Low Genetic Diversity despite Hyperendemicity of Hepatitis B Virus Genotype E throughout West Africa

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Sub-Saharan Africa suffers from an excessively high endemicity of hepatitis B virus (HBV), but little is known about the prevalent genotypes. In this study, we investigated the PreS1/PreS2/S genes of 127 viruses obtained from 12 locations in Mali, Burkina Faso, Togo, Benin, Nigeria, Cameroon, and the Democratic Republic of Congo. Except for those obtained from the Cameroon HIV cohort (18/22 HBV genotype A), 96 of 105 sequences belonged to HBV genotype E (HBV/E), and viral DNA was very similar (1.67% diversity) throughout this vast HBV/E crescent, which spans 6000 km across Africa. The low diversity suggests that HBV/E may have a short evolutionary history. Considering a typical mutation rate of DNA viruses, it would take only 200 years for the strain diversity of HBV/E viruses to develop from a single introductory event. The relatively recent introduction of HBV/E into humans would also explain its conspicuous absence in the Americas, despite the forced immigration of slaves from west Africa, until the early 19th century. Infection during infancy is mostly associated with chronic carrier status, and this combination can account for the explosive spread of virtually identical viruses within a community, but whether other routes of long-range transmissions must be considered becomes an important question.

According to the World Health Organization, >350 million people are chronic carriers of hepatitis B virus (HBV). Many develop chronic liver disease, including cirrhosis and hepatocellular carcinoma [1]. Worldwide, HBV infections account for 1 million deaths/year, most of which occur in the developing world. Excessively high incidence rates of chronic carriers have been reported in sub-Saharan populations. For instance, in Nigeria, the incidence of chronic carriers can be as high as 40%, depending on the region [2]. Genomic mutations are introduced by the viral polymerase during reverse transcription but may also occur during RNA pregenome synthesis by the cellular RNA polymerase II [3]. This explains the 15% genetic diversity of human HBV and accounts for a number of genetically distinct genotypes, designated A–H. Most genotypes show a more or less distinct geographic distribution: genotype A is prevalent in the United States [4, 5], in northern and central Europe, and in South Africa [6]; genotypes
HBV/E in West Africa

B and C (serotypes adw2 and adr/ayr) predominate in the Far East; genotype D (serotype ayw2 and ayw3) is found in the Mediterranean region, as well as in the Middle East; genotype F (serotype adw4) has been reported to mainly be in South and Central America [7–9]; genotype G has only recently been discovered in France [10] and in the United States in patients coinfected with genotype A [11, 12]; and genotype E (serotype ayw4) has been reported to be mainly in west Africa [13–17], but information on the prevalence of HBV genotypes throughout sub-Saharan Africa is very limited. Here, we report the PreS/S sequences of the 5′-end of the PreS1, PreS2, and S genes from 7 west African countries (Mali, Burkina Faso, Togo, Benin, Nigeria, Cameroon, and the Democratic Republic of Congo [DRC]). Genotype E was most prevalent and showed a conspicuously low genetic diversity.

**MATERIALS AND METHODS**

**Sample origin.** Serum samples were obtained between 2000 and 2003 from healthy individuals, patients with measles, or known HIV-infected individuals in the 7 west African countries listed above. Blood samples were obtained after informed consent was provided by the donors or, in the case of children, their parents or guardians. Characteristics of the different cohorts are shown in table 1. Serum samples from individuals from Burkina Faso, Cameroon, DRC, and Mali were tested for hepatitis B surface antigen (HBsAg) by use of the Murex kit (V3; Abbott Laboratories). Serum samples from individuals from Benin, Togo, and Mali were submitted for analysis by an automated qualitative microparticle EIA AxSYM HBsAg (V2; Abbott Laboratories), and anti–hepatitis B core (Hbc) antibodies were determined (AxSYM core; Abbott Laboratories).

HIV infections in the Cameroon cohort were confirmed by use of the Determine HIV-1/2 test (Abbott Laboratories) or by use of GenScreen HIV1/2 version 2 (Sanofi Diagnostics Pasteur). Among the 68 children reporting to the University Hospital in Yaoundé, malaria was the most common diagnosis.

In the DRC, serum samples were obtained from children reporting to hospitals in Lower Congo or Kinshasa. Most of the children were diagnosed as having measles [18]. In addition, 2 HBsAg-positive patients from Kinshasa, as well as 1 patient from the DRC from whom samples were obtained in 1992 in Luxembourg (LUX92-25059DRC), were included.

