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Hepatitis E virus: a foodborne zoonotic virus threatening the immunocompromised patient

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Hepatitis E is an acute form of liver disease caused by HEV, an RNA virus infecting humans and a number of animal species among which swine may represent a main reservoir. Large outbreaks occur sporadically in developing areas due to waterborne genotype 1 HEV, but zoonotic and foodborne transmission of genotype 3 strains is increasingly reported in developed countries, where acute hepatitis presents mild symptoms and low mortality. Recently, g3 HEV has emerged as a significant threat for immunocompromised subjects, including solid organ transplanted patients, exiting into chronic liver disease, cirrhosis and high mortality. Transmission in these patients remains unknown.

The virus and its epidemiology

The hepatitis E virus (HEV) is a single-stranded RNA virus of the Family *Hepeviridae* (Meng, 2010; Smith *et al.,* 2014), and presents an unenveloped capsid made of a single protein encoded by the Open Reading Frame 2 (ORF2). The ORF1 codes for the proteins of the viral replication complex, which are cleaved post-translationally, and the ORF3 protein is still orphan of a definite function.

As other RNA viruses, the high mutation rate during viral replication is the main cause of the broad genome diversity of HEV, based on which field strains can be distinguished in at least 7 established genotypes. Four of these, namely genotypes g1 through g4, infect humans, and g3 and g4 strains are also known to infect several animal species, including swine, wild boar, deer, rabbits, and other wild species. These viral genotypes have been recently proposed to form the novel Genus *Orthohepevirus A* (Smith *et al.,* 2014). Based on the deduced ORF2 amino acid sequence and the high cross-reactivity in laboratory testing, it is assumed that despite genetic differences all g1-4 HEV strains belong to a single serotype. However, the lack of an *in vitro* seroneutralization test has hampered fully conclusive antigenic characterization of HEV strains.

In animals such as swine, HEV causes an asymptomatic or sub-clinical infection of the liver, although focal lesions in the hepatic tissue can be shown by histology and immunohistochemistry. Differently, in man infection may result in acute hepatitis with a wide range of severity, although in most cases disease is resolved in a few weeks without permanent liver damage (Aggarwal, 2011). Whereas the lethality rate of human hepatitis E is between 0.5 – 3%, in pregnant women the rate of fatal outcome may approach 30% of cases, which is apparently restricted to HEV genotypes g1 and g2. This severe form has been reported in endemic countries with low health standards and sanitation of Africa, Asia and Central America, where large waterborne epidemic outbreaks due to g1 and g2 HEV involving hundreds to many thousands of cases have been repeatedly shown to occur (Aggarwal, 2011).

No major outbreaks of foodborne HEV infection in man have been reported this far, although g3 HEV infection is widespread among farmed swine globally, and the presence of viral genomic RNA in liver and other pork products has been reported in several studies. Nonetheless, foodborne transmission has been implicated in sporadic cases and small outbreaks of hepatitis E, which are for the most part associated with g3 HEV strains genetically related to the strains infecting pigs. The reported co-clustering of nucleotide sequences of animal and human origins from a same geographical area further supports the zoonotic transmission of these viruses. Besides the implication of swine-derived food, specific recent investigations have shown a higher risk of acquiring acute hepatitis E among coastal populations of UK and other countries, which highlight shellfish as a possible additional risk factor via foodborne transmission, although higher recreational use of seawater might also be involved.

The diagnosis of infection

Limited replication of HEV in *in vitro* systems has been reported on either conventional or three-dimensional cultures, but adaptation of field viral strains towards efficient progeny virus production has not been fully achieved yet, and the infant piglet is still the only reliable albeit problematical animal model of HEV propagation *in vivo*. Diagnosis of infection in either man or animals is usually performed serologically by search of either specific IgM or IgG serum antibodies using commercial tests based on recombinant g1 or g3 viral capsid antigens. Tests using recombinant viral proteins can be easily adapted to analysis in different animal species and are largely independent of the infecting HEV strain due to broad inter-genotype antigenic cross-reactivity. RT-PCR and, particularly, RT-qPCR assays are also largely used for rapid virus detection in both research laboratories and hospital practice, although the limited duration of the viremic phase in both humans and animals and the large nucleotide variation displayed by HEV strains represent a challenge for molecular diagnosis. Viral shedding with stools has been largely documented in swine, and more than 30% of pig feces are commonly found to be HEV-positive at any time in most swine farms throughout Europe. However, HEV shedding has no prognostic significance in swine, and is hardly useful as an indicator of risk for either professional exposure or the food chain. Although hepatitis E still seems to have a low prevalence in humans within industrialized countries, the more sensitive serodiagnostic assays recently made available commercially have detected much higher seroprevalence in the Dutch, French and other western country populations, between 30 to 50% of normal blood donors (Slot *et al.,* 2013). This leads to hypothesize that silent infection or subclinical forms of disease may in fact be largely present in “non-endemic” areas of the world, which would fit with the high risk of foodborne transmission expected on the basis of g3 HEV infection prevalence in swine but underscored using human clinical indicators.

Further optimization of highly sensitive methods for detection of HEV contamination in either foodstuff eaten raw, including shellfish, vegetables and berry fruit, in addition to pork and

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game, or surface water is desirable in order to collect data for risk assessment. Similarly, surveillance systems of acute hepatitis should be implemented to include search for HEV markers on a regular basis, as is for other hepatitis viruses.

