Hepatitis D: advances and challenges

Zhijiang Miao[1], Zhenrong Xie[2], Li Ren[3], Qiuwei Pan[1,4]

[1]Postgraduate School Molecular Medicine, Erasmus MC-University Medical Center, Rotterdam, The Netherlands;
[2]Biobank, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650032, China;
[3]Department of Obstetrics and Gynecology, The First People's Hospital of Yunnan Province, Kunming, Yunnan 650031, China;
[4]Biomedical Research Center, Northwest Minzu University, Lanzhou, Gansu 730030, China.

Abstract

Hepatitis D virus (HDV) infection causes the most severe form of viral hepatitis with rapid progression to cirrhosis, hepatic decompensation, and hepatocellular carcinoma. Although discovered > 40 years ago, little attention has been paid to this pathogen from both scientific and public communities. However, effectively combating hepatitis D requires advanced scientific knowledge and joint efforts from multi-stakeholders. In this review, we emphasized the recent advances in HDV virology, epidemiology, clinical feature, treatment, and prevention. We not only highlighted the remaining challenges but also the opportunities that can move the field forward.

Keywords: Hepatitis D virus; Hepatitis B virus; Virology; Epidemiology; Clinical impact; Treatment; Prevention

Introduction

Viral hepatitis caused by the infections of hepatotropic viruses is a major global public health problem. In 2016, the World Health Organization adopted a strategy aiming at the elimination of viral hepatitis by 2030 focusing on hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.[1] However, little attention has been reserved to the hepatitis D virus (HDV), a satellite virus coinfecting with HBV.[2] It is estimated that approximately 240 to 350 million people are living with chronic HBV infection,[3-5] but the coinfection of HDV with HBV provokes the most severe form of acute and chronic viral hepatitis.[6-9] This constitutes a major contributor to viral hepatitis-associated cirrhosis, hepatocellular carcinoma (HCC), and mortality.[2,10] Increasing awareness and research are essential for the development of specific strategies aiming at prevention and control of HDV infection. This prompted us to provide an update on the advances achieved in the HDV field and to highlight the challenges that deserve further investigation.

HDV virology

HDV was firstly identified both in liver biopsies and in the serum from chronic HBV infectedpatients experiencing severe hepatitis in the 1970s.[2,11] The International Committee on Taxonomy of Viruses has classified HDV as the archetypical member of the *Deltaviridae* family, representing the *Deltavirus* genus.[12] Recently, more HDV-like viruses (deltaviruses) have been discovered in a diverse range of hosts, including birds,[13] snakes,[14] rodents,[15] white-tailed deer,[16] Eastern woodchucks,[17] fish, amphibians, and even invertebrates.[18] This strongly indicates that HDV has a much longer evolutionary history than previously anticipated, although the origin of HDV remains uncertain.[19,20]

Human HDV is a peculiar hepatic pathogen and is a defective RNA virus. It completely relies on cellular enzymes for genome replication and dependents on HBV for its propagation in hepatocytes.[21,22] Upon entry, both the genomic RNA (gRNA) and antigenomic RNA (agRNA) are synthesized by host cellular DNA-dependent RNA polymerases via a double-rolling circle mechanism.[23] Because the HDV genome encodes only one functional open reading frame for the translation of two isoforms of hepatitis delta antigen (HDAg), small-HDAg (S-HDAg), and large-HDAg (L-HDAg).[24] Following several post-translational modifications, HDAg proteins assemble with HDV gRNA to form HDV ribonucleoproteins (RNPs), which are subsequently enveloped by three HBV envelope proteins comprised of small/middle/large HBV surface antigen (S/M/L-HBsAg) to generate infectious HDV particles.[25-27] Sharing the envelope proteins with HBV, HDV exploits not only the same particle

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002011

Zhijiang Miao and Zhenrong Xie contributed equally to the work.
Correspondence to: Qiuwei Pan, Biomedical Research Center, Northwest Minzu University, No. 1, Xibei Xincun, Lanzhou, Gansu 730030, China
E-Mail: q.pan@erasmusmc.nl

Chinese Medical Journal 2022;135(7)
Received: 12-07-2021; **Online:** 28-02-2022 **Edited by:** Yuanyuan Ji

release machinery but also the identical transmission route, especially with regard to cellular entry factors.[21,22] HDV explores the HBV receptor, sodium taurocholate cotransporting polypeptide (NTCP), for entry.[28] Of note, NTCP is a bile salt transporter exclusively expressed on the sinusoidal site of hepatocytes, which may explain the hepatic tropism of HBV and HDV.[21,28]

For HDV propagation, HBV was considered as the exclusive helper. However, a series of *in vitro* experiments have demonstrated that HDV RNPs could be artificially packaged into envelopes from several non-HBV-related viruses such as hepacivirus, flavivirus, and vesiculovirus, allowing egress of HDV RNPs from infected cells and subsequent entry into cell lines expressing their respective receptors.[21,26,29-31] In fact, in humanized mice, HDV mono-infection can persist intra-hepatically for at least 6 weeks in the absence of HBV, and infectious HDV particles can be released following HBV superinfection.[32] Furthermore, a recent study also demonstrated that coinfection of helper virus may not even be required for the horizontal transmission of rodent deltavirus.[15] Taken together, these new studies are arguing against a possible co-evolution process between deltaviruses and hepadnavirus.[15,16] Clearly, the advance in understanding HDV virology would have a major impact on the diagnosis, treatment, and prevention of the disease.

