Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Hepatitis E virus infections in humans and animals

Clin Exp Vaccine Res 2014;3:29-36
http://dx.doi.org/10.7774/cevr.2014.3.1.29
pISSN 2287-3651 • eISSN 2287-366X

Young-Jo Song[1], Woo-Jung Park[1], Byung-Joo Park[1], Joong-Bok Lee[1], Seung-Yong Park[1], Chang-Seon Song[1], Nak-Hyung Lee[1], Kun-Ho Seo[2], Young-Sun Kang[3], In-Soo Choi[1,4]

Departments of [1]Infectious Diseases and [2]Public Health, College of Veterinary Medicine, Konkuk University; [3]Department of Biomedical Science and Technology, SMART Institute of Advanced Biomedical Science, Konkuk University; [4]Veterinary Science Research Institute, College of Veterinary Medicine, Konkuk University, Seoul, Korea

Received: August 16, 2013
Revised: September 20, 2013
Accepted: September 30, 2013

Corresponding author: In-Soo Choi, PhD
Department of Infectious Diseases, College of Veterinary Medicine, Konkuk University, 263 Achasan-ro, Gwangjin-gu, Seoul 143-701, Korea
Tel: +82-2-2049-6055, Fax: +82-2-3436-5880
E-mail: ischoi@konkuk.ac.kr

No potential conflict of interest relevant to this article was reported.

This study was supported by the Veterinary Science Research Institute, College of Veterinary Medicine, Konkuk University.

Hepatitis E has traditionally been considered an endemic disease of developing countries. It generally spreads through contaminated water. However, seroprevalence studies have shown that hepatitis E virus (HEV) infections are not uncommon in industrialized countries. In addition, the number of autochthonous hepatitis E cases in these countries is increasing. Most HEV infections in developed countries can be traced to the ingestion of contaminated raw or undercooked pork meat or sausages. Several animal species, including pigs, are known reservoirs of HEV that transmit the virus to humans. HEVs are now recognized as an emerging zoonotic agent. In this review, we describe the general characteristics of HEVs isolated from humans and animals, the risk factors for human HEV infection, and the current status of human vaccine development.

Keywords: Hepatitis E virus, Zoonoses, Animals, Humans, Vaccines

Introduction

Hepatitis E virus (HEV), originally recognized as non-A and non-B hepatitis inducing agent, was recently classified into the genus *Hepevirus* and family *Hepeviridae* [1,2]. HEV is a non-enveloped virus with a diameter of 27-34 nm. It has a single-stranded and positive sense RNA genome that is approximately 7.3 kb in length. It also has a short 5' non-coding region (NCR), a 3' NCR, and 3 open reading frames (ORFs). ORF1, ORF2, and ORF3 encode nonstructural proteins including an RNA-dependent RNA polymerase, a capsid protein, and a phosphorylated small protein, respectively [3].

A number of HEVs have been isolated from humans and several animal species, but HEV has only 1 serotype. HEV isolates are classified into 4 major genotypes. The representative genotypes 1, 2, 3, and 4 are the Burma, Mexico, USA, and China strains, respectively [3-6]. Genotype 1 is most prevalent in Asia and Africa. Genotype 2 circulates in Mexico, Nigeria, and Chad. Genotype 3 is distributed globally in both industrialized and developing countries. Genotype 4 is found exclusively in Asia [7]. However, a recent study proposed classification of 6 HEV genotypes, including the 4 previously described as well as 2 additional genotypes isolated from wild boars [8]. In addition to HEVs isolated from humans and pigs, HEV variants have been identified in rats, ferrets, rabbits, bats, and chickens. While HEV genotypes 1 and 2 only infect humans, genotypes 3 and 4 have been isolated from human patients as well as from infected animals such as pigs. Therefore, HEV genotypes 3 and 4 are considered zoonotic vi-

ruses.

