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Disparate distribution of hepatitis B virus genotypes in four sub-Saharan African countries

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Abstract

Background—Hepatitis B virus (HBV) places a substantial health burden on Africa. Here, we investigated genetic diversity of HBV variants circulating in 4 countries of sub-Saharan Africa using archived samples. In total, 1492 plasma samples were tested from HIV-infected individuals and pregnant women, among which 143 (9.6%) were PCR-positive for HBV DNA (Côte d'Ivoire, 70/608 [11.5%]; Ghana, 13/444 [2.9%]; Cameroon, 33/303 [10.9%]; and Uganda, 27/137 [19.7%]).

Study design/results—Phylogenetic analysis of the *S*-gene sequences identified HBV genotypes E (HBV/E, $n = 96$) and A (HBV/A, $n = 47$) distributed as follows: 87% of HBV/E and 13% of HBV/A in Côte d'Ivoire; 100% of HBV/E in Ghana; 67% of HBV/E and 33% of HBV/A in Cameroon; and 100% of HBV/A in Uganda. The average and maximal nucleotide distances among HBV/E sequences were 1.9% and 6.4%, respectively, suggesting a greater genetic diversity for this genotype than previously reported ($p < 0.001$). HBV/A strains were classified into subgenotypes HBV/A1, HBV/A2 and HBV/A3. In Uganda, 93% of HBV/A strains belonged to HBV/A1 whereas HBV/A3 was the only subgenotype of HBV/A found in Cameroon. In Côte d'Ivoire, HBV/A strains were classified as HBV/A1 (11.1%), HBV/A2 (33.3%) and HBV/A3 (55.6%). Phylogeographic analysis of the sequences available from Africa supported earlier suggestions on the origin of HBV/A1, HBV/A2 and HBV/A3 in East, South and West/Central Africa, respectively. Using predicted amino acid sequences, hepatitis B surface antigen (HBsAg) was classified into serotype *ayw4* in 93% of HBV/E strains and *adw2* in 68% of HBV/A strains. Also, 7.7% of the sequences carried substitutions in HBsAg associated with immune escape.

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Competing interests

None declared.

Conclusions—The observations of pan-African and global dissemination of HBV/A1 and HBV/A2, and the circulation of HBV/E and HBV/A3 almost exclusively in West and Central Africa suggest a more recent increase in prevalence in Africa of HBV/E and HBV/A3 compared to HBV/A1 and HBV/A2. The broad genetic heterogeneity of HBsAg detected here may impact the efficacy of prevention and control efforts in sub-Saharan Africa.

Keywords

Hepatitis B virus; Phylogenetic analysis; Genotypes; Subgenotypes; Sub-Saharan Africa

1. Background

Hepatitis B virus (HBV) is one of the world's most widespread infectious agents. It is associated with approximately one million deaths each year [1] due to cirrhosis, liver failure and hepatocellular carcinoma. As this infection is highly endemic in sub-Saharan Africa, it imposes a substantial health burden in this part of the world [2,3]. Unfortunately, this is the region where access of the population to comprehensive vaccination and treatment is limited [4,5]. Thus, despite the availability of a safe and effective vaccine against HBV infection, many persons remain unvaccinated which hinders control and prevention efforts.

The HBV genome is highly heterogeneous and can be classified into 8 genotypes, A–H [6]. More recently, two possible new HBV genotypes, designated I and J, have been described [7–9]. HBV genotypes differ in disease severity and response to therapy [10] and have specific geographic distributions. Some are restricted to particular geographic regions while others are distributed globally [11]. Previous studies showed that HBV genotype E (HBV/E) is particularly endemic to West Africa [12–17]. This HBV genotype has not been as extensively studied as the other major genotypes [17] but several reports indicated that HBV/E has a very low intra-genotype diversity, suggesting that it emerged recently [12,15,17,18].

HBV genotype A (HBV/A) has also been reported from Africa. Subgenotype HBV/A1 has been found circulating widely in the continent [19] while HBV/A3 has been reported mainly in West and Central Africa [19,20]. The rarer tentative subgenotypes HBV/A4 and HBV/A5 were detected in Mali, Nigeria and Haiti [14,16,21]. Recently, subgenotype HBV/A6 was reported from African-Belgian patients [22] and HBV/A7 from Cameroon [14]. These observations showed a greater genetic heterogeneity of HBV/A in Africa than in any other part of the world, suggesting that genetic analysis of HBV strains from this continent is crucial for understanding routes of HBV dissemination worldwide. Several reports have shown additionally that infection with HBV/A may be associated with male homosexual activity and sexual promiscuity, and tends to persist longer following primary infection than HBV/C [23–25].

