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OF SCIENCES AND LITERATURE

RESIDUAL TRANSMISSION RISK OF OCCULT HEPATITIS B INFECTION IN BLOOD DONORS IN A SUB-SAHARAN AFRICAN HOSPITAL-BASED BLOOD BANK: THE CASE OF THE YAOUNDE UNIVERSITY TEACHING HOSPITAL, CAMEROON

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A DISSERTATION

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DECLARATION

I do hereby attest that I am the sole author of this project/thesis and that its contents are only the result of the readings and research I have done. The dissertation titled – **Residual transmission risk of Occult Hepatitis B infection in blood donors in a Sub-Saharan African Hospital-based blood bank: the case of the Yaounde University Teaching Hospital, Cameroon,** Thesis written and presented in the fulfilment of the requirements for the award of the degree Philosophy Doctorate of Immunology and Infectious Diseases at University of Selinus; is my original work. The material borrowed from similar titles other sources and incorporated in the dissertation has been duly acknowledged. The research papers published based on the research conducted out of the course of the study are also based on the study and not borrowed from other sources.

Date: 08 May 2010

DEDICATION

This work is dedicated to my family especially my father Mr. NGUEGOUE Thomas, my mother Mrs NODEM Theresa, my wife Ariane Mesmine and my daughter Maeva Dora, who gave me moral support and encouragement during my study.

This work is also particularly dedicated to the late Prof. Jean Jacques LEFRERE, former General Director of National Institute of Blood Transfusion in France and former Director of training programme of Infectious Blood Safety at the Institut Pasteur, Paris. Dear Prof., you trained me in Paris in 2014 and started mentoring me, but you died very earlier; May your soul rest in peace!

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ABREVIATIONS

anti-HBc	antibodies against the HBV core antigen
DNA	Deoxyribonucleic acid
anti-HBe	antibodies against the HBV 'e' antigen (HBeAg)
ALT :	*alanine aminotransferase*
anti-HBc	anti-HBc antibodies against the HBV Core antigen
anti-HBc :	*antibody to HBcAg*
anti-HBe :	*antibody to HBeAg*
anti-HBs :	*antibody to HBsAg*
AST :	*aspartate aminotransferase*
BSRI:	*Blood Systems Research Institute*
cccDNA :	*covalently closed circular DNA*
CHU :	Centre Hospitalier et Universitaire
EIA :	*Enzyme Immuno-Assay*
ELISAs	Enzyme Linked Immunosorbent Assays
FMSB :	Faculté de Médecine et des Sciences Biomédicales
H CC	Hepatocellular Carcinoma
HBcAg :	*Hepatitis B Core Antigen*
HBeAg :	*Hepatitis B e Antigen*
HBsAg	HBV surface antigen
HBsAg :	*Hepatitis B Surface Antigen*
HBV cccDNA	Hepatitis B viral convalently-closed-circular DNA
HBV :	*Hepatitis B Virus*
HBV-DNA	Deoxyribonucleic acid of hepatitis B virus
HBxAg :	*Hepatitis B x protein*
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HVB :	Hépatite Virale B
HVBO :	Hépatite virale B Occulte
IgM :	*Immunoglobulin M*
INTS :	Institut National de la Transfusion Sanguine
Kb :	*Kilobase*

L-HBsAg :	*large HBsAg*
M-HBsAg :	*Middle Hbsag*
NAT	Nucleic acid testing
ORF :	*Open Reading Frame*
OBI	Occult Hepatitis B Infection
PCR	Polymerase Chain Reaction
pgRNA :	*pregenomic RNA*
RT :	*Reverse Transcriptase*
TTIs	Transfusion Transmitted Infections
UCSF:	*University of California, San Franscisco*
USA:	*United States of America*

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ABSTRACT:

BACKGROUND: Most Sub-Saharan Africa Countries are highly endemic for Hepatitis B Viral infection. Despite this high endemicity in Cameroon, the prevention of HBV transmission by blood transfusion is still based on HBsAg screening alone. However, occult HBV infection (OBI) characterized by the absence of detectable HBsAg and low level of viral DNA remains a potential threat for blood safety. OBI prevalence is expected to be higher where prevalence of overt HBV infection is high. Nevertheless, there are limited data on the prevalence of OBI in Cameroonian blood donors. The prevalence of OBI was investigated in blood donors from Yaoundé to provide evidence-based recommendations to improve HBV blood safety.

STUDY DESIGN AND METHODS: Blood donations from August 1, 2016 to March 31, 2017 were routinely screened for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV investigations were performed including anti-HBc (Monolisa Anti-HBc PLUS; BIO-RAD), HBV DNA tested in minipools of two samples using the quantitative Cobas Taqman HBV assay (Roche; LoQ: 6 IU/mL) and HBV DNA genotyping by sequencing.

RESULTS: In total, 1.166 were serially included in the study. Screening for transfusion transmissible infections showed that 91 (7.80%) of total samples donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.94%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+. 1.162 were screened for total Anti-HBc IgG+IgM and 613 (52.75%) were reactive. All the 91 samples HBsAg positive were also positive for HBcAb. In 1071 HBsAg negative participants, the prevalence of HBcAb was 48,74% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48,81% (n=511). Six samples fulfilled the consensus definition of OBI: low HBV DNA load (all <6 IU/mL) with no detectable HBsAg and detectable HBcAb. Following nested PCR amplifications, HBV DNA sequences were obtained for 4 of these samples (1 nearly whole genome [3123 nt], 2 Pre-S/S regions [1356 nt], and 1 S region [445 nt]) definitively confirming OBI status.

CONCLUSIONS: HBV was confirmed highly endemic in Cameroon. More than half of blood donors were anti-HBc positive. About 1 in 100 Cameroonian blood donors who screened HBsAg negative and anti-HBc positive carried occult HBV infection. HBsAg alone for screening prospective donors is not sufficient to eliminate the risk of HBV transfusion-transmission in Cameroon and because anti-HBc screening does not appears feasible without compromising blood supply, implementation of HBV nucleic acid testing might be considered when possible.

Key words: *Occult hepatitis B viral infection, Blood donors, Yaounde-Cameroon.*

I. Rational, research question and objectives

1.1. Rational

Hepatitis B virus (HBV) is an enveloped DNA virus belonging to the Hepadnaviridae family (Daniel Candotti & Laperche, 2018).The virus is responsible for chronic hepatitis B representing a major global health problem with more than 240 million chronically infected persons worldwide, particularly in low- and middle-income countries (LMICs) (*Guidelines for the prevention, care and treatment of persons with chronic hepatitis b infection*, 2015). HBV infection remains the most common viral infection transmitted by blood transfusion (Niederhauser, 2011). For several years, the risk of transmission of HBV trough blood transfusion has been progressively reduced through the recruitment of benevolent and volunteer donors, the medical selection of donors based on risk-behavior evaluation, the development of more sensitive hepatitis B antigen (HBsAg) assays, and in some developed countries the use of hepatitis B core antibody (anti-HBc) screening, and the HBV nucleic acid testing (NAT) (Daniel Candotti & Laperche, 2018).

Occult HBV infection (OBI) is defined by detectable low level of HBV DNA (<200 IU/mL) in liver or serum with undetectable HBsAg and with/without anti-HBc or anti-HBs, excluding the pre-seroconversion window period. The molecular basis of OBI is the persistence of covalently closed circular DNA (cccDNA) in the cell(Raimondo, Caccamo, Filomia, & Pollicino, 2013). OBI have been reported among healthy asymptomatic blood donors, patients with chronic liver disease, and patients with hepatocellular carcinoma(Makvandi, 2016). The prevalence of OBI tends to be higher in regions with high HBV endemicity (Minuk et al., 2005).

Despite a considerable reduction of the risk of HBV-infected blood donation entering blood supply (residual risk) due to improved screening by HBV NAT in the developed countries, the bulk of the people with HBV living in the developing countries still needs to be screened by serologic tests such as HBsAg and anti-HBc. Many of these countries lack resources for implementing NAT and are likely to remain so in the next decade or longer, thus depending on the HBV residual risk monitoring based on serologic testing and corresponding estimation methods(Kupek, 2013).

Over the last four decades, blood safety has been continuously improved by the constant development of sensitive and specific serologic assays to detect the HBV surface antigen (HBsAg) and anti-HBc antibodies against the HBV Core antigen (anti-HBc) in blood donations. Global implementation of nucleic acid testing (NAT) for HBV DNA in 2004–2008 significantly reduced further the residual risk of HBV transfusion-transmission by reducing the diagnostic pre-sero-conversion window period and by detecting occult HBV infection/carriage(D. Candotti, Boizeau, & Laperche, 2017; Roth et al., 2012). The residual risk of HBV transfusion-transmission appears to be mainly related to blood donations negative for HBsAg but containing extremely low levels of viral DNA potentially infectious that may escape detection by the currently most sensitive NAT assays(D. Candotti et al., 2017).

In Cameroon, like in other Sub-Saharan African countries, screening for HBV in blood donors and patients relies only on serological detection of HBsAg (Bigna et al., 2017). In the absence of anti-HBc testing, blood transfusion remains at risk of transmitting HBV infection from donors with OBI(Daniel Candotti, Assennato, Laperche, Allain, & Levicnik-Stezinar, 2019). Despite the cost that is not always affordable by LMICs, HBV-DNA detection by Nucleic Acid Testing (NAT) prove to be a reliable preventive measure against HBV transmission from donors with OBI (4). In Cameroon, the only data on OBI have been reported in HIV positive patients showing prevalence rates between 5.9 and 6.9%(Gachara et al., 2017; Salpini et al., 2016). Therefore, in order to provide evidence-based recommendations to improve HBV blood safety, a study was carried out to estimate the transfusion transmission risk of OBI in blood donors from Yaoundé, Cameroon.

1.2. Research question

Considering the rational above, the research question we formulated was: What is the residual risk of OBI in blood donors in Cameroon?

1.3. Objectives

1.3.1. General objective

The general objective of this study was to determinate de residual risk of transmission of OBI through blood transfusion at the Yaounde University Teaching Hospital Blood Bank.

1.3.2. Specific objectives

- To evaluate the prevalence of HBsAg in blood donors at the YUTH Blood Bank;
- To determinate the seroprevalence of HIV and HCV and co-infections in blood donors;
- To determinate de frequency of HBcAb in HBsAg negative blood donors;
- To determinate the prevalence of OBI in blood donors and;
- To estimate the transfusion transmission risk of OBI in blood donors from Yaoundé, Cameroon.

II. Literature review

2. Review of the literature

2.2. Virology

2.2.1. The Hepatitis B Virus and HBV genome

HBV genome is a partially double-stranded relaxed circular DNA, about 3,200 nucleotides in length, and has four partially Overlapping Open-Reading Frames (ORF):

- *Pre-S/S* ORF, which correspond to HBsAg and encodes for the three viral surface proteins, pre-S1 (or Large), pre-S2 (or Middle), and S (or small);
- *Pre-C/C* ORF, encodes the soluble antigen "e" (HBeAg) and the core antigen (HBcAg);
- P ORF encodes the viral polymerase that possesses DNA polymerase and the terminal protein (TP), RNaseH activities and reverse transcriptase.
- X ORF which encodes the regulatory X protein, intervenes in transactivating the expression of cellular and viral genes (Pollicino et al., 2004).

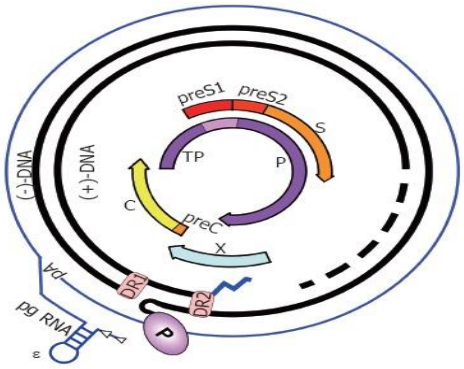

Figure 1. Organization of HBV genome (Raimondo, Pollicino, Cacciola, & Squadrito, 2007)**.**

2.2.2. The replication of HBV

The HBV replication cycle has particular characteristics that can be summarized as follows (İnan & Tabak, 2015):

a) the interaction of the virus with cell surface receptors;
b) Release of the nucleocapsid into the cytoplasm and its transport in the nuclear membrane;
c) integration into the nucleus by the HBV genome and its transformation into a covalently closed circular DNA (cccDNA);
d) Using the host RNA polymerase II, cccDNA is transcribed into all viral mRNA and a pregenomic RNA (pgRNA),
e) HBV transcripts are translocated into the cytoplasm and translated to the viral envelope, core,"e", polymerase, and X proteins;

f) nucleocapsids are assembled of and new viral DNA from pregenomic RNA is synthesized by viral reverse transcriptase;

g) nucleocapsids are recycled into the nucleus to maintain the reservoir of cccDNA stable; and coated with viral surface proteins in the endoplasmic reticulum and subsequent release of mature virions.

The figure below explains this replication process (Figure 2):

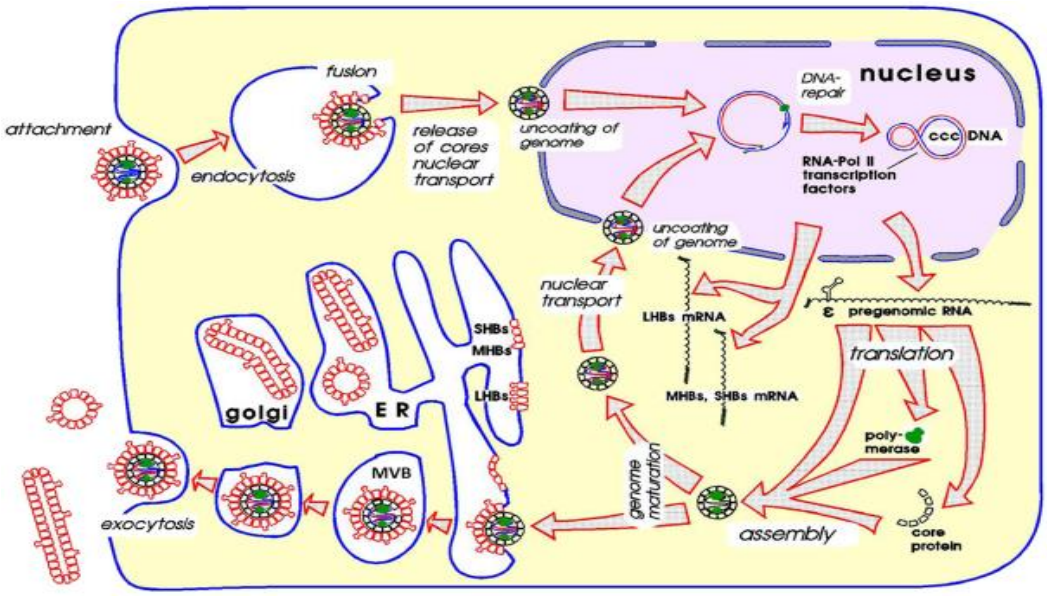

Figure 2. Life cycle of HBV(Gerlich, 2013)**.**

2.3. The virus and natural history of the infection

Two key events occur during replication of the virus in hepatocytes:

- The first is the formation of replication intermediates termed "covalently closed circular DNA" (cccDNA) in the nucleus, which are maintained throughout the life of the hepatocyte and function as a reservoir of HBV responsible for persistence.
- The second is the reverse transcription step. The enzyme reverse transcriptase, with its inherent error-prone activity, is responsible for the emergence of a highly heterogenous viral population (quasispecies) including those containing mutations that affect the production of e-Antigen (eAg)(Liaw, Brunetto, & Hadziyannis, 2010).

Symptomatic hepatitis occurs in about one-third of adults following an incubation period of 1 to 4 months in about 10% of children under 5 and only in children under 1 year of age. A fulminant phase with high mortality has been reported in the pediatric population and in approximately 1% of infected adults(Liaw et al., 2010). The resolution of infection, which is characterized by the disappearance of hepatitis B surface antigen (HBsAg) from blood within 6 months of infection, occurs in the majority of infected adults

and only in a minority of infected newborns. Resolution of the infection usually leads to lifelong protection of the disease(Ganem & Prince, 2004).

However, rather than being completely eliminated, HBV is controlled by an effective immune response; in case of significant immunosuppression, it can be reactivated. The natural course of chronic hepatitis B virus (CHB) infection is divided into four phases, arbitrarily defined as: immune tolerance, immune clearance (or immune clearance), inactivation, and reactivation.

- The **immuno-tolerant** phase with a high level of HBV replication, positivity for HBeAg and normal elevation of alanine transaminase (ALT). This period may last 20 to 30 years after perinatal acquisition, but is shorter or sometimes absent in adults.
- During the **'immune clearance phase'**, there is a reduction of the replication of HBV DNA and an increased inflammation of the liver. This phase can last for years with fluctuation of disease activity and progressive liver damage. Seroconversion of HBeAg to HBeAb is generally followed by a decrease in viral replication and liver enzyme (alamine transferase).
- The **inactive phase** is characterized by HBV DNA <2000IU/ml in the majority of patients with regression of inflammation(Liaw et al., 2010).
- The **reactivation phase** can be developed in some inactive carriers as a result of the persistence of cccDNA in hepatocytes with either the wild-type or reversion to HBeAg positivity or with HBV variants with mutations limiting HBeAg production.

  Cirrhosis and hepatocellular carcinoma (HCC) have two major complications. The risk of development of those complications varies by geographic region and may be influenced by the presence of HBsAg, HBeAg, some mutations, and higher levels of HBV DNA(Gerlich, 2013).

2.4. **Prevention through vaccination**(WHO, 2015)

Recombinant DNA-derived vaccines against HBV have been available for more than two decades. The primary hepatitis B immunization series conventionally consists of three doses of vaccine. Vaccination of infants and, in particular, delivery of hepatitis B vaccine within 24 hours of birth is 90–95% effective in preventing infection with HBV as well as decreasing HBV transmission if followed by at least two other doses. WHO recommends universal hepatitis B vaccination for all infants, and that the first dose should be given as soon as possible after birth. This strategy has resulted in a dramatic decrease in the prevalence of CHB among young children in regions of the world where universal infant vaccination programmes have been implemented. A proportion of vaccinated children (5–10%) have a poor response to vaccination, and will remain susceptible as adults to acquisition of HBV infection. In countries with intermediate or low endemicity, a substantial disease burden may result from acute and

chronic infection acquired by older children, adolescents and adults. Target groups for catch-up vaccination as well as other preventive strategies include young adolescents; household and sexual contacts of persons who are HBsAg-positive; and persons at risk of acquiring HBV infection, such as PWID, men who have sex with men, and persons with multiple sex partners.

2.5. Treatment: Antiviral therapy(WHO, 2015)

Although HBV infection can be prevented by vaccination, it is important to treat persons with CHB at high risk of progression to reduce the considerable morbidity associated with CHB. Over the past three decades, treatment outcomes have improved, first with conventional and then pegylated (PEG) interferon (IFN) and, more recently, with the advent of NAs. Currently, seven antiviral agents (lamivudine, adefovir, entecavir, telbivudine, tenofovir, emtricitabine, standard and PEG-IFN) are approved for the treatment of CHB in high-income countries, and have been shown to delay the progression of cirrhosis, reduce the incidence of HCC and improve long-term survival. Although all NAs act on HBV polymerase, their mechanism of action differs; adefovir inhibits the priming of reverse transcription; lamivudine, emtricitabine and tenofovir inhibit the synthesis of the viral (-) strand DNA; and entecavir inhibits three major stages of HBV replication. In addition to their variable mechanisms of action, their pharmacokinetics, inhibitory capacity and resistance patterns vary. Although NAs are effective inhibitors of HBV replication, they seldom result in cure, and clearance of HBsAg is rare. Therefore, at present, long-term (potentially lifelong) NA therapy is required in the majority.

