

Current Knowledge on Hepatitis E

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Abstract

Although only a single serotype of hepatitis E virus (HEV), the causative agent of hepatitis E, has been identified, there is great genetic variation among the different HEV isolates reported. There are at least four major recognized genotypes of HEV: genotypes 1 and 2 are mainly restricted to humans and linked to epidemic outbreaks in nonindustrialized countries, whereas genotypes 3 and 4 are zoonotic in both developing and industrialized countries. Besides human strains, genotype 3 and 4 strains of HEV have been genetically characterized from swine, sika deer, mongooses, sheep, and rabbits. Currently, there are approximately 11,000 human and animal sequences of HEV available at the International Nucleotide Sequence Database Collaboration. HEV is the major cause of waterborne outbreaks of hepatitis in areas of poor sanitation. Additionally, it is responsible for sporadic cases of viral hepatitis in not only endemic but industrialized countries as well. Transmission of HEV occurs predominantly by the fecal-oral route, although parenteral and perinatal routes have been reported. HEV infection develops in most individuals as a self-limiting, acute, icteric hepatitis; with mortality rates around 1%. However, some affected individuals will develop fulminant hepatic failure, a serious condition that is frequently fatal without a liver transplant. This complication is particularly common when the infection occurs in pregnant women, where mortality rates rise dramatically to up to 25%. Among the preventive measures available to avoid HEV infection, two separate subunit vaccines containing recombinant truncated capsid proteins of HEV have been shown to be highly effective in the prevention of disease. One of them, HEV 239, was approved in China, and its commercialization by Inovax began in November 2012 under the name Hecolin®.

Keywords: Hepatitis E virus; Hepatitis E; HEV; Zoonosis.

Abbreviations: ALT, alanine aminotransferase; cDNA, complementary deoxyribonucleic acid; ELISA, enzyme linked immunoassay; HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; Ig, immunoglobulin; ORFs, open reading frames; RNA, ribonucleic acid; RT-PCR, reverse transcriptase polymerase chain reaction; SFDA, State Food and Drug Administration; sIL-2R α , sIL-2 receptor α ; sIL-6, secretion of interleukin-6; Th2, T helper-2; UTRs, untranslated regions; VLPs, virus like particles; WHO, World Health Organization.

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Introduction

Hepatitis E, caused by the hepatitis E virus (HEV), is an important public-health concern and is a major cause of enterically transmitted hepatitis worldwide. It is responsible for over 50% of acute viral hepatitis cases in endemic countries, and approximately 2 billion people, a third of the world's population, live in areas endemic for HEV and are at risk of infection.^{1,2}

Hepatitis E was first described in 1983 by Balayan *et al.*,³ who reproduced HEV infection in a healthy volunteer who ingested pooled stool extracts from patients presumed to have non-A, non-B hepatitis. Stool samples from said patient were analyzed using electron microscopy, and markers of acute hepatitis A and B infection were not detected. However, virus-like particles were visualized. The virus was cloned in 1990,⁴ and the first serologic test was produced in 1991.⁵

HEV infection in developing countries is primarily a waterborne illness, associated with large epidemics due to contaminated water and water supplies and poor sanitation conditions.^{6,7} In contrast, industrialized countries, including many European countries, USA, and Japan, acute hepatitis E occurs sporadically and the contamination pathways are not fully understood.⁷ Travel to endemic countries and the presence of different HEV strains in industrialized versus developing countries support an autochthonous origin of these sporadic cases.^{8,9}

HEV is the only one of the major hepatitis viruses (A, B, C, and D) with an animal reservoir. The discovery of HEV in pigs (swine HEV), chickens (avian HEV), and, more recently, in rabbits, rodents, wild boars, ferrets, bats, sheep, and cut-throat trout as well as the successful experimental transmission of swine HEV to macaques (model for human HEV transmission) strongly support a zoonotic origin of hepatitis E. This theory is further strengthened by studies from Japan that have phylogenetically linked viruses recovered from uncooked meat (pigs and deer) and humans who became infected after consuming the meat.¹⁰ This review summarizes the current knowledge of hepatitis E.

