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Hepatitis E virus: a zoonosis adapting to humans

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Hepatitis E virus (HEV) infection is gaining global attention, not only because of the increasing burden of the disease in low endemicity countries, in terms of morbidity and mortality rates, but also due to recent advances in the molecular virology and epidemiology of this emerging pathogen. HEV infection spread can be described as the evolution of a zoonosis towards an established human infection. As known from other viruses, such as the human immunodeficiency virus or the influenza viruses, crossing the species barriers from animals to humans is a recurrent phenomenon. Albeit slow at the beginning, once the virus has adapted to humans, the person-to-person spread can proceed very quickly. Although an optimal cell culture system for HEV is not yet available, outstanding progress has been made with the *in vitro* expression of HEV-like particles. These new tools have fostered new research to understand the molecular, structural and immunological aspects of human HEV infection. Although some promising data from Phase II vaccine trials are available, recent discoveries will certainly open new avenues for HEV-specific prophylaxis and therapy.

Keywords: *Hepevirus*, vaccines, crystal structures, liver transplantations

Epidemiology

Hepatitis E virus (HEV) infection is arguably the most frequent acute viral hepatitis infection around the world. The real global burden of HEV infection is not established, but it is estimated that one-third of the world population has been infected with HEV.[1] The global spread of HEV follows the socioeconomic status of populations; thus, the seroprevalence is highest in countries where water sanitation is poor. High endemicity areas comprise: (i) Central and South East Asia, with >20% of anti-HEV seroprevalence among the general population of China, 45% in rural areas of Malaysia and >20% in India;[2,3] and (ii) North Africa and the Middle East, with up to 26% in Egypt and 17% in Saudi Arabia.[4] In Western countries, the seroprevalence within the general population is 1%–3% in Europe and ~2% in the USA (with up to 20% in certain ethnic groups). Accumulating evidence surrounding cases of HEV infection in these countries suggests that they are due to autochthonous viral strains (i.e. locally acquired),[5–7] nearly always classified as viral genotype 3. However, in these low-endemicity areas, special groups, such as farmers, veterinarians, butchers and persons handling animal meat or consumers of undercooked swine/wild deer meat, present with a considerably higher seroprevalence than the general population. This is due to the fact that HEV is mainly a zoonosis involving comestible animals, such as wild boars, deer or domestic pigs. The meat of the latter constitutes a substantial part of the human food chain, and viral spread can occur through the ingestion of infected and undercooked meat.[8] Indeed, swine and human HEV strains show extremely close genetic relatedness (especially HEV genotypes 3 and 4, as discussed below). These observations suggest, on the one hand, that animals may serve as a reservoir for human HEV infection and, on the other hand, that current human infection may originally derive from natural infection of wild boars.[9,10] The evolution from a zoonosis to a human infection is confirmed by reports showing the close relationship of full genome sequence analyses of human and swine infections for genotype 3 and 4 infections. Moreover, the routes of transmission are very interesting. The most common route of transmission of HEV is probably foodborne. This may occur either through drinking water contaminated with faeces from infected individuals or animals, or via ingestion of meat from infected animals. In addition, parenteral spread, via blood products or solid organ transplantation, has been well documented. In fact, the blood supply is vulnerable to non-enveloped viruses like HEV, because pathogen-inactivation methods are insufficient and routine serological tests are not in place.[11] Lastly, vertical mother-to-child transmission, either intrauterine or perinatal, has been described.[12] Thus, these multifaceted routes of transmission facilitate viral diffusion (and adaptation) to the human species.

