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Hepatitis E virus: reasons for emergence in humans

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Hepatitis E virus (HEV) infects both humans and other animal species. Recently, we have seen a steady increase in autochthonous cases of human HEV infection in certain areas especially in Europe, and large outbreaks in several African countries among the displaced population. This mini-review critically analyzes potential host, environmental, and viral factors that may be associated with the emergence of hepatitis E in humans. The existence of numerous HEV reservoir animals such as pig, deer and rabbit results in human exposure to infected animals via direct contact or through animal meat consumption. Contamination of drinking, irrigation and coastal water by animal and human wastes lead to emergence of endemic cases in industrialized countries and outbreaks in displaced communities especially in war-torn countries.

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Introduction

Hepatitis E virus (HEV) is a single-stranded positive-sense RNA (~7.2 kb size) virus in the family *Hepeviridae*, which comprised of two genera *Orthohepevirus* (all mammalian and avian HEV strains) and *Piscihepevirus* (Cutthroat trout virus) [1,2•]. The genus *Orthohepevirus* comprises of four species *Orthohepevirus* A–D. Within the species *Orthohepevirus* A, at least 8 distinct genotypes have been identified [2•]: genotypes 1 and 2 HEVs infect only humans, genotypes 3 and 4 HEVs infect humans and several other animal species, genotypes 5 and 6 HEVs infect wild boars, genotype 7 HEV infects dromedary camels and possibly humans [3•,4,5], and genotype 8 HEV infects Bactrian camel [6].

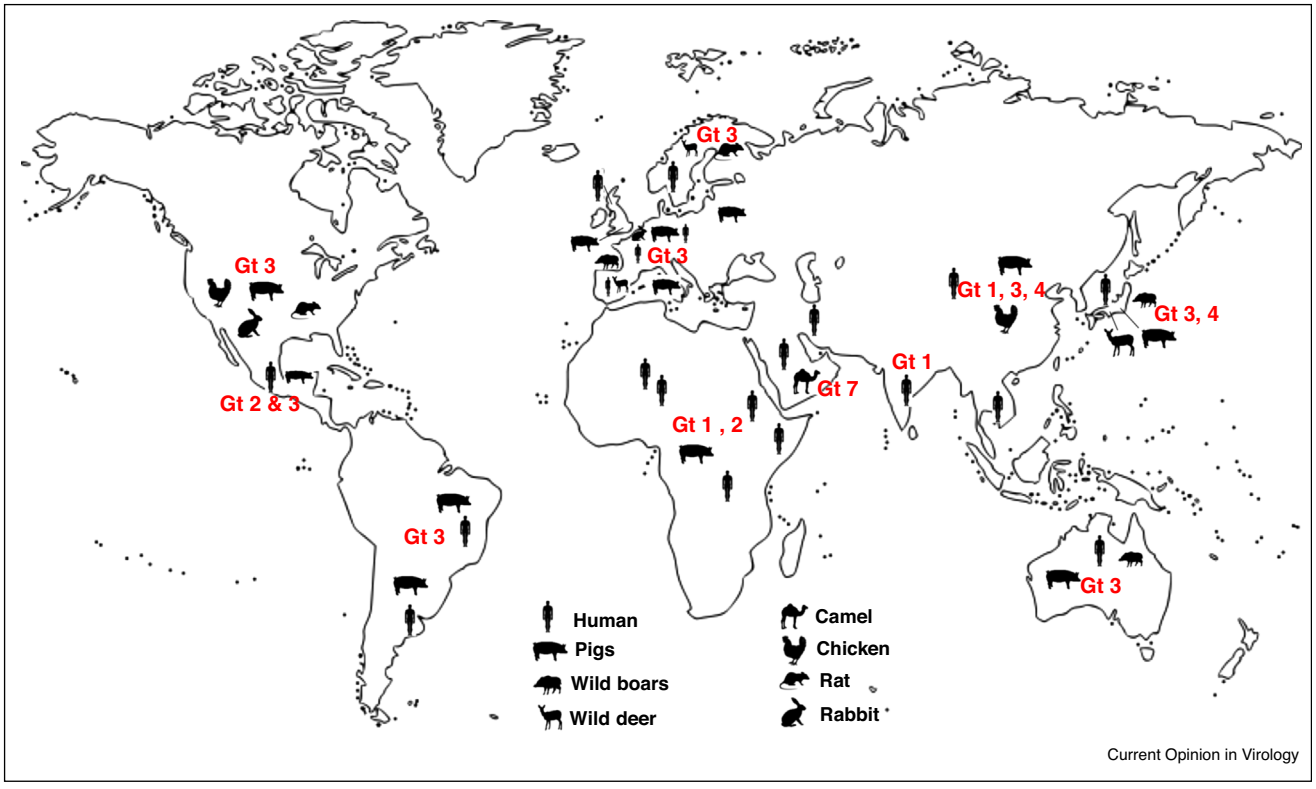
In humans, HEV infection usually results in an acute self-limiting disease, and the majority of infections are asymptomatic. The patterns of clinical disease vary among different HEV genotypes [7]. Genotypes 1 and 2 HEVs typically cause self-limiting acute infections, and are transmitted through contaminated water. Infected pregnant women, especially with genotype 1 HEV, have higher risk of developing fulminant hepatitis with a higher mortality rate of up to 30% [8]. In contrast, genotypes 3 and 4 HEVs are zoonotic, transmitted via consumption of animal meats or direct contact with infected animals, and causes mainly sporadic and cluster cases of hepatitis E [3•]. Genotype 3, and to lesser extent, genotype 4 HEVs can establish chronic infection in immunocompromised individuals such as organ transplant recipients [8].

