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High prevalence of hepatitis B virus subgenotypes A1 and D4 in Maranhão state, Northeast Brazil

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ABSTRACT

In this study, we determined the prevalence of HBV subgenotypes in Maranhão state, located in northeastern Brazil, where the population is heterogeneous, with a high proportion of African descendants. HBV was detected in 119 of 133 (89.5%) chronic hepatitis B patients, including 103 (86.5%) who were HBeAg-negative. Using phylogenetic analysis of the S/Polymerase region of HBV DNA, subgenotype A1 was found to be the most prevalent (67%), followed by genotype D (28%; subgenotype D4 was detected in 24%, D3 in 3%, and D2 in 1%). Genotype F, clustering with subgenotype F2a, was found in six (5%) patients. The topology of the phylogenetic tree showed that HBV/A1 sequences did not cluster together, suggesting that more than one strain was introduced into Maranhão. On the other hand, HBV/D4 sequences formed a monophyletic cluster, suggesting a single entry of this strain in this population. This study showed that HBV/A1 was the only subgenotype of HBV/A present in the population from Maranhão and indicated that in this region HBV/A1 was not restricted to an Afro-descendant community where it was previously reported, but is widely distributed among general population of HBV chronic carriers.

Unexpectedly, we found a high frequency of HBV subgenotype D4. Together with previously reported data on the distribution of HBV/D4 in the world, these findings suggest that this subgenotype was more prevalent in the African continent in the past and may have been introduced in Maranhão by means of the slave trade during the late XVIII century, when the largest number of African slaves arrived to this region.

1. Introduction

Globally, the prevalence of chronic hepatitis B (CHB) varies considerably, with areas of high (>8%), intermediate (2–7%) and low (<2%) endemicity (WHO, 2002). A recent epidemiological study, carried out among a representative sample of the Brazilian population showed an HBsAg prevalence of less than 1% in Northeast and Central-West regions (Pereira et al., 2009). However, in other regions, including some areas of the Amazon, south and southwest regions, hepatitis B virus (HBV) infection is hyperendemic and poses a serious health threat (Bertolini et al., 2006; Braga et al., 2012; Souto, 1999).

Brazil is a very large country and in several areas the epidemiological picture of HBV infection is unknown, especially in smaller cities or rural communities, where large reservoirs of HBV infection may persist. Maranhão state is located in the northeastern region of Brazil, is divided in 217 counties (Fig. 1), and has a population of approximately 6,794,301 inhabitants (http://www.ibge.gov.br/estadosat/perfil.php?sigla=ma). Population from Maranhão is one of the most heterogeneous in the country, with a high proportion of African descendants. In the few epidemiological studies carried

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Fig. 1. Geographic location of Maranhão state in Brazil.

out to estimate HBV prevalence in this state, was observed that it was variable, depending on the studied group: a low HBV prevalence (0.5–0.9%) was found in two studies among the urban population from São Luís, the capital of Maranhão state (Pereira et al., 2009; Souza et al., 2012); whereas an intermediate prevalence (2.8%) was found in a rural area (Buriticupu) (El Khouri et al., 2010) and a surprisingly high prevalence (12.5%) reported in an African descendant population from another rural area (Frechal) (Alvarado-Mora et al., 2011).

HBV is currently classified into ten genotypes (A–J) based on a difference in genomic sequence of 7.5% or more and some of them have been further classified into subgenotypes (Kramvis et al., 2008; Norder et al., 2004). Most HBV genotypes and some of their subgenotypes have characteristic geographic distribution around the world and a strong correlation with ethnicity (Kramvis et al., 2008; Kurbanov et al., 2010; Norder et al., 2004). Moreover, genotypes/subgenotypes can affect the natural course of infection and response to antiviral treatment (Cooksley, 2010; Kramvis and Kew, 2005; Lin and Kao, 2011).

Knowledge of HBV genotypes/subgenotypes prevalence in a geographical region is also important to understand the routes of HBV dissemination and to establish efficient strategies to prevent and control HBV infection. Genotypes B and C have been related to vertical transmission while subgenotype A1 and genotypes D, E, F and H are more related to horizontal transmission during early childhood. HBV/A2 has been frequently involved in sexual transmission and is more prone to establish chronic disease in adults. Like HBV/A2, genotype G infection is associated with sexual

transmission as a risk factor for infection and is frequently found in men who have sex with men (Araujo et al., 2011).

