Received: 23 June 2019 | Revised: 19 July 2019 | Accepted: 22 July 2019

DOI: 10.1002/rmv.2078

REVIEW

WILEY

Emerging hepatitis E virus compared with hepatitis A virus: A new sanitary challenge

Cédric Hartard[1,2,3,4] | Christophe Gantzer[2,3,4] | Jean-Pierre Bronowicki[5] | Evelyne Schvoerer[1,2,3,4]

[1]Laboratoire de Virologie, CHRU de Nancy Brabois, Vandœuvre-lès-Nancy, France

[2]Laboratoire de Chimie Physique et Microbiologie pour les Matériaux et l'Environnement (LCPME), UMR 7564, Vandoeuvre-lès-Nancy, France

[3]CNRS, LCPME UMR 7564, Nancy, France

[4]Faculté des Sciences et Technologies, Institut Jean Barriol, Université de Lorraine, Vandœuvre-lès-Nancy, France

[5]Service d'hépato-gastro-entérologie, CHRU de Nancy Brabois, Vandœuvre-lès-Nancy, France

Correspondence
Evelyne Schvoerer, Laboratoire de Virologie, CHRU de Nancy Brabois, Vandœuvre-lès-Nancy, France.
Email: evelyne.schvoerer@univ-lorraine.fr

Summary

Hepatitis A (HAV) and E (HEV) viruses are able to cause liver disease in humans. Among the five classical hepatotropic viruses, they are mainly transmitted via the fecal-oral route. Historically, many similarities have thus been described between them according to their incidence and their pathogenicity, especially in countries with poor sanitary conditions. However, recent advances have provided new insights, and the gap is widening between them. Indeed, while HAV infection incidence tends to decrease in developed countries along with public health improvement, HEV is currently considered as an underdiagnosed emerging pathogen. HEV autochthonous infections are increasingly observed and are mainly associated with zoonotic transmissions. Extra hepatic signs resulting in neurological or renal impairments have also been reported for HEV, as well as a chronic carrier state in immunocompromised patients, arguing in favor of differential pathogenesis between those two viruses. Recent molecular tools have allowed studies of viral genome variability and investigation of links between viral plasticity and clinical evolution. The identification of key functional mutations in viral genomes may improve the knowledge of their clinical impact and is analyzed in depth in the present review.

KEYWORDS

clinical relevance, genetic variability, hepatitis A virus, hepatitis E virus

1 | INTRODUCTION

Hepatitis A (HAV) and E (HEV) viruses are major causes of acute viral hepatitis worldwide with an estimate of 30 million associated infections every year.[1-3] They have variable incidence worldwide depending on the level of sanitation of countries. Their epidemiology varies also by genotype, some of which may be considered as emerging especially in industrialized countries. For example, HEV was initially described in developing countries, but today, autochthonous infection rates increase in industrialized countries and are probably underreported.

There are a number of similarities between HAV and HEV, beginning with their discovery. Initially, both were identified and subsequently described based on their common fecal-oral route of transmission during massive outbreaks.[4] As with other enteric viruses, they are nonenveloped, resulting in highly persistent infective particles conveyed by fecally polluted drinking water or food.

Despite their similarities, differences have to be underlined. One of the most notable is the ability of HEV to cross the species barrier, which is not observed for HAV. The zoonotic nature of HEV was established in the early 1990s with the discovery of a strain in pigs[5] and transmission to humans.[6] Elsewhere, atypical clinical

Abbreviations: ESCRT, endosomal sorting complexes required for transport; gt, genotype; Hel, helicase; HVR, hypervariable region; IRES, internal ribosome entry site; Met, methyltransferase; PCP, papain-like cysteine protease; PPR, polyproline region; RdRp, RNA-dependent RNA polymerase; UTR, untranslated region.

manifestations are associated with HEV infection, such as the well-known severe issue in pregnant women,[4,7] but also chronic liver infections[8] and extra hepatic manifestations.[9] Atypical signs are increasingly recognized and are now included in guidelines concerning the diagnosis and the management of HEV infections.[10] Nevertheless, their pathophysiology remains unclear.

A lot has been learned about both viruses over the past two decades, and current data seem to suggest a link between clinical features and viral genome variability.[11-14] Genomic mutations can influence virulence and lead to the distribution of both viruses within quasispecies. Detailed study of their genetic diversity is needed to increase knowledge about these viruses and to improve patient care. In this review, a comparative analysis between HAV and HEV is undertaken covering structure, epidemiology, modes of transmission, as well as genetic diversity and possible clinical consequences.

2 | VIRAL STRUCTURE

Hepatitis A and E virions both have an icosahedral capsid of 27 to 34-nm diameter containing a single-stranded RNA genome. They have a common ancestral root[15,16] but distinct evolutionary pathways. They are, respectively, the only member of the *Hepatovirus* (*Picornaviridae* family) and *Orthohepevirus* genera (*Hepeviridae* family), with the latter being divided in four species (*Orthohepevirus A* to *D*).

HAV contains a 7.5-kb RNA genome (Figure 1) including a single open reading frame (ORF) translated under the control of a type III internal ribosome entry site (IRES) within the 5′ untranslated region (UTR).[17] It may be subdivided into three major regions. The 5′ proximal region P1 encodes four proteins among which VP1, VP2, and VP3 form the external face of the capsid and VP4 is internal. The middle region P2 contains the 2A, 2B, and 2C genes encoding nonstructural proteins whose functions are not entirely defined.[18] The 2C protein may have a helicase function[18] while the 2A protein is involved in particle formation.[19] The 3′ proximal region P3 encodes four proteins including the 3C proteinase and the 3D RNA-dependent RNA polymerase (RdRp).[18] Only one serotype is defined, but based on VP1, six genotypes (gt) are described. Among them, gt1 to gt3 infect humans while others have a simian tropism.[20]

HEV has a 7.2-kb RNA genome including three overlapping ORFs[21] (Figure 1). ORF1 encodes a nonstructural polyprotein subdivided into 7/8 domains including methyltransferase, Y domain, papain-like cysteine protease, hypervariable region containing a polyproline region (HVR/PPR), X domain, helicase, and RdRp.[22-24] The functions of the Y and X domains remain unknown. The proteins encoded by ORF2 and ORF3 are produced from a bicistronic subgenomic RNA,[23] producing the viral capsid protein and a small phosphoprotein. The latter is essential for virus egress from cells.[25] HEV (meaning *Orthohepevirus A* species) are divided within eight genotypes based on the whole genome.

HAV and HEV are shed in feces as naked particles but circulate in the blood as quasienveloped virions,[26] the removal of the envelope being carried out by the detergent action of bile before the release of virions in the gut.[27] The absence of peplomers on their surface distinguishes them from conventional enveloped viruses. Quasienveloped viruses are infectious and completely resistant to neutralizing antibodies raising the question of how cell entry occurs in the absence of receptor-binding and membrane fusion activities.[26] Infection resulting from blood transmission is however much less common than that of fecal-oral transmission as quasienveloped virus attachment to the host cells is less efficient.[28-30]

The biogenesis of quasienveloped viruses is similar to that of exomes involved in mediating cellular communication. Their egress is associated with the endosomal sorting complexes required for transport (ESCRT) which is common in the release of classic enveloped

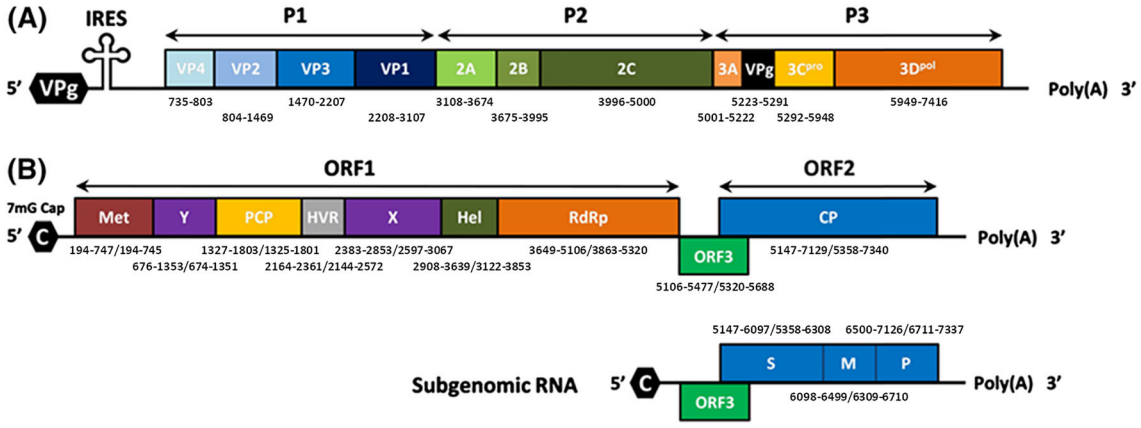

FIGURE 1 Schematic description of the HAV and HEV genomes. A, HAV HM-175 strain, gt1B (Acc. No. M14707). A single ORF under the control of an internal ribosome entry site (IRES) with three regions. P1 encodes VP1 to VP4 capsid proteins. P2 encodes nonstructural 2A to the 2C protein. P3 encodes the 3A protein, VPg (3B), protease (3C), and RNA-dependent RNA polymerase (3D). B, HEV Burmese strain, gt1 (Acc. No. M73218)/HEV 47832 strain, gt3 (Acc. No. KC618402). ORF1 encodes the nonstructural polyprotein, including methyltransferase (Met), Y-domain (Y), papain-like cysteine protease (PCP), hypervariable region (HVR), X-domain (X), helicase (Hel), and RNA-dependent RNA polymerase (RdRp). ORF2 encodes the capsid protein (CP), containing the S domain (S), M domain (M), and P domain (P). ORF3 encodes for a small multifunctional protein. ORF2 is translated from the bicistronic 2-kb subgenomic RNA

viruses. It results in the formation of structures from 50 to 110 nm in diameter containing one to four HAV particles,[28] while smaller 40-nm particles containing only one virion are described in the case of HEV.[30]

Entry of virions is dependent on clathrin-mediated endocytosis, and, unlike naked particles, quasienveloped HEV requires also Rab5 and Rab7 GTPases involved in endosomal trafficking.[29] Endosomal acidification is then responsible for lysis of the envelope, resulting in the interaction of viral capsids and their receptor on the inner leaflet of the endosome.[26] At this point, naked particles may be exposed to neutralizing antibodies if present within the same compartment.

