Review Article

Emerging Enigmatic Hepatitis E Virus: Molecular Biology, Epidemiology, Diagnosis and Management in Pregnancy

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Abstract

Early in the 1980s, an epidemic of unexplained acute hepatitis was linked to the hepatitis E virus (HEV) which is now the 5th known type of viral hepatitis. The virus is single-stranded RNA that mostly affects developing countries but has lately expanded to developed economies as well. HEV is responsible for large-scale outbreaks of acute viral hepatitis. Acute hepatitis caused by hepatitis E infection has long gone unacknowledged, and its effects on developed countries are likewise misunderstood. In unhygienic settings, HEV is transmitted by the faecal-oral route, as well as through vertical transmission and, on rare occasions, through blood transfusions. HEV genotypes 1-4 are the most prevalent genotypes eliciting infections in humans out of the eight distinct genotypes that have been so far found. Humans are more likely to get HEV1 and HEV2 infections than HEV3 and HEV4, which are zoonotic. HEV infection has a wide range of clinical phenotypes, which makes diagnosis difficult. A variety of other variables also contribute to this difficulty. Serological screening alongside molecular analysis of RNA is used for laboratory diagnosis. Acute HEV infection can resolve on its own and doesn't require any special therapy. However, in certain patients, notably women who are pregnant and those with underlying chronic liver illnesses, acute HEV infection can proceed to chronic hepatitis and liver failure. It has been proposed that the accompanying hormonal changes and ensuing immune alterations during pregnancy are secondary causes of the increased death rate in pregnancy. In this study, we included the most recent information on the virus itself, epidemiology, diagnosis, pregnancy-related HEV, and treatment options for HEV infection in pregnancy. To comprehend the HEV's natural history, enhance management, and lessen the burden of disease globally, advancements in the detection and diagnosis of HEV infection are necessary.

Keywords: Hepatitis, Genotypes, Pregnancy, Hepatitis E

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Introduction

Hepatitis E virus (HEV), which is a member of the Hepeviridae family, is the most common source of acute viral hepatitis globally.[1] Despite the fact that HEV is a significant source of hepatitis and that understanding about it is growing, its origins are still unknown.[2] In the late 1970s, India was the first country to report the presence of an outbreak of non-A, non-B hepatitis unrelated to the transfusion of blood.[3] Due to this outbreak, there were approximately 52,000 icteric illnesses and an estimated 1,700 fatalities.[4] Later, in 1983, a team of Russian virologists Balayan *et al.*,[5] consumed a pooled faecal extract from infected troops and used electron microscopy to examine a sample of their own faeces. The HEV genome was characterized

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in the early 1990s by Tam *et al*.,[6] and from then up until 2005, HEV infections were thought to be endemic exclusively in developing nations in Asia, Africa, and Central America; nevertheless, infections in high-income countries were thought to be contracted while travelling through regions where epidemics and occasional cases occurred. This impression was, however, proved false with later research.

In this review article, our aim is to provide the most recent information on HEV itself, its molecular biology, epidemiology, diagnosis, pregnancy-related HEV, and treatment options for HEV infection in pregnancy. This narrative review is based on a literature search using online databases, that included Medline, Web of Science, Scopus, Embase, PubMed, Directory of Open Access Journals.

Molecular Biology

The genome of HEV has a single-stranded positive sense ribonucleic acid (RNA) and is a small, non-enveloped virus with a diameter of 27-34 nm.[7] The HEV genome has open reading frames abbreviated as ORF, which are discontinuous regions. These include (i) ORF1 encoding nonstructural (functional) proteins including RNA-dependent RNA polymerase and methyltransferase,[8] (ii) ORF2 encoding the viral capsid protein,[9] and (iii) ORF3 encoding a functional ion channel that plays a crucial role in the release of viral particles.[10] The recently found ORF4 is exclusive to HEV genotype 1 (HEV-1) and is essential for HEV RNA polymerase's correct operation.[11] The capsid protein expressed by ORF2 shows high levels of immunogenicity and protective and neutralizing properties are present in antibodies directed against this protein.[12] As a result, it appears that the capsid protein is an appropriate target for the development of an HEV vaccine.

