Diagnostic Microbiology and Infectious Disease 95 (2019) 114873

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Comparison of anti–hepatitis D virus (HDV) ETI-AB-DELTAK-2 assay and the novel LIAISON® XL MUREX anti-HDV assay in the diagnosis of HDV infection

Caterina Rocco, Raffaele Bonavolta, Luca Vallefuoco, Umberto Braschi, Rosanna Sorrentino, Daniela Terracciano, Giuseppe Portella *

Dipartimento di Scienze Mediche Traslazionali, Università Federico II, U.O.S.D. Virologia A.O.U. Federico II, Napoli, Italy

ARTICLE INFO

Article history:
Received 1 May 2019
Received in revised form 19 July 2019
Accepted 19 July 2019
Available online 26 July 2019

Keywords:
HDV
HBV
Anti-HDV antibodies
EIA
CLIA

ABSTRACT

Hepatitis B/D virus infection leads to severe liver disease. HDV infection is not routinely investigated since the diagnosis is based on enzyme immunoassays (EIAs), which are not available in all laboratories. This study investigates the performance of new automated assay for anti HDV Ab detection: LIAISON® XL Murex anti-HDV.
HBsAg-positive samples were evaluated for HDV serology using the ETI-AB-DELTAK-2 and the new LIAISON® XL Murex with a concordance of 97.5% and 2.42% discordant results. The discordant specimens reacted negatively with EIA and positively with the new test.
Dilutions of HDV-purified antibodies and HDV-positive samples were tested with both assays, showing a lower detection limit for the new assay.
In conclusion, LIAISON® XL Murex showed a good concordance with the reference method and allowed a more rapid HDV detection. This new diagnostic tool may be useful for a more efficient approach to the HDV diagnosis and evaluation of HDV epidemiology.

1. Introduction

Hepatitis D virus (HDV) is a small, defective RNA virus that requires hepatitis B virus (HBV) co-infection for virus replication and propagation (Yurdaydin 2017). HDV was discovered and isolated in the mid-70s (Rizzetto et al. 1977) and was demonstrated to be an RNA molecule of low molecular weight, encapsidated by HBV surface antigen (HBsAg) (Rizzetto et al. 1980).

HDV leads to a liver disease either as co-infection with HBV or as superinfection of a chronic HBsAg carrier (Yurdaydin 2017). HDV co-infection increases the probability of a severe liver disease with a faster progression to cirrhosis. HDV co-infected patients experience fulminant hepatitis at least 100 times more often than those with HBV alone (Fattovich et al. 2000). Superinfected patients experience a chronicity rate reaching 70–90% and chronic hepatitis D often evolves to cirrhosis (60–70%) and hepatocellular carcinoma (Romeo et al. 2009; Buti et al. 2011; Ott et al. 2012; Manesis et al. 2013; Noureddin and Gish 2014).

Epidemiological studies performed in 80′ demonstrated that the HDV was present worldwide, however the prevalence was varying widely and regardless of HBV infection. Main areas of prevalence were the Mediterranean basin, the Middle East, Central and Northern Asia, Western and Central Africa, the Amazonian basin, Northern South America, and the Asia-Pacific region (Rizzetto et al. 1991; Navascués et al. 1995). In the same period, it was estimated that about 5% of 300 million HBV carriers were also infected by HDV, with a global burden of about 15–20 million cases of chronic HDV infection (Rizzetto et al. 1991). However, the findings of a recent meta-analysis indicate a prevalence of 10.58% among HBsAg carriers and an even higher HDV prevalence in the intravenous drug users (IVDU) and high-risk sexual behavior population (Chen et al. 2018).

Vaccination against HBV, screening of blood donors, safety procedures, use of disposable medical devices, and improved socioeconomic conditions in most countries contributed to significant changes in the prevalence of HDV infection in recent years. In Italy, the prevalence of anti-HDV antibodies (Abs) in chronic HBsAg carriers was 24.7% in 1978–1981 and 28% in 1987, declined to 14% in 1992 and to 8.3% in 1997 (Sagnelli et al. 1997; Gaeta et al. 2000), and remained at around 10% in recent years, with a large prevalence in elderly people (Stroffolini et al. 2017).