Genotypes A, D and F are the HBV genotypes more prevalent in Brazil (Bertolini et al., 2012; Gomes-Gouvea et al., 2009; Mello et al., 2007; Moura et al., 2013; Santos et al., 2010; Sitnik et al., 2004; Victoria et al., 2008). Although studies in particular regions of the country have shown the predominance of HBV subgenotypes A1, D3 and F2a (Bertolini et al., 2012; Gomes-Gouvea et al., 2009; Mello et al., 2007; Moura et al., 2013; Santos et al., 2010; Victoria et al., 2008), this information remains poorly understood in most of Brazilian regions. Specifically in Maranhão state, there is just one study that characterized the distribution of HBV subgenotypes in an Afro-descendant community where only HBV/A1 was identified (Alvarado-Mora et al., 2011), however the extent of its distribution in this state is unknown.

The aim of this study was to characterize the HBV genotypes and subgenotypes in chronic hepatitis B carriers from different regions of Maranhão state and to relate this data with demographic and clinical features of the patients.

2. Patients and methods

2.1. Patients

The study population included 133 patients with chronic hepatitis B from Maranhão state, in the northeast of Brazil. These patients were followed up at the Center for Liver Studies of the University Hospital, Federal University of Maranhão (HUUFMA) at São Luís, the capital city of Maranhão state. The local ethics committee approved this study and written informed consent was obtained from all patients included.

Demographic, clinical and laboratory data of the patients were retrieved from their medical files. All patients were HBsAg positive, for at least 6 months prior to inclusion in the study, and tested negative for hepatitis C virus or human immunodeficiency virus antibodies. The serum samples were collected during January 2008 to February 2010.

Chronic hepatitis B status was defined according to the following criteria:

(1) Inactive carrier: positive HBsAg, negative HBeAg, positive anti-HBe, normal alanine aminotransferase (ALT) levels and HBV DNA below 2000 IU/mL;
(2) Chronic hepatitis B: positive HBsAg, positive HBeAg, and high ALT levels; or positive HBsAg, negative HBeAg, positive anti-HBe and HBV DNA higher than 2000 IU/mL;
(3) Liver cirrhosis: positive HBsAg with positive or negative HBeAg regardless of the viral load; signs or symptoms of advanced liver disease (ascites and/or presence of esophageal varices) or histological diagnosis of liver cirrhosis;
(4) Immune tolerance: positive HBsAg and HBeAg with normal ALT levels and HBV DNA above 10^7 IU/mL.

2.2. Serological assays

Serum samples were tested for hepatitis B markers (HBsAg, total anti-HBc, anti-HBs, HBeAg, anti-HBe) by enzyme-linked immunosorbent assay (ELISA) using commercial kits (DiaSorin, Italy).

2.3. HBV DNA amplification and quantification

HBV DNA was extracted from 200 μL serum using the QIAamp DNA Blood Mini kit (Qiagen). Viral load was quantified by real-time PCR assay as described previously (Sitnik et al., 2010). Absolute quantification of HBV DNA was performed with an ABI PRISM 7300 (Life Technologies) real-time analyzer using universal cycling conditions. The limit of detection of this assay was established in 50 IU/mL, but it can detect samples with viral load below this value (Sitnik et al., 2010).

For genotype identification, a fragment of 1306 base pairs (bp), comprising the whole HBsAg and part of the Polymerase coding genes, was amplified by nested PCR. For this, we used the enzyme Platinum Taq DNA polymerase (Invitrogen) and the primers PS3132F (5′ CCT CCY GCH TCY ACC AAT CG 3′; nt 3132–3151 from the *Eco*R1 site) and 2920RM (5′ ACG TCC CKC GHA GRA TCC AG 3′; nt 1417–1398) for the first-round PCR, and PS3201F (5′ CAY CCH CAG GCM ATG CAG TGG 3′; nt 3201–3221) and P1285R (5′ CWA GGA GTT CCG CAG TAT GG 3′; nt 1285–1266) for the second round. PCR was carried out for 35 cycles of 94 °C for 30 s, 48 °C (first round) or 54 °C (second round) for 30 s, and 72 °C for 1 min and 40 s.