The advantage of NA therapy over IFN includes few side-effects and a onepill-a-day oral administration. The main advantages of IFN over NAs are the absence of resistance, and achievement of higher rates of HBeAg and HBsAg loss. However, the disadvantages of IFN are that less than 50% of persons treated will respond, its high cost, administration by injection and common sideeffects, which precludes its use in many persons, particularly in resource-limited settings. A number of relative and absolute contraindications to IFN also exist, which include the presence of decompensated cirrhosis and hypersplenism, thyroid disease, autoimmune diseases, severe coronary artery disease, renal transplant disease, pregnancy, seizures and psychiatric illness, concomitant use of certain drugs, retinopathy, thrombocytopenia and leucopenia. IFN also cannot be used in infants less than 1 year and in pregnant women.

Several international organizations have developed guidelines for the treatment of CHB, but the optimal timing of treatment is still debated. In general, treatment is targeted at persons with CHB and moderate or severe liver inflammation, and/or fibrosis and high viral replication, who are at high risk ofdisease

progression to cirrhosis and HCC. The benefits of treatment for those with mild inflammation or fibrosis are less certain. If HBV replication can be suppressed, the accompanying reduction in chronic liver inflammation reduces the risk of cirrhosis and HCC, but generally lifelong treatment is required.

Extrahepatic manifestations of hepatitis B such as glomerulonephritis or polyarteritis nodosa may also respond to treatment. New treatment strategies: Tenofovir alafenamide fumarate (TAF) is an orally bioavailable prodrug of tenofovir that enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes, so that the dose of tenofovir can be reduced and toxicities minimized. TAF has been evaluated in recent and ongoing clinical trials.

Research is also ongoing to develop and test new agents that can "cure" HBV by eliminating all replicative forms, including covalently closed circular DNA (cccDNA). Broadly curative antiviral strategies include agents that could directly target infected cells as well as novel immunotherapeutic strategies that boost HBV-specific adaptive immune responses or activate innate intrahepatic immunity. New molecules under investigation include entry inhibitors and shortinterfering RNAs (siRNAs), and capsid inhibitors.

2.6. Cinetic of serological marquers of HBV and OBI

The development of laboratory tests has permitted to a better understand of the natural history of the HVB infection (Table 1). There are two proteins specific to HBV that can be detected in the serum of infected patients (HBsAg and HBeAg). The HBsAg is a main viral protein that induces protective immune responses. HBsAg is found on the surface of the viral envelope and in high titers in the serum of infected patients. The persistence of HBsAg in serum for more than 6 months indicates a chronic HBV infection(Horvat, 2011).

Table I**. Diagnostic tests for HBV**(Horvat, 2011)

Type of assay	**Tests designation**	**Phase of detection**
Serology detection of HBV antigens	HBs Antigen	active HBV infection
	HBe Antigen	high levels of HBV replication
Serology detection of HBV antibodies	Anti-HBs	in individuals immunized after vaccination or infection
	IgM Anti-HBc	In recently infected persons by HBV
	Total Anti-HBc	in persons currently HBV infected or in past infection
	Total Anti-HBe	after an acute HBV infection
Molecular detection of HBV	HBV DNA	Found in early HBV infection and used to monitor antiviral treatment

2.7. Mechanisms potentially involved in HBV inhibition and induction of OBI status

The mechanisms underlying occult hepatitis B infection are not yet clear. However, there are assumptions and data regarding host factors that are generally considered to be involved in suppressing HBV replication and in controlling infection. In addition, viral factors and co-infection with other pathogens may play a role in the induction of OBI status(Akram, 2018).

2.7.1. Host related factors

Host related factors are strongly implicated in the induction and maintenance of occult B infection. An in vitro study demonstrates that the replication, transcription and protein synthesis capabilities of isolates occult viruses can be fully restored once the viruses have been removed from the microenvironment of the host's liver(Saffioti & Raimondo, 2017).

- **Immunological factors**

There is a strong direct and indirect evidence of involvement that the host's immune system (adaptive and innate) surveillance plays an important role in the development of the OBI. CD4 and CD8 long-lasting memory cells against HBV antigens were detected many years after clinical recovery of acute hepatitis B(Saffioti & Raimondo, 2017). Clinical studies have demonstrated that conditions inducing immunosuppression may induce the reactivation of OBI with the reappearance of the serological profile of overt active infection(Saffioti & Raimondo, 2017). This is an evidence of the involvement of the host's immune surveillance in the development OBI status. Effectively, besides HBV cccDNA molecules, all viral transcripts have been found in the liver of occult-infected patients (Ifeanyi & Uzoma, 2017) and real-time PCR has revealed quantifiable amounts of intrahepatic HBV mRNA in these individuals (Zoulim, 2005). Thus, the clinical recovery of HBV infection does not only imply the absence of total clearance of the virus, but also the ability of the immune system to keep the remaining viruses in the liver under control after clinical resolution of the viral infection(Ifeanyi & Uzoma, 2017).

- **Epigenetic factors**

HBV cccDNA molecules do accumulate in the nucleus of infected hepatocytes as stable mini-chromosomes packed into nucleosomes by histone and non-histone proteins, similarly to host cell chromatin (Levrero et al., 2009). Some recent findings have highlighted the importance of dynamic chromatin changes in histone composition and the regulation of gene expression and replication during the various stages of productive viral replication, latent infection and reactivation from latency, increasing the possibility that epigenetic processes may influence post-infection processes(Ganem & Prince, 2004).

2.7.2. Viral factors

Attention has been focused on viral mutations in the surface gene and its regulatory regions. Therefore, in addition to the mutations that cluster in the key immuno-dominant regions of the surface protein capable of decreasing the immune recognition of the virus, deletions in the preS1 region alter viral conditioning, structural alteration in the genomic regulatory regions causing a strong reduction in the expression of HBsAg, and mutations affecting normal post-translational modifications of HBV proteins have been demonstrated (Ganem & Prince, 2004).

2.7.3. Coinfections

In case of co-infections, HBV activity may be affected by other infectious agents. In particular, it is suspected that HCV causes a strong suppression of HBV replication, to the point of causing the development of OBI in co-infected patients. In fact, many in vitro studies have shown that the "essential" HCV protein significantly inhibits HBV replication (Caccamo, Saffioti, & Raimondo, 2014; Raimondo, Cacciamo, & Saitta, 2005) and the highest OBI prevalence are found in patients infected with HCV (Mahmoud, Ghazal, Metwally, Shamseya, & Hamdallah, 2016). However, some studies question the interaction between HCV and HBV when in vitro co-transfection experiments were performed with complete HBV genomes and an HCV replicon, resulting in no detectable interference between the two viruses(Bellecave et al., 2009).

2.8. Detection of occult viral hepatitis B infection

The identification of cases of occult infection with HBV is usually done by serum or plasma analysis. Increasing the sample volume to 1ml improves the sensitivity of DNA extraction(Raimondo et al., 2008). HBc testing should be used as a surrogate technique to identify potentially OBI individuals among blood donors, tissue or organs donors and in case of immunotherapy. However, it should be noted that not all anti-HBc positive individuals are positive for HBV DNA and that anti-HBc tests may give false positive results (Ocana, Casas, Buhigas, & Lledo, 2011; Raimondo et al., 2008).

2.8.1. Serological profile of occult B infection

The proteins released by the virus and the antibodies produced by the host make it possible to have valuable information. In patients with occult hepatitis B, there are differences depending on the results of the serological markers (table 2).

Table II. *Serological markers and HBV DNA in different states of persistence of hepatitis B virus [2,4]*

	HBsAg	anti-HBs	HBeAg	anti-Hbe	Total anti-HBc	HBV DNA
Healthy carrier	+	-	-	+	+	< 106 IU/mL
Chronic hepatitis B infection	+	-	+	-	+	+++
Chronic HBV infection with variants pre-core	+	-	-	+/-	+	++
seronegative OBI	-	-	-	-	-	< 1000 IU/mL
seropositive OBI	-	+/-	+/-	+/-	+	< 1000 IU/mL

- ***Seropositive OBI status***

Seronegative-OBI individuals are those with anti-HBc and / or anti-HBs positive in whom serum HBsAg is not detected due to the resolution of acute hepatitis B (a few months of HBsAg carriage) or after several years of chronic infection with HBsAg positive. The HBV DNA detection rate is higher in anti-HBc positive and anti-HBs negative individuals. In the presence of both antibodies, the viral load of HBV DNA is intermediate. This serologic pattern can be explained by the fact that HBV-positive HBV-positive patients who are HBV-positive remove HBsAg at an undetectable level with or without anti-HBs: this trend is associated with aging and anti-HBe(J. M. González et al., 2015).

- ***Seronegative OBI status***

Anti-HBc and anti-HBs negative individuals have very low levels of HBV DNA. This may occur early in the course of infection when patients have not yet developed antibodies specific for hepatitis B ("primary OBI") or because antibodies specific for hepatitis B are eliminated. In addition, there are so-called "false" OBI cases. They carry mutations in HBsAg (in the S gene) that are not recognized by some routine tests. Thus, the result of the DNA is the same as in other cases of HBV because they are generally positive for HBsAg(J. M. González et al., 2015).

2.8.2. Markers of the screening of OBI individuals

- **Hepatitis B surface antigen (HbsAg)**

It is essential to define the optimal methodology for detecting this marker in order to avoid false positive results, depending on the sensitivity of the HBsAg test. Mutations in the S gene are responsible for the amino acid modifications of the "a" region, which is very advantageous for inducing immunity, targeting anti-HBs. Immune pressure may lower HBsAg, but may promote selection of HBsAg mutants(*World Health Organization*, 2002).

- **Anti-core antibody (Anti-HBc)**

It is the very first antibody that appeared, even before HBsAg, that targets the nucleocapsid of HBV. This antibody can be found in almost all patients who have been in contact with HBV, even in HBV carriers without any other response. This serological regimen is called "anti-HBc alone" and may reflect an occult infection with HBV. Anti-HBc is detectable in the different phases of hepatitis, including recovery, and may be longer than anti-HBs or anti-HBe; however, this antibody is not at all protective. Anti-HBc IgM can help in the diagnosis of the acute phase of infection(*Labstract - August 2013 Viral Hepatitis Serologic Testing Changes to Test Ordering , Algorithm and Reporting*, n.d.).

- **Anti-surface antibody**

It is the last antibody to appear (about three months after the acute phase). It is the only antibody capable of neutralizing the virus. Thus, in vaccinated individuals, it is the only positive marker. This antibody can be used with an anti-HBc to study the serological status of patients with probable OBI(Liaw et al., 2010; Price et al., 2017).

- **HBV DNA**

The gold standard for the diagnosis of OBI is the study of DNA extracted by techniques that are both very sensitive and specific. Experts in Taormina recommended testing with detection limits of less than 10 copies of HBV DNA per reaction. The technologies currently used for DNA detection are: nested PCR, real-time PCR and transcription-mediated amplification (TMA). It is quite possible to reduce the lower limit of detection (<5 IU / mL of HBV DNA). This is particularly necessary in OBI because HBV DNA levels range from less than 10 to 425 copies/ml. However, false negatives and positives are close to the threshold due to the distribution of virions and specimens of blanc. According to these recommendations, primers should be specific to different genomic regions of HBV and complementary to highly conserved (genotype-shared) nucleotide sequences. Usually, the amplified PCR genes are S

and X. The use of highly sensitive methods, such as PCR, increases the risk of false positive results due to different contaminations or amplicons in the target (Price et al., 2017).

2.8.3. Performance of HBsAg assays(J.-P. Allain, 2016)

Since the first assays available in 1970, HBsAg testing has been steadily improving until enzyme immunoassays utilizing monoclonal antibodies reached in 2002 a sensitivity of 0.13–0.62 ng/mL for licensed assays and 0.07–0.12 ng/mL for three investigational assays since released for use by FDA. In 2006, a comparative evaluation of 17 HBsAg CE marked assays indicated 0.018 to 0.1 IU/mL sensitivity range for serotype AD and 0.012–0.11 IU/mL for AY. Table 1 summarizes the performance of current HBsAg assays utilized in articles reporting on OBI. Since then, several assays with higher sensitivity were developed and clinically evaluated that used chemiluminescence enzyme immunoassay (CLEIA) or chemiluminescence immunoassay (CLIA). The limit of detection (LOD) reached 0.025 ng/mL compared to 0.2 ng/mL for CLIA. A modified CLEIA claimed to reach a sensitivity of 0.005 IU/mL. These improved assays were developed for two main purposes: improve the detection of the early infection window period and monitor antiviral treatment. The latest assays claim to be able to replace HBV DNA detection in monitoring treated patients. However none of these assays specifically compared HBsAg and DNA in the circumstances of OBI.

As shown below, the hope of LOD similar to HBV DNA was defeated by the very nature of OBI. In a range of circumstances, the major hydrophobic region (MHR) of the HBV surface protein can be mutated with amino acid changes potentially affecting detection with HBsAg assays. This situation is particularly frequent in OBI of genotype A2-D, less so in genotypes A1 and E. In addition to sensitivity, ability to detect variants is critical for the diagnosis of OBI. It is recommended to retest HBV DNA positive samples, anti-HBc positive with an alternative sensitive HBsAg assay that may more effectively detect particular HBsAg variants(J.-P. Allain & Opare-Sem, 2016).

2.8.4. Performance of HBV nucleic acid testing (NAT) (J.-P. Allain, 2016)

The detection of HBV DNA was clearly key to the identification of OBI and commercial assays with increased sensitivity became recently available. The impact of sensitivity was particularly illustrated in a study conducted in Hong Kong comparing two commercial assays (Ultrio and Ultrio Plus, Grifols) from the same manufacturer with LOD of 13 and 3 IU/mL, respectively. The yield of both OBI and window period cases doubled with the more sensitive assay applied to random blood donor samples. In general, assays enabling to quantify HBV DNA are derived from the detection assays but the dynamic range of quantification is higher than the 3–5 IU/mL LOD so that a significant proportion of positive HBV DNA samples are below the limit of quantification (LOQ). Since then, a new real-time PCR based assay was

developed by Roche (Cobas CTX) with a claimed 95% LOD of 1.6 IU/mL or 7.4 copies/mL and LOD 50% of 0.3 IU/mL or 1.6 copies/mL.

Such sensitivity should considerably increase OBI detection but at the same time make the assay highly susceptible to contamination. Irrespective of this issue, all reactive results with any HBV NAT need to be confirmed with an alternative assay of similar sensitivity. Short of an alternative test with sensitivity matching the screening method, 5–10 repeats of the screening assay on the same or an alternative sample has been advocated arguing of the Poisson distribution of HBV DNA template in OBI samples. There are algorithms allowing to transform the number of repeat reactive into viral load at very low concentration.

2.8.5. Relation between HBsAg and HBV DNA levels (J.-P. Allain, 2016)

During the window period, two studies showed clearly the correlation between HBsAg and viremia. They also showed that below 300 IU/mL of HBV DNA, HBsAg was no longer detectable when tested with an assay with LOD 0.1 IU/mL. In this case, it appears that most of the detected HBsAg corresponds to full, infectious, complete virus Dane particles. In contrast, after chronic infection is established, there seem to be a switch in infected cells, enhancing S protein production in large but variable excess found in circulation as free or aggregated protein together with lipids forming pseudo-particles. These pseudoparticles are the majority of what is detected as HBsAg. Irrespective of genotype, but particularly frequent in genotype D, a substantial proportion of HBsAg positive samples (3–15%) carry no detectable HBV DNA. However, when increasing the NAT sensitivity, more of these samples are DNA positive, suggesting that the discrepancy is mostly related to assay sensitivity. In cases of OBI, among other mechanisms, specific amino acid substitutions in the S protein prevent the export of HBsAg explaining the lack of detection of HBsAg in circulation.

2.9. Epidemiology of occult hepatitis B viral infection

The prevalence of OBI varies from one region of the world to another. This variability is based on the sensitivity of HBV DNA assays, sample size, and detection of HBV DNA in liver tissue and serum by nested PCR or real-time PCR(Minuk et al., 2005). OBI cases have been reported even in some low HBV endemic areas(Minuk et al., 2005). The prevalence of BIO in the general population would have been 45.5% for B and C genotypes in China and 1.7% to 6.6% for C2 genotype in South Korea(Makvandi, 2016). In Iran, the prevalence of OBI has been reported to be 2 in 50,000 in blood donors and 14% in cryptogenic patients, while the prevalence of HIV-positive OBI was 2.27% and 0% in blood donors(Raimondo et al., 2013). In Egypt, it ranged from 4.1% to 26.8% in hemodialysis patients. In donors in Egypt, it ranged from 4.1% to 26.8% in hemodialysis patients. In healthy Egyptian blood donors, El-Sherif et al. reported a prevalence of 10% in 2007(El-She, 2007); Zeinab et al. reported 17.2% in 2017(Said et al., 2013); in a review published in 2015, Ashraf *et al.* presented other prevalence

from different studies in Egyptian blood donors: 1.3% in 1999, 1.26% in 2005, 0.4% in 2008(Elbahrawy et al., 2015).

In Burkina Faso, Diarra et al. reported in general population in 2018 a prevalence of OBI of 7.3%, all genotype E and A3(DIARRA et al., 2018). In Ghana in 2007, Zahn *et al.* reported 1.7% in blood donors and 1.5% in pregnant women(Zahn et al., 2008).

In Sudan, Mahgoub *et al*. (2011) reported a prevalence of 4.6% of OBI in blood donors (5 were genotype D and one genotype B)(Mahgoub, Candotti, El Ekiaby, & Allain, 2011). In recent studies in blood donors in Nigeria (a country that shares with Cameroon a 1,600-kilometer land boundary), Opalaye et al. (2015) found 17%, Nna et al. found 8%, Osuji et al. (2018) found 14% OBI in blood donors. In Cameroon, Salpini et al (2016) reported in HIV infected patients, a prevalence of 6.9% of OBI (12/175)(Salpini et al., 2016); and Gachara et al (2017) reported a prevalence of OBI of 5.9% in Cameroonian HIV patients(Gachara et al., 2017).

2.10. OBI and blood transfusion and transplant

Blood transfusion is a major risk factor for OBI transmission when donor screening is not conducted under strict quality assurance conditions(Seo, Whang, Song, & Han, 2015). In developed countries, the nucleic acid amplification test (NAT) is used to screen blood donors for the detection of HCV, HIV and HBV or BIO. Data show that the application of NAT for the detection of HBV DNA, HCV RNA and HIV is considerably more sensitive(Niederhauser, 2011). Therefore, the implementation of NAT testing is more sensitive than HBsAg assay as a preventive measure for HBV or OBI transmission through blood transfusion(R. González et al., 2010). In the most developing countries, HBV testing in blood donors remains relies only on serological screening of HBsAg. Although HBV NAT testing is expensive, it is effective in reducing the transmission of OBI through transfusion. Anti-HBc detection is a good test for OBI testing (Antar W, El-Shokry MH, Abd El Hamid WA, 2010). Therefore, the implementation of the anti-HBc test for blood donors may be considered as a second safeguarding policy aimed at reducing the transmission of HBV by blood transfusion(Antar W, El-Shokry MH, Abd El Hamid WA, 2010; Said et al., 2013), although NAT is more sensitive and effective than HBsAg serology as a preventive method for transferring HBV or OBI by blood transfusion. Liver transplantation is the only solution for patients with chronic end stage liver failure(Akram, 2018). However, in liver transplant recipients with OBI, HBV reactivation is favored by induced immunosuppression factors and rapidly leads to graft failure and death(Xie et al., 2015).