Despite the reduced prevalence reported in some areas, the presence of HDV infection has been discovered in new regions such as Russia,

Abbreviations: HBV, hepatitis B virus; HDV, hepatitis delta virus; HBsAg, HBV surface antigen; EIAs, enzyme immunoassays; CLIA, chemiluminescent immunoassay; anti-HDV, antibodies against HDV; anti-HBs, antibodies against HBV surface antigen; anti-HBc, total antibodies against HBV core protein; HBeAg, HBV envelope antigen; anti-HBeAb, antibodies against HBV envelope antigen.

* Corresponding author. Tel.: +39-081-7463052; fax: +39-081-7462506.
E-mail address: portella@unina.it (G. Portella).

https://doi.org/10.1016/j.diagmicrobio.2019.114873
0732-8893/

India, Albania, China, and others (Abbas et al. 2010). Moreover, an increase of the prevalence rates of HDV infection in France has been reported in blood donors (Servant-Delmas et al. 2014) likely due to the immigration of HDV infected HBsAg carriers. An increased prevalence has been also reported in the United Kingdom and in Germany (Erhardt et al. 2003; Wedemeyer et al. 2007; Cross et al. 2008; Değertekin et al. 2008; Schweitzer et al. 2015).

However, the burden of HDV infection is largely underestimated since the HDV prevalence is unknown in several areas of the world. The lack of epidemiological data is not limited to countries with low socioeconomic development. Several factors contribute to underestimate HDV infection. Hepatitis D has received the designation of orphan disease both in the United States and in the European Union, giving the misleading impression that, in these regions, HDV infection is infrequent (Yurdaydin 2017). A systematic screening for anti-HDV Abs in HBsAg carriers is not routinely performed, and therefore, HDV infection could be misdiagnosed. Technical issues also contribute to underestimation and underdiagnosis of HDV infection.

Until mid-2018, the only tests for the detection of anti-HDV available on the market were based on the enzyme immunoassays (EIAs) technique, searching for total antibodies to HDV. EIAs are excellent assays, can be automated, and sustain a high throughput. However, EIAs require working in batch, increasing the response time in laboratories performing only a small number of tests daily/weekly. Moreover, automated EIAs have an elevated testing (3.5 h) and turnaround time. The automation itself can be incomplete, with a requirement in the analytic process of high level of operator input and time, i.e., for the preparation of reagents and of the equipment. Moreover, it is critical that the manufacturer's instructions are accurately followed to ensure optimum performances; otherwise, the analytical quality and hence the overall performances of the assay may be negatively affected. Not surprisingly, in highly automated laboratories, EIAs are considered an 'old' technology and are no longer deemed efficient. As a consequence, most anti-HDV EIAs have been discontinued or are no longer available in several countries, including the US. This may ultimately contribute to underassessment and underdiagnosis of HDV infection in clinical practice.

DiaSorin S.p.A. (Saluggia, VC, Italy) has recently launched the first fully automated chemiluminescent immunoassay, i.e. the LIAISON® XL Murex, for the diagnosis of HDV infection. The full automation of the test and its high throughput may allow a better standardization of the results, as well as an easier and faster HDV diagnosis. The possibility to manage the test as a reflex one in all HBsAg-positive samples could help to improve the HDV diagnostic efficiency.

In this study, we have compared the performance of the novel LIAISON® XL Murex anti-HDV assay with the reference ETI-AB-DELTAK-2 assay in samples positive for HBsAg.

2. Materials and methods

Between November 2016 and June 2017, we have evaluated 124 serum samples of HBsAg-positive patients (median age 48.3, ranging from 13 to 82) for which laboratory diagnosis of HDV infection was required.

The patients were also characterized for HBV infection, and anti-HBs, anti-HBc, HBeAg, anti-HBe, and HBV DNA levels were evaluated. In all patients, the typical pattern of chronic HBV infection was observed: HBsAg positive, anti-HBs negative, anti-HBc positive, HBeAg negative, and anti-HBe positive. In 93 patients, HBV DNA was not detected (<10 IU/mL); in 15 patients, low levels of viral DNA were detected (ranging from 200 to 800 IU/mL); in 16 patients, high levels of viral DNA were detected (ranging from 20,000 to 1.9×10^6 IU/mL).

