

REFERENCES

1 Rizzetto M, Canese MG, Arico S *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.

2 Taylor J. Hepatitis delta virus. [www.fccc.edu/docs/research/hdv.html](http://www.fccc.edu/docs/research/hdv.html) (accessed 10 June 2005).

3 Jenkins GM, Woelk CH, Rambaut A, Holmes EC. Testing the extent of sequence similarity among viroids, satellite RNAs, and hepatitis delta virus. *J Mol Evol* 2000; **50**: 98-102.

4 Branch AD, Benenfeld BJ, Baroudy BM, Wells FV, Gerin JL, Robertson HD. An ultraviolet-sensitive RNA structural element in a viroid-like domain of the hepatitis delta virus. *Science* 1989; **243**: 649-52.

5 Brazas R, Ganem D. A cellular homolog of hepatitis delta antigen: implications for viral replication and evolution. *Science* 1996; **274**: 90-4.

6 Elena SF, Dopazo J, Flores R, Diener TO, Moya A. Phylogeny of viroids, viroidlike satellite RNAs, and the viroidlike domain of hepatitis delta virus RNA. *Proc Natl Acad Sci USA* 1991; **88**: 5631-4.

7 Gudima S, Chang J, Moraleda G, Azvolinsky A, Taylor J. Parameters of human hepatitis delta virus replication: the quantity, quality, and intracellular distribution of viral proteins and RNA. *J Virol* 2002; **76**: 3709-19.

8 Crivelli O, Arico S, Bonino F, Lavarini C, Rizzetto M. Distribution of antibodies against the delta antigen in HbsAg carriers detected by a simple immunofluorescence blocking test. *Acta Gastro-Enterol. Belg* 1978; **41**: 351-5.

9 Rizzetto M, Shih JW, Gocke DJ, Purcell RH, Verme G, Gerin JL. Incidence and significance of antibodies to delta antigen in hepatitis B virus infection. *Lancet* 1979; **ii** (8150): 986-90.

10 Crivelli O, Rizzetto M, Lavarini C, Smedile A, Gerin JL. Enzyme-linked immunosorbent assay for detection of antibody to the hepatitis B surface antigen-associated delta antigen. *J Clin Microbiol* 1981; **14**: 173-7.

11 Govindarajan S, Valinluck B, Lake-Bakkar G.. Evaluation of a commercial anti-delta EIA kit for detection of antibodies to hepatitis delta virus. *Am J Clin Pathol* 1991; **95**: 240-1.

12 Chaggar K, McFarlane IG, Smith HM, Alexander GJ, Williams R. An enzyme immunoassay for detection of IgA class antibodies against hepatitis delta virus. *J Virol Methods* 1991; **32**: 193-9.

13 Shattock AG, Morris MC. Evaluation of commercial enzyme immunoassays for detection of hepatitis delta antigen and anti-hepatitis delta virus (HDV) and immunoglobulin M anti-HDV antibodies. *J Clin Microbiol* 1991; **29**: 1873-6.

14 Jardi R, Buti M, Rodriguez F *et al*.

## 'Traditional methods for the diagnosis of HDV infection, such as detection of serum anti-HDV antibodies, are sufficient for the clinical diagnosis of hepatitis D'

Comparative analysis of serological markers of chronic delta infection: HDV-RNA, serum HDAG and anti-HD IgM. *J Virol Methods* 1994; **50**: 59-66.

15 Sumathy S, Thyagarajan SP, Latif R *et al*. A dipstick immunobinding enzyme-linked immunosorbent assay for serodiagnosis of hepatitis B and delta virus infections. *J Virol Methods* 1992; **38**: 145-52.

16 Gupta S, Valinluck B, Govindarajan S. Detection of hepatitis delta virus in serum and liver tissue by molecular hybridization. Validation of a rapid spot-hybridization technique. *Am J Clin Pathol* 1989; **92**: 218-21.

17 Smedile A, Bergmann KF, Baroudy BM *et al*. Riboprobe assay for HDV RNA: a sensitive method for the detection of the HDV genome in clinical serum samples. *J Med Virol* 1990; **30**: 20-4.

