GENERAL REVIEW

UDC: 616.9:616.36-002
https://doi.org/10.2298/VSP170815159P

# Current knowledge on Hepatitis E virus infection

## Aktuelno znanje o hepatitis E virusnoj infekciji

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

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

**Key words:**
**diagnosis; disease outbreaks; epidemiology; hepatitis e; hepatitis e virus; prevalence; therapeutics.**

**Ključne reči:**
**dijagnoza; infekcija, putevi širenja; epidemiologija; hepatitis e; hepatitis e, virus; prevalenca; lečenje.**

### 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 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 by a 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. [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 Ahmedabad, 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 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, 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 virus [1].

### 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 species [8]: *Orthohepevirus A*: isolated from human, swine, deer, mongoose, 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 hygiene [12]. Genotypes 3 and 4 (HEVgt3 and HEVgt4) infect humans and other mammals, which cause sporadic cases of Hepatitis E in industrialized countries [12].

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

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 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 Southeast Asia, in tropic and subtropical countries in Africa and Central America [1]. In the endemic area, large waterborne epidemics were described. Hepatitis E is sporadically reported in the USA and Europe [1]. In the developed world, it was thought that the infection was associated with traveling to the endemic regions [14]. 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 areas [15]. They are associated with contamination of water (water supplies) and poor sanitation conditions [15]. Both genotypes cause acute hepatitis in humans.The virus is found in feces, an environment contaminated with human's feces [15]. A study in Uganda showed that environmental factors could be much more important for transmission, than it was thought [16]. In nonendemic regions, the mechanisms of transmission are much less known, in contrast to endemic areas the contaminated water is a documented source of infection [17]. HEV is the only one among other hepatotropic viruses with zoonotic character and animal reservoir [17]. 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 world [21]. Antibodies against HEV are found in chickens, dogs, rodents, cows, sheep, goats, monkeys and other animals [22]. HEVgt3 is responsible for most of human HEV infections in Europe, North America and East Asia [23]. 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 Africa [25]. 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 Japan [30]. 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 Japan [34]. 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 boars [36]. Urine was identified as a possible source for swine HEV infection [1]. It was established that swine HEV could pass colostrum, while transplacental transmission is arguable [1]. Another possible way of HEV transmission is the direct contact of people and swine [1]. 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 products [37]. 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 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 the manure storage facilities [40].

Nowadays, wild boars are thought to be an important natural reservoir for HEVgt3 and HEVgt4 [1]. 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 Okamoto [41] 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 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 ± standard deviaton (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 ± 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 signs [14]. Sometime prolonged cholestasis could be developed or asymptomatic infection may occurred [14]. 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 abuse [81–83]. The autochthonous cases could be manifested as an acute hepatitis, asymptomatic infection, and nonspecific symptoms with anicteric diseases [48]. 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 cirrhosis [87].

Swine HEV infection does not present typical clinical symptoms and signs. Usually animal 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]. The pathological findings include viral antigen in the hepatocytes, positive immunohistochemical changes in the small and large intestines, lymph nodes, tonsils, spleen and kidneys [12]. A Spanish study reported no correlation between HEV RNA and the histological changes in the liver [88]. 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 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. The serum samples are collected for the serological tests in humans, for the pig examination it could be collected sera or meat juice [89]. In humans, anti-HEV IgM levels peak around the time of the alannine 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 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].

