

Current knowledge on Hepatitis E virus infection

Aktuelno znanje o hepatitis E virusnoj infekciji

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Introduction

Hepatitis E is an emerging viral disease affecting both humans and different kinds of domestic and wild animals. In developing countries, human hepatitis E virus (HEV) has a trend for epidemic spread with benign outcome, except pregnant women¹. The death rate due to HEV exceeds 25% in the third trimester¹. In developed countries, the autochthonous cases of human HEV infection are associated with a consumption 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 water². The infected area was characterized by a high level of viral distribution and mortality among pregnant women². The epidemic spread, the incubation period, clinical signs and biochemical results of the examined patients were similar to manifestation of Hepatitis A virus infection². A few months later Wong et al.³, 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 Ah-

medabad, India (1975–1976) and Pune, India (1978–1979). The results from that study established a few cases of acute hepatitis B and none acute hepatitis A³. 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, the first case of human HEV was described. The isolated virus had similar genomic characteristics to the swine HEV^{5,6}. That disclosure determines the zoonotic character of the virus¹.

Etiology

HEV belongs to the family of *Hepeviridae*, genus *Hepevirus*⁷. According to the current classification, the family *Hepeviridae* is divided into two genera: *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* includes four species⁸: *Orthohepevirus A*: isolated from human, swine, deer, mon-goose, rabbit, camels; *Orthohepevirus B*: isolated from birds; *Orthohepevirus C*: isolated from rats, a 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 hygiene¹². Genotypes 3 and 4 (HEVgt3 and HEVgt4) infect humans and other mammals, which cause sporadic cases of Hepatitis E in industrialized countries¹².

HEV is a small virus with a diameter approximately 27 to 32 nm, icosahedral symmetry, spherical shape and simple structure¹². The virion contains single positive-stranded RNA with size 7.2–7.5 kb¹³. HEV genome includes 5' untranslated region (UTR), three opened-reading frames (ORF1, ORF2 and ORF3) and 3' UTR, followed by poly-A tail¹³. Each reading frame has different functions^{12,13}: ORF1: is situated next to 5' and encodes unstructured proteins with enzyme function (methyltransferase, papain-like cysteine protease, macrodomain, helicase and RNA-dependent RNA polymerase); ORF2: is situated next to 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 and it also decreases inflammatory response and protects viral-infected cells.

Epidemiology and prevalence

Hepatitis E is an endemic disease in Central and South-east Asia, in tropic and subtropical countries in Africa and Central America¹. In the endemic area, large waterborne epidemics were described. Hepatitis E is sporadically reported in the USA and Europe¹. In the developed world, it was thought that the infection was associated with traveling to the endemic regions¹⁴. But 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 areas¹⁵. They are associated with contamination of water (water supplies) and poor sanitation conditions¹⁵. Both genotypes cause acute hepatitis in humans. The virus is found in feces, an environment contaminated with human's feces¹⁵. A study in Uganda showed that environmental factors could be much more important for transmission, than it was thought¹⁶. In nonendemic regions, the mechanisms of transmission are much less known, in contrast to endemic areas the contaminated water is a documented source of infection¹⁷. HEV is the only one among other hepatotropic viruses with zoonotic character and animal reservoir¹⁷. The literature search presents that most of the autochthonous human cases are associated with the consumption of raw and undercooked meat infected with HEV^{18–20}. Pigs are considered to be the main reservoirs for HEVgt3 and HEVgt4, and the two genotypes are found in pigs all over the world²¹. Antibodies against HEV are found in chickens, dogs, rodents, cows, sheep, goats, monkeys and other animals²². HEVgt3 is responsible for most of human HEV infections in Europe, North America and East Asia²³. HEVgt3 dominated in swine samples in Europe and America. The virus was detected in pork products^{19,24}. Strains of HEVgt3 were recently found in pigs in Africa²⁵. 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^{26,27}. In China, HEVgt4 is the most common virus in humans and swine^{28,29}. Also, it is endemic in Japan³⁰. 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^{31,32}.

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^{19,33}. Acute hepatitis E was described after eating pork meat infected with HEVgt4 in Japan³⁴. In Japan, it was reported a severe human case after eating raw liver from a wild boar, whereas in Europe, the severe human infections were related to consumption of pork meat^{19,35}. The phylogenetical analysis of HEV samples from Japan indicated a previous transmission of the virus from domestic pigs to wild boars³⁶. Urine was identified as a possible source for swine HEV infection¹. It was established that swine HEV could pass colostrum, while transplacental transmission is arguable¹. Another possible way of HEV transmission is the direct contact of people and swine¹. The serological studies in the 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 products³⁷. A higher rate of seroprevalence was found among foresters in comparison with the seroprevalence among blood donors^{1,21}. HEV infection could be transmitted by transfusing blood and blood products³⁸. Swine products, such as swine heparin and others, used in human medicine, could be a risk factor for HEV spread³⁹. Other possible risk for HEV source could be feces or manure⁴⁰. A study reported the presence of HEV in the manure storage facilities⁴⁰.