Genome classification

HDV is the smallest human virus ever discovered. Its genome is a circular negative single-stranded RNA (ssRNA) composed of approximately 1672 to 1697 nucleotides, depending on the strain.[33,34] As an ssRNA virus, HDV rapidly evolves resulting in high genetic diversity. The nucleotide divergence between different strains is $> 35\%$.[34,35] Further heterogeneity may result from recombination. Homologous recombination between different HDV strains has been reported in patients that suffered from multi-strain infection and has also been observed in the laboratory upon co-transfection of the genomes of different strains in cell culture systems.[36-38] Even though both mutation and recombination are important driving forces in the evolution, HDV recombinants are rare and do not spread widely as circulating recombinant forms, for reasons yet unknown.

During viral replication, three forms of HDV RNA are present: gRNA, agRNA, and messenger RNA.[39] This, to some extent, led to substantial chaos with respect to the registration of HDV sequences in public databases,[35] resulting in the definition of two inconsistent classification systems.[3,34] However, in the updated classification system when the recombinants were identified and removed and sequences were standardized, HDV strains are clearly clustered into eight genotypes, further grouped into 18 subtypes.[35] Based on comparisons of nucleotide similarity and genetic distance between strains, criteria for identifying novel HDV genotypes or subtypes have been summarized and reformulated, and reference genomes for each subtype have also been proposed.[35] Although the proposed system will likely evolve, as future epidemiological and genomic data will accumulate,[40] using standardized classification and criteria should largely avoid potential inconsistency in future research and clinical management.[41-48]

Epidemiology

The first attempt to estimate the regional epidemiology of HDV was conducted for South America in 1996, based on data published in the 1980s and 1990s. This study concluded that approximately 5% of HBV carriers in South America were coinfected with HDV, equivalent to 300,000 people in this region at the time.[49] Thereafter, this regional prevalence was extrapolated to an estimation of 15 to 20 million HDV infections worldwide.[50,51] This, however, was challenged in 2017 by an estimate of 7 million infections in sub-Saharan Africa alone.[52] In response, the global estimation was adjusted upwards to approximately 62 to 74 million in 2019,[53,54] but this has raised the concern of overestimation.[55,56] By stratifying the general population and HBV carriers, the pooled prevalence of HDV was estimated as 13.02% among HBV carriers and 0.80% in the general population, corresponding to 48 to 60 million infections worldwide.[9] However, a more recent meta-analysis has estimated HDV prevalence of 4.5% among all HBV carriers, which was translated into a prevalence of only 0.16% of the global population, corresponding to 12 million people worldwide.[57] But their underlying assumption may be questionable as HDV infected individuals are likely to be underrepresented in asymptomatic carriers.[58]

It is important to recognize the huge heterogeneities of HDV prevalence among different populations. For example, the infection rate can be much higher among particular risk populations including intravenous drug users, HCV-coinfected individuals, and human immunodeficiency virus (HIV)-coinfected patients.[9,53] The prevalence rates in intravenous drug users (IDUs) can reach as high as 80% in some countries.[53] By contrast, the prevalence appears to be much lower in blood donors and asymptomatic HBV carriers.[9,58]

There are also substantial variations of HDV prevalence among different countries and regions. Notably, China, India, and Nigeria, the major reservoirs of HBV infection, harbor one-third of total HDV infections [Figure 1].[3] Globally, HDV genotypes present distinct geographical distribution patterns: genotype 1 is widespread worldwide; genotypes 2 and 4 are mostly found in Asia; genotype 3 is found in South America; whereas genotypes 5 to 8 are found in Africa. This triggers an intriguing question of whether this is related to the genotype distribution of HBV. In the cell culture model, it has recently been demonstrated that the specific combination of HDV and HBV genotypes prominently determines the efficiency of HDV egress and entry.[48] We thus speculate that the co-existence of particular HBV and HDV genotypes in specific geographical regions substantially affects their HDV prevalence rates. Nevertheless, large-scale, well-designed epidemiological studies are required to validate this concept.

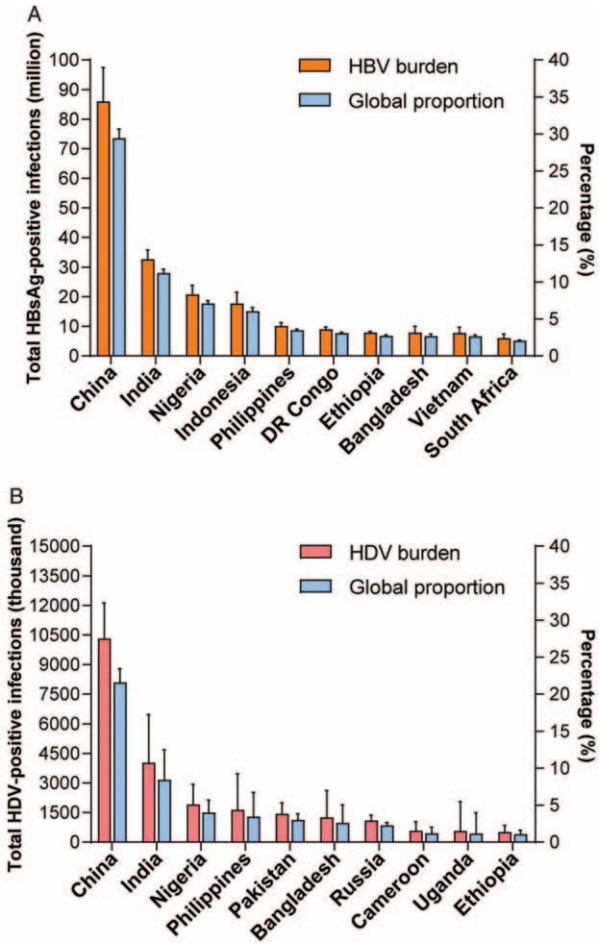

Figure 1: HDV prevalence at the country level. (A) Top ten countries of HBV burden estimates. (B) Top ten countries of HDV burden estimates in HBV carriers. The global burden of HBV infection is 292 million and the global burden of HDV infection is 48 million; data of HBV and HDV were retrieved from Polaris Observatory Collaborators[3] and Miao *et al*,[9] respectively. HDV: Hepatitis D/delta virus; HBV: Hepatitis B virus; HBsAg: HBV surface antigen.