Ultimately, HAV and HEV can take advantage of the characteristics of both forms: An envelope cloaking the viral capsid constitutes a strategy for evading the host immune system and, since nonessential for infection, does not limit viral persistence under environmental conditions. Naked particles are indeed particularly stable which promotes their transmission outside the host.

3 | EPIDEMIOLOGY AND DISTRIBUTION OF GENOTYPES

According to public health improvement, the incidence of HAV infections has been steadily decreasing as observed in China (30-fold decline of infections between 1990 and 2014).[31] The same has been earlier observed in France with a decrease of seroprevalence from 50% to 10% in 20-year-old adults between 1978 and 2000.[32] Currently, HAV endemic areas are associated with an anti-HAV IgG prevalence above 50% which corresponds to a large part of Africa as well as some areas of Asia and South America[33] (Figure 2). Vaccination is strongly recommended,[34] but nevertheless, HAV is still responsible for 10 million infections worldwide annually among which 1.5 million are symptomatic and 11 000 deaths are reported.[35,36] Regarding their distribution, HAV gt1 is the most prevalent, and subtype A is more common than B (Figure 3). HAV gt2 was initially identified in France and Sierra Leone, but its detection is currently rarely reported.[37] Finally, HAV gt3 has also a global distribution with subtype A being predominant.[1,20,37]

Making a comparison with HEV is highly informative. As for HAV, HEV genotypes are irregularly distributed worldwide (Figure 3), but their distribution is directly linked to the mode of transmission. HEV gt1 to gt4 are the major genotypes involved in human pathogenesis. We distinguish countries in which HEV gt1 (Asia and Africa) and gt2 (Mexico and some regions of Africa) are present, specific to humans, and transmitted by water. Sanitation conditions are usually poor, and HAV is highly endemic too. In contrast, when sanitation increases, circulation of human-specific HEV gt1 and gt2 tends to decrease. During Chinese public health improvements characterized by HAV decrease, HEV infections have increased 8-fold and the mortality rates have surpassed those of HAV between 2004 and 2014.[31] We can assume here that water-transmitted HEV gt1 and gt2 have progressively been replaced by other genotypes more specific to industrialized countries and associated with zoonotic transmission. Among them, HEV gt3 circulates among humans and many animals (eg, swine, rabbit, and deer) and has a worldwide distribution while gt4 circulating between humans and swine is mostly present in Southeastern Asia.[38] For the remainder, HEV gt5 and gt6 are specific to animals,[39,40] and HEV gt7 and gt8 have been identified in camels. Recently, a case of HEV gt7 transmission to a liver transplant recipient has been reported.[41,42]

Considering all of that, HEV is currently the main cause of acute hepatitis worldwide with 20 million people infected annually.[43] Among them, three million cases are symptomatic, and 50 000 to 70 000 deaths are reported.[44] Unlike HAV, HEV antibody prevalence is more challenging to evaluate due to the absence of a gold

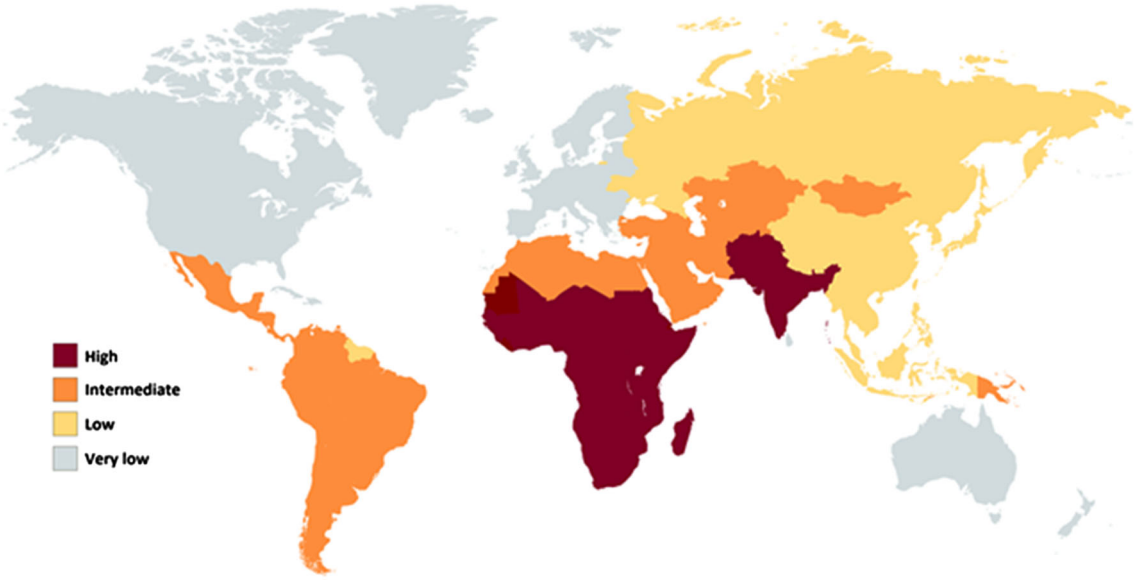

FIGURE 2 Current worldwide prevalence of HAV

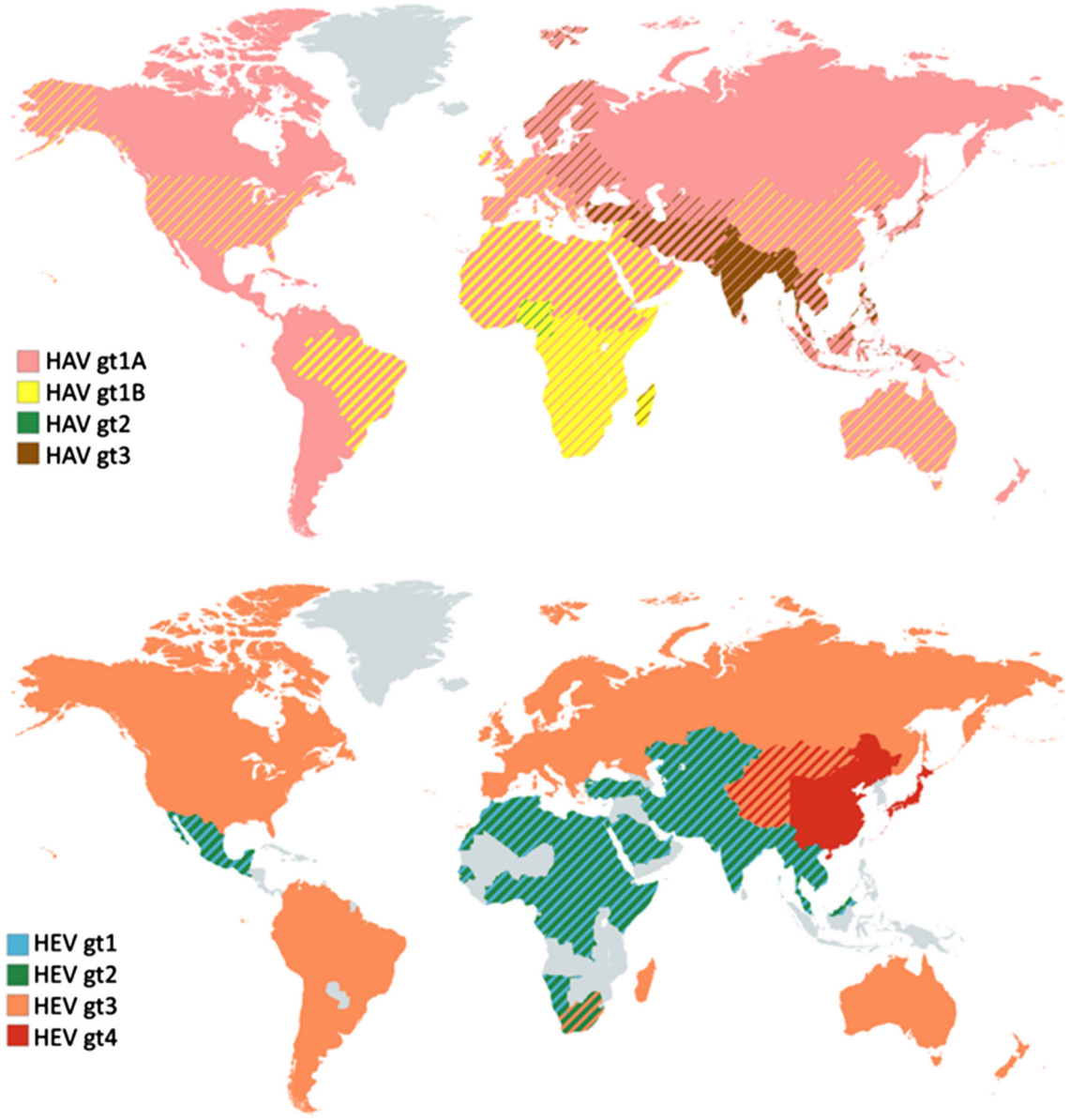

FIGURE 3 Current worldwide distribution of the major HAV and HEV genotypes

standard,[45-48] and currently, HEV RNA detection is recommended to complete the diagnosis.[10,47] In spite of this, HEV seroprevalence is constantly rising, linked at least partially to more frequent research. The number of HEV cases has increased 10-fold in Europe during the last decade,[49] and seroprevalence is close to 20% to 30% worldwide, including industrialized countries such as France, Germany, and the Netherlands.[50-52] Hyperendemic areas are recognized, as observed in Southeastern France with seroprevalence and incidence reaching occasionally 80% and 4.6%, respectively.[40,50] Insufficient data are available about these hot spots, leaving open the question of HEV transmission and circulation in developed countries. Considering their zoonotic potential, eating pattern is mainly involved, with the example of figatelli highly associated to HEV transmission.[53] Finally, we note that vaccination options against HEV are rather limited with only one licensed vaccine being available in China.[54]