In this mini-review, we critically analyze the potential host, viral, and environmental factors that are associated with the emergence of HEV infection in humans.

Epidemiology

The seroprevalence of HEV infection varies in populations and the serological assays used worldwide. For example, more than 30% in German general population [9], 60.5% blood donor population in India [10], and 52.5% in Southwestern France were tested seropositive for HEV antibodies [11]. Cases of hepatitis E in humans are associated with genotypes 1–4 HEV, and in one rare instance, genotype 7 HEV [7] (Figure 1). The genotypes 1 and 2 HEV strains are endemic to countries in Asia and Africa, and also in Mexico [7,12,13]. In addition to historical large explosive outbreaks in Asia [14–16], in recent years, genotype 1 HEV infections in humans were also reported from individuals in Israel [17], Italy [18], and Germany [19] who are usually associated with prior travel to endemic countries. Two recent large outbreaks associated with genotype 1 HEV infection have been reported from Niger [20], and Uganda [21,22]. In the Niger outbreak, a total of 1917 suspected cases were reported between January and September of 2017. During the 2013–2014 outbreak in Uganda, approximately 1359 cases were suspected with an overall mortality rate of 2.2%, while a significantly higher mortality rate of ~65% was observed in pregnant women [22]. Smaller outbreaks have also been reported from Bangladesh [23], and India [24] between 2012 and 2014. A higher HEV seroprevalence is also reported amongst refugee populations in war-torn countries such as South Sudan and Ethiopia [25•,26]. Poor sanitation condition or inadequate access to clean drinking water in overcrowded setting enhances genotype 1 HEV transmission rates in humans from developing

Figure 1



Worldwide prevalence of human hepatitis E cases associated with genotypes 1, 2, 3, 4, and 7 HEV infections.
Genotypes (Gt) 1 and 2 HEV mainly infect humans and are transmitted via fecal-oral route through contaminated water or water supplies. Gt3 and Gt4 HEVs are zoonotic in nature, and are transmitted to human through direct contact with infected animals or through eating undercooked or raw animal meat products. Pigs, wild boar and deer meats are most commonly associated with Gt3 and Gt4 transmission to humans. Gt3 rabbit HEV infection of human is reported from France, and Gt7 camel HEV infection of human is reported from Middle East.

countries, while genotype 1 HEV cases in industrialized countries are primarily associated with a history of travel to HEV endemic regions.

Autochthonous cases associated with genotypes 3 and 4 HEV infection are commonly seen in industrialized countries, but also in certain developing countries [7,27,28•]. A rise in autochthonous cases of HEV infection in humans from 2007 to 2013 was reported in Japan [29]. Interestingly, genotype 4 HEV was more predominant during 2007–2009 in Japan, whereas genotype 3 HEV infections have increased from 2010 to 2013 [29]. Importantly, genotypes 3 and 4 HEV strains are also prevalent amongst various animal species, including domestic pigs, wild boars, deer, mongoose, and rabbit in many countries including the United States, China, Japan, and numerous countries in Europe [3•,30]. Interestingly, during the last decade a decline in anti-HEV prevalence in humans has been observed in the United States [31] as well as from several European countries [32,33]. Meanwhile, in the last 25 years in China, the frequency of genotype 4 HEV infection has overtaken genotype 1 HEV-associated infections [34], therefore suggesting that a country's sanitation conditions and zoonotic HEV sources likely determine the epidemiological shift within circulating HEV genotypes in a given human population.

