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Diagnostics and importance of hepatitis E virus infections

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Abstract: The diagnosis of hepatitis E virus (HEV) infections has been recently substantially facilitated by the introduction of a whole range of new different virological assays. The increasing appearance of sporadic cases of acute hepatitis E in Germany directed the focus toward the zoonotic transmission route of the virus. The recognition of HEV genotypes differing in virulence and in pathogenic potential is not only relevant for epidemiology and the course of the disease, but also for the development and choice of diagnostic tools. A broad variety of enzymatic and protein-based assay formats detecting anti-HEV IgG or IgM antibodies directed against the different genotype variants of HEV is available (ELISA, LIA, Western blot); however, sensitivity and specificity of these assays differ notably. Today's state-of-the art technology that permits fast and reliable assay-based confirmation of HEV infections is PCR. The newly developed commercially available PCR kits will detect all four human pathogenic HEV genotypes. Further subdivision and discrimination can be achieved by sequencing, although this approach is only reasonable in the setting of specific epidemiological demands. Detection of viral antigens, cell culture, and T-cell assays are of no practical importance in a routine diagnostic setting. New insight into the pathogenesis and its clinical relevance for defined groups of patients (immunosuppressed) as well as the implementation of specific antiviral and prophylactic therapies (vaccination) will further challenge the performance of existing assay

formats and increase the technical demands for the diagnostic laboratory.

Keywords: antibody test; diagnostic methods; hepatitis E virus; RT-PCR; virus detection.

Introduction

As a result of increased attention and new, improved diagnostic methods, the number of reports received by the Robert Koch Institute about acute hepatitis E virus (HEV) infections has been rising every year. Epidemiological studies suggest, however, that not every symptomatic hepatitis E disease in Germany is detected and reported, and that the majority of infections are subclinical in their progression. Following oral intake of particles, and depending on the genotype and pre-existing conditions, the virus can cause acute or chronic hepatitis. Acutely ill patients exhibit typical clinical signs of liver inflammation, such as fever, nausea, upper abdominal pain and jaundice. Until a few years ago, HEV infections in Germany were deemed a rare disease, exclusively linked to travel history. Today we know that most of the infections occurring are acquired autochthonously [1, 2].

This paper is to reveal the current state of knowledge about hepatitis E virus infections and the options provided by virological diagnostics.

Genome organization and variability

The hepatitis E virus is a small, non-enveloped, single-stranded (+) RNA virus. The genome of the virus is formed by approx. 7200 nucleotides, and it comprises three open reading frames (ORFs) [3]. ORF1 is the largest reading frame and encodes a functional polyprotein, which consists, among other domains, of a methyltransferase, protease, helicase and polymerase [4]. ORF2 encodes the viral

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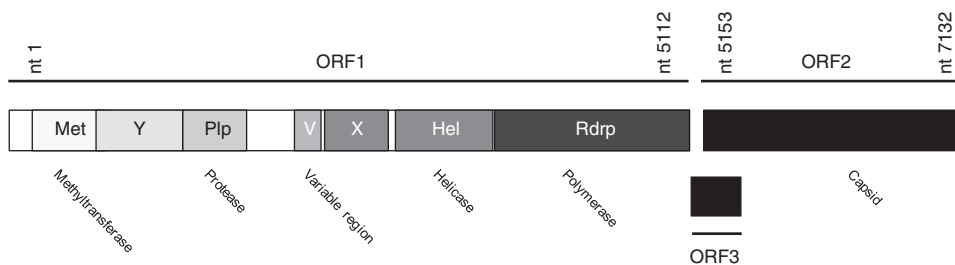


Figure 1: Overview of the genomic structure of the hepatitis E virus (example of nucleotide positions (nt) according to the genebank entry L08816 [6]).

capsid protein. ORF3 is the smallest reading frame, and many different functions are attributed to it [5] (Figure 1). Taxonomically, the hepatitis E virus is currently assigned to the Hepeviridae family as the only species of the Hepevirus genus. In total, the four human pathogenic genotypes (HEV 1-4) are divided into (at least) 24 subtypes [7]. This heterogeneity plays a role on several levels: Initial studies show that the variable region V of the HEV genome seems to affect the tropism and pathogenicity of individual genotypes [8, 9]. On the nucleotide level, the variability (up to 25% difference between the genotypes) poses a challenge in the development of NAT test systems, which ideally should cover all genotypes with the same sensitivity. This genetic variability also creates some differences in the protein sequence, resulting in a genotype-specific antigen reaction in patient samples of serological tests (see below).