4 | TRANSMISSION

HAV and HEV are spread by the fecal-oral route, and their transmission is promoted by the lasting shedding of viruses before, during, and after the symptoms. In developing countries, this transmission is primarily linked to contamination of water due to the difficulty in achieving adequate sanitation. In such countries, young infected children usually present subclinically or asymptomatically (especially described for HAV) and may thus act as viral reservoirs.[55]

In developed countries, different modes of transmission have been identified. Sporadic outbreaks may be observed in the adult population, and environmental or alimentary sources are well described. It is worth noting that viruses are the leading cause of foodborne diseases in industrialized countries,[56] and the role of fecal-borne hepatitis viruses remains of major concern in the light of the severity of the

diseases. HAV and HEV that are excreted by infected people are found in wastewater and surface waters (including irrigation water) and may contaminate food in contact with this water. Both may be detected on the surface of fresh products like leafy greens, soft red fruits, or semidried tomatoes.[57] They are characterized by a high stability not only towards factors responsible of the inactivation of viruses in the environment (eg, temperature, pH, drying, detergent) but also towards many common food-processing interventions (eg, washing, chlorination, freezing, heat, UV treatment).[58,59] Their persistence remains however difficult to estimate because of a lack of efficient cell culture systems. They are usually investigated by genome detection which leads to difficulty in interpretation considering the difference of stability in favor of genome compared with viral infectivity.[60] This phenomenon is recurring in environmental virology,[61] and new approaches (eg, the use capsid integrity markers like propidium monoazide) are proposed to improve the interpretation in the case of genome detection in foodstuff.[62] In addition to plant-based produce, shellfish are also classically involved in enteric virus transmission to humans, and HAV or HEV are regularly detected.[57,63] Finally, as already mentioned, transmission in developing countries may arise directly from contaminated waters, either through drinking or during recreational activities.[40,64]

In contrast to HAV, HEV by crossing species barrier leads to a more complex epidemiology and very specific modes of transmission. Zoonotic transmission represents the major cause of infection in developed countries and is mainly associated with gt3 and gt4, while human-specific gt1 and gt2 are responsible for the infections linked to water observed in developing countries.[8] Water may however also be contaminated with animal-infected feces and be responsible for the transmission of HEV in developed countries.[65,66] Primary animal reservoirs usually described are pigs, wild boar, and deer.[67] The consumption of their meat or derived products when raw or not sufficiently cooked to inactivate the virus represents a true health concern.[50] HEV is globally widespread among pigs with seroprevalence reaching 90% in farms, and prevalence in some pig products may reach 58%.[53,68,69] Between 0.5% and 3.8% of pigs are viremic during slaughtering explaining how HEV may enter the food chain.[67] Other animals like rabbits are also susceptible to transmitting gt3 to humans.[70] Transmission of HEV gt7 linked to consumption of camel meat and milk has been reported too.[41] An immunocompromised state of the host can undoubtedly contribute to transmission. Zoonotic transmission may also occur from contact with infected animals as observed in swine workers (ie, farmers, butchers, meat processors, retailers, and veterinarians).[71] HEV has also been detected in domestic animals,[67] and recently, the transmission of a strain belonging to *Ortohepevirus C* was documented from rat to human.[72]

Other minor modes of transmission are also described for both viruses such as HAV outbreaks reported among men who have sex with men.[73,74] It is difficult to draw conclusions relating to HEV on this topic as discordant epidemiologic outcomes exist in this population including the risk factor of HIV coinfection.[75,76] Intravenous drug users are also a population exposed to HAV infection, rather associated with poor sanitary conditions and lifestyle since HAV is not considered as a predominately blood-borne pathogen.[77]. The viremia observed during infection cannot however exclude this mode of transmission,[37] and the same can be said for HEV. Transmission by blood transfusion as well as organ donation has been reported for HEV, possibly resulting from an extended asymptomatic viremic period.[50,78] This highlights the major risk of infection facing immunocompromised patients who represent a large proportion of recipients.

HEV transmission during pregnancy is well described considering the high risk of mortality for both the fetus and mothers.[79] It is mainly associated with gt1 and gt2 and thus rarely reported in developed countries. However, it remains unclear whether this phenomenon results from the specificity of gt1 and gt2 strains or from an observation bias linked to their geographical distribution, host genetic susceptibly possibly being a contributing factor.[80,81] Concerning HAV, vertical transmission has also been reported before or at delivery.[82,83] The risk of perinatal transmission is however low given the widespread global distribution of HAV and the limited severe outcome observed.

5 | CLINICAL EXPRESSION

HEV differs from HAV by the fact that its infectivity is lower. In most cases, both viruses cause asymptomatic infections after an incubation time ranging between 14 and 28 days and 21 and 56 days for HAV and HEV, respectively. If symptomatic, acute hepatitis begins with a short prodromal phase followed by liver-specific signs including jaundice, pruritus, clay-colored stools, and darkened urine. Serum transaminase levels peak approximately 6 weeks after exposure.

Hepatocyte cytolysis is immune-mediated and involves innate and adaptive responses against viruses. It should be noted that during replication, both viruses inhibit interferon expression, through the putative domain X and PCP antagonist activity for HEV and through the 3CD protease-polymerase precursor for HAV.[84,85] Infections are usually self-limiting, but complications may occur such as fulminant hepatitis. Their incidence is associated with patient age and preexisting liver diseases or comorbidities such as chronic alcoholism, cirrhosis, or immunosuppression. HEV case fatality rate is under 0.1% in healthy patients, but may increase to 25% in pregnant women, especially in the case of infection during the third trimester.[79] The reason for this is not well established, but the high estrogen level during pregnancy is suspected.[86] The excess of mortality is mainly reported for HEV gt1 and gt2,[78] even though there have been a few cases involving gt3 and gt4.[79] More efficient replication of gt1 in placenta has been observed and is associated with a decrease of interferon production and higher tissue damage.[87]

In addition, HEV differs from HAV in the way that a chronic carrier state can develop, for now exclusively observed in immunocompromised patients. Chronic HEV infections were first observed in 2008 among kidney and liver transplant recipients[88] and are defined by the presence of RNA in the blood or feces for more than 3 months. Persistent infections have since been described in other solid organ transplant recipients,[89,90] in patients undergoing chemotherapy[91] or in

HIV-infected patients.[92] Usually, ribavirin therapy is the treatment of choice.[88,90] Infection of transplant recipients results in chronic infection for two-thirds out of them, of which one-third are symptomatic and 5% to 10% will progress to cirrhosis.[88,90] The progression is rapid when compared with typical viruses involved in chronic hepatitis, with cirrhosis occurring as early as 2 years postinfection.[8] Almost all cases of chronic infection were observed with HEV gt3, but rare persistent gt4 infections have been reported.[93] Recently, a case of chronic HEV gt7 infection has been mentioned,[41] as well as a chronic infection linked to a strain originating from rats (*Orthohepevirus C*).[72] Such persistent infections have yet to be recognized with HAV, even in immunocompromised individuals coinfected with HIV. Although relapse may be observed in 5% to 10% of acute HAV cases, ultimately, the virus is always cleared.[94,95]

Finally, HEV is associated with extra hepatic manifestations occurring both in the case of acute or chronic infection. Neurological disorders are the most common (eg, meningoencephalitis, neuromyopathy, Guillain-Barré, and Parsonage-Turner syndromes), occurring in 6% to 8% of infections and related to HEV presence in cerebrospinal fluid.[9,96-98] Impaired kidney function has been also reported (eg, membranoproliferative or membranous glomerulonephritis, IgA nephropathy), and the evidence for an association with HEV is supported by the detection of HEV in urine and kidney.[9,99] The link between HEV and hematologic disorders, myosititis, arthritis, thyroiditis, myocarditis, or pancreatitis is also suggested, even though a direct link has not been demonstrated.[9,100-103]

Kidney injuries have also been described during acute HAV infections.[95,104,105] However, no evidence of the presence of virus has been demonstrated in kidneys, and damage would likely result as an indirect complication.[104] On the other hand, the detection of HAV in organs other than the liver has been occasionally reported, but no inflammation has been observed.[106]

6 | HEV AND HAV GENETIC VARIABILITY AND BIOCLINICAL CONSEQUENCES

Genetic variability has important influence regarding the expression of infections. Variability is observed on a large scale among genotypes but may also involve specific positions within a genome. Diversity observed in the *Hepevirus* family exceeds that of the *Hepatovirus* family.[36] Moreover, human HEV gt1 and gt2 are relatively conserved compared with gt3 and gt4 which present a broader spectrum of hosts.[107]

Concerning HEV variability among genotypes, only gt1 and gt2 are associated with epidemic spread in developing countries despite the presence of the others.[108] The reason has yet to be established, as with the association between gt3 and chronic infections or extra hepatic manifestations. Infections resulting from gt3 and gt4 are generally less severe than those resulting from gt1 and gt2 which are furthermore associated with severity in pregnancy. Among gt3 (the most prevalent within developed countries), infections with subtypes 3e, 3f, and 3g are associated with a higher virus load and more severe clinical presentations.[108]

In relation to HAV, the clinical differences between subgroups are poorly described. Gt3A strains seem to cause higher liver damage compared with gt1A[109] although an association between a fulminant hepatitis and a HAV gt1A strain was observed in Japan.[110] This last case may however result from the patient's age (42 years old), which may constitute a risk factor.