Factors associated with emergence of hepatitis E cases caused by genotypes 1 and 2 HEVs

The prevalence of hepatitis E among a given human population is influenced by multi-level interactions between various factors, including living conditions and/or behavioral habits, host immune status, environmental factors that affect HEV persistence in a given geographical area, and viral factors which contribute to genetic diversity and host range of the virus. Genotypes 1 and 2 HEVs are typically transmitted to humans through contaminated water, and pregnancy increases mortality rates.

Poor sanitation and lack of clean drinking water

The first HEV outbreak was reported in 1955 from India [16,35]. During 1980s and 1990s, numerous explosive large outbreaks were reported from India, Pakistan, Bangladesh, and China [14,16]. However, recent

outbreaks have mainly been reported from war torn countries or countries with major humanitarian crises, such as Niger [20], Uganda [22], South Sudan [26], Ethiopia [26], and Chad [36], largely due to poor sanitation conditions and lack of clean drinking water [25•]. In endemic area, such as China, a spatio-temporal study predicted 4.03 infection per 100,000 person [37]. The average attack rate of HEV infection varies from 2 to 4%, however, in regions with poor sanitation conditions and without clean drinking water, as seen in overcrowded communities in slums, refugee camps, and/or displaced population, the HEV attack rate can go up to 25% [25•]. Since genotype 1 HEV is transmitted through fecal-oral route, an increased attack rate amongst humans living in a densely-populated region with poor sanitation conditions points out to the existence of human-to-human transmission. Further studies are needed to understand this mode of HEV transmission in a large population in order to develop more practical public health measures that could be implemented within economically impoverished and overcrowded communities such as refugee camps.

High mortality during pregnancy

The case fatality rate during genotype 1 HEV infection is usually between 1–3%, while pregnancy significantly increases the mortality rate. Amongst HEV-infected pregnant women from Asian and African countries, a maternal case fatality rate of 20.8%, and fetal case fatality rate of 34.2% have been reported [38]. This case fatality rate can increase up to a staggering 61.2% in mothers with fulminant hepatic failure [38]. In addition to preexisting liver condition, serum viral loads in mothers were a significant predictor of vertical transmission [39]. Sporadic cases of genotype 3 HEV-associated infection of pregnant women were also reported in some countries such as Germany [40] and France [41,42]. However, unlike the genotype 1 HEV infections, the genotype 3 HEV infection spontaneously resolved in mothers with no evidence of vertical transmission and no HEV viral RNA was detected in the newborn. The exact reason contributing to fulminant hepatitis and high mortality in genotype 1 HEV-infected pregnant women remains unknown, however changes in hormonal and immune status and/or response during pregnancy may contribute to this phenomena. However, it should be pointed out that severe hepatitis was not reproduced in pregnant rhesus monkeys experimentally infected with genotype 1 HEV [43], or in pregnant sows experimentally-infected with genotype 3 HEV [44]. Therefore, future studies are warranted to delineate the mechanism of HEV-associated fulminant hepatitis and high mortality during pregnancy.

Factors associated with emergence of human hepatitis E caused by genotypes 3 and 4 HEVs

Genotypes 3 and 4 HEVs can establish chronic infections in immunocompromised patients [45,46], and are usually transmitted through consumption of contaminated animal meat, direct contact with infected animals, or less commonly through contaminated blood products. Since genotypes 3 and 4 HEVs are zoonotic in nature, with multiple animal reservoirs, cross-species HEV infections from reservoir animals to humans also influence the emergence of human hepatitis E within a geographical area.

