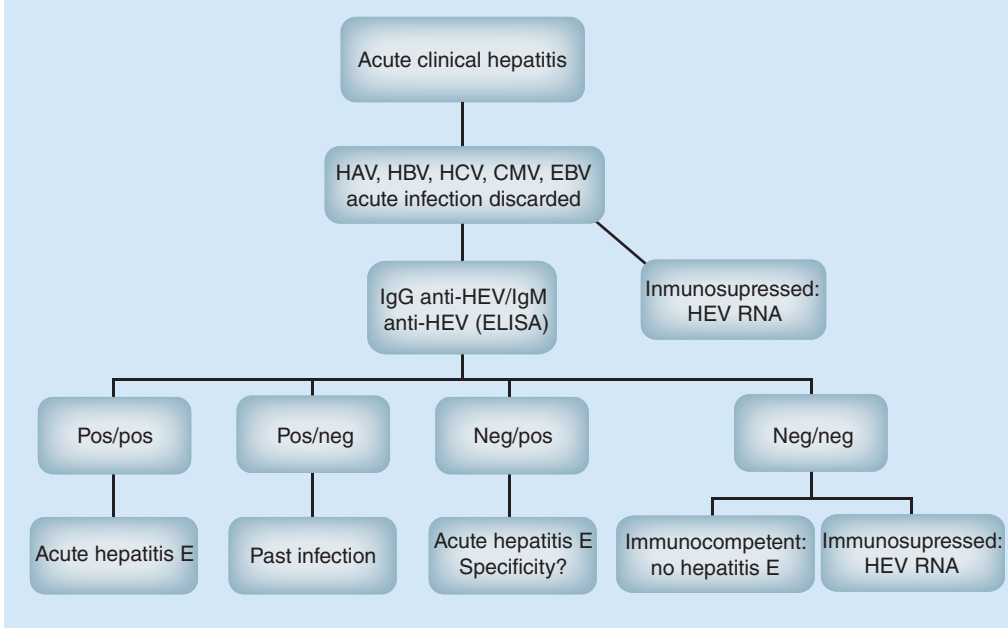

Figure 4. Proposed algorithm for the diagnosis of acute hepatitis E in low prevalence countries.
CMV: *Cytomegalovirus*; EBV: Epstein–Barr virus; HAV: Hepatitis A virus; HCV: Hepatitis C virus; HEV: Hepatitis E virus; Neg: Negative; Pos: Positive.

Table 4. Ribavirin therapy in organ transplant patients.

Transplanted organ	Patients (n)	Dose	Duration median (months)	RVR, month (%)	SVR (%)	Year	Ref.
KT/SPK	8	400–800 mg/day	3	2 weeks (50) 3 weeks (12) 2 (38) 1 (50)	5 (63)	2010	[144]
SPK	2	12 mg/kg/day	3	6 (100)	2 (100)	2010	[145]
HT	1	17 mg/kg/day	3	1 (100)	1 (100)	2011	[146]
HT	4	200–800 mg/day	5	1 (50)	3 (75)	2012	[147]
KT	1	800 mg/day	3	2 (100)	1 (100)	2012	[148]
LT	1	200 mg/day	3	1 (100)	1 (100)	2012	[149]
LT	11	600–1000 mg/day	5	1 (36) 2 (46)	9 (82)	2013	[150]
KT/LT/HT/SPK/LungT	59	600 mg/day	3	1 (64) 3 (31)	46 (78)	2014	[106]
LungT	4	600–800 mg/day	4.5	1 (25) 2 (25) 3 (25)	2 (50)	2014	[151]
LT	4	400–800 mg/day	3	3 (100)	4 (100)	2015	[152]
KT	2	600–800 mg/day	3	2 weeks (100)	2 (100)	2015	[153]
KT	1	600 mg/day	4	2 (100)	1 (100)	2015	[154]

HT: Heart transplant; KT: Kidney transplant; LT: Liver transplant; LungT: Lung transplant; RVR: Rapid viral response; SPK: Simultaneous pancreas kidney; SVR: Sustained virological response.

thus its use could be limited in the treatment of chronic hepatitis E. Ribavirin is an antiviral agent that blocks nucleic acid synthesis and is used against DNA and RNA viruses. Although several studies suggest the beneficial effect of both antivirals for chronic hepatitis E, these therapies are in their experimental phases. The time to start the treatment, the optimal dose, the duration of the treatment and its safety, are currently unknown as there are no guidelines and neither of these drugs have been approved for this use.