PCR products from the second-round reaction (nested PCR) were purified using Charge Switch® PCR Clean-Up Kit (Invitrogen) and were then sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Three inner and partially overlapping sequences were obtained in sense and antisense directions using three sets of primers: 1 – PS3201F and HBV477R (5′ GGA CAV ACG GGC AAC ATA CCT T 3′; nt 477–456); 2 – L372 (5′ – TCG YTG GAT GTR TCT GCG GCG TTT TAT – 3′; nt 370–396) and RADE2M (5′ – TGR CAN ACY TTC CAR TCA ATN GG – 3′; nt 989–970); 3 – P781F (5′ GAR TCC CTT TWT RCC KCT RTT ACC 3′; nt 781–804), and P1285R. Sequencing was carried out on an automated ABI 3500 DNA Sequencer (Applied Biosystems).

The quality of each electropherogram was evaluated using the Phred–Phrap software (Ewing and Green, 1998; Ewing et al., 1998) and consensus sequences were obtained from the alignment of these six sequences, for each single patient, using CAP3 software available at the web page *Eletropherogram quality analysis Phred* (http://asparagin.cenargen.embrapa.br/phph/).

2.4. HBV genotyping and subgenotyping

Sequences were aligned and edited using BioEdit (v. 7.0.8) and the integrated CLUSTAL W program. HBV genotypes and subgenotypes were classified by phylogenetic reconstructions using published reference sequences from the GenBank database (http://www.ncbi.nlm.nih.gov/).

Bayesian phylogenetic analyses were conducted using the Markov Chain Monte Carlo (MCMC) simulation implemented in BEAST v.1.6.1 (Drummond and Rambaut, 2007) under uncorrelated lognormal relaxed molecular clock using the model of nucleotide substitution (GTR + G + I). The MCMC chains were run for 10 million generations, and sampled every 1000 steps. The maximum clade credibility (MCC) tree was obtained from summarizing the 10,000 substitution trees and then 10% of burn-in was removed using Tree Annotator v.1.6.1 (Drummond and Rambaut, 2007).

HBV serotypes were deduced base on the presence of amino acids at positions 122, 127, 134 and 160 of S protein coding by S gene sequence: *adw*2 (Lys^{122}, Pro^{127} and Lys^{160}); *ayw*2 (Arg^{122}, Pro^{127}, Tyr^{134} and Lys^{160}); *ayw*3 (Arg^{122}, Thr^{127}, Tyr^{134} and Lys^{160}); *adw*4 (Lys^{122}, Leu^{127} and Lys^{160}) (Norder et al., 2004).

2.5. Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession numbers for the sequences described in this study are JF298901, JF298903, JN983831–JN983947 for S/POL sequences and KJ470884–KJ470898 for complete genome sequences.

2.6. Statistical analysis

Statistical analyses to compare subgenotypes and different variables were performed. HBV/F2a, D2 and D3 subgenotypes were excluded from the statistical analysis because there were too few cases. Qualitative variables (gender and chronic HBV infection stage) were compared using Chi square test, *t*-test and Mann–Whitney were used to determine for significant differences in age and viral load values between subgenotype A1 and D4 groups, respectively. Differences were considered significant for *p* values less than 0.05. The statistical analysis software used was SPSS software, version 17.0.

3. Results

3.1. Clinical and demographic characteristics of HBV DNA positive patients

Out of the 133 patients included in this study, 119 (89%) were HBV DNA positive: 53% were male with mean age 40.5 (SD = 13) years. Considering the geographical origin inside Maranhão state, most of them (76%; 90/119) were from São Luís metropolitan area, which includes four cities: São Luís (capital of the state), São José de Ribamar, Raposa, and Paço do Lumiar. The remaining patients (24%; 28/119) were from 24 different small cities located in other areas of the state.

HBeAg-negative patients were predominant (86.5%; 103/119) and most of these (85%; 88/103) had viral loads varying from <2000 to 20,000 IU/mL (mean 2, 565 IU/mL; SD 3857). On the other hand, higher viral loads (>20,000 IU/mL) were predominantly found in HBeAg-positive patients. The majority of the patients were classified as inactive carriers (46%; 55/119) or as chronic hepatitis (33%; 39/119).