1.1. Objectives

Côte d'Ivoire, Ghana, Cameroon and Uganda are countries in sub-Saharan Africa with high endemic levels of HBV infection, where the seroprevalence of hepatitis B surface antigen (HBsAg) in the general population exceeds 8% [1]. Owing to such high prevalence, liver

disease associated with HBV infection has emerged as an important cause of morbidity and mortality in this region [26]. The genotypic characteristics of HBV strains endemic to these countries, which represent West, Central and East Africa, have seldom been compared and such comparison would be crucial to understanding the epidemiology of HBV infection. We report here results of such comparison.

2. Study design

2.1. Clinical samples and ethical statement

A total of 1492 archived plasma samples were assembled for HBV testing: (a) 608 were collected in Côte d'Ivoire in 1995 from pregnant women in various hospitals; (b) 444 were from Ghana, of which 400 were collected in 1987 from pregnant women and 44 were obtained in 1997 from confirmed HIV-seropositive patients; (c) 303 samples were from Cameroon, of which 100 were collected in 1997 from confirmed HIV-positive patients while 203 were from HIV-seropositive blood donors collected in 2002; and (d) 137 samples were collected in Uganda in 1997 from HIV-seropositive patients. Côte d'Ivoire and Ghana are located in West Africa; Cameroon is located in central Africa and Uganda in East Africa. The HIV infected population was defined as high-risk because sexual transmission plays a major role in HBV transmission among adults [27,28] and because of shared modes of transmission, HBV infection is evidently more frequent in HIV-infected population [29]. The samples used in this study were anonymous with no links to personal identifiers. Prior Institutional Review Board and Ethics Committee approvals and informed consent were obtained for specimens collected in each country. In addition, a non-research determination was approved for HBV testing of anonymized archived samples by the US Centers for Disease Control and Prevention, Atlanta, Georgia, USA. All specimens were stored at −20 °C or in liquid nitrogen prior to HBV testing.

2.2. Nucleic acid extraction and amplification of the HBV S-gene

Total nucleic acids were extracted from 200 μl of plasma using the robotic Roche MagNA Pure LC system (software version 3.0.11) and the MagNA Pure LC Total Nucleic acid isolation kit (Roche Diagnostics GmbH, Mannheim, Germany), and eluted in 50 μl of elution buffer according to the manufacturer's instructions. HBV genotype/subgenotype assignment was based on phylogenetic analysis of the partial *S*-gene sequence (406 bp) as described previously but without the SP6 and T7 promoter sequences [12]. The *S*-gene is considered suitable for genotype classification [30,31]. The sense and antisense primers for the first-round PCR were HBV S1F (position 179), 5′-CTA GGA CCC CTG CTC GTG TT-3′, and HBV S1R (position 704), 5′-CG AAC CAC TGA ACA AAT GGC ACT-3′ respectively. The sense and antisense primers for second-round PCR were HBV SNF (position 217), 5′-GTT GAC AAG AAT CCT CAC AAT ACC-3′, and HBV SNR (position 658), 5′-GA GGC CCA CTC CCA TA-3′, respectively. Direct sequencing was performed with 1.0 μM of the second-round primers using the automated DNA sequencer ABI 3130xl (Applied Biosystems, Foster City, CA).

2.3. Nucleotide sequence analysis

Sequences were initially analyzed and edited using the Seq-Man and MegAlign programs of the Lasergene DNA and protein software version 7.0 (DNASTAR Inc., Madison, WI). Nucleotide sequences were aligned using the GCG (Version 11.1.2-UNIX, Accelrys Software Inc, San Diego, CA) multiple alignment program Pileup. HBV genotypes/subgenotypes were classified based on the *S*-gene sequence by comparing each sequence with published reference sequences from GenBank. The accession numbers for the HBV/E references were: AB091255, AB106564, AB194947, AB194948, AB201288, AB201289, AB201290, AB205129, AB274981, AB274983, AB274984, AF323631, AJ605025, AP007262, AR488645, AY738145, AY738147, AY739674, X75657, X75664 while those for HBV/A were: AB194949, AB194951, AB194952, AF090838, AF090839, AF090842, AF297621, AF297622, AF297623, AF297625, AF536524, AF537372, AJ309369, AJ309370, AJ309371, AM180623, AM184125, AM184126, AY034878, AY233275, AY233288, AY233290, HM363613, M57663, S50225, X02763, X51970, Z72478. Initial neighbor-Joining trees were built using the Kimura two-parameter model of nucleotide substitution [32]. Phylogenetic trees were constructed using the maximum likelihood algorithm implemented in Dnaml (PHYLIP package, v.3.6). Frequency distributions of pair-wise distances between nucleotide sequences were estimated using the evolution program in the Accelrys GCG Package (Genetic Computer Group, version 11.1-UNIX, Accelrys Inc., San Diego, CA). The ARLEQUIN software package version 3.0 [33] was used to calculate unbiased estimates of nucleotide diversity according to Nei [34] and also the MEGA5 software program [35].