Hepatitis E in Germany – a zoonotic disease

Knowing about the different characteristics of HEV genotypes is also very important in the diagnosis and treatment of hepatitis E. One of the main differences of HEV genotypes is their transmission. Genotypes 1 and 2 infect several million people in Asia and Africa every year [10]. Both genotypes are transmitted via the fecal-oral route with contaminated water and from person to person. The seroprevalence in the tropical areas of Africa and Asia is estimated to be approximately 50% [11]. In Germany it is especially genotype 3 that causes infections (autochthonous cases). Genotypes 3 and 4 have been detected both in humans and in pigs. The consumption of undercooked pork (e.g. sausages) and contact with infected animals are considered the most frequent transmission routes [12, 13]. Person-to-person transmission is irrelevant for genotype 3. Thus, a genotype 3 infection represents a zoonotic disease, endemic in Germany.

Epidemiology

The global burden of disease is concentrated in the tropical areas of Africa and Asia, with over 3 million people affected each year (genotype 1 and 2 infections). There are regular epidemic infection clusters. Mortality is reported to be about 2%. A higher risk of a fulminant progression of genotype 1 and 2 infections affects pregnant women and patients with a compromised liver function [9, 14].

In 2014, 670 HEV cases were reported to the Robert Koch Institute [15]. The majority of these genotype 3 infections were acquired in Germany (2013: 84%) (Figure 2). Children are less likely to become ill; fulminant infection progression after genotype 3 infections is the exception also for pregnant women. Seroprevalence studies with newer antibody tests (see below) have found in approximately 17% of adults in Germany anti-HEV IgG antibodies as signs of an acquired – albeit often asymptomatic – HEV infection [16]. This corresponds to estimated 250,000 new HEV infections annually. Seroprevalence, calculated incidence and reporting statistics give rise to the well-founded assumption that less than one in 100 HEV infections is clinically apparent in Germany.

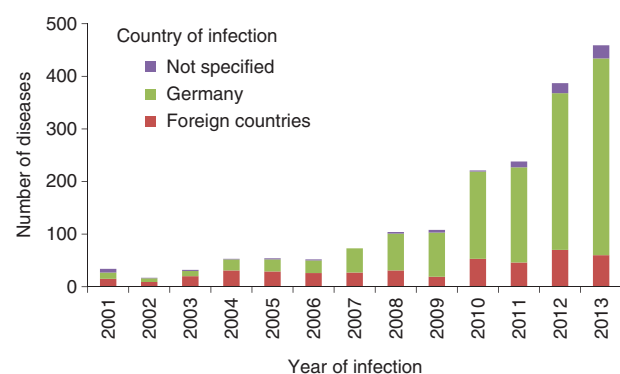


Figure 2: Reporting statistics of hepatitis E cases in Germany (Source: Infectious Disease Epidemiology yearbooks, Robert Koch Institute).

Symptomatology

The vast majority of HEV infections are asymptomatic.

Typical signs of a symptomatic HEV infection after an incubation period of up to 60 days include: fever, fatigue, nausea/vomiting, jaundice, itching and upper abdominal pain with known biochemical changes (above all, increases in transaminases and bilirubin, as well as in alkaline phosphatase and gamma GT) [1, 14]. Atypical symptoms such as arthralgia and myalgia and extrahepatic manifestations (especially neurological symptoms such as Guillain-Barré syndrome) have been reported [17]. Hepatitis E is usually a harmless and self-limiting infection.

Chronic HEV infections are described in immunocompromised patients after organ and stem cell transplantation, while they are rare in HIV patients [9]. Currently, the chronification of HEV infection is defined as a virus persistence of more than 3 months. It can lead to liver cirrhosis after a short period of time [18, 19]. Chronic progressions are described only for genotypes 3 and 4, and not for genotypes 1 and 2.

Blood donation

There is no definitive conclusion yet about the possible transmission of HEV through blood transfusions. Due to the “limited pathogen virulence”, HEV testing is deemed unnecessary for immunocompetent patients, but the possible chronification of the infection is seen as a problem in immunocompromised patients (following organ or stem cell transplantation) [20]. This is expected to be reevaluated once new research findings on the benefit of the HEV testing of blood products are available [21].

Diagnostic methods

Patient history, clinical and laboratory methods do not allow for a clear differentiation of HEV infections from hepatitis cases of a different origin. Therefore, the diagnosis is based on various direct and indirect detection methods. In general, the diagnosis of an HEV infection is asserted serologically. Molecular detection methods (PCR) can be used for confirmation, for the quantification of the viral load and for genotyping. The increasing report numbers confirm that it is necessary to consider the possibility of an HEV infection in the presence of corresponding symptoms and once other causes have been ruled out.

