

Prevalence and Genetic Diversity of Hepatitis B and Delta Viruses in Pregnant Women in Gabon: Molecular Evidence that Hepatitis Delta Virus Clade 8 Originates from and Is Endemic in Central Africa[∇]

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Hepatitis B virus (HBV) surface antigen (HBsAg) was found in 9.2% of 1,186 pregnant women from Gabon, of whom 10.1% had the HBe antigen and 89.9% had anti-HBe antibodies. Antibodies to the hepatitis delta virus (HDV) were found in 15.6% of the HBsAg-positive women. The HBV strains were of the A3 and E genotypes. The HDV strains belonged to HDV clades 1 and 8. These results provide clear evidence that HDV clade 8 is indigenous to Africa.

Hepatitis B virus (HBV) and hepatitis delta virus (HDV) are highly endemic in Africa. The prevalence of serological markers indicating exposure to HBV in sub-Saharan Africa is very high, up to 90% (3), although the prevalence of HBV carriers varies substantially between regions, from less than 7% to 35% (13).

The molecular characterization of HBV has revealed eight genomic groups, designated genotypes A to H (6, 12). Two major HBV genotypes, genotypes A and E, are predominant in central, south, and west Africa (13). Genotype A has been divided into two subgenotypes, subgenotypes A1 and A2 (8, 20); recently, a new subgenotype, subgenotype A3, was described and characterized in Cameroon and Gabon (9, 11).

HDV is highly endemic in several African countries, the Amazon region, and the Middle East (4). Recent, extensive analyses of the HDV sequences of strains isolated from patients of African origin have shown wide genetic diversity, with seven major clades; their proposed labels are HDV clade 1 (HDV-1) to HDV-7 (16). Recently, a new clade, HDV-8, was described by Le Gal et al. (10).

Perinatal HBV transmission appears to be the most important factor in determining the prevalence of infection in areas where HBV is highly endemic (3). HDV is transmissible only if the recipient is a carrier of HBV.

In Gabon, a central African country, the prevalence of hepatitis B surface antigen (HBsAg) in the general population is

TABLE 1. Prevalence of HBV and HDV in pregnant women in Gabon, by geographic area and age group^a

Variable	HBsAg positive			HBeAg positive			Antibodies to HDV		
	No. positive/no. tested (%)	OR	95% CI	No. positive/no. tested (%)	OR	95% CI	No. positive/no. tested (%)	OR	95% CI
Sentinel site									
Libreville	37/394 (9.4)	1.19	0.79–1.78	5/37 (13.5)	2.01	0.57–7.06	8/37 (21.6)	2.45	0.86–6.95
Port Gentil	10/171 (5.8)	0.64	0.33–1.25	1/10 (10.0)	1.11	0.13–9.68	0/10 (0)		
Lambaréné	29/330 (8.8)	1.07	0.69–1.67	3/29 (10.3)	1.2	0.3–4.86	0/29 (0)		
Oyem	6/65 (9.2)	1.11	0.47–2.63	0/6 (0)			3/6 (50.0)	7.57	1.39–41.22
Franceville	27/227 (11.9)	1.62	1.02–2.57	2/27 (7.4)	0.75	0.15–3.7	6/27 (22.2)	2.29	0.76–6.9
Age range (yr)									
14–20	30/372 (8.1)	0.94	0.61–1.46	7/30 (23.3)	6.54	1.76–24.28	2/30 (6.7)	0.39	0.08–1.81
21–25	34/334 (10.2)	1.34	0.88–2.05	2/34 (5.9)	0.53	0.11–2.59	7/34 (20.6)	2.13	0.74–6.14
26–30	23/259 (8.9)	1.08	0.67–1.75	1/23 (4.3)	0.4	0.05–3.29	6/23 (26.1)	2.95	0.96–9.05
31–40	22/222 (9.9)	1.25	0.76–2.04	1/22 (4.5)	0.38	0.05–3.14	2/22 (9.1)	0.59	0.12–2.79
All	109/1187 (9.2)			11/109 (10.1)			17/109 (15.6)		

^a OR, odds ratio; CI, confidence interval.