Last but not least, reported HDV screening was mainly based on the detection of anti-HDV antibodies (IgG and/or IgM). HDV RNA testing was rarely performed in the majority of epidemiological studies due to the lack of a standardized HDV RNA assay and also because of economic considerations.[51,59] Anti-HDV IgG indicates resolved HDV infection while anti-HDV IgM or viral RNA indicates acute or active infection. The positive rate of HDV RNA among anti-HDV antibody-positive patients is usually about 50%.[58] Therefore, robust estimation of HDV prevalence requires both well-designed epidemiological studies and standardized diagnostic tools with optimal performance.

Clinical feature

Compared with HBV mono-infection, HDV and HBV coinfection or superinfection usually presents with distinct clinical features [Figure 2]. Most coinfection cases (about 80%) who are immunocompetent adults can spontaneously clear the virus.[9,60-62] For those who failed to clear HBV, only a minority (around 30%) can independently clear HDV, whereas the majority will progress to chronic coinfection.[9,60-63] Superinfection is HDV infection of an individual chronically infected with HBV. Once chronic HDV infection is established, it usually exacerbates the preexisting chronic hepatitis B. In general, the clinical outcome of HDV coinfection (both viruses are usually cleared) is different from HDV superinfection (usually leads to persistent infection with both viruses). The odds ratio to recover or become chronically infected after HDV coinfection are 5.05 (95% confidence interval [CI], 1.45–17.56) and 0.05 (95% CI, 0.01–0.27), respectively, relative to HDV superinfection.[9]

HDV exposure, either via coinfection or superinfection, may involve the full range of possible outcomes from asymptomatic infection to acute liver failure and lethality.[60-62,64] In HBV patients manifested with chronic hepatitis (17%), cirrhosis (26%), and HCC (20%), HDV infection rates are extremely high but the rate is low in asymptomatic HBV carriers (<5%) [Figure 2].[9] Chronic hepatitis D is considered to be associated with the most severe form of chronic viral hepatitis, with a rapid

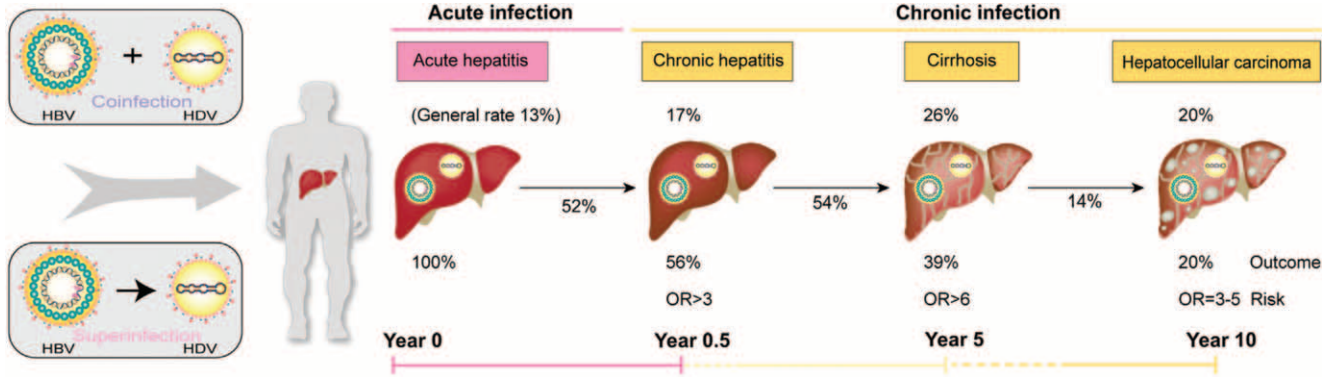

Figure 2: Schematic diagram of HDV infection patterns, disease progression, and outcome. Two distinct infection patterns of HDV (coinfection and superinfection) were shown on the left side, and the different stages of disease progression were shown on the right side. Prevalence of HDV among different chronic HBV subgroups (infection rate), the accumulated proportions to different disease categories (outcome), and the proposed significant ORs (risk, with all the lower 95% CI >1), were also shown. The synthesized data of HDV global infection were retrieved from reference.[9] CI: Confidence interval; HDV: Hepatitis D/delta virus; HBV: Hepatitis B virus; OR: Odds ratio.

progression towards fibrosis/cirrhosis and subsequent liver decompensation.[26,27,61,62] Longitudinal studies have confirmed that a large proportion of chronic hepatitis D patients swiftly progress to cirrhosis and eventually 80% of the patients will develop cirrhosis, which is significantly higher than the percentage seen in patients only infected with HBV.[6-8,64-66] In addition, the reported HCC incidence in chronic hepatitis D patients is much higher than that seen in HBV mono-infected patients.[6,67-69] Upon HDV infection, over half of the patients will have an outcome of chronic hepatitis, whereas approximately 40% and 20% of the patients will finally progress to cirrhosis and HCC, respectively [Figure 2]. On average, HDV infection progresses to cirrhosis within 5 years and to HCC within 10 years. This highlights the importance and significance of HDV screening and testing among general HBV carriers, especially in eastern Europe, Asia, and Africa, which are the regions for the high prevalence of associated liver cirrhosis and HCC. [9,52,70]

