HEPATITIS D VIRUS

1. Exposure Data

1.1 Structure and biology of hepatitis D virus (HDV)

1.1.1 *Structure of the virus*

Hepatitis delta antigen (HDAg) was first described by Rizzetto *et al.* (1977). Hepatitis D virus (HDV), also known as the 'delta agent', is a satellite agent of hepatitis B virus (HBV). HDV does not synthezise its own coat; it is enveloped by hepatitis B surface antigen (HBsAg), which occurs as large (L), middle (M) and small or major (S) surface proteins, differing in the length of the amino-terminal extension (Ueda *et al.*, 1991). In infectious hepatitis B virions, the ratio of the three HBsAgs L:M:S in the envelope is 1:1:4 (Bruss & Ganem, 1991). In comparison, the ratio L:M:S in HDV particles is 1:5:95 (Taylor, 1992). In this respect, HDV envelopes are very similar to empty spherical HBsAg particles, suggesting that HDV uses the excess production of empty HBsAg particles. It has been shown *in vitro* that the smallest HBsAg (S) is sufficient to package the HDV genome (Wang *et al.*, 1991). Interestingly, HDAg could also be packaged by woodchuck hepatitis virus (WHV) surface antigen, even in the absence of HDV RNA (Ryu *et al.*, 1992).

Naturally occurring HDV particles have a diameter of about 36 nm (Wang *et al.*, 1986) but are somewhat heterogeneous in size. Inside the HBsAg particle is the HDAg and the viral RNA genome. HDV RNA and HDAg exist as a ribonucleoprotein complex (Taylor, 1992).

1.1.2 *Structure of HDV genome and gene products*

The viral genome was first identified in 1986 in sera and liver tissue from HBV–HDV infected chimpanzees and was found to be a single-stranded (Taylor, 1990), circular RNA molecule of about 1700 base pairs (Chen *et al.*, 1986; Denniston *et al.*, 1986; Kos *et al.*, 1986; Wang *et al.*, 1986). Subsequently, HDV RNA was isolated from human sera (Makino *et al.*, 1987; Imazeki *et al.*, 1990; Saldanha *et al.*, 1990; Dény *et al.*, 1991; Imazeki *et al.*, 1991) and from liver tissue of experimentally infected woodchucks (Kuo *et al.*, 1988a; Dény *et al.*, 1991). The complete HDV RNA sequence is known for isolates from humans (Makino *et al.*, 1987; Saldanha *et al.*, 1990; Dény *et al.*, 1991; Imazeki *et al.*, 1991), chimpanzees (Wang *et al.*, 1986) and woodchucks (Kuo *et al.*, 1988a; Dény *et al.*, 1991). The domains of the HDV genome have been divided into primarily *cis*-acting sequences involved in genome replication and a region that encodes HDAg (Branch *et al.*, 1989; Chao *et al.*, 1990; Taylor, 1992). Through extensive base pairing (about 70%; Taylor, 1990), the circular HDV RNA genome forms an unbranched, rod-like structure (Kos *et al.*, 1986; Wang *et al.*, 1986; Makino *et al*, 1987; Kuo *et al.*, 1988a).

HDV is the only known animal virus with a circular RNA genome (Taylor, 1990): circular RNAs have so far been found only in higher plants, where they exist as subviral pathogens, called viroids, or as viroid-like satellite RNAs. Viroids are non-encapsidated, short, circular RNA molecules with a rod-like secondary structure, which replicate autonomously in susceptible cells and do not code for any protein. Viroid-like satellite RNAs are found within the capsid of specific helper viruses required for their replication. Phylogenetic analyses revealed that HDV RNA carries a viroid-like domain which is closely related to the viroid-like satellite RNAs of plants (Elena *et al.*, 1991).

HDV particles contain a single species of RNA, called genomic RNA. In infected liver cells, significant amounts of two other RNA species are detected: an RNA species representing the full-length complement of the genome, known as antigenomic RNA; and complementary RNAs of less than full length which encode the HDAg (Chen *et al.*, 1986, 1989; Hsieh *et al.*, 1990). Self-processing properties were first described for pre-rRNA of tetrahymena (a ciliated protozoan) (Cech & Bass, 1986); both genomic and antigenomic HDV RNAs have been shown to contain well-defined, self-cleaving, self-ligating sequences (Kuo *et al.*, 1988b; Sharmeen *et al.*, 1988, 1989; Wu & Lai, 1989; Wu *et al.*, 1989). The structural and biochemical requirements for self-cleavage and self-ligation have been defined (Wu *et al.*, 1989; Wu & Lai, 1990; Perrotta & Been, 1991; Kumar *et al.*, 1992; Suh *et al.*, 1992). Self-cleavage of HDV RNA is believed to be a central feature for the processing of viral RNA during replication (Taylor, 1990).