HEV infection occurs mainly through fecal-oral transmission. Waterborne outbreaks of HEV are often reported in endemic countries [9,10], where most infections occur in young adults aged 15-45 years [11,12]. HEV has a relatively low fatality rate, ranging 0.5-4%, but infections can induce acute liver failure in pregnant women, leading to 20-30% mortality [13-15]. Fulminant hepatitis and hepatic encephalopathy are the main causes of death [15].

Human HEVs

Clinical signs of acute HEV infection are similar to those of other viral hepatitis infections. HEV can cause jaundice, vomiting, appetite loss, fatigue, hepatalgia, and hepatomegaly [16,17]. Increases in liver enzymes such as alanine aminotransferase, aspartate transaminase, and gamma-glutamyl transpeptidase are also common [17,18]. HEV is typically diagnosed by detection of viral RNA in serum or fecal samples and testing for anti-HEV IgM or IgG antibodies in the patient sera [16]. Most HEV outbreaks are reported in developing countries in Asia, Africa, Central and South America, and the Middle East [19].

HEV infections usually occur from the consumption of contaminated or insufficiently treated drinking water [20-22]. Heavy rainfall and flooding seem to contribute to outbreaks of waterborne HEV infection in endemic regions [10,19]. Developed countries were previously assumed to be free of or non-endemic for HEV; sporadic cases of HEV in these countries have generally been associated with travel to endemic countries [23-25]. However, a considerable increase in acute HEV cases in patients who had never traveled to endemic regions prompted a study of the overall HEV infection status and the origin of infections in several developed countries [5,26]. Serological studies indicated that a considerable portion of people in developed countries had anti-HEV antibodies. For example, 19-21% of US blood donors had antibodies specific to HEV [27,28]. Studies conducted in several European counties such as England, Germany, Italy, and France demonstrated that the study populations also had relatively high rates (13-53%) of anti-HEV antibodies [29-32]. Similarly, seroprevalence studies in Asian countries such as Japan, South Korea, Hong Kong, Taiwan, and China have reported that approximately 6-43% of their populations had anti-HEV antibodies [33-37].

However, the prevalence of anti-HEV antibodies can vary depending on factors such as age, diet, type of employment, and environment. The seroprevalence studies in developed countries indicate that many HEV infections could be subclinical without development of acute hepatitis. Anti-HEV antibody levels commonly increase with age regardless of residence in endemic or non-endemic countries. Elderly people aged more than 50-60 years show higher seroprevalence of HEV infections than younger people. Similarly, swine farmers and veterinarians have much higher HEV-specific antibodies than control groups who do not have regular contact with pigs [35,38-40]. Employment in slaughterhouses is one of the highest risk jobs for HEV exposure, with the risk of infection increasing by as much as 1.5-3.5-fold [38]. Therefore, consumption of poorly sanitized water and frequent exposure to pigs may be the main risk factors for HEV infections in humans [41].

Animal HEVs

Swine HEV infection was first detected by the identification of HEV RNA in swine serum and fecal samples in 1995 [42]. However, the term "swine HEV" was first used 2 years later [43]. The infected pigs show no clinical signs but viremia and anti-HEV antibodies are detected in serum samples from infected pigs. The first isolated swine HEV showed a close similarity to human HEV, with 79-80% and 90-92% homologies in nucleotide and amino acid sequences, respectively. Subsequent serological studies have shown considerable levels of HEV infection in pigs in both HEV endemic and non-endemic countries [44,45]. Although there is significant variation between herds, anti-HEV antibodies were detected in about 20-100% of growing and adult pigs. In Korea, the overall prevalence of anti-swine HEV antibodies was approximately 15% [46]. Similarly, HEV RNA has been detected in 17.5% of fecal samples [47]. Serological studies and detection of HEV RNA in serum and fecal samples show that most swine HEV infections appear to occur at 2-3 months of age. HEV genotypes 3 and 4 have been isolated from pigs and have very similar genetic sequences to human HEV isolates [46,48,49]. These genotype isolates were tested to identify cross-species infection in pigs and humans. HEV genotype 3 isolated from pigs could infect human surrogate rhesus monkeys and a chimpanzee [50]. In a reverse experiment, genotype 3 human HEV isolate could infect pigs. Similarly, pigs and rhesus monkeys were infected by genotype 4 human HEV and genotype 4 swine HEV, respectively [51,52]. These experiments provide