The disease in the normal patient

In the developed areas of the world including European countries, clinically forms of genotype 1 HEV infection are still predominant and occur as sporadic cases, mostly in subjects with recent history of travelling to endemic countries in other continents, but an increasing number of cases of acute g3 HEV infection have been reported in recent years. These latter are considered to be authochtonous, and a possible association with food at risk, particularly swine derivatives, has been discussed in several instances.

Although pediatric hepatitis E patients are known, the disease and infections particularly involve adult males, which is more evident among the populations of industrialized countries, suggesting the risk factors for infection do not normally invest children. The reasons for the higher frequency of disease among males are not known, but both professional and/or living behavior and sex-related physiological factors might have a role.

Similar to the g1 infection in the Western populations, human hepatitis caused by g3 strains is usually a self-limiting illness that lasts from a few days to weeks in the immunocompetent patient (Kamar *et al.,* 2012). The incubation time is normally 2-6 weeks and main symptoms range from nausea and fever to vomiting, abdominal pain, malaise, up to hepatomegaly, asthenia and jaundice that affect between 40 and 75% of patients. The alanine aminotransferase (ALT) level ranges broadly, but is more frequently between 1,000 and 3,000 IU/ml of blood, and no particular ALT change seems to depend on the infecting viral genotype.

On a clinical basis, hepatitis E can be misdiagnosed as a drug-induced liver injury, particularly at older ages, and attention must be paid to possible concomitance between the onset of symptoms and the administration of poly-pharmacological therapy.

Bioptic samples are not normally made available, given the benign evolution of human disease in most cases, and a detailed description of the pathological damage and pathogenetic mechanisms is still unavailable. Details on the progression of infection are mainly derived from the pig model of HEV experimental infection in a few animal studies conducted in the last decade, although their significance is somewhat limited by the absence of clinical symptoms in the pig.

In pregnant women, infection with g1 or g2 HEV strains is particularly aggressive, and for these otherwise normal subjects fulminant liver failure is a major cause of death, which is particularly high during the last trimester, together with obstetric complication. The reasons for such a high rate of negative outcome are still unclear, but it may be related to the status of immune tolerance against the fetus, which is associated with reduced T-cell activity and cytokine production during large part of pregnancy, and the down-regulation of antigen presentation, involving significant changes in the hormone profile, in particular progesterone and estrogen and chorionic gonadotropin (Kamar *et al.,* 2014).

Hepatitis E in the immunocompromised patient

Fatal and fulminant cases of hepatitis E are more frequent in subjects with underlying chronic liver disease, or in patients with active HIV infection. During the past few years, HEV infection has also been shown to possibly evolve into chronic hepatitis and cirrhosis in subjects with compromised health conditions, in particular organ transplant (kidney, heart, liver, kidney-pancreas, bone-marrow) recipients, hematological patients receiving chemotherapy, and patients co-infected with HIV (Kamar *et al.,* 2012). Under similar circumstances, g3 HEV infection can lead to an excess of mortality throughout acute or sub-acute liver failure, affecting up to 10% of cases. In all these cases, a significant reduction in the immune status parameters can be appreciated, due to either pathogenetic mechanism of the co-infecting agent or to the pharmacologically induced immunosuppression. In fact, chronic hepatitis E is rare among AIDS patients under cART treatment, most likely because therapy allows maintain the anti-HEV immune response at an effective level.

Remarkably, chronic infections have never been reported in association with HEV genotypes other than g3.

The transmission mechanisms of HEV in patients subjected to hematological or organ transplantation have not been completely elucidated, although fecal-oral transmission through consumption of food at high risk of HEV contamination, particularly raw and undercooked pork meat or products, seems to be as important as in case of acute hepatitis E among the normal population (Legrand-Abravanel *et al.,* 2010). Although food at higher risk of HEV contamination, such as undercooked pork or contaminated water, is unlikely to be part of the transplant patient diet, the new information gathered on the long shedding period of HEV, the probable protracted infectious status of normal subjects, and the apparent large circulation of HEV among asymptomatic subjects may altogether support a pre-infection with HEV as the cause of symptomatic acute hepatitis E and its chronic evolution in this part of the population.

On the light of recently demonstrated high seroprevalence among blood-donors, the possible role of blood transfusion and blood derivative administration in transmitting HEV may not be excluded, although a clear demonstration of this transmission route has not be provided yet. Chronic HEV infection, defined as the persistence of viral RNA in the serum or feces of the patient for at least 6 months (i.e. for > 3 months after infection) in transplanted subjects, evolves into chronic liver disease and cirrhosis in approximately 60% and 10% of cases, respectively, usually within 3 to 5 years after primary infection with HEV. Therefore, occurrence of acute hepatitis E in organ transplanted patients is being considered a major risk factor for sever liver disease that needs to be carefully considered, calling for the identification of effective prevention and control measures in this category of patients.

In a future, HEV vaccination might become an important pre-requisite for a more favorable prognosis of organ transplantation or for use in controlling hepatitis E in patients with concurrent liver or immunological disorders. Given the presently low prevalence of acute symptomatic disease in the normal population, it is more difficult to think that a large use of vaccination against HEV would be accepted. Phase III vaccine trials using a recombinant HEV vaccine have been conducted in China, showing both high safety and efficacy, and this vaccine was

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eventually licensed for human use, although at present limited to that country. Anti-viral drugs might also be important tools to be used during immunosuppressive treatment of transplant recipients or in case of acute hepatitis E onset. Noteworthy, a limited number of studies have reported that drugs such as ribavirin and microphenolic acid may be efficacious to contain HEV infection and its possible chronic evolution, indicating that implementation of efforts in development of antiviral chemotherapeutical protocols should be recommended.

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