HDAg is the only known protein encoded by HDV RNA (Taylor *et al.*, 1992). HDAg is found not only inside the HDV particles but also within the nuclei of infected cells. Human HDAg has been shown to be a highly basic nuclear phosphoprotein with RNA binding activity, specific for HDV RNA (Chang *et al.*, 1988). The RNA binding activity depends, at least in part, on the rod-like structure of HDV RNA (Chao *et al.*, 1991). There are two related forms of HDAg (Bergmann & Gerin, 1986; Wang *et al.*, 1986; Weiner *et al.*, 1988): the small form (S), 195 amino acids long, which is essential for delta replication (Kuo *et al.*, 1988a, 1989); and the large form (L), 214 amino acids long (Makino *et al.*, 1987), which represents a 19-amino acid carboxy-terminal extension of the small form. During HDV replication, initiated by the S genome, a population of modified HDV genomes appears that encodes the L protein. The mutation responsible for the switch from S to L protein synthesis is an A to G mutation in the termination codon of the S form. The substrate for the sequence change is the viral genomic RNA rather than the antigenomic RNA (Luo *et al.*, 1990; Casey *et al.*, 1992; Zheng *et al.*, 1992).

The small form of HDAg (S) is required for HDV RNA replication, however, it is not sufficient for HDV virion production. While both antigenic isoforms of HDAg (S and L) have an HDV genome binding domain and are packaged into HBsAg envelopes, presence of the L form of HDAg is required for virion synthesis; this protein itself can be packaged into HBsAg particles in the complete absence of HDV RNA (Chen *et al.*, 1992). The L protein not only fails to support HDV replication but acts as a *trans*-dominant inhibitor of viral replication (Glenn & White, 1991). Experimental evidence suggests that the two main functions of the L form of HDAg, i.e. in packaging and *trans*-dominant inhibition of HDV replication, are located in different domains of the large HDAg (Chen *et al.*, 1992).

Thus, the small form of HBsAg is sufficient for HDV particle assembly; the large form of HDAg is required for HDV virion production; the small form of HDAg is necessary for HDV replication; and the large form of HDAg inhibits HDV replication.

1.1.3 *Replication of HDV*

Since the HDV genome does not carry a polymerase gene and purified preparations of HDV are not associated with polymerase activity (Wang *et al.*, 1986), the exact mode of replication of HDV and consequently the extent of the helper function of the hepadnaviruses is not known. In-situ hybridization analyses suggest that replication of HDV RNA is closely associated with HDAg expression and is located primarily in nuclei of infected cells (Gowans *et al.*, 1988).

HDV replication does not require hepatocytes and also occurs in mouse fibroblasts (Taylor, 1992) and COS7 monkey kidney cells (Kuo *et al.*, 1989). The only known HDV protein required for viral replication is the small form of HDAg (Taylor, 1992).

Studies of HDV-related nucleic acids from livers of infected hosts show clearly that HDV replicates without a DNA intermediate. Instead, HDV replicates *via* RNA-directed RNA synthesis (Taylor, 1990). The replication of HDV is in many ways similar to that of the plant viroids, virusoids and satellite RNAs (Branch *et al.*, 1990; Elena *et al.*, 1991): replication occurs in the cell nucleus (Gowans *et al.*, 1988); the enzyme is probably a redirected RNA polymerase II (MacNaughton *et al.*, 1991); and both self-cleavage and self-ligation of RNA are involved (Kuo *et al.*, 1988b; Sharmeen *et al.*, 1988, 1989; Wu & Lai, 1989; Wu *et al.*, 1989; Wu & Lai, 1990; Perrotta & Been, 1991; Kumar *et al.*, 1992; Suh *et al.*, 1992). Models have been proposed for the regulation of RNA processing and for HDV replication (Taylor, 1990; Hsieh & Taylor, 1991; Taylor *et al.*, 1992).

A number of in-vitro systems allow analysis of HDV replication. A system for the continuous replication and gene expression of the HDV genome has been established (Chen *et al.*, 1990).

1.1.4 *HDV-related animal models*

HDV has been successfully transmitted to chimpanzees (Rizzetto *et al.*, 1980a,b, 1981a; Purcell *et al.*, 1987). Serial passage of HDV in chronically HBV-seropositive chimpanzees resulted in increasingly severe liver disease without increasing markers of HDV replication or gene expression (Ponzetto *et al.*, 1988a). In addition, HDV has been propagated in WHV-infected woodchucks (Ponzetto *et al.*, 1984a, 1987a; Negro *et al.*, 1989; Dourakis *et al.*, 1991) and has been reported in ducks infected with duck hepatitis B virus (Ponzetto *et al.*, 1987b). The available evidence indicates that HDV infection does not occur naturally in these animal models, and humans are the only known natural host.

1.1.5 *HDV mutants*

Heterogeneity of HDV genomes exists between isolates from different geographic regions as well as in given isolates. A comparison of published HDV sequences from different geographic regions revealed a homology of 85–90%, regardless of their geographic origin (Lai *et al.*, 1991). The extremes are the high degree of homology (about 95%) between

an HDV isolate from southern California (Makino *et al.*, 1987) and one from England (Saldanha *et al.*, 1990) and the low degree of homology (about 80%) between two isolates from Japan (Imazeki *et al.*, 1990). Sequence divergence has also been noted within the same geographic area (Lai *et al.*, 1991). It is highest in the genome region between nucleotides 0 and 650 (Saldanha *et al.*, 1990) where no biologically important function has been localized.