Tables 4 & 5 show the studies performed using ribavirin or pegylated interferon in transplant recipients. Haagsma *et al.* [141] have published a study in which two liver transplant patients with chronic hepatitis E were treated with peginterferon during 52 and 20 weeks, respectively, observing a significant decrease in viremia, transaminase normalization and no HEV RNA detection in serum between weeks 4 and 12 of the treatment. However, one of the patients only showed positive results after lowering the immunosupressor dose. Similarly, other studies [118,142] refer to an improvement in HEV clearance when the patients' immunosupressor dose was lowered, seeing as this improves the patient's immunity [114,143].

In another study, by Kamar *et al.* [156], a patient suffering from chronic hepatitis E was treated with peginterferon, presenting a satisfactory evolution up to 3 months after the treatment. In the other investigation [144], they concluded that monotherapy with ribavirin inhibits HEV replication and may induce a sustained virologic response in patients with chronic HEV infection. Therefore, ribavirin monotherapy could be an effective and safe treatment in

Table 5. Pegylated interferon therapy in organ transplant patients.

Transplanted organ	Patients (n)	Dose (μg/kg/week)	Duration median (months)	RVR, month (%)	SVR (%)	Year	Ref.
LT	2	α-2b: 1.5	8	5 (50)	1 (50)	2010	[141]
LT	3	α-2a: 135	1.5 (67) 3 (33)	2 (67)	2 (67)	2010	[155]
KT	1	α-2a: 135	3	1 (100)	1 (100)	2010	[156]

KT: Kidney transplant; LT: Liver transplant; RVR: Rapid viral response; SVR: Sustained virological response.

immunocompromised patients with chronic hepatitis E. The use of pegylated interferon in transplant patients may lead to transplant rejection and is not recommended. Ribavirin should be the antiviral treatment of choice in chronic hepatitis E [140]. There is scarce information available regarding the administration of immunoglobulins, but the few studies existing suggest that they do not grant protection against HEV infection [157].

Vaccines

As HEV infection is significantly prevalent in developing countries, as well as among those working close to infection sources, many efforts have been placed in developing a vaccine. HEV is considered a good candidate for the development of a vaccine because it only presents one serotype and natural infection leads to protective antibodies [3]. Vaccination in developing countries could remarkably decrease the number of people infected by large waterborne HEV epidemics.

In the early stages of vaccine development, it was observed that several recombinant proteins corresponding to the HEV capsid protein induced specific antibodies in animals and protected against liver injury following subsequent challenge with the virus [158]. Additionally, an HEV DNA vaccine was tested in cynomolgus macaques, having shown induction of anti-HEV serum production and protection against rechallenging with a heterologous HEV strain [159]. Bearing this in mind, two separate subunit vaccines were developed and tested.

The first vaccine was a 56-kDa truncated HEV ORF2 protein produced from a baculovirus forming virus-like particles. In Phase I trials, an alum adjuvant was added to the formulation, which was then administered in three doses of 1, 5, 20 or 40 µg [158]. The results showed a dose-dependent production of anti-HEV antibodies. This vaccine went into Phase II and III trials, where nearly 2000 volunteers from the Nepalese Army, lacking detectable anti-HEV antibodies, received either a 20-µg vaccine or a matching placebo in three doses (at 0, 1 and 6 months) [160]. After a follow-up of >2 years, it was observed that clinically overt acute hepatitis E occurred less frequently among vaccine recipients who completed the three-dose schedule than among placebo recipients, showing a vaccine efficacy of 95.5%. A lower efficacy rate of 87% was found in individuals who only received two doses.

Meanwhile, a Chinese group prepared another vaccine named the HEV 239 vaccine. It contains a more truncated HEV capsid protein (amino acids 368–606) expressed in *Escherichia coli*, purified and absorbed on aluminum hydroxide [161]. In Phase II trials, all the volunteers lacking anti-HEV antibodies seroconverted 1 month after having received a treatment consisting of three doses of a 20-µg vaccine at 0, 1 and 6 months [162]. Following this, a randomized, double-blind, placebo-controlled, community-based, Phase III trial has been successfully completed in China [163]. For 4.5 years, 112,604 participants with ages between 16 and 65, regardless of their anti-HEV antibody status, were divided in two groups: one group received the Hecolin® vaccine while the control group received the HBV vaccine. As a result, the vaccine showed an efficacy of 86.8%, with only seven of the 60 identified cases of hepatitis E belonging to the 239 vaccine group. This vaccine has been registered in China under the name Hecolin and is already available for use.