Nowadays, wild boars are thought to be an important natural reservoir for HEVgt3 and HEVgt4¹. The 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 and Okamoto⁴¹ found HEV RNA in 1.1%–13.3% of the examined wild boars and seropositivity varied between 4.5% to 34.4%. In Germany, wild boars are considered to be one of the main sources for HEV transmission⁴². HEV RNA could be found in the serum, gall and liver from wild boars⁴³. 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 \pm standard deviation (SD) human HEV seroprevalence is 15.21 ± 14.20 (95% confidence interval (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. Regardless of the published diversity, these data confirmed the seroprevalence of HEV among blood donors in different European countries.

Worldwide, the main animal reservoir for HEVgt3 and HEVgt4 are domestic pigs and wild boars^{1,68}. 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 \pm 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.

Table 1

Seroprevalence of hepatitis E virus (HEV) in blood donors (BD) in European countries

References study	Country	Year of publication	Investigated BD (n)	HEV positive BD (%)
Macedo et al. ⁴⁴	Portugal	1998	50	4.0
Tarrago et al. ⁴⁵	Spain	2000	863	2.9
Olsen et al. ⁴⁶	Sweden	2006	108	9.3
Boutrouille et al. ⁴⁷	France	2007	1998	3.2
Dalton et al. ⁴⁸	England	2008	500	16-25
Mansuy et al. ⁴⁹	France	2008	529	16.6
Christensen et al. ⁵⁰	Denmark	2008	169	20.6
Mansuy et al. ⁵¹	France	2011	512	52.5
Kaufmann et al. ⁵²	Switzerland	2011	550	4.9
Dremsek et al. ⁵³	Germany	2012	301	11.0
Fogeda et al. ⁵⁴	Spain	2012	2305	1.08
Cleland et al. ⁵⁵	Scotland	2013	1559	4.7
Slot et al. ⁵⁶	Netherlands	2013	5239	26.7
Juhl et al. ⁵⁷	Germany	2014	1019	6.8
Petrovic et al. ⁵⁸	Serbia	2014	200	15.0
Fischer et al. ⁵⁹	Austria	2015	1203	13.55
Holm et al. ⁶⁰	Denmark	2015	504	10.7
Mansuy et al. ⁶¹	France	2015	3353	39.1
Puttini et al. ⁶²	Italy	2015	132	9.1
Aydin et al. ⁶³	Turkey	2015	327	0.92
Ricco et al. ⁶⁴	Italy	2016	199	7.0
Mansuy et al. ⁶⁵	France	2016	10569	22.4
Lucarelli et al. ⁶⁶	Italy	2016	313	49.0
Lange et al. ⁶⁷	Norway	2017	1200	14.0

Table 2

Seroprevalence of swine hepatitis E virus (HEV) antibodies in European countries

References study	Country	Year of publication	Investigated pigs (n)	HEV positive pigs (%)
Savuta et al. ⁶⁹	Romania	2007	145	42.7
Savuta et al. ⁷⁰	Romania	2008	69	49.27
Asimoula et al. ⁷¹	Greece	2009	96	80.0
Lupulovic et al. ⁷²	Serbia	2010	315	34.6
Martinelli et al. ⁷³	Italy	2011	1422	50.21
de Oya et al. ⁷⁴	Spain	2011	1141	20.4
Krumbholz et al. ³²	Germany	2013	2273	46.9
Connor et al. ⁷⁵	Ireland	2015	330	27.0
Weiner et al. ⁷⁶	Poland	2016	143	44.1
Lange et al. ⁶⁷	Norway	2017	153	90.0
Caruso et al. ⁷⁷	Italy	2017	879	50.0
Pishmisheva et al. ⁷⁸	Bulgaria	2017	85	40.0

Clinical manifestation

The most common clinical manifestation of HEV among people in the endemic areas is acute icteric hepatitis with typical clinical and laboratory signs¹⁴. Sometime prolonged cholestasis could be developed or asymptomatic infection may occurred¹⁴. A high rate of fulminant hepatic failure and death were mentioned among pregnant women in the hyperendemic areas^{79, 80}. In nonendemic areas, the virus

affected mainly elderly men, people with accompanying liver diseases and alcohol abuse⁸¹⁻⁸³. The autochthonous cases could be manifested as an acute hepatitis, asymptomatic infection, and nonspecific symptoms with anicteric diseases⁴⁸. In contrast, the severe illness does not manifest during pregnancy in the nonendemic regions^{81, 82}. Chronic HEV infection was described in solid-organ transplant recipients, patients with hematological diseases, HIV patients, people under immunosuppressive conditions and anticancer chemo-

therapy^{81, 82, 84-86}. In such case, the liver biopsy illustrated liver fibrosis, which predicts the progress to cirrhosis⁸⁷.