18 Zignego AL, Deny P, Feray C *et al*. Amplification of hepatitis delta virus RNA sequences by polymerase chain reaction: a tool for viral detection and cloning. *Mol Cell Probes* 1990; **4**: 43-51.

19 Madejon A, Castillo I, Bartolome J *et al*. Detection of HDV-RNA by PCR in serum of patients with chronic HDV infection. *J Hepatol* 1990; **11**: 381-4.

20 Simpson LH, Battegay M, Hoofnagle JH, Waggoner JG, Di Bisceglie AM. Hepatitis delta virus RNA in serum of patients with chronic delta hepatitis. *Dig Dis Sci* 1994; **39**: 2650-5.

21 Deny P, Fattovich G, Le Gal F *et al*. Polymerase-chain-reaction-based semi-quantification of hepatitis D viraemia in patients treated with high doses of alpha 2b interferon. *Res Virol* 1994; **145**: 287-95.

22 Wu JC, Chen TZ, Huang YS *et al*. Natural history of hepatitis D viral superinfection: significance of viremia detected by polymerase chain reaction. *Gastroenterology* 1995; **108**: 796-802.

23 Yamashiro T, Nagayama K, Enomoto N *et al*. Quantitation of the level of hepatitis delta virus RNA in serum, by real-time polymerase chain reaction and its possible correlation with the clinical stage of liver disease. *J Infect Dis* 2004; **189**: 1151-7.

24 Ombanza-Moussa E, Dussaix E, Roque-Afonso AM. Hepatitis delta virus RNA

detection by one-step RT-PCR (in French). *Ann Biol Clin (Paris)* 2004; **62**: 319-24.

25 Le Gal F, Gordien E, Affolabi D *et al*. Quantification of hepatitis delta virus RNA in serum by consensus real-time PCR indicates different patterns of virological response to interferon therapy in chronically infected patients. *J Clin Microbiol* 2005; **43**: 2363-9.

26 Yurdaydin C, Bozkaya H, Gurel S *et al*. Famciclovir treatment of chronic delta hepatitis. *J Hepatol* 2002; **37**: 266-71.

27 Niro GA, Rosina F, Rizzetto M. Treatment of hepatitis D. *J Viral Hepat* 2005; **12**: 2-9.

28 Kaymakoglu S, Karaca C, Demir K *et al*. Alpha interferon and ribavirin combination therapy of chronic hepatitis D. *Antimicrob Agents Chemother* 2005; **49**: 1135-8.

29 Bordier BB, Marion PL, Ohashi K *et al*. A prenylation inhibitor prevents production of infectious hepatitis delta virus particles. *J Virol* 2002; **76**: 10465-72.

30 Bordier BB, Ohkanda J, Liu P *et al*. *In vivo* antiviral efficacy of prenylation inhibitors against hepatitis delta virus. *J Clin Invest* 2003; **112**: 407-14.

31 Lok AS, Wong A, Sporton S, Lai CL, Liu V, Chung HT. Hepatitis D virus superinfection remains a rare occurrence in non-drug abusers in Hong Kong. *J Hepatol* 1992; **14**: 332-4.

32 Huo TI, Wu JC, Wu SI *et al*. Changing seroepidemiology of hepatitis B, C, and D virus infections in high-risk populations. *J Med Virol* 2004; **72**: 41-5.

33 Theamboonlers A, Hansurabhanon T, Verachai V, Chongsrisawat V, Poovorawan Y. Hepatitis D virus infection in Thailand: HDV genotyping by RT-PCR, RFLP and direct sequencing. *Infection* 2002; **30**: 140-4.

34 Zaki H, Darmstadt GL, Baten A, Ahsan CR, Saha SK. Seroepidemiology of hepatitis B and delta virus infections in Bangladesh. *J Trop Pediatr* 2003; **49**: 371-4.

35 Saady N, Sugauchi F, Tanaka Y *et al*. Genotypes and phylogenetic characterization of hepatitis B and delta viruses in Egypt. *J Med Virol* 2003; **70**: 529-36.

36 Chlabicz S, Grzeszczuk A, Lapinski TW, Prokopowicz D, Panasiuk A. Search for hepatitis delta virus (HDV) infection in hepatitis C patients in north-eastern Poland. Comparison with anti-HDV prevalence in chronic hepatitis B. *Eur J Epidemiol* 2003; **18**: 559-61.

