Prevalence and Molecular Diversity of Hepatitis B Virus and Hepatitis Delta Virus in Urban and Rural Populations in Northern Gabon in Central Africa

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The prevalence of hepatitis B virus (HBV) surface antigen was significantly higher in urban (12.9%) than in rural (7.6%) populations (P = 0.003), but no difference was found in the prevalence of hepatitis delta virus (HDV), which was high in both populations. Phylogenetic analysis showed the circulation of HBV-A3 and -E genotypes and the presence of HDV-1, HDV-7, and HDV-8 clades.

Hepatitis B virus (HBV) and hepatitis delta virus (HDV) are highly endemic in Africa (1, 2); however, little information is available on the origin, circulation, and genetic diversity of these viruses in central Africa.

We reported recently that the prevalence of HBV surface antigen (HBsAg) in pregnant women in Gabon was 9.2% and that 15.6% had antibodies to HDV. Furthermore, two genotypes of HBV (subgenotype A3 and genotype E) and HDV-1 and HDV-8 clades were found in this population (7). The previous study was, however, restricted to pregnant women, and we considered that more-extensive studies of HDV clades in the general population were needed in order to characterize the circulation of these viruses in indigenous African populations. With this aim, we assessed the prevalence and genetic diversity of HBV and HDV in urban and rural populations in Gabon and compared the distributions of the HBV genotypes and HDV clades in these two areas with those of neighboring African countries.

Two epidemiological surveys were conducted in the north part of the country, in the province of Woleu-Ntem (157,013 inhabitants), which is characterized by a high population density in the cities and a very low population density in rural areas (3). The rate of immigration from Equatorial Guinea, Cameroon, and Congo is high, representing more than 22% of the total immigration rate in Gabon (9). In the first survey, 394 samples (from 203 women and 191 men) were collected in the main city of the region, Oyem. In the second survey, 961 samples (from 565 women and 396 men) were collected in 34 villages in the same province. We used the cluster sampling method and obtained ethical clearance for the study from the local public health authorities; each person gave informed consent before blood was taken.

The presence of HBsAg was assessed with the Monolisa Ag HBs-Plus test (Bio-Rad, Marnes la Coquette, France). The presence of HDV total antibodies in all HBsAg-positive samples was determined with the Murex anti-Delta (total) assay (Abbott/Murex Diagnostic Division, Wiesbaden, Germany).

Molecular and phylogenetic characterizations of HBV and HDV were performed as described previously (7). To determine the HBV genotype and HDV clade of the new Gabonese strains, we amplified and sequenced a 377-bp fragment of the HBV-S gene and a 326-bp fragment of the HDV-shD gene. These are the fragments usually used for phylogenetic analysis and are therefore the predominant sequences in the GenBank databases. One complete HBV genome was also sequenced and characterized.

As shown in Table 1, the overall prevalence of HBsAg was significantly higher in urban (12.9%) than in rural (7.6%) areas (P = 0.03). Conversely, the prevalences of antibodies to HDV among HBsAg carriers were extremely high in both the urban and the rural areas (P not significant). Persons aged 15 to 20 years in urban areas were more frequently HBV and HDV positive than those in urban settings (P not significant), and a significant difference was found between HBsAg-positive males and females living in rural areas (P = 0.04). The prevalence of HBsAg was significantly higher (P = 0.03) among men in the urban area (16.2%) than among those in the villages (9.8%). Interestingly, only in rural areas were men more frequently HBsAg carriers (9.8%) than women (6.0%) (P = 0.04).

A 377-bp fragment of the HBV-S gene was obtained from 13 persons (7 males and 6 females), 9 in the urban area and 4 in the villages. The neighbor-joining tree method showed that the HBV strains belonged to subgenotype HBV-A3 and genotype HBV-E (Fig. 1A).

A 326-bp fragment of the shD gene of HDV was obtained from 17 HDV-infected individuals (8 males and 9 females), 7 in the urban area and 10 in the villages. The phylogenetic analyses indicated splitting of the HDV-1 clade into two subclades, one made up of HDV strains from Canada and the Central African Republic and the other subdivided into two
distinct groups (Fig. 1B). The first group contains strains from all over the world, including one rural strain from Gabon, and the second group was made up of only the Gabonese HDV strains (three urban and six rural strains). Two HDV strains originating from rural areas belonged to the HDV-7 clade, and the remaining strains from both urban and rural areas fell into the HDV-8 clade.

We present here the first analysis of the prevalences of HBV and HDV in Gabon, with wide genetic diversity. HDV are highly endemic in indigenous general populations of Gabon, with wide genetic diversity. Two new strains from Gabon within the HDV-1 clade, 9 of 10 newly characterized sequences from general population samples clustered with a strain previously described clades (6, 7, 11). The first HDV strains were isolated and characterized from Africans living in France (6, 10). We confirmed previous results from our group and others, showing that the HBV-A3 subgenotype and the HBV-E genotype are present throughout central and west Africa (5, 7, 8, 10). The new HBV strains described in this study were not restricted to a particular area, as HBV-A3 and -E strains were found in both rural and urban areas.

HDV is highly endemic in central Africa, with eight described clades (6, 7, 11). The first HDV strains were isolated and characterized from Africans living in France (6, 10). We showed recently that HDV-1 and HDV-8 clades are present in pregnant women in Gabon, and we provided the first evidence that HDV-8 is indigenous to Africa (7). In the present study, a large number of sequences were obtained, showing wide genetic diversity in the HDV-1, HDV-7, and HDV-8 clades, confirming that these HDV strains are endemic in the general population of Gabon. Two new strains from Gabon within the HDV-7 clade were closely related to HDV strains from Cameroon, indicating that this clade is also endemic in the country. In the HDV-1 clade, 9 of 10 newly characterized sequences from general population samples clustered with a strain previously described by our group, originating from pregnant Gabonese women. This Gabonese HDV-1 subclade is therefore widespread in the country. More-extensive studies are needed to confirm this clustering.

In conclusion, our data provide clear evidence that HBV and HDV are highly endemic in indigenous general populations of Gabon, with wide genetic diversity.

**Nucleotide sequence accession numbers.** Sequences were deposited in GenBank under the following accession numbers: for the new HBV-A3 and HBV-E strains, FJ349266 to FJ349277; for the complete genome sequence obtained for the
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