**Polymerase chain reaction (PCR), sequencing, and phylogenetic analysis.** HBV DNA was extracted and purified by use of the QIAamp DNA Blood mini kit (Qiagen), in accordance with the manufacturer’s instructions. Genomic amplification of the PreS1, PreS2, and S genes was performed in a nested format with overlapping PCR fragments, as described elsewhere [14]. In addition, new oligonucleotide primers were designed to improve sensitivity of the amplification reactions (P2f: 5′-CCT GCT GGT GGC TCC AGT TC-3′, position 0056–0075; P2r: 5′-GCG ACA GCA ACA TGA GGG AAA CA-3′, position 0571–0552; Mc2f: 5′-GCC CGT TTG TCC TCT AAT TCC AGG A-3′, position 0466–0490; and Mc2r: 5′-GGC AAT GAT CCC CAA CTT CCA-3′, numbering is according to HBV genotype E (HBV/E) reference strain Bas.

### Table 1. Prevalence of hepatitis B surface antigen (HBsAg) in west African cohorts from different countries.

<table>
<thead>
<tr>
<th>Country, region/province/city (date)</th>
<th>Cohort</th>
<th>Cohort size, no.</th>
<th>Age, median (range), years</th>
<th>M:F ratio</th>
<th>HBsAg prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin, Cotonou (2001)</td>
<td>Students and staff</td>
<td>120</td>
<td>27 (20–84)</td>
<td>1.2</td>
<td>16.7</td>
</tr>
<tr>
<td>Burkina Faso, Houët (2001)</td>
<td>Measles patients</td>
<td>61</td>
<td>9 (2 months to 40 years)</td>
<td>1.9</td>
<td>21.3</td>
</tr>
<tr>
<td>Cameroon (2002)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North (Garoua) and Adamawaou (Ngaoundere)</td>
<td>HIV-infected adults</td>
<td>92</td>
<td>31 (17–48)</td>
<td>15.7</td>
<td>82.6</td>
</tr>
<tr>
<td>Central (Yaoundé)</td>
<td>HIV-infected adults</td>
<td>93</td>
<td>29 (18–47)</td>
<td>1.3</td>
<td>100</td>
</tr>
<tr>
<td>Coastal (Douala)</td>
<td>HIV-infected adults</td>
<td>88</td>
<td>29 (18–49)</td>
<td>1</td>
<td>88.6</td>
</tr>
<tr>
<td>West (Bafoussam)</td>
<td>HIV-infected adults</td>
<td>89</td>
<td>29 (17–50)</td>
<td>1</td>
<td>94.4</td>
</tr>
<tr>
<td>Overall</td>
<td>HIV-infected adults</td>
<td>362</td>
<td>29 (17–50)</td>
<td>1.7</td>
<td>91.4</td>
</tr>
<tr>
<td>Yaoundé</td>
<td>Children&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68</td>
<td>2.8 (5 months to 14 years)</td>
<td>1.9</td>
<td>64.7</td>
</tr>
<tr>
<td>Democratic Republic of Congo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Congo (2000)</td>
<td>Children&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>Unknown</td>
<td>Unknown</td>
<td>30</td>
</tr>
<tr>
<td>Kinshasa (2000/2002)</td>
<td>Children&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34</td>
<td>Unknown</td>
<td>Unknown</td>
<td>9</td>
</tr>
<tr>
<td>Mali, Bamako (2002)</td>
<td>Students</td>
<td>152</td>
<td>25 (20–30)</td>
<td>2.4</td>
<td>54.6</td>
</tr>
<tr>
<td>Nigeria, Lagos (2001)</td>
<td>Liver patients</td>
<td>50</td>
<td>49 (18–84)</td>
<td>1.6</td>
<td>43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Togo (2001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast</td>
<td>Students and staff</td>
<td>89</td>
<td>32 (20–45)</td>
<td>1.9</td>
<td>30</td>
</tr>
<tr>
<td>Highlands</td>
<td>Students and staff</td>
<td>59</td>
<td>32 (20–60)</td>
<td>1.9</td>
<td>31</td>
</tr>
<tr>
<td>Overall</td>
<td>Students and staff</td>
<td>148</td>
<td>32 (20–60)</td>
<td>1.9</td>
<td>30.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Children reporting to hospital.