 http://dx.doi.org/10.7774/cevr.2014.3.1.29

strong evidence that genotypes 3 and 4 of swine HEV could be the primary sources for human infections. A recent study reported a chimeric virus containing the capsid gene of genotype 4 human HEV in the backbone of a genotype 3 swine HEV. The chimeric HEV could infect both human cells and pigs [53]. These data underscore the potential for the emergence of a chimeric virus in nature with both human and swine HEV gene sequences. In addition, wild boars have been reported to be widely infected with genotypes 3 and 4 HEV [54]. Their genomic sequences are also very similar to those of the corresponding human HEV isolates. Therefore, both domestic pigs and wild boars are major reservoirs of HEV genotypes 3 and 4 that could infect humans.

Avian HEV was isolated in the USA from chickens with hepatitis-splenomegaly [55]. A subsequent study indicated that about 71% of chicken flocks and 30% of chickens in the USA were positive for HEV antibodies [56]. A similar pattern of HEV seroprevalence was observed in Korea, with seropositivity rates of 57% in chicken flocks and 28% in chickens [57]. These studies suggest that avian HEV is enzootic in several countries. Avian HEV has about 50% sequence identity with human and swine HEVs [58]. It is currently classified into 3 genotypes that cluster by geographical region: genotype 1 (Australia), genotype 2 (USA), and genotype 3 (Europe) [59,60]. Several B-cell epitopes have been identified in the capsid protein of avian HEV [61]. At least 2 of these epitopes are common to the human HEV capsid protein, and 1 epitope is common to avian and swine HEVs.

A novel HEV was recently isolated from rabbits in China [62]. The overall full-length genetic sequence shared 74%, 73%, 78-79%, 74-75%, and 46-47% identity with genotypes 1, 2, 3, 4, and avian HEV, respectively. Other rabbit HEVs isolated from the USA and France also show high levels of homology with genotype 3 HEV [63]. Both this close genetic similarity between rabbit HEV and human HEV genotype 3 and reports of rabbit HEV replication in human cell lines imply that rabbit HEV may be another zoonotic agent [64,65]. Like other genotype 3 HEVs, rabbit HEV isolates show high genetic heterogeneity. Sera collected from HEV-positive rabbits recognized the capsid proteins of human, swine, rat, and avian HEVs. In addition, antibodies produced against the capsid proteins of human, swine, rat, and avian HEV reacted with the rabbit HEV capsid protein [64]. The cross-reactivity of rabbit HEV antibody with other HEVs prompted further cross-species infection experiments. When pigs were intravenously inoculated with rabbit and rat HEV, half of the pigs inoculated with rabbit HEV showed evidence of infection [64]. In contrast, pigs inoculated with rat HEV did not show any evidence of infection. These data indicate that rabbit HEV has antigens closely related to those of other HEV strains and may infect both pigs and humans.

Rat has been suspected to be a host of HEV based on a high prevalence of anti-HEV antibody [66]. Recently, rat HEV was isolated from wild Norway rats in Germany [67]. The comparison of partial genomic sequences demonstrated 59.9% and 49.9% homology to human and avian HEV isolates, respectively. However, rats may act as carriers to transmit HEV infection to pigs. Wild rats and Norway rats captured around a pig farm contained genotype 3 HEV that was genetically identical to swine HEV found at the same farm [68,69]. These studies support the theory of HEV transmission from rats to pigs. Rat HEVs recently reported in Vietnam and Indonesia have genetic sequences that are similar within their geographical areas but very distinct from the first reported rat HEV in Germany [70,71]. Therefore, rats may host both their own HEV and genotype 3-related HEVs. However, a recent report showed that rats are susceptible only to rat HEV but not to genotype 1, 3, and 4 HEVs [72]. It has also been demonstrated that rat HEV cannot infect rhesus monkeys, suggesting that rat HEV may not be a zoonotic agent [73]. However, more evidence is required to determine if rats may be carriers of HEVs that infect humans and pigs.