Viral particle

Hepatitis E virus (HEV) is a nonenveloped virus with an icosahedral symmetry and is between 32 and 34 nm in diameter. The buoyant density of HEV is between 1.39 and 1.40 g/cm³ in CsCl, and its sedimentation coefficient is 183 S.¹¹ HEV isolates are between 6.6 and 7.3 kb long (Fig. 1), and they are comprised of a single-stranded positive polarity ribonucleic acid (RNA) molecule with three open reading frames (ORFs).¹² ORF1 is the longest reading frame at the 5'-end of the genome. ORF1 encodes a nonstructural polyprotein that includes 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. Three glycosylation regions have been identified in ORF2, but the biological relevance of these potential modifications is unclear. ORF3 overlaps partially with ORF1 and ORF2 and encodes a phosphoprotein that modulates cellular activities.¹³ All ORFs are expressed during viral infection, as antibodies against these regions have been detected in naturally infected humans and in experimentally infected monkeys.¹⁴ Additionally, there are two untranslated regions (UTRs) in 3' and 5' terminal portions.

Classification

Although only a single serotype has been identified to date, there is great genetic diversity between the different HEV isolates reported.¹⁵ Recent studies have proposed several classifications for HEV into different genotypes and subtypes.¹⁶ The last proposed classification by the International Committee of Taxonomic Virology¹⁷ classified HEV into the family *Hepeviridae* (Table 1). This family is divided into 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, rabbit, and camel), *Orthohepevirus B* (isolates from chicken), *Orthohepevirus C* (isolates from rat, greater bandicoot, Asian musk shrew, ferret, and mink), and *Orthohepevirus D* (bat isolates).

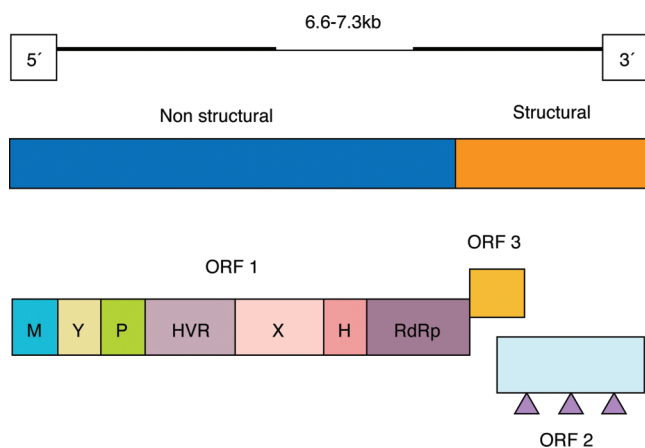


Fig. 1. Genomic organization of hepatitis E virus. M, methyltransferase; Y, Y domain; P, papain-like cysteine protease; HVR, hypervariable region; X, macro-domain; H, helicase; RdRp, RNA-dependent RNA polymerase; \blacktriangle , glycosylation site.

Within species *Orthohepevirus A*, there are four genotypes described that infect humans.

Genotype 1 comprises the human Burma strain (prototype) and strains from Asia and Africa. Genotype 2 comprises a human Mexican strain (prototype) and several strains isolated in outbreaks from Nigeria and Chad. Genotype 3 comprises human and animal strains from the USA,¹⁸ Canada,¹⁹ Argentina,²⁰ Spain,^{21,22} France,²³ UK,²⁴ Austria,²⁵ the Netherlands,²⁶ New Zealand,²⁷ and others. Genotype 4 includes human and animal strains identified in China, Taiwan, Japan, India, Vietnam, France,²⁸ and Italy.²⁹ Genotypes 1 and 2 have been responsible for outbreaks in Asian and African countries and in Mexico. On the other hand, genotype 3 and 4 are responsible for the acute autochthonous cases³⁰ reported in the USA, Argentina, European countries, Japan, and China.

These four genotypes have been divided into different subtypes based on the phylogenetic analysis of many HEV isolates.¹⁶ Genotype 1 has been divided into five subtypes, genotype 2 into two subtypes, whereas genotypes 3 and 4 show a greater diversity and have been divided into 10 and seven subtypes, respectively. Genotypes 1 and 2 have been isolated in all human epidemic outbreaks in developing countries, whereas genotypes 3 and 4 have been isolated not only in humans but also in animals in both developing and industrialized countries (Fig. 2). These data support the hypothesis that genotypes 3 and 4 have a zoonotic nature.³¹

The observed divergence among genotypes might be due to different routes of transmission: genotypes 1 and 2 produce epidemic outbreaks via efficient feco-oral transmission by means of contaminated water or food. These similar patterns of transmission may have produced similar degrees of genetic divergence. However, genotypes 3 and 4 are mainly circulating in different animal species and only infect humans occasionally, probably due to inefficient interspecies transmission of these subtypes. A possible explanation is that HEV has been circulating for a long time in different regions with varying degrees of evolution that depends on the species infected.³¹