Swine HEV infection does not present typical clinical symptoms and signs. Usually animal diseases are characterized with fluctuations in body temperature or body weight¹. After a subclinical HEV infection, mild microscopic lesions in the liver could be developed¹¹. The pathological findings include viral antigen in the hepatocytes, positive immunohistochemical changes in the small and large intestines, lymph nodes, tonsils, spleen and kidneys¹². A Spanish study reported no correlation between HEV RNA and the histological changes in the liver⁸⁸. So it is 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 the serological examination. The laboratory diagnostics use serum samples for detection of HEV antibodies by enzyme-linked immunosorbent assays (ELISA) and western blot assays¹. 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 infection¹. After that antibodies of class IgG follow up and show a recent or past infection. The serum samples are collected for the serological tests in humans, for the pig examination it could be collected sera or meat juice⁸⁹. In humans, anti-HEV IgM levels peak around the time of the alanine aminotransferase (ALT) peak and may persist up to 5 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 reconvalescent 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}.

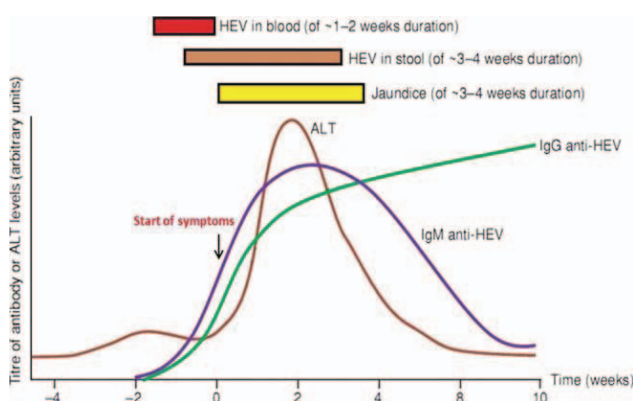


Fig. 1 – The development of hepatitis E virus (HEV) infection in humans.

The majority of pigs are naturally infected with HEV at the age of 2 to 4 months⁴. Eighty six percentage of pigs are naturally infected with the virus until their eighteenth week⁹². The maternal antibodies decline at the age of 8 to 10

weeks¹⁷. After the reduction of them, the piglets could be attacked by the virus around the second week after birth¹¹. The swine HEV infection is accompanied with a transient viremia lasting 1 to 2 weeks and a fecal-oral emission of the pathogen continuing three to 7 weeks⁹. The number of viremic pigs increase from 9 weeks with peaking around 15 weeks, following decline to slaughter age⁹³. 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 4 months (Figure 2)¹⁷. Interestingly enough, the presence of antibodies does not always assure the absence of 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-reservoirs²¹.

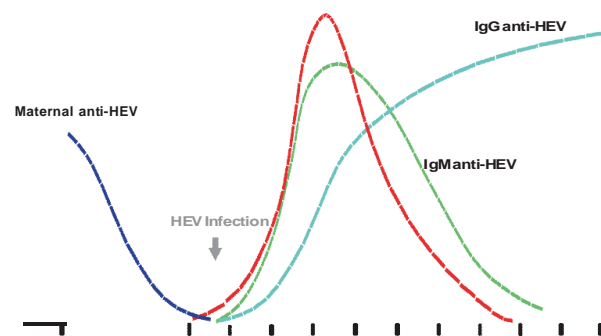


Fig. 2 – The course of a typical swine hepatitis E virus (HEV) infection.

Nowadays, the detection of HEV RNA using molecular-genetic methods is considered as the “golden standard” in the laboratory diagnosis¹. The detection of RNA is performed by different RT-PCR methods, amplifying genomic fragments in one of the three ORFs^{11,21}. HEV RNA could be found in the patients’ blood and/or feces in the prodromal period, and after that, the virus could be detected in feces for another 2 weeks¹². The viremic period is very short, therefore HEV RNA could not be always 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^{94,95}. However, the test has a low sensibility compared to the methods that use the amplification of nucleic acid⁹⁶. The presence of HEV antigen in different swine tissues using immunohistochemistry was recently demonstrated⁹⁷. The detection of HEV antigen in the liver tissue representing a valuable tool for the viral establishment in biopsy, autopsy and explant liver tissues⁹⁸.

Therapy and prophylaxis

There is no specific therapy for the acute HEV infection in humans, because in most cases the illness is self-limiting. The management of acute illness in the 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 is needed⁹⁹. In chronic HEV infection, the

administration of pegylated interferon alpha-2a/alpha-2b, or ribavirin for 3–12 months were applied as a specific antiviral therapy^{100, 101}. A recombinant vaccine showed 94%–100% efficacy in a phase III study of > 100,000 Chinese adults¹⁰². The vaccine protected from HEVgt1 and HEVgt2¹⁰².

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

The prevention measures are guided to improving sanitation and hygiene in developing countries¹⁷. In developed countries, the population with a high risk could be informed about the virus and his zoonotic characteristic, and, consequently, should be asked to reduce and/or avoid consumption of raw, or undercooked meat and meat products from pigs, wild boars, deer and direct contact with infected animals.

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.

Conflict of interest

None.

R E F E R E N C E S

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