Epidemiology of Mammalian Hepatitis E Virus Infection

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Key Words

Hepatitis E virus • Epidemiology • Autochthonous hepatitis E • Zoonosis • Zoonotic transmission • Foodborne transmission • Pig • Prevention • Vaccine

Abstract

Mammalian hepatitis E virus (HEV), the etiological agent of hepatitis E in humans, is a recently discovered infectious agent. It was identified for the first time in 1983 using electron microscopy on a faecal specimen of a person infected with non-A, non-B enterically-transmitted hepatitis. Based on retrospective and prospective studies, HEV was long described as one of the leading causes of acute viral hepatitis in tropical and subtropical countries, whereas in developed countries hepatitis E was considered an imported disease from HEV hyperendemic countries. Data from studies conducted during the past decade have greatly shifted our knowledge on the epidemiology and clinical spectrum of HEV. Recently, it has been shown that contrary to previous beliefs, hepatitis E is also an endemic disease in several developed countries, particularly in Japan and in Europe, as evidenced by reports of high anti-HEV immunoglobulin G prevalence in healthy individuals and an increasing number of non-travel-related acute hepatitis E cases. Moreover, a porcine reservoir and growing evidence of zoonotic trans-

mission have been reported in these countries. This review summarizes the current knowledge on the epidemiology and prevention of transmission of mammalian HEV.

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Introduction

Mammalian hepatitis E virus (HEV), the etiological agent of hepatitis E in humans (previously known as non-A, non-B enterically-transmitted hepatitis virus), is a disease of worldwide distribution [1], usually transmitted by the oral-faecal route via contaminated water [2–6]. The first indication of the circulation of an enterically-transmitted non-A, non-B hepatitis virus came from two independent studies conducted in India in the 1980s [7, 8]. In these studies, retrospective analyses of serum samples collected during epidemics of jaundice that occurred from December 1955 through January 1956 in New Delhi [8], and November 1978 through January 1979 in Kashmir [7], indicated the existence of a new enterically-transmitted hepatitis agent circulating in India. The viral hepatitis agents A, and/or B, were excluded, and anti-HEV antibodies were detected using serological assays [9, 10].

HEV was visualized for the first time in 1983 by Balayan et al. [11] using electron microscopy on faeces of a vol-

Fig. 1. Number per year of publications and nucleotide HEV sequences found in the NCBI PubMed database and GenBank nucleotide sequence database, respectively, using the keywords 'hepatitis E virus' and 'hepatitis E'.

unteer (Balayan himself) who ingested extracts of purified pooled faeces collected from patients suspected of non-A, non-B enterically-transmitted hepatitis contracted during a 1981 hepatitis epidemic in a Soviet military camp in Afghanistan. Its genetic organisation was only characterized 7 years later, in 1990 by Reyes et al. [12] through the cloning and sequencing of an isolate of HEV from Burma.

Based on retrospective and prospective studies, HEV was long described as one of the leading causes of acute viral hepatitis in tropical and subtropical countries, whereas in developed countries hepatitis E was considered as an imported disease from developing countries [13]. Data from studies conducted during the past decade have greatly shifted our knowledge on the epidemiology and clinical spectrum of HEV [14–23]. Recently, it has been shown that contrary to previous beliefs, hepatitis E is also an endemic disease in several developed countries [14, 18–23], especially in Japan and parts of Europe. This new face of hepatitis E has been indicated by reports of high anti-HEV immunoglobulin (Ig) G prevalence in healthy individuals and an increasing number of acute hepatitis E in patients with non-A, non-B, non-C hepatitis who did not visit HEV-hyperendemic countries during a period compatible with the incubation of the disease (40 days on average), or have contact with travellers from these geographical areas [14, 15, 18–23]. Moreover, a porcine reservoir and growing evidence of zoonotic transmission have been reported in developed countries [14, 15, 24–29]. Furthermore, possible progression towards chronic hepatitis E and hepatitis E-associated cirrhosis were observed in severely immunocompromised patients [16, 17, 30–34]. These findings led to a resurgence of the interest in research on HEV, as indicated by nearly a doubling of the

number of papers published annually in peer-reviewed journals referenced in the NCBI PubMed database over the last 5 years (fig. 1). Concurrently, the number of HEV RNA sequences available in the NCBI GenBank database showed a similar trend (fig. 1). This review summarizes the current knowledge on the epidemiology and prevention of transmission of mammalian HEV.

Taxonomy, Basic Virology and Genome Organisation

Until 2005, the taxonomy of HEV remained unresolved [35]. At first, HEV was tentatively classified into the *Caliciviridae* family based on its morphological similarities and physicochemical properties [36]. However, because the genomic organisation of HEV is significantly different from caliciviruses and any other viruses, HEV has been reclassified in a new family, the *Hepeviridae* family, genus *hepevirus* [35, 37].

HEV is a small non-enveloped icosahedral in shape virus; its diameter is approximately 27–34 nm [11, 35, 36]. The HEV genome is a single-stranded, linear, positive-sense RNA of approximately 7.5 kilobases (kb) in length [12, 35, 38]. This genome (fig. 2) is capped at the 5'-end, polyadenylated at the 3'-end, and contains a short 5' untranslated region of 27 nucleotides, three discontinuous partially overlapping open reading frames (ORF) and a short 3' untranslated region of 65 nucleotides [35, 38, 39]. ORF1, which extends about 5 kb from the 5'-end of the HEV genome and constitutes the most variable region of the genome, codes for a non-structural polypeptide (including methyltransferase, papain-like cysteine protease,

Fig. 2. Schematic of the genome of HEV. Nucleotide positions correspond to the HEV strain described by Tam et al. [38]. Met = Methyltransferase; Y = domain Y; PCP = papain-like cysteine protease; Pr = proline; X = domain X; H = RNA helicase; RdRp = RNA-dependent RNA polymerase; ▲ = glycosylation site.