Foodborne HEV infection via consumption of contaminated animal meats

It has been demonstrated that consumption of raw or undercooked meats or meat products from reservoir animals such as pig and deer represents an important risk factor for the emergence of hepatitis E in human population [8,28•]. In liver transplant patients, consumption of undercooked game meat or pork products was associated with HEV infection and chronic liver disease [47]. Autochthonous HEV infection from Australia was reported in people who ate pork products in one restaurant during 2013–2014 [48]. A familial HEV outbreak was definitively linked to consumption of HEV-infected wild-boar meat in Spain [49]. Autochthonous cases of genotype 3 HEV from Germany and Italy have also been attributed to consumption of pork products. Pork liver sausages have been documented as sources for numerous cluster cases of foodborne hepatitis E. For example, figatelli, a traditional pig liver sausage widely consumed in France, was linked to cluster cases of HEV infection [50•]. Similarly, in Japan, cluster cases of autochthonous hepatitis E are linked to consumption of raw meat [51,52]. Four patients who consumed raw deer meat were infected with genotype 3 HEV [52]. Autochthonous genotype 4 HEV infection due to consumption of raw deer meat have also been reported from South Korea [53]. It has been reported that a significant proportion of wild deer population is infected by HEV [54]. Recently, chronic/persistent HEV infections caused by genotype 4 HEV have been reported in a child with acute lymphoblastic leukemia in China [55], in a woman post-liver transplant from Taiwan [45], and in an individual from the United States with a fatally accelerated cirrhosis [56]. Clearly, foodborne transmission is partially responsible for the emergence of hepatitis E in humans, and the prevalence of diverse HEV genotypes in various farm and wild food animal populations is a risk factor to humans who consume raw or undercooked animal meat products.

Since HEV can contaminate animal meat and meat products, it is important to properly cook the meats before consumption. Incubation of HEV at 60 °C or greater could inactivate up to 80% infectious virions [57], and can reduce HEV RNA levels [58,59]. Infectious HEV present in contaminated pig livers could be inactivated by cooking the meat product to an internal temperature of at least 71 °C for 5 min [60]. Understanding the thermal stability of HEV will enable consumers to properly cook animal meats to reduce the chance for the emergence of foodborne hepatitis E.

Cross-species infection via direct contact with HEV-infected reservoir animals

Under experimental conditions, several animal strains of HEV can infect across species barriers. Swine HEV infects both rhesus macaques and chimpanzee [61•,62], the surrogates of man. Pigs may serve as a mixing vessel for different HEV strains due to their susceptibility to multiple HEV genotypes including genotypes 3, 4, 5 and 6 strains, and thus pose a public health risk [3•]. Avian HEV infect chickens and turkeys but not rhesus monkeys [63,64], suggesting that avian HEV likely does not infect humans. The rabbit HEV can be experimentally transmitted to cynomolgus macaques [65], and infections of humans by rabbit HEV have been reported [66,67].

A high rate of anti-HEV seropositivity has been reported amongst veterinarians, swine farmers and hunters from many countries including the United States [68], Finland [69], China [70], Italy, and Poland [71,72]. For example, U.S. swine veterinarians were 1.51 times more likely to be IgG anti-HEV seropositive than blood donor controls. Importantly, subjects from traditionally major swine states such as Minnesota are more likely to be seropositive than those from traditionally non-swine states such as Alabama [68]. A significantly higher prevalence of IgG anti-HEV was also reported in individuals with occupational exposure to wild animals and environmental sources of animal wastes [73]. However, there was no association between anti-HEV seropositivity and exposure to pigs in the Netherlands [74]. Therefore, zoonotic HEV transmission in human population would be dependent on frequency and nature of contact exposure, the amount of viral loads in the infected animals, and the animal species.

Blood transfusion

Transfusion-transmitted cases of hepatitis E have been reported from many countries including Japan [75], U.K [76], France [77], Germany [78], Australia [79], and Canada [80]. HEV seroprevalence among blood donors varies from 3.4% in Japan [81], 5.9% in Canada [82], 6.8% in Germany [78,83], to 22.4% in France [84]. A seroprevalence of 18.8% was reported in blood donors in the United States, however, during the follow-up study, there was no transfusion-transmitted HEV infection among the recipients [85]. A recent large-scale survey study from United States (N = 128,020) reported a 0.002% HEV RNA positivity amongst the plasma donors [86]. The risks associated with transfusion-transmitted HEV depend upon the frequency of viremia in blood donors, the viral loads in the donor blood, and the volume of plasma in the final transfused blood component [87].