Despite being based on the G1 virus, the Chinese vaccine has proven to provide protection against G4 HEV infections. Further studies are required to determine whether these vaccines provide protection against G3 virus strains, prevalent in developed countries.

How this vaccine should be used is still unclear. In nonendemic regions, a vaccine could be used in cases of residents who are planning to travel to an endemic area. On the other hand, endemic areas could use the vaccine for pregnant women and patients with pre-existing chronic liver disease. However, cost considerations and the duration of protection afforded will need to be taken into account when deciding whether HEV vaccines should be used for the general population in endemic regions.

The WHO has developed a three-stage approach to the threat of hepatitis E infections in which steps 2 and 3 refer to Hecolin as the only vaccine available in the market [164]. After evaluating the safety, immunogenicity, efficacy, cost–effectiveness and programmatic considerations of said vaccine for its use in prevention, control and treatment HEV, the WHO did not recommend the use of this vaccine routinely in countries where HEV is endemic. However, WHO also specifies that there might be special situations (outbreaks, travelers, pregnant women) in which individual countries can

decide to use the vaccine as part of a prevention program [165].

Moreover, the use of these vaccines in animals should be considered seeing as they act as a reservoir and are becoming one of the main transmission routes, especially in developed countries. The efficacy of the HEV 239 vaccine was tested in 12 specific pathogen-free rabbits divided randomly into two groups and inoculated with HEV 239 and placebo (phosphate-buffered saline), respectively [166]. All animals were exposed to swine G4 HEV or rabbit HEV 7 weeks after the initial dose. Infection was monitored for 10 weeks measuring parameters, such as, duration of viremia, viral presence in stool, HEV antibody response and serum ALT levels. The group immunized with the HEV 239 vaccine showed no signs of HEV infection for the duration of the experiment, while those inoculated with placebo developed viral hepatitis. The results of this study show that the HEV 239 vaccine is also effective in rabbits and could possibly be extended to other animals, such as pigs.

The positive results obtained with the HEV 239 vaccine have encouraged researchers to develop vaccines that will provide combined protection against viruses sharing the same transmission route. This is the case of Wang *et al.* [167] who are developing a vaccine to protect simultaneously against Norovirus (NoV) and HEV. This bivalent vaccine is still in the early stages of development.

Conclusion & future perspective

For the time being, HEV infection can be considered an emerging disease for chronic and progressive severe hepatitis in immunosupressed patients in developed countries. Several studies report that the different available immunosuppressants may play a role on the severity of the infection, mainly in the transplant setting. Regarding these controversial issues, more data are needed to control the high HEV replication in these patients [168,169].

The newly marketed vaccine (HEV 239 or Hecolin) is a giant leap in the prevention of hepatitis E in developing countries. However, WHO does not recommend its use routinely in countries where HEV is endemic. Moreover, its activity is yet to be proven in the target population of this vaccine in developed countries: immunosuppressed patients and pregnant women. Therefore, further efficacy studies are necessary.

Financial & competing interests disclosure

Work cited in this review from the author's laboratory was supported in part by grants from the Universidad CEU Cardenal Herrera (PRUCH 25/10, PRUCH 39/11 and Santander-PRUCH 19/12; INDI1420). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

EXECUTIVE SUMMARY

- Hepatitis E is a highly prevalent disease in developing countries.
- According to the WHO, 20 million cases of Hepatitis E virus (HEV) infections are registered annually, of which >3 million are acute cases and approximately 56,600 result in death.
- HEV classification is being constantly reviewed; the last classification was developed in 2014 by the International Committee of Taxonomic Virology.
- More data about replication and cell culture systems are constantly being developed, which will result in a better understanding of the damages this virus may cause.
- The main transmission route for HEV is the fecal–oral route, but the parenteral route has also been described.
- HEV can cause chronic hepatitis in organ transplant recipients and immunocompetent patients.
- The diagnosis is based in serological studies and detection of HEV-RNA in blood and stool samples.
- There is no specific treatment against HEV, however, ribavirin monotherapy may be an effective and safe treatment in immunocompromised patients with chronic hepatitis E.
- The development and commercialization of the first vaccine in China may help reduce future devastating epidemics caused by this virus. Vaccination is a good option to prevent infection, especially in countries where HEV is endemic.

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