37 Smith HM, Alexander GJ, Webb G, McManus T, McFarlane IG, Williams R. Hepatitis B and delta virus infection among "at risk" populations in south east London. *J Epidemiol Community Health* 1992; **46**: 144-7.

38 Terrault N. Chronic viral hepatitis in the United States (2000). [www.hepnet.com/hepc/aasld00/terrault.html](http://www.hepnet.com/hepc/aasld00/terrault.html) (accessed 10 June 2005).

**Table 2.** Prevalence of hepatitis D in selected populations.

Population		Prevalence (%)	n	Ref
Hong Kong	IV drug users with HBV-related chronic liver disease	93	13/14	31
	Non-drug users with chronic HBV infection	0.15	1/664	
Taiwan	HbsAg <sup>+</sup> female sex workers	4.5	5/111	32
	HbsAg <sup>+</sup> IV drug users	13.8	12/87	
	HbsAg <sup>+</sup> male sex workers	15.6	7/45	
Thailand	HbsAg <sup>+</sup> IV drug users	21.8	12/55	33
Bangladesh	HbsAg <sup>+</sup> adults and children	24.4	44/180	34
Egypt	HbsAg <sup>+</sup> subjects 20 15/75 35			
Poland	HbsAg <sup>+</sup> subjects 3.9 4/102 36			
UK	IV drug users with chronic HBV infection (1979–1988)	70.6	12/17	37
USA	Chronic HBV infection	5.6	70,000/ 1,250,000	38
IV: intravenous				

as a function of the number of PCR cycles.

The significance of viraemia during HDV superinfection was studied using PCR.<sup>22</sup> This showed that superinfection could be divided into three phases: acute (active HDV replication, suppression of HBV, high alanine aminotransferase [ALT] levels); chronic (decreasing HDV, reactivating HBV, moderate ALT levels); and late (development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or remission resulting from marked reduction of both viruses).

A sensitive method to quantify the concentration of HDV RNA in serum using real-time reverse-transcriptase PCR (RT-PCR) was developed in the hope that it would shed light on the pathophysiology of HDV infection.<sup>23</sup> In addition, a one-step method to detect HDV RNA in which reverse transcription and amplification were carried out in the same tube, reducing the handling time and contamination risk, was developed.<sup>24</sup> The sensitivity of the technique was estimated at 420 copies per reaction using serial dilutions of a titred sample.

A real-time RT-PCR assay was developed to quantify the HDV RNA load in serum.<sup>25</sup> Its efficacy was evaluated with 160 serum samples. The assay was sensitive (100

copies/mL serum) and efficiently detected all HDV genotypes. Such assays may help investigators understand the natural history of HDV infection and to define guidelines for the management of chronic hepatitis D.

Traditional methods for the diagnosis of HDV infection, such as detection of serum anti-HDV antibodies, are sufficient for the clinical diagnosis of hepatitis D. However, such techniques lack the sensitivity and specificity required to characterise more accurately the nature of HDV infection and to assess the efficacy of treatment. Molecular techniques, such as RT-PCR, provide increased diagnostic precision and a more thorough understanding of the natural course of HDV infection. These advances have enhanced the clinician's ability to accurately evaluate the stage of HDV infection, response to therapy and occurrence of re-infection after orthotopic liver transplant.

The increasing pace of development in molecular biology during the last decade has had a direct effect on mass testing and diagnostic applications, including blood screening. Prototype DNA microarrays are in development for HDV testing and such systems might soon be useful as diagnostic techniques in the clinical laboratory.

**Table 3.** Hepatitis D virus proteins.

	Size		Shared functions	Unique functions
	aa	kDa		
Large delta antigen	214	27	RNA binding Nuclear localisation	Mediates HDV assembly. Inhibits HDV RNA replication. Associates with membrane when farnesylated
Small delta antigen	195	24	Oligomerisation	Transactivation of HDV RNA replication

aa: amino acid residues

## Treatment

There is no pre- or post-exposure prophylaxis available for HDV. As HDV depends on HBV for replication, HBV-HDV co-infection can be prevented with either pre- or post-exposure prophylaxis for HBV. However, no products exist to prevent HDV superinfection of persons with chronic HBV infection. Thus, prevention of HDV superinfection depends primarily on education to reduce high-risk behaviour.