Clinical manifestations

The clinical manifestations of HEV infection span a large array of symptoms, i.e. from a totally asymptomatic course to life-threatening severe hepatitis with acute liver failure (ALF) and elevated mortality. Endemic disease associated with genotype 1 or 2 infections in developing countries occurs commonly in

young adults, whereas clinical hepatitis E associated with infection with genotypes 3 or 4, as seen in industrialized countries, peaks in patients ≥60 years of age.[13] In most patients, acute infection manifests after an incubation period of 3–6 weeks, and is characterized by a subclinical to severe picture of cholestatic hepatitis, with jaundice, anorexia, nausea, vomiting and occasionally fever, lasting for 1–6 weeks. Severe, life-threatening hepatitis with ALF may occur in ~1% of cases, considered overall, in both high- and low-endemicity areas. However, severe morbidity and mortality rates are highest in pregnant women. In a recent retrospective study from India on ALF in pregnancy, it was shown that a significantly higher proportion of ALF was caused by HEV, but also that the overall outcome of ALF in pregnant women was unrelated to the cause of the disease.[14] Thus, the mortality for ALF in HEV-infected pregnant women (51%) was not higher than that in HEV-uninfected pregnant women (54%).

Until recently, it was believed that HEV was a self-limited infection. However, persistent HEV infection accompanied by chronic liver disease, in some rare cases progressing to cirrhosis, has been described in immune-suppressed patients. In particular, different cases of HEV infection in solid organ transplant recipients (liver, kidney or pancreas) have been reported with persistent detection of HEV RNA in stools or serum up to 15 months after the acute infection.[15–17] Liver histology may show portal hepatitis with lymphocytic infiltrates and fibrosis, with progression over time to cirrhosis even requiring retransplantation of a liver graft in one case. Of note is another report of a reactivation of HEV infection 3 months after complete recovery and allogenic stem cell transplantation, leading to speculation that the virus might persist in hepatocytes.[18] Taken together, these reports have changed our view on the natural history of HEV infection. Persistent HEV infection, accompanied by chronic, progressive liver damage that can evolve towards cirrhosis, is an infrequent but clinically significant occurrence in immune-suppressed patients.

Diagnosis

Diagnostic tools to assess HEV infection are available and encompass serological assays to detect anti-HEV antibodies capable of differentiating recent from past infection (IgG and IgM). ELISA for antibodies to HEV genotypes 1–4 are commercially available, and are based on the detection of antibodies against the highly conserved and immunogenic capsid protein [encoded by open reading frame (ORF)2]. These ELISA are characterized by a broad activity and good reproducibility. Some concerns have been raised on the specificity of these assays, especially when assessing the HEV prevalence in countries with low endemicity rates.[19] It is, however, unlikely that seroprevalence figures will change dramatically in the future upon the introduction of new generation assays.

The appearance of anti-HEV-specific IgM without IgG indicates very recent infection, whereas the presence of anti-HEV IgG without IgM points towards past infection. Anti-HEV-specific IgG are produced early in infection; thus, both IgG and IgM might be detected together, indicating an early state of the infection. Anti-HEV-specific IgA has been used in the past to define acute infection, but its use today is questionable. Diagnosis in immune-suppressed patients is more difficult, because in these patients seroconversion can be late or even lacking altogether.[16,17] Anti-HEV antibodies are detectable 1–3 weeks after acute infection of immune-competent persons (IgM and IgG). On the contrary, in immune-suppressed patients, seroconversion might be delayed by up to 6–10 months. Thus, if the patient is immune suppressed, a combination of serology and nucleic acid testing, the most sensitive test for acute or ongoing HEV infection, should be used. Nested or real-time PCR amplifying a 189 bp stretch located in ORF2 may detect viral genomic RNA in serum or stools, especially in acute infection and within the first week after exposure. However, the period of detectable HEV RNA in serum may be short-lived (10–30 days after the onset of symptoms), although faecal shedding of the virus may last for up to 2–3 months.