HDV genotype can also influence the outcome of chronic hepatitis D. Patients chronically infected with HDV-1 and HDV-3 are associated with more severe hepatitis than those infected with genotype HDV-2 and HDV-4.[41-45] European HDV-1 and African HDV-5 patients have a greater risk of developing cirrhosis.[46] Interestingly, HDV isolates from different genotypes exhibited remarkable differences in their replication efficiency and envelopment preferences in experimental models *in vitro*.[48] Furthermore, it has been indicated that HDV may cause liver fibrosis through the modulation of transforming beta-growth factor-induced signaling and the activation of epithelial-mesenchymal transition, whereas the exact underlying mechanisms of HDV pathogenesis remain largely elusive and require further investigation.[71,72]

Therapeutic development

For decades, pegylated interferon (IFN) alpha is the only treatment regimen currently recommended by the international guidelines.[73] However, a long-term sustained virological response can only be achieved in minority patients,[2,31,62,73] although patients with HDV genotype 5 seem to have a better response to IFN treatment.[46,47] With advanced knowledge recently gained on HDV virology, novel therapeutic options are increasingly explored by targeting different stages of the HDV life cycle. Several candidates are currently under clinical investigation.[2,31,62,73] Bulevirtide (previously named Myrcludex-B) was approved in Europe in 2020 under the branded name of Hepcludex. It is a subcutaneously delivered lipopeptide that specifically inhibits HBV/HDV entry into hepatocytes.[25,74-76] Lonafarnib is an orally administered farnesyl-transferase inhibitor that blocks the prenylation of L-HDAg, thus resulting in the inhibition of virion assembly in hepatocytes. A dose-dependent reduction of HDV RNA was observed in the serum of treated patients.[77,78] REP 2139 is a nucleic acid polymer that selectively blocks the assembly of subviral particles derived from covalently closed circular DNA or integrated HBV DNA and also decreases serum HDV RNA levels with yet unknown mode-of-action.[79,80] Recently, the infectious clones of HDV 1 to 8 and HBV envelope protein expression constructs of genotype A-H have been successfully established. Based on this complete tool set, bulevirtide and lonafarnib, for the first time, were confirmed as potent and pan-genotypic antiviral regimens against HBV/HDV or HDV.[48] With the development of novel cell culture models (eg, HuH7-END and HepNB2.7) to support the full life cycle of HDV,[81] many more novel antivirals that target any stage of the HDV cycle, especially the later stages of HDV replication and release and HBsAg secretion, are expected to be discovered in the near future.

Prevention

Because HDV is thought to be transmitted only in the context of HBV infection, HBV immunization in the general population should have a protective effect against HDV transmission.[82-87] Since the first hepatitis B vaccine was licensed by the Food and Drug Administration of the United States of America in 1981, the subsequent worldwide implementation of the hepatitis B vaccine has led to a dramatic decline in the number of HBsAg carriers. Although the initial vaccination uptake was low on a global scale, by 2018, 108 countries had adopted HBV vaccination for infants and 189 countries had adopted the HepB3 vaccine, which involves three doses of hepatitis B vaccine, in their population-wide vaccination programs.[88,89] Despite the HBV vaccine having been estimated to prevent approximately 12 million individuals from HDV infection, the global burden of HDV remains substantial at present, which may be partially attributed to global population growth.[90] Thus, effective prevention of HDV infection requires multi-dimensional approaches by enhanced awareness, screening, and interventions, as well as specific attention for high-risk populations.

Summary and perspective

As a peculiar satellite virus, understanding the unique aspects of HDV virology is essential. There has been major progress in deciphering the HDV life cycle, but further research is required to deepen the knowledge of virus-host interactions and the possible origin of HDV. The field is realizing the importance of understanding HDV epidemiology, which is the cornerstone of any effective public health response. Unfortunately, there are many pitfalls of the currently available epidemiological data making it difficult to accurately estimate the global burden. Only when higher quality epidemiology studies incorporating improved diagnostic tools become available in the future, the burden could be more precisely estimated. Mathematical modeling could be used to further assist the research in this respect. Fortunately, therapeutic development has achieved major breakthroughs, with the entry-inhibitor bulevirtide being approved in the European Union in 2020 as a successful example. Given the recent advance of HDV *in vitro* models, many new therapeutic options are expected to emerge, providing hope to millions of HDV-infected patients. Nevertheless, many challenges remain in combating hepatitis D, requiring joint efforts from multi-stakeholders to enhance research, public health,

and patient care, thereby contributing to the global mission of eliminating viral hepatitis by 2030.

Funding

This work was supported by the China Scholarship Council for funding PhD fellowships to Zhijiang Miao (No. 201708530234); the Project of Yunnan Provincial Science and Technology Department to Zhenrong Xie (No. 2017FE468[-142]); and the National Natural Science Foundation of China to Li Ren (No. 82060608).

Conflicts of interest

None.