2.11. HBV residual risk via blood transfusion

Predominantly, the residual risk of HIV, HBV or HCV infections in blood or plasma donations is defined as the probability of collecting a donation from an asymptomatic viraemic donor infected with one of these bloodborne viruses, and this not being detected by the routine screening assays.

The residual risk of missing viral infections using any screening assay is mainly due to the viraemic phase of the diagnostic window period (vDWP) for each assay – the mean size of which varies between different assay categories. Another component of the residual risk is the virus epidemiology of the donor population (consisting of repeat and first-time donors) with the rate of new infections (incidence) in donors determining the probability of window-period donations.

2.11.1. **Estimation of incidence and window period modelling of risks**

- **Incidence**(World Health Organization, 2016)

The rate of new infections of repeat donors (incidence) is defined as the number of NAT conversions or seroconversions (number of infected donors) divided by the total number of person years of observation of all donors during the study period. Determining the person years of observation requires a computer system that records the follow-up periods for each individual donation. This kind of information management system is often not available in resource-limited blood establishments.

For the purpose of these WHO Guidelines, both the estimation of incidence and the estimation of the residual risk per blood donation are derived from data from the repeat-donor population for the period of one calendar year (365 days). Incidence is calculated by dividing the number of newly infected repeat donors by the total number of repeat donors, usually expressed as the number of new infection cases per 100 000 repeat donors. If one calendar year is taken as the observation period then the incidence is expressed as per 100 000 person years. This simplification assumes that each repeat donor has been followed for one year during the calendar year and that differences in follow-up periods for individual donors will average out at one person year of observation per donor.

In low-incidence regions the number of positive donors may show strong year-to-year variation. For these situations longer periods may be chosen for the calculation of residual risks.

Screening-positive donations that were excluded for other reasons (for example, donor self-exclusion) may be excluded from the calculation (adjusted incidence).

Formula 1: Incidence (per 100 000 person years)

$$\text{Incidence} = \frac{\text{number of repeat donors tested positive during one year}}{\text{total number of repeat donors in the year}} \times 100\,000$$

- **Residual risk per blood donation in repeat donors**(World Health Organization, 2016)

For calculating the probability that a blood donation has been collected during the vDWP different factors are involved:

- the rate of new infections (incidence) in the repeat-donor population,
- the length of the vDWP for the assay used.

The residual risk of a blood donation from a repeat donor having been collected during the vDWP of the screening assay used can be calculated as follows:

Formula 2: Residual risk (RR) per donation

$$\text{RR per donation} = \text{vDWP} \times \text{incidence}$$

RR is usually expressed as per million donations (for which one has to multiply the RR figure calculated above by 1 million.

Formula 2 can be directly used to calculate RR for HIV and HCV infections in repeat donors; for HBV infections RR calculated by this formula has to be multiplied by an HBV incidence adjustment factor.

An adjustment factor of ≥ 1 is necessary because HBV (sero) conversions in repeat donors may be missed due to the transient nature of viraemia and antigenaemia in HBV infections that resolve after the acute phase. Such a transient infection course is seen in adults for the majority of HBV infections (95%) while 5% become chronic carriers. The probability of missing transiently detectable HBsAg or HBV DNA in repeat donors by respective screening assays depends on the length of the IDIs and on assay sensitivity. The donation frequency of repeat donors (average number of donations per repeat donor) determines the average length of the IDI. The average IDI (in days) can be calculated by dividing the observation period of one calendar year (365 days) by the average number of donations per repeat donor.

2.11.2. HBV Residual Risk Reports Worldwide

We review in this section the reports on the HBV residual risk from all over the world. As the majority of the reports referred to the developed countries, a separate review was made regarding the level of economic development.

- **Developed Countries**(Kupek, 2013)

The USA have been leading the residual risk research since the REDS study provided its initial impulse(Kleinman et al., 2014). In the beginning of the 2000 decade, residual risk and NAT yield for HIV, HCV, and HBV were mainly estimated by the incidence/window-period models. For the period 1995–2001, the repeat donors HBV incidence was estimated by HBsAg (surface HBV antigen) alone at

1.27 per 100.000, with this figure twice as high for the first-time donors(Loureiro et al., 2014). Although the HBV residual risk was relatively high (1 : 205.000) in the beginning of the 2000 decade, other infections transmissible by blood transfusion, such as variant Creutzfeld-Jacobs disease, West Nile encephalitis, malaria, Chagas, severe acute respiratory syndrome, and babesiosis, were pointed out as targets for the residual risk research(Corrêa et al., 2018; De Mendoza, Altisent, Aznar, Batlle, & Soriano, 2012; Kleinman et al., 2014). Using revised HBV window period of 30–38 days, a marked reduction in the HBV residual risk was calculated from 1 : 86.000–1 : 110.000 in 1997–99 to 1 : 280.000–1 : 355.000 in 2006–8(S A Glynn et al., 2000). The most recent estimates of the HBV residual risk in the USA were approximately 1 : 300.000(Kleinman et al., 2014).

In Europe, a mathematical model of residual risk and NAT yield for HIV, HCV, and HBV projected the yield of 1.2 per million by ID (individual donation) NAT for the year 1997, considered a rather small gain(S Laperche, S., & Laperche, 1998). Another mathematical modeling of the cost per life-year gained with enhanced sensitivity HBsAg tests and HBV ID NAT was evaluated at 0.73 and 5.8 million euros p/10 million donations, respectively, for Europe in year 2000(Kiely et al., 2017). The former option was deemed acceptable and the latter too costly at the time. After the introduction of NAT screening for the EU countries, more precise cost-effectiveness measures such as ICER (incremental cost-effectiveness ratio) per quality adjusted life-year became available, showing a value of 303.000 euros and 519.000 euros for HBV MP (minipool) NAT and ID NAT, respectively, for the Netherlands by the end of the 2010 decade(Annambhotla, Gurbaxani, Kuehnert, & Basavaraju, 2017; Chandrashekar, 2014). The former option was thought acceptable and the latter too expensive.

- **Developing Countries**(Kupek, 2013)

Among developing countries, the African continent has shown the most dramatic situation regarding residual risk not only for HIV but also for HBV. In the Sub-Saharan Africa, about half of the blood donors were deferred because of HBsAg-reactive rapid test result (Owusu-Ofori et al., 2005). Mathematical modeling of the HBV residual risk in the same region used the WHO and published incidence and prevalence data to arrive at HBV infection risk by transfusion of 4.3%(Jayaraman, Chalabi, Perel, Guerriero, & Roberts, 2010). In Senegal, the HBV residual risk was estimated at 1:1000 in the first half of the 2010 decade (Jayaraman et al., 2010). In Abidjan, the capital of the Ivory Coast, the HBV residual risk was estimated as 1 : 383 (Jayaraman et al., 2010), and in Conackry, the capital of Guinée, it reached staggeringly 1:121 . More recently, a pooled analysis of the data from 51 blood banks in 17 African countries showed low sensitivity (75.6%) of serological HBV testing by HBsAg and anti-HBc due

to widespread use of rapid tests(Loua, Sow, Magassouba, Camara, & Baldé, 2004). The specificity of the tests was also lower (94.5%) compared to the developed countries.

South Africa was the first African country to introduce HBV ID NAT screening, thus being able to observe in the first year of its implementation a yield of 1 : 36.612 for HBV and 1 : 5.200 for OBI among HBsAg-positive donors(Loua et al., 2004). More recently, the HBV ID NAT yield was estimated more precisely at 1 : 19.608, of which 1 : 25.627 in pre-anti-HBc and 1 : 83.473 in post-anti-HBc window period (Vermeulen et al., 2009).

In the Middle East, an analysis from Egypt showed that, in highly endemic region with anti-HBc prevalence of 7.8%, adding this marker to routine HBsAg screening of blood donors yielded 0.5% HBV-DNA-positive test results (Antar, El-Shokry, Abd El Hamid, & Helmy, 2010). Another research showing the utility of this HBV marker for routine screening in hyperendemic areas was realized in Shiraz, Iran, where the anti-HBc yield reached 6.55% and that of HBV DNA testing 12.2% among HBsAg-negative blood donors. On the other hand, this yield was shown to generate a very high discard of likely noninfectious blood donors in Turkey as 99% of the yield cases were HBV DNA negative(Behzad-Behbahani et al., n.d.). Similar concern was raised in New Delhi where almost 20% of HBsAg-negative donors were reactive to anti-HBc and 37% of these tested anti-HBs negative but 41.4% had anti-HBs titer exceeding 100 UI/L, considered protective against HBV infection(Antar et al., 2010; Jayaraman et al., 2010).

2.11.3. Methods for Estimating the HBV Residual Risk(Kupek, 2013)

The Retroviral Epidemiology Donor Study (REDS) has extensively used the incidence/window-period model in the second half of the 1990 decade to estimate the risk of an infected donation entering the blood supply despite negative results of the serologic screening(Behzad-Behbahani et al., n.d.). The risk became known as “residual risk” and was calculated as a product of two independent probabilities: (a) the probability of a donor being infected during the study period, that is, the incidence of a viral infection in the donor population, and (b) the probability of detecting an infectious donation by available serologic markers, which tends to zero in the early phase of infection when serologic screening cannot detect specific viral antigens or antibodies, known as immunologic window period. This approach has been widely applied to HIV, HCV, and HBV, with some adjustments in the latter case. In the beginning of the 2000 decade, it became an internationally accepted method, applied in various countries(Simone A. Glynn, Kleinman, Wright, & Busch, 2002).

A synthesis of the methods for calculating the HBV residual risk based on routinely used serological markers HBsAg and/or anti-HBc (IgG + IgM) is presented. Other methods using anti-HBc IgM or anti-

HBe are not considered here as these are mainly research driven and unlikely to be used for routine residual risk monitoring in blood bank setting in the near future(Syria Laperche et al., 2008).

- **The Standalone HBsAg**(Kupek, 2013)

This method is based solely on HBsAg screening and is restricted to repeat blood donors. It includes an adjustment proposed by Korelitz and colleagues that takes into account the transient nature of the marker by dividing its average duration (63 days for the second generation EIA, 77 days for ChLIA|PRISM) by the length of the average interdonation interval (IDI) among HBsAg seroconverting repeat donors. The seroconversion is defined as the first positive test result after the last negative result, and the time between the two makes the IDI in question. Over time, more stringent case definitions have been adopted, such as repeatedly positive serologic test results after the first one, sample to cutoff values greater than six, and confirmation of HBV DNA by NAT , but the core model remained the same. In addition, this model assumes that 5% of the repeat donors may be chronic HBV carriers whose antigens or antibodies are always detected by serologic screening and that 25% of the donors never produce detectable HBsAg levels due to the primary antibody response, thus leading to the following calculation of the probability of detecting an incident HBV case by HBsAg testing:

$$P = 0.7\left(\frac{\sum s1}{\mathrm{Mdn}\left(\mathrm{IDI}_{s1}\right)}\right) + 0.05, \qquad (1)$$

where and are the number of HBsAg seroconverting repeat donors and their median IDI, respectively(Vermeulen et al., 2012). Although most applications so far have used median IDI, mean IDI has been suggested more recently.

The adjustment factor for the incidence density (rate) is then, and the incidence is the number of HBsAg seroconverting repeat donors divided by all the repeat donor person-time at risk, assuming that the seroconversion occurred at the midpoint of the IDI. Finally, the HBV residual risk is given by multiplying the incidence by its adjustment factor and the WP duration:

$$\text{Residual risk} = \left(\frac{\sum s1}{P\left(\sum \mathrm{IDI}_{\mathrm{ad}} - 0.5\sum \mathrm{IDI}_{s1}\right)}\right)\mathrm{WP}. \qquad (2)$$

It is worth noticing that various parameters of the above equation may be highly variable between the donor populations. IDI depends on the donation frequency; the larger the proportion of the repeat donors, the higher the precision of this parameter. Often the definition of repeat donors is restricted to

those who donated within last 12 months, whereas those with larger time span between donations are considered “lapsed donors”. A variety of social, demographic, and cultural factors influence IDI. Some authors have argued that the assumptions about the incidence adjustment factor may not hold for some donor populations, although its overall impact on the residual risk is likely to be limited. Modifications of the WP model should also include assumed duration of the HBsAg detectability, extended from 63 days for the second generation EIA to 77 days for CHLIA.

- **Müller-Breitkreutz Model**(Kupek, 2013)

The probability of an infected repeat donor may be estimated by cumulative incidence, that is, the proportion of incident cases among all repeat donors, and the probability of an infected WP donation may be estimated by dividing the window duration by the median for HBsAg seroconverting donors , thus leading to the following expression(Sang & 2000, n.d.):

$$\text{Residual risk} = \frac{\sum s1}{n_{\text{rd}}\left(\text{WP}/\text{Mdn}\left(\text{IDI}_{s1}\right)\right)}. \qquad (3)$$

Again, median is motivated by a small number of HBsAg seroconverting donors, and average preseroconversion interval depends on the test sensibility. The cumulative incidence estimate used here is a proportion which does not take into account a variable person-time denominator as opposed to the incidence density or rate.

- **HBsAg Yield**(Kupek, 2013)

Another method for estimating HBV incidence and residual risk was named “HBsAg yield method”. It requires both HbsAg and anti-HBc serologic markers. It modifies the case definition in the Müller-Breitkreutz model by considering as seroconverters only those HBsAg-positive repeat donors who were also anti-HBc negative, thus excluding those who tested positive on both markers. Then the probability of an infected repeat donor is again estimated by cumulative incidence and the probability of an infected WP donation by dividing the window duration by the median IDI for HBsAg seroconverting donors, thus leading to the following expression for calculating the residual risk (RR):

$$\text{Residual risk} = \frac{\sum s2}{n_{\text{rd}}\left(\text{WP}/\text{Mdn}\left(\text{IDI}_{s2}\right)\right)}. \qquad (4)$$

The cumulative incidence is called the “yield rate”, although it is not a density-type incidence estimator to which the expression “rate” is normally applied in epidemiology(Kupek, 2013). The HBsAg

preseroconversion interval is restricted by the onset time for anti-HBc and is named “yield window”. The ratio of the cumulative incidence between the repeat and the first-time donors is denominated the “yield rate ratio” and is used as a risk ratio estimate for HBV seroconversion, that is, as a multiplying factor which allows to extrapolate the residual risk calculation to the first-time donors as well. This is an important gain over the methods restricted to repeat donors only whose residual risks are known to be significantly lower when compared to the first-time donors from the past research. However, it is worth noticing that the use of cumulative incidence ratio between the groups under comparison may be applied to any method willing to assume the accuracy of this approximation.

- **Extended Yield Method (HBsAg and Anti-HBc Yield)** (Kupek, 2013)

Recently, an extension of the yield method was proposed to include anti-HBc (IgM and IgG) seroconverting donors within last year in addition to the HBsAg only seroconverters. In other words, the repeat donor who had tested anti-HBc negative on previous donation not longer than a year ago and tested anti-HBc positive after that is considered an incident HBV case, despite negative HBsAg test results on both occasions. By analogy, such cases may be considered “anti-HBc yield”. These donors are most likely recently infected due to the relatively short IDI. As recrudescent HBV infection is a very rare event for repeat donors due to previous screening history and low HBV incidence in this group, the appeal of adding anti-HBc is twofold:

(a) it reduces the probability of false negative screening result due to the transient nature of HBsAg marker and propensity to primary antibody response, and

(b) it increases the precision of the incidence estimates by including more cases; typically, the anti-HBc yield is greater than the HBsAg one in a blood bank setting. This simply reflects the higher probability of finding a stable and often permanent marker of HBV infection such as core antibody compared to a transient marker such as surface antigen whose average duration is only 63 days.

- **Other Methodological Considerations** (Kupek, 2013)

There are several additional methodological issues to be considered. First, the misclassification of seroconverting cases depends on their confirmation algorithm. For a HBsAg reactive test, it may require the same result on subsequent independent blood samples or anti-HBc or HBV NAT positive results. In addition, values exceeding six may also be used to reduce false positive fraction for HBsAg. Some authors have proposed HBV confirmation criteria based on reactive anti-HBc and a variety of other markers of HBV infection, such as anti-HBc IgM, anti-HBe, anti-HBs without receiving the HBV vaccine, or HBV DNA. However, many blood banks in the developing countries use, routinely only, only anti-HBc total (IgM +IgG) and HBsAg with subsequent testing for anti-HBs if any of the former were reactive. It is

worth noticing that self-reported HBV immunization may be inaccurate, particularly regarding the number of doses received. In order to overcome these limitations, a simplified criterion of repeatedly reactive HBsAg or anti-HBc has been proposed for the confirmation of an incident HBV infection(Syria Laperche et al., 2008).

For the first-time donors, a variety of methods to calculate the HBV seroconversion risk ratio of these to repeat donors were reviewed by Soldan et al. First, the ratio of the HBsAg seroconverting fractions can be calculated between the two donor subpopulations. Second, the anti-HBc prevalence may be used as an estimate of cumulative HBV incidence because this marker is persistent in the vast majority of HBV-infected individuals since the sixth month after the infection(Zou, Musavi, Notari, Stramer, & Dodd, 2010). Third, the time at risk for the first-time donors may be estimated by assuming that they were exposed to HBV since birth and that the infection occurred at the mid-point of exposure time, so that it equals half their age at the time of HBV-seropositive test result. Fourth, it is possible to estimate probability of the HBV infection for the first-time donors during lifetime and their probability of donating blood during infectious window period, so that multiplying these probabilities (assuming that they are independent) gives an estimate of a window-period donation for this group. The next step is to multiply this estimate by the HBV-seropositive fraction among the first-time donors. The same procedure can be applied to the repeat donors, so that the risk ratio between two donor subpopulations estimates the ratio of the window-period donation probabilities, that is, the HBV residual risk ratio.

Most of the above estimation methods have been developed for the HIV whose pandemic circulation could be estimated with reasonable precision for the purpose of these calculations. However, no such analogy exists for the HBV, thus assuming that the time of risk for HBV starting at birth is heavily dependent on the HBV vertical transmission rate which varies hugely between the countries and regions. For example, in the countries with low HBV prevalence and universal child vaccination, the risk of HBV infection in childhood is extremely low as compared to hyperendemic areas with high vertical transmission rate. Given all these uncertainties regarding the first-time donors time at risk for HBV infection, a simulation of various parameters seems a sensible approach(Soldan, Barbara, Ramsay, & Hall, 2003).

III. Materials and methods

III. Materials and methods

3.1. Framework of the study

They investigations were made in Yaoundé (Cameroon) and in Paris (France), precisely at:

- The Blood Bank of the Yaounde University Teaching Hospital (YUTH), Cameroon, where samples were collected and screened for HBsAg, HIV and HCV serology. The YUTH is one of the main reference hospitals and the unique teaching hospital in the country. Its Blood Bank realizes different activities such as blood collection, processing and testing, preparation of blood products, conservation and distribution to health facilities;
- The Reference Laboratory of Virology of the National Institute of Blood Transfusion in Paris (France) where further investigations were made (screening for HBcAb, western blot for HIV and HCV, HBV DNA PCR and sequencing, confirmation of HBsAg for DNA positive samples). The National Institute of Blood Transfusion (INTS) is an actor of the French transfusion landscape, its missions are the exercise of reference activities, research and training to help improve blood safety. Its activities contribute to blood transfusion safety, prevention of known or emerging risks and the adaptation of transfusion activity to scientific and technological developments.