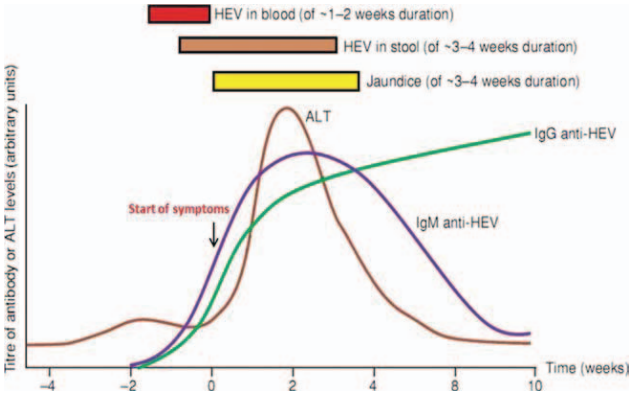


**Fig. 1 – The development of hepatitis E virus (HEV) infection in humans.**

The majority of pigs are naturally infected with HEV at the age of 2 to 4 months [4]. Eighty six percentage of pigs are naturally infected with the virus until their eighteenth week [92]. The maternal antibodies decline at the age of 8 to 10 weeks [17]. After the reduction of them, the piglets could be attacked by the virus around the second week after birth [11]. 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 weeks [9]. The number of viremic pigs increase from 9 weeks with peaking around 15 weeks, following decline to slaughter age [93]. 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-reservoirs [21].

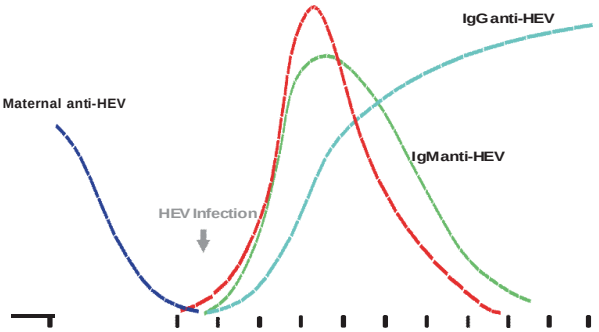


**Fig. 2 – The course of a typical swine hepatitis E virus (HEV) infection.**

Nowadays, the detection of HEV RNA using molecular-genetic methods is considered as the "golden standard" in the laboratory diagnosis [1]. 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 weeks [12]. 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 acid [96]. The presence of HEV antigen in different swine tissues using immunohistochemistry was recently demonstrated [97]. The detection of HEV antigen in the liver tissue representing a valuable tool for the viral establishment in biopsy, autopsy and explant liver tissues [98].

### 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 needed [99]. 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 adults [102]. The vaccine protected from HEVgt1 and HEVgt2 [102].

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 countries [17]. 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