The HEV virus has long been regarded as non-enveloped. This was determined based on the naked virus particles that were isolated from faeces. The virions seen in bile also appear similar. However, the viral particles in blood appear enveloped and wrapped in host-cell membranes.[13] Although the mechanism by which HEV particles are enveloped is not entirely understood, ORF3 seems to be an important element.[14] It is hypothesized that HEV virions' envelopes may be degraded by the detergent action of bile, producing the non-enveloped particles seen in bile and faeces.[15] Since the surface of the enveloped HEV virus particles lacks any antigenic proteins, they are immune to the neutralizing effects of anti-ORF2 antibodies. It's interesting to note that HEV isolated from serum is less contagious than HEV isolated from faeces.[16] The enveloped and non-enveloped viral particles penetrate host cells by different pathways.[17] Hence, HEV may both increase the infectivity of new hosts and circumvent the immune response of an existing host by taking on these two different forms.

Epidemiology

The most prevalent cause of acute viral hepatitis is now understood to be HEV. An estimated 20 million infections occur annually, leading to three million symptomatic cases and around 70,000 fatalities from HEV.[18] Despite how huge these numbers are, the real global burden of HEV is probably far higher.[19] According to seroprevalence studies, up to one-third of the global population may contract the disease at some point in their lifespan.[20] The case-to-infection ratio in endemic areas ranges from 1:3 to 1:4; when combined, these numbers show that more than 500 million people have had clinically evident hepatitis E.[21] At least 63 nations have been found to have hepatitis E outbreaks or sporadic cases, with almost half of them reporting significant epidemics.[22]

Geographical variances in circulating HEV genotypes are principally responsible for the wide variations in the epidemiology and clinical manifestation of HEV infection. It is believed that hepatitis E has been plaguing people for a very long time since mediaeval literature depicting jaundice supports this theory.

The HEV progenitor split into anthropotropic and enzootic forms around 1,344 years ago. As we know them now, the anthropotropic variation developed into genotypes 1 and 2, and the enzootic variant became genotypes 3 and 4.[23] So, HEV1, HEV2, HEV3, and HEV4 are the four HEV genotypes that may infect people of the eight different HEV genotypes. HEV1 and HEV2 are mostly found in humans, and no cases of HEV1 or HEV2 transmission from animals to humans have yet been documented. Periodically, HEV1 and HEV2 infections appear in various parts of Asia, Africa, Mexico, and the Middle East.[24] Large waterborne epidemics in these areas can be brought on by unintentional faecal contamination of water sources, especially after flooding and periods of high rainfall.[25] In endemic locations, sporadic HEV infections have also emerged in addition to the pandemic illness.[26] Although infections with genotypes 1 and 2 often result in self-limiting acute infectious hepatitis, they nonetheless

place a significant problem on healthcare services in low-income nations. While vertical transmission from mother to foetus during pregnancy is well-defined,[27] person-to-person transmission of HEV1 and HEV2 is uncommon in both sporadic and epidemic settings.[28] Additionally, it was noted that HEV1 can be transmitted by blood transfusions.[29] The rate of mortality in acute HEV1 or HEV2 infection is notably high in pregnant women and babies for unidentified processes.

Most HEV3 and HEV4 infections arise via zoonotic transmission, which is brought on by intimate contact with sick animals or by eating infected food (frequently raw or undercooked meat). The pig is the primary reservoir for these two genotypes, but they have also been found in wild boars, rabbits, goats, sheep, deer, horses, cats, and dogs.[30,31]Therefore, it is possible to think of these animals as probable zoonotic carriers for human transmission. They can also be found in significant amounts in the milk of sick cows and the faeces of asymptomatic animals.[32]

HEV was formerly thought to be exclusive to several underdeveloped countries. As a zoonotic infectious agent, it is currently recognized being endemic in the majority of high-income nations. In some affluent nations, locally acquired (autochthonous) HEV infections due to genotypes 3 and 4 have emerged as the leading cause of acute viral hepatitis. China, where genotype 1 (HEV1) was formerly the most prevalent genotype, has been surpassed by genotype 4, most likely as a result of better sanitation and hygiene practices.[33]

HEV genotypes 5 and 6 (HEV5 and HEV6) are not linked to diseases in people and have only been identified in wild boars thus far.[34] Camels have been found to carry HEV7 and HEV8.[35] The only case of human infection of HEV7 was in 2016 reported in a patient who consistently consumed camel milk and meat.[36] According to phylogenetic research, the viruses discovered in patient samples as well as samples from camel meat and milk were HEV7. Since then, no other human incidences have been found, though.