3.2. Materials

3.2.1. Biological material

The biological material was constituted of blood sampled collected from each participant in two dry tubes of 5 ml.

3.2.2. Reagents

The different reagents used were:

- Murex HIV Ag/Ab combination (Diasorin),
- Murex HBsAg Version 3 (Diasorin),
- Murex HCV Ag/Ab Combination (Diasorin),
- Monolisa™ Anti-HBc PLUS (Biorad),
- INNO-LIA™ HCV Score,
- HIV BLOT 2.2 Genelabs Diagnostic,
- DNA extraction : High Pure Viral Nucleic Acid Kit (Roche Life Science),
- COBAS® TaqMan® HBV Test For Use With The High Pure System (Roche Molecular Diagnostics).

3.2.3. Equipement

- ELISA chain biotek in Cameroon
- ELISA chain tecan in France
- GeneAmp PCR System 9700 (Applied Biosystems, USA)

Figure 3. GeneAmp PCR System 9700 (Applied Biosystems, USA)

3.2.4. Other materials

Micropipettes, Vortex, cone or filter tip, incubator, centrifuge

3.3. Methods

3.3.1. Study design and duration

We conducted a descriptive and cross-sectional study from the 1st August 2016 to the 31st March 2017.

3.3.2. Studied population

The study subjects were blood donors received at the Blood Bank of the Yaoundé University Teaching Hospital, Yaoundé.

- **Inclusion criteria:** The target population was all consent blood donors
- **Exclusion criteria:**
  - Donors having withdrawn their consent during recruitment;
  - Donors with HBsAg reactive;
  - Donors reactive for HIV and HCV;
  - Donors HBsAg false negative after confirmatory testing.

3.3.3. Sample size

The table below provides the number of samples required for the study according to the prevalence expected and the confidence intervals around this prevalence expressed in number of donors and number of HBsAg negative donors (HBsAg prevalence estimated at 15 %).

Table III. Sample size determination

Expected OBI prevalence	+/-95% CI	Total Number of donors	Number of donors HBsAg Negative (Prevalence of 15%)
1%	1%	366	311
1%	2%	94	80
1%	3%	42	36
2%	1%	700	595
2%	2%	185	157
2%	3%	83	71
3%	**1%**	**1005**	**854**
3%	2%	272	231
3%	3%	123	105

We planned to recruit 1005 HBsAg-negative donors in order to detect a prevalence of OBI between 1% and 3% with an accuracy of +/- 1%.

3.3.4. Measures

- **Demographic caracteristics :** Sex and age
- **Type of donation:** Family or Benevolent
- **Serological testing results of:** HIV, HBsAg and HCV
- **Screening for Occult B infection**: HBcAb and HVB DNA

3.3.5. Ethical issues, recruitment of participants and sample collection and processing

We applied and obtained an authorization for research from the General Directorate after the approval of the hospital ethical committee of the Yaounde University Teaching Hospital (annexe xx).

Prior to recruitment at the Blood Bank of YUTH, each participant was informed about the study through an information leaflet (annexe xx); they read and sign the consent form (annexe xx). Then, a questionnaire (annexe xx) was administered to each individual.

Two dry tubes of blood were collected from each consent participant from derived blood bag during blood donation. After clot formation, the tubes were centrifuged at 3.000 rpm for 5 minutes and two

aliquots of 2 ml of serum were made and stored at – 30°C. Later on, they were transported to Paris in the dry ice in the triple packing system and stored at – 80°C before screening.

We followed the algorithm below:

3.3.6. Initial screening of blood donors

Blood donors were initially screened for HBV, HIV and HCV (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]) at the YUTH.

3.3.7. Screening for HBcAb

All donor negative for HBsAg were screened for HBcAb using Monolisa™ Anti-HBc PLUS (Biorad) in Paris, France(Biorad, n.d.).

- **Clinical interest of the test**

The detection of antibodies against the main hepatitis B nucleocapsid or antigen is the main way to diagnose the presence of past (anti-HBc Total) or recent (anti-HBc IgM) infections by the hepatitis B virus. Hepatitis B. Patients infected with chronic hepatitis B virus in general have high levels of anti-HBc that can persist for a long time and can be observed in at least three clinical situations: associated with HBsAg, associated with anti-HBs antibodies and, in some cases, alone.

- **Principle of the test**

Monolisa ™ Anti-HBc PLUS is an enzyme immunoassay (indirect ELISA) for the detection of total antibodies to hepatitis B virus in human serum or plasma. Monolisa Anti-HBc PLUS is based on the use of a solid phase prepared with a recombinant HBc antigen.

- **Different steps of the manipulation:**

01. The sera samples to be tested and the controls are added to the wells. If anti-HBc antibodies are present, they will bind to the antigens attached to the solid phase.
02. Peroxidase-labeled human anti-IgG and IgM antibodies are added after a washing step. They bind in turn to specific antibodies captured on the solid phase.
03. After removal of unbound enzymatic conjugate, the antigen-antibody complex is revealed by adding substrate.
04. After the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-700 nm. The absorbance measured for a sample allows the presence or absence of antibodies to HBc to be determined. The colour intensity is proportional to the quantity of anti-HBc antibodies bound on the solid phase.

05. After stopping the reaction, the absorbance values are read with a spectrophotometer at 450/620-700 nm. The absorbance measured for a sample determines the presence or absence of anti-HBc antibodies. The intensity of the color is proportional to the amount of anti-HBc antibodies bound to the solid phase.

➢ **Calculation and interpretation of the results**

The presence or absence of anti-HBc antibodies was determined by comparing for each sample the absorbance recorded with that of the calculated cut-off value.

- The mean of the absorbance values for the positive control serum was calculated as follows (OD R4) Example: Positive control R4

Well with positive control serum	Optical density
C1	1.796
D1	1.802
E1	1.852
Total	5.450
Mean	**5.450/3=1.817**

- **Calculation of the cut-off value (Vs)**

Vs=Mean of OD R4/5= 1.817/5=**0.363**

The validation criteria were as follows:

- For the Negative Control: Each measured absorbance value must be less than 0.100.
- For the positive control: each absorbance value must be greater than or equal to 1 000 and less than or equal to 2,900.

- **Interpretation of the results**

Samples with optical density below the cut-off value were considered negative with Monolisa ™ Anti-HBc PLUS test. Samples with optical density greater than or equal to the cut-off value were initially considered positive with the Monolisa ™ Anti-HBc PLUS test. However, for the initial samples that were either reactive or questionable (0.9 <ratio <1), after a new test, the sample was considered positive with the Monolisa ™ Anti-HBc PLUS test if it was greater than or equal to the cut-off value. The sample was considered negative with the Monolisa ™ Anti-HBc PLUS test if both values were below the cut-off value.

3.3.8. HIV and HCV confirmation

Positive samples for HIV and HCV were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively.

3.3.9. HBV DNA extraction and amplification

For HBcAb positive cases, HBV DNA was tested mini-pools of two samples (MP-2) using the Cobas Taqman HBV Quantitative Test (Roche, 95% LQ <6 IU / mL). Concerning the minipools (MP-2) positive positive for DNA, the initial sera were tested separately using the quantitative Cobas Taqman HBV assay.

3.3.9.1. HBV DNA extraction using High Pure Viral Nucleic Acid Kit (Roche Life Science)(Roche, 2007)

- **Test Principle**

As a pre-condition for analysis of viral nucleic acids by polymerase chain reaction (PCR) or RT-PCR, it is necessary to isolate the DNA from serum, plasma or whole blood. The virus is lysed by incubating the sample in a special lysis / binding buffer in the presence of proteinase K. Subsequently, the nucleic acids bind specifically to the surface of the glass fibers in the presence of a chemotropic salt. The binding reaction occurs in a few seconds due to the disruption of the organized structure of the molecules and the interaction of the nucleic acids with the surface of the glass fibers. Thus, the adsorption on the glass fiber is favored. Since the binding process is nucleic acid specific, the bound nucleic acids are purified from salts, proteins and other impurities by a washing step and are eluted in a low salt buffer or in water.

- **Procedure used for preparation of nucleic acids from 200 µl samples of plasma or serum**

01. In a nuclease free microcentrifuge tube of 1.5 ml:

- Add 200 µl serum or plasma
- Add 200 µl of freshly prepared working solution of carrier RNA-supplemented Binding Buffer,
- Add 50 µl of proteinase K solution, and mix immediately.
- Incubate at +72°C for 10 min.

02. Add 100 µl of Binding Buffer and mix

03. To transfer the sample to a High Pure Filter Tube:

- Insert one High Pure Filter Tube in one Collection Tube.
- Pipette the entire sample into the upper reservoir of the Filter Tube.

04. Insert the entire High Pure filter tube into a standard tabletop centrifuge. Centrifuge 1 min at 8000×g
05. After centrifugation: Remove the filter tube from the collection tube, discard the through-flowing liquid and the collection tube. Combine the filter tube with a new collection tube.
06. After combining the Filter Tube with a new Collection Tube, add 500 µl Inhibitor Removal Buffer to the upper reservoir of the Filter Tube and centrifuge 1 min at 8,000 × g.
07. After centrifugation: Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and the Collection Tube. Combine the Filter Tube with a new Collection Tube.
08. After removal of the inhibitors: Add 450 µl of wash buffer to the upper reservoir of the filter tube. Centrifuge 1 min at 8000 × g and eliminate the flow.
09. After the first washing and centrifugation:

- Remove the filter tube from the collection tube, discard the flow liquid and the collection tube.
- Combine the filter with a new collection tube.
- Add 450 µl Wash Buffer to the upper reservoir of the filter tube.
- Centrifuge 1 min at 8000 × g and eliminate the flow.
- Leave the filter tube and filter tube assembly in the centrifuge and rotate it for 10 seconds at maximum speed (13,000 × g) to remove any residual wash buffer.

10. Discard the collection tube and insert the filter tube into a sterile 1.5 ml nuclease-free centrifuge tube.
11. To elute viral nucleic acids: Add 50 µl of elution buffer to the upper reservoir of the filter tube. Centrifuge the tube for 1 min at 8000 × g.
12. The centrifuge tube now contains eluted viral nucleic acids. The viral DNA was used directly in the PCR.

3.3.9.2. HBV DNA amplification using COBAS® TaqMan® HBV Test(Roche, 2017)

- **Intended use**

The COBAS® TaqMan® HBV Assay for use with the High Pure System (HPS) is an in vitro nucleic acid amplification assay for the quantification of hepatitis B virus (HBV) DNA human plasma or serum, using the High Pure System Kit viral nucleic acid kit for manual sample preparation and the COBAS® TaqMan® 48 analyzer for automated amplification and detection.

- **Principles of the procedure**

The COBAS® TaqMan® HBV Test is based on two main processes: (1) manual sample preparation to obtain HBV DNA; (2) Automated PCR amplification of the target DNA using complementary HBV-

specific primers, and detection of dual-cleaved fluorescent dye-labeled oligonucleotide probes that allow quantification of the HBV target amplified product (amplicon) and Quantitation Standard HBV DNA, processed, amplified, and detected simultaneously with the sample.

- **Specimen Preparation**

The treated specimen, containing HBV DNA and standard HBV quantification DNA, was added to the amplification / detection mixture. The HBV target DNA and standard HBV quantification DNA were then amplified and detected on the COBAS® TaqMan® 48 Analyzer using the amplification and detection reagents provided in the test kit.

- **PCR Amplification**
  - **Target Selection**

The selection of the target DNA sequence for HBV depends on the identification of regions of the HBV genome that exhibits maximum conservation of the sequence among all genotypes. Therefore, proper selection of primers and probe is essential to the ability of the assay to detect all clinically relevant HBV genotypes. A genome region of the partially single-stranded circular DNA of HBV has been shown to have maximum retention of DNA sequences among genotypes. The COBAS® TaqMan® HBV assay uses PCR amplification primers that define a sequence within the highly conserved pre-core / core region of the HBV genome.

The complete HBV genome was amplified using nested primers and validated with Sanger sequencing using specific primers. The HBV genome was divided into 2 fragments (Fig.) and both rounds of nested PCR were performed in 25 µL reaction system.

Frag*	Primer	Sequences (5′–3′)	Position (nt)	Size† (bp)
A	P3	CTCGCTCGCCCAAATTTTTCACCTCTGCCTAATCA	1825–1841	2092
	AR1	ACAGTGGGGGAAAGC	759–745	
	AR2	AGAAACGGRCTGAGGC	702–687	
B	P4	CTGGTTCGGCCCAAAAAGTTGCATGGTGCTGG	1823–1806	1320
	AF1	GTCTGCGGCGTTTTATC	419–435	
	AF2	TGCCCGTTTGTCCTCTA	503–519	

* Whole genome of HBV was divided into 2 fragments: A and B. For the fragment A, P3 and AR1 were mixed in the first round of PCR, P3 and AR2 were mixed in the second round of PCR. For the fragment B, P4 and AF1 were mixed in the first round of PCR, P4 and AF2 were mixed in the second round of PCR.

† Size represented the products of second round of nested PCR.

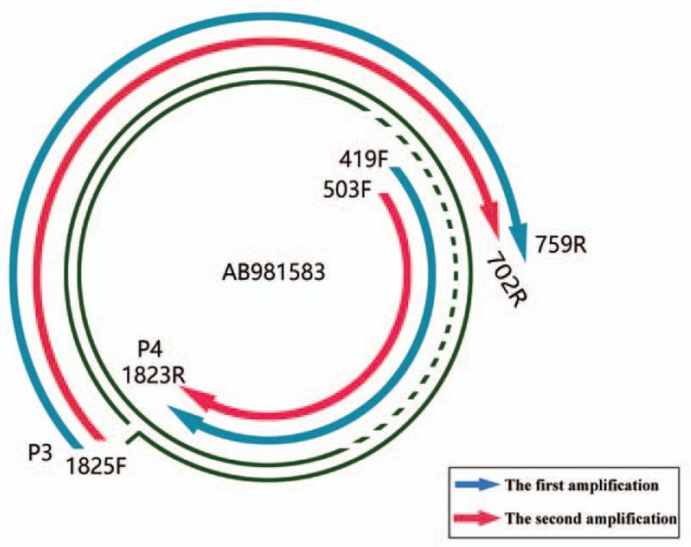

Figure 4. The structure of HBV genome and nested PCR

The blue arrow indicates the first round of amplification and the red arrow indicates the second round of amplification. The primers are depicted along with the HBV genome.

- **Target Amplification**

Treated samples were added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. The COBAS® TaqMan® 48 analyzer thermal cycler heated the reaction mixture to denature the double-stranded DNA and exposed the target sequences of the specific primer on the genome of the HBV circular DNA and HBV standard DNA. When the mixture cooled, the primers were annealed on the target DNA. The thermostable DNA polymerase Thermus specie Z05 (Z05) in the presence of $Mn2^{+}$ and deoxynucleotide in excess triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine and deoxyuridine (in place of thymidine), extended the ring finger initiated along the target matrix to produce double-stranded DNA molecule called amplicon. The COBAS® TaqMan® 48 analyzer automatically repeated this process for a defined number of cycles, each cycle being intended to double the amount of amplicon DNA. The number of cycles required was preprogrammed in the COBAS® TaqMan® 48 Analyzer. Amplification occurred only in the region of the HBV genome between the primers.

- **Detection of PCR Products in a COBAS® TaqMan® Test**

The amplification of HBV DNA and HBV Quantitation Standard DNA were measured independently at different wavelengths. This process was repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent

identification of HBV DNA and HBV Quantitation Standard DNA. The intensity of the signals was related to the amount of starting material at the beginning of the PCR.

- **HBV DNA Quantification**

The COBAS® TaqMan® HBV assay quantifies the viral DNA of HBV using a second target sequence (HBV quantification standard) that is added to each sample at a known concentration.

During the PCR annealing phase on the COBAS® TaqMan® 48 analyzer, samples are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each sample. The readings of each sample are then corrected for instrumental fluctuations. These fluorescence measurements are sent by the instrument to the AMPLILINK software and stored in a database. Screening checks were used to determine whether the HBV DNA and standard HBV quantification data were valid sets, and indicators were generated when the data were outside the predefined limits. Once all the pre-checks are completed and successful, the fluorescence measurements are processed to generate the Ct values for HBV DNA and standard HBV DNA. The batch-specific calibration constants provided with the COBAS® TaqMan® HBV assay were used to calculate the titre value for the samples and controls based on the Ct values of HBV DNA. The COBAS® TaqMan® HBV Test complies with the WHO International Standard for Testing the 97/74629 HBV Assay and titration results are expressed in International Units (IU / mL).

3.3.10. HBV DNA sequencing and genotyping

3.3.10.1. HBV DNA sequencing

Following nested PCR amplifications, HBV DNA sequences were obtained definitively confirming OBI status. Three additional minipools tested DNA low positive (<6 IU/mL) but the 6 anti-HBc positive samples included in these pools were DNA non-reactive when tested individually. Considering the extremely low DNA signal and the lack of sample volume to perform multiple repeat testing or viral particle concentration.

3.3.10.2. HBV DNA genotyping

The Viral Epidemiology Signature pattern Analysis program (VESPA; https://www.hiv.lanl.gov/content/sequence/VESPA/vespa) was used. No OBI-specific mutation was observed within the S and Core. In the Pol region (sample OBI-0312), two unusual mutations were present within the catalytic area of the RT/polymerase: V518A and A731G. However, impossible to speculate on potential functional consequence

3.3.11. Analyses statistiques

The Statistical Package of Social Sciences (SPSS) version 18 was used in statistical analysis. Cross tabulation, Chi square (X^2), and ANOVA test were used to detect the significant differences between the various groups, *P-value* less than 0.05 was considered significant.

IV. RESULTS AND DISCUSSION

IV. Results and Discussion

4.1. Results

4.1.1. Demographic characteristics and serological profile of participants

The mean age of the 1166 was 29.24 (SD= 8.214), the median was 27 years, the minimum was 18 years and the maximum 64 years. The table below shows the distribution of gender in different age groups.

- **Age and gender**

Table IV. Distribution of gender according to age groups

Age groups	Gender		
	Male	Female	Total n(%)
[18;20]	84	14	98(8,40)
[21;30]	557	108	665(57,03)
[31;40]	249	26	275(23,58)
[41;50]	90	8	98(8,40)
[51;60]	24	4	28(2,40)
[60;65]	2	0	2(0,17)
Total	1006	160	1166(100)

1006 were male and 160 female, with a sex ratio of 6,28. The majority of participants (57,03%) were aged between 21 to 30 years old.

- **Donor status**

The donor status distribution according to age groups is shown in the table below (table II).

Table V. Donor status according to age groups

Age groups	Donor status			
	Volontary		Family	
	Effectif	N %	Effectif	N %
[18;20]	40	40.82	58	59.18
[21;30]	209	31.43	456	68.57
[31;40]	51	18.55	224	81.45
[41;50]	22	22.45	76	77.55
[51;60]	6	21.43	22	78.57
[60;65]	1	50.00	1	50.00
Total	329	28.22	837	71.78

Volontary and family donors were respectively 329 (28.22%) and 837 (71.78%).

4.1.2. Serological profile of donors

The 1.166 donors were initially screened for HBsAg, HCVAb and HIVAb. The table below (table III) shows the serological profile according to gender, donor status and age groups.