1. *Walsh SR.* Hepatitis E virus. In: *Bennett JE, Dolin R, Blaser MJ*, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Philadelphia, USA: Elsevier Saunders; 2015. p. 2131–41.
2. *Khuroo MS*. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. Am J Med 1980; 68(6): 818–24.
3. *Wong DC, Purcell RH, Sreenivasan MA, Prasad SR, Pavri KM.* Epidemic and endemic hepatitis in India: Evidence for a non-A, non-B hepatitis virus aetiology. Lancet 1980; 316(8200): 876–9.
4. *Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS,* et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci U.S.A. 1997; 94(18): 9860–5.
5. *Kwo PY, Schlauder GG, Carpenter HA, Murphy PJ, Rosenblatt JE, Dawson GJ,* et al. Acute hepatitis E by a new isolate acquired in the United States. Mayo Clin Proc 1997; 72(12): 1133–6.
6. *Schlauder GG, Dawson GJ, Erker JC, Kwo PY, Knigge MF, Smalley DL*, et al. The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. J Gen Virol 1998; 79( Pt 3): 447–56.
7. *Hoofnagle JH, Nelson KE, Purcell RH.* Hepatitis E. N Engl J Med 2012; 367(13): 1237–44.
8. *Smith DB, Simmonds P, Jameel S, Emerson SU, Harrison TJ, Meng XJ*, et al. Consensus proposals for classification of the family Hepeviridae. J Gen Virol 2014; 95(Pt 10): 2223–32.
9. *Clemente-Casares P, Ramos-Romero C, Ramirez-Gonzalez E, Mas A.* Hepatitis E virus in industrialized countries: The silent threat. BioMed Res Int 2016; 2016: 9838041.
10. *Hartl J, Wehmeyer MH, Pischke S.* Acute hepatitis E: Two sides of the same coin. Viruses 2016; 8(11). pii: E299.
11. *Doceul V, Bagdassarian E, Demange A, Pavio N.* Zoonotic hepatitis E virus: Classification, animal reservoirs and transmission routes. Viruses 2016; 8(10). pii: E270
12. *Khuroo MS, Khuroo MS, Khuroo NS.* Hepatitis E: Discovery, global impact, control and cure. World J Gastroenterol 2016; 22(31): 7030–45.
13. *Nan Y, Zhang YJ.* Molecular biology and infection of hepatitis E virus. Front Microbiol 2016; 7: 1419.
14. *Aggarwal R, Jameel S.* Hepatitis E. Hepatology 2011; 54(6): 2218–26.
15. *Hazam RK, Singla R, Kishore J, Singh S, Gupta RK, Kar P.* Surveillance of hepatitis E virus in sewage and drinking water in a resettlement colony of Delhi: What has been the experience? Arch Virol 2010; 155(8): 1227–33.
16. *Howard CM, Handzel T, Hill VR, Grytdal SP, Blanton C, Kamili S,* et al. Novel risk factors associated with hepatitis E virus infection in a large outbreak in Northern Uganda: Results from a case-control study and environmental analysis. Am J Trop Med Hyg 2010; 83(5): 1170–3.
17. *Pavio N, Meng XJ, Renou C.* Zoonotic hepatitis E: Animal reservoirs and emerging risks. Vet Res 2010; 41(6): 46.
18. *Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ.* Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. J Gen Virol 2007; 88(Pt 3): 912–7.
19. *Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P,* et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 2010; 202(6): 825–34.
20. *Berto A, Grierson S, Hakze-Honing R, Martelli F, Johne R, Reetz J,* et al. Hepatitis E virus in pork liver sausage, France. Emerging Infect Dis 2013; 19(2): 264–6.
21. *Kmush BL, Nelson KE, Labrique AB.* Risk factors for hepatitis E virus infection and disease. Expert Rev Anti Infect Ther 2015; 13(1): 41–53.
22. *Lhomme S, Marion O, Abravanel F, Chapuy-Regaud S, Kamar N, Izopet J.* Hepatitis E pathogenesis. Viruses 2016; 8(8). pii: E212.
23. *Pérez-Gracia MT, García M, Suay B, Mateos-Lindemann ML.* Current Knowledge on Hepatitis E. J Clin Transl Hepatol 2015; 3(2): 117–26.
24. *Wedemeyer H, Pischke S, Manns MP.* Pathogenesis and treatment of hepatitis E virus infection. Gastroenterology 2012; 142(6): 1388–1397.e1
25. *Paula VS, Wiele M, Mbunkah AH, Daniel AM, Kingsley MT, Schmidt-Chanasit J.* Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. Emerg Infect Dis 2013; 19(4): 666–8.
26. *Hsieh SY, Yang PY, Ho YP, Chu CM, Liaw YF.* Identification of a novel strain of hepatitis E virus responsible for sporadic acute hepatitis in Taiwan. J Med Virol 1998; 55(4): 300–4.
27. *Hsieh SY, Meng XJ, Wu YH, Liu ST, Tam AW, Lin DY,* et al. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. J Clin Microbiol 1999; 37(12): 3828–34.
28. *Liu P, Li L, Wang L, Bu Q, Fu H, Han J,* et al. Phylogenetic analysis of 626 hepatitis E virus (HEV) isolates from humans and animals in China (1986-2011) showing genotype diversity and zoonotic transmission. Infect Genet Evol 2012; 12(2): 428–34.
29. *Geng Y, Zhao C, Fan J, Harrison TJ, Zhang H, Lian H,* et al. Genotype analysis of hepatitis E virus from sporadic hepatitis E cases in Northern China. Infect Genet Evol 2013; 20: 413–7.