Diagnosis

The diverse clinical profile of HEV infection makes diagnosis challenging, and a variety of other variables add to this difficulty. Acute hepatitis E has biochemical test findings similar to acute hepatitis caused by other viruses. Recent appearance or several-fold rise in titres of particular IgG antibodies or the presence of HEV RNA in clinical specimens are used in the laboratory to make a diagnosis of recent HEV infection.[37]

HEV infections typically have a two to six weeks incubation period.[38] An essential indicator of acute HEV infection is the detection of anti-HEV IgM antibodies in the blood. Anti-HEV IgM antibodies are positive for a brief period of time (about 3-4 months), however, it might sometimes last for a year.[39] The length of the anti-HEV IgG response is yet unknown, despite it being comparatively long-lasting and an indicator of past-infection. After three weeks of exposure, HEV RNA may be found in the blood, and stool viral shedding lasts for around four to six weeks.[40]

The most commonly used serological approach for determining anti-HEV IgG and IgM antibodies in the assessment of HEV infection is enzyme-linked immunosorbent assays. In addition to detecting anti-HEV antibodies, enzyme immunoassays may also detect the HEV capsid antigen. The sensitivity and specificity of several currently used commercial tests for anti-HEV antibodies, however, are not up to standard. The sensitivity of each assay in one research that examined 6 different assays ranged from 72% to 98%, while the specificity ranged from 78% to 96%.[41] Additionally, anti-HEV antibodies are frequently not detected in individuals with chronic infections who are immunosuppressed. HEV RNA can be found in blood or stool by using molecular laboratory testing. Although these methods provide a more accurate diagnostic test, there are clear cost restrictions.

The sensitivity of various molecular diagnostic assay types varies in the detection of HEV RNA. As a result, the WHO created an international reference panel and standard for genotypes 1-4.[42] This allowed laboratories to release the findings by means of a common unit, namely the international unit (IU), and also allows them to compare the results acquired by various molecular assays.

Treatment

There are no recommendations from the World Health Organization for treating hepatitis E. Hepatitis E treatment is often supportive in nature. Immunosuppressive medication withdrawal or reduction, ribavirin administration, interferon administration, or a combination of these treatments have all been proven beneficial in treating chronic hepatitis E in solid organ transplant recipients.[43] The best part is that the vast majority of the time, acute HEV infection can resolve on

its own and doesn't require any special therapy. However, in certain patients, notably pregnant women and those with underlying chronic liver illnesses, acute HEV infection can proceed to chronic hepatitis and liver failure. Ribavirin or immunosuppressive drugs like corticosteroids are used to treat extra-hepatic symptoms of HEV infections.

HEV in Pregnancy

HEV is typically transmitted faeco-orally, even though the person-to-person transmission has also been documented.[44] There is mounting evidence that HEV infection particularly with HEV1 during the third trimester of pregnancy significantly increases the risk of fulminant hepatic failure, maternal morbidity and mortality in South Asian countries.[45] HEV infection during pregnancy has been linked to miscarriage, preterm labour, or stillbirth in addition to maternal morbidity. Hepatitis E mortality in the general population ranges from 0.1 - 4%, while among pregnant women in the third trimester, it can vary from 10 - 50%. Uncertainty exists regarding the precise mechanism causing the significantly high death rate among pregnant women.[46]