Epidemiology

Like Hepatitis A, hepatitis E has been traditionally thought to be enterically transmitted. This epidemiological pattern has been recorded in developing countries where epidemic outbreaks linked to contaminated drinking water have been reported. However, the epidemiology of hepatitis E in industrialized countries is different and has changed over time. When it was first reported in developed countries, it was linked to travel to endemic areas, but now, it is becoming an infection linked to an animal reservoir. Besides, the seroprevalence in industrialized countries is greater than it was previously thought when compared to the number of autochthonous cases reported. Indeed, several studies already define a clear separation between the two different types of HEV infection. Genotypes 1 and 2, prevalent in developing countries, are responsible for water-borne disease; whereas genotypes 3 and 4, prevalent in industrialized countries, produce food-borne zoonotic infection.³²

HEV infection is endemic in the Center and Southeast of Asia. Several outbreaks have been reported in the Middle East, North and West Africa, and Central America (Mexico). In the rest of the world, HEV infection is considered infrequent and is restricted to people who have travelled to areas where HEV is

Table 1. Classification of the family *Hepeviridae*

Family	Genus	Species	Genotype	Source	
<i>Hepeviridae</i>	<i>Orthohepevirus</i>		1		
			2		
			3		
			<i>Orthohepevirus A</i>	4	
			5		
			6		
			7		
		<i>Orthohepevirus B</i>	-		
		<i>Orthohepevirus C</i>	C1		
	C2				
		<i>Orthohepevirus D</i>	-		
	<i>Piscihepevirus</i>	<i>Piscihepevirus A</i>	-		

endemic. HEV outbreaks are long lasting and can affect from hundreds to thousands of people. Outbreaks may vary from acute outbreaks to long lasting epidemics (>1 year). During these outbreaks, the percentage of the affected population may vary between 1% and 15%. The illness is more frequent in adults (3–30%) than in children (0.25–10%). These numbers suggest that anicteric and/or subclinical infections may be more frequent in children. Men are more commonly infected than women. Person-to-person transmission is rare and is more frequent in members of the same family who are infected by a common watersource.³³

Sporadic hepatitis E is observed in nonepidemic countries where it constitutes 1–11% of acute cases of hepatitis and is

mainly related to travelling to endemic zones,³⁴ although an increasing number of autochthonous hepatitis has been recently reported.^{35,36}

In industrialized countries, HEV seroprevalence rates between 1% and 5% have been reported in the healthy population. The high prevalence found in these countries does not correlate to the low incidence of acute hepatitis E cases in these areas. Therefore, whether seroreactivity in endemic areas is a reflection of subclinical infections, cross-reactions to other agents, false positives, a combination of all of them, or none remains uncertain. Another possibility is that the high seroprevalence found among healthy individuals is related to a subclinical infection with swine HEV, but further