No effective antiviral therapy is currently available for HDV infection. The nucleoside analogue famciclovir (500 mg, three times per day for six months) was evaluated in a group of 15 adult patients with chronic hepatitis D. Although HBV DNA levels decreased in nine of the patients and rose again after treatment, famciclovir had no effect on ALT, HbsAg levels, serum HDV RNA or liver histology.<sup>26</sup> The clinical trial authors concluded that famciclovir had no significant effect on disease progression.

Currently, interferon- $\alpha$  (IFN $\alpha$ ) is the only recommended treatment for HDV infection, but its usefulness is limited. The rate of response is proportional to the dose of IFN $\alpha$ , with nine million units three times a week being more effective than three million units thrice weekly. Sustained responses are unusual and are accompanied by clearance of serum HbsAg, seroconversion to anti-HBs and improvement of liver histology.<sup>27</sup>

Efficacy of IFN $\alpha$  and ribavirin combination therapy for chronic hepatitis D was investigated in a group of 19 adults with chronic hepatitis D who were treated with IFN $\alpha$ 2b (10 million units, three times per week, subcutaneously) and ribavirin (1000 to 1200 mg/day, orally) for 24 months.<sup>28</sup> The investigators concluded that combination therapy did not induce virological responses sufficiently, despite its partial effectiveness in improving biochemical responses, and was not superior to IFN $\alpha$  monotherapy.

Liver transplantation may be considered for cases of fulminant acute and end-stage chronic hepatitis D infection. Therapeutic approaches utilising small interfering RNA (siRNA) may be useful in future but are currently only in the experimental stage.

Targeting the post-translational modification of the large delta antigen with specific prenylation/farnesyltransferase inhibitors has been shown to disrupt HDV assembly *in vitro* and *in vivo*.<sup>29,30</sup> These compounds represent an attractive class of antiviral agents; however, no human clinical trials have been reported.

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A comparative study of the sensitivity and specificity of HDV-RNA determination by molecular hybridisation, serum HDAg by immunoblot and anti-HD IgM by commercial EIA compared with intrahepatic HDAg detection by an immunoperoxidase method was conducted.<sup>14</sup> Serum HDAg determination by immunoblot was the most specific test, followed by HDV RNA analysis. The least specific was the anti-HD IgM technique. The results suggested that the determination of HDV RNA by the hybridisation method could be of great value in the diagnosis and monitoring of chronic delta hepatitis and presaged the introduction of more sensitive nucleic acid-based technologies for detecting HDV infection.

## ‘Hepatitis D virus, which is nearly spherical and 36–43 nm in diameter, is the only member of the genus Deltavirus’

and 100%, respectively, for anti-HDV. Based on the simplicity and economical nature of the test system, DIA was recommended as a diagnostic tool for field surveys and small laboratories in developing countries.