HDV sequence variations are also seen in individual patients. In a study from Japan (Imazeki *et al.*, 1990), the mutation rate of HDV was calculated to be about 0.6 per kilobase and year; and in one from southern California (USA) (Lai *et al.*, 1991), the rate was 7–20 mutations per kilobase and year. This mutation frequency, inherently high for RNA viruses owing to lack of a proof-reading function of RNA polymerases, results in the coexistence of different genotypes, called quasispecies, in HDV-infected individuals. The significance of HDV mutations and HDV mutants is unknown.

1.1.6 *HDV–HBV interaction*

HDV replication can proceed in the absence of a hepadnavirus both *in vitro* (Taylor *et al.*, 1987) and *in vivo* (Ottobrelli *et al.*, 1991). For propagation and expression of its pathogenetic potential, HDV requires the presence of genetic information that encodes the major HBsAg (Wang *et al.*, 1991) from a hepadnavirus. Therefore, concurrent infection with a hepadnavirus is a prerequisite for the natural life cycle of HDV. Although HBV expression is a prerequisite for HDV propagation, it is significantly reduced by HDV both *in vivo* (Arico *et al.*, 1985; Hadziyannis *et al.*, 1985; Krogsgaard *et al.*, 1987; Chen *et al.*, 1988; Chu & Liaw, 1988; Wu *et al.*, 1990a) and *in vitro* (Wu *et al.*, 1991).

Clinically, the highest levels of HDV replication are found in patients with the highest level of HBV replication, usually in the serological setting of HBsAg and HBeAg positivity; these patients also had the most serious course of liver disease (Smedile *et al.*, 1991a).

In patients who had undergone liver tranplantation for end-stage HDV-associated chronic liver disease, HDV reinfection was detected early with no evidence of HBV infection or liver pathology. Reappearance of liver disease was preceded by reactivation of HBV replication and by intrahepatic propagation of HDV (Ottobrelli *et al.*, 1991). These findings suggest that HDV–HBV cooperation is necessary for HDV-associated liver disease. Therefore, a third mechanism in addition to HDV coinfection with HBV and HDV superinfection of HBV carriers may be HBV superinfection of HDV carriers. Whether HDV infection exists in HBV-seronegative individuals who are potentially at risk of becoming superinfected with HBV is unclear.

1.1.7 *Host range and target cells of HDV infection*

The host range of HDV is very narrow and follows that of HBV. Current evidence indicates that natural HDV infection occurs only in humans. This host range is believed to reflect the specificity of the liver-cell receptor for the HBV envelope protein, which binds to an epitope in the pre-S1 region 21–47 (Neurath *et al.*, 1986) and which is also found on cells of extrahepatic origin (Neurath *et al.*, 1990). It is not clear whether the ligand on HDV used for virus adsorption is identical to HBV.

In infected individuals, HDAg and nucleic acids are found primarily in liver cells. HDV RNA copy numbers are estimated to be about 300 000 per infected cell (Taylor, 1990). In

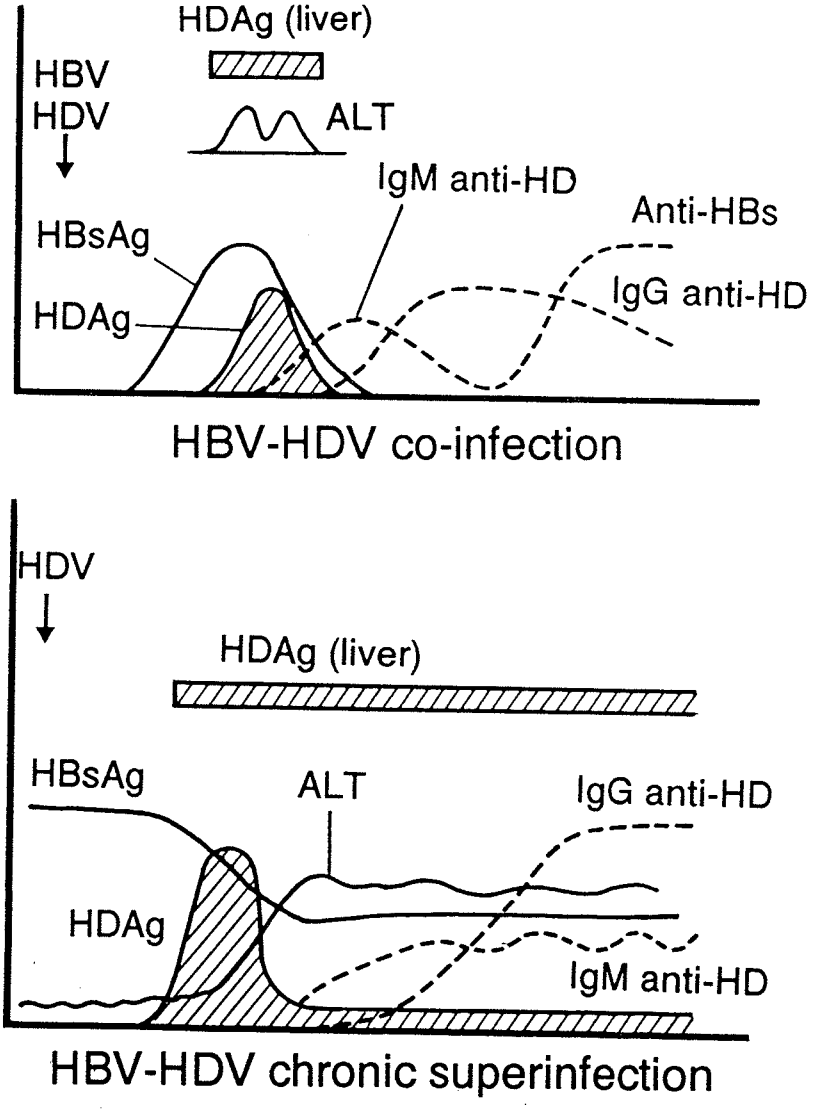
infected chimpanzees, as many as 10^{11} virions can be detected per millilitre of blood (Ponzetto *et al.*, 1988a). In contrast to HBV (Blum *et al.*, 1983; Halpern *et al.*, 1983; Tagawa *et al.*, 1985; Yoffe *et al.*, 1990), HDAg and HDV RNA have not been detected conclusively in tissues other than liver.