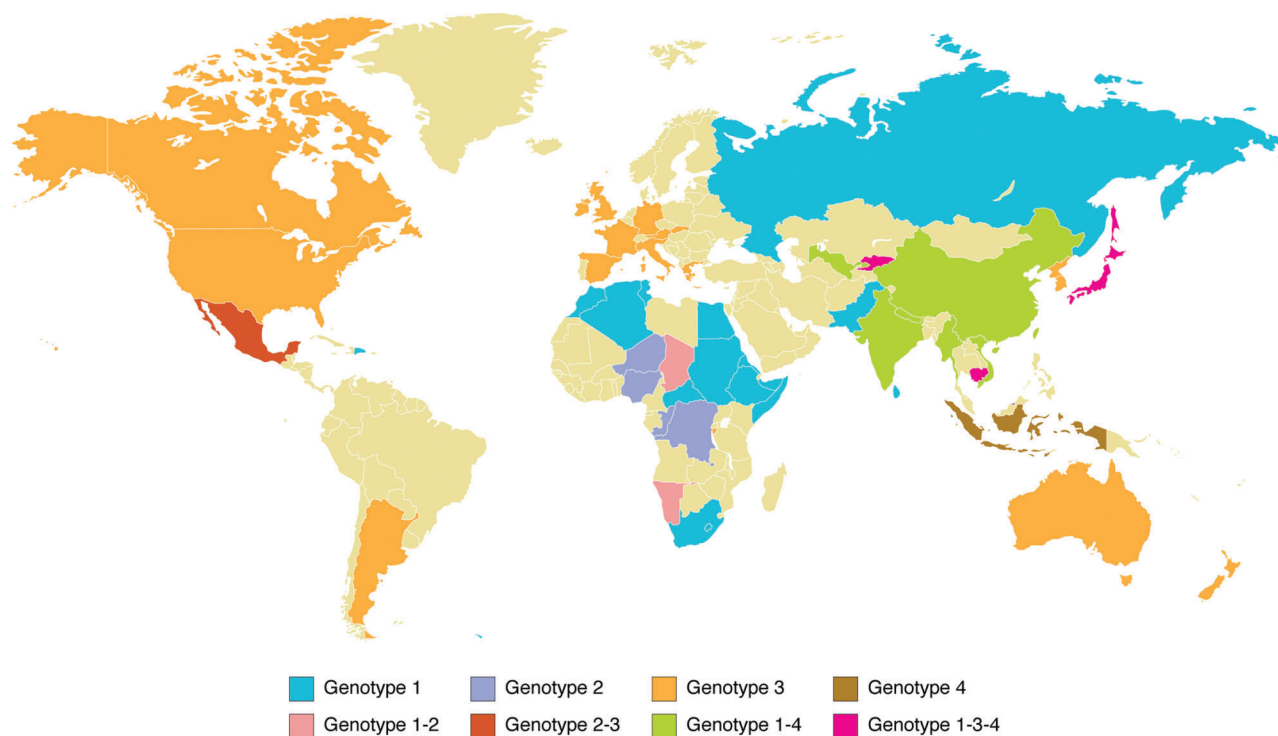


Fig. 2. Geographical distribution of hepatitis E virus genotypes.

studies are required to evaluate the role of swine HEV in the epidemiology in developed areas.

An increasing number of studies suggest that hepatitis E is a zoonosis.³⁷ Antibodies for HEV have been found in human population from nonendemic countries.³⁸ These data raised the suspicion of animals as HEV reservoirs. Consistent with this, it has been reported by means of experimental infections that HEV is capable of crossing the species-barrier. Thus, primates have been infected with swine HEV;³⁷ while pigs, lambs,³⁹ and rats⁴⁰ have infected with human HEV. Experimental inoculated pigs with a human isolate from the USA were rapidly viremic and seroconverted. These data suggest that this strain was well adapted in pigs and perhaps of swine origin. HEV is considered enzootic in a wide range of animals, including wild and domestic species.

Regarding serological data in animals, there are a number of studies reporting detection of antibodies against HEV in pigs from developing countries such as Nepal⁴¹ and Thailand.⁴² Antibodies against HEV have been found in swine from industrialized countries, including the USA,¹⁸ Canada,¹⁹ Korea,⁴³ Taiwan,⁴⁴ Australia, and New Zealand.²⁷ Detection of antibodies in other species, such as poultry,⁴⁵ dogs,^{44,46} cattle,^{46,47} deer,⁴⁸ cats,⁴⁹ rodents,^{47,50,51} and mongooses,⁵² have been reported as well. All this serological information suggests that certain animal species are exposed to HEV (or HEV-like agent), although the epidemiology of infection remains unclear.

The HEV genome has been detected in poultry,⁵³ horses,⁵⁴ pigs,¹⁸ deer, wild boars,⁵⁵ rats,⁵⁶ rabbits,⁵⁷ and sheep.⁵⁸ One possible explanation for the great genomic variability of HEV is an animal origin. Genomic sequencing and phylogenetic analysis of many HEV strains have shown a close relationship between swine and human isolates of the same geographic

area (Fig. 2) in industrialized countries like Japan,⁵⁵ Korea,⁵⁹ United Kingdom,⁶⁰ Taiwan,⁶¹ and the USA.¹⁸ Hepatitis E infection related to poorly cooked or raw swine liver consumption has been reported.⁶² There have been cases of hepatitis E infection by deer and wild boar meat ingestion as well.^{55,63} Because of its ability to cross the species-barrier, HEV constitutes an important Public Health problem, especially for swine workers who are at a higher risk of infection.⁶⁴⁻⁶⁶

Transmission

Hepatitis E is generally transmitted by the fecal-oral route, usually due to the consumption of contaminated water or food. In developing countries, this is the route of infection that leads to outbreaks. In contrast to the hepatitis A virus and other enteric viruses, human-to-human transmission of HEV is rare.²⁸

Alternative transmission routes, such as transfusions and vertical transmission, are becoming increasingly relevant as more cases are reported each year. Transmission via blood transfusion has become one of the main transmission routes in developed countries. A research group in southeast England retrospectively screened a total of 225,000 blood donors, of which 79 were found to be viremic with genotype 3 HEV.⁶⁷ Follow-up of 43 recipients showed 18 (42%) had evidence of infection, where only immunosuppressed patients presented acute morbidity. Another increasingly common transmission route is vertical transmission from pregnant women to their children, especially since these women susceptible to HEV infection.⁶⁸ A recent study suggests in developing countries that HEV may be responsible for more than 3,000 stillbirths each year, including fetal deaths linked to antenatal maternal mortality.⁶⁹