<sup>b</sup> PreS/S polymerase chain reaction.
The MinElute Gel Extraction Kit (Qiagen). The products were purified by use of the JetQuick Gel Extraction Spin kit (Genomed) or the MinElute Gel Extraction Kit (Qiagen). The products were sequenced in both directions on an ABI Prism 377 (Applied Biosystems), using the PCR primers in a 10-μL reaction volume containing 2 μL of premix (DYEnamic ET terminator cycle sequencing kit [Amersham Biosciences] or BigDye Terminator Cycle Sequencing Ready Reaction kit [Applied Biosystems]), 2 μL of dilution buffer (200 mmol/L Tris-HCl [pH 9.0] and 5 mmol/L MgCl2), and 5.5 μL of purified PCR product and sequencing primer (final concentration of primer, 0.5 μmol/L). Sequencing data were analyzed by use of the following computer programs: ABI Sequencing Analysis (version 3.4.1 and Sequence Navigator (version 1.0.1) (both Applied Biosystems), ClustalX (version 1.81 [19]), and Treeview (version 1.6.6 [20]). ClustalX uses neighbor-joining algorithms to build a tree from the distance matrix, which is the preferred method for a large data set. MEGA (version 2.1 [21]) was used as a second phylogenetic tool, to confirm the reliability of the data. Sequences included part of the PreS1 gene and the entire PreS2 and S genes, with a total length of ~1066 nt (genome position 3123–3212/0001–0976). For phylogenetic comparisons, only the 1016-nt coding region for the PreS/S gene has been used (genome position 3123–3212/0001–0835; numbering is according to HBV/E reference strain Bas [13]). Viruses that seemed to display re-combinations in positions located between PCR fragments were not included in the phylogenetic analysis but were included in the diversity estimate. No out-group sequences were included. Sequences have been submitted under GenBank accession numbers AJ604931–AJ605047.

RESULTS

Prevalence of HBV markers. In Mali, Togo, and Benin, 80%–82% of a total of 420 young, healthy adults had previous contact with HBV, as determined by the presence of anti-HBc antibodies. Table 1 shows that, among children or young, healthy adults, the prevalence of HBsAg was mostly 20%–30%, but ranged from 9% in DRC to ~50% in Mali. Although it is difficult to compare the cohorts, prevalence of antigen was highest in Cameroon, with two-thirds (44/68) of children who presented to the hospital (mostly with malaria) being HBsAg positive. In the HIV cohorts from Cameroon, overall HBs an-tigenemia was 91.4%, with some regional differences: it was highest (100%) in the capital of Yaoundé and lowest (82%) in the most-distant provinces. The serum samples obtained from outpatients with chronic liver disease in Lagos, Nigeria, were only submitted for analysis by the PreS/S PCR; 19 of 44 gave a positive signal.

Genotypes. Serum samples containing viral DNA were selected for sequence analysis. Sequence analysis was based on a 1016-nt stretch of the PreS/S gene. A total of 127 sequences from 7 west African countries were obtained: 22 strains from Cameroon HIV cohorts (9 from the North province and the province of Adamawa, 6 from the Central province, 1 in the Coastal province, and 6 from the West province, as well as 18 strains from the child cohort); 22 strains from Togo (15 from the highlands and 7 from the coastal region); 13 strains from Benin; 20 from Mali; 6 strains from the DRC; 11 strains from Burkina Faso; and 15 strains from Lagos, Nigeria. For phylogenetic analysis, 19 sequences from our previous study that focused on Ibadan, Nigeria, were included [14], as well as previously published sequences of HBV/E and representative sequences of the other genotypes (A–H; figure 1).

Except for sequences obtained from the Cameroon HIV cohort, 96 of 105 sequences that were obtained in the present study belonged to HBV/E (figure 1). The overall genetic variability in the PreS/S gene region of these sequences was maximally 1.67%, or 17 nt across the 1016-nt fragment of the most distantly related strains within HBV/E (data not shown). When all known HBV/E sequences available in GenBank were included, the genetic variability did not increase. Identical viruses were found in locations as distant as Bamako (MAI140), Lomé (TOG116), Lagos (554), and Cameroon (CAE312 and others; figure 1) or Kinshasa (DRC14578), Lagos (508), Con- tonou (BEN017 and BEN002), and Bamako (MAI029).