Several studies have shown that a wide variety of animals can act as HEV hosts. In addition to pigs, wild boars, deer, and rats, novel HEV strains have also been found in ferrets and bats [74,75]. Ferret HEV is genetically close to rat HEV but distinct from other HEVs. Genetic sequence analysis indicated that bat HEV is also distinct from previously reported HEVs. A serological study revealed that about 16% of goats had anti-HEV antibodies; however, goat HEV RNA could not be isolated from the sera [76].

Zoonosis

Accumulating data indicate that human HEV infections are mediated by the consumption of uncooked or undercooked animal meat or foods made with pig organs such as liver. In Japan, considerable cases of fulminant hepatitis E have been reported after consumption of meats and entrails of pigs, wild boars, and deer [77-79]. A study reported that the full genome of an HEV isolated from wild boars had a nearly identical sequence (99.7% identity) to that of HEV isolated from wild

deer and patients who contracted HEV after eating raw deer meat [80]. These data imply that pigs, wild boar, and wild deer are an important source of HEV infection in humans. Recently, European countries have also reported sporadic cases of HEV infection. Consumption of raw seafood, pork liver sausage, and exposure to wild boars are proposed as major risk factors associated with HEV infection in Italy [25]. One of the risk factors for HEV infection in France is the ingestion of raw pork liver sausage, which has been reported to contain infectious HEV particles [81,82]. In addition, HEV was detected in muscle samples in experimentally infected pigs [83]. In Korea, there has been a single instance of fulminant HEV infection in a patient who consumed raw bile juice from wild boar [84]. The genetic sequence of HEV isolated from the patient matched genotype 4 swine HEV. These data clearly suggest swine HEV is mainly transmitted to humans through the consumption of under-cooked or raw pork meat and liver products.

Eating raw shellfish is another risk factor for human HEV infections. About 9% of oysters collected from the coastal regions of Korea harbored HEV whose sequence matched genotype 3 swine HEV [85]. These and other data indicate that raw shellfish cultivated in sewage-contaminated waters may be a significant source of HEV infection in humans [86].

Vaccine Development

The HEV capsid protein encoded by ORF2 is a structural unit that is assembled into virus particles. The capsid proteins of several HEV isolates contain neutralizing antigenic epitopes [87-89]. Therefore, several experimental HEV vaccines for humans have been developed using the capsid protein expressed by *Escherichia coli*, baculovirus, or plasmid DNA [90-92]. One of these vaccine candidates, "Hecolin," was recently approved by the Chinese government after successful phase III clinical trials and is now available for use in China [93-95].

Conclusion

Accumulating research data indicate that HEV is not an old disease restricted to developing countries. It is now generally accepted that HEV is endemic in both developing and industrialized countries and that HEV is a serious public health threat worldwide. Animal HEVs appear to cause most HEV infections in developed countries. Therefore, effective animal HEV vaccines should be developed to prevent cross-species HEV infection to humans.