3.2. HBV genotype and subgenotype distribution

Genotype A was the most prevalent (67%; 80/119), followed by genotypes D (28%; 33/119) and F (5%; 6/119). The phylogeny of genotype A sequences showed that all strains belonged to subgenotype A1, but grouped in different clusters of the HBV/A1 branch. HBV/A1 sequences from Maranhão mainly grouped together with sequences isolated from other regions of Brazil, but only some clusters were well supported (Fig. 2). All HBV/A1 strains were classified as serotype *adw*2.

HBV/D4 was the most frequent subgenotype of genotype D from Maranhão (24%; 29/119). Subgenotypes D2 (*ayw*2) and D3 (*ayw*2/*ayw*3) were found in a few cases with a frequency of 0.8% (1/119) and 2.4% (3/119), respectively (Fig. 3a and Supplementary Fig. S1). The HBV/D4 (*ayw*2) sequences isolated in Maranhão grouped in a monophyletic cluster with strong support (a posterior probability = 0.93) and clustered closer to HBV/D4 strains previously isolated in other Brazilian regions, but more distantly to other HBV/D4 strains from other countries. To confirm the subgenotype classification of the D4 isolates from Maranhão state, the complete genomes of fifteen isolates were sequenced and the phylogenetic analysis of the complete genomes showed similar grouping to that of S/POL genes (Fig. 3b). Moreover, the genetic diversity based on complete genome was less than 4.0% compared to previously characterized HBV/D4, confirming their subgenotype classification as D4.

HBV/F2a (adw4) was the unique subgenotype of genotype F found in Maranhão state. Most sequences clustered together with other F2a sequences previously isolated from two other Brazilian regions: Rondônia and Amazonas states (Supplementary Fig. S1).

3.3. HBV subgenotypes and demographical, clinical and laboratory characteristics of the patients

Table 1 shows HBV subgenotype distribution of 119 patients according gender, age, race (based in skin color), geographical origin, HBeAg profile, clinical status and HBV DNA levels. Overall, subgenotypes A1 and D4 comprised 92% of study population and a statistical comparison of demographical (gender and age), clinical and laboratorial data of these patients was performed. Patients infected with HBV/D4 presented a higher frequency of HBeAg-positive status than those infected with HBV/A1 [8/29 (28%) vs. 8/80 (10%), $p = 0.02$] and were more frequently found in the immune tolerance stage of chronic HBV infection [7/29 (24%) vs. 3/80 (4%), $p = 0.003$]. There was no significant difference in gender, age and viral load median.

4. Discussion

This study determined the distribution of HBV genotypes and subgenotypes in Maranhão state. This state is the fourth most populous state of the Northeast region and its population is one of the most heterogeneous in Brazil, with a high proportion of African descendants, who live in isolated or semi-isolated rural communities.

The HBV genotype distribution in Maranhão state was similar to that reported in other Brazilian regions, showing a higher prevalence of genotype A, followed by genotypes D and F (Bertolini et al., 2012; Gomes-Gouvea et al., 2009; Mello et al., 2007; Moura et al., 2013; Santos et al., 2010; Sitnik et al., 2004; Victoria et al., 2008). However, molecular characterization at the subgenotype level revealed a relatively high prevalence of subgenotypes A1 and D4. It is noteworthy, that the population analyzed in this study was representative of HBV carriers from whole Maranhão state because the patients were recruited from a liver disease referral center that monitors the entire state.

To date only two subgenotypes of genotype A (A1 and A2) have been found in Brazil and in Brazilian regions, where data is available, HBV/A1 has been the single or most prevalent subgenotype of genotype A (Bertolini et al., 2012; Gomes-Gouvea et al., 2009; Mello et al., 2007; Moura et al., 2013; Santos et al., 2010; Victoria et al., 2008). The present study showed that under the period of study only HBV/A1 was circulating in Maranhão state and was the most prevalent (67%) subgenotype in this state. This result indicates that in Maranhão HBV/A1 was not restricted to an Afro-descendant community, where it was previously reported (Alvarado-Mora et al., 2011), but was widely distributed among the general population of HBV chronic carriers.

Phylogeographic studies of HBV sequences from different countries suggest that genotypes/subgenotypes can be used to trace human migrations (Kramvis and Paraskevis, 2013). Because of the presence of HBV/A1 in some regions of Africa and its distribution among Brazilian communities that are formed mostly by descendants of African slaves (Quilombos), it has been postulated that HBV/A1 was introduced in Brazil by African slaves, who arrived in Brazil between the 16th and 19th centuries (Alvarado-Mora et al., 2011; Araujo et al., 2004; Motta-Castro et al., 2008).