The Cameroon HIV cohort was a conspicuous exception, since 18 of 22 strains belonged to HBV genotype A (HBV/A); the others belonged to HBV/E. In contrast, among the children from Cameroon, 4 were infected with HBV/A and 14 with HBV/E. HBV sequences isolated from HIV-positive children were of genotypes E and A. A single sporadic case of HBV/A was also detected in Burkina Faso (BFA-S121). The HBV/A strains from Cameroon form a cluster of their own, distinct from the HBV/A’ viruses [22] from Europe and the United States and are grouped in a cluster designated A’ (figure 2). The diversity among these sequences is 1.87%, which is slightly higher than the total diversity of HBV/E. The overall diversity of HBV/A viruses from the 3 continents is 3.44%, which is significantly higher than that of HBV/E.

Genotype diversity among the few strains from the DRC was surprising. Strains DRC14574 and DRC020033 from Kinshasa belonged to HBV/E, whereas Lux92-25059DRC, isolated in Luxembourg in 1992 from an individual born in the DRC, was
Figure 1. Dendrogram of hepatitis B virus (HBV) genotype E sequences, on the basis of 1016 nt of the PreS1 gene and the entire PreS2 and S genes. The insert shows a phylogenetic tree of the known human and primate genotypes (A–H). New strains are in italic and bold type, earlier strains from Nigeria are in italic type, and accession numbers of GenBank sequences are shown. BEN, Benin; BFA, Burkina Faso; CAE, Cameroon HIV cohort; CAEch, Cameroon child cohort; CIV, Ivory Coast; DRC, Democratic Republic of Congo; MAI, Mali; SEN, Senegal; TOG, Togo.
most closely related to a Malawian HBV/A' strain [23]. Strain DRC0300441, isolated from a 48-year-old woman, also belonged to HBV/A and is most closely related to the Cameroon HBV/A' strains. Her 28-year-old son was infected with an HBV genotype D virus (DRC0300442; data not shown).

Interestingly, 2 strains were found in Mali (MAI036 and MAI042) that formed outliers to both genotypes E and A (figure 2), even when phylogenetic analysis was performed on the individual PCR fragments. Further analysis (e.g., by cloning) needs to be performed to exclude mixed variants.

Deletions in PreS1. In the PreS1 fragment, deletions are rare and, so far, have only been found in some strains belonging to D or nonhuman primate genotypes (33 nt [24]). Among HBV/E viruses, only Ibadan010.98 had a so-far unique single codon deletion at the C-terminal end of PreS1. Similarly, strain MAI095 displayed a unique 6 aa deletion in position 85–90 of PreS1.

PreS2 mutations and deletions. Seven HBV/E strains, including Ibadan024.97, described elsewhere [14], displayed a mutated ATG start codon in the PreS2 gene, precluding the synthesis of the corresponding M surface protein. The methionine codon was replaced by 1 of the aliphatic, hydrophobic amino acids: valine (MAI136, CAE071, CAE075, CAE085, CAE108, and CAE182), isoleucine (CAE050, MAI140, and Ibadan024.97), alanine (CAE094), or threonine (DRC0300441). These substitutions were found both in HBV/A (DRC0300441, CAE085, CAE108, and CAE182) and HBV/E (MAI136, MAI140, and Ibadan024.97) strains in at least 4 countries (Cameroon, the DRC, Mali, and Nigeria). These mutations are frequently associated
with chronic infection [25], as well as fulminant hepatitis [26] and hepatocellular carcinoma [27, 28].

A sizeable proportion of sequences reported here (33/108 HBV/E) contained deletions in the PreS2 region, varying in length between 1 and 7 codons, similar to those of the Ibadan strains included in table 2. Most of these deletions were found in HBV/E sequences obtained from asymptomatic carriers from Togo, Benin, and Mali. Three HBV/A strains showed similar but much longer PreS2 deletions (10 or 17 codons; table 2). The deleted region carries neutralizing B-cell epitopes [29] and is often, but not exclusively [14], found in chronically infected individuals [30].

