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Food and environmental routes of Hepatitis E virus transmission

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Hepatitis E virus (HEV), genus Hepevirus, family hepeviridae is a main cause of epidemic hepatitis in developing countries and single cases of hepatitis in higher income countries. There are at least four HEV genotypes which have different epidemiologic and clinical features. Hepatitis E viruses are often transmitted via food and environmental routes. The actual role of these transmission routes in the spread of HEV can depend on the virus genotype, the environmental conditions, the hygienic conditions and the types of foods consumed. In this review food and environmental routes of HEV transmission are discussed to raise the awareness regarding the focal points for the development of accurate prevention and control strategies of HEV infection, food safety and public health protection

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Hepatitis E virus background

Hepatitis E virus (HEV) is a small, non-enveloped, single-stranded, positive-sense RNA virus. The genome size is approximately 7.2 kb [1]. In 2004 Hepatitis E virus (HEV) was designated as the sole member of the genus Hepevirus in the family hepeviridae [2]. The HEV genome has three open reading frames (ORFs): ORF1 encoding the non-structural polyprotein (nsp), ORF2 encoding the viral capsid protein and ORF3 encoding a small regulatory phosphoprotein [3]. Hepatocytes are assumed to be the primary target cells in which the virus replicates in the cytoplasm. On the basis of the lack of an efficient cell culture system, the mechanism by which HEV enters the cells and how the virion is released from the cells is not fully understood yet [4]. In humans HEV infections often run an asymptomatic course but after an incubation period of 4–5 weeks HEV disease can present like an acute icteric viral hepatitis. Frequently observed symptoms include anorexia, nausea, jaundice, fever and abdominal pain [5]. Mortality rates are generally under 0.5% but may reach up to 25% in pregnant women for at least genotype 1 [6].

HEV variants most closely related to those infecting humans can be divided into at least four genotypes [7] and up to six genotypes as two lineages have been detected in wild boar which have been demonstrated to be more divergent [8•]. Within genotypes 1–4 subdivisions into subtypes have been suggested by different authors, based on whole genome sequences or (partial) sequences derived from different open reading frames of the virus genome. An increasing number of HEV sequences are reported and based on findings of divergent lineages in an increasing number of animal species deeper taxonomic groupings and genera have been proposed [8•,9]. Reported lineages of HEV strains within genotypes 1 and 2 are less divergent and seem to be more conserved compared to HEV strains from genotype 3 and 4. Genotypes 1 and 2 only seem to affect humans. Genotype 1 viruses are predominantly isolated from outbreaks and sporadic cases in Asia and Africa, whereas genotype 2 strains mainly have been observed in outbreaks in Mexico and Africa. Genotypes 3 and 4 are zoonotic and are observed in different animal species and sporadic human cases, worldwide for HEV genotype 3 and mainly in Asia for HEV genotype 4. The proposed genotypes 5 and 6 this far have only been detected in wild boar in Japan.

HEV in infected individuals is shed enterically. Before the onset of disease symptoms up to 10E8 HEV genome copies per milligram faeces can be excreted for several days [10]. In swine HEV RNA has also been detected in urine and it has been suggested that this may play a role in HEV transmission within this animal reservoir [11,12]. Aerosol transmission of Hepatitis E virus has not been reported. However, if infectious HEV is excreted via urine, this route of transmission might be possible too. The concentration of viable virus in an environmental or food matter inoculum may be an important factor in the outcome of clinical Hepatitis E infection [13].

For Hepatitis E virus acquired locally in developed countries, it is generally very difficult to definitely identify the source of infection. Because of the long incubation period of up to 60 days, potentially implicated foods or environmental samples for analyses often will not be available for analysis. Cell culture propagation of HEV has been difficult [14••]. Using PLC/PRF/5 cells, derived from human hepatocellular carcinoma, and A549 cells, derived from human lung cancer, as host cells, several mainly genotype 3 and 4 HEV field strains have been

cultured *in vitro* but few laboratories have reported to be successful in doing this [15,16].