Brazil is a country with continental dimensions and different regions have diverse population histories, cultural habits and environmental characteristics. These features can influence the HBV genotype/subgenotype distribution and/or the spread of a specific HBV strain. Therefore it is important not to only know the genotype/subgenotype distribution in each region but also to study the phylogenetic relationship among the isolates identified.

The clustering pattern of HBV/A1 sequences from Maranhão state suggests different entries of this subgenotype into the state.

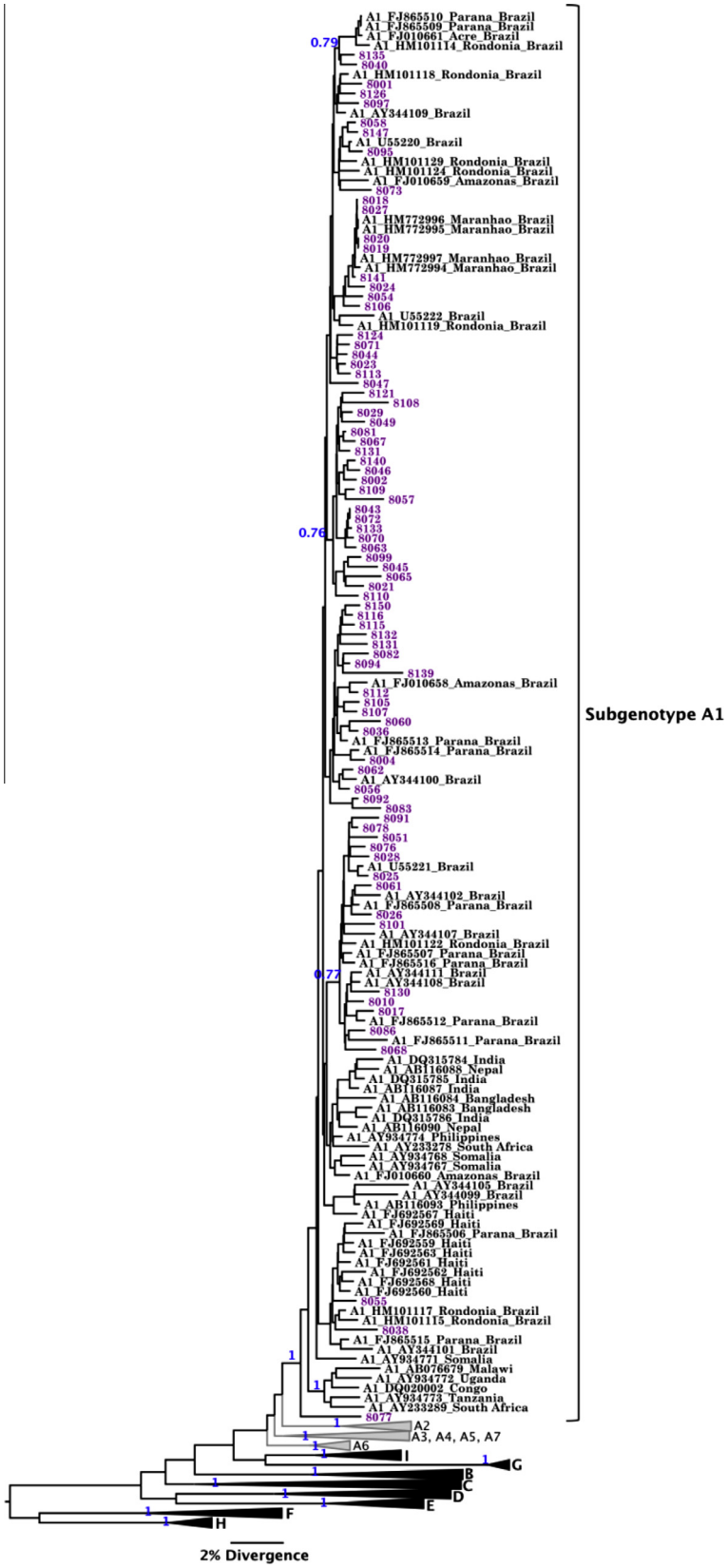

Fig. 2. Maximum clade credibility (MCC) tree estimated by Bayesian analysis of HBV sequences that partially comprise HBsAg and DNA polymerase coding regions (S/POL). HBV/A1 sequences characterized in this study are highlighted in red and sequences obtained from GenBank are identified by their accession number followed by geographic origin. The collapsed clades correspond to sequences of the other HBV genotypes (B, C, D, E, F, G, H) and subgenotypes of genotype A (A2–A7). Posterior probability values are shown above their respective key nodes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