RNA helicase and RNA-dependent RNA polymerase) involved in viral genome replication [37–39]. ORF2, located at the 3'-end of the HEV genome, is about 2 kb in length and codes for the viral capsid protein and is involved in most capsid-related functions, such as virion assembly, interaction with host cells and immunogenicity [38, 39]. In addition, ORF2 contains a typical signal peptide sequence and three potential glycosylation sites [39]. Mutations in these glycosylation sites have been shown to prevent the formation of infectious particles from transfected replicons [39]. The third and the smallest ORF of the HEV genome (ORF3, of approximately 369 bases in size) overlaps the first ORF by one nucleotide at the 5'-end and shares most of the remaining sequence (328 bp) with the ORF2 [38, 39]. ORF3 codes for an immunogenic phosphoprotein with several functions, such as virion morphogenesis and release [39].

Epidemiology

HEV Genotypes and Their Geographic Distribution

All HEV strains belong to a single serotype [40], but based on genetic diversity of HEV sequences, four phylogenetically distinct HEV genotypes (1–4) [35] and 24 subtypes [41] have been defined. However, due to the recent identification in various animal species (wild Norway rats, Rex rabbits, wild boars and bats) of HEV strains genetically distinct to the four recognized HEV genotypes, the genotypic classification of HEV might evolve [42]. For example, the HEV recovered recently from rats and bats in studies conducted in Germany appear to belong to two new mammalian HEV genotypes based on phylogenetic analysis [42].

The geographical distribution of HEV genotypes is complex and changing (table 1). Genotypes 1 and 2 only

infect humans and are responsible for both epidemic and sporadic hepatitis E cases occurring in tropical and subtropical countries [1, 4–6, 41, 43–47]. Genotypes 3 and 4 have been shown to infect not only humans, but also domestic animals throughout the world, especially pigs and wild boars [1, 14, 15, 18, 19, 23, 25–28, 48, 49]. Genotypes 3 or 4 are responsible for autochthonous (i.e. locally-acquired) sporadic hepatitis E cases in America, Europe, Oceania and Asia [1, 14, 15, 18, 19, 21–23, 50]. HEV genotype 4, which is indigenous to Asia, was recently described in swine in Belgium [51] and in travel-unrelated hepatitis E cases in Germany and France [23, 49].

Evolutionary Relationship among HEV

The discovery in a pig in 1997 of genotype 3 HEV with close relationship to strains recovered in humans [52] raised questions regarding the origin and evolution of HEV. HEV is currently the sole member of the *Hepeviridae* family [35]. Twenty years ago, in 1992, based on the analysis of the polymerase and helicase sequences of positive-strand RNA viruses, Koonin et al. [37] grouped HEV with rubella virus and beet necrotic yellow vein virus (a plant virus). Recently, Purdy and Khudyakov [53] performed a Bayesian analysis on curated sets of 43 ORF1 sequences, 48 ORF2 sequences and 54 ORF3 sequences with known dates of collection [53]. They tentatively estimated that the most recent common ancestor for modern HEV genotypes 1–4 existed between 536 and 1,344 years ago [53]. They described that HEV ancestors may have evolved into anthroptropic and enzootic viruses, which gave rise to genotypes 1 and 2, and genotypes 3 and 4, respectively [53]. Moreover, Purdy and Khudyakov [53] estimated the time of divergence from the ancestor of avian or rat and genotype 1–4 HEV sequences at about $1.4 \cdot 10^6$ and $7.4 \cdot 10^4$ years ago, respectively. In addition,

Table 1. Geographical distribution of HEV infections according to the genotypes

Continent	Country	Genotype of hepatitis E virus									
		1		2		3			4		
		humans	water	humans	water	humans	animals ^b	water	humans	animals ^c	water
Africa	Algeria	X									
	CAR	X		X							
	Chad	X		X							
	DRC			X			X				
	Djibouti	X									
	Egypt	X		X							
	Morocco	X									
	Namibia	X		X							
	Nigeria			X							
	Tunisia	X									
	South Africa		X				X			X	
	Sudan	X									
	Uganda	X					X				
Asia	Bangladesh	X									
	Cambodia						X	X			
	China	X				X	X		X	X	
	India	X	X						X	X	
	Indonesia								X	X	
	Japan	X ^a				X	X		X	X	
	Kyrgyzstan	X					X				
	Myanmar	X									
	Nepal	X									
	Pakistan	X									
	South Korea					X	X				
	Taiwan						X		X	X	
	Thailand					X	X				
	Uzbekistan	X									
	Vietnam	X							X		
	Europe	Austria					X				
Belgium						X	X			X	
Denmark		X ^a				X	X				
Czech Republic						X	X				
Finland		X ^a									
France		X ^a				X	X	X	X		
Greece						X					
Germany						X	X		X		
Hungary		X ^a				X	X				
Italy		X ^a	X			X	X	X			
The Netherlands						X	X	X			
UK		X ^a				X	X	X			
Russia		X				X					
Spain		X ^a	X			X	X	X			
Sweden		X ^a				X	X				
Switzerland						X					
America	Argentina					X	X				
	Brazil					X	X				
	Canada						X				
	Cuba	X									
	Mexico			X			X				
	USA					X	X	X			
	Uruguay					X					
Oceania	Australia						X				
	New Zealand					X	X				

CAR = Central African Republic; DRC = Democratic Republic of the Congo.