2.4. Inference of HBV serotypes

HBV serotypes were predicted based on amino acid sequences at positions 122, 160, 127 and 134 in the *S*-gene as previously described [12,36].

2.5. Detection of mutations within the ‘a’ determinant of the HBsAg sequences

Mutations associated with immune escape located within the HBsAg immunodorminant ‘a’ determinant (residues 124–147): (I/T126A/N, A128V, Q129H/R, G130N, M133L/T, K141E, D144A/H, and G145R) [37,38] were searched for manually. The first and most common ‘vaccine escape mutant’ described [37] is the substitution of Gly at position 145 by Arg (G145R).

2.6. Accession numbers

All the *S*-gene sequences reported here have the GenBank accession numbers KC174559–KC174701.

3. Results

3.1. HBV DNA detection

Among the 1492 samples, 143 (9.6%) were positive for HBV DNA (Table 1). Prevalence of 11.5%, 2.9%, 10.9% and 19.7% was found in Côte d’Ivoire, Ghana, Cameroon and Uganda, respectively. Two sets of specimens from Ghana and Cameroon were used in this study.

However, while both sets were from HIV-infected patients in Cameroon and showed a similar HBV DNA detection rate of 10% and 11.3%, only one set was collected from HIV-infected patients in Ghana with HBV DNA detection rate of 6.8%, with the second set with a lower rate of 2.5% being collected from HIV-negative pregnant women. Additionally, the two specimen sets from Ghana were collected 10 years apart, with the more recent set being collected from HIV-infected patients (Table 1). Differences in risk factors and collection dates may account for the disparity in HBV prevalence between sets from this country. The finding of low detection rates in these archived samples from Ghana reported here are less than the rate reported for HBV infection in Ghana occurring more recently [39,40], and may indicate an increase in HBV infection in Ghana. Although the high HBV prevalence in these four countries, especially Uganda, is compatible with the HBV hyperendemicity in sub-Saharan Africa [1], testing of specimens from the high-risk HIV-infected persons may also contribute to the high HBV DNA detection rates in this study.

3.2. Geographical distribution of HBV genotypes

All 143 HBV strains were classified into genotypes E (n = 96) and A (n = 47) (Fig. 1). In Côte d'Ivoire, HBV/E was found in 61 (87.1%) patients and HBV/A in 9 (12.9%); in Ghana, all 13 (100%) HBV strains belonged to HBV/E; in Cameroon, 22 (66.7%) belonged to HBV/E and 11 (33.3%) to HBV/A; in Uganda, all (100%) belonged to HBV/A.

3.3. HBV/A subgenotypes

The HBV/A strains belonged to subgenotypes HBV/A1, HBV/A2 and HBV/A3 (Fig. 2). All 3 subgenotypes were found in Côte d'Ivoire: 1 (11.1%) HBV/A1; 3 (33.3%) HBV/A2; and 5 (55.6%) HBV/A3. In Cameroon, all 11 (100%) HBV/A strains belonged to HBV/A3. In Uganda, 25 (92.6%) belonged to HBV/A1 and 2 (7.4%) to HBV/A2. Comparison of HBV/A *S*-gene sequences obtained here (n = 47) and those from other studies in Africa (n = 271) showed exclusive circulation of HBV/A3 in West and Central Africa, while HBV/A1 and HBV/A2 can be found in all regions of sub-Saharan Africa (Fig. 3).

3.4. HBV/E genetic diversity

The mean and maximal genetic distances among the HBV/E *S*-gene sequences obtained here were calculated to be 1.9% and 6.4%, respectively. The corresponding HBV/E sequences recovered from GenBank (n = 700) have the mean and maximal genetic distances of 0.7% and 4.8%, respectively. Analysis of variance (ANOVA, IBM SPSS Statistics version 21) showed statistically significant difference ($p < 0.001$) between the mean genetic distances calculated for sequences sampled in this study and those obtained from GenBank. Considered by country of origin, the mean and maximal genetic distances for the Côte d'Ivoire, Ghana and Cameroon HBV/E strains were 1.8% and 5.2%, 2.7% and 4.9%, and 1% and 3%, respectively.