30. *Sato Y, Sato H, Naka K, Furuya S, Tsukiji H, Kitagawa K,* et al. A nationwide survey of hepatitis E virus (HEV) infection in wild boars in Japan: Identification of boar HEV strains of genotypes 3 and 4 and unrecognized genotypes. Arch Virol 2011; 156(8): 1345–58.
31. *Dremsek P, Joel S, Baechlein C, Pavio N, Schielke A, Ziller M,* et al. Hepatitis E virus seroprevalence of domestic pigs in Germany determined by a novel in-house and two reference ELISAs. J Virol Methods 2013; 190(1–2): 11–6.
32. *Krumbholz A, Joel S, Neubert A, Dremsek P, Dürrwald R, Johne R,* et al. Age-related and regional differences in the prevalence of hepatitis E virus-specific antibodies in pigs in Germany. Vet Microbiol 2013; 167(3–4): 394–402.
33. *Li T, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y*, et al. Hepatitis E virus transmission from wild boar meat. Emerging Infect. Dis 2005; 11(12): 1958–60.
34. *Miyashita K, Kang J, Saga A, Takahashi K, Shimamura T, Yasumoto A,* et al. Three cases of acute or fulminant hepatitis E caused by ingestion of pork meat and entrails in Hokkaido, Japan: Zoonotic food-borne transmission of hepatitis E virus and public health concerns. Hepatol Res 2012; 42(9): 870–8.
35. *Matsuda H, Okada K, Takahashi K, Mishiro S*. Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. J Infect Dis 2003; 188(6): 944.
36. *Nakano T, Takahashi K, Arai M, Okano H, Kato H, Ayada M,* et al. Identification of European-type hepatitis E virus subtype 3e isolates in Japanese wild boars: Molecular tracing of HEV from swine to wild boars. Infect Genet Evol 2013; 18: 287–98.
37. *Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE*, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol 2002; 40(1): 117–22.
38. *Lewis HC, Wichmann O, Duizer E.* Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: A systematic review. Epidemiol Infect 2010; 138(2): 145–66.
39. *Crossan C, Scobie L, Godwin J, Hunter JG, Hawkes T, Dalton HR*, et al. Hepatitis E virus and porcine-derived heparin. Emerging Infect Dis 2013; 19(4): 686–8.
40. *Kasorndorkbua C, Opriessnig T, Huang FF, Guenette DK, Thomas PJ, Meng XJ,* et al. Infectious swine hepatitis E virus is present in pig manure storage facilities on United States farms, but evidence of water contamination is lacking. Appl Environ Microbiol 2005; 71(12): 7831–7.
41. *Takahashi M, Okamoto H.* Features of hepatitis E virus infection in humans and animals in Japan. Hepatol Res 2014; 44(1): 43–58.
42. *Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A,* et al. Phylogenetic and case-control study on hepatitis E virus infection in Germany. J Infect Dis 2008; 198(12): 1732–41.
43. *Schielke A, Sachs K, Lierz M, Appel B, Jansen A, Johne R.* Detection of hepatitis E virus in wild boars of rural and urban regions in Germany and whole genome characterization of an endemic strain. Virol J 2009; 6(1): 58.
44. *Macedo G, Pinto T, Sarmento JA, Vale AM, Ribeiro T.* The first assessment of hepatitis E virus seroprevalence in northern Portugal. Acta Med Port 1998; 11(12): 1065–8. (Portugal)
45. *Tarragó D, López-Vélez R, Turrientes C, Baquero F, Mateos ML.* Prevalence of Hepatitis E Antibodies in Immigrants from Developing Countries. Eur J Clin Microbiol Infect Dis 2000; 19(4): 309–11.
46. *Olsen B, Axelsson-Olsson D, Thelin A, Weiland O.* Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. Scand J Infect Dis 2006; 38(1): 55–8.
47. *Boutrouille A, Bakkali-Kassimi L, Crucière C, Pavio N.* Prevalence of anti-hepatitis E virus antibodies in French blood donors. J Clin Microbiol 2007; 45(6): 2009–10.