Molecular biology

Hepatitis E virus is a small, spherical, non-enveloped virus and a member of the *Hepevirus* genus. Initially, based on structural similarities, HEV was classified as a *Calicivirus*. However, according to phylogenetic analyses, HEV is now grouped apart in the *Hepeviridae* family. This comprises the human and swine HEV, which is further divided into 4 genotypes, and the avian HEV, which probably should be classified in a genus distinct from the mammalian HEV. Similarities and differences of *Caliciviridae* and *Hepeviridae* are summarized in Table 1. *Hepeviridae* and *Caliciviridae* can infect different animals and provoke diverse disease patterns. For example, HEV infection in piglets remains asymptomatic, whereas the avian HEV can cause the hepatitis-splenomegaly syndrome in chickens, with increased mortality. In the *Caliciviridae* family, the rabbit haemorrhagic disease virus causes severe pulmonary haemorrhage and extended liver necrosis, leading to up to 90% mortality in infected animals.[20] Of note is that a vaccine against avian HEV has been developed to avoid pandemics that threaten the chicken industry.[21]

Recent insights about the viral structure of HEV have led to great advances in the understanding of the molecular and immunological features of this virus. The viral genome is a 7.2 kb positive stranded linear RNA that contains three ORFs. ORF1 encodes non-structural proteins, ORF2 encodes the viral capsid, the target for humoral and cellular immunity, and ORF3 encodes a small non-structural phosphoprotein that interacts at various levels with the cell physiology, thus contributing to HEV replication and pathogenesis, and possibly accounting for its role in *in vivo* infectivity.[22]

Because there is no closely related, well-characterized virus, little is known about the structure, life cycle and pathogenic mechanisms of HEV. Moreover, an efficient cell culture system for HEV is still lacking. Even though Tanaka *et al.*[23] succeeded to establish a persistent viral replication of a genotype 3 strain in human hepatoma and carcinoma epithelial cell lines, the system was hampered by the production of insufficient amounts of viral particles. Thus, the size and structure of infectious virions can only be deduced based on electron microscopy imaging, sucrose gradient measurements and knowledge of the structural components. HEV particles appear spherical, with indentations on the surface, with a diameter of ~32–34 nm.

Table 1. Similarities and differences of *Caliciviridae* and *Hepeviridae*

	Caliciviridae[a]	*Hepeviridae*
Phylogenetic classification	Norwalk virus (NV) Sapporo-like viruses (SV) Rabbit haemorrhagic disease virus (LV) European brown hare syndrome virus (LV) Rabbit calicivirus (LV) San Miguel sea lion virus (VV) Vesicular exanthema of swine virus (VV)	the human and swine HEV is further classified into four major genotypes; the avian HEV should probably be classified in a genus distinct from the mammalian HEV
Viral structure	non-enveloped viruses with a single-stranded positive-sense RNA genome having a size varying between 7.3 and 8.3 kb	non-enveloped virus with a single-stranded positive-sense RNA genome of 7.2 kb
Viral proteins	two to three ORFs encoding structural and non-structural proteins	three ORFs: ORF1 and 3 encode non-structural proteins, whereas ORF2 encodes the major capsid protein
Capsid protein	the capsid protein of both families has a highly exposed domain that is recognized by neutralizing antibodies; this protruding region is structurally different among the viruses, but might have similar roles in the host–virus interaction	

ORF, open reading frame.
[a]The family of *Caliciviridae* is divided into four genera: *Norovirus* (NV); *Sapovirus* (SV); *Lagovirus* (LV); and *Vesivirus* (VV).

The outer shell, composed of 60 capsid proteins with an icosahedral symmetry, surrounds a single-stranded 7.2 kb RNA genome.[24–26]