6.1 | HAV genome variability

A mutation rate close to 10^{-4} substitutions/site/year is estimated for the HAV genome.[108] The 5′ UTR region containing the IRES is the most impacted by nucleic acid substitutions which subsequent modulate pathogenicity. Fujiwara et al reported substitutions associated with severe or fulminant hepatitis (especially between nucleotides 200 and 500).[111] G324A and C372G/T have been associated with mild hepatitis while U164C, U255C, and U278C can lead to an increased stability of the IRES and a concomitant reduction of its activity in benign cases.[11,12] However, Ajmera et al allude to the absence of specific substitutions associated with acute liver failure among gt1A.[112] Concerning gt1B, a greater number of nucleic acid substitutions have been noted among variants involved in acute failure. Other mutations associated with cell culture adaptation or attenuation have also been identified (A161G, U214del, A598G, G653A, C676U, and A694G), but their clinical significance remains unknown.[12,113]

Regarding the coding region, no significant variation in disease severity was observed in the VP1-P2B region.[112] In HAV gt1A, Fujiwara et al suggest that numerous mutations in the central part of 2B could be associated with fulminant hepatitis.[13] Substitutions in the VP1 (K/R29E, R91S), VP3 (Y50H), and VP4 (K/R5S) have been related to a long-lasting viremia and the occurrence of neurological complications (Guillain-Barré syndrome) in a patient infected with HAV gt3A.[114]

Finally and despite the high degree of conservation of the capsid, mutations leading to the emergence of variants resistant to the protective effect of vaccines have been suggested (V1166G, W1170C, V1171A, Y1181S, A1187P, R1189T, A1280V).[115] Considering their low fitness, they would rapidly be outcompeted by wild type, and their selection would only result in a considerable amount of viruses ingested by patients showing low IgG levels.

6.2 | HEV genome variability

A mutation rate close to 10^{-3} substitutions/site/year is estimated for HEV genome,[116] and more data are available regarding mutations and their clinical relevance compared with HAV[14,117] (Table 1).

Among ORF1, F179S, A317T, T735I, L1110F, and V1120I have been associated with fulminant hepatic failure,[119] V27A and D29N have been identified in patients with acute liver failure and higher viremia while H105R was related to a low viral loads.[118] Elsewhere, some silent mutations were also associated with fulminant hepatitis

TABLE 1 HEV mutations associated with human clinical relevance (adapted from van Tong et al[14] and Ikram et al[117])

Nucleotide Substitution	Amino Acid Change	Genotype	Domain/Region	Clinical Manifestation	Reference
T107C	V27A	HEV gt1	Met/ORF1	ALF	Borkakoti et al[118]
G115A	D29N	HEV gt1	Met/ORF1	ALF	Borkakoti et al[118]
A341G	H105R	HEV gt1	Met/ORF1	ALF	Borkakoti et al[118]
T563C	F179S	HEV gt1	Met/ORF1	FH	Mishra et al[119]
G977A	A317T	HEV gt1	Y/ORF1	FH	Mishra et al[119]
C2232T	T735I	HEV gt1	HVR/ORF1	FH	Mishra et al[119]
75 to 186 nt insertion	No	HEV gt3	HVR/ORF1	CH	Lhomme et a[120]
186 nt insertion	No	HEV gt3	HVR/ORF1	CH	Johne et al[121]
171 nt insertion	No	HEV gt3	HVR/ORF1	CH	Shukla et al[122]
174 nt insertion	No	HEV gt3	HVR/ORF1	CH	Shukla et al[122]
117 nt insertion	No	HEV gt3	HVR/ORF1	CH	Nguyen et al[123]
282 nt insertion	No	HEV gt3	HVR/ORF1	RTF	Debing et al[124]
V1213A	V239A	HEV gt3	Hel/ORF1	ALF	Takahashi et al[125]
C1816	No	HEV gt4	?/ORF1	FH	Inoue et al[126]
U3148	No	HEV gt4	Hel/ORF1	FH	Inoue et al[126,127]
T3355C	L1110F	HEV gt1	Hel/ORF1	FH	Mishra et al[119]
G3386A	V1120I	HEV gt1	Hel/ORF1	FH	Mishra et al[119]
n/a	Y1320H	HEV gt3	RdRp/ORF1	RTF	Debing et al[124]
n/a	K1383N	HEV gt3	RdRp/ORF1	RTF	Debing et al[124]
n/a	D1384G	HEV gt3	RdRp/ORF1	RTF	Todt et al[128]
n/a	K1398R	HEV gt3	RdRp/ORF1	RTF	Todt et al[128]
T4344A	F1439Y	HEV gt1	RdRp/ORF1	FH	Mishra et al[119]
n/a	V1479I	HEV gt3	RdRp/ORF1	RTF	Todt et al[128]
C4476G	C1483W	HEV gt1	RdRp/ORF1	FH	Borkakoti et al[129]
A4616C	N1530T	HEV gt1	RdRp/ORF1	FH	Borkakoti[129]
n/a	Y1587F	HEV gt3	RdRp/ORF1	RTF	Todt et al[128]
n/a	G1634R/K	HEV gt3	RdRp/ORF1	RTF	Debing et al,[124,130]
C5907	No	HEV gt4	ORF2	FH	Inoue et al[127]
C5927T	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
C5933T	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
T6014C	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
C6032T	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
G6098A	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
C6104T	n/a	HEV gt1	ORF2	FH	Borkakoti et al[131]
n/a	P259S	HEV gt1	ORF2	FH	Borkakoti et al[131]

Abbreviations: n/a, not available; ALF, acute liver failure; CH, chronic hepatitis; FH, fulminant hepatitis; RTF, ribavirin treatment failure.

(eg, U3148 for which an additive effect was observed with the silent mutation C5907 located in ORF2).[126,127] The authors concluded that the genome secondary structure may be favorable for translation of the viral proteins. Such observations have allowed definition of a hot spot region in ORF1 meaning HVR/PPR, Hel, and RdRp domains[14,23]: Insertions within the HVR/PPR domain may result in chronic hepatitis or ribavirin treatment failure. One of the most relevant insertions was observed in the HEV strain Kernow-C1 isolated from a HIV coinfected patient suffering from a chronic HEV-related infection. This strain contains an insertion of a 174-nt gene fragment of human ribosomal protein S17 allowing it to replicate 500 times more effectively in cell culture systems.[132] In the same way, a 282-nt insertion (ie, duplicated fragment from the HVR and a RdRp-derived fragment) coupled to A173V has been associated with increased viral replication and ribavirin treatment failure.[124] Concerning the Hel domain, V239A has been associated with increased virulence[125] while L1110F and V1120I were linked to fulminant hepatic failure.[133] Concerning the RdRp domain, F1439Y, C1483W, and N1530T have been associated with

acute liver failure and high mortality[119,129] while Y1320H, K1383N, D1384G, K1398R, V1479I, Y1587F, and G1634R/K have been linked to ribavirin treatment failure.[124,128,130] In contrast, K1383N is associated with an in vitro decreased replication rate and an increase in ribavirin sensitivity, suggesting a multifactorial mechanism.[124]

Mutations in the ORF2 region, encoding the capsid protein, may have important consequences on virus stability, immune escape, clinical evolution, and also on vaccine efficiency. Synonymous C5927T, C5933T, T6014C, C6032T, G6098A, and C6104T and nonsynonymous P259S have been associated with fulminant liver failure.[131]

Mutations in ORF3 may also have significant impact because of its interaction with the "membrane" of quasienveloped virions. No clinical mutation has yet been reported while modifications in the two conserved PSAP motifs were associated with a decrease in virus egress.[134] It should be noted that the ORF2/ORF3 overlapping is of special interest because mutations may influence the two functions of both proteins. It is thus also considered as a hot spot,[14,128] quite conserved in some portions allowing the design of universal primers[135] but which might rapidly evolve in other segments.