3.5. Vaccine-escape substitutions

Amino acid substitutions within the "*a*" determinant of HBsAg associated with vaccine escape were identified in 11 of 143 HBV sequences (7.7%), with 6 (6.3%) belonging to

HBV/E (n = 96) and 5 (10.6%) to HBV/A (n = 47). The following substitutions were detected: T126 M/S, A128G, Q129R, M133L, D144A/E, and G145R (Table 3).

3.6. HBV serotypes

HBV serotypes were predicted from amino acid positions 122, 127, 134 and 160 of HBsAg as previously described [12,20,36]. HBsAg sequences were classified into serotypes *ayw4* (89/143; 62.2%), *ayw1* (*22/143*; 15.4%) and *adw2* (*32*/143; 22.4%); 92.7% (n = 89/96) of all *ayw4* strains were HBV/E and 68.1% (n = 32/47) of HBV/A strains were *adw2*. Serotype *ayw1* was identified in 7.3% of HBV/E and 31.9% of HBV/A strains (Table 2).

4. Discussion

HBV variants detected from archived specimens from Côte d'Ivoire, Ghana, Cameroon, and Uganda were found to belong to two genotypes, HBV/A and HBV/E. The prevalence of HBV/E strains was remarkably higher in the West African countries of Côte d'Ivoire and Ghana than in Cameroon (Central Africa), and Uganda (East Africa) (Fig. 1 and Table 2). HBV/E was detected in all PCR-positive cases identified from Ghana while HBV/E was not found in specimens from Uganda. A downward gradient of HBV/E distribution from west to east suggested by this observation has been noted in other studies [41]. The predominance and almost exclusive circulation of HBV/E in West and Central Africa were interpreted as suggesting the West African origin of this genotype [12,13,15–18,21]. The vast majority of HBV/E HBsAg were classified into serotype *ayw4* (Table 2), lending support to previous findings of *ayw4* in geographic regions where HBV/E is dominant [12,15].

A low genetic diversity of HBV/E strains had been repeatedly noted [17,18,20]. The mean genetic diversity of 1.71% was reported for HBV/E full-genome sequences, while the estimate of diversity of the *S*-gene sequences, similar in size and genomic location to sequences used here, was found to be only 0.73% [13]. The mean diversity calculated for sequences obtained from GenBank for the same HBV/E genomic region studied here was 0.7%. The mean genetic distance among HBV/E sequences reported in this study was 1.9%, which is statistically different ($p < 0.001$) from the value of 0.7% for the GenBank sequences; the maximal genetic distance was 6.4%. HBV/E strains from Côte d'Ivoire and Ghana exhibited the mean and maximum diversity of 1.8% and 5.2%, 2.7% and 4.9%, respectively, thus presenting the highest detected genetic diversity for HBV/E. Taking into consideration that the region studied here is one of the most conserved in HBV genome, the detected level of genetic heterogeneity reflects unusually high genetic diversity of HBV/E, potentially consistent with the existence of HBV/E subgenotypes. However, such a conclusion would need to be drawn after phylogenetic analysis of whole-genome sequences rather than being based on sub-genomic fragments.

The most genetically distant *S*-gene sequences were identified in HBV/E strains sampled from pregnant women in Ghana in 1987 and Côte d'Ivoire in 1995, while all other strains were collected in 1997 and 2002 from HIV-coinfected patients (Table 1, Fig. 1). Significant genetic diversity observed in HBV from these countries supports the hypothesis that HBV/E originated from the North-Western region of sub-Saharan Africa [13]. Additionally, the observation of genetically distant lineages in archived samples collected from a group of

pregnant women without knowledge of risk factors of HBV exposure in both countries suggest complex temporal patterns of HBV/E dissemination in human subpopulations in this region of Africa. Further studies might lead to a more precise understanding of how this HBV genotype had diverged and evolved over time.

HBV/A predominated in Uganda and exhibited a downward gradient from East to West Africa, the direction opposite to HBV/E. Previous studies have also shown that HBV/A is the main genotype endemic in Uganda [19], similar to other countries in East Africa like Kenya where 88% of HBV strains belong to genotype A [42]. This observation is in concert with the HBV genotype divide, with HBV/E dominating in the west and HBV/A in the east of Africa [41,43].