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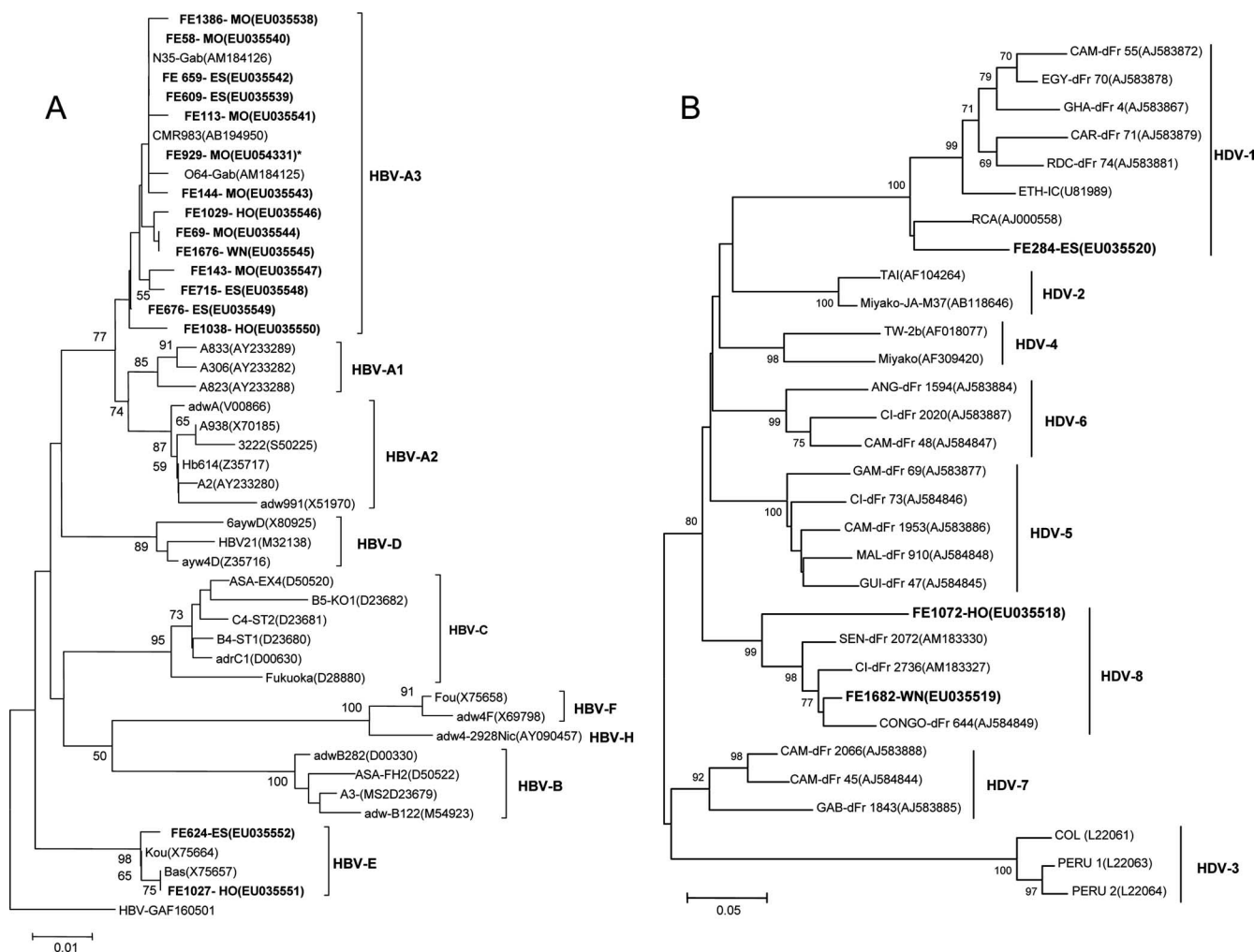


FIG. 1. Phylogenetic analysis of HBV and HDV strains obtained from pregnant women in Gabon, central Africa. (A) Phylogenetic analysis of a 315-bp fragment of the HBV-S gene from different HBV isolates by the neighbor-joining method with HBV-G (GenBank accession number AF160501) as the outgroup. Fourteen HBV-A3-FE sequences and 2 HBV-E sequences (highlighted in boldface) were compared with 31 HBV sequences from GenBank. *, a complete genome sequence was obtained for the FE-929-MO sample (GenBank accession number EU054331). This HBV subgenotype A3 clustering was confirmed by a separate phylogenetic analysis of the partially overlapping open reading frames coding for the polymerase-reverse transcriptase protein Pol (bootstrap value, 99%); envelope proteins S, M, and L (bootstrap value, 91%); the core protein (bootstrap value, 65%); and the transcriptional *trans*-activator protein (bootstrap value, 67%). No mutations in *cis*-acting elements were observed in the FE-929-MO isolate. pFDW294 (GenBank accession number M57663) was used as the reference strain (data not shown). (B) Phylogenetic analysis of a 326-bp fragment of the sHD gene from various HDV isolates by the neighbor-joining method, with HDV-3 used as the outgroup. One HDV-1 sequence (strain FE284-ES) and two HDV-8 sequences (strains FE1072-HO and FE1682-WN) (in boldface) were analyzed with 28 HDV sequences from GenBank.

often greater than 8 to 10% (1, 2, 17), but nothing is known about the prevalence and genetic diversity of HBV or HDV in pregnant women. There are no data on the prevalence and geographic distributions of the HDV clades in central Africa.