References

1. Emerson SU, Purcell RH. Hepatitis E virus. In: Knipe DM, Howley PM, Griffin DE, et al., editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p.3047-58.
2. Reyes GR, Purdy MA, Kim JP, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. Science 1990;247:1335-9.
3. Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology 1991;185:120-31.
4. Huang CC, Nguyen D, Fernandez J, et al. Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). Virology 1992;191:550-8.
5. Schlauder GG, Dawson GJ, Erker JC, 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.
6. Wang Y, Ling R, Erker JC, et al. A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. J Gen Virol 1999;80(Pt 1):169-77.
7. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol 2006;16:5-36.
8. Smith DB, Purdy MA, Simmonds P. Genetic variability and the classification of hepatitis E virus. J Virol 2013;87:4161-9.
9. Arankalle VA, Chadha MS, Tsarev SA, et al. Seroepidemiology of water-borne hepatitis in India and evidence for a third enterically-transmitted hepatitis agent. Proc Natl Acad Sci U S A 1994;91:3428-32.
10. Corwin AL, Khiem HB, Clayson ET, et al. A waterborne outbreak of hepatitis E virus transmission in southwestern Vietnam. Am J Trop Med Hyg 1996;54:559-62.
11. Clayson ET, Shrestha MP, Vaughn DW, et al. Rates of hepatitis E virus infection and disease among adolescents and adults in Kathmandu, Nepal. J Infect Dis 1997;176:763-6.
12. Kumar S, Subhadra S, Singh B, Panda BK. Hepatitis E virus: the current scenario. Int J Infect Dis 2013;17:e228-33.
13. Gurley ES, Halder AK, Streatfield PK, et al. Estimating the burden of maternal and neonatal deaths associated with jaundice in Bangladesh: possible role of hepatitis E infec-

tion. Am J Public Health 2012;102:2248-54.
14. Hamid SS, Jafri SM, Khan H, Shah H, Abbas Z, Fields H. Fulminant hepatic failure in pregnant women: acute fatty liver or acute viral hepatitis? J Hepatol 1996;25:20-7.
15. Rayis DA, Jumaa AM, Gasim GI, Karsany MS, Adam I. An outbreak of hepatitis E and high maternal mortality at Port Sudan, Eastern Sudan. Pathog Glob Health 2013;107:66-8.
16. Goumba AI, Konamna X, Komas NP. Clinical and epidemiological aspects of a hepatitis E outbreak in Bangui, Central African Republic. BMC Infect Dis 2011;11:93.
17. Terzic D, Dupanovic B, Mugosa B, Draskovic N, Svirtlih N. Acute hepatitis E in Montenegro: epidemiology, clinical and laboratory features. Ann Hepatol 2009;8:203-6.
18. Turner J, Godkin A, Neville P, Kingham J, Ch'ng CL. Clinical characteristics of hepatitis E in a "Non-Endemic" population. J Med Virol 2010;82:1899-902.
19. Piper-Jenks N, Horowitz HW, Schwartz E. Risk of hepatitis E infection to travelers. J Travel Med 2000;7:194-9.
20. Benjelloun S, Bahbouhi B, Bouchrit N, et al. Seroepidemiological study of an acute hepatitis E outbreak in Morocco. Res Virol 1997;148:279-87.
21. Coursaget P, Buisson Y, Enogat N, et al. Outbreak of enterically-transmitted hepatitis due to hepatitis A and hepatitis E viruses. J Hepatol 1998;28:745-50.
