ACCEPTED MANUSCRIPT

Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article: CURRENT KNOWLEDGE ON HEPATITIS E VIRUS INFECTION

Authors: Roman Pepovich*, Magdalena Baymakova†, Maria Pishmisheva‡, Plamen Marutsov§, Liliya Pekova§, Ilia Tsachev§; Vojnosanitetski pregled (2017); Online First November, 2017.

UDC:

DOI: https://doi.org/10.2298/VSP170815159P

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
Current knowledge on *Hepatitis E virus* infection

Roman Pepovich*, Magdalena Baymakova†, Maria Pishmisheva‡, Plamen Marutsov§,
Liliya Pekova§, Ilia Tsachev§

* University of Forestry, 1797 Sofia, Bulgaria; † Military Medical Academy, 1606 Sofia, Bulgaria; ‡ Hospital of Pazardjik, 4400 Pazardjik, Bulgaria; § Trakia University of Stara Zagora, 6000 Stara Zagora, Bulgaria

**Correspondence to:** Magdalena Baymakova, MD, PhD; Department of Infectious Diseases, Military Medical Academy, 1606 Sofia, Bulgaria; Phone: +359 898 767594; E-mail: dr.baymakova@gmail.com
Abstract

The knowledge about epidemiology and genomic characteristics of Hepatitis E virus (HEV) have developed and changed over the past 30 years. HEVgt1 and HEVgt2 are basically met in South-East Asia and Africa and their transmission is by waterway. HEVgt3 and HEVgt4 are mainly established in Europe and North America, they have native character and transmitted by consummation of contaminated food. The current article presents systematic analysis of the epidemiology, etiology, clinical signs, diagnosis, therapy and prevention of HEV infection.

Key words:

hepatitis E virus; prevalence; mechanism of transmission
Introduction

Hepatitis E is an emerging viral disease affecting both humans and different kinds of domestic and wild animals. In developing countries, human HEV has trend for epidemic spread with benign outcome, except pregnant women \(^1\). The death rate due to HEV exceeds 25\% in the third trimester \(^1\). In developed countries, the autochthonous cases of human HEV infection are associated with a consummation of poorly heat-treated meat and meat products (mostly domestic and wild swine) \(^1\).

The current article presents systematic analysis of the epidemiology, etiology, clinical signs, diagnosis, therapy and prevention of HEV infection.

History

Hepatitis E is „recognized” in 1980 during the epidemic in the valley of Kashmir (India) \(^2\). The affected people were between 11–40 years old, native citizen of the valley with common source of water \(^2\). The infected area was characterized with high level of viral distribution and mortality among pregnant women \(^2\). The epidemic spread, the incubation period, clinical signs and biochemical results of the examined patients were similar to manifestation of Hepatitis A virus infection \(^2\). A few months later Wong et al, published results from retrospective serological study of stored samples from a large hepatitis epidemic in Delhi, India (1955–1956) and two smaller infected areas in Ahmedabad, India (1975–1976) and Pune, India (1978–1979) \(^3\). The results from that study established a few cases of acute hepatitis B and none acute hepatitis A \(^3\). Owing that fact was given the idea for existence of „non-A, non-B hepatitis agent” \(^3\). The next serious
breakthrough was in USA (1997), when was found a swine virus, named “swine hepatitis virus”\(^4\). At the same time has been described the first case of human HEV, the isolated virus had similar genomic characteristics to the swine HEV\(^5,6\). That disclosure determines the zoonotic character of the virus\(^1\).

**Etiology**

Hepatitis E virus belongs to family *Hepeviridae*, genus *Hepevirus*\(^7\). According to the current classification, family *Hepeviridae* is divided into two genera: *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* includes four species\(^8\):

- **Orthohepevirus A**: isolated from human, swine, deer, mongoose, rabbit, camels;
- **Orthohepevirus B**: isolated from birds;
- **Orthohepevirus C**: isolated from rats, big Indian rat, Asian kind of mole, ferret and mink;
- **Orthohepevirus D**: isolated from bats.

