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Hepatitis B immune escape and drug resistance mutations among blood donors in Gabon during the year 2022

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Hepatitis B virus (HBV) and specifically occult HBV infection (OBI) remains concerning in transfusion medicine in many countries. This study aimed to determine the burden of overt and occult HBV infections and characterize the circulating viral genotypes among blood donors in Gabon. A facility-based study was conducted among blood donors at the Gabon National Blood Transfusion Centre in 2022. Screening for HBsAg and anti-HBc was done using ELISA; characterization of OBI (defined serologically as HBsAg- but anti-HBc+) was done using HBV DNA viral load (VL); and HBV Pol/S sanger-sequencing was performed for the analysis of immune-escape and drug resistance mutations. Data were analyzed with $p < 0.05$ considered significant. Overall, 283 participants were enrolled: 218 (77.0%) males, median age 31 (26–35) years and 264 (93.3%) frequent donors. HBV seropositivity revealed 25.1% (71/283) overt infection (i.e. HBsAg + and anti-HBc+) and 5.7% (16/283) OBI (HBsAg- but anti-HBc+). All OBI cases (16/16) had HBV DNA VL < 10 IU/ml versus 37.5% (6/16) VL < 10 IU/ml, 25% (4/16) VL between]10–500] IU/ml, and 37.5% (6/16) VL > 500 IU/ml among controls (overt infections).

Genotyping was successful for 15.6% (5/32) participants with VL > 500 IU/ml ($p = 0.02$). Following genotyping, 80% (4/5) participants (VL > 2000 IU/ml) had at least one immune escape mutation (sT131N, sR122K, sG145A) and rtI169L mutation associated with drug resistance was detected in one donor. Molecular phylogeny revealed genotypes A (80%; 4/5) and E (20%; 1/5). HBV seropositivity was 25.1%, markedly higher than the estimated prevalence in the general population and therefore of significant concern. Likewise, the observed OBI prevalence of 5.7% represents a substantial risk to transfusion safety.

Keywords HBV, Occult HBV infection, HBV genotype, Transfusion risks, Blood donors, Gabon

Abbreviations

Anti-HBc	Hepatitis B Core Antibodies
CIRCB	Chantal BIYA International Reference Centre for research on
HIV/AIDS	Prevention and management
HBV-DNA	Hepatitis B viral DNA
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
NBTC	National Blood Transfusion Centre
NCER	National Commitee of Ethics for Research
OBI	Occult B Infection
PCR	Polymers Chain Reaction

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Hepatitis B virus (HBV) infection is a major public health threat in the world. Indeed, nearly 350 million people worldwide are exposed or infected, and a great majority is also carrier of chronic HBV[1]. HBV is a leading cause of chronic liver disease, cirrhosis, liver failure and hepatocellular carcinoma, with nearly 1.4 million deaths each year[2]. The global spread of HBV is disproportionate, with sub-Saharan Africa (SSA) contributing to more than 70% of new infections globally. In Gabon, 7% of the population is infected and about 160,000 people have been in contact with HBV, with the virus being responsible for 405 deaths per year in the country[3]. Recently, HBV has been phylogenetically classified into nine genotypes (A–I) and one putative genotype (J)[4]. The HBV genotypes and sub-genotypes exhibit distinct geographical distributions, reflecting differences in disease transmission, natural history, and response to therapy[4]. Occult HBV infection (OBI) on the other hand is usually defined as the presence of HBV DNA in the serum and/or liver, with surface antigen (HBsAg) seronegativity[5]. Of note, OBI can be grouped into two types: (i) seropositive OBI is defined when there is the presence of HBV DNA with antibodies against the core antigen (anti-HBc) and/or antibodies against the surface antigen (anti-HBs); (ii) seronegative OBI, defined as the presence of HBV DNA without anti-HBc and anti-HBs[6]. However, within the frame of blood transfusion in limited resource settings, even though overt infection is diagnosed on the basis of HBsAg seropositivity (i.e. HBsAg+), OBI is most often identified among donors with HBsAg- but anti-HBc + given the limited resources available at health facilities, especially in remote areas[6,7].