1.2 Methods of detection

1.2.1 *In serum and plasma*

Infection is detected on the basis of assays for viral antibodies or viral RNA in serum or HDAg in liver tissue. Typical serological markers of acute and chronic hepatitis D are shown in Figure 1.

Fig. 1. Typical serological markers of acute and chronic hepatitis D



HBV, hepatitis B virus; HDV, hepatitis D virus; HDAg, hepatitis D antigen; HBsAg, hepatitis B surface antigen; Ig, immunoglobulin; anti-HD, hepatitis D antibody; anti-HBs, hepatitis B surface antibody; ALT, alanine aminotransferase

(*a*) *Hepatitis D antigen*

Several commercial assay systems are available for the detection of HDAg but are not used clinically (Rizzetto *et al.*, 1991). HDAg can be detected by alternative western blot assay (Rizzetto *et al.*, 1991) or by blocking radioimmunoassay (Smedile *et al.*, 1981).

(*b*) *Hepatitis D antibody*

Hepatitis D antibody (anti-HD) immunoglobulin (Ig) M class or total anti-HD is detected by enzyme immunoassay and radioimmunoassay (Rizzetto *et al.*, 1981b).

(*c*) *HDV RNA*

HDV RNA can be detected by dot (northern) blot hybridization (Smedile *et al.*, 1986) or reverse transcriptase with the polymerase chain reaction (PCR). PCR is much more sensitive than conventional hybridization techniques or immunological analysis. PCR also allows cloning and sequencing of HDV in order to determine genomic structure (Denniston *et al.*, 1986; Zignego *et al.*, 1992).

1.2.2 *In liver tissues*

Liver HDAg can be tested by staining in frozen or fixed paraffin sections using fluorescence or peroxidase-labelled antibody to HDAg (Rizzetto, 1983; Rizzetto *et al.*, 1983; Di Biceglie & Negro, 1989).

1.2.3 *Serological markers of HDV infection*

There are two main patterns of delta virus infection (Fig. 1; Table 1; Rizzetto *et al.*, 1991).

Table 1. Typical serological patterns for HBV–HDV infection

HBsAg	Anti-HBc		Anti-HBs	Anti-HDV		Comments
	IgM	Total		IgM	Total	
+	+	+	–	+	+	Acute co-infection
–	–	+	+	–	(+ then –)	Recovered co-infection
+	–	+	–	+	+	Acute superinfection
+	–	+	–	–	+	Chronic superinfection

In the first pattern, called co-infection, an individual susceptible to HBV infection is infected simultaneously with HBV and HDV. These individuals often develop severe acute hepatitis, which progresses to fulminant hepatitis more frequently than HBV infection alone. The disease may be biphasic, with a course of hepatitis B followed by a brief period of recovery, and a relapse representing hepatitis D, which may be quite severe. If patients recover, they usually clear both HBV and HDV infection and rarely become HBV or HBV–HDV carriers. HDV infection in these individuals is characterized by seropositivity for IgM class anti-HD during acute infection, followed by a weak IgG class anti-HD response (measured as total anti-HDV), which persists for one to five years. For this reason,

serological surveys of anti-HD prevalence do not reflect adequately past history of HDV coinfection.