At the point where maternal and foetal circulation converges, the placenta releases certain enzymes and cytokines that decrease cell-mediated immunity, providing a route for HEV transmission.[46] Furthermore, evidence of HEV replication in the placenta has been reported.[47] The only genotypes known to cause complications in pregnancy are genotypes 1 and 2, hence the higher virulence of genotypes 1 and 2 as well as immunological and hormonal changes during pregnancy are proposed mechanisms.[48]

Pregnancy is thought to enhance steroid hormone synthesis, which enhances virus replication and suppresses cell-mediated immunity, particularly inhibition of CD4 cells.[46] Although HEV is ostensibly linked to pregnant mortality, there are important geographical variations that should not be disregarded. In northern India, hepatitis E is highly prevalent. According to a prospective research conducted in New Delhi, India, hepatitis E infection is responsible for over 60% of viral hepatitis cases in pregnant women. The probability of developing fulminant hepatic failure was 2.7 times greater in HEV-infected women (55%) than in non-HEV-infected women (20%). Maternal mortality due to fulminant hepatic failure was significantly higher in the HEV-infected group (41%) compared to 7% in the non-HEV-infected group.[49] Contrarily, some regions where HEV is endemic, including Egypt and the southern part of India, have seen little to no HEV-related mortality.[46] Anti-HEV prevalence in one research of 2,428 pregnant women in Egypt was 84.3%. There were no cases of acute viral hepatitis recorded.[50] These geographical differences raise the possibility that additional elements, such as the age of exposure, nutritional condition, or inadequate access to healthcare, are potentially having a crucial but underappreciated impact on disease prognosis.

A study including 469 pregnant women looked at the high likelihood of vertical transmission of the HEV infection from mother to child and found that 100% of babies had been exposed, albeit there may have been some selection bias. The significant transmission rate nonetheless highlights the significance of vertical transmission of HEV infection.[51]

Studies on animals have not added to our knowledge of the pathogenesis of HEV in pregnancy. The course of liver damage was comparable in both groups of primates in an animal investigation to examine the effects brought on by HEV in pregnant and non-pregnant animals.[52] This is not unexpected, though, given that primates only experience a lesser kind of liver damage as a result of HEV infection. One of several potential host variables, such as variations in immunological and hormonal factors happening during pregnancy, and the genetic and environmental factors, may be linked to the severe liver damage caused by HEV infection during pregnancy.

Despite the colostrum's anti-HEV antibodies and HEV RNA, breastfeeding is regarded as safe in asymptomatic HEV-infected mothers. However, it is deemed risky if the mother has a high viral load or severe hepatitis. Formula feeding is indicated in these situations due to the risk of transmission from contaminated breast milk or sores on the nipple during sucking.[53]

Management of HEV in Pregnancy

The Food and Drug Administration (FDA) placed ribavirin in pregnancy category X due to its embryocidal and teratogenic impacts in animal studies.[54,55] Ribavirin is thus not advised for usage in women who are pregnant. The FDA placed IFN-α in pregnancy category C due to its severe effects and ability to cause abortion in animals.[55] As a result, it was advised against giving IFN-α to pregnant women.[56] Sofosbuvir recently demonstrated antiviral effectiveness against HEV both in vitro and in vivo, and as a pregnancy category B medicine, maybe a potential antiviral medication against

HEV in pregnancy.[57] Interferon λ1-3 was demonstrated to prevent HEV replication when compared to other antiviral options.[58] Before sofosbuvir may be advised for HEV infection during pregnancy, further well-controlled research is required. There is still more work to be done on the other antiviral possibilities described above. As a result, careful monitoring and intense treatment are presently encouraged in the management of HEV infection in pregnant women.[59] Preventing HEV infection during pregnancy is possibly the most crucial management strategy given that there is no proven therapy for HEV infection in pregnant women.[60]

HEV Vaccine

In 2010, a phase-3 trial with more than 100,000 individuals from China evaluated an HEV vaccine based on a protein encoded by ORF 2 of HEV genotype 1. The long-term effectiveness and safety of this vaccination were investigated over a period of more than 4 years in reference to a control group (n = 56302 individuals). Only 60 instances of hepatitis E were confirmed by the authors, and seven of those belonged to the immunized group. Moreover, there were no reported severe vaccine-related side effects.[61] Additionally, Hecolin vaccine (from Innovax, China) is being tested in a significant ongoing study in Bangladesh on more than 20000 pregnant women. To fully comprehend the efficiency and safety of Hecolin in pregnant women who are at high risk of HEV1 infections, the findings of this research will be crucial.[62]