Pathogenesis

From a clinical point of view, hepatitis E is similar to hepatitis A, with acute self-limiting and symptomatic presentation that varies in severity from subclinical to fulminant cases.³¹ Incubation period ranges from 2 weeks to 2 months with an average of 40 days, and viremia is transient, occurring mainly during the prodromic phase, and disappears at the onset of clinical symptoms (Fig. 3). Fecal excretion of the virus begins a few days (5 days on average) prior to jaundice and slows down at the onset of jaundice within 2 to 3 weeks.⁷⁰

Most autochthonous cases of hepatitis E were reported in middle-aged and elderly men.⁷ Fulminant hepatitis was observed in 1–4% of the cases, which is a higher rate than that of hepatitis A.⁷¹ The mortality rate reached up to 25% in pregnant woman in some endemic areas (northern and central India, Pakistan).^{72,73}

Host and viral factors that determine the severity of illness caused by HEV infection are not fully understood. Viral factors, such as the HEV strain (genotype or subtype), viral load, and other coinfections, might play a role in pathogenesis.⁷⁴ It appears that genotype 3 and 4 strains are less pathogenic in humans relative to genotypes 1 and 2. A report from Japan found that patients infected with genotype 4 HEV (n=538) had a more severe illness than those infected with genotype 3.⁷⁵ The patients with genotype 4 HEV infection had significantly higher peaks of alanine aminotransferase (ALT) levels and notably lower prothrombin activity. Fulminant hepatitis has not been described in genotype 2 HEV infection; however, this might merely be due to the limited data available for this genotype. Therefore, the differences in HEV genotypes between different geographical regions might explain the severity of infection during pregnancy. The viral inoculum dose may be relevant. In a study in primates, a larger viral inoculum dose was associated with more marked liver injury.⁷⁰

Other factors involved in the severity of the disease are related to the host. In particular, pregnancy, use of contraceptives,⁹ age, and pre-existing liver disease clearly appear to be important. In addition, host immune response may also play a significant role.

More recently, other clinical forms of HEV diseases have been observed in patients under immunosuppressive conditions, such as transplant recipients⁷⁶ or patients suffering from leukemia.⁷⁷ Some of these patients developed chronic HEV infection progressing to cirrhosis.⁷⁸ This clearly

suggested that the atypical clinical and virological outcome of HEV infection in these cases could be related to immunosuppressive treatments, which might have resulted in suboptimal anti-HEV-specific immune responses and subsequent viral persistence. Such hypothesis has been evoked by Kamar et al.⁷⁶ who found significantly reduced CD2, CD3, and CD4-lymphocyte counts in patients with chronic disease and in populations at risk of developing fulminant hepatic failure. Another population that might be at risk of chronic HEV infection are human immunodeficiency virus (HIV) patients with advanced immunodeficiency.^{79,80} In other studies, no increase in the prevalence of HEV infection was observed in this population,^{81,82} and only a few cases were reported,⁸³ including a pregnant woman who recovered completely.⁸⁴ In non-endemic regions, the number of hepatitis E cases in humans seems to be rapidly rising due to the inclusion of HEV among the differential diagnosis of hepatitis.⁸⁵ Several studies have evaluated the consistency of HEV diagnostic tools^{86,87} in order to achieve a more reliable diagnostic. One retrospective study reported that the incidence of hepatitis E in France was stable over a period of 5 years, suggesting that hepatitis E is generally under-diagnosed.²³

Most patients with autochthonous hepatitis E do not require a liver biopsy since they have a self-limiting disease. Some patients may present a more severe hepatitis with worsening liver blood tests and, therefore, a liver biopsy is sometimes helpful. There are few data on the hepatic histopathology of acute autochthonous hepatitis E, and such reports are limited to patients with severe disease.

Liver pathology of acute autochthonous hepatitis E in the noncirrhotic liver was similar to that seen in acute viral hepatitis, presenting lobular disarray with reticulin framework distortion. Portal tracts were expanded by a severe mixed polymorph and lymphocytic inflammatory infiltrate. Moderate to severe interface hepatitis and cholangitis were also present.^{88,89} In one study, three patients with autochthonous hepatitis E showed polymorphs concentrated at the periphery and interface of the liver, with lymphocytes, including aggregates, concentrated centrally.⁸⁸ These findings might be helpful in distinguishing autochthonous hepatitis E from other causes of hepatitis such as autoimmune hepatitis. However, these findings were based on a small number of cases and require confirmation.