Total antibodies to HDV were detected using ETI-AB-DELTAK-2 (reference method) according to manufacturer instructions. The ETI-AB-DELTAK-2 (DiaSorin S.p.A., Italy) is a competitive assay based on the enzyme-linked immunosorbent assay technique for the qualitative determination of anti-HDV. The cutoff value was determined by adding the mean absorbance of negative control multiplied by 0.5 to the mean absorbance for the positive control multiplied by 0.5. Samples with absorbance values within ±10% of the cutoff value were retested to confirm the initial result. Samples repeatedly reactive were considered as positive, samples nonreactive at the second test were considered as negative, and samples repeatedly testing within ±10% of the cutoff were considered as indeterminate. According to the manufacturers' instructions, the test requires different manual steps to be performed, and about 3.5 h is needed to obtain the result.

All samples collected in the study period were also tested with the new LIAISON® XL Murex anti-HDV assay (DiaSorin S.p.A., Italy), an indirect chemiluminescent immunoassay run on the fully automated LIAISON® analyzer family, according to the manufacturer's recommendations. Based on the vendor instructions, this assay is intended as an aid in the diagnosis of HDV infection and as a screening test for organ, tissue, and cells postmortem donors. It allows the qualitative determination of total anti-HDV in a range of 0.100 to 30.0 AU/mL value and in less than 35 min. Specimens with results above or equal to 1.00 AU/mL were considered reactive for HDV antibodies.

All discordant samples were further tested by another EIA assay (Diagnostic Bioprobes Srl, Italy). The assay was performed and interpreted according to the manufacturers' instructions.

To evaluate the sensitivity of EIA ETI-AB-DELTAK-2 and LIAISON® XL Murex anti-HDV assays, the human anti-HDV positive control from ETI-AB-DELTAK-2 assay and anti-HDV positive samples were diluted in phosphate-buffered saline and tested in parallel with both assays.

A total of 50 HBsAg-negative samples obtained from patients with autoimmune diseases (Stroffolini et al. 2017) and pregnant women (35) were also tested with LIAISON® XL Murex anti-HDV assays.

3. Results

We collected 124 HBsAg-positive serum samples (Table 1). All samples were analyzed for the presence of anti-HDV using the ETI-AB-DELTAK-2. Thirty-nine (31.45%) and 85 (68.55%) specimens were anti-HDV positive and negative (Table 2), respectively, whereas 42 samples evaluated with the new LIAISON® XL Murex anti-HDV assay gave a positive result (33.87%). Three samples (2.42%) reacting negatively with EIA repeatedly tested positive with the new assay, albeit with a low reactivity ranging between 2.3 and 3.0 AU/mL. Eighty-two samples tested negative with the LIAISON® XL Murex anti-HDV assay (66.13%). The level of concordance between the reference method and the new anti-HDV assay was 97.5%.

The discordant samples were further evaluated with EIA assay by Diagnostics Bioprobes, and a nonreactive result was obtained in all samples.

According to the manufacturers' instructions, the LIAISON® XL Murex anti-HDV assay has a better sensitivity with respect to the EIA

Table 1
Demographic, serological, and virological characteristic of the patients.

Median age, 48 (13–82)		Males 68, females 56	
		N	%
	HBsAg positive	124	100
	HBsAg positive	124	100
	Anti-HBsAg negative	124	100
	Anti-HBc positive	124	100
	HBeAg negative	124	100
	Anti-HBe Ag positive	124	100
	HBV-DNA not detected	93	75
	HBV-DNA low levels	15	12
	HBV-DNA high levels	16	13

HBV-DNA not detected: <10 IU/mL; low levels: ranging from 200 to 800 IU/mL; high levels: ranging from 20,000 to 1.9×10^6 IU/mL.

Table 2
Comparison of the results.

		LIAISON® XL MUREX anti-HDV	
		Neg	Pos
ETI-AB-DELTAK-2	Neg	82	3
	Pos	0	39

assays (100% vs. 99%). To assess whether the discrepant results were due to a differential detection limit between assays, dilutions of HDV-purified antibodies and HDV-positive samples were tested with both assays(Table 3).