Until now, there are four main genotypes, with more than 24 subtypes and only one serotype\(^9\)--\(^11\). Genotypes 1 and 2 (HEVgt1 and HEVgt2) are linked with large human epidemics in countries with poor hygiene\(^12\). Genotypes 3 and 4 (HEVgt4 and HEVgt3) infect humans and other mammals, which cause sporadic cases of Hepatitis E in industrialized countries\(^12\).

HEV is a small virus with a diameter approximately 27 to 32 nm, icosahedral symmetry, spherical shape and simple structure\(^12\). The virion contains single positive-stranded RNA with size 7.2–7.5 kb\(^13\). HEV genome includes 5′ untranslated region (UTR), three opened-reading frames (ORF1, ORF2 and ORF3) and 3′ UTR, followed by poly-A tail\(^13\). Each one reading frame has different functions\(^12,13\).
• ORF1: is situated next do 5′ and encodes non-structured proteins with enzyme function (Methyltransferase, Papain-Like Cysteine Protease, Macrodomain, Helicase and RNA-dependent RNA Polymerase);

• ORF2: is situated next do 3′ and encodes viral capsid protein, build from 660 amino acids, which is responsible for the viral cutting, interaction with target cells and immunogenicity properties;

• ORF3: encodes small protein, build from 113-114 amino acids, which is responsible for replication and building cytoskeleton, also decreases inflammatory response and protects viral-infected cells.

**Epidemiology and prevalence**

Hepatitis E is an endemic disease for Central and Southeast Asia, for tropic and subtropical countries in Africa and Central America. In the endemic area large waterborne epidemics were described. Hepatitis E is sporadically reported in USA and Europe. In the developed world, it has been thought that the infection was associated with traveling to the endemic regions. But in nowadays it is known that it is a local, autochthonous transmission.

HEVgt1 and HEVgt2 are responsible for enterically-transmitted epidemics in tropical and some subtropical areas. They are associated with contamination of water (water supplies) and poor sanitation conditions. Both genotypes cause acute hepatitis in humans, the virus is found in feces, an environment contaminated with human’s feces. A study in Uganda showed that environmental factors could be much more important for transmission, than it was thought until now.
In non-endemic regions the mechanisms of transmission are much less known, in contrast in endemic areas the contaminated water is a documented source of infection. HEV is the only one among other hepatotropic viruses with zoonotic character and animal reservoir. The literature search presents that most of the autochthonous human cases are associated with the consumption of raw and undercooked meat infected with HEV. Pigs are considered to be the main reservoirs for HEVgt3 and HEVgt4, and the two genotypes are found in pigs all over the world. Antibodies against HEV are found in chickens, dogs, rodents, cows, sheep, goats, monkeys and other animals. HEVgt3 is responsible for most of human HEV infections in Europe, North America and East Asia. HEVgt3 is dominated in swine samples in Europe and America. The virus was detected in pork products. Strains of HEVgt3 were recently found in pigs in Africa. In 1998 HEVgt4 was responsible for sporadic human HEV cases in Taiwan, and after that the virus was found in pigs at the same geographical area. In China, HEVgt4 is the most common virus in humans and swine. Also it is endemic in Japan. In Europe, Japan and USA, specific antibodies against HEV are often detected in domestic pigs, which prove their role as a source of HEV infection. Studies done in Japan and France presented the transmission of the virus through a consumption of meat and sausages, made of domestic pigs, wild boars and deer. Acute hepatitis E was described after eating pork meat infected with HEVgt4 in Japan. In Japan was reported severe human case after eating raw liver from a wild boar, whereas in Europe the severe human infections were related to a consumption of pork meat. A phylogenetical analysis of HEV samples from Japan indicated a previous transmission of the virus from domestic pigs to wild boars. Urine was identified as a possible source for swine HEV infection. It has been established that swine HEV could pass colostrum, while transplacental transmission is arguable.
possible way of HEV transmission is the direct contact between people and swine \(^1\). Serological studies in USA reported that veterinaries and people, who are working in slaughterhouses had high positive results for anti-HEV IgG compared to population with lower risk for direct contact with pigs and pork products \(^{37}\). A higher rate of seroprevalence among foresters was found in comparison with the seroprevalence among blood donors \(^{1,21}\). HEV infection could be transmitted by transfusing blood and blood products \(^{38}\). Swine products, such as swine heparin and others, used in human medicine, could be a risk factor for HEV spread \(^{39}\). Other possible risk for HEV source could be feces or manure \(^{40}\). A study reported the presence of HEV in manure storage facilities \(^{40}\).