Furthermore, in SSA as in many other countries worldwide, transfusion therapy is an integral part of the management of various diseases[8], and must be executed at a low frequency both for volunteers and family donors, especially considering the high risk of HBV transmission during the transfusion[7]. In effect, nucleic acid assays are usually not employed within screening algorithms before transfusion and the risk is usually estimated at 4300 individuals newly infected per million donations (predominantly volunteers and family donors)[9]. In Gabon specifically, only 21% of donations are deemed safe after all blood safety measures have been performed[9,10]. Of note, conditions for blood ineligibility usually include: (i) unprotected sex with new partners few days before blood donation; (ii) heart disease conditions; (iii) high blood pressure; (iv) acute malaria; (v) recent surgery; (vi) pregnancy; (vii) dizziness and intense fatigue (etc.…) The main threat in the safety of blood-

supply is related to pre-seroconversion phase, which is defined as one of the following scenarios: (i) HBsAg carriers with undetectable HBV DNA viral load; (ii) the presence of HBV DNA among seronegative HBsAg individuals or OBI; (iii) the co-circulation of different HBV genotypes and sub-genotypes (QS-A3, D and/or E); and (iv) the non-/low expression of the one of the four HBV genes, namely pre-S/S (surface proteins), pre-C/C (capsid proteins or HBc), X (transcriptional co-activator) and P (DNA polymerase) which would significantly undermine the diagnosis[11]. Also, circulating genotypes of HBV may habour genotypic mutations which can negatively impact the diagnosis through routine assays and thus lead to an increased risk of HBV transmission[11]. Importantly, as mentioned above, suspected cases of OBI among blood donors in many SSA countries are usually defined on the basis of serology as the presence of anti-HBc among seronegative HBsAg individuals[7]. To the best of our knowledge, only few African countries have described the distribution of genotypes in acute HBV infection and its relationship to patterns of disease transmission in SSA so far[12–15]. There is currently no clear description of the incidence of OBI among blood donors in Gabon which is highly endemic for HBV infections in Africa.

The objective of this study was therefore to determine the prevalence of overt and occult HBV infection among blood donors in Gabon and the distribution of HBV genotypes and sub-genotypes.

Results

Figure 1 below illustrates all the candidates for blood donation during the study period and the sample eventually recruited into the study.

Socio-demographic characteristics of study participants

Among the eligible 283 donors in the study (see clarifications on Fig. 1), 219 (77%) were males and 64 (23%) were females. Majority of donors were aged[16–25] years and 87.6% (248/283) were not family donors.

Serological results

Following serology, no donor was found positive to HIV and HCV. As concerns HBV, we found 30.7% anti-HBc+. Specifically, 25.1% (71/283) were HBsAg + and anti-HBc+ (i.e. overt infection) whereas 5.6% (16/283) were classified as OBI (i.e. HBsAg- and anti-HBc+). Table 1 summarizes the distribution of HBV markers according to socio-demographic features.

Characterization of overt and occult HBV infection among blood donors

Interestingly, HBV DNA was detectable < 10 IU/ml for all (16/16) cases of OBI whereas we found 37.5% (6/16) with VL < 10 IU/ml, 25% (4/16) with VL between [10–500 IU/ml] and 37.5% (6/16) with VL > 500 IU/ml among overt HBV cases (the control group; see Table 2). Most OBI cases were male (10/16), with an average age of 28.4 years and all were family donors (Table 2).