^a Probable cases imported from an endemic area for genotype 1 HEV. ^b At least one positive sample from domestic pigs, wild boars, deer, mongoose or any combination. ^c At least one positive sample from domestic pigs, wild boars or any combination.

they explained that HEV genotypes 3 and 4 may have expanded starting around the end of the 19th century until World War II, and that genotype 3 experienced an additional increase in population size until approximately 1960. Both genotypes 3 and 4 may have undergone a rapid decline in population size to pre-expansion levels since approximately the year 1990 [53]. Regarding genotype 1, its expansion was dated approximately 30–35 years ago [53]. Overall, results of this study suggested that the HEV ancestors may have adapted in a stepwise manner to various successive animal hosts, which led to human hosts. Besides, Xia et al. [54] analysed 80 full-length HEV genomes using Bayesian analysis and reported that genotypes 3 and 4 strains recovered from swine were paraphyletic with regard to strains recovered from humans, which appeared phylogenetically nested among pig strains. Recombination events in HEV have been reported [55]. The recombination events could be a consequence of co-infection or superinfection of the host. The dual infection of a single host by HEV of two different genotypes and two different subtypes has been documented in humans [56]. The possibility of superinfection with HEV has recently been documented in pigs in Spain by de Deus et al. [57], showing that the same pig can be infected by at least two different strains of HEV during its productive life. The nucleotide similarity between the two HEV sequences obtained from the same pig at 1 and 15 weeks of age was 84% [57]. Moreover, HEV quasi-species have been described [58]. Inpatient HEV sequence diversity of the Tanefdour (Algeria) hepatitis E epidemic (between 1986 and 1987) ranged between 0.11 and 3.4% [58].

Routes and Sources of HEV Transmission

The route of HEV transmission during outbreaks is well established, and was usually associated with the consumption of faecally contaminated drinking water [2–6, 9, 10, 43, 59]. In contrast, it is poorly documented for sporadic hepatitis E cases reported in both developing and developed countries [14, 21, 44, 60]. To date, four major documented routes of HEV transmission have been reported: waterborne transmission [2–8, 61]; foodborne transmission [29, 48]; bloodborne transmission [62, 63], and vertical transmission [64–66]. Person-to-person HEV transmission is rarely reported and is considered as an uncommon event, both in the context of outbreaks and sporadic infections [67, 68]. HEV nosocomial transmission [69] and accidental transmission in the laboratory [70] were also reported. Finally, a case of HEV infec-

tion transmitted via an infected graft after orthotopic liver transplantation has been recently reported in Germany [71]. The infectious titer for HEV is not known precisely but could be estimated through several experimental studies, and may be approximately 10^4 – 10^7 genome equivalents per millilitre [1, 72].

Waterborne Transmission

In developing countries of Asia, Africa and Latin America, the faecal-oral route through drinking contaminated water is the principal route of HEV transmission, and is responsible for the majority of hepatitis E outbreaks occurring in these areas [2–8, 59, 61, 73–75]. These outbreaks usually occur during the rainy season, floods or the monsoon, conditions facilitating contamination of pipes, groundwater and sources of drinking water by human excreta [6–8, 59]. The common characteristic for the majority of the hepatitis E outbreaks is drinking unchlorinated water containing an elevated rate of coliform organisms [74, 75]. Nevertheless, drinking chlorinated water does not exclude the occurrence of hepatitis E outbreaks, as shown in a refugee camp in Darfour, Sudan [61]. Therefore, further studies are needed to evaluate the concentration of chlorine required to inactivate HEV in drinking water, but also to identify other appropriate environmental measures enabling the reduction of the risk of HEV transmission by the faecal-oral route. Case-control studies have shown that consumption of boiled water significantly reduced the risk of epidemic HEV infection [6, 59]. Strong arguments supporting the faecal-oral transmission of HEV during hepatitis E outbreaks came from recent studies conducted in India and Uganda [3, 4, 73]. Environmental investigations showed the presence of HEV RNA (of genotype 1) in river water samples collected during hepatitis E outbreaks in India [3, 73], and the presence of HEV RNA in common hand washing water collected during the Kitgum District (Uganda) hepatitis E outbreak [4]. Interestingly, in the Alandi (India) hepatitis E outbreak, partial HEV sequences from patients infected with HEV and water samples were identical [3]. Besides, HEV of genotype 1 has been detected in drinking water treatment plants in Egypt and India, and sewage in India [73, 76]. In addition, a 12-fold (range, 2–78) higher risk of anti-HEV seropositivity was found in Turkish agricultural workers (35%) who used untreated waste water for irrigation compared to a control group (4%) [77]. Of note, HEV of genotype 3 has been recently detected in river water in Cambodia [78]. This report suggests that waterborne hepatitis E of genotype 3 might occur in developing countries.

Fig. 3. Schematic drawing of documented and possible sources and routes for autochthonous HEV transmission to humans in developed countries.

In developed countries, evidence of HEV transmission through the faecal-oral route has not been reported until now, although infectious HEV of genotype 3 has been isolated from sewage or a drinking water treatment plant [60, 79, 80]. However, this mode of contamination cannot be excluded, and could be related to drinking water contaminated by HEV or consumption of food items receiving sewage sludge, such as shellfish (fig. 3). In South Korea, an oyster HEV strain of genotype 3 phylogenetically close to HEV recovered from a South Korean pig has been reported [81]. In Canada, HEV RNA has been detected on irrigated, field-grown strawberries [82]. In addition,

drinking water from non-public supplies was identified as a risk factor for acquiring hepatitis E in Spain [83] and France [20]. Therefore, as in developing countries, the environment should be considered as a potential source of HEV transmission in developed countries. This hypothesis is supported by the finding of Borgen et al. [60] in the Netherlands, who showed a 100% identity between HEV RNA detected in a patient infected with HEV and that of a surface water sample taken in the close surroundings of the patient. In another study conducted in the Netherlands, HEV RNA was detected in 17% (2/12) of surface water samples collected from the Meuse River, and

showed high nucleotide similarity with HEV recovered from humans in the same geographical area [79]. Finally, a hepatitis E case acquired following intense exposure to waste-water sludge has been reported in France [84]. Of note, HEV RNA of genotype 1 has been detected in urban sewage and biosolids (treated sludge) in Spain [80]. This finding was unexpected, and therefore suggests that contracting of non-travel-related hepatitis E of genotype 1 cannot be excluded in developed countries.