HBV/A is significantly heterogeneous and has been classified into seven subgenotypes, HBV/A1, HBV/A2, HBV/A3, HBV/A4, HBV/A5, HBV/A6 and HBV/A7 [13,16,21,22,44]. Here, we identified 3 subgenotypes, HBV/A1, HBV/A2 and HBV/A3. They were not found to be uniformly distributed in the countries of Côte d'Ivoire, Cameroon and Uganda where HBV/A strains were detected. HBV/A1 was found almost exclusively in Uganda, with only 1 HBV/A1 strain detected in Côte d'Ivoire; while HBV/A3 was predominant in Côte d'Ivoire and in Cameroon. HBV/A2 was found as a minority HBV/A variant in Côte d'Ivoire and Uganda. Thus, the data suggest predominance of HBV/A1 in East Africa, and of HBV/A3 in West/Central Africa. Phylogenetic analysis of the African HBV/A sequences obtained from GenBank (Figs. 2 and 3) confirmed that HBV/A3 isolates were almost exclusively detected in West and Central Africa. Taken together, HBV/A3 appears to have originated in this African region, as was suggested earlier [12,44]. By contrast, HBV/A1 and HBV/A2 are widespread across Africa, albeit with varying prevalence.

As reported earlier [45], HBV/A variants from South Africa are divided between HBV/A1 and HBV/A2. The large number of Gen-Bank sequences from South Africa analyzed here (Fig. 3) may represent oversampling of HBV strains from this geographic region rather than actual prevalence. Nevertheless, despite the limited sampling of HBV/A2 variants from other African countries, the tight phylogenetic clustering of *S*-gene sequences by geographic regions outside of South Africa (Fig. 3) contrasting with the wide genetic heterogeneity of HBV/A2 strains in South Africa suggests introduction of this subgenotype from South Africa to other regions.

HBV/A1 was found to be endemic in East Africa (Figs. 2 and 3). HBV variants from East and South Africa are broadly represented among the HBV/A1 strains examined here, while variants from other countries cluster in individual branches, suggesting that HBV/A1 originated from South or East Africa. However, the finding that East-African HBV/A1 variants were located predominantly at the long tips of the phylogenetic tree rather than as clusters as for the South African variants (Fig. 3) supports the hypothesis that HBV/A1 originated from East Africa [21,46], which then spread to the South, becoming endemic there. Had HBV/A1 been introduced from South to East Africa, HBV/A2 would also have been endemic in East Africa as HBV/A1. The low prevalence of HBV/A2 in East Africa lends additional support to the East African origin of HBV/A1.

Among all 4 major HBV genetic groups, HBV/E, HBV/A1, HBV/A2 and HBV/A3, identified in the four countries here, only HBV/A1 and HBV/A2 have disseminated broadly across sub-Saharan Africa [19,20,47]. HBV/A2 has been observed also in North Africa where HBV genotype D is predominant [48–50]. Besides Africa, HBV/A1 was observed also in South Asia [46,47]. HBV strains identified in acute cases of hepatitis B in Europe and North America belong predominantly to HBV/A2 [51]. The distinct geographic segregations of these two HBV/A subgenotypes may reflect disparate routes of HBV spread across diverse populations in Africa and globally. Assuming that HBV/A emerged from Africa as suggested here and had previously been hypothesized [46], it would be reasonable to infer that HBV/A1 spread to South Asia from East Africa whereas HBV/A2 spread from South Africa to Europe and North America.

Contrasting with HBV/A1 and HBV/A2, HBV/E and HBV/A3 circulate almost exclusively in West and Central Africa. Such geographical restriction has occurred despite the high endemicity of HBV infections in this region [1] and the translocation of large numbers of West Africans across the Atlantic Ocean from the slave trade that lasted for >200 years from the 17th century and ended during the early 19th century. Phylogenetic analysis of HBV/E from Nigeria indicated that this genotype rapidly expanded during the second half of the 20th century [12], which was most probably associated with mass vaccination programs implemented in West/Central Africa [12,52,53]. Should similar epidemiological mechanisms be responsible for the dissemination of other HBV strains, HBV/A3 would have experienced a very recent sharp increase in prevalence as well. Thus, the limited global presence of HBV/E and HBV/A3 is likely associated with an extremely low HBV prevalence in West/Central Africa before the 20th century, in contrast to HBV/A1 and HBV/A2 which should have been sufficiently prevalent much earlier, thus having facilitated broad dissemination of these subgenotypes through long-term trades and exploration across Africa and globally.