We evaluated the seroprevalence of HBV and HDV in a large population cohort of pregnant women in the five main cities of the country (Table 1), and we characterized the circulating genotypes.

Between January and March 2005, a total of 1,186 samples were obtained from pregnant women. The mean age of the women was 25.0 ± 6.4 years (range, 14 to 40 years). Plasma samples were assessed for the presence of HBsAg as described previously (11). HBsAg was detected in 109 plasma samples (9.2%). The prevalence of HBsAg did not differ significantly by age. However, the prevalence of HBe antigen positivity was

significantly higher (23.3%; *P* = 0.045) in the 14- to 20-year-old age group than in the other age groups (Table 1).

The presence of HDV antibodies was determined by the Murex anti-delta assay (Abbott, Wiesbaden, Germany). Of the 109 HBsAg-positive samples, 17 (15.6%) had antibodies to HDV. Antibodies were detected in women in all age groups. The percentage of HDV antibodies was lower in women aged 14 to 20 years (6.7%) than in the women in the other age groups (Table 1).

A 315-bp fragment of the HBV-S gene was amplified and sequenced for 16 HBsAg-positive isolates, as described previously (11). After alignment with reference isolates (HBV genotypes A to H), 14 of the new HBV isolates were shown to belong to subgenotype A3 and 2 were found to be closely related to HBV genotype E (99.4% and 99.6% similarities, respectively). Phylogenetic analysis showed that the strains

from Gabon belonged to HBV subgenotype A3 or genotype E (Fig. 1A). In the subgenotype A3 cluster, our new strains clustered with strains from Cameroon and with other strains from Gabon described previously (11). The strains in genotype E from Gabon were closely related to other strains of African origin (bootstrap value, 98%).

A fragment of 326 bp in the sHD gene of HDV from the three regions in which HDV was detected was amplified and sequenced as described previously (16). After alignment of the sequences with those of HDV isolates representing HDV-1 to HDV-8, one strain belonged to HDV-1 with an 84.4% similarity. The other two strains showed strong similarity with the newly described HDV-8 (84.9% and 95.7%). Phylogenetic analysis showed that these two strains clustered with HDV-8 with a bootstrap value of 99% (Fig. 1B).

In our study, the prevalence of HBsAg in pregnant women was as high as that in other African countries (5, 14, 18, 19). However, the hepatitis B envelope antigen (HBeAg) level was higher in pregnant women in Gabon than in pregnant women in other African countries, suggesting the earlier exposure and transmission of HBV in pregnant women in central Africa than in other regions of Africa.

We also showed that the HBV genotypes circulating in pregnant women belong to HBV subgenotype A3 and genotype E. Subgenotype A3 predominates in five widely separated geographical regions of Gabon. HBV genotype E has previously been reported mainly in West Africa (7, 13, 15), although it was also recently described in Cameroon (9). The E genotype was first suspected of circulating in Gabon when we found an HBV genotype A-E recombinant strain in a rural population (11). In the present study, we clearly demonstrated the occurrence of HBV genotype E in pregnant women in at least two regions of the country.

Few data on the prevalence of HDV in the general population in central Africa are available, although one study (17) showed a prevalence of 8.5% in three villages in a rural area of Gabon. In the present study, the overall prevalence of HDV among pregnant women was high (15.6%).

Up to September 2006, seven major clades, HDV-1 to HDV-7 (16), had been identified, with strong phylogenetic support. Recently, Le Gal (10) described a new clade, HDV-8, the strains of which were isolated from patients of African origin living in France. These strains clustered with another strain (strain dFr-644), previously closely related to HDV-7, isolated from a patient originating from Brazzaville, Congo, but living in France (16). Therefore, Le Gal and coauthors proposed the identification of a new clade with a probable African origin (10). To date, few sequences have been identified directly from indigenous African individuals, and no HDV-8 sequences have been identified in Africa. In this study, we showed that the new HDV strains circulating in various regions of Gabon belong to the new clade, HDV-8, and are indigenous to Africa.

This study was restricted to pregnant women, and more extensive studies of the HDV clades in central Africa are needed to better characterize the circulation of HDV in autochthonous African populations.

Nucleotide sequence accession numbers. The GenBank accession numbers of the new HBV subgenotype A3 and HBV genotype E strains from Gabon are EU035538 to EU035552.

The GenBank accession numbers of the new HDV strains are EU035518, EU035519, and EU035520.

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