22. Guthmann JP, Klovstad H, Boccia D, et al. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan, 2004: the role of water treatment methods. Clin Infect Dis 2006;42:1685-91.
23. Centers for Disease Control and Prevention (CDC). Hepatitis E among U.S. travelers, 1989-1992. MMWR Morb Mortal Wkly Rep 1993;42:1-4.
24. Johansson PJ, Mushahwar IK, Norkrans G, Weiland O, Nordenfelt E. Hepatitis E virus infections in patients with acute hepatitis non-A-D in Sweden. Scand J Infect Dis 1995;27:543-6.
25. La Rosa G, Muscillo M, Vennarucci VS, Garbuglia AR, La Scala P, Capobianchi MR. Hepatitis E virus in Italy: molecular analysis of travel-related and autochthonous cases. J Gen Virol 2011;92:1617-26.
26. Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC, Mushahwar IK. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. J Med Virol 1999;57:243-51.
27. Thomas DL, Yarbough PO, Vlahov D, et al. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. J Clin Microbiol 1997;35:1244-7.
28. Xu C, Wang RY, Schechterly CA, et al. An assessment of hepatitis E virus (HEV) in US blood donors and recipients: no detectable HEV RNA in 1939 donors tested and no evidence for HEV transmission to 362 prospectively followed recipients. Transfusion 2013;53(10 Pt 2):2505-11.
29. Faber MS, Wenzel JJ, Jilg W, Thamm M, Hohle M, Stark K. Hepatitis E virus seroprevalence among adults, Germany. Emerg Infect Dis 2012;18:1654-7.
30. Ijaz S, Vyse AJ, Morgan D, Pebody RG, Tedder RS, Brown D. Indigenous hepatitis E virus infection in England: more common than it seems. J Clin Virol 2009;44:272-6.
31. Mansuy JM, Bendall R, Legrand-Abravanel F, et al. Hepatitis E virus antibodies in blood donors, France. Emerg Infect Dis 2011;17:2309-12.
32. Romano L, Paladini S, Tagliacarne C, Canuti M, Bianchi S, Zanetti AR. Hepatitis E in Italy: a long-term prospective study. J Hepatol 2011;54:34-40.
33. Chiu DM, Chan MC, Yeung AC, Ngai KL, Chan PK. Seroprevalence of hepatitis E virus in Hong Kong, 2008-2009. J Med Virol 2013;85:459-61.
34. Dong C, Dai X, Shao JS, Hu K, Meng JH. Identification of genetic diversity of hepatitis E virus (HEV) and determination of the seroprevalence of HEV in eastern China. Arch Virol 2007;152:739-46.
35. Lee JT, Shao PL, Chang LY, et al. Seroprevalence of hepatitis E virus infection among swine farmers and the general population in rural Taiwan. PLoS One 2013;8:e67180.
36. Li RC, Ge SX, Li YP, et al. Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. Emerg Infect Dis 2006;12:1682-8.
37. Taniguchi M, Kim SR, Mishiro S, et al. Epidemiology of hepatitis E in Northeastern China, South Korea and Japan. J Infect 2009;58:232-7.
38. Krumbholz A, Mohn U, Lange J, et al. Prevalence of hepatitis E virus-specific antibodies in humans with occupational exposure to pigs. Med Microbiol Immunol 2012;201:239-44.
39. Meng XJ, Wiseman B, Elvinger F, 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;40:117-22.
40. 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;38:55-8.