Our study also showed that the majority of HBV/A isolates belonged to serotype *adw2* (Table 2). This is concordant with previous reports that showed the dominance of *adw* in East Africa where HBV/A1 is highly prevalent [54]. The circulation of many HBV/A variants could be a reflection of the long history of viral persistence and transmission through many human generations in this region.

The finding of 7.7% of strains among HBV/E, HBV/A1 and HBV/A3 carrying substitutions that confer immune escape properties (Table 3) presents a potentially important public health issue. Since HBV vaccination of newborns in Africa started in 2003–2005 and specimens used in our study were collected from adults between 1987 and 2002, the substitutions would not have been selected by vaccination and can be considered as naturally occurring. Infections with strains carrying these substitutions may be less preventable through vaccination programs [55], and may be inefficiently detected with conventional diagnostic assays for HBsAg [56–59]. These findings need to be taken into consideration for implementation of public health interventions in Africa.

In conclusion, HBV strains from Côte d’Ivoire and Ghana from West Africa, Cameroon from Central Africa and Uganda from East Africa were classified into HBV/E and HBV/A1,

HBV/A2 and HBV/A3. Significant genetic heterogeneity of both HBV genotypes and broad variation in prevalence of these genotypes and subgenotypes of HBV/A in these countries suggest a dynamic evolutionary history of HBV in sub-Saharan Africa and may present unique challenges to public health in this part of the world.

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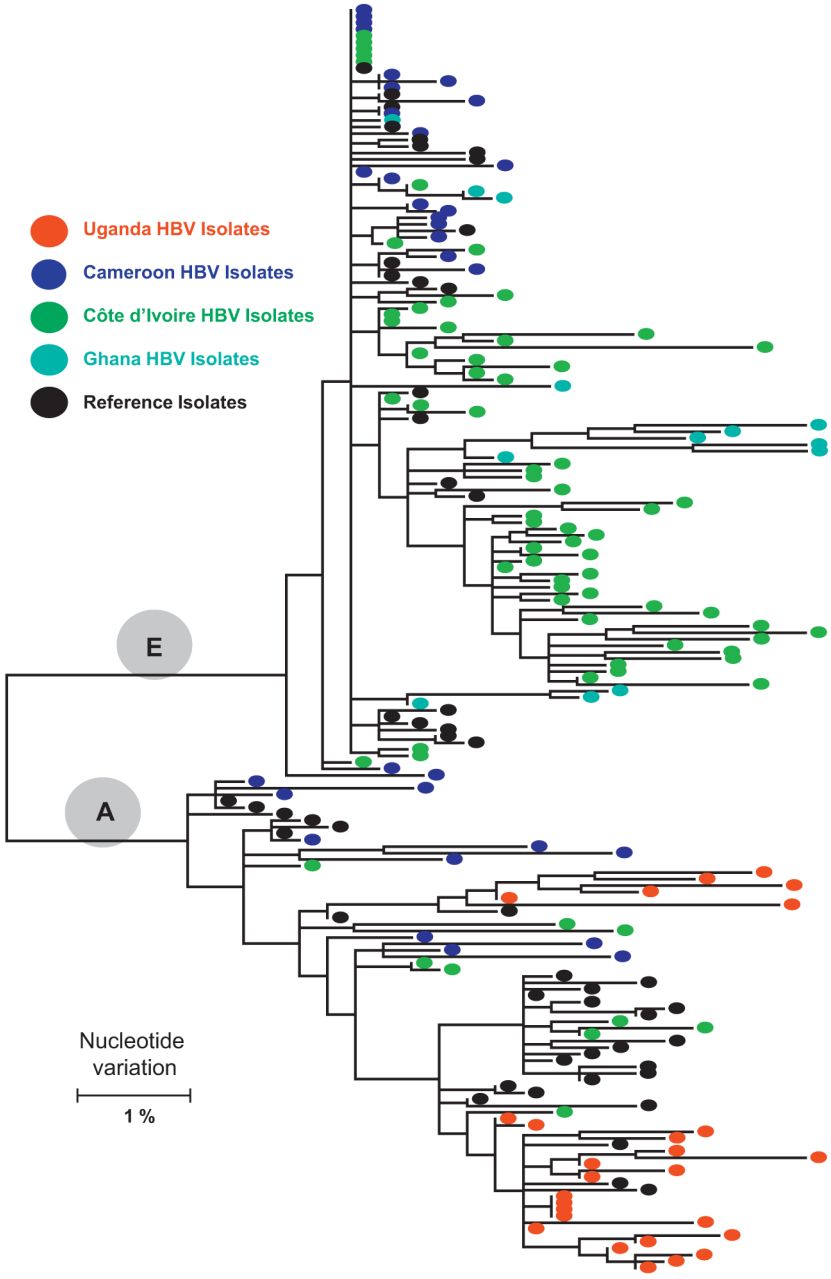

Fig. 1.