In patients with hepatitis E who have underlying cirrhosis, liver pathology findings were nonspecific⁹⁰ and could easily be mistaken for alcoholic hepatitis in the context of established ethanolic cirrhosis.

In the small number of immunosuppressed transplant patients who have developed chronic infection with HEV, liver pathology showed progressive fibrosis and portal hepatitis with lymphocytic infiltration and piecemeal necrosis^{78,91} with progression to cirrhosis.

Pregnancy

Pregnant women are at increased risk of complications in HEV infection, with the risk increasing as the pregnancy progresses, often leading to fulminant hepatic failure and death in a high number of cases.⁹² Acute HEV infection is especially severe during second and third trimesters of pregnancy, and it may lead to fulminant hepatic failure and death in 30–100% of patients.⁹³ There is also increased number of obstetric complications, e.g., premature rupture of membranes, postpartum hemorrhage, spontaneous abortions, and intrauterine

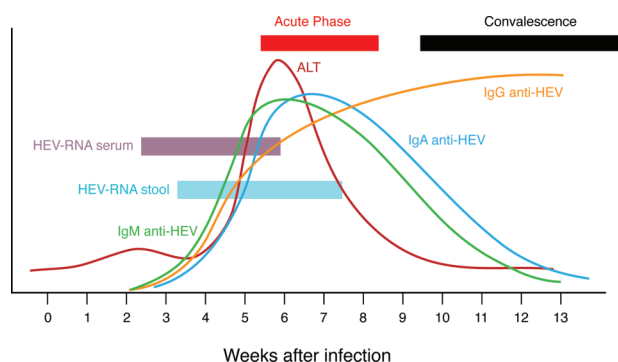


Fig. 3. Schematic summary of main pathogenetic events (virology, serology, disease) during acute HEV infection.

fetal death. The fetal complications include prematurity and low birth weight. In a study from Bangladesh, it was observed that 19–25% of all maternal deaths and 7–13% of all neonatal deaths were associated with jaundice in pregnant women. Further, 58% of deaths in pregnant women with acute liver disease in hospitals was associated with HEV.⁹⁴

The high mortality rate of acute hepatitis E in pregnancy is postulated to result from associated hormonal (estrogen and progesterone) and immunological changes, including down-regulation of nuclear factor-kappa-B and shift in T helper-1 cells/T helper-2 cells (Th2) balance toward Th2, and host susceptibility factors that occur in pregnant state.^{95–97} Devhare *et al.*⁹⁸ studied the immune response to HEV infection and observed early cellular response in HEV infection and associated molecular mechanisms, suggesting a potential role of HEV infection triggered inflammatory response in host immune response and pathogenesis. They demonstrated up-regulation of cytokine and chemokine genes and secretion of interleukin-6 (sIL-6), IL-8 and tumor necrosis factor- α in human epithelial cells infected with HEV. Tripathy *et al.*⁹⁹ found that elevated IL-1 α and sIL-2 receptor α (sIL-2R α) levels in the blood are pivotal in the pathogenesis of HEV. They also demonstrated involvement of the innate immune response at the site of infection. Furthermore, Srivastava *et al.*¹⁰⁰ suggested that interferon- γ secreting CD4 lymphocytes are involved in immune response and are related to intrahepatic sequestration of immune response. All these studies suggested that immune mediated destruction of hepatocytes is important in the pathogenesis of hepatitis E.

Moreover, a recent study¹⁰¹ demonstrated that HEV replicates in placenta, which could explain the high fetal and maternal mortality rate. Xia *et al.*¹⁰² confirmed these data in pregnant rabbits with a rabbit HEV isolate “*in vitro*”. They concluded that HEV infection could lead to adverse outcomes in pregnancy and vertical transmission, suggesting the necessity for pregnant women at risk of HEV infection to be vaccinated.

Laboratory diagnosis

Laboratory diagnosis tests to detect HEV infection include molecular techniques and electronic immunomicroscopy in feces and serum as well as serological assays to detect anti-HEV immunoglobulin IgM and IgG. HEV RNA is detectable in feces using reverse transcriptase polymerase chain reaction (RT-PCR) from 1 week before the onset of clinical signs to 2 weeks, although sometimes it has been detected up to 52 days after the onset of clinical signs. There are some real-time PCR assays to quantify HEV RNA in fecal and serum samples.^{103,104} These techniques have the advantage to reduce the diagnostic time (3 h) and show a high sensitivity (10 molecules complementary deoxyribonucleic acid (cDNA)/PCR). Electronic immunomicroscopy in feces is too arduous to perform and its sensitivity is very low, therefore, it is not used for routine diagnosis. HEV antigen has been detected in hepatic tissue from experimentally infected primates. Serologic diagnosis of HEV infection is usually carried out by means of enzyme linked immunoassays (ELISA). Synthetic peptides or recombinant proteins are used as antigens in these assays and correspond to immunodominant epitopes from ORF2 and/or ORF3 belonging to Mexico and/or Burma strains.