Zoonotic Transmission

Under non-experimental conditions, the hypothesis of zoonotic transmission of HEV was suggested for the first time in 1997 by Meng et al. [52]. They described a genotype 3 HEV strain from a pig in the USA, which showed high nucleotide and amino acid similarity with human HEV sequences [52]. The zoonotic transmission of HEV was confirmed by the experimental demonstration of cross-species transmission of human HEV to pigs and of pig HEV to non-human primates [72], but also by several studies that showed a strong phylogenetic link between HEV RNA recovered from humans and other mammals (especially pigs, wild boars and deer), including meat and derived products (fig. 3) [15, 24, 25, 27, 29, 48]. Furthermore, HEV has been detected in pig livers or pig liver sausages purchased in supermarkets [15, 24, 85], and the infectivity of some of them was proven [85, 86].

Foodborne Zoonotic Transmission

The strongest evidence of zoonotic transmission of HEV has come from studies conducted in Japan, and was associated with eating not thoroughly cooked deer or wild boar meat [29, 48]. HEV sequences recovered from leftover frozen meats and from patients infected with HEV were identical [29, 48]. Further evidence supporting the zoonotic transmission of HEV is through the consumption of contaminated raw or undercooked food products from pigs purchased in supermarkets or from wild boars, although the leftover meats were not available for HEV testing [15, 23, 24, 60, 87]. HEV sequences recovered from suspected commercial pig livers or pig liver sausages were closely related, or identical in some cases, with those recovered from patients infected with HEV [15, 24, 62]. For example, in Marseille (France), HEV RNA sequences recovered from hepatitis E patients who ate uncooked pig liver sausage were closely related to those recovered from figatelli, with nucleotide identity >99% [24]. In addition, based on case-control studies conducted in France and Germany, consumption of uncooked pig liver sausage,

game meats or offal was identified as a risk factor for HEV infection [23, 24, 88]. Another argument in favour of zoonotic foodborne transmission of HEV is the finding in Indonesia of significantly higher HEV seroprevalence in Hindu people, who eat pig meat, than in Moslem people, who do not eat pig meat [89]. In addition, in a study conducted in Japan by Toyoda et al. [90], anti-HEV antibodies detection was significantly different between hunters who reported the consumption of undercooked or raw boar meat and those who did not ($p = 0.002$). Thus, the prevention of foodborne zoonotic transmission of HEV could consist of avoiding consumption of undercooked meats and other derived products from animals identified as potential reservoirs of HEV. Of note, it has been shown that HEV in contaminated pig livers can be inactivated if cooked at an internal temperature of 71° for 5–20 min, boiled in water for 5 min, or fried at 191° for 5 min; in contrast, incubation at 56° for 1 h can be insufficient for viral inactivation [86].

Non-Foodborne Zoonotic Transmission

Direct contact with animals, such as pigs, wild boars, deer and cats, working in a pig slaughterhouse or being exposed to pig blood during surgical training have been identified as potential risks for zoonotic transmission of HEV (fig. 3) [14, 15, 90–92]. A high prevalence of anti-HEV antibodies has been found in veterinarians and staff of pig farms as opposed to the general population [14, 15]. In studies conducted in France and Japan, hunting has been found to be associated with the higher prevalence of anti-HEV antibodies in blood donors [90, 92]. For instance, in the Japanese study, anti-HEV seroprevalence was significantly higher in wild boar hunters than in the control group ($p < 0.0001$) [90]. Finally, contact with horses and pets have been associated with a higher prevalence of anti-HEV antibodies in multivariate analysis [93, 94]. Besides, due to the fact that pigs raise major interest in xenotransplantation [95] and are concurrently considered as a potential reservoir of HEV, the xenografts of tissue, organs and cells of pigs have been considered as a possible source of HEV transmission in transplant patients, but no evidence of such transmission has been published so far.

Non-Zoonotic Foodborne Transmission

Foodborne transmission of HEV linked to shellfish consumption has been suspected in Italy, the UK and France [14, 20, 96]. In a study conducted in the UK by Said et al. [96], consumption of shellfish was identified as the only risk factor during a hepatitis E outbreak in patients

returning from a cruise around the world in March 2008. Epidemiological investigation of this outbreak showed that of 789 individuals who provided blood samples, 195 (25%) were seropositive, 33 (4%) had IgM anti-HEV antibodies and 162 (21%) had anti-HEV IgG antibodies. The sequencing of HEV RNA from 3 case patients showed that they belonged to genotype 3 and were genetically close to the genotype 3 HEV circulating in Europe [96]. Additionally, evidence that shellfish can be a source of HEV transmission to humans was provided by the detection of HEV RNA of genotype 3 in bivalve molluscs in Japan [97] and in oysters in South Korea [81].