41. Galiana C, Fernandez-Barredo S, Garcia A, Gomez MT, Perez-Gracia MT. Occupational exposure to hepatitis E virus (HEV) in swine workers. Am J Trop Med Hyg 2008;78:1012-5.
42. Clayson ET, Innis BL, Myint KS, et al. Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. Am J Trop Med Hyg 1995;53:228-32.
43. Meng XJ, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci U S A 1997;94:9860-5.
44. Chandler JD, Riddell MA, Li F, Love RJ, Anderson DA. Serological evidence for swine hepatitis E virus infection in Australian pig herds. Vet Microbiol 1999;68:95-105.
45. Meng XJ, Dea S, Engle RE, et al. Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. J Med Virol 1999;59:297-302.
46. Choi IS, Kwon HJ, Shin NR, Yoo HS. Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. J Clin Microbiol 2003;41:3602-8.
47. Kim SE, Kim MY, Kim DG, et al. Determination of fecal shedding rates and genotypes of swine hepatitis E virus (HEV) in Korea. J Vet Med Sci 2008;70:1367-71.
48. Huang FF, Haqshenas G, Guenette DK, et al. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. J Clin Microbiol 2002;40:1326-32.
49. Takahashi M, Nishizawa T, Miyajima H, et al. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol 2003;84(Pt 4):851-62.
50. Meng XJ, Halbur PG, Shapiro MS, et al. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. J Virol 1998;72:9714-21.
51. Arankalle VA, Chobe LP, Chadha MS. Type-IV Indian swine HEV infects rhesus monkeys. J Viral Hepat 2006;13:742-5.
52. Feagins AR, Opriessnig T, Huang YW, Halbur PG, Meng XJ. Cross-species infection of specific-pathogen-free pigs by a genotype 4 strain of human hepatitis E virus. J Med Virol 2008;80:1379-86.
53. Feagins AR, Cordoba L, Sanford BJ, et al. Intergenotypic chimeric hepatitis E viruses (HEVs) with the genotype 4 human HEV capsid gene in the backbone of genotype 3 swine HEV are infectious in pigs. Virus Res 2011;156:141-6.
54. Sato Y, Sato H, Naka 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;156:1345-58.
55. Haqshenas G, Huang FF, Fenaux M, et al. The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and chicken big liver and spleen disease virus. J Gen Virol 2002;83(Pt 9):2201-9.
56. Huang FF, Haqshenas G, Shivaprasad HL, et al. Heterogeneity and seroprevalence of a newly identified avian hepatitis E virus from chickens in the United States. J Clin Microbiol 2002;40:4197-202.
57. Kwon HM, Sung HW, Meng XJ. Serological prevalence, genetic identification, and characterization of the first strains of avian hepatitis E virus from chickens in Korea. Virus Genes 2012;45:237-45.
58. Huang FF, Sun ZF, Emerson SU, et al. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. J Gen Virol 2004;85(Pt 6):1609-18.
59. Bilic I, Jaskulska B, Basic A, Morrow CJ, Hess M. Sequence analysis and comparison of avian hepatitis E viruses from Australia and Europe indicate the existence of different genotypes. J Gen Virol 2009;90(Pt 4):863-73.
60. Marek A, Bilic I, Prokofieva I, Hess M. Phylogenetic analysis of avian hepatitis E virus samples from European and Australian chicken flocks supports the existence of a different genus within the Hepeviridae comprising at least three different genotypes. Vet Microbiol 2010;145:54-61.
61. Guo H, Zhou EM, Sun ZF, Meng XJ, Halbur PG. Identification of B-cell epitopes in the capsid protein of avian hepatitis E virus (avian HEV) that are common to human and swine HEVs or unique to avian HEV. J Gen Virol 2006;87(Pt 1):217-23.
62. Zhao C, Ma Z, Harrison TJ, et al. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. J Med Virol 2009;81:1371-9.
63. Cossaboom CM, Cordoba L, Cao D, Ni YY, Meng XJ. Complete genome sequence of hepatitis E virus from rabbits in the United States. J Virol 2012;86:13124-5.
64. Cossaboom CM, Cordoba L, Sanford BJ, et al. Cross-species infection of pigs with a novel rabbit, but not rat, strain