Nowadays, wild boars are thought to be an important natural reservoir for HEVgt3 and HEVgt4 \(^1\). Recent study done across Asia and Europe showed a high rate of HEV seroprevalence likewise a molecular evidence of HEV infection in wild boars \(^{1,12,21}\). Takahashi et al found HEV RNA in 1.1–13.3% of examined wild boars and seropositivity varied between 4.5% to 34.4% \(^{41}\). In Germany, wild boars are considered as one of the main sources for HEV transmission \(^{42}\). HEV RNA could be found in the serum, gall and liver from wild boars \(^{43}\). HEV samples collected from the wild boars showed great genetic variability \(^{9,11}\).

In many European countries different serological studies for human HEV seroprevalence were conducted over the past years. We present the results of HEV seropositivity in blood donors from 24 studies (Table 1) \(^{44-67}\). The calculated Mean±SD human HEV seroprevalence is 15.21±14.20 (95%CI = 12.61–43.04). The great variety of positive results are affected by geographic location, national traditions and customs, design of the study, year of projects conducted and type of diagnostic tests. Nevertheless of the
published diversity, these data confirmed the seroprevalence of HEV among blood donors in different European countries.

In worldwide, the main animal reservoir for HEVgt3 and HEVgt4 are domestic pigs and wild boars \(^1,^6,^8\). Data for swine HEV seroprevalence in European countries are summarized in Table 2 \(^32,^67,^69–^78\). There is a broad spectrum of variety in seropositivity among different countries. The evaluated Mean±SD swine HEV seroprevalence is 47.93±19.75 (95%CI = 9.23–86.64). The presented average percentage for seropositivity illustrates the existence and persistence of the virus among pigs and their potential animal reservoir.

**Clinical manifestation**

The most common clinical manifestation of HEV among people in endemic areas is acute icteric hepatitis with typical clinical and laboratory signs \(^14\). Sometime prolonged cholestasis could be developed or asymptomatic infection may occurred \(^14\). High rate of fulminant hepatic failure and death were mentioned among pregnant women in hyperendemic areas \(^79,^80\). In non-endemic areas the virus affected mainly elderly men, presence of accompanying liver diseases and alcohol abuse \(^81–^83\). The autochthonous cases could be manifested as an acute hepatitis, asymptomatic infection, and nonspecific symptoms with anicteric diseases \(^48\). In contrast, severe illness does not show during pregnancy in non-endemic regions \(^81,^82\). Chronic HEV infection has been described in solid-organ transplant recipients, patients with hematological diseases, HIV patients, people under immunosuppressive conditions and anticancer chemotherapy \(^81,^82,^84–^86\). In such case of patients the liver biopsy illustrated liver fibrosis, which predicts the progress to cirrhosis \(^87\).
Swine HEV infection does not present with typical clinical symptoms and signs. Usually animals’ diseases are characterized with fluctuations in body temperature or body weight \(^1\). After a subclinical HEV infection mild microscopic lesions in the liver could be developed \(^{11}\). Pathological findings include viral antigen in the hepatocytes, positive immunohistochemical changes in the small and large intestines, lymph nodes, tonsils, spleen and kidneys \(^{12}\). Spanish study reported no correlation between HEV RNA and the histological changes in the liver \(^{88}\). So it’s arguable whether or not a natural HEV infection causes any histological changes in the liver.