Parenteral Transmission

Direct and indirect arguments in favour of transmission of HEV through blood transfusions in humans have been reported [62, 63, 98–100]. Direct evidence was reported in France, Japan and the UK [62, 63]; HEV sequences from the donor and recipient were identical [62, 63]. Indirect evidence of HEV transmission through blood transfusion is the finding of individuals who have seroconverted to anti-HEV antibodies (IgG or IgM) after a blood transfusion [98], the high prevalence of anti-HEV IgG antibodies in blood donors in several other studies [92, 100, 101] and statistically significantly higher prevalence of anti-HEV IgG antibodies among multitransfused patients compared to control groups [102]. Transmission of hepatitis E virus through blood transfusion may be favoured by the existence of a phase of viraemia of at least 2 weeks during the pre-icteric phase of infection [103–105] and the existence of symptom-free HEV infections [103–106]. Of note, in most countries, including developed countries, ALT level is not tested in blood donors. The interest of ALT testing in blood donors was demonstrated in several studies conducted in Japan [99], and one study in Germany [104], showing that on-going (defined by the HEV RNA detection) and/or recent HEV infection (defined by anti-HEV IgM detection) can be detected in blood donors with elevated ALT level [99]. In China, Guo et al. [106] found HEV RNA in 0.07% (30/44,816) of eligible blood donors. Also, it has been found that HEV viraemia in blood donors can exceed 7 log₁₀ HEV RNA copies/ml [99]. Recently, Baylis et al. [107] screened 95,835 Swedish, 18,100 German and 51,075 American plasma donations for the presence of HEV RNA in plasma mini-pools of up to 96 donations. They found that 12 (representing 1:7,986 donations), 4 (representing 1:4,525 donations) and 0 donations for Swedish, German and American donors, respectively, were HEV RNA positive. Molecular characterization of 12 out of the 16 HEV RNA-

positive samples indicated that all cases belonged to HEV of genotype 3. Viral loads varied between 3.2 and 5.7 log₁₀ IU/ml. Noteworthy, using the solid-phase enzyme-linked immunosorbent assays from the Wantai Pharmaceutical Company (Beijing, China), one viraemic sample was anti-HEV IgM-positive and another was anti-HEV IgG-positive [107]. In another work, of 75 plasma fractionation pools analysed for the presence of HEV RNA, 8 were positive including 3/34 from Europe, 1/4 from North America, 0/11 from the Middle East and 4/23 from Southeast Asia [108]. Genotype 3 viruses were found in European and North American pools and genotype 4 viruses were identified in Asian pools. None of the positive pools exceeded a load of 1,000 copies/ml. Only Asian pools were found to have detectable anti-HEV IgG antibodies [108]. In England, Ijaz et al. [109] detected HEV RNA in 6/880 (0.7%) plasma mini-pools collected in 2007 and made up from 48 individual blood donors (42,000 individual donors). In HEV RNA-positive pools, HEV load was low (<2,000 GEq/ml). Anti-HEV IgG and IgM antibodies were detected in 6 and 1 cases, respectively. These previous results raise concern on the consequences of HEV contamination of blood and plasma. It is noteworthy that in the UK, about 75% of blood or blood components are administered to immunocompromised patients, who are at risk of chronic HEV infection [109].

Apart from transfusion of blood or derived products, a nosocomial hepatitis E outbreak with an overall ward attack rate of 15.9% (18 out of 113 patients) was reported in a neurosurgery ward in Karachi, Pakistan; inappropriate practice of shared intravenous administration was identified as the risk factor [69]. Furthermore, the possibility of parenteral transmission of HEV in groups at risk, such as haemodialysis patients, haemophilia patients and intravenous drug users, has been suggested, although conflicting data exist [110–112].

Vertical Transmission

The transmission of HEV from mother to child has been reported in India and in the United Arab Emirates based on serological and/or virological evidence [64–66]. The transmission rate ranged from 33 to 100% [64–66], and the neonatal mortality can reach up to 40% [64, 65]. On the experimental level, vertical transmission of HEV infection was not reproduced in Rhesus monkeys and in gilts [113]. Besides, although anti-HEV IgG antibodies and HEV RNA have been detected in the colostrum of HEV-infected mothers, breastfeeding was not associated with HEV transmission to infants [114].

Burden of HEV Infection

HEV Infection in Humans

It is difficult to estimate the burden of HEV infection in a population due to the significant variability in sensitivity and specificity between the assays used for the diagnosis of HEV infection [101, 108, 115, 116]. Another difficulty is that there is limited access to HEV diagnosis assays in several countries worldwide, and misdiagnosis of some symptomatic hepatitis E cases is a possibility, as shown in recent studies [115, 116]. Moreover, the duration of persistence of antibodies against HEV is poorly understood [34, 117–120]. Similarly, data concerning the seasonality of sporadic hepatitis E cases occurring throughout the year in both developed and developing countries are scarce [21, 22, 121, 122]. A seasonal variation in the occurrence of hepatitis E cases has been observed in studies conducted in the UK [22], Japan [121] and China [122]. In contrast, no seasonal variation was observed in the occurrence of hepatitis E cases diagnosed between 2003 and 2007 in the Midi-Pyrénées region of France [21]. In South-west England, from 1999 through 2006, the peak of hepatitis E cases was recorded in spring and summer [22]. In Japan, at Tohoku University Hospital in Sendai (between January 1999 and August 2008), the majority of hepatitis E cases were diagnosed from September through December [121]. In China, the highest incidence rate of hepatitis E in patients from Wuhan Tongji Hospital (between 2007 and 2008) was observed from January through March [122]. Further studies are needed in order to elucidate the seasonality of HEV.