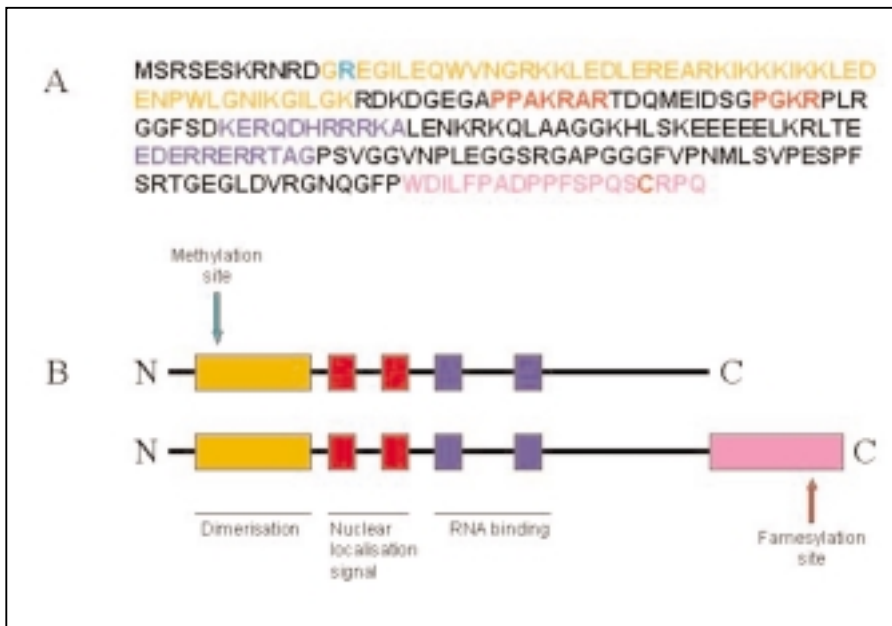
HDV RNA, a spot-hybridisation method utilising a HDV complementary DNA (cDNA) probe was developed.<sup>16</sup> The method proved to be quick, sensitive and specific for HDV RNA on a small sample ( $n=12$ ) of HDV-infected subjects. The authors concluded that spot-hybridisation permitted detection of HDV RNA with relative ease and was applicable to the evaluation of large numbers of cases with hepatitis D.

A hybridisation assay for the detection of the HDV genome in serum using a strand-specific RNA probe obtained by transcription of a recombinant riboprobe has been reported.<sup>17</sup> The assay was tested on a panel of 30 sera from HBsAg carriers with HDAg in the liver. The riboprobe assay detected HDV RNA in the serum of 83% of the patients, while 63% were positive using a DNA hybridisation assay. Antigen (HDAg) was detected in 73% of the same serum samples by immunoblotting. The riboprobe assay was the most sensitive of available methods to measure hepatitis D viraemia.

The first polymerase chain reaction (PCR) technique to detect HDV was described in 1990.<sup>18</sup> Total RNA from HDV-infected liver and serum samples was purified and reverse-transcribed. HDV cDNA was then amplified directly using three pairs of specific primers. The entire HDAg open reading frame was amplified. A DNA fragment of the expected size was obtained repeatedly from an initial sample of less than 0.1 pg liver RNA and from 10 pL infected serum. Using serial dilutions of serum samples containing known concentrations of HDV-RNA, PCR was found to be 10,000-times more sensitive than slot-blot hybridisation.<sup>19</sup>

A hot-start PCR method, in which the reaction begins at 60–80°C, showed a higher sensitivity than conventional PCR. Hepatitis D viral RNA was detected in 26/28 (93%) patients with chronic hepatitis D.<sup>20</sup> Detection of HDV RNA correlated well with detection of HDAg by immunostaining in the liver. Detection of HDV RNA with PCR was highly sensitive and specific, demonstrating that virtually all patients with chronic HDV infection had ongoing viral replication.

Semi-quantitative PCR-based detection of HDV RNA was developed to study the antiviral efficacy of high doses of interferon- $\alpha$ 2b for chronic hepatitis D treatment.<sup>21</sup> The semi-quantification method was based on the appearance of a positive amplification signal



**Fig 3.** Amino acid sequence and structural organisation of hepatitis delta antigen. A) Primary amino acid sequence of hepatitis delta antigen (GenBank accession number AF540888). B) Structural organisation of the small (upper) and large (lower) delta antigens. Note the C-terminal extension in the large delta antigen. The residues comprising the structural motifs are highlighted by the corresponding colour in the primary sequence.

In 1992, a simple, specific and economical dipstick immunobinding enzyme-linked immunosorbent assay (DIA) for detecting HBsAg and anti-HDV utilising a cellulose nitrate membrane was described.<sup>15</sup> The positive and negative predictive values of DIA for HBsAg were 100% and 99.6% and 96.7%

### Nucleic acid-based techniques

In the late 1980s serological tests to detect HDAg and anti-HDV were supplemented by a Northern blot hybridisation assay for HDV RNA. However, this technique was cumbersome for the analysis of multiple samples. In order to simplify detection of