Table VI. Serological profile of blood donors' participants after initial screening

Characteristics	ELISA Murex HBsAg Version 3 N=1166 n(%)		ELISA Murex HCV Ag-Ab Combo N=1166 n(%)		**ELISA Murex HIV Combo** N=1166 n(%)	
	Positive 91(7,80)	**Negative 1075(92,20)**	**Positive 11(0,94)**	**Negative 1155(99,06)**	**Positive 14(1,2)**	**Negative 1151(98,8)**
Gender	***p-value=0,63***		***p-value=0,18***		***p-value=0,42***	
Male	81(8,05)	925(91,95)	11(1,09)	995(98,91)	13(1,39)	992(98,61)
Female	10(6,25)	150(93,75)	0(0,00)	160(100,00)	1(0,63)	159(99,38)
Type of donor	***p-value=0,68***		***p-value=0 ,45***		***p-value=0,06***	
Voluntary	25(7,60)	304(92,40)	2(,61)	327(99,39)	1(0,30)	328(99,70)
Family	66(7,89)	770(92,11)	9(1,08)	828(98,92)	13(1,67)	823(98,33)
Age group (year)	**p-value=0,24**		**p-value=0,52**		**p-value=0,89**	
[18;20]	9(9,18)	89(90,82)	0(0,00)	98(100,00)	2(2,04)	96(97,96)
[21;30]	51(7,67)	614(92,33)	4(0 ,60)	661(99,40)	6(1,05)	658(98,95)
[31;40]	21(7,64)	254(92,36)	2(0,73)	273(99,27)	4(1,45)	271(98,55)
[41;50]	10(10,20)	88(89,80)	2(2,04)	96(97,96)	2(2,04)	96(97,96)
[51;60]	0(,00)	28(100,00)	2(7,14)	26(92,86)	0(0,00)	28(100,00)
[60;65]	0(,00)	2(100,00)	1(50,00)	1(50,00)	0(0,00)	2(100,00)

The prevalences of HBsAg, HIVAb and HCVAb after Enzyme ImmunoAssay screening of 1166 samples were respectively 7.80% (n=91), 0.94% (n=11), 1.2 %(n=14). There was a statistical difference between HCV infection and age (*p=0.0001*).

After retesting of HCV and HIV positive samples by western blot and AgHBs by ELISA Murex version 3, data below were obtained (table IV).

Table VII. Serological profile of blood donors' participants with HBsAg retested by ELISA and confirmed HIV and HCV by Western Blot

Characteristics	HBsAg N=1162 n(%)		INNO-LIA HCV N=1162 n(%)		HIV BLOT 2.2 N=1162 n(%)	
	Positive 91(7,83)	**Negative 1071(92,17)**	**Positive 03(0,26)**	**Negative 1159(99,74)**	**Positive 4 (0,34)**	**Negative 1158(99,66)**
Gender	***p-value=0,630***		***p-value=0,12***		***p-value=0,424***	
Male	81(8,08)	921(91,92)	3(0,30)	999(99,70)	4(0,40)	998(99,60)
Female	10(6,25)	150(93,75)	0(0.00)	160(100.00)	0(0,00)	160(100,00)
Type of donor	***p-value=0,686***		***p-value=0,062***		***p-value=0,209***	
Voluntary	25(7,62)	303(92,38)	00(0.00)	327(100.00)	0(0,00)	328(100,00)
Family	66(7,91)	768(92,09)	3(0,36)	831(99,64)	4(0,48)	830(99,52)
Age group (year)	**p-value=0,243**		***p-value=0,52***		**p-value=0,288**	
[18;20]	9(9,18)	89(90,82)	0(0,00)	98(100)	1(1,02)	97(98,98)
[21;30]	51(7,70)	611(92,30)	1(0 ,15)	661(99,85)	0(0,00)	662(100,00)
[31;40]	21(7,66)	253(92,34)	0(0,00)	274(100)	2(0,73)	272(99,27)
[41;50]	10(10,20)	88(89,80)	0(0,00)	98 (100)	1(1,02)	97(98,98)
[51;60]	0(0,00)	28(100,00)	1(3,57)	27(96,43)	0(0,00)	28(100,00)
[60;65]	0(0,00)	2(100,00)	1(50)	1(50)	0(0,00)	2(100,00)

The prevalence of HIVAb and HCVAb after Western-blot screening of samples were respectively 0.26% (n=3), 0.34 %(n=4).

4.1.2. Hepatitis B Virus core antibody (*HBcAb*) screening

The table below shows the prevalence of HBcAb according to demographic and serological characteristics (including HBsAg).

Table VIII. Frequency of HBcAb according to demographic and serological characteristics (including HBsAg)

HBcAb					
Characteristics		**Positive N=613 (52,75)**	**Negative N=549(47,25)**	**Total N=1162(100)**	**P value**
Gender	Male	536(53,49)	466(46,51)	1002	**0,2**
	Female	77(48,13)	83(51,88)	160	
Type of donor	Benevolent	158(48,17)	170(51,83)	328	**0,05**
	Family	455(54,56)	379(45,44)	834	
Age groups (years)	[18;20]	43(43,88)	55(56,12)	98	**0,001**
	[21;30]	326(49,24)	336(50,76)	662	
	[31;40]	165(60,22)	109(39,78)	274	
	[41;50]	58(59,18)	40(40,82)	98	
	[51;60]	21(75,00)	7(25,00)	28	
	[60;65]	0(,00)	2(100,00)	2	
HBsAg	Positive	91(100,00)	0(0,00)	91	**0,0001**
	Negative	522(48,74)	549(51,26)	1071	
Murex HIV1/2	Positive	7(50.00)	7(50.00)	14	**0,61**
	Negative	606(52,79)	542(47,21)	1148	
HIV BLOT 2.2	Positive	2(50,00)	2(50,00)	4	***0,99***
	Negative	611(52,76)	547(47,24)	1158	
ELISA Murex HCV Ag-Ab	Positive	5(45,45)	6(54,55)	11	**0,31**
	Negative	608(52,82)	543(47,18)	1151	
INNO-LIA HCV	Positive	1(33,33)	2(66,67)	3	***0,50***
	Negative	612(52,80)	547(47,20)	1159	

The overall prevalence of Total Anti-HBc IgG+IgM was 52,75% (n=613), with a statistical difference significative with age groups(p=0.001). All 91 samples HBsAg positive were also positive for HBcAb (p=0.0001); thus, were excluded from the study.

The table (table VI) below shows the prevalence of HBcAb according to demographic characteristics (excluding HBsAg positive cases).

Table IX. Frequency of HBcAb according to demographic characteristics (excluding HBsAg positive cases)

Characteristics		HBcAb			
		Positive N=522 (48,74)	**Negative N=549 (51,26)**	**Total N=1071 (100)**	**P value**
Gender	Male	455(49,40)	466(50,60)	921	**0,**282
	Female	67(44,67)	83(55,33)	150	
Type of donor	Voluntary	133(43,89)	170(56,11)	303	0,046
	Family	389(50,65)	379(49,35)	768	
Age groups (years)	[18;20]	34(38,20)	55(61,80)	89	**0,0001**
	[21;30]	275(45,01)	336(54,99)	611	
	[31;40]	144(56,92)	109(43,08)	253	
	[41;50]	48(54,55)	40(45,45)	88	
	[51;60]	21(75,00)	7(25,00)	28	
	[60;65]	0(0,00)	2(100,00)	2	

In HBsAg negative participants, the prevalence of HBcAb was 48,74% (n=522). 50,65% of family donors were positive for HBcAb compared to benevolent (49,35%). The statistical difference was significative (p=**0,046).**

After excluding the HIV and HCV positive samples, the frequency of HBcAb only was obtained as indicated in the table below.

Table X. Frequency of HBcAb according to demographic characteristics in seronegative individuals

Characteristics		HBcAb			
		Positive N=511(48,81)	**Negative N=536(51,19)**	**Total N=1047(100)**	**P value**
Gender	Male	444(49,44)	454(50,56)	898	**0,**31
	Female	67(44,97)	82(55,03)	149	
Type of donor	Benevolent	130(43,33)	170(56,67)	300	0,025
	Family	381(51,00)	366(49,00)	747	
Age groups (years)	[18;20]	34(38,64)	54(61,36)	88	**0,0001**
	[21;30]	270(44,93)	331(55,07)	601	
	[31;40]	141(57,09)	106(42,91)	247	
	[41;50]	46(54,76)	38(45,24)	84	
	[51;60]	20(76,92)	6(23,08)	26	
	[60;65]	0(0,00)	1(100,00)	1	

In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48,81% (n=511). The majority of family donors were positive for HBcAb (51,00%) compared to benevolent (49,00%). The statistical difference was significative (p=**0,025). The prevalence of** HBcAb increases with the age, from 38,64% in age group 20-30 years old to 76,92% in the age group 51-60 years old. The statistical difference was significative (p=0001).

4.1.3. Nucleic acid testing by Polymerase Chain Reaction

The Nucleic acid testing (NAT) done on HBsAg negative/HBcAb positive individuals (OBI) gave the following results in the table below.

Table XI. Frequency of HBcAb according to demographic characteristics

Characteristics		HBV DNA in HBsAg negative/HBcAb positive			
		Positive **N=**6(1,15)	**Negative** **N=**516(98,85)	**Total** **N=**522(100)	**P value**
Gender	Male	5(1,10)	450(98,90)	455	**0,**77
	Female	1(1,49)	66(98,51)	67	
Type of donor	Voluntary	0(,00)	133(100,00)	133	0,15
	Family	6(1,54)	383(98,46)	389	
Age groups (years)	[18;20]	0(,00)	34(100,00)	34	**0,049**
	[21;30]	1(,36)	274(99,64)	275	
	[31;40]	5(3,47)	139(96,53)	144	
	[41;50]	0(,00)	48(100,00)	48	
	[51;60]	0(,00)	21(100,00)	21	
	[60;65]	0(,00)	0(,00)	0	

Six on 522 samples (1.15%) were detected as OBI positive; all the six were family donors, 5 were male and age between 31 to 40 years.

Table XII. HBV DNA in HBsAg negative/HBcAb positive individual (Excluding the HIV and HCV positive samples)

Characteristics		HBV DNA in HBsAg negative/HBcAb positive			
		Positive **N=**6(1,17)	**Negative** **N=** 505(98,83)	**Total** **N=**511**(100,00)**	**P value**
Gender	Male	5(1,13)	439(98,87)	444	0,67
	Female	1(1,49)	66(98,51)	67	
Type of donor	Voluntary	0(,00)	130(100,00)	130	0,06
	Family	6(1,57)	375(98,43)	381	
Age groups (years)	[18;20]	0(,00)	34(100,00)	34	0,04
	[21;30]	1(,37)	269(99,63)	270	
	[31;40]	5(3,55)	136(96,45)	141	
	[41;50]	0(,00)	46(100,00)	46	
	[51;60]	0(,00)	20(100,00)	20	
	[60;65]	0(,00)	0(,00)	0	

Excluding the HIV and HCV positive samples, the risk of transmission of OBI in HBcAb positive samples was 1.17.

Table XIII. Demographic and viral characteristics of 613 HBcAb positive blood donors

parameters	HBsAg positive (n = 91)	HBsAg negative (n = 522)
Gender	9 F/82 M	63 F/ 459 M
Age (mean [years])	28.5	30.6
HBV DNA		
Positive	20	6
Negative	1	514
Not tested	70	2
Viral load (IU/mL)		
Median	293.5	<6
Range	<6 - 75933	<6

Six samples fulfilled the consensus definition of OBI: low HBV DNA load (all <6 IU/mL) with no detectable HBsAg and detectable HBcAb. Following nested PCR amplifications, HBV DNA sequences were obtained for 4 of these samples (1 nearly whole genome [3123 nt], 2 Pre-S/S regions [1356 nt], and 1 S region [445 nt]) definitively confirming OBI status.

Three additional minipools tested DNA low positive (<6 IU/mL) but the 6 anti-HBc positive samples included in these pools were DNA non-reactive when tested individually. Considering the extremely low DNA signal and the lack of sample volume to perform multiple repeat testing or viral particle concentration, it is not possible to differentiate between false-positive result and non-repeat reactive OBI. Based on minipool+individual testing DNA positivity, the prevalence of OBI can be estimated to 0.5% (6/1164) in blood donors irrespective of HBcAb status or 0.98% (6/613) in HBcAb+ only donors. Despite limited numbers, OBI carriers appeared older (35 y) than HBsAg+ donors (28.5y), HBcAb+/DNA- donors (30.6 y), and non-infected donors (28 y).

Table XIV. Characteristics of donors with OBI

Samples	Gender	Age	Type	HIV	AcHBc	HBV DNA	Viral load (IU/mL)
OBI-0312	M	40	F	Négative	Positive	Positive	<6
OBI-0547	M	39	F	Negative	Positive	Positive	<6
OBI-0554	M	31	F	Negative	Positive	Positive	<6
OBI-0597	F	30	F	Negative	Positive	Positive	<6
OBI-0612	M	33	F	Negative	Positive	Positive	<6
OBI-0797	M	*37*	F	Negative	Positive	Positive	<6

This table shows that 6 participants were identified as OBI. Among them, 5 were men; all the 6 were family donors and aged between 30 to 40 years old.

4.1.4. Genotyping and phylogenetic analysis

Phylogenetic analysis identified genotype E in all OBI samples whereas previous epidemiological data from Cameroon are contradictory about the genotype A/genotype E ratio: 65% A vs 35% E or 67% E vs 33% A. Maybe useful to consider sequencing HBsAg+ samples from the present study to verify this difference between genotype distributions according to populations.

Partial S sequences (640 nt) from 3 OBI samples were compared with 66 and 70 corresponding sequences obtained from genotype E-infected HBsAg+ blood donors collected in France and Guinea, respectively. The Viral Epidemiology Signature pattern Analysis program (VESPA; https://www.hiv.lanl.gov/content/sequence/VESPA/vespa) was used. No OBI-specific mutation was observed within the S and Core. In the Pol region (sample OBI-0312), two unusual mutations were present within the catalytic area of the RT/polymerase: V518A and A731G. However, impossible to speculate on potential functional consequences **(Figure 1).**

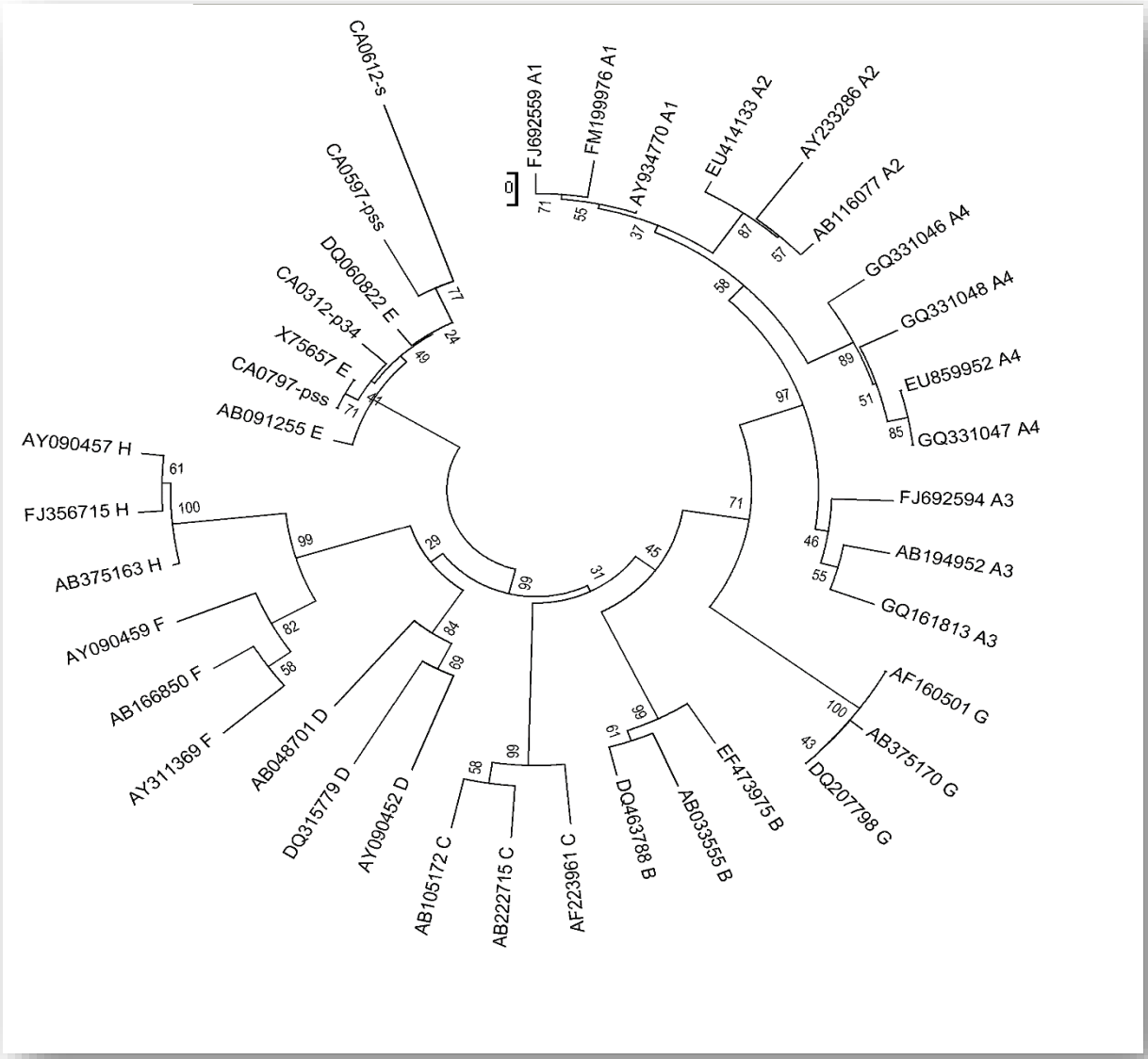

Figure 5. Phylogenetic analysis

4.2. Discussions

This survey determines the prevalence of occult hepatitis B infection in blood donors at the Blood Bank of the Yaounde University Teaching Hospital, Cameroon.

Overall, 52.7% (613/1162) of blood donors in Yaounde, Cameroon, had serological markers of HBV infection. The 7.83% prevalence of HBsAg+ chronic carriers was lower than the overall 10.5% prevalence recently reported from the meta-analysis of 12 studies conducted in Cameroonian blood donors between 2001 and 2016 (Bigna et al., 2017).

This study also found OBI in approximately 1% of HBsAg negative/HBcAb positive blood donors.
The prevalence of HBcAb has been reported to be elevated in areas highly endemic for HBV(Badrawy & Bakry, 2013). In sub-Saharan African countries (such as Burkina Faso, Ghana, Sudan, Nigeria), the prevalence of OBI varies from 1.7% to 14% (10–14). The high rate of HBcAb is found in people aged > 50(J. P. Allain & Candotti, 2009). This may be related to the vertical transmission and horizontal transmission before the age of five, resulting in frequent chronic infections which leads to occult infection. In areas of high endemicity such as Cameroon, the value of information is limited because the majority of the population of blood donation age is anti-HBc. Thus, In Cameroon, blood donors seem to be highly prevalent to anti-HBc compared to blood donors in Egypt, whereas both countries are highly endemic for HBV.