EIA assay gave a reactive result up to 1:10 dilution of anti-HDV purified antibodies, whereas the new anti-HDV assay tested reactive up to 1:75 dilution. A similar result was obtained diluting anti-HDV–positive samples (Table 3), confirming a lower detection limit of the new LIAISON® XL Murex anti-HDV assay.

HBsAg-negative samples (50) were tested to evaluate the specificity of the new assay; a negative result was obtained, confirming the high performance of the LIAISON® XL Murex anti-HDV test.

4. Discussion

HBV-HDV infection is underdiagnosed, and its epidemiological burden is underestimated, as confirmed by several studies. The lack of fully automated assays has contributed to hamper the screening for HDV infection in HBsAg positive patients. It has been reported that only 42% of patients with chronic hepatitis B (CHB) were tested, showing HDV infection in 8% of the individuals (Gish et al. 2013), suggesting that a relevant percentage of HBsAg carriers is not correctly evaluated for HDV co-infection or superinfection. Overall, HDV testing rates are low despite the guidelines recommending universal testing of HBV-infected patients (El Bouzidi et al. 2015).

The diagnosis of HDV infection patients is important to avoid inappropriate management of HBV/HDV infection. The current treatment based on of pegylated interferon provides a sustained virological response in only 25%–40% of patients (Noureddin and Gish 2014; Yurdaydin 2017). Novel compounds such as entry and farnesylation inhibitors or nucleic acid polymers are currently in phase II clinical trials with promising results, further emphasizing the necessity to correctly diagnose HBV/HDV infected subjects (Botelho-Souza et al. 2017; Yurdaydin 2017).

Even in regions with a low prevalence, rapid and accurate diagnostic assays are needed since high-risk groups need to be evaluated for the presence of HDV antibodies. These include intravenous drugs users and immigrants from HDV-endemic countries. More attention should be addressed to HDV infection testing for HBsAg positive patients. This strategy requires fast and easy-to-perform assays. However, the diagnosis of HDV infection so far has been based on serological EIAs. EIA methods have some limitations, as they require high-performance laboratory infrastructures and equipment, and do not allow reflex testing in HBsAg carriers. Moreover, the decreasing endemicity of HDV infection in the Western world has led to a reduced alert about the HDV infection, and anti-HDV assays are not available in all laboratories; consequently, not all HBsAg carriers are evaluated for HDV infection.

In the present study, we have evaluated a new assay, the LIAISON® XL Murex anti-HDV on the fully automated LIAISON XL platform. This instrument allows a random access of the samples, with a result time of ≤35 min and a remarkable reduction of turnaround time. Moreover, this platform could allow performing anti-HDV as a reflex test in HBsAg-positive patients, thus allowing a more efficient diagnosis of HDV infection in all potentially infected individuals.

LIAISON® XL Murex anti-HDV assay, compared to the reference method, has detected all EIA anti-HDV–positive samples. However, 3 specimens reacted only with the new assay, albeit at a low reactivity.

Comparing the detection limits, we demonstrated a lower detection limit of LIAISON® XL Murex anti-HDV assay with respect to the reference method. This observation strongly suggests that the automated

Table 3
Dilutions of purified anti-HDV Ab and positive anti-HDV Ab samples.

Type of sample	Dilution	ETI-AB-DELTAK-2		LIAISON® XL MUREX anti-HDV	
		OD	Result	AU	Result
Purified anti-HDV	Undiluted	0.09	Positive	13	Positive
	1:10	0.128	Positive	7.87	Positive
	1:50	0.411	Indeterminate	1.96	Positive
	1:75	0.583	Negative	1.35	Positive
	1:100	0.578	Negative	0.705	Negative
Positive sample 1	Undiluted	0.280	Positive	3.74	Positive
	1:10	0.420	Negative	1.85	Positive
Positive sample 2	Undiluted	0.03	Positive	13	Positive
	1:20000	0.411	Negative	2.6	Positive
	1:25000	0.520	Negative	1.4	Positive
Positive sample 3	Undiluted	0.025	Positive	13	Positive
	1:10000	0.37	Positive	3.5	Positive
	1: 12500	0.458	Negative	2.34	Positive