References

1. Thomas DL. Global elimination of chronic hepatitis. N Engl J Med 2019;380:2041–2050. doi: 10.1056/NEJMra1810477.
2. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. Lancet 2011;378:73–85. doi: 10.1016/S0140-6736(10)61931-9.
3. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol 2018;3:383–403. doi: 10.1016/S2468-1253(18)30056-6.
4. Soriano V, Young B, Reau N. Report from the International Conference on Viral Hepatitis - 2017. AIDS Rev 2018;20:58–70.
5. Basnayake SK, Easterbrook PJ. Wide variation in estimates of global prevalence and burden of chronic hepatitis B and C infection cited in published literature. J Viral Hepat 2016;23:545–559. doi: 10.1111/jvh.12519.
6. Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, de Franchis R, *et al*. A 28-year study of the course ofhepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. Gastroenterology 2009;136:1629–1638. doi: 10.1053/j.gastro.2009.01.052.
7. Manesis EK, Vourli G, Dalekos G, Vasiliadis T, Manolaki N, Hounta A, *et al*. Prevalence and clinical course of hepatitis delta infection in Greece: a 13-year prospective study. J Hepatol 2013;59:949–956. doi: 10.1016/j.jhep.2013.07.005.
8. Buti M, Homs M, Rodriguez-Frias F, Funalleras G, Jardi R, Sauleda S, *et al*. Clinical outcome of acute and chronic hepatitis delta over time: a long-term follow-up study. J Viral Hepat 2011;18:434–442. doi: 10.1111/j.1365-2893.2010.01324.x.
9. Miao Z, Zhang S, Ou X, Li S, Ma Z, Wang W, *et al*. Estimating the global prevalence, disease progression, and clinical outcome of hepatitis delta virus infection. J Infect Dis 2020;221:1677–1687. doi:10.1093/infdis/jiz633.
10. Alfaiate D, Clement S, Gomes D, Goossens N, Negro F. Chronic hepatitis D and hepatocellular carcinoma: a systematic review and meta-analysis of observational studies. J Hepatol 2020;73:533–539. doi: 10.1016/j.jhep.2020.02.030.
11. Rizzetto M, Canese MG, Arico S, Crivelli O, Trepo C, Bonino F, *et al*. Immunofluorescence detection of new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. Gut 1977;18:997–1003. doi: 10.1136/gut.18.12.997.
12. Magnius L, Taylor J, Mason WS, Sureau C, Dény P, Norder H, *et al*. ICTV virus taxonomy profile: deltavirus. J Gen Virol 2018;99:1565–1566. doi: 10.1099/jgv.0.001150.
13. Wille M, Netter HJ, Littlejohn M, Yuen L, Shi M, Eden JS, *et al*. A divergent hepatitis D-like agent in birds. Viruses 2018;10:720. doi: 10.3390/v10120720.
14. Hetzel U, Szirovicza L, Smura T, Prahauser B, Vapalahti O, Kipar A, *et al*. Identification of a novel deltavirus in Boa Constrictors. mBio 2019;10:e00014–e00019. doi: 10.1128/mBio.00014-19.
15. Paraskevopoulou S, Pirzer F, Goldmann N, Schmid J, Corman VM, Gottula LT, *et al*. Mammalian deltavirus without hepadnavirus coinfection in the neotropical rodent Proechimys semispinosus. Proc Natl Acad Sci U S A 2020;117:17977–17983. doi: 10.1073/pnas.2006750117.
16. Bergner LM, Orton RJ, Broos A, Tello C, Becker DJ, Carrera JE, *et al*. Diversification of mammalian deltaviruses by host shifting. Proc Natl Acad Sci U S A 2021;118:e2019907118. doi: 10.1073/pnas.2019907118.
17. Iwamoto M, Shibata Y, Kawasaki J, Kojima S, Li YT, Iwami S, *et al*. Identification of novel avian and mammalian deltaviruses provides new insights into deltavirus evolution. Virus Evol 2021;7:veab003. doi: 10.1093/ve/veab003.
18. Chang WS, Pettersson JHO, Le Lay C, Shi M, Lo N, Wille M, *et al*. Novel hepatitis D-like agents in vertebrates and invertebrates. Virus Evol 2019;5:vez021. doi: 10.1093/ve/vez021.
19. Perez-Vargas J, de Oliveira RP, Jacquet S, Pontier D, Cosset FL, Freitas N. HDV-like viruses. Viruses 2021;13:1207. doi: 10.3390/v13071207.
20. Rasche A, Sander AL, Corman VM, Drexler JF. Evolutionary biology of human hepatitis viruses. J Hepatol 2019;70:501–520. doi: 10.1016/j.jhep.2018.11.010.
21. Lempp FA, Ni Y, Urban S. Hepatitis delta virus: insights into a peculiar pathogen and novel treatment options. Nat Rev Gastroenterol Hepatol 2016;13:580–589. doi: 10.1038/nrgastro.2016.126.
22. Sureau C, Negro F. The hepatitis delta virus: replication and pathogenesis. J Hepatol 2016;64:S102–S116. doi: 10.1016/j.jhep.2016.02.013.
23. Chang J, Nie X, Chang HE, Han Z, Taylor J. Transcription of hepatitis delta virus RNA by RNA polymerase II. J Virol 2008;82:1118–1127. doi: 10.1128/JVI.01758-07.
24. Weiner AJ, Choo QL, Wang KS, Govindarajan S, Redeker AG, Gerin JL, *et al*. A single antigenomic open reading frame of the hepatitis delta virus encodes the epitope(s) of both hepatitis delta antigen polypeptides p24 delta and p27 delta. J Virol 1988;62:594–599. doi: 10.1128/JVI.62.2.594-599.1988.
25. Urban S, Neumann-Haefelin C, Lampertico P. Hepatitis D virus in 2021: virology, immunology and new treatment approaches for a difficult-to-treat disease. Gut 2021;70:1782–1794. doi: 10.1136/gutjnl-2020-323888.
26. Lucifora J, Delphin M. Current knowledge on hepatitis delta virus replication. Antiviral Res 2020;179:104812. doi: 10.1016/j.antiviral.2020.104812.
27. Pascarella S, Negro F. Hepatitis D virus: an update. Liver Int 2011;31:7–21. doi: 10.1111/j.1478-3231.2010.02320.x.
28. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, *et al*. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. Elife 2012;1:e00049. doi: 10.7554/eLife.00049.
29. Perez-Vargas J, Amirache F, Boson B, Mialon C, Freitas N, Sureau C, *et al*. Enveloped viruses distinct from HBV induce dissemination of hepatitis D virus in vivo. Nat Commun 2019;10:2098. doi: 10.1038/s41467-019-10117-z.
30. Chemin I, Pujol FH, Scholtes C, Loureiro CL, Amirache F, Levrero M, *et al*. Preliminary evidence for hepatitis delta virus exposure in patients who are apparently not infected with hepatitis B virus. Hepatology 2021;73:861–864. doi: 10.1002/hep.31453.
31. Zhang Z, Urban S. New insights into HDV persistence: the role of interferon response and implications for upcoming novel therapies. J Hepatol 2021;74:686–699. doi: 10.1016/j.jhep.2020.11.032.
32. Giersch K, Helbig M, Volz T, Allweiss L, Mancke LV, Lohse AW, *et al*. Persistent hepatitis D virus mono-infection in humanized mice is efficiently converted by hepatitis B virus to a productive co-infection. J Hepatol 2014;60:538–544. doi: 10.1016/j.jhep.2013.11.010.
33. Le Gal F, Gault E, Ripault MP, Serpaggi J, Trinchet JC, Gordien E, *et al*. Eighth major clade for hepatitis delta virus. Emerg Infect Dis 2006;12:1447–1450. doi: 10.3201/eid1209.060112.
34. Le Gal F, Brichler S, Drugan T, Alloui C, Roulot D, Pawlotsky JM, *et al*. Genetic diversity and worldwide distribution of the deltavirus genus: a study of 2152 clinical strains. Hepatology 2017;66:1826–1841. doi: 10.1002/hep.29574.
35. Miao Z, Zhang S, Ma Z, Hakim MS, Wang W, Peppelenbosch MP, *et al*. Recombinant identification, molecular classification and proposed reference genomes for hepatitis delta virus. J Viral Hepat 2019;26:183–190. doi: 10.1111/jvh.13010.
36. Wu JC, Chiang TY, Shiue WK, Wang SY, Sheen IJ, Huang YH, *et al*. Recombination of hepatitis D virus RNA sequences and its implications. Mol Biol Evol 1999;16:1622–1632. doi: 10.1093/oxfordjournals.molbev.a026075.