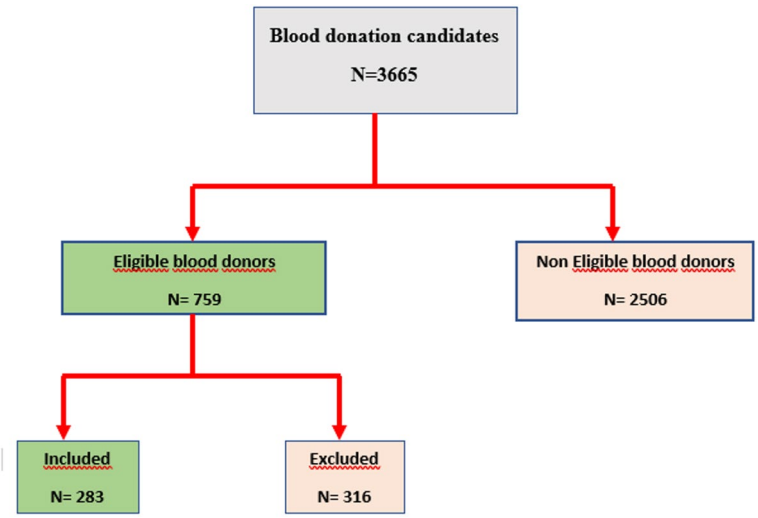

Fig. 1. Flow-chart of samples from the study population. Legend: Briefly, from the 3665 candidates for blood donation that attended the Gabonese NBTC during the study period 2,506 were not eligible. Conditions for ineligibility included: (i) non-respect of the conditions for blood donation (1478/2506); (ii) denial to participate in the study (853/2506); (iii) some others desisted from giving their blood at the end (175/2506). From the 759 that were eligible for blood donation, only 283 were included in the study. Principal reasons for exclusion where: (i)incomplete data on the donor (88/316); insufficient plasma volume for lab analyses or discomfort during phlebotomy (143/316); and invalid results without the possibility of resampling (85/316).

Characteristics	HBsAg+/anti-HBc+	HBsAg-/anti-HBc+	HBsAg-/anti-HBc-	HBsAg+/anti-HBc-
N donors	71 (25.1%)	16 (5.7%)	196 (69.3%)	00 (0.0%)
Age (Years)				
20–29	35 (49.3%)	11 (68.8%)	86 (43.9%)	/
30–39	22 (31%)	3 (18.8%)	80 (40.8%)	/
40–49	14 (19.7%)	2 (12.5%)	30 (15.3%)	/
Gender				
Female	11 (15.5%)	6 (37.5%)	47 (24%)	/
Male	60 (84.5%)	10 (62.5%)	149 (76%)	/
Donor type				
Volunteer	56 (78.9%)	0 (0%)	192 (98%)	/
Family	15 (21.1%)	16 (100%)	4 (2%)	/

Table 1. Distribution of HBV markers according to socio-demographic characteristics.

Characteristics	OBI	Control group (i.e. overt HBV)
No. of donors	16	16
Mean age (years)	28.4 ± 2.7	27.7 ± 3.1
Male gender, n (%)	10 (62.5%)	11 (68.8%)
Family donors, n (%)	16 (100%)	13 (81.3%)
HBV titer (IU/mL), n (%)		
Detectable but low (i.e. VL < 10)	16 (100%)	6 (37.5%)
10 ≤ viral load < 200	0 (%)	3 (18.8%)
200 ≤ viral load < 500	0 (%)	1 (6.3%)
500 ≤ viral load < 1000	0 (%)	2 (12.5%)
1000 ≤ viral load < 2000	0 (%)	1 (6.3%)
Viral load ≥ 2000	0 (%)	3 (18.8%)

Table 2. HBV DNA viral load among occult and overt infection ($N = 32$). NB : The HBeAg status of patients with overt infection was unknown.

HBV genotyping and phylogenetic analysis

From the 32 samples collected for HBV DNA viral load, only 10 were found with HBV DNA titers > 10 IU/mL and were thus processed for genotyping; of which 50% (5/10) were successfully genotyped (yielding an overall success rate of 15.6%; 5/32). Notably, all successful genotyping was found in the control group with VL > 500 IU/ml ($p = 0.02$) (Table 3). Phylogenetic analysis of the partial RT and S gene sequences from the five samples successfully processed revealed three main genotypes: A1 (2/5), A3 (2/5) and E (1/5) as shown in Fig. 2. Sequencing data showed that none of them was identical to the known sequences generated at CIRCB and reference sequences retrieved from GenBank.

Drug resistance and immune escape profiles interpretation

Following genotyping, 80% (4/5) participants (100% with VL > 2000UI/ml) had at least one vaccine or immune escape mutation (sT131N, sR122K, sG145A) as presented in Table 3. Only one patient was found with a drug resistance mutation (rtI169L; leading to resistance to entecavir).

Discussion

To the best of our knowledge, there is currently no clear description of the incidence of overt and occult HBV infection among blood donors in Gabon which is known as a highly endemic region for HBV in Africa[3,26,27]. Also, studies on the effect of HBV genetic diversity in blood transfusion and resource-limited settings are scarce worldwide[28]. The objective of this study was to determine the burden of overt and occult HBV infections and characterize the circulating viral genotypes among blood donors in Gabon.