**Laboratory diagnostics**

The most common method for routine diagnosis of HEV infection is serological examination. Laboratory diagnostics use serum samples for detection of HEV antibodies by enzyme-linked immunosorbent assays (ELISA) and western blot assays \(^1\). The tests estimate the presence of antibodies of class IgM and IgG (rarely IgA) against HEV. In the first stage of the infection antibodies of class IgM appear and mark acute present infection \(^1\). After that antibodies of class IgG follow up and show a recent or past infection. Serum samples are collected for serological tests in humans, for swine examination it could be collected sera or meat juice \(^{89}\). In humans, anti-HEV IgM levels peak around the time of the ALT peak and may persist up to five months after the onset of the illness (Figure 1) \(^{14}\). A little later anti-HEV IgG begin to produce, they remain during the acute phase, the recovalescent period and also maintain high levels at least one year after the recovering (Figure 1). Commercially available immunoassays differ substantially in their sensitivity and specificity, and the false-positive results varying from 0.3% to 2.5% \(^{90,91}\).
The majority of pigs are naturally infected with HEV at the age of two to four months. Eighty six percentage of pigs are naturally infected with the virus until their eighteenth week. The maternal antibodies decline at the age of 8 to 10 weeks. After the reduction of them, the piglets could be attacked by the virus around the second weeks after birth. Swine HEV infection is accompanied with a transient viremia lasting one to two weeks and a fecal-oral emission of the pathogen continuing three to seven weeks. The number of viremic pigs increase from nine weeks with peaking around 15 weeks, following decline to slaughter age. Seroconversion of anti-HEV IgM, which is related to the peak of the virus excretion through feces, is followed by seroconversion of anti-HEV IgG with the highest concentrations at the age of four months (Figure 2). Interestingly enough, the presence of antibodies does not always assure the absence the virus because HEV RNA and the anti-HEV antibodies are found in pigs together. This leads to the conclusion that these animals are HEV-reservoirs.

Nowadays, the detection of HEV RNA using molecular-genetic methods is considered as the „golden standard” in the laboratory diagnosis. The detection of RNA is performed by different RT-PCR methods, amplifying genomic fragments in one of the three ORFs. HEV RNA could be found in patients’ blood and/or feces in the prodromal period, after that the virus could be detected in feces for another two weeks. The viremic period is very short, wherefore HEV RNA not always could be found in sera. The presence of HEV RNA is a definitive marker for a current infection.

Recently, the establishment of HEV antigen is introduced as an early diagnostic method. However, the test has a low sensibility compared to methods that use the amplification of nucleic acid. The presence of HEV antigen in different swine tissues using immunohistochemistry was recently demonstrated. The detection of HEV antigen
in liver tissue representing a valuable tool for the viral establishment in biopsy, autopsy and explant liver tissues\textsuperscript{98}.

\textbf{Therapy and prophylaxis}

There is no specific therapy for acute HEV infection in humans, because in most cases the illness is self-limiting. The management of acute illness in immunocompetent patients include a strict diet, administration of fluids and hepatoprotective medications. In case of acute liver failure intensive care treatment is required and sometime liver transplantation needs \textsuperscript{99}. In chronic HEV infection the administration of Pegylated interferon alpha-2a/alpha-2b or Ribavirin for 3–12 months were applied as specific antiviral therapy \textsuperscript{100,101}. A recombinant vaccine showed 94–100\% efficacy in a phase III study of >100 000 Chinese adults \textsuperscript{102}. The vaccine protected from HEVgt1 and HEVgt2 \textsuperscript{102}.

There are no specific therapeutic medications for animals. Swine HEV vaccine has not been developed yet.

The prevention measures are guided to improving sanitation and hygiene in developing countries \textsuperscript{17}. In developed countries, population with high risk could be informed for the virus and his zoonotic characteristic, consequently should be asked to reduce and/or avoid consummation of raw or undercooked meat and meat products from pigs, wild boars, deer and direct contact with infected animals.

\textbf{Conclusion}

Swine are defined as the main reservoirs for the zoonotic HEVgt3 and HEVgt4. The infection is widely spread in pigs all around the world. Just like in humans, the fecal-oral mechanism of the transmission is thought to be the main one in animals as well. The nature
course of swine HEV is subclinical manifestation, therefore the sick animals are hard to be focused and isolated. The animal reservoir and the lack of specific prophylaxis transform HEV as a potential threat to public health.