In the second pattern, called superinfection, an HBV carrier is infected with HDV. At this point, the patient may develop clinical, biochemical and histological signs and symptoms of acute viral hepatitis; however, most patients are unable to clear the virus and become chronic carriers of both HBV and HDV, with a clinical course of chronic active hepatitis progressing to cirrhosis, which may be much more aggressive than HBV infection alone. Total anti-HDV antibodies persist at high titres for prolonged periods, often for life, in these patients. HDV infection may suppress HBV replication, and some patients become HBsAg seronegative, leading to potential diagnostic confusion unless testing for anti-HBc and anti-HDV is done concurrently. HDV RNA may be used to follow these patients; HDAg is not routinely measured in serum (Salassa *et al.*, 1991).

1.3 Epidemiology of infection

1.3.1 *Prevalence*

The interpretation of epidemiological studies of infection should take into account the fact that it requires the presence of HBV. In many studies, only those individuals seropositive for HBV are tested for HDV. Since HDV infection suppresses the expression of HBsAg, the prevalence of infection may be underestimated. Secondly, in people who resolve their HDV infection, there is no long-lasting serological marker of past infection. Thus, prevalence studies reflect only recent and chronic infection. Superinfection is associated with significantly increased morbidity and mortality from chronic liver disease, and this effect on survival also influences the interpretation of prevalence data. For these reasons, the studies of geographic variation described below indicate the characteristics of the groups being tested.

Since HDV infection is so dependent on HBV infection, the descriptive data have been classified by level of infection with HBV. Table 2 shows the prevalence of infection with HDV in countries with a low endemicity of hepatitis B. There was no significant difference in infection rates between men and women in these studies. The reports from Norway and Sweden both showed no HDV infection in populations of intravenous drug users in the early 1970s but a rise in the prevalence of infection from the mid-70s onwards. HDV was introduced in Scandinavia in 1970–75 (Hansson *et al.*, 1982; Siebke *et al.*, 1986). The study of blood donors in the USA showed no association between seroprevalence for HDV and age, sex or blood group, but there was a significantly higher prevalence of anti-HD in HBV-carrier donors from California (12.1%) than from other areas of the USA (1.4–6.7%) (Nath *et al.*, 1985).

Table 3 shows the prevalence of infection with HDV in various populations in countries with intermediate levels of hepatitis B endemicity. There is a consistently higher prevalence among people with chronic hepatitis than among asymptomatic carriers. There is also marked geographical variability. HDV was introduced in Greece in 1965; a decrease in HDV infection has been observed subsequently (Hadziyannis *et al.*, 1991).

The prevalences of HDV in countries with a high endemicity for hepatitis B are shown in Table 4. There is again geographical variability between countries and also marked varia-

Table 2. Prevalence of HDV antibody (HDAg) in various hepatitis B surface antigen (HBsAg)-seropositive populations of countries and regions with low endemicity of hepatitis B

Country or region	Period	Group studied	No. of HBsAg carriers	Prevalence (%) of HDAg sero-positivity among HBsAg sero-positives	Reference
France	1984–88	Pregnant women	52	13.5	Ranger *et al.* (1990)
Germany	1982–84	Blood donors	301	0.3	Roggendorf *et al.* (1986)
		Haemodialysis patients	298	0.7	
		Chronic liver disease patients	220	2.7	
		Intravenous drug users	13	38.5	
		Haemophiliacs	16	50.0	
Northern Ireland	1970–89	Haemophiliacs	28	7.1	Curran *et al.* (1991)
		Foreign-born adults/contacts	153	2.6	
Sweden	1970–81	Chronic carriers			Hansson *et al.* (1982)
		Intravenous drug users	80	51.3	
		Non-intravenous drug users	101	3.0	
		Acute carriers			
		Intravenous drug users	291	17.2	
		Non-intravenous drug users	308	1.6	
Norway	1972–82	Chronic carriers	108	0	Siebke *et al.* (1986)
		Intravenous drug users	64	32.8	
Poland	NR	Asymptomatic carriers	123	1.6	Boron *et al.* (1987)
		Acute hepatitis B patients	35	2.8	
		Chronic hepatitis B or cirrhosis patients	15	6.6	
Canada	1983–85	Chronic carriers	121	0	Ratnam *et al.* (1986)
		Acute hepatitis B patients	22	0	
USA	1979	Blood donors	1915	3.8	Nath *et al.* (1985)

NR, not reported

bility within countries both geographically and between ethnic groups. Thus, Greenfield *et al.* (1986) in 1982–84 found virtually no HDV infection in 123 HBsAg-seropositive patients from the southern part of Kenya but a prevalence of 31% among healthy individuals in the north. Among these northern tribes, there was marked variation in the prevalence of HDV infection: 22% in the Turkana, 65% in the Rendille and 72% in the Samburn. A study in Djibouti in 1987 (Abbatte *et al.*, 1989) found marked differences by ethnic group, with a prevalence of HDV of 7.7% in male Afars and 0.7% in the remainder of the population. In China (Roggendorf *et al.*, 1987), prevalences of HDV infection in the general population vary by province, from 0 to 9%, in HBsAg-seropositive individuals. The reasons for these local variations are not understood. HDV infection, and particularly superinfection of HBV carriers, appears to be an ominous occurrence that may develop in populations among whom

HBV infection is endemic, e.g. in Venezuela. The age at infection in these populations is dependent on the usual age at infection with HBV (Hadler *et al.*, 1984). In the study of Bensabath *et al.* (1987) in the Amazon Basin, Brazil, children were infected under the age of 10 years, clearly indicating that a horizontal, non-sexual route is involved.