Conclusion

HEV infection is not just prevalent in some developing nations; it is also prevalent in many developed countries and is mostly zoonotic in origin. In the third trimester of pregnancy, infection with HEV especially with HEV1 has a more serious consequence that may result in fulminant hepatitis, causing maternal and foetal death. With the exception of individuals who have symptomatic HEV infection, breastfeeding is generally safe for mothers with hepatitis E infection. Many aspects involving HEV, particularly HEV infection during pregnancy, remain unresolved despite growing understanding through research. Genomic diversity and HEV variants could well be related to the extent of HEV infection in pregnancy, in addition to immunological and hormonal aspects. Investigating the common HEV genotypes, their virulence, and pregnant morbidity may thus be crucial.

References

1. Fierro NA, Realpe M, Meraz-Medina T, Roman S, Panduro A. Hepatitis E virus: An ancient hidden enemy in Latin America. World J Gastroenterol. 2016;22(7):2271-83. doi: 10.3748/wjg.v22.i7.2271.
2. Nimgaonkar I, Ding Q, Schwartz RE, Ploss A. Hepatitis E virus: advances and challenges. Nat Rev Gastroenterol Hepatol. 2018;15(2):96-110. doi: 10.1038/nrgastro.2017.150.
3. 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. doi: 10.1016/0002-9343(80)90200-4.
4. Khuroo MS. Discovery of hepatitis E: the epidemic non-A, non-B hepatitis 30 years down the memory lane. Virus Res. 2011;161(1):3-14. doi: 10.1016/j.virusres.2011.02.007.
5. Balayan MS, Andjaparidze AG, Savinskaya SS, Ketiladze ES, Braginsky DM, Savinov AP, Poleschuk VF. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. Intervirology. 1983;20(1):23-31. doi: 10.1159/000149370.
6. Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology. 1991;185(1):120-31. doi: 10.1016/0042-6822(91)90760-9.
7. Kenney SP, Meng XJ. Hepatitis E Virus Genome Structure and Replication Strategy. Cold Spring Harb Perspect Med. 2019;9(1):a031724. doi: 10.1101/cshperspect.a031724.
8. Szkolnicka D, Pollán A, Da Silva N, Oechslin N, Gouttenoire J, Moradpour D. Recombinant Hepatitis E Viruses Harboring Tags in the ORF1 Protein. J Virol. 2019;93(19):e00459-19. doi: 10.1128/JVI.00459-19.
9. Pezzoni G, Stercoli L, Pegoiani E, Brocchi E. Antigenic Characterization of ORF2 and ORF3 Proteins of Hepatitis E Virus (HEV). Viruses. 2021;13(7):1385. doi: 10.3390/v13071385.
10. Ding Q, Heller B, Capuccino JM, Song B, Nimgaonkar I, Hrebikova G, Contreras JE, Ploss A. Hepatitis E virus ORF3 is a functional ion channel required for release of infectious particles. Proc Natl Acad Sci U S A. 2017;114(5):1147-1152. doi: 10.1073/pnas.1614955114.
11. Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, et al. Endoplasmic Reticulum Stress Induced Synthesis of a Novel Viral Factor Mediates Efficient Replication of Genotype-1 Hepatitis E Virus. PLoS Pathog. 2016;12(4):e1005521. doi: 10.1371/journal.ppat.1005521.
12. Debing Y, Moradpour D, Neyts J, Gouttenoire J. Update on hepatitis E virology: Implications for clinical practice. J Hepatol. 2016;65(1):200-212. doi: 10.1016/j.jhep.2016.02.045.
13. Nagashima S, Takahashi M, Kobayashi T; Tanggis, Nishizawa T, Nishiyama T, et al. Characterization of the Quasi-Enveloped Hepatitis E Virus Particles Released by the Cellular Exosomal Pathway. J Virol. 2017;91(22):e00822-17. doi: 10.1128/JVI.00822-17.
14. Glitscher M, Hildt E. Hepatitis E virus egress and beyond - the manifold roles of the viral ORF3 protein. Cell Microbiol. 2021;23(12):e13379. doi: 10.1111/cmi.13379.
15. Takahashi M, Tanaka T, Takahashi H, Hoshino Y, Nagashima S, Jirintai, et al. Hepatitis E Virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the