HEV Infection in Developing Countries

In developing countries of Asia, Africa and Latin America, HEV infection is considered to be a major cause of viral hepatitis [1, 45–47], and occur either in the epidemic or sporadic form [2, 5, 6, 43, 45–47, 59, 61, 74, 75]. The seroprevalence of IgG antibody to HEV in these areas is high, and can exceed 70% in Egypt [123]. The prevalence of hepatitis E cases in hospital-based studies is also high, and can exceed 37% in South Asia [1, 45–47]. During hepatitis E outbreaks, the attack rate varied from 0.3 to 25% [2, 5, 43, 61, 74, 124]. The symptomatic attack rate in pregnant women in general is high [2, 5, 45–47, 61] and can reach up to 81% [5].

The majority of the HEV infections in developing countries are subclinical in presentation, with an estimated two to five times greater frequency compared to symptomatic infections for both sporadic and epidemic cases [61, 120, 125]. In addition, the frequency of symp-

tomatic infection appears to be higher in men than in women [126]. The first documented outbreak (based on a retrospective study) of hepatitis E occurred in 1955 in New Delhi, India, following faecal contamination of the drinking water supply system by the floods of the Yamuna river [8]. During this epidemic, over 29,000 cases of jaundice were recorded, representing nearly 2% of the population affected [8]. Since then, numerous other outbreaks of hepatitis E have been reported, especially in Asia and in Africa [2, 4–7, 43, 59, 74, 75, 124]. The most recent hepatitis E outbreaks have occurred on the African continent, especially in Uganda (>10,196 hepatitis E cases and 160 deaths recorded in the Kitgum district between October 2007 and June 2009) [5], Sudan (2,621 hepatitis E cases with a case-fatality rate of 1.7% recorded in a refugee camp in Darfur between July and December 2004) [61] and Central African Republic (222 hepatitis E cases and four deaths recorded in the Begoua district between July 2 and October 2002) [2].

The population mostly affected by HEV infection in developing countries is composed of young adults, men, patients with chronic liver diseases and pregnant women [5, 43, 45–47, 61, 74, 122]. The mortality rate is estimated to be less than 4% in the general population [2, 43, 61, 124], exceeding 20% in pregnant women [5, 46, 47, 61] and in patients with chronic liver diseases [122, 127]. The reasons for the higher mortality in pregnant women remain unknown. Immunological and hormonal factors have been hypothesized to explain the pathogenesis of HEV infection during pregnancy [128, 129].

HEV Infection in Developed Countries

In developed countries, hepatitis E cases usually occur sporadically [14, 18, 19, 21–23, 60], although clusters of acute hepatitis E cases have been reported [24, 62, 87]. The high prevalence rates of on-going HEV infection (which can reach up to 1%) and of anti-HEV antibody prevalence in blood donors suggest that HEV is responsible for several cases of subclinical HEV infections in industrialized countries [99, 107, 109, 130]. For instance, in the UK it has been estimated in a study conducted by Ijaz et al. [131] that 60,000 hepatitis E cases occur per year.

Until the end of the 1990s, hepatitis E in developed countries was considered to be an imported disease from HEV hyperendemic areas [13]. However, HEV has emerged (especially in Europe and Japan) over the past decade as a significant cause of non-travel-associated acute hepatitis [14, 18–20, 22, 23, 60, 67, 132]. In some countries, the prevalence of autochthonous acute hepatitis E may exceed 16% in undiagnosed non-A, non-B, non-

C acute hepatitis patients [14, 22, 67, 132]. In Japan, the number of reported annual domestic hepatitis E cases increased from 2 in 2000 to 54 in 2006, then plateaued [18]. In England, Dalton et al. [22] observed in Southwest England that the number of hepatitis E cases increased from 1 in 2000 to 13 in 2006. In France, according to data collected by the National Reference Center of Enterically Transmitted Hepatitis created in 2002, the number of autochthonous hepatitis E cases increased by a factor of 9 from 2006 through 2010 [133]. For the year 2010 only, 238 autochthonous hepatitis E cases have been registered by the National Reference Center of Enterically Transmitted Hepatitis [133].

Compared to that of the developing countries, the population mainly affected by HEV infection in developed countries is composed by the elderly, men, immunocompromised patients and patients with chronic liver diseases [14, 18, 20–22, 100, 134]. The mortality rate in these areas is high and ranges between 2 and 13% according to the data from hospital-based studies [20–22, 134]. These mortality data should be interpreted with caution, because they are mostly based on the mortality data from patients with clinical symptoms and more severe forms of HEV disease. Striking data have been reported recently by Dalton et al. [135] about the statistically significant independent association between pig meat consumption and mortality from chronic liver diseases in developed countries in the 1990–2000 period. This was revealed by the analysis of data on mortality related to chronic liver diseases, alcohol consumption and hepatitis B and C virus seroprevalence for 18 developed countries from 1990 through 2000, and, concurrently, data on national pork and beef consumption. Multivariate regression showed that alcohol, pig meat consumption and hepatitis B virus seroprevalence were independently associated with mortality from chronic liver diseases. A 10-kg increase in national annual average per capita consumption of pig meat was associated with an increase in mortality related to chronic liver diseases that represented 4–5 deaths/100,000 population [135]. The high mortality found in pregnant women in developing countries is not observed in developed countries. To date, to the best of our knowledge, only three autochthonous hepatitis E cases of genotype 3 have been reported in pregnant women in developed countries [136–138].

The seroprevalence of anti-HEV IgG varies from 2 to over 25% in developed countries [14, 15, 92–94, 99–101, 131]. In addition, regional differences of HEV seroprevalence have been observed within countries, as shown in the USA [94] and France [92, 139]. In France, seroprevalence of anti-HEV IgG ranged from approximately 3% in

the north to approximately 16% in the south as assessed using a same serologic assay [92, 139]. In certain developed countries, the seroprevalence of anti-HEV IgG in the general population is generally much higher than would be expected given the low prevalence of acute symptomatic hepatitis E cases. For instance, in the USA, the seroprevalence of HEV is 21% in blood donors [94], while only five autochthonous hepatitis E cases have been reported so far [140].