37. Wang TC, Chao M. RNA recombination of hepatitis delta virus in natural mixed-genotype infection and transfected cultured cells. J Virol 2005;79:2221–2229. doi: 10.1128/JVI.79.4.2221-2229.2005.
38. Chao M, Wang TC, Lee SE. Detection of hepatitis delta virus recombinants in cultured cells co-transfected with cloned genotypes I and IIb DNA sequences. J Virol Methods 2006;137:252–258. doi: 10.1016/j.jviromet.2006.06.022.
39. Hsieh SY, Taylor J. Regulation of polyadenylation of hepatitis delta virus antigenomic RNA. J Virol 1991;65:6438–6446. doi: 10.1128/JVI.65.12.6438-6446.1991.
40. Karimzadeh H, Usman Z, Frishman D, Roggendorf M. Genetic diversity of hepatitis D virus genotype-1 in Europe allows classification into subtypes. J Viral Hepat 2019;26:900–910. doi: 10.1111/jvh.13086.
41. Smedile A, Rosina F, Saracco G, Chiaberge E, Lattore V, Fabiano A, *et al*. Hepatitis B virus replication modulates pathogenesis of hepatitis D virus in chronic hepatitis D. Hepatology 1991;13:413–416. doi: 10.1002/hep.1840130305.
42. Strauss E. Hepatitis delta virus infection and Labrea hepatitis. JAMA 1988;259:3559–3560. doi: 10.1001/jama.1988.03720240021021.
43. Su CW, Huang YH, Huo TI, Shih HH, Sheen IJ, Chen SW, *et al*. Genotypes and viremia of hepatitis B and D viruses are associated with outcomes of chronic hepatitis D patients. Gastroenterology 2006;130:1625–1635. doi: 10.1053/j.gastro.2006.01.035.
44. Casey JL, Brown TL, Colan EJ, Wignall FS, Gerin JL. A genotype of hepatitis D virus that occurs in northern South America. Proc Natl Acad Sci U S A 1993;90:9016–9020. doi: 10.1073/pnas.90.19.9016.
45. Wu JC, Chen TZ, Huang YS, Yen FS, Ting LT, Sheng WY, *et al*. Natural history of hepatitis D viral superinfection: significance of viremia detected by polymerase chain reaction. Gastroenterology 1995;108:796–802. doi: 10.1016/0016-5085(95)90453-0.
46. Roulot D, Brichler S, Layese R, BenAbdesselam Z, Zoulim F, Thibault V, *et al*. Origin, HDV genotype and persistent viremia determine outcome and treatment response in patients with chronic hepatitis delta. J Hepatol 2020;73:1046–1062. doi: 10.1016/j.jhep.2020.06.038.
47. Spaan M, Carey I, Bruce M, Shang D, Horner M, Dusheiko G, *et al*. Hepatitis delta genotype 5 is associated with favourable disease outcome and better response to treatment compared to genotype 1. J Hepatol 2020;72:1097–1104. doi: 10.1016/j.jhep.2019.12.028.
48. Wang W, Lempp FA, Schlund F, Walter L, Decker CC, Zhang Z, *et al*. Assembly and infection efficacy of hepatitis B virus surface protein exchanges in 8 hepatitis D virus genotype isolates. J Hepatol 2021;75:311–323. doi: 10.1016/j.jhep.2021.03.025.
49. Torres JR. Hepatitis B and hepatitis delta virus infection in South America. Gut 1996;38 (Suppl 2):S48–S55. doi: 10.1136/gut.38.suppl_2.s48.
50. Hadziyannis SJ. Review: hepatitis delta. J Gastroenterol Hepatol 1997;12:289–298. doi: 10.1111/j.1440-1746.1997.tb00424.x.
51. Wedemeyer H, Manns MP. Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. Nat Rev Gastroenterol Hepatol 2010;7:31–40. doi: 10.1038/nrgastro.2009.205.
52. Stockdale AJ, Chaponda M, Beloukas A, Phillips RO, Matthews PC, Papadimitropoulos A, *et al*. Prevalence of hepatitis D virus infection in sub-Saharan Africa: a systematic review and meta-analysis. Lancet Glob Health 2017;5:e992–e1003. doi: 10.1016/S2214-109X(17)30298-X.
53. Chen HY, Shen DT, Ji DZ, Han PC, Zhang WM, Ma JF, *et al*. Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. Gut 2019;68:512–521. doi: 10.1136/gutjnl-2018-316601.
54. Shen DT, Ji DZ, Chen HY, Goyal H, Pan S, Xu HG. Hepatitis D: not a rare disease anymore: global update for 2017-2018. Gut 2020;69:786–788. doi: 10.1136/gutjnl-2019-318691.
55. Wedemeyer H, Negro F. Devil hepatitis D: an orphan disease or largely underdiagnosed? Gut 2019;68:381–382. doi: 10.1136/gutjnl-2018-317403.
56. Stockdale AJ, Kreuels B, Henrion MRY, Giorgi E, Kyomuhangi I, Geretti AM. Hepatitis D prevalence: problems with extrapolation to global population estimates. Gut 2020;69:396–397. doi: 10.1136/gutjnl-2018-317874.