Finally, it is important to note that little is known concerning the impact of mutation on the efficiency of HEV vaccine. Current research is focused on their efficiency against all genotypes since the commercial Chinese vaccine was derived from a gt1 recombinant protein and has only been tested in a very low endemic area mainly affected by gt4.[54] The use of envelope-associated ORF3 protein as a target for viral neutralization has also been investigated.[136]

6.3 | Viral quasispecies

Quasispecies relate to intrahost viral genetic heterogeneity linked to a lack of RdRp proofreading activity and the absence of postreplication nucleic acid repair mechanisms.[137] This means that HAV and HEV both circulate in infected individuals as a pool of genetically distinct but closely related variants.[8,138] Thus, amino acid modifications discussed above may affect only a minority, and a positive selection pressure may be involved. If little is known concerning HAV, important facts have been described regarding HEV quasispecies. For example, a correlation between HEV quasispecies heterogeneity (ie, M and P domains of ORF2 as well as HVR/PPR domain) and the development of chronic HEV infection in solid organ transplant recipients was reported.[139,140]

Moreover, the emergence of variants associated with resistance (eg, G1634R/K, Y1320H, K1383N) has been observed in the case of ribavirin administration, leading to a nonsustained virological response.[128,130,141] Interestingly, a second long-term treatment usually appears effective despite resistant viral variants.

Finally, quasispecies heterogeneity may also impact viral tropism. Kamar et al described a genetically distinct HEV variant in cerebrospinal fluid from a patient with neurological symptoms suggesting a functional compartmentalization.[98]

7 | CONCLUSIONS

Historically, epidemic jaundice has often been attributed to HAV, masking the true incidence of HEV. However, recent data suggest a strong involvement of both viruses. The scientific community has often grouped HAV and HEV together; however, recent advances have provided new insights, and the gap is widening. HEV is currently considered as an emerging pathogen, associated with common autochthonous infections in developed countries, and serious cases are reported while HAV incidence is usually low. The virological diagnosis of both viruses is essential, and after the serological window period, the detection of antibodies is an efficient method to identify HAV infection.[142] HEV diagnosis is more troublesome, and genome detection is widely recommended to confirm HEV-related infections.[10]

If host genetic polymorphism can impact the clinical outcome,[80,81] viral genetic diversity may also interfere. The occurrence of viral mutations has two important consequences: (1) the selection of viral variants during evolution leading to the diversification of geographical genotypes and (2) the distribution within a quasispecies for a single infected individual.[143] This should be considered when discussing vaccine development or diagnostic tools, especially in the case of severe or atypical symptoms linked to HEV infections. It is also important to keep in mind when tracking viruses in the environment since a possible association between molecular signatures and transmission route has been suggested.[66,125,144] These recent discoveries have changed our mind and lead to new questions. Despite the existence of a quasispecies in infected individuals and the potential contribution of HEV variants to pathogenesis, the use of next-generation sequencing is currently poorly investigated and has yet to be developed to investigate minor variants.[124,128,141] The HEV genome hot spot regions are identified (ie, Hel, HVR, and RdRp of ORF1, M, and P domain of ORF2 and ORF2/ORF3 overlap), and next-generation sequencing should be considered for possible biological markers helping to predict progression of disease.

ACKNOWLEDGEMENT

We are indebted to Carina O'Brien for her rereading of the manuscript.

CONFLICT OF INTEREST

The authors have no competing interest.

ORCID

Cédric Hartard https://orcid.org/0000-0001-5942-2345

REFERENCES

1. Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis A virus infection: a molecular approach. *Clin Microbiol Rev*. 2006;19(1):63-79. https://doi.org/10.1128/CMR.19.1.63-79.2006

2. Wasley A, Fiore A, Bell BP. Hepatitis A in the era of vaccination. *Epidemiol Rev*. 2006;28(1):101-111. https://doi.org/10.1093/epirev/mxj012
3. Abravanel F, Lhomme S, Dubois M, et al. Hepatitis E virus. *Med Mal Infect*. 2013;43(7):263-270. https://doi.org/10.1016/j.medmal.2013.03.005
4. Lemon SM, Walker CM. Enterically transmitted non-A, non-B hepatitis and the discovery of hepatitis E virus. *Cold Spring Harb Perspect Med*. 2018;9:a033449. https://doi.org/10.1101/cshperspect.a033449
5. Meng X-J, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human. *Proc Natl Acad Sci U S A*. 1997;94:9860-9865.
6. Kow PY, Schlauder GG, Carpenter HA, et al. Acute hepatitis E by a new isolate acquired in the United States. *Mayo Clin Proc*. 1997;72(12):1133-1136. https://doi.org/10.4065/72.12.1133
7. Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *Am J Med*. 1981;70(February):252-255.
8. Kamar N, Izopet J, Pavio N, et al. Hepatitis E virus infection. *Nat Rev Dis Primers*. 2017;3(1):17086. https://doi.org/10.1038/nrdp.2017.86
9. Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus: infection beyond the liver? *J Hepatol*. 2017;66(5):1082-1095. https://doi.org/10.1016/j.jhep.2016.11.016
10. Dalton HR, Kamar N, Baylis SA, Moradpour D, Wedemeyer H, Negro F. EASL Clinical practice guidelines on hepatitis E virus infection. *J Hepatol*. 2018;68(6):1256-1271. https://doi.org/10.1016/j.jhep.2018.03.005
11. Fujiwara K, Yokosuka O, Fukai K, Imazeki F, Saisho H, Omata M. Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. *J Hepatol*. 2001;35(1):112-119. https://doi.org/10.1016/S0168-8278(01)00074-5
12. Mackiewicz V, Cammas A, Desbois D, et al. Nucleotide variability and translation efficiency of the 5′ untranslated region of hepatitis A virus: update from clinical isolates associated with mild and severe hepatitis. *J Virol*. 2010;84(19):10139-10147. https://doi.org/10.1128/JVI.02598-09
13. Fujiwara K, Yokosuka O, Imazeki F, et al. Analysis of hepatitis A virus protein 2B in sera of hepatitis A of various severities. *J Gastroenterol*. 2007;42(7):560-566. https://doi.org/10.1007/s00535-007-2039-7
14. van Tong H, Hoan NX, Wang B, Wedemeyer H, Bock C-T, Velavan TP. Hepatitis E virus mutations: functional and clinical relevance. *EBioMedicine*. 2016;11:31-42. https://doi.org/10.1016/j.ebiom.2016.07.039
15. Koonin EV, Dolja VV, Krupovic M. Origins and evolution of viruses of eukaryotes: the ultimate modularity. *Virology*. 2015;479-480:2-25. https://doi.org/10.1016/j.virol.2015.02.039
16. Lemon SM, Walker CM. Hepatitis A virus and hepatitis E virus: emerging and re-emerging enterically transmitted hepatitis viruses. *Cold Spring Harb Perspect Med*. 2018;9(6):a031823. https://doi.org/10.1101/cshperspect.a031823
17. Brown EA, Zajac AJ, Lemon SM. In vitro characterization of an internal ribosomal entry site (IRES) present within the 5′ nontranslated region of hepatitis A virus RNA: comparison with the IRES of encephalomyocarditis virus. *J Virol*. 1994;68(2):1066-1074.
18. Emerson SU, Huang YK, Nguyen H, et al. Identification of VP1/2A and 2C as virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. *J Virol*. 2002;76(17):8551-8559. https://doi.org/10.1128/JVI.76.17.8551-8559.2002
19. Probst C, Jecht M. Gauss-Müller V. *Intrinsic signals assem hepat A virus part*. 1999;274(8):4527-4531.
20. Costa-Mattioli M. Genetic variability of hepatitis A virus. *J Gen Virol*. 2003;84(12):3191-3201. https://doi.org/10.1099/vir.0.19532-0
21. Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): Molecular cloning and sequencing of the full-length viral genome. *Virology*. 1991;185(1):120-131. https://doi.org/10.1016/0042-6822(91)90760-9
22. Debing Y, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis e virology: implications for clinical practice. *J Hepatol*. 2016;65(1):200-212. https://doi.org/10.1016/j.jhep.2016.02.045
23. Cao D, Meng X-J. Molecular biology and replication of hepatitis E virus. *Emerg Microbes Infect*. 2012;1(1):1-10. https://doi.org/10.1038/emi.2012.7
24. Koonin EV, Gorbalenya AE, Purdy MA, Rozanov MN, Reyes GR, Bradley DW. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc Natl Acad Sci*. 1992;89(17):8259-8263. https://doi.org/10.1073/pnas.89.17.8259
25. Yamada K, Takahashi M, Hoshino Y, et al. ORF3 protein of hepatitis E virus is essential for virion release from infected cells. *J Gen Virol*. 2009;90(8):1880-1891. https://doi.org/10.1099/vir.0.010561-0
26. Feng Z, Hirai-Yuki A, McKnight KL, Lemon SM. Naked viruses that aren't always naked: quasi-enveloped agents of acute hepatitis. *Annu Rev Virol*. 2014;1(1):539-560. https://doi.org/10.1146/annurev-virology-031413-085359
27. Hirai-Yuki A, Hensley L, Whitmire JK, Lemon SM. Biliary secretion of quasi-enveloped human hepatitis A virus. *MBio*. 2016;7(6):1-11. https://doi.org/10.1128/mBio.01998-16
28. Feng Z, Hensley L, McKnight KL, et al. A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature*. 2013;496(7445):367-371. https://doi.org/10.1038/nature12029
29. Yin X, Ambardekar C, Lu Y, Feng Z. Distinct entry mechanisms for nonenveloped and quasi-enveloped hepatitis E viruses. *J Virol*. 2016;90(8):4232-4242. https://doi.org/10.1128/JVI.02804-15
30. Nagashima S, Takahashi M, Kobayashi T, et al. Characterization of the quasi-enveloped hepatitis E virus particles released by the cellular exosomal pathway. *J Virol*. 2017;91(22):e00822-17. https://doi.org/10.1128/JVI.00822-17
31. Ren X, Wu P, Wang L, et al. Changing epidemiology of hepatitis A and hepatitis E viruses in China, 1990–2014. *Emerg Infect Dis*. 2017;23(2):276-279. https://doi.org/10.3201/2302.161095
32. Joussemet M, Depaquit J, Nicand E, et al. Seroepidemiological shift of hepatitis A in French youth. *Gastroentérologie Clin Biol*. 1999;23(4):447-452. GCB-05-1999-23-4-0399-8320-101019-ART98
33. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine*. 2010;28(41):6653-6657. https://doi.org/10.1016/j.vaccine.2010.08.037
34. Ogholikhan S, Schwarz K. Hepatitis vaccines. *Vaccine*. 2016;4(1):6. https://doi.org/10.3390/vaccines4010006
35. Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. *J Hepatol*. 2018;68(1):167-184. https://doi.org/10.1016/j.jhep.2017.08.034
36. Sander A-L, Corman VM, Lukashev AN, Drexler JF. Evolutionary origins of enteric hepatitis viruses. *Cold Spring Harb Perspect Med*. 2018;8:a031690. https://doi.org/10.1101/cshperspect.a031690
37. Vaughan G, Goncalves Rossi LM, Forbi JC, et al. Hepatitis A virus: host interactions, molecular epidemiology and evolution. *Infect Genet Evol*. 2014;21:227-243. https://doi.org/10.1016/j.meegid.2013.10.023
38. Kamar N, Pischke S. Acute and persistent hepatitis E virus genotype 3 and 4 infection: clinical features, pathogenesis, and treatment. *Cold Spring Harb Perspect Med*. 2018;9:a031872. https://doi.org/10.1101/cshperspect.a031872