48. *Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W,* et al. Autochthonous hepatitis E in Southwest England: Natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol 2008; 20(8): 784–90.
49. *Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S*, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from South West France. J Med Virol 2008; 80(2): 289–93.
50. *Christensen PB, Engle RE, Hjort C, Homburg KM, Vach W, Georgsen J,* et al. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: A potential zoonosis in Denmark. Clin Infect Dis 2008; 47(8): 1026–31.
51. *Mansuy J, Bendall R, Legrand-Abravanel F, Sauné K, Miédouge M, Ellis V*, et al. Hepatitis E virus antibodies in blood donors, France. Emerging Infect. Dis 2011; 17(12): 2309–12.
52. *Kaufmann A, Kenfak-Foguena A, André C, Canellini G, Bürgisser P, Moradpour D*, et al. Hepatitis E virus seroprevalence among blood donors in southwest Switzerland. PLoS ONE 2011; 6(6): e21150.
53. *Dremsek P, Wenzel JJ, Johne R, Ziller M, Hofmann J, Groschup MH*, et al. Seroprevalence study in forestry workers from eastern Germany using novel genotype 3- and rat hepatitis E virus-specific immunoglobulin G ELISAs. Med Microbiol Immunol 2012; 201(2): 189–200.
54. *Fogeda M, Avellón A, Echevarría JM.* Prevalence of specific antibody to hepatitis E virus in the general population of the community of Madrid, Spain. J Med Virol 2012; 84(1): 71–4.
55. *Cleland A, Smith L, Crossan C, Blatchford O, Dalton HR, Scobie L,* et al. Hepatitis E virus in Scottish blood donors. Vox Sang 2013; 105(4): 283–9.
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 2013; 18(31): pii: 20550.
57. *Juhl D, Baylis SA, Blümel J, Görg S, Hennig H.* Seroprevalence and incidence of hepatitis E virus infection in German blood donors. Transfusion 2014; 54(1): 49–56.
58. *Petrović T, Lupulović D, Jiménez ON, Vojvodić S, Blázquez A, Escribano-Romero E*, et al. Prevalence of hepatitis E virus (HEV) antibodies in Serbian blood donors. J Infect Dev Ctries 2014; 8(10): 1322–7.
59. *Fischer C, Hofmann M, Danzer M, Hofer K, Kaar J, Gabriel C.* Seroprevalence and Incidence of hepatitis E in blood donors in Upper Austria. PLoS ONE 2015; 10(3): e0119576.
60. *Holm DK, Moessner BK, Engle RE, Zaaijer HL, Georgsen J, Purcell RH,* et al. Declining prevalence of hepatitis E antibodies among Danish blood donors. Transfusion 2015; 55(7): 1662–7.
61. *Mansuy JM, Saune K, Rech H, Abravanel F, Mengelle C, Homme LS*, et al. Seroprevalence in blood donors reveals widespread, multi-source exposure to hepatitis E virus, southern France, October 2011. Euro Surveill 2015; 20(19): 27–34.
62. *Puttini C, Riccio ML, Redi D, Tordini G, Cenerini M, Romanello F,* et al. Seroprevalence of hepatitis E virus (HEV) infection in blood donors and renal transplant recipients: A retrospective study from central Italy. Infez Med 2015; 23(3): 253–6.
63. *Aydın NN, Ergünay K, Karagül A, Pınar 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)
64. *Ricco G, Bonino F, Lanza M, Scatena F, Alfieri CM, Messa P*, et al. New immunoassays for total, IgA and IgM antibodies against hepatitis E virus: Prevalence in Italian blood donors and patients with chronic liver or kidney diseases. Dig Liver Dis 2016; 48(5): 536–41.
65. *Mansuy JM, Gallian P, Dimeglio C, Saune K, Arnaud C, Pelletier B,* et al. A nationwide survey of hepatitis E viral infection in French blood donors. Hepatology 2016; 63(4): 1145–54.