57. Stockdale AJ, Kreuels B, Henrion MYR, Giorgi E, Kyomuhangi I, de Martel C, *et al*. The global prevalence of hepatitis D virus infection: systematic review and meta-analysis. J Hepatol 2020;73:523–532. doi: 10.1016/j.jhep.2020.04.008.
58. Miao Z, Pan Q. Revisiting the estimation of hepatitis D global prevalence. J Hepatol 2020;73:1279–1280. doi: 10.1016/j.jhep.2020.05.019.
59. Ahn J, Gish RG. Hepatitis D virus: a call to screening. Gastroenterol Hepatol (N Y) 2014;10:647–686.
60. Yurdaydin C, Idilman R, Bozkaya H, Bozdayi AM. Natural history and treatment of chronic delta hepatitis. J Viral Hepat 2010;17:749–756. doi: 10.1111/j.1365-2893.2010.01353.x.
61. Farci P, Niro GA. Clinical features of hepatitis D. Semin Liver Dis 2012;32:228–236. doi: 10.1055/s-0032-1323628.
62. Mentha N, Clement S, Negro F, Alfaiate D. A review on hepatitis D: from virology to new therapies. J Adv Res 2019;17:3–15. doi: 10.1016/j.jare.2019.03.009.
63. Negro F. Hepatitis D virus coinfection and superinfection. Cold Spring Harb Perspect Med 2014;4:a021550. doi: 10.1101/cshperspect.a021550.
64. Flodgren E, Bengtsson S, Knutsson M, Strebkova EA, Kidd AH, Alexeyev OA, *et al*. Recent high incidence of fulminant hepatitis in Samara, Russia: molecular analysis of prevailing hepatitis B and D virus strains. J Clin Microbiol 2000;38:3311–3316. doi: 10.1128/JCM.38.9.3311-3316.2000.
65. Fattovich G, Boscaro S, Noventa F, Pornaro E, Stenico D, Alberti A, *et al*. Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B. J Infect Dis 1987;155:931–935. doi: 10.1093/infdis/155.5.931.
66. Saracco G, Rosina F, Brunetto MR, Amoroso P, Caredda F, Farci P, *et al*. Rapidly progressive HBsAg-positive hepatitis in Italy. The role of hepatitis delta virus infection. J Hepatol 1987;5:274–281. doi: 10.1016/s0168-8278(87)80032-6.
67. Ji J, Sundquist K, Sundquist J. A population-based study of hepatitis D virus as potential risk factor for hepatocellular carcinoma. J Natl Cancer Inst 2012;104:790–792. doi: 10.1093/jnci/djs168.
68. Kushner T, Serper M, Kaplan DE. Delta hepatitis within the Veterans Affairs medical system in the United States: prevalence, risk factors, and outcomes. J Hepatol 2015;63:586–592. doi: 10.1016/j. jhep.2015.04.025.
69. Benegiamo G, Vinciguerra M, Guarnieri V, Niro GA, Andriulli A, Pazienza V. Hepatitis delta virus induces specific DNA methylation processes in Huh-7 liver cancer cells. FEBS Lett 2013;587:1424–1428. doi: 10.1016/j.febslet.2013.03.021.
70. Sarin SK, Kumar M, Eslam M, George J, Al Mahtab M, Akbar SMF, *et al*. Liver diseases in the Asia-Pacific region: a Lancet Gastroenter-ology & Hepatology Commission. Lancet Gastroenterol Hepatol 2020;5:167–228. doi: 10.1016/S2468-1253(19)30342-5.
71. Choi SH, Jeong SH, Hwang SB. Large hepatitis delta antigen modulates transforming growth factor-beta signaling cascades: implication of hepatitis delta virus-induced liver fibrosis. Gastroenterology 2007;132:343–357. doi: 10.1053/j.gastro.2006.10.038.
72. Liang YJ, Sun CP, Hsu YC, Chen YW, Wang IA, Su CW, *et al*. Statin inhibits large hepatitis delta antigen-Smad3-twist-mediated epitheli-al-to-mesenchymal transition and hepatitis D virus secretion. J Biomed Sci 2020;27:65. doi: 10.1186/s12929-020-00659-6.
73. Koh C, Heller T, Glenn JS. Pathogenesis of and new therapies for hepatitis D. Gastroenterology 2019;156:461–476. doi: 10.1053/j.gastro.2018.09.058.
74. Schieck A, Schulze A, Gahler C, Muller T, Haberkorn U, Alexandrov A, *et al*. Hepatitis B virus hepatotropism is mediated by specific receptor recognition in the liver and not restricted to susceptible hosts. Hepatology 2013;58:43–53. doi: 10.1002/hep.26211.
75. Urban S, Bartenschlager R, Kubitz R, Zoulim F. Strategies to inhibit entry of HBV and HDV into hepatocytes. Gastroenterology 2014;147:48–64. doi: 10.1053/j.gastro.2014.04.030.
76. Bogomolov P, Alexandrov A, Voronkova N, Macievich M, Kokina K, Petrachenkova M, *et al*. Treatment of chronic hepatitis D with the entry inhibitor myrcludex B: first results of a phase Ib/IIa study. J Hepatol 2016;65:490–498. doi: 10.1016/j.jhep.2016.04.016.
77. Koh C, Canini L, Dahari H, Zhao X, Uprichard SL, Haynes-Williams V, *et al*. Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised,