Generally, the prevalence of OBI is higher in the area of high endemicity of HBV(Gutiérrez-García, 2011); But the prevalence of OBI in Cameroonian blood donor is lower than in other Sub-Saharan African countries: 1.7% in Ghana(Zahn et al., 2008), 4.6% in Sudan (Mahgoub et al., 2011), 7.3% in Burkina Faso (DIARRA et al., 2018), in Nigeria 8% to 14% in blood donors(A et al., 2018; Oluyinka et al., 2015). This difference can be explained by the techniques used and the screening strategy. The OBI may be lower in blood donors compared to patients as reported in the study conducted in the same setting. This can be explained by the medical selection, the collection of blood among healthier subjects. Moreover, HIV has been demonstrated to be a risk factor of HBV(Gachara et al., 2017; Salpini et al., 2016). The implementation of NAT testing for HBV at the Yaoundé University teaching Hospital Blood Bank may prevent 56 HBV transmission per 10,000 donations. This implementation remains a challenge in Cameroon were resources are limited for equipment, reagents and few staff qualified.

The prevalence of Anti-HBc has been reported high in HBV high endemic areas(Badrawy & Bakry, 2013). Other studies in Egyptian blood donors reported a prevalence of 7.8-16.6% (Antar W, El-Shokry MH, Abd El Hamid WA, 2010; Said, 2013). The high rate of Anti-HBc is found in people aged > 50(J. P. Allain & Candotti, 2009). This may be related to the vertical transmission and horizontal transmission before age five, resulting in frequent chronic infections which leads to occult infection. This marker is associated with contact with HBV whether resolved or chronic infection results from such contact. It becomes detectable in post-acute infection some days or weeks after the peak of HBsAg and HBV DNA has been reached(20). In high endemic areas like Cameroon, the value of the information is limited since a majority of the population of blood donation age carries anti-HBc. Thus, In Cameroon, blood donors seem to be highly prevalent to anti-HBc compared to blood donors in Egypt, whereas both countries are highly endemic for HBV.

All OBI identified were genotype E. The circulation of three HBV genotypes A, E and D was reported in patients in Cameroon, however, genotypes A and E are predominantly found(Kramvis & Kew, 2007). In sub-Saharan African countries (such as Burkina Faso, Ghana, Sudan, Nigeria), the prevalence of OBI in blood donors are of genotypes A3, B, D and E (10–14). Several factors are involved in the recognition of the occult HBV infection, including viral variants carrying mutant HBsAg that are not recognized by specific antibodies used in assays for HBsAg(Zhu, Li, Li, & Zhang, 2016) and low level expression of HBV genes(Pollicino et al., 2004). As in the present study, the viral loads of the six samples were <6 IU/mL strong suppression of viral replication and gene expression may have resulted from the host immune responses(Samal, Kandpal, & Vivekanandan, 2012).

This study had some limitations. First, our study is conducted in a specific hospital based blood service and may not reflect the prevalence of OBI in the entire country. However, the prevalence of HBsAg and HBC Ab are similar to those found in various areas in Cameroon and even in Africa. Second, we did not screen HBsAg negative samples for HBsAb, and we cannot rule out false HBcAb positive that could have overestimated anti-HBc only prevalence but the volume of samples was limited and it was not possible to test with another test. Also, it would have been useful to consider sequencing HBsAg+ samples from the present study to verify the difference between genotype distributions according to populations. Finally, we do not have risk factors of HBV infection to investigate HBV infection in our blood donors' population and the number of OBI was too low to identify specific characteristics of them.
High HBcAb prevalence excludes the use of HBcAb screening to avoid blood shortage which is a critical issue in Africa. HBV nucleic acid amplification testing is effective in reducing the risk of HBV transmission if performed on individual donations; however, the cost of such a strategy is prohibitive in a

resource-limited setting like Cameroon. Alternatively, better strategies to identify risk factors for HBV in blood donors and defer those at risk may also limit the risk of transfusion-transmitted OBI.

V. PUBLICATIONS AND PRESENTATIONS

V. Publications and presentations

5.1. Articles

5.1.1. **Article 1:** Occult Hepatitis B infection from blood donors from Cameroon in *Blood Transfusion*

Occult hepatitis B infection among blood donors from Yaoundé, Cameroon

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Background. In Cameroon, the prevention of hepatitis B virus (HBV) transmission by blood transfusion is still only based on hepatitis B surface antigen (HBsAg) screening. However, occult HBV infection (OBI) characterised by the absence of detectable HBsAg and low level of viral DNA remains a potential threat for blood safety. The prevalence of OBI was investigated in blood donors from Yaoundé to provide evidence-based recommendations to improve HBV blood safety.

Material and methods. Blood donations from August 1st, 2016 to March 31st, 2017 were routinely screened for HBV, human immunodeficiency virus (HIV), and hepatitis C virus (HCV) infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV investigations were performed, including hepatitis B core antibody ([HBc] Monolisa Anti-HBc PLUS; BIO-RAD) and HBV DNA tested in minipools of two samples using the quantitative Cobas Taqman HBV assay (Roche; LoQ: 6 IU/mL) and HBV DNA genotyping by sequencing.

Results. Of 1,162 donations analysed, 91 (7.8%) were reactive for HBsAg. All of them were also anti-HBc positive. Among the 1,071 HBsAg negative samples, 522 (48.7%) were reactive for anti-HBc. Six (0.56% of all donations) samples fulfilled the consensus definition of OBI and showed low HBV DNA loads (all <6 IU/mL). Following nested polymerase chain reaction amplifications, HBV DNA sequences were obtained for 4 of these samples (1 nearly whole genome [3123 nt], 2 Pre-S/S regions [1,356 nt], and 1 S region [445 nt]). Phylogenetic analysis identified genotype E in all samples.

Discussion. Around 1 in 100 Cameroonian blood donors screened who resulted HBsAg negative and anti-HBc positive carried occult HBV infection. HBsAg alone for screening prospective donors is not sufficient to eliminate the risk of HBV transfusion transmission in Cameroon, and because anti-HBc screening does not seem to be feasible without compromising blood supply, implementation of HBV nucleic acid testing could be considered when possible.

Keywords: occult hepatitis B infection, blood donors, Yaoundé-Cameroon.

Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus belonging to the *Hepadnaviridae* family[1]. The virus is responsible for the fact that chronic hepatitis B represents a major global health problem with more than 240 million chronically infected persons worldwide, particularly in low- and middle-income countries (LMICs)[2]. HBV infection remains the most common viral infection transmitted by blood transfusion[3]. Over the past decades, the risk of HBV transfusion transmission has been steadily reduced through the recruitment of volunteer donors, the selection of donors based on behavioural-risk assessment, the development of increasingly more sensitive hepatitis B antigen (HBsAg) assays, and, in some countries, the use of hepatitis B core antibody (anti-HBc) screening and HBV nucleic acid testing (NAT)[1].

Occult HBV infection (OBI) is defined by detectable low level of HBV DNA (<200 IU/mL) in liver or serum with undetectable HBsAg and with/without anti-HBc or anti-HBs, excluding the pre-seroconversion window period (WP). The molecular basis of OBI is the persistence of covalently closed circular DNA (cccDNA) in the cell[4]. OBI has been reported among healthy asymptomatic blood donors, patients with chronic liver disease, and patients with hepatocellular

carcinoma[5]. The prevalence of OBI tends to be higher in regions with high HBV endemicity[6].

In Cameroon, as in most developing countries, screening for HBV among blood donors and patients relies only on serological detection of HBsAg[7]. In the absence of anti-HBc testing, blood transfusion carries the risk of transmitting HBV infection from donors with OBI[8,9]. Besides the cost, that is not always affordable by LMICs, HBV-DNA detection by NAT has been proven to be a reliable preventive measure against HBV transmission from donors with OBI[4]. In Cameroon, the only data on OBI have been reported in human immunodeficiency virus (HIV) positive patients showing prevalence rates between 5.9 and 6.9%[10,11]. Therefore, in order to provide evidence-based recommendations to improve HBV blood safety, a study was carried out to establish OBI prevalence in blood donors from Yaoundé, Cameroon.

Materials and methods

Samples

A total of 1,162 blood donors were included consecutively in the study at the Blood Bank of the Yaoundé University Teaching Hospital (YUTH), Cameroon. Two 1 mL aliquots of serum were obtained from each donor sample. These were stored at –20°C, and later transported under appropriate conditions to the National Reference Centre (NRC) for Infectious Risks in Blood Transfusion of the National Institute of Blood Transfusion in Paris, France, where further HBV investigations were performed. Prior to recruitment at the Blood Bank of the YUTH, we received research authorisation from the General Manager of the YUTH and ethical clearance from the Regional Ethical Committee in Yaoundé. Each participant was informed about the study through an information leaflet and was invited to sign a consent form. Furthermore, to collect information on demographics, each blood donor was asked to fill in a questionnaire as per routine practice. Before the samples were transported for serological and molecular biology testing in France, the research authorisation of the French Ministry of Health was obtained by the National Reference Center for Infectious Risks in Blood Transfusion, Paris, France.

Blood donation testing

Donations were screened routinely for HBV, HIV and hepatitis C virus (HCV) infections in the blood bank of YUTH using the Murex® HBsAg Version 3, Murex® HIV Ag/Ab Combination, and Murex® HCV Ag/Ab Combination (DiaSorin SpA, Saluggia, Italy), respectively. Additional testing performed at the National Institute of Blood Transfusion in Paris, France, included anti-HBc (Monolisa™ Anti-HBc PLUS; Biorad, Marnes-la-Coquette, France) and HBV DNA in minipools of two samples (MP-2) using the quantitative Cobas® Taqman® HBV assay (Roche Diagnostics; Meylan, France; 95% LOQ of 6 IU/mL). When positive, minipools were resolved by testing each individual sample in the pool with the same NAT assay.

Hepatitis B virus DNA analysis

The nearly whole genome (3,123 nt), and the Pre-S/S (1,356 nt) and S (445 nt) regions of HBV were amplified by nested PCR as previously described[12]. Amplified products were purified and directly sequenced using the Sanger method (GATC Eurofins Biotech, Constance, Germany). Multiple S sequence alignment and phylogenetic analysis were performed as previously described[13]. The Viral Epidemiology Signature pattern Analysis programme[14] was used to compare the deduced amino acid sequences obtained in this study with 66 and 70 corresponding sequences obtained from genotype E-infected HbsAg-positive blood donors collected in France and Guinea, respectively[15,16].

Statistical analysis

The Statistical Package of Social Sciences (IBM SPSS® Statistics, Armonk, NY, USA) version 21 was used in statistical analysis. Cross tabulation and χ^2 test were used to detect the significant differences between HBV parameters (HBsAg, anti-hepatitis B virus core antibody [HBcAb], HBV DNA) and the donor characteristics (gender, age groups and donor type). $p<0.05$ was considered statistically significant.

Results

Blood donors included in the study were 1,002 males (86.2%) and 160 females (13.7%) with a mean age of 29.24 years (standard deviation [SD]=8.21). The distribution of voluntary and family donors was 328 (28.2%) and 834 (71.8%), respectively.

Of these 1,162 donors, 91 (7.8%) tested HbsAg positive. All HbsAg positive samples and 522 (48.7%) out of 1,071 HbsAg negative samples were reactive for HBcAb (Figure 1). Repeat testing to confirm HBcAb reactivity was not possible due to limitations of sample volume. There was no relationship between the presence of HBsAg and demographic characteristics (age, gender and donor type). In contrast, HBcAb reactivity was significantly associated with age (p=0.0001) (Table I).

Hepatitis B virus DNA was tested in the 522 $HBsAg^-/HBcAb^+$ donors by using MP-2 NAT and 6 out of 255 (2.3%) mini-pools were found reactive. Pool resolution identified 6 HBV DNA reactive samples (one reactive in each initially reactive pool) with individual HBV DNA load <6 IU/mL that were classified as OBI. Five were males, aged 33-40 years

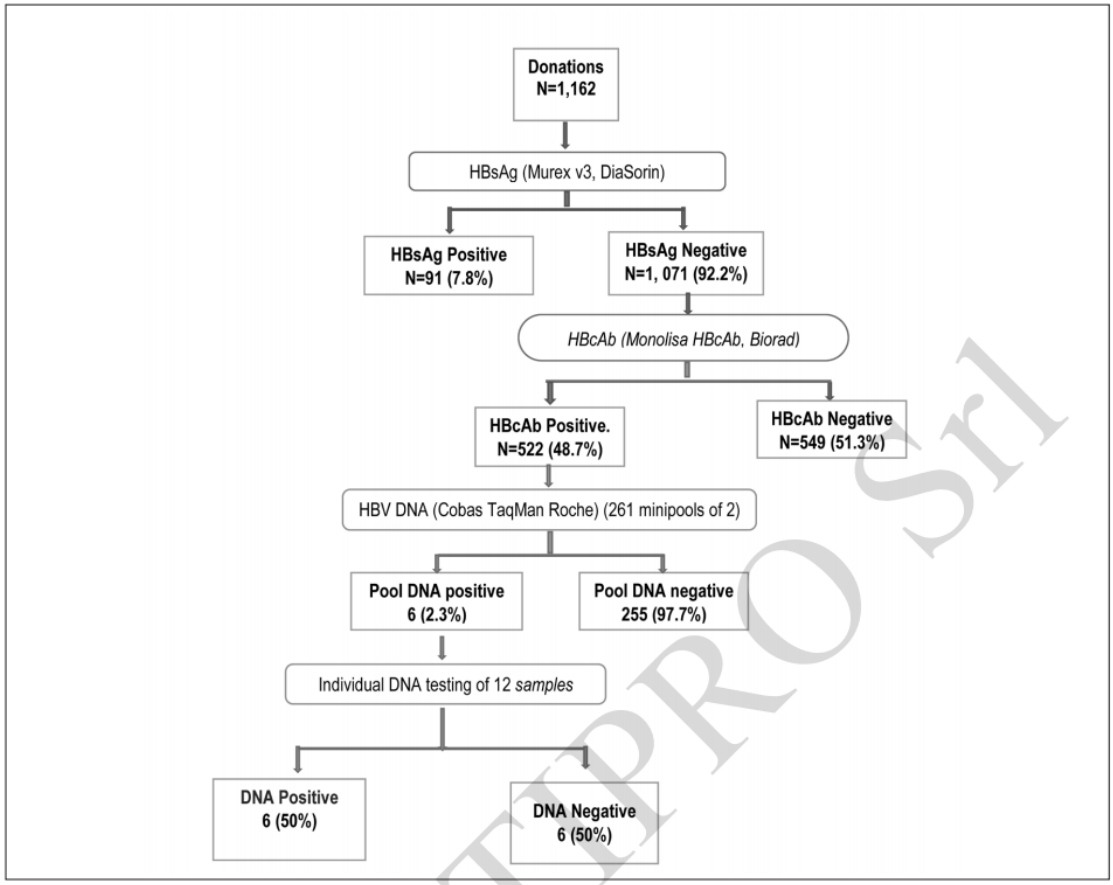

Figure 1 - Flow chart of serological and molecular investigations of hepatitis B in blood donations collected at the University Teaching Hospital Blood Service, Yaoundé, Cameroon.

Table I - Demographic and viral characteristics of 1,162 Cameroonian blood donors at the Yaoundé University Teaching Hospital in 2017.

Characteristics	HBsAg		HBcAb		HBV DNA	
	Tested	*Positive*	*Tested*	*Positive*	*Tested*	*Positive*
N donors	1,162	91 (7.8%)	1,071[a]	522 (48.7%)	522[b]	6 (1.1%)
Gender						
Male	1,002 (86.2%)	81 (8.1%)	921 (86.0%)	455 (49.4%)	455 (87.2%)	5 (1.1%)
Female	160 (13.8%)	10 (6.3%)	150 (14%)	67 (44.7%)	67 (12.8%)	1 (1.5%)
p-value		*NS*		*NS*		*NA*[a]
Donor type						
Benevolent	328 (28.2%)	25 (7.6%)	303 (28.3%)	133 (43.9%)	133 (25.5%)	0 (-)
Family	834 (71.8%)	66 (7.9%)	768 (71.7%)	389 (50.7%)	389 (74.5%)	6 (1.5%)
p-value		*NS*		*NS*		*NA*
Age groups (years)						
18-20	98 (8.4%)	9 (9.2%)	89 (8.3%)	34 (38.2%)	34 (6.5%)	0 (-)
21-30	662 (57.0%)	51 (7.7%)	611 (57.1%)	275 (45.0%)	275 (52.7%)	1 (0.4%)
31-40	274 (23.6%)	21 (7.7%)	253 (23.6%)	144 (56.9%)	144 (27.6%)	5 (3.5%)
41-50	98 (8.4%)	10 (10.2%)	88 (8.2%)	48 (55.0%)	48 (9.2%)	0 (-)
51-60	28 (2.4%)	0 (-)	28 (2.6%)	21 (75.0%)	21 (4.0%)	0 (-)
61-65	2 (0.2%)	0 (-)	2 (0.2%)	0 (-)	0 (-)	0 (-)
p-value		***NS***		***0.0001***		***NA***

HBsAg: hepatitis B antigen; HBcAb: anti-hepatitis B virus core antibody; HBV DNA: hepatitis B virus deoxyriboinucleic acid; NS: not significant; NA: not applicable. [a]Only HBsAg negative samples were tested; [b]Only HBcAb positive samples were tested.

6.

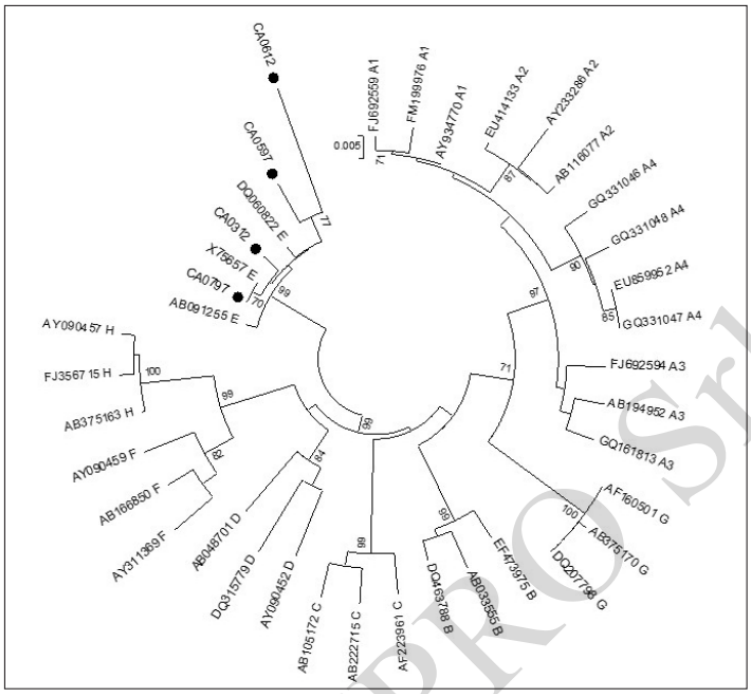

Figure 2 - Phylogenetic tree of Cameroonian occult hepatitis B virus (HBV) infection (OBI) strains based on partial S sequences (445 nt).
Phylogenetic analysis was performed with the neighbour-joining algorithm based on Kimura two-parameter distance estimation method. Only bootstrap values ≥75% are shown (1,000 replicates). Cameroonian sequences are identified by a black dot and HBV reference sequences of genotypes/subgenotypes A1-4, B, C, D, E, F, G, and H are identified by their GenBank accession numbers.

old, and one was a 30-year old female. Mean age of OBI carriers (35 years) was higher than those of HBsAg positive (28.5 years) and non-infected donors (28 years) *(*p=0.7*)* but not significantly different compared to HBcAb positive only donors (30.6 years). All were family donors and none were infected with HCV or HIV. Nested PCR products and the corresponding sequences were obtained for four OBI samples (1 whole genome, 2 Pre-S/S, and 1 S) definitively confirming OBI carriage. Phylogenetic analysis identified genotype E in all OBI samples (Figure 2). OBI sequences were compared to 66 and 70 sequences obtained from HBV genotype E-infected HBsAg-positive blood donors collected in France and Guinea, respectively. No OBI-specific mutation was observed within the S (4 strains) or Core (1 strain). In the Pol region, sample CA0312 showed two unusual amino acid substitutions (V518A and A731G) within the catalytic area of the RT/polymerase.