The study included both male and female participants. Male participants were almost three times more than the females. In low-and middle-income countries, men are generally most likely to donate blood[29]. Women were less represented because of certain contraindications to blood donation, such as breastfeeding and menstruation. In 15 of the 113 countries worldwide reporting blood donations, less than 10% of donors are women[29]. Similar results have been reported in some sub-Saharan African countries, notably Cameroon (87% versus 13%), Kenya (60% versus 40%) and Burkina Faso (53.4% versus 40%)[11,30,31]. Similarly, majority of blood donors were of the younger age group (aged 20–29) reflecting the Gabonese population. This finding could probably be linked to the dynamism and capacity of the young population to donate blood. This observation has been reported in other African studies, notably in Kenya[11] and Burkina Faso[32].

	Total ($n=5$)	HBV DNA (IU/mL)		*P*-value
		>2000 ($n=3$)	<2000 ($n=2$)	
Age (Years)				
20–29	3	2 (66.7%)	1 (50%)	0.2
30–39	1	0 (0%)	1 (50%)	
40–49	1	1 (33.3%)	0 (0%)	
Donor type				
Volunteer	1	1 (33.3%)	0 (0%)	0.5
Family	4	2 (66.7%)	2 (100%)	
HBV DNA viral load, mean	1836	2277.6	1314.6	/
HBV DNA S region (+)	5 (100%)	3/3 (100%)	2/2 (100%)	/
Vaccine or immune escape mutations (%)				
sT131N	4/5 (80%)	2/3 (67%)	2/2 (100%)	/
sR122K	1/5 (20%)	1/3 (33.3%)	0/2 (0%)	/
sG145A	1/5 (20%)	1/3 (33.3%)	0/2 (0%)	/
Drug resistance mutation (%)				
rtI169L	1/5 (20%)	1/3 (33.3%)	0/2 (0%)	/
HBV genotype (%)				
A1	2/5 (40%)	0/3 (0%)	2/2 (100%)	/
A3	2/5 (40%)	2/3 (67%)	0/2 (0%)	/
E	1/5 (20%)	1/3 (33%)	0/2 (0%)	/

Table 3. Profile of HBV sequences generated.

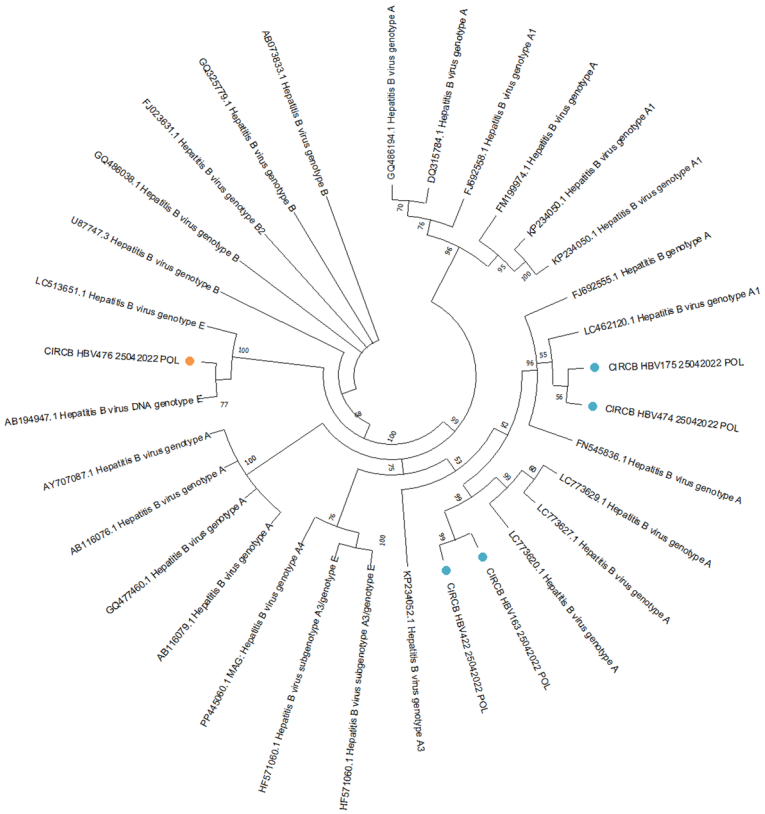

Fig. 2. Phylogenetic tree of HBV generated sequences. Legend: The study strains A1, A3 and E have been identified with colored circle symbols in blue for genotypes A1 and A3 and orange for genotype E. The ML (Maximum Likelihood) phylogenetic tree of the HBV RT/S region. The origin of the reference sequences is indicated in parenthesis. The bootsrap values > 70% (used to assess the robustness of the test or method) are indicated in the roots of the tree. The tree was constructed using our sequences from the samples of interest. The reference sequences were downloaded from a bio bank (NCBI GenBank) by blasting in Fasta format and these sequences were fed into MEGAv11 which constructed the tree according to Maximum Likelihood.