Anti-HEV IgM antibodies can be detected during the acute phase of the illness and can last approximately 4 or 5 months. IgG antibodies appear just after IgM levels rise, and they

increase from the acute phase until the convalescent phase. IgG have been detected up to 4.5 years after the acute phase. Therefore, increased IgM levels are indicative of acute infection, whereas IgG levels are related to previous contact with HEV.⁴¹

Prevention and control

Efforts to prevent HEV concentrate on improving the sanitary status in developing countries, in order to limit contamination of drinking water by infected materials. Particular information and surveillance must be taken from populations at higher risk for fulminant hepatitis such as pregnant women and people with underlying liver conditions. In industrialized countries, where zoonotic transmissions may be the main route of hepatitis E infection, raw or undercooked meat consumers should be aware of the potential risk.

Other populations at risk, such as pregnant women, transplant recipients and individuals with underlying liver conditions, should be informed about the possible risk of consuming raw pork, deer, or wild boar meat or contact with infected pigs. Pork in these areas should be cooked thoroughly (71 °C/20 min) and appropriate safety measures should be taken during the storage, handling, and preparation of uncooked pork.¹⁰⁵

Pig handlers and veterinarians who are exposed to HEV must take hygienic measures after handling animals. In swine, since HEV seems to be very contagious between animals,¹⁰⁶ further investigation must be performed to determine which preventive biosecurity procedures would limit HEV dissemination. There are several other prevention strategies that could be considered, including the removal or reduction of viral burden eliminated by pigs via early weaning.¹⁰⁷ Vaccination of pigs seems a less appropriate control option for the moment since there is no firm data available regarding the incidence of infection in human beings.

Another method of prevention is to enhance the surveillance strategy in commercial importation of swine. Recently, a genotype 4 virus was isolated in swine in Europe.¹⁰⁸ Investigations on the origin of this genotype in Europe are needed. Since swine represent a large reservoir of HEV in nonendemic regions, pigs are probably a source of infection for sporadic cases of acute hepatitis E. Surveillance of swine, along with wild boar and deer reservoirs, should be performed until all the routes of human exposure to HEV are identified.

In order to prevent and control HEV waterborne outbreaks, the World Health Organization (WHO) has developed guidelines to make sure countries manage these situations as rapidly and effectively as possible. These control strategies are grouped in four major categories (Table 2) depending on the stage of the outbreak: prevention of exposure, prevention of infection, prevention of disease, and prevention of death.¹⁰⁹

Vaccine

HEV infection can also be prevented with an effective human immunization program.¹¹⁰ Since HEV has only one serotype and natural infection leads to protective antibodies,¹¹⁰ HEV is a good candidate for the development of an effective vaccine.¹¹¹ A large vaccination campaign in developing countries would reduce large waterborne epidemics for thousands of people.

Table 2. WHO strategies for prevention and control of waterborne HEV outbreaks

Exposure	Infection	Disease	Death
<ul style="list-style-type: none"> Improving quality and quantity of drinking water 	<ul style="list-style-type: none"> Prenatal tests in pregnant women 	<ul style="list-style-type: none"> Development of vaccines such as the Chinese 239 vaccine Hecolin® 	<ul style="list-style-type: none"> Prompt diagnosis and management of cases
<ul style="list-style-type: none"> Treating and disposing of human wastes correctly 	-	-	<ul style="list-style-type: none"> Timely referral to a health-care facility
<ul style="list-style-type: none"> Improving personal hygiene 	-	-	<ul style="list-style-type: none"> Avoid administration of unnecessary hepatotoxic drugs
<ul style="list-style-type: none"> Preparing safe and clean food 	-	-	-

In animal studies, several truncated recombinant HEV capsid proteins have been found to induce specific antibodies and to protect against liver injury following subsequent challenge with homologous and heterologous strains of the virus. An HEV DNA vaccine has also been shown to induce serum anti-HEV antibodies in cynomolgus macaques and protect against a heterologous challenge.¹¹² These findings have led to the development of two separate subunit vaccines, which have been tested in clinical trials.