Contrary to what has been reported in developing countries, since February 2008, several chronic hepatitis E cases and cases of hepatitis E-associated cirrhosis have been described in immunocompromised patients in Europe, including solid-organ transplant recipients (in the majority of cases), HIV-infected patients and patients with haematological diseases [16, 17, 30–33, 141]. All chronic hepatitis E cases reported so far belonged to genotype 3 [16, 17, 30–33, 141]. Studies are needed to determine whether infection with other HEV genotypes can evolve towards chronic hepatitis.

Possible Impairment of Serologic Assessment of HEV Prevalence and Incidence

There is no gold standard test for the diagnosis of HEV infection. In the setting of routine diagnosis, HEV infection is usually diagnosed using enzyme immunoassays for the detection of specific IgM and IgG antibodies and/or RT-PCR assays for viral RNA detection (standard or, mostly, real-time RT-PCR) [5, 19, 20, 22, 43, 44, 60, 67, 108, 122, 132]. The diagnosis of acute hepatitis E is based on the detection of IgM antibody to HEV and/or HEV RNA detection (in serum, or faeces when available). No study has performed comparative analysis of all commercial assays that are currently available for the detection of antibodies to HEV. Studies evaluating the performance of some ELISA assays have provided controversial results. Thus, the capability of some assays to detect anti-HEV IgG or IgM antibodies has been found to vary considerably in non-immunocompromised individuals [33, 115]. Bendall et al. [115] reported that anti-HEV IgG testing provided positive results in 98% of 50 sera from 18 proven cases with the Wantai assay (Beijing, China) versus in 56% of sera using the Genelabs assay (Genelabs Diagnostics, Singapore). In addition, the Wantai assay resulted in a substantially higher estimate of seroprevalence in blood donors than the test of Genelabs (16.2 vs. 3.6%). Moreover, anti-HEV IgG were detected in all 12 sera collected >1 year after the hepatitis E onset using the Wantai assay, compared with in 50% of these sera using the Genelabs kit. Dalton et al. [33] also reported that anti-HEV IgG and

IgM antibodies in a patient chronically infected with HIV (as attested by PCR) were either positive or negative when assessed by the Wantai or the Genelabs assay, respectively. Strikingly, considerable differences were recently pointed out in Southwest France between anti-HEV IgG prevalence measured by different serologic assays. Indeed, anti-HEV IgG antibodies were detected in 52 or 16% of blood donors over the same time period with the Wantai assay or the Adaltis assay, respectively [92, 101]. In addition, anti-HEV IgG prevalence among patients who underwent haematopoietic stem cell transplantation was 36 and 12% using the Wantai assay or the Adaltis assay (Ingen, France), respectively [142]. Besides, a progressive decrease of anti-HEV IgG over time, and rapid HEV seroreversion in some cases have been reported [34, 117–119]. Thus, Khuroo et al. [117] reported that anti-HEV IgG remained detectable in around half of patients over a 14-year period, by performing longitudinal testing on serum samples collected from hepatitis E cases. The specificity of all different serological assays currently used for the detection of antibody to HEV has not been evaluated. Commercial ELISA HEV kits from Adaltis and MP Diagnostics available on the market showed a fairly good sensitivity and an excellent specificity, but specificity was assessed using serum samples from blood donors and not samples collected from patients with different pathologies, including patients presenting infectious diseases or increased levels of transaminases [115, 143].

HEV Infection in Other Mammals

The major animal reservoir for HEV appears to be pigs, although the presence of HEV RNA has been reported in wild boars, deer, rabbits, rats, cows, sheep, bats and mongooses [14, 15, 25–27, 48, 132, 144–146]. HEV infection is ubiquitous in pigs worldwide [14, 15, 25, 26, 28] and the proportion of farms with at least 1 pig found positive for anti-HEV antibodies and/or HEV RNA can reach up to 100% in some studies [48, 147]. The oldest samples from pigs tested to date show that HEV has been circulating in the population of pigs in Spain and India since at least 1985 [148, 149]. Pigs are usually HEV RNA-positive when they are young (2–3 months), but high prevalence of HEV RNA (reaching 41%) have also been found in pigs at the age (6 months) at which they are slaughtered [15, 25, 28, 147]. Similarly, anti-HEV antibody detection in pigs varies with age [48, 52, 147]. Most pigs under two months of age are anti-HEV-negative, while the majority of pigs older than 3 months are seropositive [48, 52, 147]. In wild boars (Europe, Japan and Australia), the prevalence of anti-HEV antibodies ranges

from 9 to 43% and that of HEV RNA ranges from 3 to 25% [27, 48, 146, 150]. Anti-HEV IgG was detected in 2–35% of deer in Japan [48] and 5% of deer in the Netherlands [146]. The prevalence of HEV RNA in deer was 15% in red deer in the Netherlands [146] and 34% in roe deer in Hungary [132]. In Japan, the seroprevalence of anti-HEV IgG antibodies in mongooses ranged from 8 to 21% [145, 151]. In rats, anti-HEV antibodies have been detected in animals captured in Germany [152], Japan [153], Vietnam [154] and USA [155]. The prevalence of anti-HEV IgG antibodies in rats is high and ranged from 21 to 84% [152–155] and that of HEV RNA ranged from 0.7 to 18% [152–154]. Anti-HEV IgM have been detected in 4% (5/139) of wild rats trapped in Vietnam [154]. Little is known about the HEV infection in rabbits and only 3 independent studies have been conducted so far [144, 156, 157]. In Virginia, USA, HEV RNA was detected in 22% (19/85) of rabbits and anti-HEV antibodies was detected in 36% (31/85) [156]. In China, the overall prevalence of anti-HEV antibody in rabbits ranged from 15 to 57% and that of HEV RNA ranged from 2 to approximately 8% [144, 157]. In addition, a high prevalence of anti-HEV antibodies was detected in various other mammalian groups, including cattle, dogs, cats, horses, goats [48, 81] and in non-human primates [158].