**Table 1.** Hepatitis viruses.

	Hepatitis A (HAV)	Hepatitis B (HBV)	Hepatitis C (HCV)	Hepatitis D (HDV)	Hepatitis E (HEV)	Hepatitis G (HGV)
Family	<i>Picornaviridae</i>	<i>Hepadnaviridae</i>	<i>Flaviviridae</i>	<i>Deltaviridae</i>	<i>Hepeviridae</i>	<i>Flaviviridae</i>
Genus	<i>Hepatovirus</i>	<i>Orthohepadnavirus</i>	<i>Hepacivirus</i>	<i>Deltavirus</i>	<i>Hepevirus</i>	unassigned genus
Genome	ssRNA (+)	dsDNA	ssRNA (+)	ssRNA (-)	ssRNA (+)	ssRNA (+)
Genome size	4500 nt	3200 bp	9600 nt	1700 nt	7500 nt	10,000 nt
Virus isolated	1973	1969	1989	1977	1990	1996

nt: nucleotides; bp: base pairs; ss: single-stranded; ds: double-stranded

## HDV-HBV superinfection

Superinfection occurs when an individual with established HBV infection subsequently acquires HDV. Several characteristic serological features generally occur in such patients (Fig 2b). These include: hepatitis B surface antigen (HbsAg) titre declines at the time HDAg appears in the serum; HDAg and HDV RNA remain detectable in the serum, as chronic HDV infection generally occurs in most patients with HDV superinfection, unlike the case with co-infection; and high titres of both IgM and IgG anti-HDV are detectable and persist indefinitely. In long-term studies of chronic HBV carriers with HDV superinfection, 70–80% developed evidence of chronic liver disease (eg cirrhosis) compared with 15–30% of patients with chronic HBV infection alone.

## The 'virus'

Hepatitis D virus is the only member of the genus *Deltavirus*. The virus particles are nearly spherical and 36–43 nm in diameter. Internally, a ribonucleoprotein complex comprising the 1679-nucleotide, single-stranded, negative-sense circular RNA genome is encased in 60 molecules of delta antigen (Fig 1). The HBV helper virus is the source of HbsAg, which is incorporated into the HDV envelope, facilitating subsequent hepatocellular infection. The HDV genome encodes two isoforms (large and small delta antigen) of a single protein (Table 3). No other protein products are encoded by the HDV genome.

## Origin

It is proposed that HDV is related to viroids and satellite RNA because they share features. Viroids and satellite RNAs are small (200–400 nucleotide) plant pathogens, both of which lack open reading frames.<sup>3</sup> Viroids (eg tomato apical stunt) are unencapsidated, circular and replicate autonomously. Satellite RNAs (eg tobacco ringspot) are encapsidated, may be circular or linear and require the assistance of a non-homologous helper virus. Notably, HDV is different in that the genome is considerably larger and contains a viroid-like region as well as an open reading frame encoding the delta antigen. It is postulated that the open reading frame for delta antigen may have been acquired through capture of a cellular messenger RNA (mRNA) transcript by a viroid-like RNA, an hypothesis supported by the identification of a cellular homologue of the delta antigen.<sup>4,5</sup> However, the evolutionary origins of these pathogens remain unclear.

The most comprehensive phylogenetic study of viroids, satellite RNAs and HDV suggests that they have a monophyletic origin consistent with the hypothesis that they may be 'living fossils' of a precellular RNA world.<sup>6</sup> However, later sequence similarity studies indicate that viroids, satellite RNAs and HDV do not share any basic sequence similarity and the sequences of these pathogens cannot

# 'Liver transplantation may be considered for cases of fulminant acute and end-stage chronic hepatitis D infection'

be aligned in any statistically significant way.<sup>3</sup> This undermines any attempt at reconstructing phylogenetic relationships using sequence data.

## Replication

After the HDV genomic RNA has entered the cell, it migrates to the nucleus where it replicates via a rolling circle mechanism catalysed by a combination of delta antigen and a recruited host-cell RNA polymerase. During this RNA-dependent RNA replication, an anti-genomic form of the RNA is synthesised, which serves as the template for production of more genomic RNA. The anti-genomic RNA also carries the open reading frame for delta antigen.