Table 3. Prevalence of HDV antibody (HDAg) in various hepatitis B surface antigen (HBsAg)-seropositive populations of countries or regions with intermediate endemicity of hepatitis B

Country or region	Period	Group studied	No. of HBsAg carriers	Prevalence (%) of HDAg sero-positivity among HBsAg sero-positives	Reference
Former USSR	NR	Asymptomatic carriers			Ketiladze *et al.* (1987)
		European	274	3.3	
		Asian	257	14.4	
		Chronic hepatitis B patients			
		European	36	13.9	
		Asian	63	41.3	
Greece	1983	General population	260	27.3	Hadziyannis *et al.* (1987)
Spain	1974–86	Acute hepatitis B patients			Buti *et al.* (1988)
		Intravenous drug users	155	65	
		Non-intravenous drug users	102	8.8[a]	
		Chronic hepatitis B patients			
		Intravenous drug users	105	67	
		Non-intravenous drug users	319	5.6	
		Asymptomatic carriers	68	0	
Italy	1975–85	Asymptomatic carriers	210	6.0	Smedile *et al.* (1987)
		Acute hepatitis B patients	238	5.4	
		Chronic hepatitis B patients	171	16.3	
Argentina	NR	Blood donors	1168	1.4	Fay *et al.* (1987)
		Acute hepatitis B patients	130	0.77	
		Chronic hepatitis B patients	135	2.2	
		Cirrhosis patients	52	5.8	
Jordan	1978–85	Asymptomatic carriers	136	1.5	Toukan *et al.* (1987)
		Acute hepatitis B patients	108	15.7	
		Chronic hepatitis B patients	79	22.8	
Egypt	1986	Chronic hepatitis B patients	44	47.7	El Zayadi *et al.* (1988)
		Asymptomatic carriers	48	8.3	
Yemen	1988	General population	112	1.8	Scott *et al.* (1990)
Saudi Arabia	NR	Pregnant women	185	9.7	Ramia & Bahakim (1988)
India	NR	Commercial donors	135	5.9	Arankalle *et al.* (1992)
		Voluntary donors	243	2.1	

NR, not reported
[a]HDAg or anti-HD

Table 4. Prevalence of HDV antibody (HDAg) in various hepatitis B surface antigen (HBsAg)-seropositive populations of countries or regions with high endemicity of hepatitis B

Country or region	Period	Group studied	No. of HBsAg carriers	Prevalence (%) of HDAg sero-positivity among HBsAg sero-positives	Reference
China	1983–85	Asymptomatic carriers	246	0.4	Wang *et al.* (1987)
		Acute hepatitis B patients	65	3.1	
		Chronic hepatitis B patients	104	0.9	
Indonesia	1986	Pregnant women	26	0	Vranckx *et al.* (1988)
Pacific Islands	1985–86	General population	646	33	Brindle *et al.* (1988)
Somalia	NR	General population	220	16.8	Aceti *et al.* (1989)
Kenya	1986–87	General population	132	42.4	Okoth *et al.* (1991)
Central African Republic	1984–88	Hospital patients	34	41.2	Crovari *et al.* (1991)
Brazil (Amazon)	NR	General population	232	45.2	da Fonseca *et al.* (1987)
Brazil (Amazon)	NR	Asymptomatic carriers	99	24	Bensabath *et al.* (1987)
		Acute hepatitis B patients	25	28	
		Chronic hepatitis B patients	21	100	
		Fulminant hepatitis B patients	27	74	

1.3.2 *Transmission*

In all populations studied, intravenous drug users have some of the highest prevalences of infection with HDV. In the USA, some 42% of HBsAg-seropositive intravenous drug users were found to be infected with HDV in the early 1970s (Ponzetto *et al.*, 1984b), 35% of imprisoned intravenous drug users who were HBV carriers in Greece were positive for HDV (Roumeliotou-Karayannis *et al.*, 1987) and 65% of a similar group in Scotland. As in Scandinavia, the virus appears to have first affected intravenous drug users in Scotland around 1975 (McCruden & Follett, 1989). In Poland, serial prevalence studies of intravenous drug users suggest that introduction was later, in the mid-1980s (Laskus *et al.*, 1992). The introduction of HDV into the intravenous drug user population has been shown to have led to outbreaks of infection among the non-user population (Mijch *et al.*, 1987) through both non-sexual and sexual routes (Lettau *et al.*, 1987).