Antibody detection

Methods

Tests for HEV-specific antibodies in the patient serum are available commercially today in various test formats. Common methods are: Western blot, line immunoassay (LIA), enzyme linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA) [22]. Most of these tests are based on recombinant antigens of ORFs 2 and 3, which, depending on the manufacturer, utilize different genotypes. In particular, the C-termini of the viral proteins encoded in ORF2 and 3 have been shown to be highly immunogenic [6]. Confusion arises from the marked differences in diagnostic sensitivity and specificity of different hepatitis E ELISAs and immunoblots [23]. Studies in Germany reflect these problems as well, as seroprevalence estimates vary depending on the test manufacturer [24] (Table 1). While only one serotype is postulated for human pathogenic hepatitis E viruses, there is still indication of different, genotype-specific antibody reactions. Several studies from Europe and Asia suggest that the genotype of the antigen used may have a significant influence on the sensitivity of a test, and therefore it is recommended that the local distribution of HEV genotypes should be taken into account when selecting a test kit [23, 27].

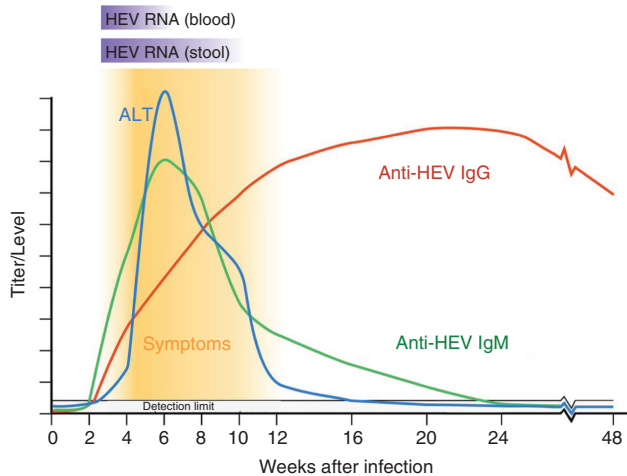
Antibody classes

The detection of IgM and IgG is suitable for the diagnosis of a new or past HEV infection in immunocompetent patients. In combination, a sensitivity of up to 99% can be reached for an acute HEV infection. IgM antibodies are detectable shortly after the onset of symptoms, and subsequently for a period of 3–5 months. Shortly after the first detection of IgM, the IgG titer increases as well and can then be detected as a titer indicative of a past infection for several years [14, 28] (Figure 3). A solitary IgM should be verified or supplemented by a PCR analysis, since IgM is considered a very sensitive marker of acute hepatitis E, but its specificity is still subject to critical discussions in the literature [29, 30]. In the context of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infections, false positive results for anti-HEV IgM antibodies are frequently observed [31]. The analysis of IgA has been proposed in the literature as an alternative to IgM or as a possible addition to serological diagnosis [32]. This is to increase specificity and allow for a new HEV infection to remain detectable over a longer period of time. However, commercial test

Table 1: Overview of IgG seroprevalence rates detected by different commercial hepatitis E virus antibody assays.

Name	Manufacturer	Seroprevalence		
		Southern Germany ^a	Southern England ^b	Southern France ^c
MP Diagnostik HEV IgG ELISA	Genelabs technology	4.5%	3.6%	16.6%
Axiom Diagnostics HEV IgG EIA	Wantai	29.5%	16.2%	52.5%
RecomLINE HEV IgG immunoblot	Mikrogen	18%	–	–

^aWenzel et al. [24], ^bBendall et al. [23], ^cMansuy et al. [25, 26].

**Figure 3:** Schematic of a hepatitis E infection (adapted from Krain et al. [9]).

systems available up to now can be used only to determine antibodies of the IgG and IgM classes.

Nucleic acid diagnostics

Real-time PCR

Thanks to new sensitive PCR methods, the HEV genome detection succeeds in around 90% of all acute HEV infections. HEV-RNA can be detected in blood 2–4 weeks and

even up to 6–8 weeks in stool after the onset of symptoms [28]. The success of genome detection through NAT detection systems still requires that samples of viral RNA be transported correctly and that samples be taken at the appropriate time. The nucleic acid of the hepatitis E virus can even be detected in biopsy material. This might play a role in the future, for example in diagnosing chronic hepatitis E after transplantation and immunosuppression [33]. Modern PCR systems based on real-time methods have been optimized for several genotypes. In some cases, they can detect the HEV genome in patient material also quantitatively [34]. This specific issue is addressed by several commercially available test kits (Table 2), but the use of so-called “in-house” PCR systems is widespread. Inter-laboratory testing of all samples and methods shows, overall, an average rate of >92% in terms of correct test results. However, it seems that commercial assays perform slightly better than in-house systems. This high success rate also applies to samples with rather low virus concentrations between 10^3 and 10^4 IU/mL [35]. A “loop-mediated isothermal amplification”-based test has been developed for countries with limited resources [36]. Given the various genotypes, there are differences in sensitivity and, thus, also quantification in a large number of PCR assays used. A WHO standard (genotype 3), which was released recently, represents an important step towards the harmonization and comparability of PCR assays used throughout the world [37]. The question whether genotype-specific detection and/or type differentiation could become relevant outside the scope of epidemiology is hard to answer at this point.