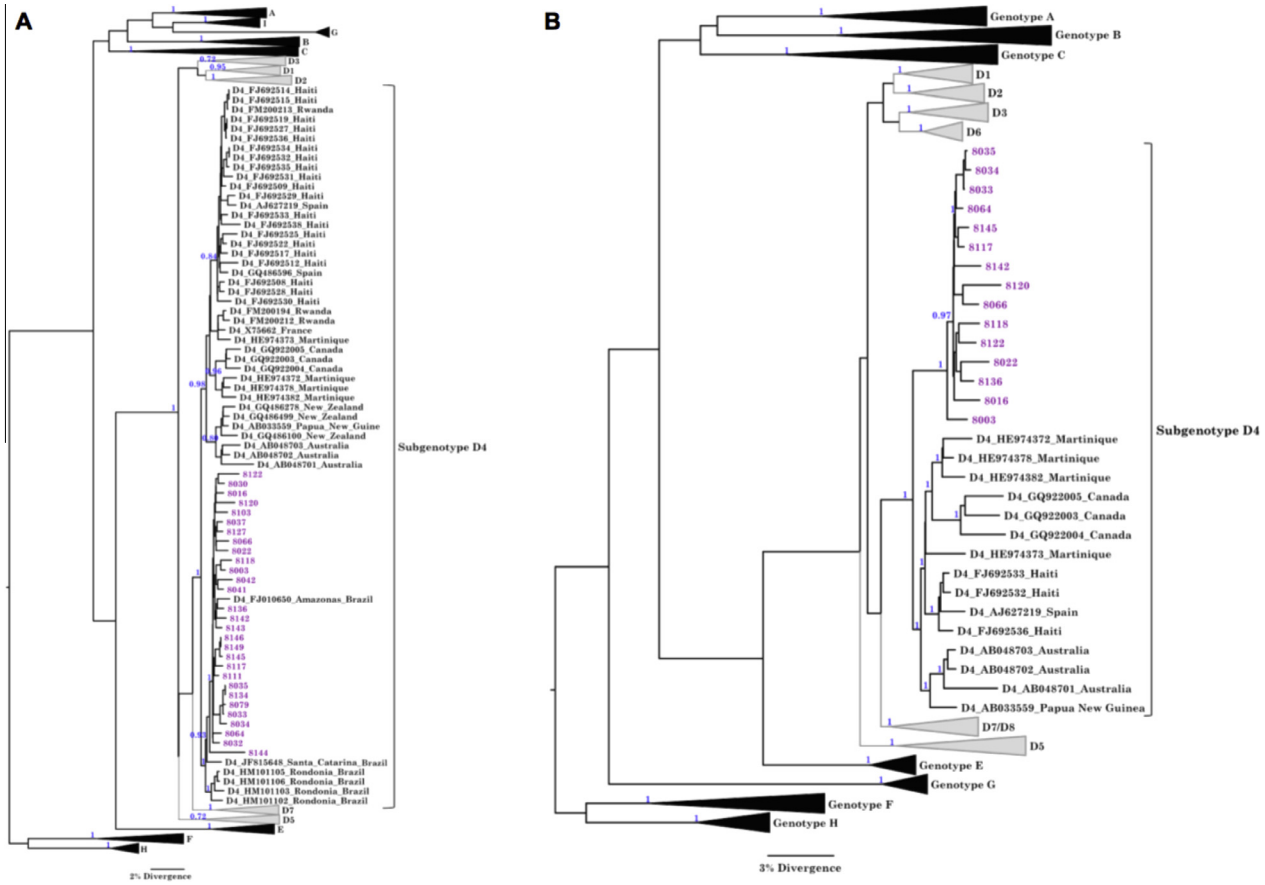

Fig. 3. Maximum clade credibility (MCC) tree estimated by Bayesian analysis of (A) HBV sequences that partially comprise HBsAg/DNA polymerase coding regions (S/POL) and (B) complete genome sequences. HBV/D4 sequences characterized in this study are highlighted in red and sequences obtained from GenBank are identified by their accession number followed by geographic origin. The collapsed clades correspond to sequences of the other HBV genotypes (A, B, C, E, F, G, H) and the subgenotypes of genotype D (D1, D2, D3, D5, D6, D7/D8). Posterior probability values are shown above their respective key nodes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characteristics and HBV genotypes/subgenotypes of 119 chronic HBsAg carriers from Maranhão state.