HEV Vaccine

Since all HEV strains belong to the same serotype [40], a hepatitis E vaccine that shows efficacy to one HEV genotype may be able to provide protection against other genotypes of HEV [159, 160]. Two recombinant hepatitis E vaccines produced with a genotype 1 HEV strain showed promising results concerning the prevention of hepatitis E in humans [161, 162]. The phase III clinical trial of the first vaccine, HEV 239 (expressed in *Escherichia coli*), has been completed in healthy men and non-pregnant women aged 16–65 years from Jiangsu Province, China [161]. The HEV 239 vaccine was well tolerated and effective in preventing new HEV infections. Three doses (30 µg per dose) of this vaccine administered intramuscularly at 0, 1 and 6 months were 100% (95% CI, 72.1–100.0) efficacious in 48,693 vaccinated persons, 1 year after receiving the third dose [161]. In comparison, 15 of 48,663 individuals who received the placebo (hepatitis B vaccine) developed hepatitis E [161]. Of note, the HEV 239 vaccine has recently (in 2012) been approved for marketing in China [163]. The second vaccine, named rHEV, was produced in insect cells (*Spodoptera frugiper-*

da) and its safety and efficacy has been evaluated in a study conducted in Nepal [162]. The rHEV vaccine was well tolerated, and the efficacy of a three-dose vaccination (intramuscular administration of 20 µg per dose at months 0, 1 and 6) was 95.5% (95% CI, 85.6–98.6) in a group of volunteers from the Nepalese army [162]. Two years after the administration of the third dose of the rHEV vaccine, the efficacy of the vaccine was only 56.3%, suggesting the necessity to evaluate the maximum duration of protection afforded by the rHEV vaccine [162]. Although the efficacy and safety of the rHEV vaccine has been proven, there are limitations of the study that need to be addressed. The majority of the study participants were men (>99%) with a mean age of 25.2 years (standard deviation 6.25). The efficacy of the rHEV vaccine for the prevention of asymptomatic HEV infection was not assessed because the endpoint was clinical disease confirmed by laboratory diagnosis of HEV. Of note, a pre-clinical study conducted in monkeys using the rHEV vaccine showed that this vaccine can protect all monkeys tested against the hepatitis E disease but only partially protected against HEV infection [160]. The use and efficacy of these vaccines can be questioned in immunocompromised patients, as post-exposure prophylaxis in order to determine whether they may be used to control hepatitis E outbreaks, and in the porcine reservoir. The maximum duration of protection afforded by the two hepatitis E vaccines needs to be determined. Finally, a major issue to be addressed is related to obtaining of funding to implement HEV vaccine strategies for developing countries where HEV is hyperendemic.

Conclusion

HEV has unveiled a new face over the last decade. The data reviewed here show that HEV undoubtedly has its place alongside other hepatitis viruses A, B and C, and the delta agent as one of epidemiological and clinical significance. In developed countries this virus is no more an anecdotal agent responsible for acute hepatitis imported from hyperendemic geographical areas. Therefore, physicians should consider HEV infection as a possible aetiology when facing acute hepatitis. The emergence of chronic hepatitis E and cirrhosis in solid-organ transplant recipients in Europe during the last 5 years has promoted the use of drugs for HEV eradication. The efficacy of pegylated interferon-alpha [141] and of ribavirin, which is an antiviral drug with broad-spectrum activity against several DNA and RNA viruses, has already been

evaluated in France in this special population [31, 164]. Fortunately, these drugs, especially ribavirin, have shown promising results.

Our knowledge on the epidemiology of HEV in developed countries has certainly improved considerably (fig. 3), although this should not obscure the fact that a specific route of HEV transmission is identified in a minority of HEV infections. Implementing hospital-based surveillance or mandatory notification of HEV infection, as is the case currently in Germany, China, Australia and Japan [18, 23, 165], might improve our knowledge of the HEV epidemiology. The porcine reservoir of HEV has been largely described and identified as a source of HEV in Asia and Europe. Measures for the surveillance and control of HEV infections in pigs or pig food items (such as meat) could be implemented to increase food safety. In 2009, in an attempt to prevent the transmission of HEV through the zoonotic route in France, the French Agency for Food, Environmental and Occupational Health and Safety compelled manufacturers of pig liver sausages to note on the packaging that the sausages should be cooked thoroughly [24]. The emergence of hepatitis E in developed countries must not lead us to forget that the majority of the clinical burden related to HEV stands in developing countries. The availability and efficacy of the HEV vaccines in these HEV hyperendemic areas are important issues. Besides, globalization might enable the worldwide circulation of HEV strains of different genotypes through human travel and migration, and pig international trading. In this view, whether or not HEV genotypes may be associated with different clinical presentation and outcome should be specifically addressed in the future. Also, the reasons for the higher morbidity or mortality associated with HEV infection in some populations (pregnant women, patients with chronic liver diseases, immunocompromised patients) needs to be deciphered. Finally, the accuracy of our assessment of HEV seroprevalence and incidence using currently commercialized serologic assays is not clear. Therefore, studies to assess the performance of all currently available commercial serological assays for the detection of antibodies against HEV are needed.

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