Authors’ contributions

R. Pepovich, M. Baymakova and I. Tsachev designed the study. R. Pepovich, M. Baymakova and I. Tsachev collected data. R. Pepovich, M. Baymakova and I. Tsachev interpreted data. R. Pepovich, M. Baymakova and I. Tsachev prepared the article. R. Pepovich, M. Baymakova, M. Pishmisheva, P. Marutsov, L. Pekova and I. Tsachev performed the literature search. All authors approved the final version of the article.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

REFERENCES


Time trend of the prevalence of hepatitis E antibodies among farmers and blood 

17(12): 2309–12.
D, et al. Hepatitis E virus seroprevalence among blood donors in southwest 
Seroprevalence study in forestry workers from eastern Germany using novel 
genotype 3- and rat hepatitis E virus-specific immunoglobulin G ELISAs. Med 
54. Fogeda M, Avellan A, Echevarria JM. Prevalence of specific antibody to hepatitis 
56. Slot E, Hogema BM, Riezebos-Brilman A, Kok TM, Molier M, Zaaijer HL. Silent 
hepatitis E virus infection in Dutch blood donors, 2011 to 2012. Euro Surveill 
57. Juhl D, Baylis SA, Blunel J, Gorg S, Hennig H. Seroprevalence and incidence of 
hepatitis E virus infection in German blood donors. Transfusion 2014; 54(1): 49–56.


63. Aydin NN, Ergunay K, Karagul A, Pinar A, Us D. Investigation of the hepatitis E virus seroprevalence in cases admitted to Hacettepe University Medical Faculty Hospital. Mikrobiyol Bul 2015; 49(4): 554–64. (Turkish)


Table 1

Seroprevalence of *hepatitis E virus* in blood donors in European countries

<table>
<thead>
<tr>
<th>References study</th>
<th>Country</th>
<th>Year of publication</th>
<th>Investigated BD, (n)</th>
<th>HEV positive BD, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macedo et al</td>
<td>Portugal</td>
<td>1998</td>
<td>50</td>
<td>4.0</td>
</tr>
<tr>
<td>Tarrago et al</td>
<td>Spain</td>
<td>2000</td>
<td>863</td>
<td>2.9</td>
</tr>
<tr>
<td>Olsen et al</td>
<td>Sweden</td>
<td>2006</td>
<td>108</td>
<td>9.3</td>
</tr>
<tr>
<td>Boutrouille et al</td>
<td>France</td>
<td>2007</td>
<td>1998</td>
<td>3.2</td>
</tr>
<tr>
<td>Dalton et al</td>
<td>England</td>
<td>2008</td>
<td>500</td>
<td>16-25</td>
</tr>
<tr>
<td>Mansuy et al</td>
<td>France</td>
<td>2008</td>
<td>529</td>
<td>16.6</td>
</tr>
<tr>
<td>Christensen et al</td>
<td>Denmark</td>
<td>2008</td>
<td>169</td>
<td>20.6</td>
</tr>
<tr>
<td>Mansuy et al</td>
<td>France</td>
<td>2011</td>
<td>512</td>
<td>52.5</td>
</tr>
<tr>
<td>Kaufmann et al</td>
<td>Switzerland</td>
<td>2011</td>
<td>550</td>
<td>4.9</td>
</tr>
<tr>
<td>Dremsek et al</td>
<td>Germany</td>
<td>2012</td>
<td>301</td>
<td>11.0</td>
</tr>
<tr>
<td>Fogeda et al</td>
<td>Spain</td>
<td>2012</td>
<td>2305</td>
<td>1.08</td>
</tr>
<tr>
<td>Cleland et al</td>
<td>Scotland</td>
<td>2013</td>
<td>1559</td>
<td>4.7</td>
</tr>
<tr>
<td>Slot et al</td>
<td>Netherlands</td>
<td>2013</td>
<td>5239</td>
<td>26.7</td>
</tr>
<tr>
<td>Juhl et al</td>
<td>Germany</td>
<td>2014</td>
<td>1019</td>
<td>6.8</td>
</tr>
<tr>
<td>Petrovic et al</td>
<td>Serbia</td>
<td>2014</td>
<td>200</td>
<td>15.0</td>
</tr>
<tr>
<td>Fischer et al</td>
<td>Austria</td>
<td>2015</td>
<td>1203</td>
<td>13.55</td>
</tr>
<tr>
<td>Holm et al</td>
<td>Denmark</td>
<td>2015</td>
<td>504</td>
<td>10.7</td>
</tr>
<tr>
<td>Mansuy et al</td>
<td>France</td>
<td>2015</td>
<td>3353</td>
<td>39.1</td>
</tr>
<tr>
<td>Puttini et al</td>
<td>Italy</td>
<td>2015</td>
<td>132</td>
<td>9.1</td>
</tr>
<tr>
<td>Aydin et al</td>
<td>Turkey</td>
<td>2015</td>
<td>327</td>
<td>0.92</td>
</tr>
<tr>
<td>Ricco et al</td>
<td>Italy</td>
<td>2016</td>
<td>199</td>
<td>7.0</td>
</tr>
<tr>
<td>Mansuy et al</td>
<td>France</td>
<td>2016</td>
<td>10569</td>
<td>22.4</td>
</tr>
<tr>
<td>Lucarelli et al</td>
<td>Italy</td>
<td>2016</td>
<td>313</td>
<td>49.0</td>
</tr>
<tr>
<td>Lange et al</td>
<td>Norway</td>
<td>2017</td>
<td>1200</td>
<td>14.0</td>
</tr>
</tbody>
</table>