double-blind, placebo-controlled phase 2A trial. Lancet Infect Dis 2015;15:1167–1174. doi: 10.1016/S1473-3099(15)00074-2.
78. Yurdaydin C, Keskin O, Kalkan C, Karakaya F, Caliskan A, Karatayli E, *et al*. Optimizing lonafarnib treatment for the management of chronic delta hepatitis: the LOWR HDV-1 study. Hepatology 2018;67:1224–1236. doi: 10.1002/hep.29658.
79. Bazinet M, Pantea V, Cebotarescu V, Cojuhari L, Jimbei P, Anderson M, *et al*. Persistent control of hepatitis B virus and hepatitis delta virus infection following R E2139-Ca and Pegylated interferon therapy in chronic hepatitis B virus/hepatitis delta virus coinfection. Hepatol Commun 2021;5:189–202. doi: 10.1002/hep4.1633.
80. Bazinet M, Pantea V, Cebotarescu V, Cojuhari L, Jimbei P, Albrecht J, *et al*. Safety and efficacy of REP 2139 andpegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and R E301-LTFa non-randomised, open-label, phase 2 trial. Lancet Gastroenterol Hepatol 2017;2:877–889. doi: 10.1016/S2468-1253(17)30288-1.
81. Ni Y, Zhang Z, Engelskircher L, Verch G, Tu T, Lempp FA, *et al*. Generation and characterization of a stable cell line persistently replicating and secreting the human hepatitis delta virus. Sci Rep 2019;9:10021. doi: 10.1038/s41598-019-46493-1.
82. Gaeta GB, Stroffolini T, Chiaramonte M, Ascione T, Stornaiuolo G, Lobello S, *et al*. Chronic hepatitis D: a vanishing disease? An Italian multicenter study. Hepatology 2000;32:824–827. doi: 10.1053/jhep.2000.17711.
83. Huang YH, Wu JC, Tao MH, Syu WJ, Hsu SC, Chi WK, *et al*. DNA-based immunization produces Th1 immune responses to hepatitis delta virus in a mouse model. Hepatology 2000;32:104–110. doi: 10.1053/jhep.2000.8348.
84. Huang YH, Wu JC, Hsu SC, Syu WJ. Varied immunity generated in mice by DNA vaccines with large and small hepatitis delta antigens. J Virol 2003;77:12980–12985. doi: 10.1128/jvi.77.24.12980-12985.2003.
85. Fiedler M, Roggendorf M. Immunology of HDV infection. Curr Top Microbiol Immunol 2006;307:187–209. doi: 10.1007/3-540-29802-9_10.
86. Rizzetto M. Hepatitis D: the comeback? Liver Int 2009;29 (Suppl 1):140–142. doi: 10.1111/j.1478-3231.2008.01937.x.
87. Rizzetto M, Ponzetto A, Forzani I. Hepatitis delta virus as a global health problem. Vaccine 1990;8 (Suppl):S10–S14. doi: 10.1016/0264-410x(90)90207-3.
88. Casey RM, Dumolard L, Danovaro-Holliday MC, Gacic-Dobo M, Diallo MS, Hampton LM, *et al*. Global routine vaccination coverage, 2015. MMWR Morb Mortal Wkly Rep 2016;65:1270–1273. doi: 10.15585/mmwr.mm6545a5.
89. Peck M, Gacic-Dobo M, Diallo MS, Nedelec Y, Sodha SV, Wallace AS. Global routine vaccination coverage, 2018. MMWR Morb Mortal Wkly Rep 2019;68:937–942. doi: 10.15585/mmwr.mm6842a1.
90. Miao Z, Li Y, Yu P, Yu B, Peppelenbosch MP, Pan Q. The dynamics of hepatitis delta virus prevalence and its potential association with hepatitis B virus vaccination. Clin Res Hepatol Gastroenterol 2021;45:101677. doi: 10.1016/j.clinre.2021.101677.

How to cite this article: Miao Z, Xie Z, Ren L, Pan Q. Hepatitis D: advances and challenges. Chin Med J 2022;135:767–773. doi: 10.1097/CM9.0000000000002011