Maximum likelihood tree of HBV *S*-gene sequences from Uganda (red), Cameroon (blue), Côte d'Ivoire (green) and Ghana (cyan). Reference sequences are shown as black dots.

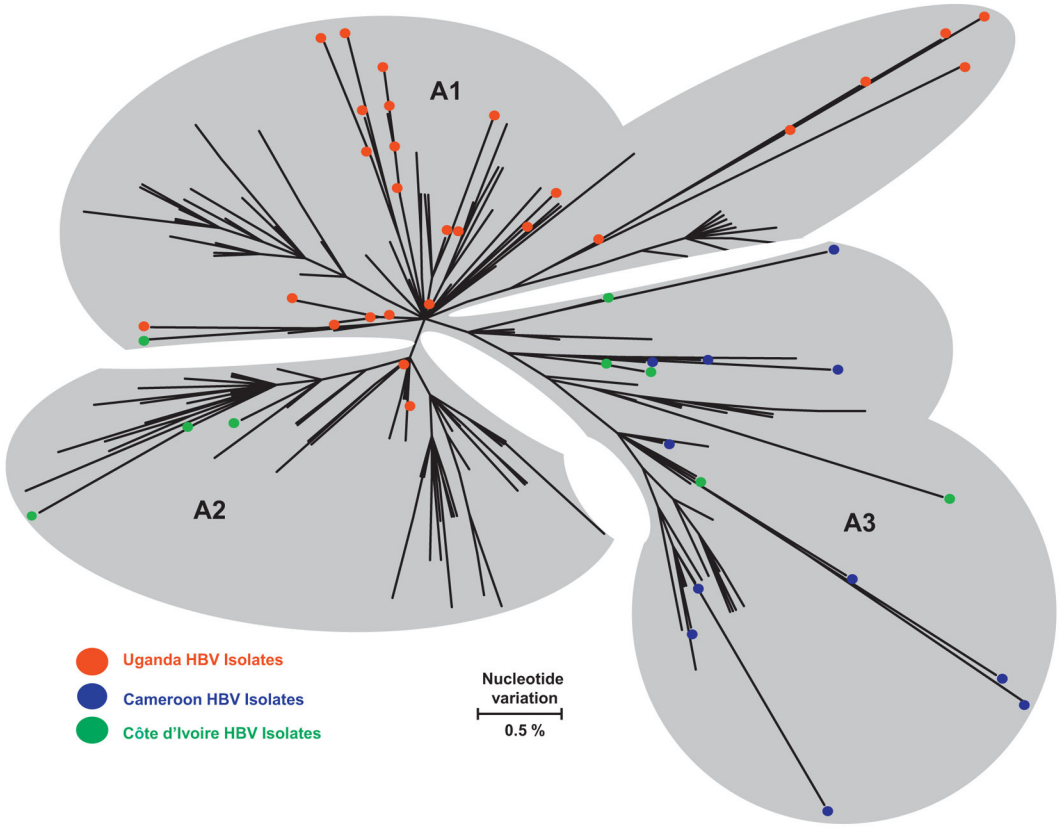

Fig. 2.

Maximum likelihood tree of the HBV/A *S*-gene sequences. HBV/A1, HBV/A2 and HBV/A3 are subgenotypes of HBV/A. Sequences from Uganda, Cameroon and Côte d'Ivoire are noted with red, blue or green dots, respectively. Reference sequences are shown as branches without dots.