Sexual transmission of HDV infection appears to be relatively inefficient. A study of 1368 US female prostitutes, $\geq$ 18 years old, showed that only 6% of the 18 non-drug users who were HBV carriers were seropositive for HDV; seropositivity rose to 21% of the 21 HBV carriers among intravenous drug users (Rosenblum *et al.*, 1992). A study of acute hepatitis due to HDV in Taiwanese men showed a significant association with sexual contact with

prostitutes (odds ratio, 5.5); the prevalence of HDV infection amongst HBV-carrier prostitutes was 59% (Wu *et al.*, 1990b). In a similar study in Taiwan, which included women with acute HDV hepatitis, one of the four women seropositive for HDV was a prostitute and another was the wife of a patient with active HDV infection (Liaw *et al.*, 1990). These studies suggest that the major risk for HDV infection of the general population in some parts of the world is heterosexual transmission.

The prevalence of HDV among male homosexual HBV carriers in Italy was 40% (4/10) and that in male heterosexual HBV carriers was 17% (6/36) (Mele *et al.*, 1988). Studies of four cohorts of homosexual men in the USA showed that rates of HDV infection among HBsAg-seropositive men varied significantly by geographic location, from 15.1% in Los Angeles to 0 in Chicago. HDV infection was associated with intravenous drug use, number of sexual partners and rectal trauma (Solomon *et al.*, 1988). Incident studies of homosexual male participants in HBV vaccine trials and in the 'Sentinel Counties Study' of acute hepatitis in the USA revealed three men with HDV infections among 290 with newly diagnosed HBV in the trials and none among 63 men with acute HBV infections in the Sentinel Counties Study, suggesting that HDV is an infrequent cause of acute hepatitis in homosexual men in the USA (Weisfuse *et al.*, 1989).

Blood transfusion and in particular administration of pooled blood products carried a high risk of HDV infection, as attested by the prevalence of 17–100% HDV infection in HBsAg-seropositive haemophiliacs (Lemon *et al.*, 1991; see also Table 2). The risk for transfusion-associated HDV infection has been estimated at one in 3000 transfusions, on the basis of sensitive tests for HBV markers (Rosina *et al.*, 1985).

Familial transmission of HDV was studied in Italy. Female sex of the index case in a household (odds ratio, 5.5) and HDV infection in that index HBV carrier (odds ratio, 71) were both independently associated with HDV superinfection of household members (Craxì *et al.*, 1991).

A study of 1556 HBV carriers who first presented to 35 liver units in Italy showed that the prevalence of HDV was independently related to young age (peak, 30–40 years), residence in the south of the country, intravenous drug use, large families and household contact with a HDV carrier. No association was found with blood transfusion or male homosexuality (Sagnelli *et al.*, 1992).

1.4 Clinical diseases (other than cancer)

HDV can either co-infect with HBV or superinfect HBV carriers (Rizzetto, 1983). In early studies, a link between HDV and severe liver disease was consistently demonstrated, leading to the widely accepted concept that this virus was invariably a highly pathogenic agent (Rizzetto *et al.*, 1983). Contrary to that hypothesis was a recent observation that patients in Greece (Hadziyannis *et al.*, 1991) and several liver transplant recipients in Italy and Belgium (Ottobrelli *et al.*, 1991) had clinically latent HDV infections which were associated with minor liver damage and even normal histology (Rizzetto *et al.*, 1992).

1.4.1 *Acute infection*

Acute hepatitis D results either from coinfection or superinfection and is of differing degrees of clinical severity. Many patients with acute HDV–HBV co-infection have a delayed

immune response to HDV (anti-HD), requiring that serial blood samples be tested over months after the onset of hepatitis in order to establish the correct diagnosis (Salassa *et al.*, 1991). In these patients, the hepatitis had a biphasic course, characterized by two peaks in serum alanine aminotransferase levels a few weeks apart (Caredda *et al.*, 1987). Co-occurrence of IgM anti-HBc and IgM anti-HD is a typical serological feature of acute co-infection (Di Bisceglie & Negro, 1989) (see also Table 1). HDV superinfection of HBsAg carriers often results in a monophasic hepatitis with seroconversion to IgM anti-HD. Severe hepatitis is common in both co-infected and superinfected patients (Craig *et al.*, 1986; Caredda *et al.*, 1987).

Fulminant hepatitis is more frequent in HDV-infected patients than in patients with hepatitis B alone (Smedile *et al.*, 1982). During the years 1979–82, 55 cases of fulminant hepatitis were seen in a unit in Milan, Italy: 28 were serologically related to HDV, and four of these were cases of superinfection of chronic HBsAg carriers (Rizzetto *et al.*, 1992). During the period 1986–88, HDV infection was found to account for 13 of 25 cases (52%) of fulminant hepatitis B in adults seen at the Western Attica General Hospital in Athens, Greece; five of the 13 were intravenous drug users (Tassopoulos *et al.*, 1990). Of the 71 patients admitted to the University of Southern California Liver Service (USA) in 1969–83 with fulminant hepatitis B, HDV infection was demonstrated in 33.8%, and 79% of these cases were due to HBV–HDV co-infection (Govindarajan *et al.*, 1984a).