Following HBV serology, we found a very high prevalence (> 30%) of anti-HBc positivity among blood donors attending the NBTC with an important proportion of overt infection (here defined as co-presence of HBsAg and anti-HBc, 25.1%) and a lower but significant proportion of suspected cases of occult HBV (here defined as presence of anti-HBc antibodies in a HBsAg-negative participants, 5.6%)[5,6]. As concerns the circulation of anti-HBc antibodies, our observations are higher that what reported in other African countries like Mozambique (0.98%) and in other high-income settings (0.56% in the UK, 0.84% in the USA, 1.4% in Germany, 15.03% in Greece, 16.4% in Saudi Arabia), but low compared to what reported in countries like Nigeria (46.1%) and Ghana (76%)[33–35]. Nevertheless, whether it is the circulation of anti-HBc or the high carriage of HBsAg, this study confirmed the high endemicity for HBV in the country as reported before[3,26,27,36].

Surprisingly, all suspected cases of occult infection had very low levels of HBV DNA compared to overt cases and this led to genotyping being successful only among overt cases with high viral loads (> 500UI/ml). Interestingly, other studies carried among blood donors in Cameroon, Mozambique, and Burkina Faso also revealed low viral loads levels in the frame of occult HBV[17,18,33]; results supported by a systematic review by Takiussu et al.,[16] who found low viremia detectable in 0% to 20% among HBsAg-negative/anti-HBc-positive blood donors in Africa. This may be partially attributed to the assay's sensitivity and target. Indeed, the assay targets a very conserved region of S gene, coding for the surface antigen[19]; in other words, the presence of mutations in the surface antigen's may have alter the detection of HBV-DNA and even prevented samples from reacting with "HBsAg"[20]. If this observation is true, both serological screening and genomic assays at blood banks will be required to effectively guarantee blood safety before transfusion in our setting.

Furthermore, the current study found three genotypes circulating among overt HBV cases, with genotypes A1 and A3 (40% each) prevailing over genotype E (20%). These results can be superimposed on several other studies carried across the continent[11,13,15,17,21,22,33]. Of note, the circulation of genotypes A among blood donors in Gabon could be attributed to migration from various populations; and this goes along with important public health threat as carriers of this genotype are more likely to develop hepato-cellular carcinoma given its particularly aggressive nature[13,17]. Notably, three immune escape mutations, sR122K, sT131N and sG145A, were found in this study among donors infected with genotypes A. Even though these results are comparable to that of previous studies on blood donors in Botswana, Rwanda, Kenya, and Mozambique[11,15,20,23,33], serious clinical implications can be drawn. In effect, these mutants usually common among asymptomatic HBV carriers with no history of vaccination, were found in 60% (3/5) of sequences from blood donors in the current study, suggesting a wider prevalence in the general population and indicating limited efficacy of HBV immunization in the country and potentially also in the setting of mother-to-child transmission. This therefore advocates for in-depth assessment of anti-HBs antibodies' titers to measure the effectiveness of HBV vaccines in the country as well as urgent revision of the locally immunization program. Still among a donor with genotypes A, we found an amino acid substitution linked to HBV antiviral resistance (rtI169L), which is thought to encourage the replication of variants resistant to entecavir. Notably, tenofovir, adefovir, lamivudine and entecavir are used to treat hepatitis B in Gabon[23]. Although none of the study participants—blood donors—had received antiviral therapy in the past, treatment options are expected to be compromised among those eligible to treatment (i.e. with higher viral loads). As circulation of genetic drug resistance was already documented in HIV-1 co-infected patients[23,24], it is worth nothing that the spread of specific complex mutations to Entecavir under sequential use of HBV antivirals' pressure will inevitably increase the risk of treatment failure and progression to cirrhosis and hepatocellular carcinoma[25].