Asymptomatic viremia has been reported in healthy blood donors from many countries, and the rate of HEV viremia in blood donors ranges from 1:600 to 1:42,000 [86,87]. The majority of viremic donors remained seronegative at the time of blood donation in Germany [88•] and U.K [89], and had spontaneous virus clearance within a median time of 6–8 weeks. The lowest viral dose required for transmission was reportedly at 2×10^4 IUs, and 55% of the components containing this dose transmitted HEV infection [90•]. Immunosuppressed patients with an extensive need for blood transfusion are at higher risk of developing transfusion-transmitted chronic HEV sequel [75,90•]. Interestingly, a severe case of genotype 3 HEV infection was reported from France in a previously healthy male trauma patient who received massive transfusion of HEV-contaminated platelet pool [91]. Taken together, although blood transfusion is associated with the emergence of hepatitis E in humans especially in immunosuppressed patients requiring extensive transfusion, such cases are low. Therefore, this raises a question as to whether blood donors should be required to screen for HEV RNA. Although some European countries have already implemented screening of blood donors for acute HEV infection marker such as HEV RNA [92•], however, this practice is not yet implemented universally.

Environmental factors contributing to the persistence and emergence of HEV in humans

Studies on the environmental factors affecting persistence and emergence of HEV is very limited. HEV has been detected in environmental samples such as urban wastewater effluents, soil, and effluents from animal slaughterhouses. For example, approximately 25% of urban wastewater effluent was tested positive for genotype 3 HEV in France [93], and approximately 4.9% soil samples near river were tested positive for genotype 1 HEV in India [94]. Infectious swine HEV was detected in pig manure storage facilities in U.S. swine farms including lagoons and concrete pits, although evidence of drinking or surface water contamination on or near pig farms was not found [95]. Swine HEV has also been detected in effluents from pig farms [93,96], slaughter houses [93,96], and pig manure composite plants [97]. In regions with large operations of hog farms, swine manure or sewage water could potentially contaminate irrigation water or coastal waters with concomitant contamination of produce or shellfish. For example, genotype 3 HEV of swine origin was detected in mussels from Spain [98]. In Canada, genotype 3 HEV of swine origin was detected on farm-grown strawberries and irrigation water is suspected as the source of contamination [99]. Taken together, the available data clearly suggest a conducive fecal-contaminated wet environment that potentially aids in emergence of hepatitis E in humans.

Viral factors contributing to the host range and cross-species infection of HEV

Two major events, point mutations and recombination, attribute to the genetic variations and evolution of HEV genome. Accumulating evidence suggest that the ORF1 appears to be responsible for determining the host range

and cross-species infection. It has been documented that point mutations affect HEV heterogeneity, and contribute to viral pathogenesis and susceptibility [100•]. Recombination events can occur within different HEV genotypes, and between human and other animal HEV strains. An extensive heterogeneity in the hypervariable region (HVR) and macro domain of HEV genome is seen amongst the immunocompromised patients developing a chronic disease as compared to patients with resolving HEV infections [101]. Insertion and/or deletions in HVR of HEV ORF1 contributes to viral replication efficiency and host adaptations [102,103]. By utilizing intergenotypic chimeric viruses between human-exclusive genotype 1 HEV, and zoonotic genotypes 3 and 4 HEVs, it was found that the HEV ORF2 capsid gene does not determine host range [104,105]. Instead, the ORF1 is thought to be involved in host tropism of HEV. It has been shown that insertion of host RPS17 gene within the HEV HVR led to increased adaptability and infection of the virus in cells from different animal origins [106•]. A clinical isolate of HEV from a chronically-infected patient with a 258 bp duplicated HVR fragment and a 24 bp RdRp-derived fragment was associated with increased viral replication [107•]. HVR appears to be exchangeable between different HEV genotypes, however the chimeric viruses displayed a reduced virus replication kinetics [103]. Thus, it appears that HVR may contain genotype-specific sequences that are crucial for viral RNA replication and host adaptability, which may further influence the disease outcome.

Conclusion

Human behavioral and/or living conditions, as well as the presence of zoonotic HEV strains among animal species at a given geographic area determine changes in epidemiology of human HEV infection. More extensive studies are needed to understand the ecology and natural history of animal HEV strains as well as various modes of HEV transmission among host species, in order to formulate public health policies to prevent and control HEV infection in humans.

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