Variables	HBV Subgenotypes					
	A1	D2	D3	D4	F2a	Total
N (%)	80 (67%)	1 (1%)	3 (3%)	29 (24%)	6 (5%)	119
Male Gender	42 (53%)	0	2 (67%)	10 (34%)	3 (50%)	57 (52%)
Mean age (years)	41.8	49	38	37.6	38.3	40.6
Race[*]						
Pardo	50 (63%)	0	2 (67%)	22 (76%)	4 (67%)	78 (66%)
Black	18 (23%)	0	0	4 (14%)	1 (17%)	23 (19%)
White	12 (15%)	1 (100%)	1 (33%)	3 (10%)	1 (17%)	18 (15%)
Origin						
Metropolitan area of São Luís[**]	63 (79%)	0	3 (100%)	19 (66%)	6 (100%)	91 (76%)
Other cities	17 (21%)	1 (100%)	0	10 (34%)	0	28 (24%)
HBeAg						
Positive	8 (10%)	0	0	8 (28%)	0	16 (13%)
Negative	72 (90%)	1 (100%)	3 (100%)	21 (72%)	6 (100%)	103 (87%)
Diagnosis						
Inactive carrier	37 (46%)	1 (100%)	2 (67%)	12 (41%)	3 (50%)	55 (46%)
Chronic hepatitis	29 (36%)	0	1 (33%)	6 (21%)	3 (50%)	39 (33%)
Cirrhosis	11 (14%)	0	0	4 (14%)	0	15 (13%)
Immune tolerance	3 (4%)	0	0	7 (24%)	0	10 (8%)
HBV DNA levels						
<2000 IU/mL	41 (51%)	1 (100%)	2 (67%)	13 (45%)	3 (50%)	60 (50%)
2000–20,000 IU/mL	24 (30%)	0		4 (14%)	2 (33%)	30 (25%)
>20,000 IU/mL	7 (9%)	0	1 (33%)	5 (17%)	1 (17%)	14(12%)
>10,000.000 IU/mL	8 (10%)	0	0	7 (24%)	0	15 (13%)

[*] Based on the skin color.
[**] São Luís, São José de Ribamar, Raposa and Paço do Lumiar.

The phylogenetic analysis shows that HBV/A1 sequences isolated from other regions of Brazil also have this characteristic. This pattern may be a result of the introduction of different strains by slaves originating from diverse African regions or may be as a result of internal migration of infected slaves in Brazil (Klein, 1986). On the other hand, a recent study shows that HBV/A1 sequences, isolated in Pernambuco, another state in Northeastern Brazil, have a different phylogenetic grouping pattern, indicating that most of HBV/A1, introduced in this region, came from a common strain (Moura et al., 2013).

HBV genotype D, the second most common (28%) genotype found in Maranhão, is highly prevalent in southern Brazil and in some southeastern areas (Bertolini et al., 2012; Mello et al., 2007; Sitnik et al., 2004). This genotype has been observed throughout the world, but it predominates in northeastern Europe, the Mediterranean basin, northern Africa, and the Middle East (Schaefer, 2007; Yousif and Kramvis, 2013). The presence of genotype D in Brazil, especially in southern and in southeastern regions has been mainly attributed to the European migration that occurred during the years 1872–1975 (Bertolini et al., 2012; Mello et al., 2007).

In Maranhão state, three subgenotypes of genotype D were found, D2, D3 and D4. Unexpectedly subgenotype D4 was found in 88% of the cases harboring genotype D, whereas D1 and D2 were found in 3% and 9%, respectively. Subgenotype D4 has previously not been found frequently in Brazil (Mello et al., 2007). Subgenotype D4 has been identified in only a few cases in the Amazonas (Gomes-Gouvea et al., 2009) and Rondônia (Santos et al., 2010), states in the Amazon region of Brazil.