Discussion

Overall, 52.7% (613 of 1,162) of blood donors in Yaoundé, Cameroon, had serological markers of HBV active or past infection. This study reports a very high prevalence of HBsAg carriers (approximately 8%), which confirms that hepatitis B infection is still a major problem in the region. But this prevalence was lower than the overall prevalence of 10.5% (95% CI: 8.7-12.4) recently reported in a meta-analysis of 12 studies conducted in Cameroonian blood donors between 2001 and 2016[7]. Difference may be related to the medical selection procedure of blood donors at the YUTH Blood Bank and the evolution of the methodologies used over the 15-year period and/or to regional variations in HBV seroprevalence[7].

In the present study, the prevalence of occult HBV carriage was 1.1% (6 of 522) in HbcAb positive donors suggesting an estimated 0.52% (6 of 1,162) prevalence in the total blood donor population of Yaoundé. After elimination of 91 donors who tested HBsAg positive, the potential infectivity of OBI$^+$ donations can be estimated at 0.56% (6 of 1,071). These frequencies are significantly lower than data collected in West and East Africa that showed OBI prevalence of 4.6-17% in selected cohorts

of HBsAg-negative donors and 1.5-1.7% in the general blood donor populations[17-19]. Nevertheless, OBI prevalence also remained lower than that recently reported in HIV co-infected Cameroonian patients (5.9-6.9%)[10,11]. The heterogeneity of data between studies may be explained by differences in the methods and the strategy used. In the present study, HBV DNA was tested in minipools of two samples in a selected subset of the donor population. The dilution introduced by pooling may affect the efficiency of DNA testing, as suggested by the extremely low viral DNA loads (<6 IU/mL) observed in all OBI samples. Strong suppression of viral replication and gene expression may have resulted from the host immune responses. But it could also be due to viral characteristics (e.g., genotype E)[20] and/or to assay performance[21].

In the present study, HBV DNA was not investigated in HBcAb-negative donors and a possible underestimation of the number of HBV DNA carriers cannot be ruled out. Cameroonian OBI donors were predominantly males with a mean age of 35 years, and were infected with HBV genotype E (HBV_E). Genotype E is the most prevalent genotype in western Africa throughout the crescent from Mauritania to Namibia[22]. In Cameroon, HBV genotype A was also prevalent in particular populations including Pygmies, Bantus, and HIV-infected individuals[22,23]. However, the age distribution in Cameroonian OBI donors was similar to that observed in South African OBI donors infected with genotype A1 (30-40 years)[21]. Occurrence of OBI at a younger age does not seem to be related to any particular African HBV genotype but rather to the specificity of the natural history of HBV infection in sub-Saharan Africa that includes mainly horizontal but also vertical transmission in early childhood[24].

The analysis of the limited number of HBV sequences available showed a very limited genetic variability in the OBI strains, similar to that usually observed in non-OBI genotype E strains[12,13].

In particular, the S protein was similar to comparison sequences from genotype E-infected HBsAg-positive chronic carrier blood donors. The host immune pressure did not seem to play a major role in the occurrence of OBI in these HBV_E-infected donors. However, this analysis is limited by the absence of anti-HBs antibody testing. Many different mechanisms are potentially associated with OBI[25]. It is tempting to hypothesize that the presence of the unusual V518A and A731G amino acid substitutions within the catalytic area of the RT/polymerase may affect the efficiency of the viral replication, leading to an OBI phenotype. However, further functional analyses are needed to explore this.

Nucleic acid testing for HBV DNA reduces the risk of HBV transfusion-transmission by detecting OBI as well as acute WP infections[21]. According to the present data, the implementation of HBV NAT at the Yaoundé University Teaching Hospital Blood Bank may intercept at least 52 HBV-infected donations per 10,000 donations. However, the implementation of viral nucleic acid testing is still challenging for low-income countries like Cameroon due to its considerable cost, the limited logistical resources available, and the few adequately trained staff. HBcAb testing might be a potentially affordable alternative for low-income countries, despite the fact it cannot detect WP infections[26]. In addition, the yield of HBcAb in negative HBsAg Cameroonian donors was 48.7%. Despite the lack of confirmatory testing in the present study, this was similar to the 35.1% and 48.4% values reported previously in Ghanaian donors under 20 years of age and older than 40 years, respectively[27]. In contrast, lower HBcAb reactivity rates have been observed in Sudanese (33%) and Egyptian (8%) donors[19,28,29]. Therefore, the introduction of HBcAb screening in the Yaoundé Blood Bank would cause a significant shortage in the blood supply.

In summary, HBsAg screening alone is not sufficient to eliminate the risk of HBV transfusion-transmission in Cameroon. Anti-HBc screening does not seem to be feasible without compromising blood supply and implementation of HBV nucleic acid testing might be considered. However, further studies are needed for a more precise evaluation of the prevalence of HBV DNA in the total blood donor population, the cost-effectiveness, and the specific feasibility issues before considering NAT implementation in Cameroon.

Conclusions

In summary, this study provides clear evidence of OBI infection in blood donors at the Yaoundé University Teaching Hospital, Cameroon. HBV was confirmed highly endemic in Cameroon. More than 50% of blood donors were anti-HBc positive. Around 1 in 100 Cameroonian blood donors screened who tested HBsAg negative and anti-HBc positive carried occult HBV infection. HBsAg alone for screening prospective donors is not sufficient to eliminate the risk of HBV transfusion-transmission in Cameroon, and because anti-HBc screening does not seem to be feasible without compromising blood supply, implementation of HBV nucleic acid testing could be considered when possible. Introducing HBV NAT would certainly reduce transfusion transmitted cases, but the overall impact in limiting HBV epidemic in Cameroon is expected to be negligible in the absence of effective vaccination and treatment campaigns.

Funding and resources

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Authorship contributions

DF and DC are co-first authors. DF, CTT, DM, SL, DC and ELM conceived and designed the study, analysed and interpreted the data, wrote the manuscript and approved the final version. HIK and FEC designed the study, interpreted the data, reviewed the manuscript and approved the final version. CTT, SL and ELM provided study material. DF, CD and DC carried out laboratory testing. DF, DC and SL collected, assembled, analysed and interpreted the data.

The Authors declare no conflicts of interest.

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Serological confirmation of viral infections trends in blood donors in Yaoundé Cameroon

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ABSTRACT

Background. The high prevalence of transfusion-transmissible infections (TTIs) is the most important challenge of safe blood supply in Cameroon. The seroprevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) was determined among prospective blood donors at blood bank Yaoundé University Teaching Hospital (YUTH), Yaoundé, Cameroon.

Material and Methods. Blood donors were consecutively screened for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV testing including anti-HBc (Monolisa Anti-HBc PLUS; BIO-RAD) were performed. HIV and HCV serology were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively.

Results. In total, 1.162 donors were serially included in the study. Screening for viral infections showed that 91 (7.80%) of total sample donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.95%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+. Screening samples for total Anti-HBc IgG+IgM revealed that 613(52.75%) samples were reactive. All of the 91 samples positive HBsAg positive were also positive for HBcAb. In the 1071 HBsAg negative participants, the prevalence of HBcAb was 48.7% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48.8% (n=511). Testing of 13 HIV and 9 of HCV reactive samples revealed that 4 and 3 were confirmed positive by western blot, respectively.

Discussion. This study clearly showed a high prevalence of viral infections among Cameroonian blood donors at the YUTH. Strategies to increase voluntary and regular donors should be intensified to improve the medical selection of blood donors and reduce the frequency of TTIs found in donated blood. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infection prevalence in Cameroonian blood donors.

Key words: Serological confirmation, Viral infections, *Blood donors, Yaoundé-Cameroon.*

Abbreviations

anti-HBc	antibodies against the HBV core antigen
DNA	Deoxyribonucleic acid
anti-HBc	anti-HBc antibodies against the HBV Core antigen
cccDNA :	*covalently closed circular DNA*
EIA :	*Enzyme Immuno-Assay*
HBsAg :	*Hepatitis B Surface Antigen*
HBV cccDNA	Hepatitis B viral convalently-closed-circular DNA
HBV :	*Hepatitis B Virus*
HBV-DNA	Deoxyribonucleic acid of hepatitis B virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HVB :	Hépatite Virale B
INTS :	Institut National de la Transfusion Sanguine
NAT	Nucleic acid testing
OBI	Occult Hepatitis B Infection
PCR	Polymerase Chain Reaction
pgRNA :	*pregenomic RNA*
TTIs	Transfusion Transmitted Infections

BACKGROUND

In sub-Saharan Africa(SSA) the prevalence of viral infections such as Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in

blood donors is high considering their epidemiology and especially the blood safety process and procedures implemented by blood services (Hagos et al., 2018; Heyredin, Mengistie, & Weldegebreal, 2019; Syria Laperche et al., 2009; Tagny et al., 2009; Tayou Tagny et al., 2009). Therefore screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infections from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible(World Health Organization, 2009). According to World Health Organization (WHO) recommendations, screening of all blood donations should be mandatory for the following markers: screening for either a combination of HIV antigen-antibody or HIV antibodies for HIV-1 and HIV-2, screening for hepatitis B surface antigen (HBsAg) for Hepatitis B, screening for either combination of HCV antigen-antibody or HCV antibodies and screening for specific treponemal antibodies(World Health Organization, 2009).

Globally, there are approximately 71 million individuals chronically infected with hepatitis C virus (HCV), more than 257 million with hepatitis B virus (HBV), and 37.9 million human immunodeficiency virus (HIV)-infected people(World Health Organization, 2017, 2018). According to WHO reports, the prevalence of *HBV*, *HCV* and *HIV* varies from 0.008% to 6.08%, 0.004% to 1.96%, and 0.0004% to 2% respectively in different parts of the *world* (Heyredin et al., 2019; Mohsenizadeh, Mollaei, & Ghaziizadeh, 2017).

In SSA the incidence of HIV attributable to transfusion is uncertain; but some findings suggested that only 1% of new HIV infections are attributable to transfusion(Weimer et al., 2019). The prevalence of HBV in blood donors is above 10%, Occult HBV infection remains unaddressed. Also, there is an increase in donor HCV prevalence, despite dedicated donor selection and educational efforts(Weimer et al., 2019).

In Cameroon, there is a National Programme of Blood Transfusion that coordinates blood transfusion activities at the Ministry of Health in collaboration with hospital blood banks. This programme elaborates policies and guidelines in order to harmonize the blood banks practices for blood safety. But there National Centre of Blood Transfusion has been created in 2018 and still yet to function. Despites those measures taken in Cameroon, there are lots of critical challenges to improve supply and safety of blood through voluntary donors (Tagny et al., 2009; Weimer et al., 2019).

Provision of constant and safe blood has been a public health challenge in SSA with high prevalence of TTIs. Monitoring of the magnitude of TTIs in blood donors is important

for determining the risk of transmission of infections and optimizing donor recruitment strategies. Therefore, this study aimed to determine seroprevalence of HIV, HBV, HCV and associated factors among blood donors at the Yaoundé University Teaching Hospital Blood Bank.

Materials and Methods

Study setting and population

Blood donors' sera were collected serially at the Blood Bank of the Yaounde University Teaching Hospital (YUTH) (Cameroon) and were screened for HBsAg, HIV and HCV serology. Two aliquots of recruited donor were made and stored at –20°C and later on transported to the Reference Laboratory of Virology of the National Institute of Blood Transfusion in Paris (France) where further investigations were made (screening for HBcAb, western blot for HIV and HCV, retesting of HBsAg). The study obtained an authorization for research for the General Directorate and the Ethical Clearance from the Regional Ethical Committee for Center Region, Yaounde. Prior to recruitment at the Blood Bank of YUTH, each participant was informed about the study through an information leaflet where they read and signed the consent form. A questionnaire was then answered by each consent participant.

Serological screening and confirmation

Blood donors were screened initially for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]) at the YUTH.

Further serological investigations of viral infections were done at the Reference Laboratory of Virology of the National Institute of Blood Transfusion in Paris (France). All samples were retested by Murex HBsAg Version 3 and HBsAg positive samples were screened for anti-HBc (Monolisa™ Anti-HBc PLUS; Biorad, Marne la Coquette, France). Reactive samples on Murex HCV Ab/Ag combo were retested by Monolisa HCV Ag/Ab ULTRA and then confirmed by INNO-LIA HCV (Fujirebio). HIV positive samples were confirmed using HIV BLOT 2.2 (Genelabs Diagnostic).

Statistical analysis

The Statistical Package of Social Sciences (IBM SPSS® Statistics, Armonk, NY, USA) version 21 was used in statistical analysis. Cross tabulation and χ^2 test were used to detect the significant differences between serological markers (Hepatitis B surface antigen,

HIV antibodies and HCV antibodies) and the donor characteristics (Gender, age groups and donor type). $p<0.05$ was considered statistically significant

Results

A descriptive and cross-sectional study was conducted on 1162 blood donors (1002 male and 160 female) with known status for HBsAg, HIV Ab and HCV Ab. The mean age was 29.24 (SD= 8.21). Voluntary and family donors were 328 (28.2%) and 834 (71.8%), respectively. The prevalence of HBsAg, HIVAb and HCVAb after Enzyme ImmunoAssay screening of 1162 samples were 7.8% (n=91), 1.2% (n=14) and 0.95% (n=11), respectively. The prevalence of HIVAb and HCVAb after Western-blot screening of samples were respectively 0.34 % (n=4) and 0.26% (n=3) (figure 1).

The prevalence of HIVAb and HCVAb after Western-blot screening of samples were respectively 0.26% (n=3), 0.34 % (n=4) (table 1).

Screening samples for total Anti-HBc IgG+IgM revealed that 613 (52.75%) samples were reactive. All of the 91 samples positive for HBsAg were also positive for HBcAb. In the 1071 HBsAg negative participants, the prevalence of HBcAb was 48.7% (n=522). In seronegative participants for HBsAg, HCV and HIV, the prevalence of HBcAb was 48.8% (n=511).

Discussion

Cameroon experiences critical challenges in blood safety and availability. The high prevalence of blood borne viruses (including HIV, HBV, and HCV) remains a major concern. Although articles published in recent years show that significant change has been made in settings where WHO guidelines and quality system have been implemented, blood safety is still an important issue in Cameroon. Also, the trend of TTIs can be well monitored only it an effective quality system is put in place. That is why the prevalence of TTIs can be influenced by the screening algorithm and the tests used.

This survey determines the trends of hepatitis B, HIV and hepatitis C infection with serological confirmation in blood donors at the Blood Bank of the Yaounde University Teaching Hospital, Cameroon. In that setting, HBV screening relies only on detection of HBsAg. The seroprevalence of HBV infection in Cameroon is high. Studies show that the overall pooled seroprevalence is 11.2% (95% CI 9.7% to 12.8%) with high heterogeneity between studies (I^2=97.9%)(Bigna et al., 2017).

The mean age was 29.24 (SD= 8.214), the sex ratio male/female was 6.3. The majority of participants (57.03%) were aged between 21 to 30 years old. Voluntary and family donors were 328 (28.2%) and 834 (71.8%), respectively. In 2009, Tagny et al. (Tagny et al., 2009), reported in Cameroon the following characteristics of blood donors: 63.7% aged between 18 to 30, 28.3% female, 25.5 % benevolent. Up till now, the Yaounde University Teaching Hospital Blood Bank is still far from the strategy of the WHO Regional Committee for Africa (Regional Committe for Africa, 2001).

After confirmation, the prevalence of serological markers for HBV, HIV, and HCV were respectively 7.83%, 0.34 % and 0.26%. These prevalence have dropped compared to those obtained in the same setting by Tayou Tagny et al. in 2009: 10.3% for HBV, 2.9% for HIV, 3.9 % for HCV(Tagny et al., 2009). This is due to the implementation of WHO recommendations in terms of screening blood donation for TTIs and also the implementation of the Standards of the African Society for Blood Transfusion. Then, the reduction of the prevalence of HBV can also lead to the reduction of OBI in blood donors.

From the 1072 HBsAg negative participants, 522 were positive for HBcAb (48.8%) and the frequency of HBcAb only positive donors was 44.4% (516/1162). In Egypt, Antar et al (Antar W, El-Shokry MH, Abd El Hamid WA, 2010) reported that 7.8% of blood samples negative for HBsAg found to be reactive to anti-HBc. In 2013, Zaid et al reported that 16.6% of HBsAg negative blood donors were positive for HBcAb in Egypt (Said, 2013). Thus, In Cameroon, blood donors seem to be highly prevalent to anti-HBc compared to blood donors in Egypt, whereas both countries are highly endemic for HBV. This may be related to the vertical transmission and horizontal transmission before age five, resulting in frequent chronic infections which lead to occult infection. This marker is associated with contact with HBV whether resolved or chronic infection results from such contact. It becomes detectable in post-acute infection some days or weeks after the peak of HBsAg and HBV DNA has been reached(J. P. Allain & Candotti, 2009). In high endemic areas like Cameroon, the value of the information is limited since a majority of the population of blood donation age carries anti-HBc.

This study clearly showed a high prevalence of HBV but lower prevalences of HIV and HCV among Cameroonian blood donors at the YUTH, but the trends of viral infections were lower than those observed in the same setting by Tayou Tagny et al. in 2009. Strategies to increase voluntary and regular donors should be intensified as such medical selection of

blood donors may reduce the frequency of TTIs in blood donors. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infections prevalence in Cameroonian blood donors. At the national level, a screening algorithm should be developed for each TTI. The design of an algorithm will be determined by the specific infection marker to be screened for, the expertise of the users, the infrastructure, testing conditions and quality systems of individual screening facilities. Once an algorithm has been defined, this will guide the procurement of the specific test kits, reagents and laboratory testing systems required.

Conclusions

This study clearly showed a high prevalence of HBV but lower prevalences of HIV and HCV among Cameroonian blood donors at the YUTH, but the trends of viral infections were lower than those observed in the same setting by Tayou Tagny et al. in 2009. Strategies to increase voluntary and regular donors should be intensified as such medical selection of blood donors may reduce the frequency of TTIs in blood donors. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infections prevalence in Cameroonian blood donors. At the national level, a screening algorithm should be developed for each TTI. The design of an algorithm will be determined by the specific infection marker to be screened for, the expertise of the users, the infrastructure, testing conditions and quality systems of individual screening facilities. Once an algorithm has been defined, this will guide the procurement of the specific test kits, reagents and laboratory testing systems required.
These findings underline the need of confirmatory strategies to avoid blood wastage and to reevaluate viral infections prevalence in African blood donors that may be overestimated

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Authorship contributions

Diderot Fopa, Claude Tayou Tagny, Dora Mbanya, Syria Laperche, Daniel Candotti, Edward. L. Murphy: conception and design of the study; analysis and interpretation of data; manuscript writing and final approval of the manuscript.

Hany Ibrahim Kenawy and Farha El Chenawi: design of the study, interpretation of data, manuscript review and final approval of the manuscript.