Hepatitis E virus hosts and reservoirs

Hepatitis E viruses are identified in an increasing number of animal species. First detections in animals were reported in swine, chicken and deer in 1997, 2001 and 2003 respectively [17,18,19•]. More recently HEV sequences have also been detected in rats, wild boar, monkeys, mongoose, rabbits, ferrets, cutthroat trout and bats [20–28]. All of these animal species potentially are natural hosts of HEV but just a few of these species have been identified as true reservoirs of HEVs. Defining a true reservoir as a population in which the pathogen can be permanently maintained and from which it is shed to a defined target population [29], presumably only domestic swine, wild boar and deer should be regarded as true reservoirs of zoonotic HEVs. Only for domestic swine has it been proven in an experimental setting that HEV can persistently circulate within a closed animal population [30•]. HEV does not seem to cause clinical disease in animals other than primates but subclinical infection and mild hepatitis due to HEV genotype 3 and 4 have been reported in swine [31•,32]. Since HEV prevalences in pig production regions as well as within herds of domestic swine often are very high (>60%), domestic swine are almost certainly the main source of direct zoonotic transmission. HEV genotype 3 viruses are detected in domestic swine in nearly all developed countries where this has been looked for, and in a number of studies it has been shown that contact exposure to swine is a main risk factor for HEV infection. Seroprevalences in swine farmers, slaughterhouse workers and swine veterinarians are significantly higher compared to the general population [33,34].

On the basis of studies on HEV epidemiology in endemic regions there is no doubt that within the human reservoir genotype 1 and 2 viruses are predominantly transmitted via the faecal-oral route, usually through contamination of drinking water [35,36]. Regarding HEV human-to-human transmission there is an increasing number of reports of infection following blood transfusion, the use of blood products or solid organ transplantations [37]. In just a fraction of these reports HEV strains were characterized; all four major genotypes were detected but genotypes 3 and 4 seem to be involved more frequently. This is likely to be due to the fact that most of these reports are from higher income countries where HEV genotypes 1 and 2 are not endemic. Interfamilial spread of HEVs is not common but multiple cases in one family have been reported [36,38–41]. It is suggested that this is due to shared contaminated water rather than person-to-person transmission as the time interval between cases was too short.

Within the swine reservoir faecal-oral transmission has been demonstrated repeatedly [12,31•] and it is assumed that this is the major mode of transmission within this species. Since high concentrations of HEV genotype 3 RNA were detected in swine urine also [11,12], it is likely that HEVs in swine are transmitted via urine also. Solid data of HEV transmission routes within wildlife reservoirs are not available. An estimation of the likelihood of HEV transmission has only been made for the faecal-oral route in swine [42]. From that work it was concluded that the faecal oral route is likely to be a main route of transmission but may not be the only route of transmission for HEV genotype 3 viruses in swine.

It has been estimated that the infectivity titre of HEV for macaques is 10 000-fold higher when inoculated intravenously compared with when it is ingested [43]. Clinical signs of Hepatitis E are dose-dependent in these animal models and production of disease may require challenge doses 1000 times or more greater than that required for infection [2]. It has been postulated that blood borne HEVs may be more infectious than faecally excreted viruses because ORF3 proteins and cellular membranes are dissociated from the virion after shedding into the bile duct [16].