coexistence of HEV antibodies: characterization of HEV virions in blood circulation. J Clin Microbiol. 2010;48(4):1112-25. doi: 10.1128/JCM.02002-09.
16. Sayed IM, Verhoye L, Cocquerel L, Abravanel F, Foquet L, Montpellier C, et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. Gut. 2017;66(5):920-929. doi: 10.1136/gutjnl-2015-311109.
17. Yin X, Ambardekar C, Lu Y, Feng Z. Distinct Entry Mechanisms for Nonenveloped and Quasi-Enveloped Hepatitis E Viruses. J Virol. 2016;90(8):4232-4242. doi: 10.1128/JVI.02804-15.
18. World Health Organization. Hepatitis E: Fact Sheet. Available at https://www.who.int/news-room/fact-sheets/detail/hepatitis-e accessed September 29, 2022).
19. Mihalčin M, Husova L, Vasickova P, Bena M, Husa P. Hepatitis E - epidemiology and clinical course in the largest cohort in the Czech Republic. Arch Med Sci. 2022;18(5):1395-1398. doi: 10.5114/aoms/152338.
20. Purcell RH and Emerson SU. Prevention. In: Thomas HC, Lemon S and Zuckerman AJ (eds) Viral hepatitis. 3rd ed. Malden, MA: Blackwell Publishing, 2005, pp.635–645.
21. Webb GW, Dalton HR. Hepatitis E: an underestimated emerging threat. Ther Adv Infect Dis. 2019;6:2049936119837162. doi: 10.1177/2049936119837162.
22. Aggarwal R. The global prevalence of hepatitis E virus infection and susceptibility: a systematic review. Geneva: World Health Organization; 2010 (Available at http://whqlibdoc. who.int/hq/2010/WHO_IVB_10.14_eng.pdf) accessed on Aug 25, 2022.
23. Khuroo MS, Khuroo MS, Khuroo NS. Hepatitis E: Discovery, global impact, control and cure. World J Gastroenterol. 2016;22(31):7030-45. doi: 10.3748/wjg.v22.i31.7030.
24. Gunsar F. Hepatitis E update. Hepatol Forum. 2020;1(1):37-40. doi: 10.14744/hf.2020.0001.
25. Tripathy AS, Sharma M, Thorat NC, Jadhav S, Koshatwar KA. An outbreak of hepatitis E in Yavatmal, India, 2019. J Med Virol. 2021;93(6):3761-3768. doi: 10.1002/jmv.26377.
26. Gupta E, Agarwala P. Hepatitis E virus infection: An old virus with a new story! Indian J Med Microbiol. 2018;36(3):317-323. doi: 10.4103/ijmm.IJMM_18_149.
27. Okwara VC, Mbachu II, Ndububa VI, Okpara HC, Mbachu CP. Seroprevalence, Associated Factors, and Fetomaternal Outcome in Pregnant Women That Tested Positive to Hepatitis E Antibodies in Nigeria. Obstet Gynecol Int. 2021;2021:9341974. doi: 10.1155/2021/9341974.
28. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, Dalton HR. Hepatitis E virus infection. Nat Rev Dis Primers. 2017;3:17086. doi: 10.1038/nrdp.2017.86.
29. Saba N, Waheed U, Mohammad I, Wazeer A. Hepatitis E virus RNA in Pakistani blood donors: An emerging transfusion transmitted infection. Glob J Transfus Med 2020;5(2):233. doi: 10.4103/GJTM.GJTM_85_20
30. Barroso P, Risalde MA, García-Bocanegra I, Acevedo P, Barasona JÁ, Caballero-Gómez J, et al. Long-Term Determinants of the Seroprevalence of the Hepatitis E Virus in Wild Boar (*Sus scrofa*). Animals (Basel). 2021;11(6):1805. doi: 10.3390/ani11061805.