66. *Lucarelli C, Spada E, Taliani G, Chionne P, Madonna E, Marcantonio C,* et al. High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy, February to March 2014. Euro Surveill 2016; 21(30). doi: 10.2807/1560-7917.ES.2016.21.30.30299.
67. *Lange H, Øverbø J, Borgen K, Dudman S, Hoddevik G, Urdahl AM,* et al. Hepatitis E in Norway: Seroprevalence in humans and swine. Epidemiol Infect 2017; 145(1): 181–6.
68. *Meng XJ.* Hepatitis E virus: Animal reservoirs and zoonotic risk. Vet Microbiol 2010; 140(3–4): 256–65.
69. *Savuta G, Anita A, Anita D, Ludu L, Pavio N.* Preliminary epidemiological investigations regarding hepatitis E virus infection in swine from the North-East of Romania. Bulletin USAMV-CN 2007;64(1-2):356-358.
70. *Savuta GH, Anita A, Anita D, Ludu L, Duca E, Pavio N.* Seroepidemiological researches regarding swine and human hepatitis E in Romania. Lucrari Stiintifice Med Vet (Timisoara) 2008; 41: 309–13.
71. *Asimoula S, Tzika E, Alexopoulos C, Kyriakis SC, Froesner G.* First report of serological evidence of hepatitis E virus infection in swine in Northern Greece. Acta Vet Beograd 2009; 59(2–3): 205–11.
72. *Lupulovic D, Lazic S, Prodanov-Radulovic J, de Oya JN, Escribano-Romero E, Saiz JC*, et al. First serological study of hepatitis E virus infection in backyard pigs from Serbia. Food Environ Virol 2010; 2(2): 110–13.
73. *Martinelli N, Luppi A, Cordioli P, Lombardi G, Lavazza A.* Prevalence of hepatitis E virus antibodies in pigs in Northern Italy. Infect Ecol Epidemiol 2011; 1. doi: 10.3402/iee.v1i0.7331.
74. *de Oya JN, de Blas I, Blazquez AB, Martin-Acebes MA, Halaihel N, Girones O*, et al. Widespread distribution of hepatitis E virus in Spanish pig herds. BMC Res Notes 2011; 4: 412.
75. *O'Connor M, Roche S, Sammin D.* Seroprevalence of Hepatitis E virus infection in the Irish pig population. Ir Vet J 2015; 68(1): 8.
76. *Weiner M, Tokarska-Rodak M, Plewik D, Panczuk A, Szepeluk A, Krajewska M.* Preliminary study on the detection of hepatitis E virus (HEV) antibodies in pigs and wild boars in Poland. J Vet Res 2016; 60(4): 385–9.
77. *Caruso C, Peletto S, Rosamilia A, Modesto P, Chiavacci L, Sona B*, et al. Hepatitis E virus: A cross-sectional serological and virological study in pigs and humans at zoonotic risk within a high-density pig farming area. Transbound Emerg Dis 2017; 64(5): 1443–53.
78. *Pishmisheva M, Baymakova M, Golkocheva-Markova E, Kundurzhiev T, Pepovich R, Popov GT, et al.* First serological study of hepatitis E virus infection in pigs in Bulgaria. C R Acad Bulg Sci 2018; 71(7): 1001–8.
79. *Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI.* Incidence and severity of viral hepatitis in pregnancy. Am J Med 1981; 70(2): 252–5.
80. *Viswanathan R, Sidhu AS, Pradhan SK, Magar NG.* Infectious hepatitis; clinical findings. Indian J Med Res 1957; 45(Suppl): 49–58.
81. *Borgen K, Herremans T, Duizer E, Vennema H, Rutjes S, Bosman A,* et al. Non-travel related Hepatitis E virus genotype 3 infections in the Netherlands: A case series 2004-2006. BMC Infect Dis 2008; 8: 61.
82. *Mansuy JM, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M,* et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol 2004; 74(3): 419–24.
83. *Baymakova M, Sakem B, Plochev K, Popov GT, Mihaylova-Garnizova R, Kovaleva V*, et al. Epidemiological characteristics and clinical manifestations of hepatitis E virus infection in Bulgaria: A report on 20 patients. Srp Arh Celok Lek 2016; 144(1–2): 63–8.
84. *Kamar N, Selves J, Mansuy J, Ouezzani L, Péron J, Guitard J,* et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med 2008; 358(8): 811–7.