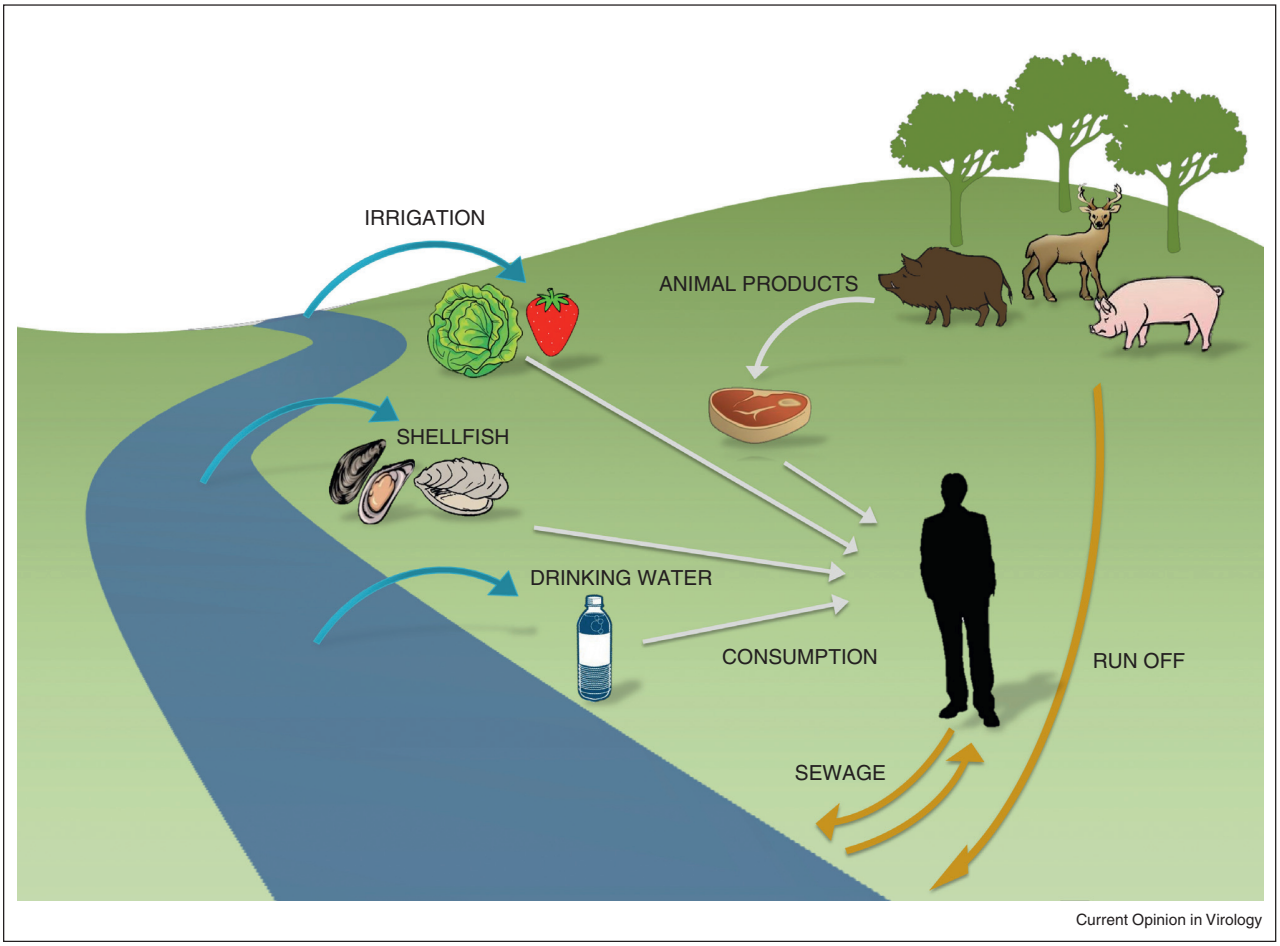
Hepatitis E virus transmission via foods

HEV sequences have been detected in various tissues and organs of swine [11] deer and wild boar [19,44], and also in bivalves such as mussels, cockles and oysters [45–47]. Commercial pig livers purchased as food from local grocery stores can be contaminated with HEVs [48–50] and some of such HEV-contaminated commercial pig livers may contain infectious virus [50].

Foodborne transmission of HEV was first demonstrated in clusters of Japanese patients after eating raw or undercooked meat from swine, wild boar or Sika deer [19,44]. The genomic sequences of HEVs identified from the infected patients were identical to those recovered from the frozen leftover meat [19,49].

Through either detection of HEV sequences or epidemiological study more and more Hepatitis E virus cases have been linked to the consumption of HEV contaminated food products. This includes infection via locally produced meat products [51] but also from game meat, processed pork [52] mussels, shellfish and other bivalves [44,46] (Figure 1). Eating raw or undercooked meat products has been identified as a higher risk factor [53]. Bivalves are known transmitters of enteric viruses and especially oysters are eaten worldwide as raw seafood. In a case–control study by Wichmann *et al.* [54•] in Germany, it was shown that consumption of raw or undercooked wild boar meat, and offal (liver, kidney, and intestine) was statistically significantly associated with autochthonous HEV infection. More recently HEV sequences have been detected on soft fruits and vegetables, with irrigation water as the suspected contamination origin [55,56] (Maunula L, Kaupke A, Vasickova P,

Figure 1



Potential routes for food and environmental transmission of hepatitis E viruses.