Table 2: Overview of commercial hepatitis E virus PCR detection kits.

Detection kit	Manufacturer	Specifications (according to manufacturer)
Real Star® HEV RT-PCR Kit	Altona Diagnostics	CE-IVD, detection limit: 0.31 IU/μL eluate
AmpliCube HEV	Mikrogen	CE-IVD, detection limit: >10 ⁴ copies/mL; 100%
COBAS TaqScreen HEV	Roche	Detection HEV 1–4, detection limit: 19 IE/mL
Hepatitis E Virus Assay	Ceeram Tools	Detection limit: >1–10 copies/PCR solution
HEV Real Time-PCR Kit IVD	Geno-Sen's	Detection limit: 80 copies/mL (for RotorGene)

Sequencing

The sequencing of the hepatitis E virus genome provides an insight into the epidemiology of hepatitis E. Thus, it has been possible to map the geographical distribution of the genotypes [7] and confirm transmission from pigs to humans in some cases of autochthonous HEV infections [13]. The sub-genotyping and phylogenetic analyses necessary to address these questions are mainly performed in laboratories with certain epidemiological specializations.

Special diagnostics

Antigen detection

Studies done in Asia have shown that the antigen detection from serum and stool constitutes a sensitive method during the viremic phase of an acute hepatitis E. Especially when resources are limited, or in outbreak situations, this method may be a diagnostic alternative to genome detection [38]. To what extent this can be applied to tests in industrialized countries with HEV infections of a different genotype and different epidemiology, and whether it is suitable for routine processes (such as for blood donations in Germany), has not been revealed yet [21, 39].

Histology

In clarifying an unclear case of hepatitis, a liver biopsy may sometimes be taken, which, to an experienced pathologist, may indicate the presence of an HEV infection. Immuno-histochemical staining of HEV antigen (especially the capsid antigen) has been described experimentally, but has not yet found its way into routine pathological diagnostics – particularly, as rising awareness of hepatitis E has often caused diagnoses to be made more quickly by serological or molecular-biological tests [40].

Virus-specific T-cells

Meanwhile it also possible to detect hepatitis E virus-specific T-cells in patients with a new infection and to correlate their activation and cytokine release with the progression of the condition [9, 41]. The extent to which these findings may be diagnostically relevant to the prognosis for immunocompromised patients has yet to be evaluated.

Direct virus detection

Experimentally, the virus can be detected directly following the inoculation and infection of primates or pigs. Recently it has also become possible to grow the virus in cell culture (PLC/PRF/5, A549) deploying positive patient material [42, 43]. However, these cell culture systems are not used for diagnostic purposes, but rather help explore the molecular biology of the virus and possible treatments options.

Treatment and prevention

Clinically apparent HEV infections are treated purely on the basis of symptoms. A collection of case reports, recently published by Kamar et al. [44], provides proof of the possible HEV elimination (genotype 3) in connection with ribavirin therapy in the case of organ transplant recipients. Pischke et al. [45] have confirmed the effectiveness of a ribavirin therapy in connection with HEV infections. Therefore, drug treatment should also be considered in the rare cases of HEV-associated, fulminant liver failure.

In recent years, a genotype 1 subunit vaccine against HEV was developed and approved in China (Hecolin®) [46]. The WHO Strategic Advisory Group of Experts on Immunization (SAGE) has not yet issued a general recommendation for the use of the vaccine; there have not been sufficient studies yet to assess its benefit. Only in the case

Table 3: Varying significance of different HEV genotypes.

	Genotypes 1 and 2	Genotype 3 (and 4)
Spread	Tropics of Asia and Africa	Western industrialized countries (genotype 4 also sporadically in Asia)
Transmission	Drinking water	Pork
Seroprevalence	50%	17% (Germany)
Clinical progression	Asymptomatic/acute	Asymptomatic/acute/chronic
Risk groups	Pregnant women – fulminant progression	Immunocompromised/chronically ill patients – chronic progression

of infection clusters (outbreaks) can the use of the HEV vaccine be considered, according to SAGE [47].

Conclusions

The data collected in recent years on the hepatitis E virus suggest that there are considerable variations between the different hepatitis E virus genotypes with respect to epidemiology, transmission and clinical progression (Table 3).

This genetic heterogeneity was also taken into account during the further development of immunological and molecular-biological HEV test methods. This will now allow one to detect the infection with certainty, provided one keeps in mind the differential diagnosis of hepatitis E.

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