39. Takahashi M, Nishizawa T, Sato H, et al. Analysis of the full-length genome of a hepatitis E virus isolate obtained from a wild boar in Japan that is classifiable into a novel genotype. *J Gen Virol*. 2011;92 (4):902-908. https://doi.org/10.1099/vir.0.029470-0
40. Mansuy JM, Gallian P, Dimeglio C, et al. A nationwide survey of hepatitis E viral infection in French blood donors. *Hepatology*. 2016;63 (4):1145-1154. https://doi.org/10.1002/hep.28436
41. Lee G-H, Tan B-H, Chi-Yuan Teo E, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150(2):355-357. e3. https://doi.org/10.1053/j.gastro.2015.10.048
42. Woo PCY, Lau SKP, Teng JLL, et al. New hepatitis E virus genotype in bactrian camels, Xinjiang, China, 2013. *Emerg Infect Dis*. 2016;22 (12):2219-2221. https://doi.org/10.3201/eid2212.160979
43. Hakim MS, Wang W, Bramer WM, et al. The global burden of hepatitis E outbreaks: a systematic review. *Liver Int*. 2017;37(1):19-31. https://doi.org/10.1111/liv.13237
44. Nimgaonkar I, Ding Q, Schwartz RE, Ploss A. Hepatitis E virus: advances and challenges. *Nat Rev Gastroenterol Hepatol*. 2017;15(2): 96-110. https://doi.org/10.1038/nrgastro.2017.150
45. Shrestha AC, Flower RLP, Seed CR, Stramer SL, Faddy HM. A comparative study of assay performance of commercial hepatitis E virus enzyme-linked immunosorbent assay kits in Australian blood donor samples. *J Blood Transfus*. 2016;2016:1-6. https://doi.org/10.1155/2016/9647675
46. Abravanel F, Lacipière A, Lhomme S, et al. Performance of a commercial assay for detecting and quantifying HEV RNA in faeces. *J Clin Virol*. 2018;109(October):1-5. https://doi.org/10.1016/j.jcv.2018.10.003
47. Norder H, Karlsson M, Mellgren Å, et al. Diagnostic performance of five assays for anti-hepatitis E virus IgG and IgM in a large cohort study. *J Clin Microbiol*. 2016;54(3):549-555. https://doi.org/10.1128/JCM.02343-15
48. Abravanel F, Goutagny N, Joffray R, et al. Performance characteristics of the VIDAS® ANTI-HEV IgM and IgG assays. *J Clin Virol*. 2019; 112(January):10-14. https://doi.org/10.1016/j.jcv.2019.01.005
49. EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, Allende A, et al. Public health risks associated with hepatitis E virus (HEV) as a foodborne pathogen. *EFSA J*. 2017;15(7). https://doi.org/10.2903/j.efsa.2017.4886
50. Dalton HR, Izopet J. Transmission and epidemiology of hepatitis E virus genotype 3 and 4 infections. *Cold Spring Harb Perspect Med*. 2018;8(11):a032144. https://doi.org/10.1101/cshperspect.a032144
51. Slot E, Hogema BM, Kok TM, Molier M, Zaaijer HL. Silent hepatitis E virus infection in Dutch blood donors, 2011 to 2012. *euro*. 2013;18 (31):20550.
52. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. *Lancet*. 2012;379(9835):2477-2488. https://doi.org/10.1016/S0140-6736(11)61849-7
53. Colson P, Borentain P, Queyriaux B, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis*. 2010;202(6):825-834. https://doi.org/10.1086/655898
54. Wu X, Chen P, Lin H, Hao X, Liang Z. Hepatitis E virus: current epidemiology and vaccine. *Hum Vaccin Immunother*. 2016;12(10):2603-2610. https://doi.org/10.1080/21645515.2016.1184806
55. Pelletier AR, Mehta PJ, Burgess DR, et al. An outbreak of hepatitis A among primary and secondary contacts of an international adoptee. *Public Health Rep*. 2010;125(5):642-646. https://doi.org/10.1177/003335491012500505
56. EFSA. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA J*. 2015;13(12):4329. https://doi.org/10.2903/j.efsa.2015.4329
57. Yeargin T, Gibson KE. Key characteristics of foods with an elevated risk for viral enteropathogen contamination. *J Appl Microbiol*. 2019; 126(4):996-1010. https://doi.org/10.1111/jam.14113
58. Cook N, Bertrand I, Gantzer C, Pinto RM, Bosch A. Persistence of hepatitis A virus in fresh produce and production environments, and the effect of disinfection procedures: a review. *Food Environ Virol*. 2018;10(3):253-262. https://doi.org/10.1007/s12560-018-9349-1
59. Cook N, van der Poel WHM. Survival and elimination of hepatitis E virus: a review. *Food Environ Virol*. 2015;7(3):189-194. https://doi.org/10.1007/s12560-015-9196-2
60. Gassilloud B, Schwartzbrod L, Gantzer C. Presence of viral genomes in mineral water: a sufficient condition to assume infectious risk ? *Appl Environ Microbiol*. 2003;69(7):3965-3969. https://doi.org/10.1128/AEM.69.7.3965-3965.2003
61. Hartard C, Banas S, Rivet R, Boudaud N, Gantzer C. Rapid and sensitive method to assess human viral pollution in shellfish using infectious F-specific RNA bacteriophages: Application to marketed products. *Food Microbiol*. 2017;63:248-254. https://doi.org/10.1016/j.fm.2016.12.002
62. Randazzo W, Vasquez-García A, Aznar R, Sánchez G. Viability RT-qPCR to distinguish between HEV and HAV with intact and altered capsids. *Front Microbiol*. 2018;9:1-8. https://doi.org/10.3389/fmicb.2018.01973
63. Bellou M, Kokkinos P, Vantarakis A. Shellfish-borne viral outbreaks: a systematic review. *Food Environ Virol*. 2013;5(1):13-23. https://doi.org/10.1007/s12560-012-9097-6
64. Hunter JG, Madden RG, Stone AM, et al. Coastal clustering of HEV; Cornwall. *UK Eur J Gastroenterol Hepatol*. 2016;28(3):323-327. https://doi.org/10.1097/MEG.0000000000000518
65. Grodzki M, Schaeffer J, Piquet J-C, et al. Bioaccumulation efficiency, tissue distribution, and environmental occurrence of hepatitis E virus in bivalve shellfish from France. *Appl Environ Microbiol*. 2014;80(14):4269-4276. https://doi.org/10.1128/AEM.00978-14
66. Fenaux H, Chassaing M, Berger S, Gantzer C, Bertrand I, Schvoerer E. Transmission of hepatitis E virus by water: an issue still pending in industrialized countries. *Water Res*. 2019;151:144-157. https://doi.org/10.1016/j.watres.2018.12.014
67. King NJ, Hewitt J, Perchec-Merien A-M. Hiding in plain sight? It's time to investigate other possible transmission routes for hepatitis E virus (HEV) in developed countries. *Food Environ Virol*. 2018;10(3):225-252. https://doi.org/10.1007/s12560-018-9342-8
68. Pavio N, Merbah T, Thébault A. Frequent hepatitis E virus contamination in food containing raw pork liver. *France Emerg Infect Dis*. 2014;20(11):1925-1927. https://doi.org/10.3201/eid2011.140891
69. Szabo K, Trojnar E, Anheyer-Behmenburg H, et al. Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. *Int J Food Microbiol*. 2015;215:149-156. https://doi.org/10.1016/j.ijfoodmicro.2015.09.013
70. Abravanel F, Lhomme S, El Costa H, et al. Rabbit hepatitis E virus infections in humans France. *Emerg Infect Dis*. 2017;23(7):1191-1193. https://doi.org/10.3201/eid2307.170318
71. Huang X, Huang Y, Wagner AL, Chen X, Lu Y. Hepatitis E virus infection in swine workers: a meta-analysis. *Zoonoses Public Health*. 2018; 66:155-163. https://doi.org/10.1111/zph.12548
72. Sridhar S, Yip CCY, Wu S, et al. Rat hepatitis E virus as cause of persistent hepatitis after liver transplant. *Emerg Infect Dis*. 2018;24(12): 2241-2250. https://doi.org/10.3201/eid2412.180937
73. Bordi L, Rozera G, Scognamiglio P, et al. Monophyletic outbreak of hepatitis A involving HIV-infected men who have sex with men, Rome, Italy 2008–2009. *J Clin Virol*. 2012;54(1):26-29. https://doi.org/10.1016/j.jcv.2012.01.009
74. Stene-Johansen K, Tjon G, Schreier E, et al. Molecular epidemiological studies show that hepatitis A virus is endemic among active