An RNA editing event occurs at the translation stop codon for the small delta antigen, extending the reading frame to the next downstream stop codon. This generates the large delta antigen, which is a protein 19 amino acid residues longer at the C-terminus than the small delta antigen. Both protein isoforms have common nuclear localisation, oligomerisation and RNA binding domains (Fig 3). However, the large delta antigen inhibits HDV genome replication and promotes virion formation through interaction with HbsAg, an activity dependent

on addition of a 15-carbon farnesyl group to a cysteine residue during a post-translational isoprenylation modification.

Delta antigen and antigenomic RNA are largely nuclear, but most of the genomic RNA is found in the cytoplasm.<sup>7</sup> This enables it to be packaged efficiently into budding HDV particles.

## Detection

### Virology

Hepatitis D virus is notoriously difficult to culture in the laboratory. However, advances in hepatitis B virus cell culture techniques have led directly to increased knowledge of the natural history of HDV. Chimpanzees and woodchucks are used as the principal animal models. Cell culture systems for *in vitro* infection or transfection using both primary cultures of human and non-human hepatocytes and non-hepatocytes and cell lines have also been identified.

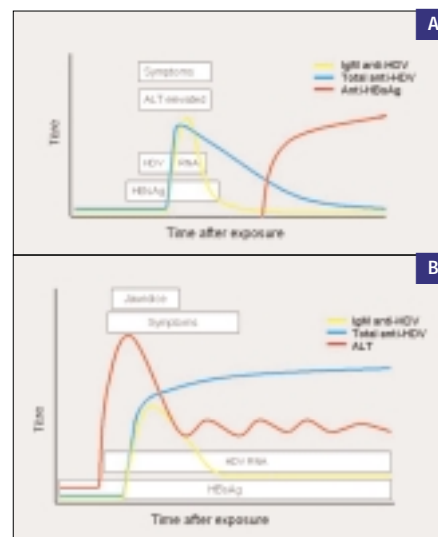
### Serology

One of the first assays to detect HDAg was a simple immunofluorescence blocking test.<sup>8</sup> This method had very low sensitivity and was quickly superseded by a solid-phase radioimmunoassay (RIA).<sup>9</sup> This was sensitive but required expensive equipment and repeated preparation of radioiodinated probe. In 1981 the first enzyme-linked immunosorbent assay (ELISA) for HDAg was described.<sup>10</sup> The sensitivity of the ELISA was between that of the immunofluorescence and RIA systems but was simpler and more convenient to perform. The RIA and ELISA assays for HDV continued to be developed and modified throughout the 1980s and contributed significantly to advances in the epidemiology of the disease (Table 2).

Anti-HDV antibodies were measured by solid-phase IgG and IgM capture RIA, as well as by competitive binding enzyme immunoassay (EIA) in both acute and chronic HDV infection.<sup>11</sup> The presence of high-titre rheumatoid factor in the serum and lipaemic samples produced false-positive results by EIA. Use of undiluted serum samples for EIA probably exaggerated the factors contributing to false-positive reactions.

A sensitive and reproducible ELISA for IgA-class antibodies against HDAg has been described.<sup>12</sup> Positive results were obtained with sera from 11/14 patients with chronic HDV infection (seropositive for HbsAg and IgM anti-HDV, negative for IgM anti-HBc) at serum dilutions up to 1 in 1,000,000.

Fourteen commercial EIA from six different companies were evaluated for detecting HDV markers (HDAg, anti-HDV and IgM anti-HDV).<sup>13</sup> The authors noted a substantial improvement in sensitivity and specificity over previously evaluated commercial assays for anti-HDV detection, and the sensitivities of IgM anti-HDV assays were also comparable. However, major differences in sensitivity remained among some assays for HDAg detection.