Söderberg K, Kozyra I, Lazic S, Van der Poel WH, Bouwknegt M, Rutjes S, Willems KA, *et al.*: **Tracing enteric viruses in the European berry fruit supply chain 2013**; submitted for publication).

Using a 3D cell culture system viable Hepatitis E virus has been demonstrated in a Figatelli sausage, a traditional food product from the south of France containing dried, cold-smoked raw pig liver [57]. This underlines that consumption of food products containing raw pig liver should be considered a high risk for foodborne transmission of HEV.

Environmental routes of HEV transmission

Shedding of enterically excreted HEVs into the environment plays a major role in HEV transmission. For human-to-human transmission of HEV genotype 1 and genotype 2 strains in regions without good drinking water sanitation this has been identified as the main mode of transmission [58]. In higher income countries the viability of HEVs in water and sewage will need to be studied to support the hypothesis that water-borne transmission can play a significant role. In sewage, surface water and waste water, HEV sequences can be detected which cluster with sequences found in indigenous cases and in swine and wildlife from the same geographical region. HEVs may contaminate surface waters and enter food production chains, in particular via shellfish culture areas and irrigation waters (Figure 1). HEV contamination of irrigation and drinking water via animal manure or sewage with concomitant contamination of vegetables, fruits, or shellfish which concentrate the virus by filter feeding, may implicate a food safety risk. In recent studies Hepatitis E virus sequences have been detected in water drained from fields where pig slurry was applied (Krog JS, Schultz AC, Larsen LE, Dalsgaard A, Kjaer J, Olsen P, Forslund A: **Leaching of viruses naturally occurring in pig slurry to field drains and their correlation with other microorganisms**; submitted for publication) and on vegetables and fruits from fields irrigated with surface water [55,56]

(Maunula L, Kaupke A, Vasickova P, Söderberg K, Kozyra I, Lazic S, Van der Poel WH, Bouwknegt M, Rutjes S, Willems KA, *et al.*: **Tracing enteric viruses in the European berry fruit supply chain** 2013; submitted for publication). Whether environmental contaminations result in HEV infections will depend on the level of contamination, HEV stability and the HEV infectious dose. This may be different for the different HEV genotypes.

Conclusion

In general, both foodborne and environmental transmission of HEV play a major role. However, the actual contribution of either or both of these routes to HEV spread during outbreaks will be different depending on the virus genotype and strain, the level of sanitation and the environmental conditions. Sewage water treatment and drinking water sanitation is very important to prevent HEV (genotype 1 and 2) transmission in developing countries. In developed countries HEV genotype 1 (and presumably also genotype 2) infections are almost exclusively related to travel into hyperendemic regions. This difference between genotypes can be explained by the fact that much higher levels of contamination are reached in drinking water in developing countries. HEV genotype 3 and 4 infections occur as sporadic infections after foodborne zoonotic transmission, animal contact and/or environmental transmission. Viable HEV genotype 3 viruses have been detected in food products implicated in infections but the level of involvement of environmental transmission in HEV genotype 3 infections is uncertain because the viability and the stability of HEVs in water, sewage and soil are unknown.

For Hepatitis E risk assessment it is important to determine at what points in the potential transmission routes HEV reaches sufficiently high doses to cause infection and clinical disease. This means that it is important to test for virus in samples all along food production chains and also in environmental samples and do quantitative analyses as much as possible. In such surveillance studies HEV genotyping is also very important because of the differences in transmission routes for the different genotypes.

To study HEV transmission routes an efficient cell culture system is definitely needed. Such a system would be a major tool for virus viability and stability studies. Moreover such a system can be helpful to elucidate the mechanisms by which HEV enters the cells and is released from the cells. Information on HEV stability and virus-cell interaction will also shed more light on the likelihood of different HEV transmission routes in contributing to HEV spread in susceptible populations.

Further study of food and environmental routes of Hepatitis E virus transmission is of major importance for the development of accurate prevention and control strategies.

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