The first human vaccine contained virus like particles (VLPs) made up of a 56 kD truncated HEV ORF2 protein (amino acids 112–607) produced in *Spodoptera frugiperda* cells infected with a recombinant baculovirus. In a phase 1 trial, three doses of 1, 5, 20, or 40 µg of this recombinant protein, administered in an aluminium-adsorbed formulation, induced production of anti-HEV antibodies among healthy volunteers.¹¹⁰ The antibody response was found to be dose-dependent. In a phase 2–3 efficacy trial, nearly 2,000 volunteer Nepalese soldiers who lacked detectable anti-HEV antibodies randomly received either 20 µg of this vaccine or a matched placebo (given as three doses at 0, 1, and 6 months) and were followed-up for a median of 804 days.¹¹³ The study subjects were overwhelmingly (>99%) male and mostly young (mean age, 25 years). Clinically overt acute hepatitis E occurred less frequently among vaccine recipients who completed the three dose schedule than among placebo recipients, with a vaccine efficacy rate of 95%. Administration of two doses was associated with a somewhat lower efficacy rate of 86%. Adverse reactions were similar except for more frequent injection-site pain with the vaccine. This vaccine has not been commercialized.

The second vaccine, the HEV 239 vaccine, contains a more truncated HEV capsid protein (corresponding to amino acids 368–606) expressed in *Escherichia coli*, which has been purified and adsorbed on aluminum hydroxide suspended in buffered saline solution.¹¹⁴ In a phase 2 human trial, all volunteers who lacked anti-HEV antibodies showed seroconversion 1 month after three doses of 20 µg each, administered at 0, 1, and 6 months, respectively.¹¹⁵ A large, community-based, randomized, double-blind, placebo-controlled, phase 3 trial of this vaccine has recently been completed in China.¹¹⁶ This study enrolled nearly 113,000 participants, aged 16–65 years and of either gender, irrespective of their anti-HEV antibody status. Among the approximately 97,000 participants who received three dose of the vaccine (30 µg each, at 0, 1, and 6 months), the protective efficacy rate was 100% during the next year. Even after two doses of the

vaccine, 100% protection was noted, although these data were more limited.

The Chinese vaccine was shown to provide protection against genotype 4 HEV infections, even though it is based on genotype 1 of the virus. Whether these vaccines provide protection against genotype 3 virus strains prevalent in developed countries remains to be determined. The HEV 239 vaccine was approved in China by the State Food and Drug Administration (SFDA) in January 2012, and its commercialization by Innovax (Xiamen Innovax Biotech) started in November 2012 under the name of Hecolin®.^{117,118}

A recent study published in the New England Journal of Medicine addressed the long-term efficacy of this vaccine.¹¹⁹ During 4.5 years, this group studied efficacy, immunogenicity, and safety of the vaccine compared to a control group receiving the hepatitis B virus (HBV) vaccine. As a result of this study, 60 cases of hepatitis E were identified, of which only seven belonged to the 239 vaccine group, proving the efficacy of the vaccine to be 87%.

This vaccine appears very useful for travelers from non-endemic countries to areas with high prevalence of hepatitis E. In industrialized countries, such vaccines may be useful for individuals who are at a high risk of severe disease following HEV infection, such as pregnant women, transplant recipients, people with pre-existing chronic liver disease, and immunosuppressed patients at risk of chronic HEV infection. Since patients with chronic liver disease are most at risk if infected, vaccination should be targeted at these individuals. Patients with chronic liver disease are currently being advised to receive vaccination against hepatitis A virus (HAV) and HBV. Including HEV vaccine in this program would seem logical, since superinfection with any of these viruses in the context of pre-existing chronic liver disease carries a higher mortality rate. The problem with this approach is that unidentified compensated chronic liver disease is common in the community; so all at-risk individuals would not be vaccinated. A more vigorous approach would be to vaccinate whole populations, possibly at the age of 40. This would be a huge undertaking and would need careful cost-benefit analysis before introduction. In addition, the vaccination might help to interrupt outbreaks of hepatitis E in highly endemic areas and among disadvantaged groups, such as flood affected and displaced populations.

Conclusions

Hepatitis E is an important public-health concern and is a major cause of enterically transmitted hepatitis worldwide.

HEV mainly causes large outbreaks of acute hepatitis in endemic areas and sporadic cases in industrialized countries. Although most infections are subclinical, the clinical symptoms may range from acute hepatitis, chronic infection in immunosuppressed and transplant patients to severe acute failure in pregnant women. Transmission may occur via water, food and blood. Laboratory diagnosis relies on serological assays and testing for HEV RNA in blood. A vaccine has been developed in China and the results are promising.

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Conflict of interest

None

Author contributions

Manuscript writing (MTPG, MG, BS, MLML).

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