Less than half of the study's total data from blood donors was gathered due to resource limitations at the NBTC. It should also be mentioned that the high expense of real-time PCR testing precluded HBV DNA testing among donors both negative for HBsAg and anti-HBc. Due to financial constraints also, testing for hepatitis B surface antibodies was not possible in this particular study; which would have helped to confidently know vaccinated individuals. Finally, the control of circulating virus strains in Gabon was limited because few samples were suitable for genetic sequencing. However, as aforementioned, findings of the current study first confirmed the high endemicity of the HBV in Gabon as already described and also highlighted clinical concerns of blood transfusion safety that merit urgent attention. Unfortunately, we could not trace back data on infant HBV vaccination among young blood donors in Gabon, and this limits the understanding of the high prevalence of HBsAg + described in this study.

In conclusion, there is low prevalence of occult infection and high overt HBV infection among blood donors attending the NBTC in Gabon; with prevailing genotype A and high viral loads observed among cases of overt infection. Importantly, immune escape and antiviral resistance mutations were found and are potentially circulating in the general population. Consequently, serological and molecular testing should both be required and complementary in order to maximize transfusion safety. Furthermore, these findings also call for urgent revision of the Gabonese immunization program to ensure effective protection against HBV and to curve down the progression of the disease in the region.

Materials and methods

Study design and setting

A cross-sectional and analytical study was conducted throughout 2022 among blood donors attending the Gabonese National Blood Transfusion Centre (NBTC). The objectives were explained to each participant over the age of 18 years, and a written informed consent was obtained before enrollment into the study. Eligibility criteria included in the study: (i) unprotected sex with new partners few days before blood donation; (ii) pregnancy; (iii) being positive to malaria the past few days; (v) recent surgery; (vi) HIV and/or HCV co-infection and (vii) high blood pressure. A questionnaire was filled out for all eligible participants, which contained the basic information about the individual like age, gender, blood donor type, residence. Following the consecutive sampling on eligible donors, the serological analysis (4th generation ELISA) was carried out at the NBTC in Gabon, targeting HIV,

HCV, syphilis and HBV (i.e. HBsAg and anti-HBc); molecular assays (i.e. HBV DNA viral load and sequencing) were done at the Chantal BIYA International Reference Centre (CIRCB) for research on HIV/AIDS prevention and management in Yaoundé-Cameroon. All authorizations were obtained from each facility within various countries (Gabon and Cameroon) prior to the start of the study and methods were all performed in accordance with the relevant guidelines and regulations.

HBV serological assays at the NBTC

Specifically, screening for HBV at the Gabonese NBTC follows the national guidelines which involved detection of HBsAg using the enzyme linked immunosorbent assay (ELISA) following the manufacturers' instructions[36]. Serum HBV total anti-HBc was performed by ELISA technique [Monolisa Anti-hepatitis B core Plus-Bio-Rad (Bio-Rad™ – 3, boulevard Raymond Poincaré 92430 Marnes-la-Coquette - France)] according to manufacturer's instructions. Anti-HBc-positive plasma were confirmed with the same assay and by enzyme immunoassay (EIA) as per the manufacturers' instructions[37].

HBV viral load quantification

Quantification of HBV DNA viral load (VL) was carried out using the real time PCR technique (Abbott *m2000* System, USA) by searching for HBV nucleic acid (DNA) in the plasma of donors, with extraction being done on Abbott *m2000rt* instrument. Molecular analysis was performed according to the protocol of the manufacturer. Of note, the linear range of the kit used (i.e. Abbott HBV DNA by real-time detection PCR based on Taq Man) was 10^1-10^9 IU/ml.