**Serotypes.** With a single exception, all HBV/E strains belonged to serotype ayw4, on the basis of the deduced amino acid composition at positions 122 (d for K and y for R), 160 (w for K and r for R), and 127 (w1l2 for P, w3 for T, and w4 for L) of the S protein [8]. The only aberrant strain was CAE040, with a deduced serotype adw1/2. The HBV/A strains belonged to serotype ayw1/2 (BFA-S121, CAE012, CAE050, CAE075, CAE083, CAE085, CAE088, CAE094, CAE151, CAE154, CAE168, CAE182, CAE193, CAE384, and DRC0300441) or ayw4 (CAE071, CAE108, CAE353, and CAE371), 3 strains belonged to serotype adw1/2 (CAE357, CAECh052, and CAECh055), and 1 strain belonged to serotype adw4 (CAECh044).

**DISCUSSION**

Except for those sequences from our earlier study that focused on Nigeria [14], only a few single sequences were available from west Africa [15, 31]. Here, we have reported 127 new sequences on Nigeria [14], only a few single sequences were available from Nigeria [14], and have added 12 new

Table 2. Lengths and positions of amino acid deletions (-) in the PreS2 gene of hepatitis B virus (HBV) genotype E (HBV/E) and HBV genotype A (HBV/A) strains.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Amino acid sequence example</th>
<th>Length</th>
<th>No.</th>
<th>Geographical distribution^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>E^b</td>
<td>MQWNSTTFHQALQDPRVRGLYFPAGSSS &lt;br&gt; MQWNSTTFHQALQDPRVRGLYPAGSSSG</td>
<td>0</td>
<td>1</td>
<td>NIE 1/35</td>
</tr>
<tr>
<td>E^c</td>
<td>MQWNSTTFHQALQDPRVRGLY-PAGSSSG</td>
<td>1</td>
<td>1</td>
<td>CAE 1/19, TOG 1/22</td>
</tr>
<tr>
<td>E^d</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>3</td>
<td>2</td>
<td>CAE 1/19, TOG 1/22</td>
</tr>
<tr>
<td>E^e</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>4</td>
<td>27</td>
<td>BEN 7/13, CAE 1/19, MAI 2/18, NIE 11/25, TOG 6/22</td>
</tr>
<tr>
<td>E^f</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>5</td>
<td>4</td>
<td>BEN 2/13, MAI 1/18, NIE 1/35</td>
</tr>
<tr>
<td>E^g</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>6</td>
<td>4</td>
<td>DRC 1/3, MAI 1/18, NIE 1/35, TOG 1/22</td>
</tr>
<tr>
<td>A^b</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>7</td>
<td>3</td>
<td>DRC 1/3, NIE 1/35, TOG 1/22</td>
</tr>
<tr>
<td>A^c</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>14</td>
<td>1</td>
<td>CAE 1/13</td>
</tr>
<tr>
<td>A^d</td>
<td>MQWNSTTFHQALQDPRVRRPAGSSSG</td>
<td>17</td>
<td>1</td>
<td>CAE 1/13</td>
</tr>
</tbody>
</table>

**NOTE.** BEN, Benin; BFA, Burkina Faso; CAE, Cameroon; DRC, Democratic Republic of Congo; MAI, Mali; NIE, Nigeria; TOG, Togo.

^a No. of sequences with deletions over total no. per country and genotype.

^b Amino acid sequence HBV Kou_SEN (GenBank accession no. X75664) shown as reference.

^c A total of 120 HBV/E sequences included, including those from Nigeria [14].

^d A total of 27 HBV/A African sequences included.

This is the same genotype that, before, we and others found to the west (Senegal, Ivory Coast, and Gambia [15, 31, 47]), east (Niger [31]), and south (Angola [31]) of this region, as well as in southwest Nigeria [14]. Both in Gambia and in Ivory Coast, 87% of strains were of HBV/E, although, among strains obtained from Africans living in Sweden, only 20% were of this genotype [48]. Throughout this HBV/E crescent, which covers one-third of the African continent and >40% of its population, prevalence of HBV antibody is excessively high: in our unselected (non-HIV) cohorts, seroprevalence was >80%. Similar but, also, lower seroprevalence rates were reported by other investigators [32, 33]. We have found a prevalence of chronic carriers in the general population, estimated by HBsAg, of 9%–65%. Although a bias due to point-source introductions cannot be excluded with ~70–140 million chronic carriers (20%–40% of the estimated 350 million population), genotype E may well be the most important genotype worldwide. It is therefore critical to reassess the sensitivity of diagnostic tests, the efficacy of vaccines, and the clinical and therapeutic aspects, with respect to this virus [34–36].

