Hepatitis E in Europe

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Etiology

Hepeviruses are nonenveloped viruses with a single stranded RNA genome of positive polarity containing three major open reading frames (ORF). The current taxonomy classifies Hepatitis E virus (HEV) as the sole member of the genus *Hepevirus* within the *Hepeviridae* family. Avian HEV is also classified as a member of the family *Hepeviridae*, but not assigned to a genus. Rat HEV represents an additional tentative member of the family. Phylogenetic investigations demonstrated clearly separated human pathogenic genotypes 1, 2, 3 and 4. Genotypes 3 and 4 are zoonotic and found in several animal species including domestic pig, wild boar and deer. HEV sequences found in rabbits show a high similarity to human pathogenic genotype 3. Additional HEV genotypes have been detected in wild boars from Japan, which are closely related to human genotypes 1-4. The recent findings of novel hepeviruses in bats, carnivores, like ferret, fox and mink, and moose underlines the necessity of a new taxonomic classification. A distantly related virus was identified in cutthroat trout (*Oncorhynchus clarki*) and related species.

Affected species (wildlife, domestic animals, humans)

Wildlife: Wild boar (*Sus scrofa*) were found to be frequently affected by HEV infection with genotype 3, but also with genotype 4, and represent the major reservoir for transmission to human. An additional reservoir with the potential of human transmission seems to be the Sika deer (*Cervus nippon*). HEV-like viruses of unknown zoonotic potential were molecularly detected in other terrestrial wildlife species, like rats, fox and moose. Further, antibodies to HEV have been detected in other wildlife species without parallel detection of HEV-specific nucleic acid. Thus, HEV-reactive antibodies has been detected in several rodent species (e.g. in Brazil and USA) and in primates in an outdoor breeding facility (Japan).

Domestic animals: Domestic pigs are susceptible to infection and the major animal reservoir. Seroepidemiological studies documented seropositive domestic pigs in several European countries. Molecular investigations confirmed infections by HEV genotype 3. In addition, genotype 4 has been detected in swine in China, Japan and India. Rabbits (*Oryctolagus cuniculus*) in China, Europe and the USA have been found to harbor a HEV which is closely related to the human pathogenic genotype 3 HEV. Avian HEV strains were detected to cause in chicken big liver and spleen disease (BLS) in Australia, and Hepatitis-Splenomegaly (HS) syndrome in the United States and Canada. HEV has been serologically detected in sheep (Spain, north India), goats (Spain, north India, China), cats (Spain), dogs (China, Brazil), horses (China), cattle (Brazil, China), ducks (China) and pigeons (China), but no HEV RNA was detected in those species. In addition, HEV-like viruses have been detected in ferret and mink husbandries.

Humans: Human HEV infections have been reported for almost all European countries. The seroprevalence in the human population differs between the countries, with prevalences of more than 10% in several countries. The differences of the reported seroprevalences might be, at least in part, due to the application of different serological assays. The number of recorded human cases seems to be quite low, but increasing. Chronic infections have been detected in immunosuppressed patients.

Imported infections by genotypes 1 and 2 and autochthonous infections almost exclusively by genotype 3 have been identified in Europe.

Epidemiological characteristics and disease course

Human: The epidemiologic characteristics of the human infections are determined by the causative genotype. In developing countries, hepatitis E is caused by infections with genotypes 1 and 2. The main transmission route by contaminated drinking water results in outbreaks with large number of cases. In developed countries an increasing number of autochthonous HEV infections have been recorded with a zoonotic transmission of genotypes 3 and 4. The main route of transmission seems to be exposure to pigs and consumption of undercooked meat from domestic pig and wild boar, although large numbers of autochthonous cases did not report about these risk factors. In addition, HEV has been demonstrated in commercially available pig liver and sausage. Additional transmission routes are consumption of shellfish and blood transfusions. The majority of cases are self-limiting; only in very rare cases chronic infections have been reported. These chronic infections have been found in immunocompromised persons, i.e. organ-transplant recipients, cancer chemotherapy patients and HIV-infected individuals.
Chicken: three different genotypes of avian HEV have been described with different geographical distribution: genotype 1 in Australia, genotype 2 in USA, and genotype 3 in Europe and China. Pathogenic and non pathogenic avian HEV have been described in several chicken flocks.