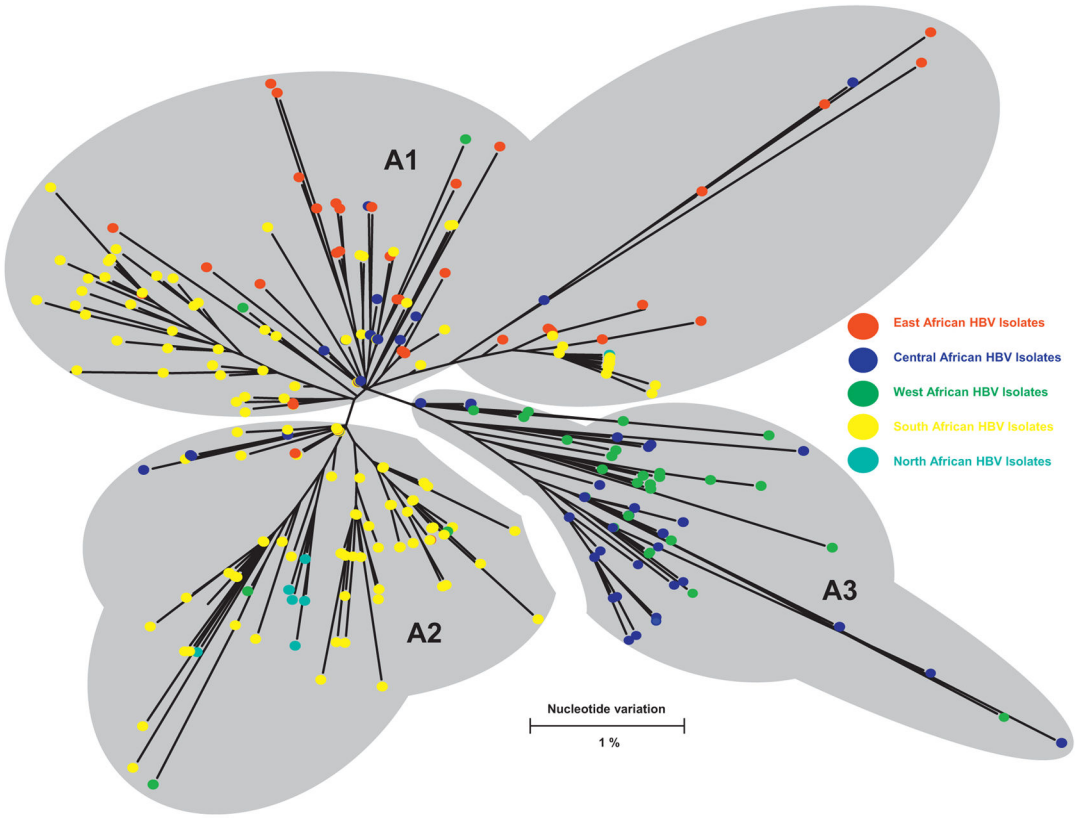

Fig. 3.

Maximum likelihood tree of the HBV/A *S*-gene sequences determined in this study and those available in GenBank from other African countries: East Africa (red), Central Africa (Blue), West Africa (green), South Africa (yellow) and North Africa (cyan).

Table 1

Detection rates of HBV DNA in four countries from sub-Saharan Africa.

Country	Location	Population	Date of collection	Number tested	Number positive (*S*-gene)	Prevalence (%)	Overall prevalence (%)
Côte d'Ivoire	West Africa	Pregnant women	1995	608	70	12	12
Ghana	West Africa	Pregnant women	1987	400	10	3	3
		HIV positive patients	1997	44	3	7	
Cameroon	Central Africa	Blood donors (HIV positive)	2002	203	23	11	11
		HIV positive patients	1997	100	10	10	
Uganda	East Africa	HIV positive patients	1997	137	27	20	20
Overall total				1492	143	10	10

Table 2

HBV serotype distribution in four countries in sub-Saharan Africa.

Location	Genotype	Total	Serotypes		
			ayw4	*ayw1*	*adw2*
Ivory Coast	E	61	54 (89%)	7 (19%)	0
	A	9	0	2 (22%)	7 (78%)
Ghana	E	13	13 (100%)	0	0
Cameroon	E	22	22(100%)	0	0
	A	11	0	6 (55%)	5 (46%)
Uganda	A	27	0	7 (26%)	20 (74%)

Table 3

Vaccine escape substitutions in HBsAg.

Location	Genotype	Isolate ID	Accession numbers	Mutations	Subgenotype	Serotype
Ivory Coast	E	CIV12	KC174562	D144E	–	*ayw1*
		CIV13	KC174563	D144E	–	*ayw4*
		CIV28	KC174578	D144E	–	*ayw4*
		CIV90	KC174628	A128G/G145R	–	*ayw1*
		CIV92	KC174626	T126M	–	*ayw4*
Cameroon	E	CMR20	KC174656	T126S	–	*ayw4*
	A	CMR167	KC174652	M133L	HBV/A3	*ayw1*
		CMR174	KC174655	G145R	HBV/A3	*adw2*
Uganda	A	UG2191	KC174677	D144E	HBV/A1	*ayw1*
		UG2214	KC174681	Q129R	HBV/A1	*adw2*
		UG2223	KC174684	D144A	HBV/A1	*adw2*

No mutations in the ‘a’ determinant were identified in Ghana.