homosexual men in Europe. *J Med Virol*. 2007;79(4):356-365. https://doi.org/10.1002/jmv.20781
75. Payne BAI, Medhi M, Ijaz S, et al. Hepatitis E virus seroprevalence among men who have sex with men. *United Kingdom Emerg Infect Dis*. 2013;19(2):333-336. https://doi.org/10.3201/eid1902.121174
76. Alberts CJ, van der Loeff MF S, Sadik S, et al. Hepatitis E virus seroprevalence and determinants in various study populations in the Netherlands. *PLoS One*. 2018;13(12):e0208522. https://doi.org/10.1371/journal.pone.0208522
77. Ngui S-L, Granerod J, Jewes LA, Crowcroft NS, Teo C-G. Outbreaks of hepatitis A in England and Wales associated with two co-circulating hepatitis A virus strains. *J Med Virol*. 2008;80(7):1181-1188. https://doi.org/10.1002/jmv.21207
78. Lhomme S, Marion O, Abravanel F, Chapuy-Regaud S, Kamar N, Izopet J. Hepatitis E pathogenesis. *Viruses*. 2016;8(8):212. https://doi.org/10.3390/v8080212
79. Pérez-Gracia MT, Suay-García B, Mateos-Lindemann ML. Hepatitis E and pregnancy: current state. *Rev Med Virol*. 2017;27(3):e1929. https://doi.org/10.1002/rmv.1929
80. Deka M, Mounita B, Baruah B, et al. Role of CYP2E1 gene polymorphisms association with hepatitis risk in Northeast India. *World J Gastroenterol*. 2010;16(38):4800-4808. https://doi.org/10.3748/wjg.v16.i38.4800
81. Gu M, Qiu J, Guo D, et al. Evaluation of candidate genes associated with hepatitis A and E virus infection in Chinese Han population. *Virol J*. 2018;15(1):47. https://doi.org/10.1186/s12985-018-0962-2
82. Renge R, Dani V, Chitambar S, Arankalle VA. Vertical transmission of hepatitis A. *indian J pediatry*. 2002;69(6):535-536.
83. Tanaka I, Shima M, Kubota Y, Takahashi Y, Kawamata O, Yoshioka A. Vertical transmission of hepatitis A virus. *Lancet*. 1995;345(8946):397. https://doi.org/10.1016/S0140-6736(95)90389-5
84. Qu L, Feng Z, Yamane D, et al. Disruption of TLR3 signaling due to cleavage of TRIF by the hepatitis A virus protease-polymerase processing intermediate, 3CD. *PLoS Pathog*. 2011;7(9):e1002169. https://doi.org/10.1371/journal.ppat.1002169
85. Nan Y, Yu Y, Ma Z, Khattar SK, Fredericksen B, Zhang Y-J. Hepatitis E virus inhibits type I interferon induction by ORF1 products. *J Virol*. 2014;88(20):11924-11932. https://doi.org/10.1128/JVI.01935-14
86. Yang C, Yu W, Bi Y, et al. Increased oestradiol in hepatitis E virus-infected pregnant women promotes viral replication. *J Viral Hepat*. 2018;25(6):742-751. https://doi.org/10.1111/jvh.12865
87. Gouilly J, Chen Q, Siewiera J, et al. Genotype specific pathogenicity of hepatitis E virus at the human maternal-fetal interface. *Nat Commun*. 2018;9(1):4748. https://doi.org/10.1038/s41467-018-07200-2
88. Kamar N, Selves J, Mansuy J-M, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*. 2008;358(8):811-817. https://doi.org/10.1056/NEJMoa0706992
89. Luciani L, Deharo P, Aherfi S, Chalvignac V, Borentain P, Colson P. Hepatitis E virus infection in heart transplant recipients. *Southeastern France Clin Res Hepatol Gastroenterol*. 2019;43(1):108-111. https://doi.org/10.1016/j.clinre.2018.09.010
90. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140(5):1481-1489. https://doi.org/10.1053/j.gastro.2011.02.050
91. Tavitian S, Péron J-M, Huynh A, et al. Hepatitis E virus excretion can be prolonged in patients with hematological malignancies. *J Clin Virol*. 2010;49(2):141-144. https://doi.org/10.1016/j.jcv.2010.06.016
92. Dalton HR, Keane F, Ijaz S. Treatment of chronic hepatitis E in a patient with HIV infection. *Ann Intern Med*. 2011;155(7):479-480. https://doi.org/10.1001/jama.1962.03050420147057
93. Geng Y, Zhang H, Huang W, et al. Persistent hepatitis E virus genotype 4 infection in a child with acute lymphoblastic leukemia. *Hepat Mon*. 2013;14(1):10-13. https://doi.org/10.5812/hepatmon.15618
94. Walker CM. Adaptive Immune responses in hepatitis A virus and hepatitis E virus infections. *Cold Spring Harb Perspect Med*. 2018;9:a033472. https://doi.org/10.1101/cshperspect.a033472
95. Schiff ER. Atypical clinical manifestations of hepatitis A. *Vaccine*. 1992;10:S18-S20. https://doi.org/10.1016/0264-410X(92)90534-Q
96. Dalton HR, van Eijk JJJ, Cintas P, et al. Hepatitis E virus infection and acute non-traumatic neurological injury: a prospective multicentre study. *J Hepatol*. 2017;67(5):925-932. https://doi.org/10.1016/j.jhep.2017.07.010
97. Kamar N, Marion O, Abravanel F, Izopet J, Dalton HR. Extrahepatic manifestations of hepatitis E virus. *Liver Int*. 2016;36(4):467-472. https://doi.org/10.1111/liv.13037
98. Kamar N, Izopet J, Cintas P, et al. Hepatitis E virus-induced neurological symptoms in a kidney-transplant patient with chronic hepatitis. *Am J Transplant*. 2010;10(5):1321-1324. https://doi.org/10.1111/j.1600-6143.2010.03068.x
99. Taton B, Moreau K, Lepreux S, et al. Hepatitis E virus infection as a new probable cause of de novo membranous nephropathy after kidney transplantation. *Transpl Infect Dis*. 2013;15(6):E211-E215. https://doi.org/10.1111/tid.12143
100. Shah SAR, Lal A, Idrees M, et al. Hepatitis E virus-associated aplastic anaemia: the first case of its kind. *J Clin Virol*. 2012;54(1):96-97. https://doi.org/10.1016/j.jcv.2012.02.002
101. Del Bello A, Arné-Bes MC, Lavayssière L, Kamar N. Hepatitis E virus-induced severe myositis. *J Hepatol*. 2012;57(5):1152-1153. https://doi.org/10.1016/j.jhep.2012.05.010
102. Dumoulin FL, Liese H. Acute hepatitis E virus infection and autoimmune thyroiditis: yet another trigger? *Case Reports*. 2012;2012:bcr1220115441-bcr1220115441. https://doi.org/10.1136/bcr.12.2011.5441
103. Martínez-Artola Y, Poncino D, García ML, Munné MS, González J, García DS. Acute hepatitis E virus infection and association with a subacute thyroiditis. *Ann Hepatol*. 2015;14(1):141-142. https://doi.org/10.1136/bcr.12.2011.5441.6
104. Jeong S-H, Lee H-S. Hepatitis A: clinical manifestations and management. *Intervirology*. 2010;53(1):15-19. https://doi.org/10.1159/000252779
105. Choi HK, Song YG, Han SH, et al. Clinical features and outcomes of acute kidney injury among patients with acute hepatitis A. *J Clin Virol*. 2011;52(3):192-197. https://doi.org/10.1016/j.jcv.2011.07.013
106. Cullen JM, Lemon SM. Comparative pathology of hepatitis A virus and hepatitis E virus infection. *Cold Spring Harb Perspect Med*. 2019;9(4):a033456. https://doi.org/10.1101/cshperspect.a033456
107. Brayne AB, Dearlove BL, Lester JS, Kosakovsky Pond SL, Frost SDW. Genotype-specific evolution of hepatitis E virus. Ou J-HJ, ed. *J Virol*. 2017;91(9):1-16. https://doi.org/10.1128/JVI.02241-16
108. Smith DB, Simmonds P. Classification and genomic diversity of enterically transmitted hepatitis viruses. *Cold Spring Harb Perspect Med*. 2018;8(9):a031880. https://doi.org/10.1101/cshperspect.a031880
109. Yun H, Lee HJ, Jang J-H, et al. Hepatitis A Virus genotype and its correlation with the clinical outcome of acute hepatitis A in Korea: 2006-2008. *J Med Virol*. 2011;83(12):2073-2081. https://doi.org/10.1002/jmv.22229