HBV/E was first described in 1993 [31]. Except for single sporadic cases in Africans living in northern Europe [37], Belgium [16], and The Netherlands [17], HBV/E has not been found outside of Africa. Despite the forced migration of slave labor from west Africa to the western hemisphere, genotype E has not been reported in that continent. By 1 account, genotype D/E (19.9%) was found in Texas and California in an ethnically mixed cohort also including African Americans, but the assay could not distinguish between genotypes D and E, and no other genotyping results were available. The genotype has also not been reported from Central and South America [9, 38].

In Cameroon, most HIV-positive adults (80%–100%) were chronic carriers of HBV. Surprisingly, with a few exceptions (1 from the North province [CAE040], 1 from the Coastal prov-
Figure 3. Hepatitis B virus (HBV) genotype distribution across the African continent. HBV genotype A, black; HBV genotype D, stripes; and HBV genotype E, gray. Incidental genotypes are shown as inserted circles.

ince [CAE283], and 2 from the West province [CAE312 and CAE382]), these individuals were infected with genotype A. Within Africa, genotype A has been found extensively in southern Africa, including South Africa [22, 39], Malawi [23], Somalia, Kenya, and Ethiopia (figure 3). Although HBV/A may be the predominant genotype in Cameroon, the HBV/A bias (10/14) may also be caused by HIV-typical horizontal transmission (sexual intercourse/sharing needles), as suggested by the predominance of HBV/E (15/18) in the child cohort. Children positive for both HIV and HBsAg were infected with either HBV/A or HBV/E.

An intriguing finding of the present study is the low sequence diversity of HBV/E (1.67%) throughout the expanses of the HBV/E crescent, which covers almost 6000 km from Senegal to Angola. This suggests that HBV/E has a short evolutionary history in humans. Such a conclusion would also be supported by the conspicuous absence of this genotype in African Americans and would be incompatible with an evolution from the closest known human virus, a genotype D virus. Such an evolution would have taken an estimated 700 years, assuming that the 5.7% genetic distance across PreS/S evolved at an evolutionary rate of $4.2 \times 10^{-3}$/site and year [40, 41]. Therefore, the relatively recent introduction from an animal reservoir must be seriously considered. If HBV/E was introduced by a single introductory event, it would have taken ~200 years to develop the 1.67% diversity. In contrast, introduction of HBV from other host species into primates (including humans) is thought to have occurred 6000 years ago [40]. Cross-species infections from humans to monkeys have been shown experimentally, but there is no evidence of natural transmission of HBV from primates to humans. HBV/E has been found once in a chimpanzee, but the direction of transmission could not be established [24]. Thus, similar to HIV, different genotypes of HBV may have been introduced independently into humans.

Under the simplest assumption of a single introductory event, the very high antibody seroprevalence (>80%) of HBV
in the HBV/E crescent is surprising and would require an explosive spread of HBV in a naive population. Transmission during early childhood is supposed to be the most common mode of infection in Africa, and most children infected before the age of 6 months become chronic carriers [42]. Early age of infection and high probability of chronic carrier status results in high rates of transmission [43]. Although this may explain the explosive spread of virtually identical viruses within a community, it is critical to understand whether it explains also the similarity of viruses across the vast expanses of the HBV/E crescent. Perhaps, the most important question raised by the low diversity of HBV/E is whether known modes of transmission can account for the high viral prevalence and the low genetic diversity or whether new routes of transmission must be discussed, including blood-feeding insects [44–46]. Early contaminated childhood vaccines should also be considered, particularly since most vaccines are given during infancy, when chronic carrier status is most likely to develop.

Acknowledgments

Without the important contributions of many health workers and physicians throughout west Africa, this comprehensive study would not have been possible. We acknowledge the following as representatives for the many unnamed individuals: O. B. Salu (Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos, Nigeria), J. P. Bronowicki (Service d’Hépatogastro-Entérologie, Hôpital d’Adultes, Vandoevere-les-Nancy, France), A. Sanni (Département de Biochimie et Biologie Cellulaire, Université d’Abomey-Calavi, Abomey-Calavi, Benin), E. K. Amouzou (University of Sciences, Lome, Togo), and Mathias Opp and François Schneider (Laboratoire National de Santé, Luxembourg, Luxembourg).

References

30. Policicino T, Campo S, Raimondo G. PreS and core gene heterogeneity

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