ETI-AB-DELTAK-2: negative control >/= 0.6 OD, positive control </= 0.08 OD, cutoff <0.409 OD.
LIAISON® XL MUREX ANTI-HDV: negative control = 0.02 AU, positive control >13 AU, cutoff = 1.0 AU.

assay has correctly detected low reactive samples not identified by the EIA assay. It is not possible to exclude a false-positive reactivity in these 3 samples, although the manufacturer reports a diagnostic specificity of 99.35%. Due to the unavailability of further serum, it was not possible to search HDV RNA, whose presence would have confirmed an active infection.

To better assess the specificity of the assay, 50 HBsAg-negative samples obtained from patients with autoimmune diseases and from pregnant women were tested, yielding negative results and confirming the high specificity of the assay.

A differential sensitivity and specificity among anti-HDV EIAs have been described. Chow et al. have evaluated 2 commercial assays: the DiaSorin and the Cusabio (Chow et al. 2016). The DiaSorin assay demonstrated 100% sensitivity and specificity with respect of the reference assay, whereas the Cusabio assay demonstrated a sensitivity of 81.3% and specificity of 90.9%. The authors explained these differences with the different origin of HDV strain used to generate the assay, Italy and China, respectively. This hypothesis can be excluded in the present study since both assays were generated using the same HDV strain. It is not possible to exclude that the new automated assay might show a different sensitivity toward antibodies raised against different HDV strains.

The aim of the present study was to compare, in a real-life diagnostic laboratory, the reference method for the detection of anti-HDV antibodies with the new automated assay. Therefore, to avoid any bias, only routine samples with an order for the detection of anti-HDV antibodies were used.

A limitation of our study could be represented by the number of the samples tested even if it represents the routine of the HDV diagnosis requests in our center. Moreover, the HDV prevalence is about 10% among HBsAg carriers, with a large prevalence in elderly people (Gaeta et al. 2000), making it difficult to evaluate a larger number of HDV-positive samples.

5. Conclusions

The results of this study suggest that the novel LIAISON® XL Murex anti-HDV assay represents an easy-to-perform fully automated tool that ensures the standardization of the results and a rapid response. This new diagnostic tool may be used for a more efficient HDV diagnosis and for an optimized evaluation of HDV epidemiology. Furthermore, it can be applied as routine approach for the screening of high-risk population, as well as organ and tissue donors, and thus might contribute to improve management of HDV infection.

Acknowledgments

We thank Dr. Daniela Bernasconi (DiaSorin S.p.A., Italy) for the useful suggestions and critical reading of the manuscript.

Conflict of interest

The study has been performed with the support of DiaSorin s.r.l. We do not have any other confict of interest.

Author contributions

All authors confirmed they have contributed to the intellectual content of the study and have met the following requirements: 1) significant contributions of the conception and design, acquisition of data, or analysis and interpretation of the data; 2) drafting or revising the manuscript for intellectual content and gave a substantial contribution to the acquisition and interpretation of data, in revising critically the article and 3) in giving final approval of the version to be published.

Disclosure statement

All authors have no relevant affiliations or financial involvement with any organization or entity with financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Ethical approval

The study did not require the specific approval of the Ethical Committee of A.O.U. Federico II, since the samples were tested for the same assay requested by a medical order (Total anti HDV Ab-, HBV makers and HBV DNA). The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Research funding sources

This work was possible thanks to the financial support provided by DiaSorin S.p.A., Italy.