31. Tialla D, Cissé A, Ouédraogo GA, Hübschen JM, Tarnagda Z, Snoeck CJ. Prevalence of hepatitis E virus antibodies in cattle in Burkina Faso associated with swine mixed farming. J Vet Sci. 2022;23(3):e33. doi: 10.4142/jvs.21235.
32. Huang F, Li Y, Yu W, Jing S, Wang J, Long F, et al. Excretion of infectious hepatitis E virus into milk in cows imposes high risks of zoonosis. Hepatology. 2016;64(2):350-9. doi: 10.1002/hep.28668.
33. Dai X, Dong C, Zhou Z, Liang J, Dong M, Yang Y, et al. Hepatitis E virus genotype 4, Nanjing, China, 2001-2011. Emerg Infect Dis. 2013;19(9):1528-30. doi: 10.3201/eid1909.130013.
34. Li TC, Kataoka M, Takahashi K, Yoshizaki S, Kato T, Ishii K, et al. Generation of hepatitis E virus-like particles of two new genotypes G5 and G6 and comparison of antigenic properties with those of known genotypes. Vet Microbiol. 2015;178(1-2):150-7. doi: 10.1016/j.vetmic.2015.04.020.
35. Rasche A, Saqib M, Liljander AM, Bornstein S, Zohaib A, Renneker S, et al. Hepatitis E Virus Infection in Dromedaries, North and East Africa, United Arab Emirates, and Pakistan, 1983-2015. Emerg Infect Dis. 2016;22(7):1249-52. doi: 10.3201/eid2207.160168.
36. Lee GH, Tan BH, Teo EC, Lim SG, Dan YY, Wee A, et al. Chronic Infection With Camelid Hepatitis E Virus in a Liver Transplant Recipient Who Regularly Consumes Camel Meat and Milk. Gastroenterology. 2016;150(2):355-7.e3. doi: 10.1053/j.gastro.2015.10.048.
37. Khudyakov Y, Kamili S. Serological diagnostics of hepatitis E virus infection. Virus Res. 2011;161(1):84-92. doi: 10.1016/j.virusres.2011.06.006.
38. Abravanel F, Chapuy-Regaud S, Lhomme S, Miedougé M, Peron JM, Alric L, et al. Performance of anti-HEV assays for diagnosing acute hepatitis E in immunocompromised patients. J Clin Virol. 2013;58(4):624-8. doi: 10.1016/j.jcv.2013.10.003.
39. Ishida S, Yoshizumi S, Sakata H, Matsubayashi K. Broadly Reactive Real-Time RT-PCR Assay for the Detection of Hepatitis E Virus and Simultaneous Genotyping by Single Nucleotide Polymorphism Analysis. Microbiol Spectr. 2022;10(1):e0191221. doi: 10.1128/spectrum.01912-21.
40. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on hepatitis E virus infection. J Hepatol. 2018;68(6):1256-1271. doi: 10.1016/j.jhep.2018.03.005.
41. 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. doi: 10.1086/654801.
42. Baylis SA, Blümel J, Mizusawa S, Matsubayashi K, Sakata H, Okada Y, Nübling CM, Hanschmann KM; HEV Collaborative Study Group. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. Emerg Infect Dis. 2013;19(5):729-35. doi: 10.3201/eid1905.121845.
43. Péron JM, Abravanel F, Guillaume M, Gérolami R, Nana J, Anty R, et al. Treatment of autochthonous acute hepatitis E with short-term ribavirin: a multicenter retrospective study. Liver Int. 2016;36(3):328-33. doi: 10.1111/liv.12911.
44. Dalton HR, Izopet J. Transmission and Epidemiology of Hepatitis E Virus Genotype 3 and 4 Infections. Cold Spring Harb