The occurrence of the few cases of HBV/D2 and D3 in Maranhão may be attributed to the internal migration of carriers from other Brazilian regions, where these subgenotypes are prevalent, as occurred in some regions of the Central West and North of the country (Mello et al., 2007; Santos et al., 2010; Souto, 2004). On the other hand, the presence of HBV/D4 in Maranhão is not as a result of European immigration because this subgenotype has not been found in Europe (Schaefer, 2007). Subgenotype D4 is not very common, with a few sequences identified from patients in Australia, Papua New Guinea (Norder et al., 2004), Rwanda (Hubschen et al., 2009) and Canada (Osiowy et al., 2011). In Haiti, HBV/D4 was found as the most frequent subgenotype of genotype D, corresponding to 80% (29/36) of samples classified as HBV/D (36/179) (Andernach et al., 2009) and to 57% (4/7) in another study (Brichler et al., 2013). The presence of HBV/D4 in two different regions in Latin America might reflect its dispersal as a result of the slave trade routes from Africa to Haiti and Brazil (Silva, 2008). The possible African origin of HBV/D4 circulating in Maranhão is supported by the fact that we found an African genotype of hepatitis delta virus (HDV-8) co-infecting two of the patients who were HBV/D4 carriers (Barros et al., 2011). Although currently HBV/D4 has not been reported frequently in Africa, having been described only in Rwanda (Hubschen et al., 2009), it is possible that this subgenotype was distributed in different regions of the African continent in the past. A recombinant of subgenotype D4 has been identified in the Sudan (Yousif et al., 2013). It is possible that D4 originated in Africa but has subsequently been replaced by other subgenotypes of D and the recombinant is a remnant of the original strain/s (Yousif et al., 2013). The fact that HBV/D4 is found in aboriginal populations in Papua New Guinea and Australia (Norder et al., 2004) and in the Canadian Inuit population (Osiowy et al., 2011) suggests that this is a an early subgenotype dating from the time of the early human intercontinental migrations (Hudjashov et al., 2007). The HBV/D4 sequences from Maranhão grouped in a specific monophyletic cluster (with high posterior probability), indicating that a single virus entered this population, establishing a founder effect. It is possible that subgenotype D4 has a wider global distribution and more research is required, especially in Africa, the Americas and Australasia. Moreover, the effect of subgenotype D4 on clinical manifestation has not been researched. In this study we observed that among HBV/D4 carriers the frequency of immune tolerant status was higher than among HBV/A1 carriers. Since there is not a statistically significant difference between the mean age of HBV/A1 and D4 carriers (42 ± 12 vs. 38 ± 13, $p = 0.13$), HBeAg seroconversion to anti-HBe was a late event in cases of HBV/D4 infection and we may infer that perinatal transmission of the virus occurred in these cases. Studies with larger numbers of individuals infected with this subgenotype are required to confirm this observation.

As expected, the frequency of genotype F in Maranhão was low, corresponding to 5% of the cases. Accordingly, the prevalence of this genotype is low in most of the Brazilian regions and increases in the western Amazon region (Bertolini et al., 2012; Gomes-Gouvea et al., 2009; Mello et al., 2007; Santos et al., 2010; Sitnik et al., 2004; Victoria et al., 2008). Genotype F is considered indigenous to America because it has been found among Amerindians, mainly in South America, and is a most prevalent genotype in some countries of this region (Alvarado Mora et al., 2011; Devesa et al., 2008; Mello et al., 2007; Nakano et al., 2001; Pezzano et al., 2011; Venegas et al., 2008). The low prevalence of HBV genotype F in most of regions of Brazil may be related to a lower contribution of Amerindians to the formation of the Brazilian population, as demonstrated by genomic ancestry studies (Pena et al., 2011).

5. Conclusions

This study shows the distribution of HBV genotypes and subgenotypes in Maranhão state. In this region, the prevalence of HBV genotypes is similar to those reported in other Brazilian regions, where this was studied, with a predominance of genotypes A and D, and a lower prevalence of genotype F. However, the subgenotype distribution shows some specific characteristics, with a high frequency of HBV subgenotype D4 that is uncommon in other regions of Brazil. The great genetic variability of HBV strains circulating in Brazil needs to be taken into account when national guidelines, on how to treat patients with chronic hepatitis B, are formulated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.03.007.

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