85. *Tamura A, Shimizu YK, Tanaka T, Kuroda K, Arakawa Y, Takahashi K,* et al. Persistent infection of hepatitis E virus transmitted by blood transfusion in a patient with T-cell lymphoma. Hepatol Res 2007; 37(2): 113–20.
86. *Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S.* Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med 2009; 361(10): 1025–7.
87. *Gérolami R, Moal V, Colson P.* Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. N Engl J Med 2008; 358(8): 859–60.
88. *Casas M, Cortés R, Pina S, Peralta B, Allepuz A, Cortey M,* et al. Longitudinal study of hepatitis E virus infection in Spanish farrow-to-finish swine herds. Vet Microbiol 2011; 148(1): 27–34.
89. *Wacheck S, Werres C, Mohn U, Dorn S, Soutschek E, Fredriksson-Ahomaa M*, et al. Detection of IgM and IgG against hepatitis E virus in serum and meat juice samples from pigs at slaughter in Bavaria, Germany. Foodborne Pathog Dis 2012; 9(7): 655–60.
90. *Legrand-Abravanel F, Thevenet I, Mansuy J, Saune K, Vischi F, Peron J,* et al. Good performance of immunoglobulin M assays in diagnosing genotype 3 hepatitis E virus infections. Clin Vaccine Immunol 2009; 16(5): 772–4.
91. *Drobeniuc J, Meng J, Reuter G, Greene-Montfort T, Khudyakova N, Dimitrova Z,* et al. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: Pangenotypic evaluation of performances. Clin Infect Dis 2010; 51(3): e24–7.
92. *Leblanc D, Ward P, Gagné M, Poitras E, Müller P, Trottier Y*, et al. Presence of hepatitis E virus in a naturally infected swine herd from nursery to slaughter. Int J Food Microbiol 2007; 117(2): 160–6.
93. *Deus N, Casas M, Peralta B, Nofrarías M, Pina S, Martín M,* et al. Hepatitis E virus infection dynamics and organic distribution in naturally infected pigs in a farrow-to-finish farm. Vet Microbiol 2008; 132(1–2): 19–28.
94. *Gupta E, Pandey P, Pandey S, Sharma MK, Sarin SK.* Role of hepatitis E virus antigen in confirming active viral replication in patients with acute viral hepatitis E infection. J Clin Virol 2013; 58(2): 374–7.
95. *Majumdar M, Singh MP, Pujhari SK, Bhatia D, Chawla Y, Ratho RK.* Hepatitis E virus antigen detection as an early diagnostic marker: Report from India. J Med Virol 2013; 85(5): 823–7.
96. *Vollmer T, Knabbe C, Dreier J.* Comparison of real-time PCR and antigen assays for detection of hepatitis E virus in blood donors. J Clin Microbiol 2014; 52(6): 2150–6.
97. *Ha S, Chae C.* Immunohistochemistry for the detection of swine hepatitis E virus in the liver. J Viral Hepat 2004; 11(3): 263–7.
98. *Gupta P, Jagya N, Pabhu SB, Durgapal H, Acharya SK, Panda SK.* Immunohistochemistry for the diagnosis of hepatitis E virus infection. J Viral Hepat 2012; 19(2): e177–83.
99. *Somani SK, Aggarwal R, Naik SR, Srivastava S, Naik S.* A serological study of intrafamilial spread from patients with sporadic hepatitis E virus infection. J Viral Hepat 2003; 10(6): 446–9.
100. *Kumar N, Rostaing L, Abravanel F, Garrouste C, Esposito L, Cardeau-Desangles I,* et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. Clin Infect Dis 2010; 50(5): e30–3.
101. *Kamar N, Rostaing L, Abravanel F, Garrouste C, Lhomme S, Esposito L*, et al. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis e virus infection. Gastroenterology 2010; 139(5): 1612–8.
102. *Zhu F, Zhang J, Zhang X, Zhou C, Wang Z, Huang S,* et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. Lancet 2010; 376(9744): 895–902.

Received on August 15, 2017.
Accepted on October 10, 2017.
Online First November, 2017.