110. Miura Y, Kanda T, Yasui S, et al. Hepatitis A virus genotype IA-infected patient with marked elevation of aspartate aminotransferase levels. *Clin J Gastroenterol*. 2017;10(1):52-56. https://doi.org/10.1007/s12328-016-0700-5
111. Fujiwara K, Yokosuka O, Ehata T, et al. Association between severity of type A hepatitis and nucleotide variations in the 5` non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut*. 2002;51(1):82-88. https://doi.org/10.1136/gut.51.1.82
112. Ajmera V, Xia G, Vaughan G, et al. What factors determine the severity of hepatitis A-related acute liver failure? *J Viral Hepat*. 2011;18(7):e167-e174. https://doi.org/10.1111/j.1365-2893.2010.01410.x
113. Schultz DE, Honda M, Whetter LE, McKnight KL, Lemon SM. Mutations within the 5′ nontranslated RNA of cell culture-adapted hepatitis A virus which enhance cap-independent translation in cultured African green monkey kidney cells. *J Virol*. 1996;70(2):1041-1049.
114. Joshi MS, Cherian SS, Bhalla S, Chitambar SD. Longer duration of viremia and unique amino acid substitutions in a hepatitis A virus stain associated with Guillain-Barré syndrome (GBS). *J Med Virol*. 2010;82(6):913-919. https://doi.org/10.1002/jmv.21757
115. Pérez-Sautu U, Costafreda MI, Caylà J, et al. Hepatitis A virus vaccine escape variants and potential new serotype emergence. *Emerg Infect Dis*. 2011;17(4):734-737. https://doi.org/10.3201/eid1704.101169
116. Purdy MA, Khudyakov YE. Evolutionary history and population dynamics of hepatitis E virus. *PLoS One*. 2010;5(12):e14376. https://doi.org/10.1371/journal.pone.0014376
117. Ikram A, Hakim MS, Zhou J, Wang W, Peppelenbosch MP, Pan Q. Genotype-specific acquisition, evolution and adaptation of characteristic mutations in hepatitis E virus. *Virulence*. 2018;9(1):121-132. https://doi.org/10.1080/21505594.2017.1358349
118. Borkakoti J, Ahmed G, Rai A, Kar P. Report of novel H105R, D29N, V27A mutations in the methyltransferase region of the HEV genome in patients with acute liver failure. *J Clin Virol*. 2017;91:1-4. https://doi.org/10.1016/j.jcv.2017.03.017
119. Mishra N, Walimbe AM, Arankalle VA. Hepatitis E virus from India exhibits significant amino acid mutations in fulminant hepatic failure patients. *Virus Genes*. 2013;46(1):47-53. https://doi.org/10.1007/s11262-012-0833-7
120. Lhomme S, Abravanel F, Dubois M, et al. Characterization of the polyproline region of the hepatitis E virus in immunocompromised patients. *J Virol*. 2014;88(20):12017-12025. https://doi.org/10.1128/JVI.01625-14
121. Johne R, Reetz J, Ulrich RG, et al. An ORF1-rearranged hepatitis E virus derived from a chronically infected patient efficiently replicates in cell culture. *J Viral Hepat*. 2014;21(6):447-456. https://doi.org/10.1111/jvh.12157
122. Shukla P, Nguyen HT, Torian U, et al. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. *Proc Natl Acad Sci*. 2011;108(6):2438-2443. https://doi.org/10.1073/pnas.1018878108
123. Nguyen HT, Torian U, Faulk K, et al. A naturally occurring human/-hepatitis E recombinant virus predominates in serum but not in faeces of a chronic hepatitis E patient and has a growth advantage in cell culture. *J Gen Virol*. 2012;93(3):526-530. https://doi.org/10.1099/vir.0.037259-0
124. Debing Y, Ramière C, Dallmeier K, et al. Hepatitis E virus mutations associated with ribavirin treatment failure result in altered viral fitness and ribavirin sensitivity. *J Hepatol*. 2016;65(3):499-508. https://doi.org/10.1016/j.jhep.2016.05.002
125. Takahashi K, Okamoto H, Abe N, et al. Virulent strain of hepatitis E virus genotype 3. *Japan Emerg Infect Dis*. 2009;15(5):704-709. https://doi.org/10.3201/eid1505.081100
126. Inoue J, Nishizawa T, Takahashi M, et al. Analysis of the full-length genome of genotype 4 hepatitis E virus isolates from patients with fulminant or acute self-limited hepatitis E. *J Med Virol*. 2006;78(4):476-484. https://doi.org/10.1002/jmv.20565
127. Inoue J, Takahashi M, Mizuo H, et al. Nucleotide substitutions of hepatitis E virus genomes associated with fulminant hepatitis and disease severity. *Tohoku J Exp Med*. 2009;218(4):279-284. https://doi.org/10.1620/tjem.218.279
128. Todt D, Gisa A, Radonic A, et al. In vivo evidence for ribavirin-induced mutagenesis of the hepatitis E virus genome. *Gut*. 2016;65(10):1733-1743. https://doi.org/10.1136/gutjnl-2015-311000
129. Borkakoti J, Ahmed G, Kar P. Report of a novel C1483W mutation in the hepatitis E virus polymerase in patients with acute liver failure. *Infect Genet Evol*. 2016;44:51-54. https://doi.org/10.1016/j.meegid.2016.06.030
130. Debing Y, Gisa A, Dallmeier K, et al. A Mutation in the hepatitis E virus RNA polymerase promotes its replication and associates with ribavirin treatment failure in organ transplant recipients. *Gastroenterology*. 2014;147(5):1008-1011.e7. https://doi.org/10.1053/j.gastro.2014.08.040
131. Borkakoti J, Ahmed G, Hussain SA, Rai A, Kar P. Novel molecular alterations in the ORF 2 capsid gene of hepatitis E virus in patients with acute liver failure in North India. *Arch Virol*. 2014;159(12):3391-3394. https://doi.org/10.1007/s00705-014-2198-9
132. Kenney SP, Meng X-J. Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb Perspect Med*. 2019;9(1):a031724. https://doi.org/10.1101/cshperspect.a031724
133. Devhare P, Sharma K, Mhaindarkar V, Arankalle V, Lole K. Analysis of helicase domain mutations in the hepatitis E virus derived from patients with fulminant hepatic failure: Effects on enzymatic activities and virus replication. *Virus Res*. 2014;184:103-110. https://doi.org/10.1016/j.virusres.2014.02.018
134. Nagashima S, Takahashi M, Jirintai TT, Yamada K, Nishizawa T, Okamoto H. A PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from infected cells. *J Gen Virol*. 2011;92(2):269-278. https://doi.org/10.1099/vir.0.025791-0
135. Inoue J, Takahashi M, Yazaki Y, Tsuda F, Okamoto H. Development and validation of an improved RT-PCR assay with nested universal primers for detection of hepatitis E virus strains with significant sequence divergence. *J Virol Methods*. 2006;137(2):325-333. https://doi.org/10.1016/j.jviromet.2006.07.004
136. Nan Y, Wu C, Zhao Q, Sun Y, Zhang Y-J, Zhou E-M. Vaccine development against zoonotic hepatitis E virus: open questions and remaining challenges. *Front Microbiol*. 2018;9:1-16. https://doi.org/10.3389/fmicb.2018.00266
137. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. *Microbiol Mol Biol Rev*. 2012;76(2):159-216. https://doi.org/10.1128/MMBR.05023-11
138. Costa-Mattioli M. Analysis of sequential hepatitis A virus strains reveals coexistence of distinct viral subpopulations. *J Gen Virol*. 2006;87(1):115-118. https://doi.org/10.1099/vir.0.81286-0
139. Lhomme S, Abravanel F, Dubois M, et al. Hepatitis E virus quasispecies and the outcome of acute hepatitis E in solid-organ transplant patients. *J Virol*. 2012;86(18):10006-10014. https://doi.org/10.1128/JVI.01003-12
140. Lhomme S, Garrouste C, Kamar N, et al. Influence of polyproline region and macro domain genetic heterogeneity on HEV persistence in immunocompromised patients. *J Infect Dis*. 2014;209(2):300-303. https://doi.org/10.1093/infdis/jit438
141. Lhomme S, Kamar N, Nicot F, et al. Mutation in the hepatitis E virus polymerase and outcome of ribavirin therapy. *Antimicrob Agents Chemother*. 2016;60(3):1608-1614. https://doi.org/10.1128/AAC.02496-15

142. Shin EC, Jeong SH. Natural history, clinical manifestations, and pathogenesis of hepatitis A. *Cold Spring Harb Perspect Med*. 2018;8(9):1-14. https://doi.org/10.1101/cshperspect.a031708
143. Pawlotsky J-M. Hepatitis C virus genetic variability: pathogenic and clinical implications. *Clin Liver Dis*. 2003;7(1):45-66. https://doi.org/10.1016/S1089-3261(02)00065-X
144. Fenaux H, Chassaing M, Berger S, et al. Molecular features of hepatitis E Virus circulation in environmental and human samples. *J Clin Virol*. 2018;103:63-70. https://doi.org/10.1016/j.jcv.2018.04.003

How to cite this article: Hartard C, Gantzer C, Bronowicki J, Schvoerer E. Emerging hepatitis E virus compared with hepatitis A virus: A new sanitary challenge. *Rev Med Virol*. 2019;e2078. https://doi.org/10.1002/rmv.2078