1.4.2 *Chronic infection*

In Milan, hepatitis progressed to chronicity in 1.2% of patients with acute hepatitis B alone, in 2.4% of HBV–HDV co-infected patients and in as many as 91% of those super-infected with HDV (Caredda *et al.*, 1987). While progression to chronicity was frequent in the superinfected patients, the risk in the co-infected patients was the same as for patients with HBV infection alone.

Several lines of evidence support the existence of carriers of HBsAg and anti-HD with persistently normal serum alanine aminotransferase and essentially normal livers (Hadziyannis *et al.*, 1991; Rizzetto *et al.*, 1992). Follow-up of 27 HDV-seropositive liver transplant recipients showed that HDV recurred in 22 (81%); in nine patients, the virus recurred without recrudescence of liver disease (Ottobrelli *et al.*, 1991).

Anti-HD was commoner in patients with chronic active hepatitis or cirrhosis than in other patient groups (Sagnelli *et al.*, 1992). In highly endemic areas, chronic liver failure was largely caused by HDV superinfection and exposure to HDV early in life (Bensabath *et al.*, 1987). Chronic hepatitis D presents a wide range of symptoms, none of which is sufficiently specific to provide a diagnosis. Characteristic features of these patients are the prevalence of anti-HBe in serum and a frequent history of acute hepatitis. In contrast, a clinical episode of hepatitis was reported in fewer than 5% of the HDV-seronegative carriers with anti-HBe in Mediterranean countries (Rizzetto *et al.*, 1983). Additional features noted in southern European patients were the presence of splenomegaly and, in advanced cases, the presence of cholestasis out of proportion to other indices of liver failure (Rizzetto *et al.*, 1992).

A long-term study of 176 Italian patients with chronic hepatitis D showed that the course of the disease is bimodal (Bonino *et al.*, 1987). In about 15% of the patients, the disease progressed to liver failure within two years; in about 70%, the disease evolved slowly over

10–20 years; and the remainder had spontaneous remission of the disease. Using an HDV RNA riboprobe to detect the serum virological pattern and PCR for amplification of serum HBV DNA, chronic hepatitis D patients were divided into three groups: those with simultaneously replicating HDV and HBV, those with replicating HDV alone and those with neither HDV nor HBV replication (Smedile *et al.*, 1991b). Histological examination showed active disease in all patients of the first group, with a tendency for rapid evolution to cirrhosis and liver failure. More cases of inactive cirrhosis were observed in the second and third groups. It is possible that the virological states of the three subgroups represent different phases of the same disease course.

These data clearly indicate that active infection with HBV and HDV results in more severe liver damage than with either virus alone. Studies of liver transplant patients demonstrated that HDV infection in the transplanted liver is not in itself pathogenic and the virus becomes hepatotoxic only when HBV is reactivated (Ottobrelli *et al.*, 1991). Serial analyses in patients demonstrate rapid development of genotypic heterogeneity of HDV. The rates of evolution of the HDV isolates may correlate with the changes in the clinical pictures of hepatitis: the more drastic the change in the symptom of hepatitis, the more nucleotide changes were detected (Lee *et al.*, 1992).

1.5 Therapy and immunoprophylaxis

1.5.1 *Therapy*

(*a*) *Acute fulminant HDV infection*

In symptomatic acute fulminant HDV infection, therapy is aimed at relieving the signs and symptoms associated with the acute phase of the disease. Therapy includes parenteral nutrition in cases of dehydration and inanition due to nausea and vomiting, replacement of coagulation factors in cases of bleeding due to impaired synthetic liver functions and liver transplantation in cases of advanced liver failure and hepatic coma (Maddrey & Van Thiel, 1988). Except for an anecdotal report on the use of adenosine arabinoside monophosphate (Garcia *et al.*, 1990), no trial of antiviral agents in acute HDV infection has been published.

(*b*) *Chronic HDV infection*

Because of the severe natural course of HDV infection, several therapeutic modalities have been explored: prednisone, azathioprine, levamisole (Arrigoni *et al.*, 1983; Rizzetto *et al.*, 1983) and adenine arabinoside (Rosina *et al.*, 1991) were shown to be ineffective. Interferon-α has been explored in several trials (Hoofnagle *et al.*, 1986, 1987; Rosina *et al.*, 1987; Thomas *et al.*, 1987; Rosina & Rizzetto, 1989; Rosina *et al.*, 1991). While interferon-α was of some value for the treatment of chronic hepatitis D, long-term follow-up of HDV carriers revealed a high relapse rate after cessation of therapy.

1.5.2 *Immunoprophylaxis*

There is no specific vaccine against HDV. Vaccines against hepatitis B provide protection to individuals susceptible to both HBV and HDV but provide protection to HBV carriers against HDV. The degree of protection afforded by HBV vaccine is presumed to be similar to

that against HBV carriage, but the protective efficacy against HDV has never been evaluated. HBV immunoglobulin does not prevent superinfection with HDV.