HBV sequencing

All plasma samples with detectable HBV DNA levels were used for the sequencing at the CIRCB in Cameroon following an in-house procedure as described elsewhere[38]. Briefly, HBV-DNA was extracted using Qiagen kits (QIAmp DNA mini-kit, Qiagen Inc., USA) as per manufacturer's instructions. HBV-DNA extracts were then amplified with AmpliTaq-Gold polymerase using the specific primers (5'-GGTCACCATATTCTTGGGAA-3' and 5'-GTGGGGGTTGCGTCAGCAAA-3') and PCR conditions (one cycle 93 °C 12 min, 40 cycles [94 °C 50 s, 57 °C 50 s, 72 °C 90 s], and a final cycle 72 °C for 10 min). The expected 1400 bp band in the polymerase/S region was resolved on 1% agarose gel. Direct sequencing was done using eight overlapping primers (Fwd [5'-GTTG ACAAGAATCCTCACAATA-3', 5'-GGTCACCATATTCTTGGGAA-3', 5'-GGCATGGGGACGAATCTTT-3', and 5'-CTCAGTTTACTAGTGCCATT-3']; Rev [5'-GAGGTTGGGGACTGCGAATT-3'; 5'-GTGGGGGTTG CGTCAGCAAA-3'; 5'-CCTCTTGTTGCTGCTGTACAAAA-3'; and 5'-GGTGGACTTCCTCTCAATTTT])[38]. Sequences were detected by capillary electrophoresis on ABI 3500 automated sequencer (Applied Biosystems™, USA). Sequences were analyzed using SeqScape v2.7, with the quality endpoint being ensured by coverage of at least two bidirectional primers. Sequence with a mixture of wild type and mutant at a given position was considered having the mutant. Duplicate tests were performed on a donor sample. The sample was considered a positive control and the manipulation was validated if the same result (PCR amplicons and identical sequences generated) was detected in both reactions. At the same time, a negative control was amplified at the same time as the samples, and the manipulation was validated in case of absence of amplicon in the latter. The positive control was used to check for the absence of inhibitor, while the negative control was used to check for the absence of contamination in the reaction medium.

Interpretation of HBV DNA sequences and molecular phylogeny

Mutations in the HBV polymerase (primary and secondary mutations) and the HBsAg gene (immune/diagnostic-escape HBsAg mutations) were interpreted using geno2pheno algorithm (https://hbv.geno2pheno.org)[38]. Phylogenetic analysis was performed using MEGAv11[39], and a Maximum Likelihood (ML) tree was inferred using Kimura two-parameter and Gamma-distributed Rate models. An analysis of the genetic diversity of RT/HBsAg sequences from our samples was conducted and compared with HBV sequences from NCBI[38].

Statistical analysis

Categorical variables (median-age, gender, donor type) were assessed using Chi-squared or Fisher's exact test where appropriate. We filled in missing data, corrected for duplicates in the database, and performed analyses using SPSS Statistic v21.0 (IBM Inc., Armonk, NY) and EPI info v7.2. For all statistical tests, all p-values < 0.05 were considered significant.

Definition of cases

Donors were prospectively classified by the consultant medical virologist as having an overt or occult HBV infection based on confirmatory testing and clinical history[9]. We used these definitions to define HBV infection states. For this study, overt infection was defined as individuals with detectable HBsAg and/or detectable HBV DNA. Individuals who were negative on ELISA (HIV-Ab/Ag, anti-HCV, HBsAg and Syphilis) and who had no detectable viremia were considered to be free of infection (no prior/current infection). Occult HBV infection was defined as the presence of HBV DNA (even in low quantity) in blood with undetectable HBsAg[6]. Seropositive occult HBV included those with anti-HBc antibodies[6].

Data availability

All sequences described herein were deposited in the GenBank under the accession numbers PP550135 – PP550139. Data generated and analyzed during the current study are provided within the manuscript.

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Author contributions
DMB and JF designed the study; DMB, AGW and CMA collected the data; DT, GAB, NKE, CM, SCDN, SMS, CAC, ADN, MT, NF, RK, were responsible for lab analysis and validated the results; DMB, NKE, GAB, DT and ENJS analyzed the data and prepared figures; TN, HKN, VC, CFP, AN, and VP supervised the study; DMB and ENJS initiated the manuscript. DMB, JF, ENJS, VS, RS, DT, GAB, NKE, CM, SCDN, AGW, SMS, CAC, AND, MCTT, NF, RK, VAM, HKG, GEHE, RAA, CT, DM, ACZKB, VP, ORP, TN, VC, NN, CFP and AN commented and upgraded the first draft of the paper. All the authors revised and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
Informed consent was taken from donors and controls and ethical clearance was taken from the Gabonese National Ethical Committee for research (Clearance n° 0088/2019/PR/SG/CNER). The study was explained to each participant, and a written informed consent was obtained before enrollment to the study. No obligation on the patients to participate in the study. Participants who were found to be seropositive for hepatitis B virus were channeled to the appropriate clinics for further evaluation and management.

Additional information
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