Perspect Med. 2018;8(11):a032144. doi: 10.1101/cshperspect.a032144.
45. Kar P, Sengupta A. A guide to the management of hepatitis E infection during pregnancy. Expert Rev Gastroenterol Hepatol. 2019;13(3):205-11. doi: 10.1080/17474124.2019.1568869.
46. Navaneethan U, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: understanding the pathogenesis. Liver Int 2008;28(9):1190-1199. doi: 10.1111/j.1478-3231.2008.01840.x.
47. Bose PD, Das BC, Hazam RK, Kumar A, Medhi S, Kar P. Evidence of extrahepatic replication of hepatitis E virus in human placenta. J Gen Virol. 2014;95(Pt 6):1266-1271. doi: 10.1099/vir.0.063602-0.
48. Sayed IM, Vercouter AS, Abdelwahab SF, Vercauteren K, Meuleman P. Is hepatitis E virus an emerging problem in industrialized countries? Hepatology. 2015;62(6):1883-92. doi: 10.1002/hep.27990.
49. Patra S, Kumar A, Trivedi SS, Puri M, Sarin SK. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. Ann Intern Med. 2007;147(1):28-33. doi: 10.7326/0003-4819-147-1-200707030-00005.
50. Stoszek SK, Abdel-Hamid M, Saleh DA, El Kafrawy S, Narooz S, Hawash Y, et al. High prevalence of hepatitis E antibodies in pregnant Egyptian women. Trans R Soc Trop Med Hyg. 2006;100(2):95-101. doi: 10.1016/j.trstmh.2004.12.005.
51. Kumar RM, Uduman S, Rana S, Kochiyil JK, Usmani A, Thomas L. Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. Eur J Obstet Gynecol Reprod Biol. 2001;100(1):9-15. doi: 10.1016/s0301-2115(01)00448-1.
52. Aggarwal R. Hepatitis E and pregnancy. Indian J Gastroenterol. 2007;26(1):3-5.
53. Khuroo MS. Hepatitis E and Pregnancy: An Unholy Alliance Unmasked from Kashmir, India. Viruses. 2021;13(7):1329. doi: 10.3390/v13071329.
54. Sinclair SM, Jones JK, Miller RK, Greene MF, Kwo PY, Maddrey WC. The Ribavirin Pregnancy Registry: An Interim Analysis of Potential Teratogenicity at the Mid-Point of Enrollment. Drug Saf. 2017;40(12):1205-1218. doi: 10.1007/s40264-017-0566-6.
55. Spera AM, Eldin TK, Tosone G, Orlando R. Antiviral therapy for hepatitis C: Has anything changed for pregnant/lactating women? World J Hepatol. 2016;8(12):557-65. doi: 10.4254/wjh.v8.i12.557.
56. Kinast V, Burkard TL, Todt D, Steinmann E. Hepatitis E Virus Drug Development. Viruses. 2019;11(6):485. doi: 10.3390/v11060485.
57. van der Valk M, Zaaijer HL, Kater AP, Schinkel J. Sofosbuvir shows antiviral activity in a patient with chronic hepatitis E virus infection. J Hepatol. 2017;66(1):242-243. doi: 10.1016/j.jhep.2016.09.014.
58. Nishiyama T, Kobayashi T, Jirintai S, Kii I, Nagashima S, Prathiwi Primadharsini P, et al. Screening of novel drugs for inhibiting hepatitis E virus replication. J Virol Methods. 2019;270:1-11. doi: 10.1016/j.jviromet.2019.04.017.
59. Kar P, Sengupta A. A guide to the management of hepatitis E infection during pregnancy. Expert Rev Gastroenterol Hepatol. 2019;13(3):205-211. doi: 10.1080/17474124.2019.1568869.
60. Terrault NA, Levy MT, Cheung KW, Jourdain G. Viral hepatitis and pregnancy. Nat Rev Gastroenterol Hepatol. 2021;18(2):117-130. doi: 10.1038/s41575-020-00361-w.
61. Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, 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. doi: 10.1016/S0140-6736(10)61030-6.
62. US National Library of Medicine. Clinical Trials. Available at https://clinicaltrials.gov/ct2/show/NCT02759991 accessed on July 25, 2022.