*Note:* BD – Blood donors;
Table 2

Seroprevalence of swine HEV antibodies in European countries

<table>
<thead>
<tr>
<th>References study</th>
<th>Country</th>
<th>Year of publication</th>
<th>Investigated pigs, (n)</th>
<th>HEV positive pigs, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savuta et al</td>
<td>Romania</td>
<td>2007</td>
<td>145</td>
<td>42.7</td>
</tr>
<tr>
<td>Savuta et al</td>
<td>Romania</td>
<td>2008</td>
<td>69</td>
<td>49.27</td>
</tr>
<tr>
<td>Asimoula et al</td>
<td>Greece</td>
<td>2009</td>
<td>96</td>
<td>80.0</td>
</tr>
<tr>
<td>Lupulovic et al</td>
<td>Serbia</td>
<td>2010</td>
<td>315</td>
<td>34.6</td>
</tr>
<tr>
<td>Martinelli et al</td>
<td>Italy</td>
<td>2011</td>
<td>1422</td>
<td>50.21</td>
</tr>
<tr>
<td>Jimenez de Oya et al</td>
<td>Spain</td>
<td>2011</td>
<td>1141</td>
<td>20.4</td>
</tr>
<tr>
<td>Krumbholz et al</td>
<td>Germany</td>
<td>2013</td>
<td>2273</td>
<td>46.9</td>
</tr>
<tr>
<td>O'Connor et al</td>
<td>Ireland</td>
<td>2015</td>
<td>330</td>
<td>27.0</td>
</tr>
<tr>
<td>Weiner et al</td>
<td>Poland</td>
<td>2016</td>
<td>143</td>
<td>44.1</td>
</tr>
<tr>
<td>Lange et al</td>
<td>Norway</td>
<td>2017</td>
<td>153</td>
<td>90.0</td>
</tr>
<tr>
<td>Caruso et al</td>
<td>Italy</td>
<td>2017</td>
<td>879</td>
<td>50.0</td>
</tr>
<tr>
<td>Pishmisheva et al</td>
<td>Bulgaria</td>
<td>2017</td>
<td>85</td>
<td>40.0</td>
</tr>
</tbody>
</table>
Fig. 1 – The development of HEV infection in humans

Fig. 2 – The course of a typical swine HEV infection