To overcome the current limitations, *in vitro* systems expressing HEV-like particles have been developed. These baculovirus-based expression systems have fostered new research avenues to unravel the molecular, structural and immunological features of HEV infection. In particular, the nucleocapsid protein encoded by ORF2 is of particular interest. The N-terminus of this protein is a signal peptide followed by an arginine-rich domain that is well conserved. This capsid protein can be produced either in *Escherichia coli* or in insect cells and expressed as HEV-like particles by recombinant baculovirus. These virus-like particles of the capsid protein, with protruding spikes on the surface, can be analysed by cryoelectron microscopy and the crystal structure can be studied. As a consequence, Guu *et al.*[27] have shown that the capsid protein contains three linear domains (S, P1 and P2) that play different roles in organizing the icosahedral capsid. The S domain forms the capsid shell, and P1 and P2 contain the receptor-binding domains; the highly exposed P2 domain probably plays an important role in immunogenicity and virus neutralization. Using the same system, Yamashita *et al.*[28] have determined the crystal structure of HEV-like particles from a genotype 3 strain at a 3.5 Å resolution and found differences in the folding of the capsid protein compared with genotype 1. Furthermore, cellular receptor binding regions and epitopes for neutralizing antibodies were identified in the crystal structure of the HEV-like particles of the capsid protein. This 3D structure provides valuable information for understanding the mechanisms of virus entry and assembly. Lastly, Li *et al.*[29] studied the structure of the protruding domain of the capsid protein (domain E2s). This region harbours the neutralizing antibody-binding site and it seems that its dissociation abolishes antibody recognition. This domain appears to be essential for viral–host interaction and the propagation of the viral infection, and may be an important target for new antivirals.

Translational applications of the 3D structure of the viral capsid protein

An open question remains: how can we treat severe HEV infection today? Specific treatments are lacking and therapy is supportive. However, since the majority of infections are self-limited, specific interventions may be needed only in the infrequent severe, life-threatening cases. In ALF cases, an urgent liver transplant should be considered. However, available data about the outcome are scanty.[30,31]

An unresolved issue remains the treatment of persistent infection (and hepatitis) in immune-suppressed patients. Because chronic HEV infection has been reported only recently, the optimal therapy has not yet been established. In one case series from Kamar *et al.*,[32] three patients with chronic HEV after liver transplantation were treated with pegylated interferon-α: two of them reached a sustained virological response, while the third patient relapsed after the end of treatment.

What about vaccines? A Phase II study in volunteers of the Nepalese Army, organized by GlaxoSmithKline and the US Army, showed promising results.[33] The vaccine was a genotype 1 recombinant capsid protein buffered with aluminium hydroxide. The vaccination afforded a good protection, with an efficacy of 95% after three doses and 85.7% after two doses over a median follow-up of 2.5 years. Another interesting vaccine is the HEV 239, whose Phase II study results were reported recently.[34] Vaccine 239 is a recombinant HEV capsid protein that is truncated to 239 amino acids, expressed in *E. coli* and absorbed with aluminium hydroxide. The Phase II study evaluated safety and immunogenicity with a dose-finding design. With three doses of 10 μg or two doses of 20 μg, a 100% and 98% seroconversion rate was induced. Lastly, another vaccine from France, tested so far only in mice, uses the above-described approach of capsid protein expression in the baculovirus system.[35] HEV virus-like particles of the ORF2 gene products

(capsid protein) were injected intradermally into mice, either directly or as human papillomavirus (HPV) type 31 virus-like particles. Two ORF gene products were used: one expressing amino acids 112–660; and the other expressing amino acids 112–608. The ORF2 virus-like particles (112–660) in the HPV 31 appeared strongly immunogenic and evoked antibody responses in all mice at very high titres.

Taken together, the recent advances in the knowledge of the HEV life cycle and structural features of the HEV capsid protein, obtained by crystallographic analyses, facilitate vaccine and new drug developments. The next and even more difficult question will be how and where to use such vaccines. Undoubtedly, travellers from low-endemicity to higher endemicity areas should be protected. However, a real issue is how feasible can be a nationwide campaign in high-endemicity areas. Shouldn't the sanitation conditions be tackled first? Undoubtedly, a universal paediatric vaccination in high-endemicity countries would be the best and most effective solution, but is this realistic? In the most recent viral hepatitis reports of the WHO, it is emphasized that HEV vaccines should be developed, but how a global vaccine programme should be deployed is not discussed. And yet, as in the case of other examples of zoonoses that became human infections, the burden of HEV will certainly increase in low-endemicity areas with both autochthonous and imported viral strains.

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Transparency declarations

None to declare.

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