

# Frequency of Occult Hepatitis B Infection in Blood Donors from Maputo, Mozambique

Zicai, AFM; Mello, FCA; Costa, VD; Lago, BV; Lampe, E; Mabunda, NJ; Lewis-Ximenez, LL

## BACKGROUND

Hepatitis B virus (HBV) is one of the three most important agents responsible for infections transmitted by blood transfusion. Occult hepatitis B is characterized by the presence of HBV DNA in serum, plasma or hepatic tissue in subjects with negative serology for the surface antigen (HBsAg). Occult HBV is associated with increased risk of hepatocellular carcinoma, reactivation to chronic HBV during immune suppression, and transmission during blood transfusion and liver transplant. There are several mechanisms leading to occult HBV infection, and viral mutations that alter HBsAg epitopes and its recognition by diagnostic tests antibodies is likely a significant factor. Mozambique is considered an endemic country for chronic hepatitis B infection, with more than 8% of chronic carriers in the total population. Currently, there is no available data of occult hepatitis B in blood donors of Mozambique. The knowledge of the impact of this infection in the blood supply would be crucial for the implementation of NAT tests in blood compounds in order to avoid viral dissemination. The aim of this study was to determine the frequency of occult hepatitis B virus infection among blood donors in a blood bank located in the Central Hospital of Maputo and to perform a molecular characterization of the viral strains found, phylogenetically determining viral genotypes and identifying possible HBsAg diagnostic-escape mutations.

Table 1 – Study population socio-demographic and serological characteristics

Characteristics	Total	HBsAg + (%)	HBsAg – (%)	Occult infection (%)
<b>N</b>	1500	64 (4.3)	1436 (95.7)	17 (1.2)
<b>Mean Age</b>	32 [16-65]	30 [16-65]	32 [16-65]	32 [16-65]
<b>Gender</b>				
Male	1173 (78.2)	50 (78.1)	1122 (78.2)	14 (82.4)
Female	327 (21.8)	14 (21.9)	314 (21.8)	3 (17.6)
<b>Donor Type</b>				
Repository	1120 (74.6)	57 (89.1)	1062 (74)	16 (94.1)
Voluntary	381 (25.4)	7 (10.9)	374 (26)	1 (5.9)

## METHODS

1,500 blood donors were recruited and tested for serological markers HBsAg, HBeAg, anti-HBc, and anti-HBs with commercial ELISA assays. HBsAg-negative serum samples were tested for the presence of HBV DNA using commercial real-time PCR technique. HBV DNA-positive samples were submitted to a semi-nested PCR for amplification of a fragment of ~900 bp comprising partial S/P genes for direct nucleotide sequencing and subsequent sequence and phylogenetic analyses.

## RESULTS

Serological tests indicated that the frequency of hepatitis B infection (HBsAg-positive serum samples) among blood donors was 4.3% (64/1500). HBV DNA detection in the 1,436 HBsAg-negative serum samples indicated a frequency of occult hepatitis B infection of 1.2% (17/1436). Of these, 10 individuals had anti-HBc as the unique serological marker for HBV infection, 3 were positive for both anti-HBc and anti-HBs, one was solely anti-HBs-positive, and 3 were seronegative for all serological markers evaluated. PCR amplification and nucleotide sequencing was successful for ten HBV DNA-positive samples whereas the remaining seven could not be amplified due to low viral load (< 20 IU/mL) indicated by commercial real-time PCR test. Phylogenetic analysis of the 10/17 sequences of occult hepatitis B infection obtained in this study revealed that 9 isolates belonged to genotype A and one to genotype E. HBV/A strains presented a close relationship with sequences isolated in South Africa, Kenya and Rwanda while the HBV/E isolate clustered with sequences from Angola, South Africa, and Namibia. Mechanisms others than nucleotide substitution in HBsAg epitope are involved in the occult character of these infections since molecular characterization of HBV strains did not revealed any known escape mutation in S gene nor evidences of drug resistance mutations in P gene that could contribute to evasion of recognition by antibodies used in commercial ELISA assays.

## CONCLUSIONS

This is the first report of occult hepatitis B infection in blood donors in Mozambique. HBV DNA found in different serological profiles, including individuals seronegative for all HBV serological markers, encourages the discussion about the necessity of adoption of more rigorous screening methods in routine processing of blood derivatives to minimize the potential risk of transmission of viral pathogens by blood transfusion in Mozambique.

## REFERENCES

- Pondé RA. Molecular mechanisms underlying HBsAg negativity in occult HBV infection. *Eur J Clin Microbiol Infect Dis*. 2015;34(9):1709-31.
- Viegas EO, Tembe N, Macovela E, *et al*. Incidence of HIV and the prevalence of HIV, hepatitis B and syphilis among youths in Maputo, Mozambique: a cohort study. *PLoS One*. 2015;10(3):e0121452.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

INSERT TEXT, DIAGRAM, GRAPH OR IMAGE HERE

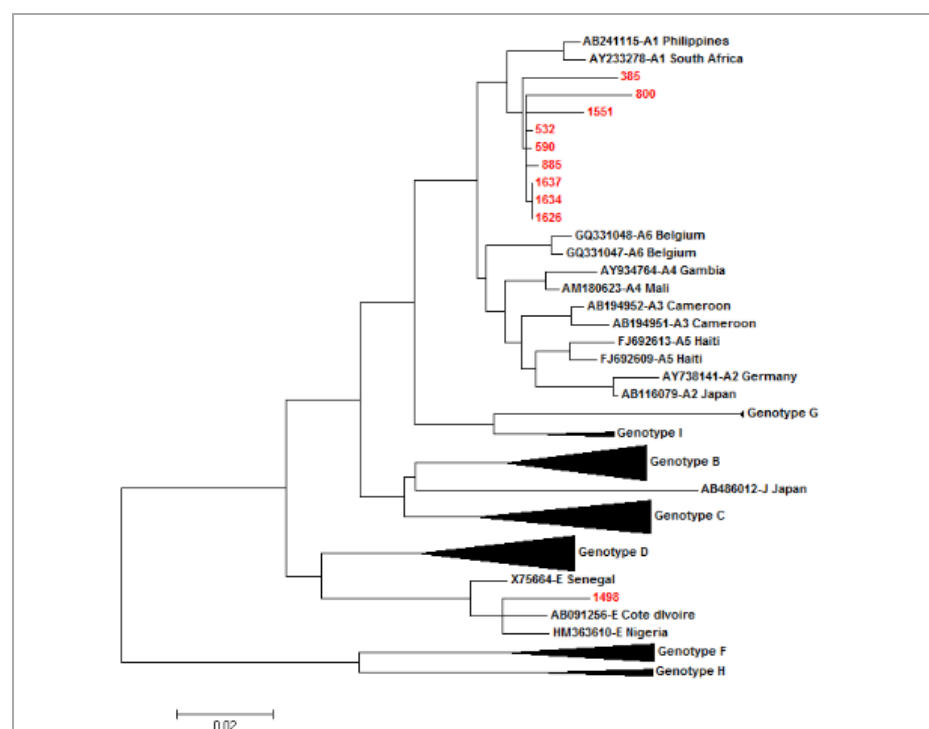


Figure 1 – Phylogenetic analysis based on the S gene from individuals with occult hepatitis B infection from Mozambique and sequences retrieved from Genbank. Phylogenetic tree was generated using maximum likelihood method under GTR+G+I nucleotide substitution model with a bootstrap of 1000 replicates. The Mozambican sequences are represented in red.

## Contact Information

NAME Francisco Mello  
 TEL NO +55 21 988925219  
 EMAIL fcamello@ioc.fiocruz.br