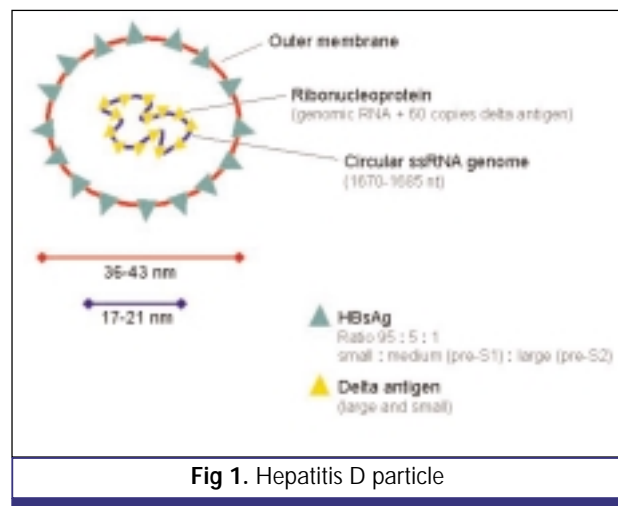
**Fig 2.** Typical serological course of A) HDV-HBV co-infection and B) HDV-HBV superinfection (redrawn from CDC data).

Hepatitis D virus is a unique human pathogen responsible for some 20 million infections globally. A long history of clinical laboratory testing and development has resulted in a range of rapid and sensitive assays to detect this intriguing subviral satellite agent. Here, Richard A Collins provides a comprehensive overview of current knowledge.

# Hepatitis D: the parasite's parasite

**In 1977, Italian scientists discovered a new antigen in patients infected with hepatitis B virus (HBV) that was different from the surface (s), core (c) and e antigens. The new antigen was called delta antigen and was thought to belong to a new virus.<sup>1</sup> This hypothesis was confirmed some years later by experimentally infecting chimpanzees and obtaining a new viral particle that was named hepatitis D virus (HDV; Fig 1).**

Hepatitis D virus is one of the most unusual pathogens in nature, as it can only infect hepatocytes in the presence of a helper virus, usually hepatitis B virus (HBV). Strictly speaking, HDV is not a virus at all. Owing to its helper requirement, HDV does not satisfy the definition of a virus and should be called a 'subviral' agent. In addition, as it shares no sequence similarity to the genome of its helper virus, it should be regarded as a 'satellite' of HBV. In full, HDV should be termed a subviral satellite agent with HBV its natural helper virus.<sup>2</sup> Table 1 shows the general properties of HDV and of the other viruses that cause hepatitis.



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## Pathogenesis

In addition to its intriguing genetic makeup, HDV poses a considerable health hazard, with clinical course varying from an acute self-limiting infection to acute fulminant liver failure. Chronic infection with HDV can lead to end-stage liver disease and associated complications. Detection is difficult because HDV infection is clinically indistinguishable from other forms of viral hepatitis, and up to 90% of patients are asymptomatic. The incubation period for HDV is 21–45 days and symptoms include jaundice, fever, lack of energy, dark (tea-coloured) urine, abdominal pain, nausea with vomiting, confusion, bruising, bleeding (rare) and pruritus (itching).

## Prevalence

Prevalence of HDV varies widely (Table 2). Of the estimated 400 million hepatitis B carriers worldwide, about 5% are also infected with HDV. Thus, some 20 million people may be at increased risk from exacerbated liver disease.

## Mode of transmission

Hepatitis D virus is transmitted in much the same way as HBV, with percutaneous exposure the most efficient. Sexual

transmission of HDV is less efficient than for HBV. Blood is potentially infectious during all phases of active HDV infection. Peak infectivity probably occurs just before the onset of acute disease. Infection can be acquired either as a co-infection with HBV or as a superinfection of persons with chronic HBV infection.

## HDV-HBV co-infection

Co-infection occurs when an individual acquires HBV and HDV simultaneously. Persons with HBV-HDV co-infection may have more severe acute disease and a higher risk of fulminant hepatitis (2%-20%) compared with those infected with HBV alone. In most persons with HBV-HDV co-infection, both IgM antibody to HDV (anti-HDV) and IgG anti-HDV are detectable during the course of infection (Fig 2a). However, in about 15% of patients the only evidence of HDV infection may be the detection of either IgM anti-HDV alone during the early acute period of illness or IgG anti-HDV alone during convalescence. Anti-HDV generally declines to subdetectable levels after the infection resolves and there is no serological marker that persists to indicate that the patient was ever infected with HDV. Hepatitis D antigen (HDAg) can be detected in serum in only about 25% of patients with HBV-HDV co-infection. When HDAg is detectable, it generally disappears as HBsAg disappears and most patients do not develop chronic infection.