 http://dx.doi.org/10.7774/cevr.2014.3.1.29

of hepatitis E virus isolated in the United States. J Gen Virol 2012;93(Pt 8):1687-95.
65. Lhomme S, Dubois M, Abravanel F, et al. Risk of zoonotic transmission of HEV from rabbits. J Clin Virol 2013;58:357-62.
66. Kabrane-Lazizi Y, Fine JB, Elm J, et al. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. Am J Trop Med Hyg 1999;61:331-5.
67. Johne R, Plenge-Bonig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. J Gen Virol 2010;91(Pt 3):750-8.
68. Lack JB, Volk K, Van Den Bussche RA. Hepatitis E virus genotype 3 in wild rats, United States. Emerg Infect Dis 2012;18:1268-73.
69. Kanai Y, Miyasaka S, Uyama S, et al. Hepatitis E virus in Norway rats (Rattus norvegicus) captured around a pig farm. BMC Res Notes 2012;5:4.
70. Li TC, Ami Y, Suzaki Y, et al. Characterization of full genome of rat hepatitis E virus strain from Vietnam. Emerg Infect Dis 2013;19:115-8.
71. Mulyanto, Depamede SN, Sriasih M, et al. Frequent detection and characterization of hepatitis E virus variants in wild rats (Rattus rattus) in Indonesia. Arch Virol 2013;158:87-96.
72. Li TC, Yoshizaki S, Ami Y, et al. Susceptibility of laboratory rats against genotypes 1, 3, 4, and rat hepatitis E viruses. Vet Microbiol 2013;163:54-61.
73. Purcell RH, Engle RE, Rood MP, et al. Hepatitis E virus in rats, Los Angeles, California, USA. Emerg Infect Dis 2011;17:2216-22.
74. Drexler JF, Seelen A, Corman VM, et al. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. J Virol 2012;86:9134-47.
75. Raj VS, Smits SL, Pas SD, et al. Novel hepatitis E virus in ferrets, the Netherlands. Emerg Infect Dis 2012;18:1369-70.
76. Sanford BJ, Emerson SU, Purcell RH, et al. Serological evidence for a hepatitis E virus-related agent in goats in the United States. Transbound Emerg Dis 2013;60:538-45.
77. Masuda J, Yano K, Tamada Y, et al. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. Hepatol Res 2005;31:178-83.
78. Miyashita K, Kang JH, Saga 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;42:870-8.
79. Tei S, Kitajima N, Ohara S, et al. Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. J Med Virol 2004;74:67-70.
80. Takahashi K, Kitajima N, Abe N, Mishiro S. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. Virology 2004;330:501-5.
81. Colson P, Borentain P, Queyriaux B, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 2010;202:825-34.
82. Berto A, Grierson S, Hakze-van der Honing R, et al. Hepatitis E virus in pork liver sausage, France. Emerg Infect Dis 2013;19:264-6.
83. Bouwknegt M, Rutjes SA, Reusken CB, et al. The course of hepatitis E virus infection in pigs after contact-infection and intravenous inoculation. BMC Vet Res 2009;5:7.
84. Kim YM, Jeong SH, Kim JY, et al. The first case of genotype 4 hepatitis E related to wild boar in South Korea. J Clin Virol 2011;50:253-6.
85. Song YJ, Jeong HJ, Kim YJ, et al. Analysis of complete genome sequences of swine hepatitis E virus and possible risk factors for transmission of HEV to humans in Korea. J Med Virol 2010;82:583-91.
86. Balayan MS. Hepatitis E virus infection in Europe: regional situation regarding laboratory diagnosis and epidemiology. Clin Diagn Virol 1993;1:1-9.
87. Meng J, Dai X, Chang JC, et al. Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. Virology 2001;288:203-11.
88. Schofield DJ, Glamann J, Emerson SU, Purcell RH. Identification by phage display and characterization of two neutralizing chimpanzee monoclonal antibodies to the hepatitis E virus capsid protein. J Virol 2000;74:5548-55.
89. Zhang H, Dai X, Shan X, Meng J. Characterization of antigenic epitopes of the ORF2 protein from hepatitis E virus genotype 4. Virus Res 2009;142:140-3.
90. Jimenez de Oya N, Escribano-Romero E, Blazquez AB, et al. Characterization of hepatitis E virus recombinant ORF2 proteins expressed by vaccinia viruses. J Virol 2012;86:7880-6.
91. Kamili S, Spelbring J, Carson D, Krawczynski K. Protective efficacy of hepatitis E virus DNA vaccine administered by

gene gun in the cynomolgus macaque model of infection. J Infect Dis 2004;189:258-64.
92. Li SW, Zhang J, Li YM, et al. A bacterially expressed particulate hepatitis E vaccine: antigenicity, immunogenicity and protectivity on primates. Vaccine 2005;23:2893-901.
93. Wu T, Li SW, Zhang J, Ng MH, Xia NS, Zhao Q. Hepatitis E vaccine development: a 14 year odyssey. Hum Vaccin Immunother 2012;8:823-7.
94. Zhang J, Shih JW, Wu T, Li SW, Xia NS. Development of the hepatitis E vaccine: from bench to field. Semin Liver Dis 2013;33:79-88.
95. Zhu FC, Zhang J, Zhang XF, 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;376:895-902.

 http://dx.doi.org/10.7774/cevr.2014.3.1.29