Clinical signs

Humans: HEV infection seems to be frequently asymptomatic. The incubation period of acute, self-limited hepatitis E is 2 to 10 weeks with a short prodromal phase and a symptomatic period lasting days to weeks. The prodromal phase is characterized by fever, anorexia, dysguesia, vomiting, nausea, bowel alterations, and abdominal pain and lasts. The “icteric” period symptoms include jaundice, hepatomegaly, and often a splenomegaly. Among hospitalized patients with hepatitis E, case fatality rates have been reported to be 0.5%-4%, but only 0.07%-0.6% in population surveys during outbreaks. For pregnant women a high attack rate and higher rates of occurrence of fulminant hepatic failure and death have been reported.

Domestic pig and wild boar reservoir: subclinical infections.

Rabbits: no disease reported.

Chicken: Depending on avian strains, increased mortality which could cause significant economic loss in poultry industry. Clinical signs include fatty liver hemorrhage syndrome, hepatitis and splenitis or blood-stained fluid in the abdomen and drop in egg production (up to 20%).

Cutthroat trout (Oncorhynchus clarki): no disease reported.

Gross lesions

Hepatitis E patients: hepatomegaly.

Hepatitis E in chicken: enlarged liver with hemorrhage and sometimes subcapsular hematomas and mild to severely enlarged spleen, and regressive ovaries.

Histological lesions

Hepatitis E patients: The histological lesions differ in acutely and chronically infected patients, after long-term infection. Several histopathologic features of acute hepatitis E have been reported including enlarged and apoptotic hepatocytes, acidophilic bodies, focal parenchymal necrosis, and polymorphonuclear leukocyte infiltrates in the lobules and enlarged portal tracts. However, in portal tracts lymphycytes represent the predominant cell type. In patients with severe liver injury submassive or massive necrosis and collapse of liver parenchyma have been detected. Focal hepatocyte necrosis was observed with prominent accumulations of mononuclear macrophages and activated Kupffer cells.

Hepatitis E in chicken: Liver lesions have been characterized microscopically to vary from multifocal hemorrhage to large areas of necrosis and hemorrhage and infiltration of heterophils and mononuclear inflammatory cells.

Differential diagnosis

Hepatitis A and other acute viral hepatitis.

Criteria for diagnosis

Human: serological detection of HEV-specific IgM antibodies or molecular detection of HEV is needed for diagnosis of acute infections, as clinical signs of hepatitis E cannot be distinguished from that of hepatitis A and other hepatitides.

Chicken: RT-PCR for detection of avian HEV in acute diseased animals, for retrospective studies and flock screenings ELISAs using *E. coli*-expressed recombinant avian HEV antigen might be applied for serological detection of anti-HEV IgG antibodies.

Wild boar and domestic pig: Although infections in these reservoirs are asymptomatic, animals might be screened for HEV infection to determine the prevalence or to identify infection risks. Diagnosis by (real-time) RT-PCR and/or serological detection; identification of the virus genotype and strain requires RT-PCR and subsequent sequence determination.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

(a) Serological detection of HEV-specific antibodies

- immunoblot assay: commercially available line assay using recombinant proteins of selected HEV genotypes for detection of HEV-specific IgM and IgG antibodies in humans
- ELISA: commercially available and in-house assays using recombinant antigens for detection of IgM and IgG antibodies in humans and swine. In addition, host species-independent ELISAs are available. Usually bacterially or baculovirus-expressed ORF2- and ORF3-derived gene products are used as antigens

→ Samples: blood, serum (from live individuals), stored at -20 °C. The minimum amount of serum or blood needed for the assays is usually 10 - 50 µl.

(b) Molecular detection of HEV

- conventional RT-PCR: mostly RT-PCR assays targeting the ORF2 are used, for detection of unknown HEV-like viruses broad-spectrum consensus RT-PCR assays were developed targeting a conserved region within the ORF1
- real-time RT-PCR: both Sybr Green- and probe-based real-time RT-PCR assays have been developed targeting ORF 2. Commercially available assays exist.
- reverse transcription loop-mediated isothermal amplification (LAMP) targeting ORF3

→ Samples: Blood, liver tissue, bile or fecal suspension (10%PBS) is needed for RNA isolation. In general the viral load is significantly higher in liver tissue, bile and fecal sample compared to blood/serum. The samples should be stored at -20 °C and the RNA at -70 °C.

(c) Virus isolation

Virus isolation from RNA positive samples is rarely obtained in hepatoma cell lines.

APHAEA protocol (for harmonization at large scale)

The protocol depends on the diagnostic or research question. For standardized serological investigations the use of commercial ELISAs is recommended. Virus RNA detection should use real-time or conventional RT-PCR assays; for molecular epidemiological investigations conventional RT-PCR assays and sequence analysis should be performed.

Laboratories that can be contacted for diagnostic support

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Recommended literature

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