References

Abbas Z, Jafri W, Raza S, Hepatitis D. Scenario in the Asia-Pacific region. World J Gastroenterol 2010;16:554–62.
Botelho-Souza LF, Vasconcelos MPA, Dos Santos AO, Salcedo JMV, Vieira DS. Hepatitis delta: virological and clinical aspects. Virol J 2017;14:177.
Buti M, Homs M, Rodriguez-Frias F, Funalleras G, J ardí 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;18:434–42.
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 2018. https://doi.org/10.1136/gutjnl-2018-316601. Sep 18. pii:gutjnl-2018-316601.
Chow SK, Atienza EE, Cook L, Prince H, Slev P, Lapé-Nixon M, et al. Comparison of enzyme immunoassays for detection of antibodies to hepatitis D virus in serum. Clin Vaccine Immunol. 2016;23:732–4.
Cross TJ, Rizzi P, Horner M, Jolly A, Hussain MJ, Smith HM, et al. The increasing prevalence of hepatitis delta virus (HDV) infection in South London. J Med Virol 2008;80:277–82.
Değertekin H, Yalçin K, Yakut M, Yurdaydin C. Seropositivity for delta hepatitis in patients with chronic hepatitis B and liver cirrhosis in Turkey: a meta-analysis. Liver Int 2008;28:494–8.
El Bouzidi K, Elamin W, Kranzer K, Irish DN, Ferns B, Kennedy P, et al. Hepatitis delta virus testing, epidemiology and management: a multicentre cross-sectional study of patients in London. J Clin Virol 2015;66:33–7.
Erhardt A, Knuth R, Sagir A, Kirschberg O, Heintges T, Häussinger D. Socioepidemiological data on hepatitis delta in a German university clinic—increase in patients from Eastern Europe and the former Soviet Union. Z Gastroenterol 2003 Jun;41:523–6.
Fattovich G, Giustina G, Christensen E, Pantalena M, Zagni I, Realdi G, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). Gut 2000;46:420–6.
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;32:824–7.
Gish RG, Yi DH, Kane S, Clark M, Mangahas M, Baqai S, et al. Coinfection with hepatitis B and D: epidemiology, prevalence and disease in patients in Northern California. J Gastroenterol Hepatol 2013;28:1521–5.
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;59:949–56.
Navascués CA, Rodríguez M, Sotorrío NG, Sala P, Linares A, Suárez A, et al. Epidemiology of hepatitis D virus infection: changes in the last 14 years. Am J Gastroenterol 1995;90:1981–4.
Noureddin M, Gish R. Hepatitis delta: epidemiology, diagnosis and management 36 years after discovery. Curr Gastroenterol Rep 2014 Jan;16(1):365–72.
Ott JJ, Stevens GA, Groeger J, Wiersma ST. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. Vaccine 2012;30:2212–9.
Rizzetto M, Canese MG, Aricò S, Crivelli O, Trepo C, Bonino F, et al. Immunofluorescence detection of new antigen-antibodys ystem (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. Gut 1977;18:997–1003.
Rizzetto M, Hoyer B, Canese MG, Shih JW. Purcell RH. Gerin JL. Delta agent: association of delta antigen with hepatitis B surface antigen and RNA in serum of delta-infected chimpanzees. Proc Natl Acad Sci USA 1980;77:6124–8.
Rizzetto M, Ponzetto A, Forzani I. Epidemiology of hepatitis delta virus: overview. Prog Clin Biol Res 1991;364:1–20.

Romeo R, Del Ninno E, Rumi M, Russo A, Sangiovanni A, de Franchis R, et al. A 28-year study of the course of hepatitis delta infection: a risk factor for cirrhosis and hepatocellular carcinoma. Gastroenterology 2009;136:1629–38.

Sagnelli E, Stroffolini T, Ascione A, Chiaramonte M, Craxì A, Giusti G, et al. Decrease in HDV endemicity in Italy. J Hepatol 1997;26:20–4.

Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet 2015;386:1546–55.

Servant-Delmas A, Le Gal F, Gallian P, Gordien E, Laperche S. Increasing prevalence of HDV/HBV infection over 15 years in France. J Clin Virol 2014;59:126–8.

Stroffolini T, Sagnelli E, Sagnelli C, Russello M, De Luca M, Rosina F, et al. Hepatitis delta infection in Italian patients: towards the end of the story? Infection 2017;45:277–81.

Wedemeyer H, Heidrich B, Manns MP. Hepatitis D virus infection—not a vanishing disease in Europe! Hepatology 2007;45(5):1331–2.

Yurdaydin C. Recent advances in managing hepatitis D. F1000Res. 2017; 6:1596.