Claude Tayou Tagny, Syria Laperche and Edward. L. Murphy: provision of study material

Diderot Fopa, Camille Doux and Daniel Candotti: Laboratory testing

Diderot Fopa, Daniel Candotti and Syria Laperche: collection, assembly, analysis and interpretation of data.

Diderot Fopa is the first author.

Competing interests

The authors declare that they have no competing interests.

Tables and Figures

Table 1. Demographic and serological characteristics of 1,162 Cameroonian blood donors at the Yaounde University Teaching Hospital in 2017

Characteristics	HBsAg[a] N=1162 n(%)		INNO-LIA HCV [b] N=1162 n(%)		HIV BLOT 2.2 [c] N=1162 n(%)	
	Positive 91(7,83)	**Negative 1071(92,17)**	**Positive 03(0,26)**	**Negative 1159(99,74)**	**Positive 4 (0,34)**	**Negative 1158(99,66)**
Gender						
Male	81(8,08)	921(91,92)	3(0,30)	999(99,70)	4(0,40)	998(99,60)
Female	10(6,25)	150(93,75)	0(0.00)	160(100.00)	0(0,00)	160(100,00)
p-value	*NS*		*NS*		*NS*	
Type of donor						
Benevolent	25(7,62)	303(92,38)	00(0.00)	327(100.00)	0(0,00)	328(100,00)
Family	66(7,91)	768(92,09)	3(0,36)	831(99,64)	4(0,48)	830(99,52)
p-value	*NS*		*NS*		*NS*	
Age group (year)						
[18;20]	9(9,18)	89(90,82)	0(0,00)	98(100)	1(1,02)	97(98,98)
[21;30]	51(7,70)	611(92,30)	1(0 ,15)	661(99,85)	0(0,00)	662(100,00)
[31;40]	21(7,66)	253(92,34)	0(0,00)	274(100)	2(0,73)	272(99,27)
[41;50]	10(10,20)	88(89,80)	0(0,00)	98 (100)	1(1,02)	97(98,98)
[51;60]	0(0,00)	28(100,00)	1(3,57)	27(96,43)	0(0,00)	28(100,00)
[60;65]	0(0,00)	2(100,00)	1(50)	1(50)	0(0,00)	2(100,00)
p-value	*NS*		*NS*		*NS*	

HBsAg: hepatitis B antigen; NS: not significant; NA: not applicable.

[a]All samples were retested by Murex HBsAg Version 3

[b]Only reactive samples with Murex HCV Ab/Ag combo and Monolisa HCV Ag/Ab ULTRA were confirmed with INNO-LIA HCV (Fujirebio).

[c] Only HIV positive samples with Murex HIV Ag/Ab Combination were confirmed using HIV BLOT 2.2 (Genelabs Diagnostic).

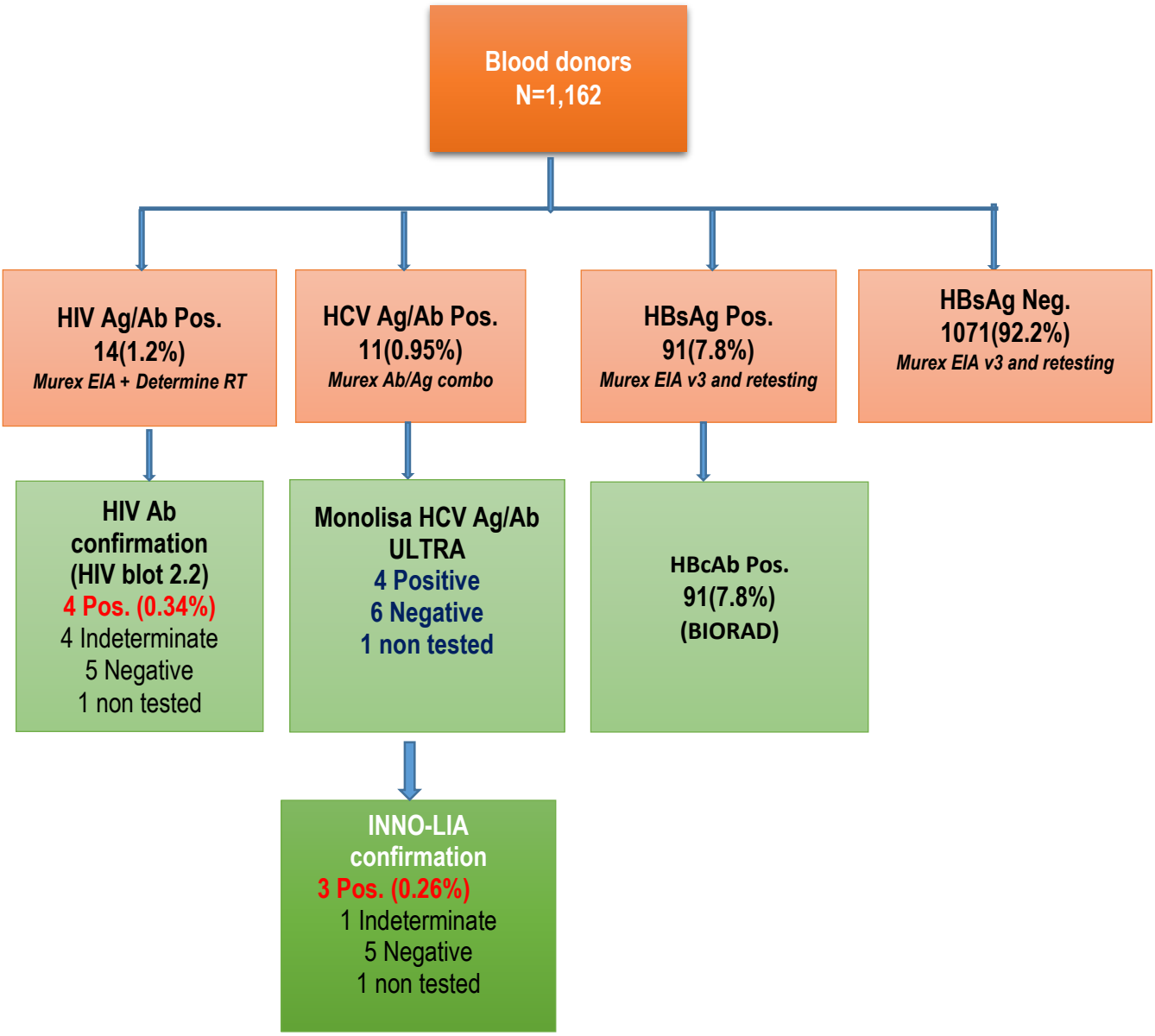

Figure 1 - Flow chart of serological investigations of viral infections in blood donations collected at the University Teaching Hospital Blood Service, Yaoundé, Cameroon.

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5.2. Presentations

5.2.1. **Poster 1** (International Society of Blood Transfusion, Toronto Congress, Canada, 2018)

P–396

OCCULT HEPATITIS B INFECTION AMONG BLOOD DONORS FROM YAOUNDE, CAMEROON

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Background: In Cameroon, which is highly endemic for HBV infection, the prevention of HBV transmission by transfusion is still based on HBsAg screening alone. However, occult HBV infection (OBI) characterized by the absence of detectable HBsAg and low level of viral DNA remains a potential threat in blood safety. OBI prevalence tends to be higher where prevalence of overt HBV infection is high.

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Nevertheless, there is limited data on the prevalence of OBI in Cameroonian blood donors.

Aims: The prevalence of OBI was investigated in blood donors from Yaounde to provide evidence-based recommendations to improve HBV blood safety.

Methods: 1,167 blood donors were screened initially for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). Additional HBV testing included anti-HBc (Monolisa Anti-HBc PLUS; BIO-RAD). HBV DNA was tested in minipools of two samples using the quantitative Cobas Taqman HBV assay (Roche; LOD of <6 IU/ml).

Results: Initial screening showed 85 (7.3%) donations reactive for HBsAg, 13 (1.1%) for HIV, 11 (0.92%) for HCV, and 1 (0.08%) for HBsAg and HIV. Among 1,057 donors negative for these markers, 545 (51.6%) were anti-HBc reactive: 35 (6.4%) showed low reactivity (12. When 30 of the anti-HBc low reactive samples were retested, 13 were non-reactive, 8 remained low reactive, and 9 showed S/CO values >2. Ongoing molecular testing showed that 4/134 (3%) anti-HBc positive/HBsAg negative samples carried detectable levels of HBV DNA with viral loads ranging between <6 IU/ml and 927 IU/ml. Of the 13 HIV reactive samples, 4 were confirmed positive by WB and PCR and 2 of them had anti-HBc and 1 of 3/10 HCV confirmed samples was anti-HBc positive.

Summary/Conclusions: HBV is confirmed highly endemic in Cameroon with a HBsAg positive rate of 7% and an overall anti-HBc prevalence of 53% (593/1119) in blood donors. Preliminary data suggest that approximately 1.6% % of Cameroonian blood donors screened HBsAg negative carry occult HBV infection. Further serological and molecular testing is currently ongoing to confirm these data. HBsAg alone for screening prospective donors is not sufficient to eliminate the risk of HBV transfusion-transmission in Cameroon and because anti-HBc screening appears not feasible without compromising blood supply, implementation of HBV nucleic acid testing might be considered when possible.

5.2.2. Poster 2 (68th Annual Meeting of American Society of Tropical Medicine and Hygiene, Washington DC, USA, 2019)

PRESENTATION NUMBER: 222

Seroprevalence of viral infections in blood donors in Yaounde, Cameroon

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ABSTRACT

The high prevalence of transfusion-transmissible infections (TTIs) is the most important challenge of safe blood supply in Cameroon. The seroprevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) was determined among prospective blood donors at blood bank Yaoundé University Teaching Hospital (YUTH), Yaoundé, Cameroon. Blood donors were consecutively screened for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]). HIV and HCV serology were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively. In total, 1.162 were serially included in the study. Screening for transfusion transmissible infections showed that 91 (7.80%) of total samples donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.94%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+. Of 14 HIV and 11 HCV reactive samples, 4 and 3 were confirmed positive by western blot, respectively. This study clearly showed a high prevalence of viral infections among Cameroonian blood donors at the YUTH. Strategies to increase voluntary and regular donors should be intensified to improve the medical selection of blood donors and reduce the frequency of TTIs found in donated blood. The confirmatory results of HIV and HCV underline the need to re-evaluate viral infection prevalence in Cameroonian blood donors.

***Key words*: Viral infections, Blood donors, Yaoundé-Cameroon.**

INTRODUCTION

- In sub-Saharan Africa (SSA), the prevalence of viral infections such as Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) in blood donors is high due to high background prevalence and blood safety processes and procedures implemented by blood donation services.
- Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infections from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible.
- The high prevalence of TTIs is the most important threat to ensuring safe blood supplies in Cameroon. In 2009, Tayou Tagny et al. reported a prevalence of 10.3% for HBV, 2.9% for HIV, and 3.9 % for HCV in donated blood. A quality system has been put in place to reduce the trends of TTIs in our setting.
- The seroprevalence of Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV) was determined among prospective blood donors at blood bank of the Yaoundé University Teaching Hospital (YUTH), Yaoundé, Cameroon.

METHODS

- Blood donors' sera were consecutively collected at the Blood Bank of the Yaoundé University Teaching Hospital (YUTH) (Cameroon) and were screened for HBsAg, HIV and HCV serology from August 1, 2016 to February 28, 2017.
- Two aliquots were taken from each recruited donor and stored at –20°C. Later, these aliquots were transported to the Virology Reference Laboratory of the National Institute of Blood Transfusion in Paris (France) where further investigations were made (screening for HBcAb, western blot for HIV and HCV, retesting of HBsAg).
- Blood donors were screened initially for HBV, HIV and HCV infections (Murex HBsAg Version 3, Murex HIV Ag/Ab Combination, and Murex HCV Ag/Ab Combination [DiaSorin]).
- HIV and HCV serology were confirmed with HIV BLOT 2.2 (Genelabs Diagnostic) and INNO-LIA HCV (Fujirebio), respectively.

RESULTS

- In total, 1,162 donors were serially included in the study.
- Screening for transfusion transmissible infections showed that 91 (7.80%) of total samples donations were reactive for HBsAg+, 14 (1.2%) for HIV+, 11 (0.94%) for HCV+, and 1 (0.08%) for HBsAg+ and /HIV+.
- All the 1,162 participants were screened for total Anti-HBc IgG+IgM and 613 (52.75%) were reactive. Of these, the 91 HBsAg-positive samples were also positive for HBcAb.
- In 1,071 HBsAg-negative participants, the prevalence of HBcAb was 48.7% (n=522).
- Of 14 HIV and 11 HCV reactive samples, 4 and 3 were confirmed positive by western blot, respectively.
- Confirmed seroprevalence for the three viruses is shown in Table 1.

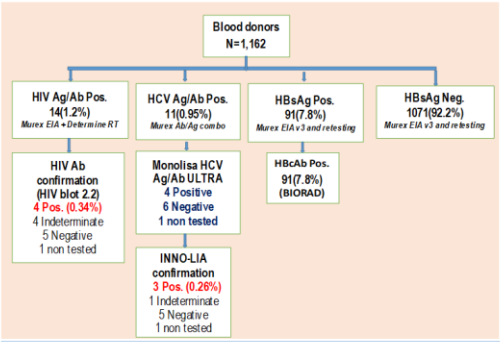

Figure 1 - Flow chart of serological investigations of viral infections in blood donations collected at the University Teaching Hospital Blood Service, Yaoundé, Cameroon

Table 1. Viral markers prevalence in 1,162 blood donors from Yaoundé, Cameroon

Viral markers	N	%
HIV Ab +	4	0.3
HCV Ab +	3	0.2
HBsAg +	91	7.8

CONCLUSION

- This study clearly showed a high prevalence of HBV but lower prevalences of HIV and HCV among Cameroonian blood donors at the YUTH.
- Disease prevalence were lower than those observed in the same setting by Tayou Tagny et al. in 2009.
- Strategies to increase voluntary and regular donors should be intensified as such medical selection of blood donors may reduce the frequency of TTIs in blood donors.
- The confirmatory results of HIV and HCV underline the need to re-evaluate viral infections prevalence in Cameroonian blood donors.

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CONCLUSIONS AND RECOMMANDATIONS

CONCLUSIONS AND RECOMMANDATIONS

In summary, this study provides clear evidence on occult hepatitis B infection in blood donors at the Yaounde University Teaching Hospital, Cameroon. The prevalence of anti-HBc in Cameroonian blood donors was so high. Among those positive anti-HBc individuals, the prevalence of occult HBV was not high; then rejection of anti-HBc positive blood units does not seems feasible because it will lead to elimination of considerable blood units which are not contaminated. Thus, the implementation of NAT testing can be evaluated as well risk factors for HBV infection in blood donors in general and OBI in particular. Surprisingly, all OBI identified were of genotype E; then it would be useful to consider sequencing HBsAg+ samples from the present study to verify this difference between genotype distributions according to populations.

A high prevalence of anti-HBc antibodies was found among blood donors highlighted the need to develop new strategies for blood donors' selection and screening for hepatitis B virus through:

- Evaluation of risk factors for HBV infections and OBI in Cameroonians blood donors,
- Increase the proportion of voluntary donors since this will reduce drastically the blood rejection rate and also improve the cost effectiveness of blood collection in Cameroon, since all OBI were family donors,
- The establishment of nucleic acid tests (NAT) for the purpose of screening donors to reduce the residual risk of transmission of HBV by blood transfusion by allowing infections to be detected during the so-called "seroconversion" period (before the onset of HB) as well as the detection of OBI, both characterized by the presence of HBV DNA in the absence of HBsAg.

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APPENDIX

Annexe 1: Fiche d'information

1. **Thème de l'étude** :

L'étude est intitulée : « ***Prévalence de l'Hépatite Virale B occulte chez les donneurs de sang au CHU de Yaoundé*** ». Cette étude est menée en vue de l'obtention du diplôme de Master en Immunologie et Médecine Régénérative à l'Université de Mansoura en Egypte.

2. Investigateurs :

- **Investigateur principal :** Diderot FOPA (étudiant à l'Université de Mansoura en Egypte),
- **Co-**Investigateurs (Superviseurs) : Prof. Dr. Farha El Chenawi, Professor, University of Mansoura, Egypt ; Prof. Hany Kenawy, Associate Professor, University of M-ansoura, Egypt; Prof. Dora MBANYA, FHS/University of Bamenda, Cameroon and Prof. TAYOU TAGNY Claude Bertrand, FMSB/UY1, Cameroon.

3. Objectifs de l'étude

Déterminer la prévalence de l'Hépatite Virale B occulte chez les donneurs de sang au CHU de Yaoundé.

4. Procédure

Si vous acceptez librement à participer à cette étude, les étapes ci-après seront suivies :

- Répondre anonymement au questionnaire sur une page de papier. Ces questions concernent l'âge, le sexe, le statut marital, la profession, les antécédents médicaux et chirurgicaux, la vie reproductives pour les femmes.
- Prélèvement du sang dans un tube de 5 ml pour des analyses du laboratoire. Sérologie de l'hépatite virale B au laboratoire d'hématologie du CHU de Yaoundé et dépistage génomique viral à l'Institut National de Transfusion Sanguine.

5. Bénéfice :

- Tout participant bénéficiera des résultats gratuits du dépistage des marqueurs suivants : Antigène de surface du virus de l'Hépatite B (AgHBs), la recherche de l'ADN du virus de l'hépatite B.

6. Risque

- Il y a un risque minimal en matière de collecte de sang comme une enflure au point piqûre d'aiguille (hématome) et de la douleur. La banque de sang vous donnera la même assistance donnée aux donneurs de sang au cas où cela vous arrive.
- Il y a un petit risque que vos renseignements personnels ne puissent pas être gardés de manière confidentielle. Cependant, nous allons travailler dur pour garder secret les résultats de cette étude. Le questionnaire, ainsi que les échantillons seront identifiés par des codes et non par votre nom.

7. Avantages:

Vous n'êtes pas obligé de participer à cette étude et à tout moment, vous pouvez retirer votre consentement à participer. L'avantage personnel de participer à cette étude est d'avoir le test de dépistage génomique effectué, et au-delà, vous allez contribuer à mieux comprendre l'épidémie de l'Hépatite Virale B Occulte dans notre pays.

8. Respect des participants :

Les résultats des examens que je subirais et qui devront m'être communiqués resteront confidentielles.

NB : Celui ou celle qui refusera de signer le formulaire ne sera pas pénalisé d'une manière ou d'une autre, mais ne pourra pas participer à cette étude.

Annexe 2. Fiche de consentement volontaire et éclairé

1. **Thème de l'étude** :

   Prévalence de l'Hépatite Virale B occulte chez les donneurs du sang au CHU de Yaoundé.

2. **Objectif de l'étude**

- Déterminer la prévalence de l'Hépatite Virale B occulte chez les donneurs da sang au CHU de Yaoundé.

3. **Procédure**

J'accepte de participer à cette étude et m'engage à :

- Répondre aux questions qui me seront posées
- Accepter les prélèvements sanguins nécessaires à la réalisation de cette étude qui me seront faits.

J'ai lu la notice d'information qui m'a été en plus expliqué et en ai reçu une copie.

J'ai compris le but de l'étude, ses avantages et contraintes.

J'accepte librement de participer à cette étude.

Signature du participant Signature de l'investigateur

Annexe 3. Questionnaire de recherche

Prévalence de l'Hépatite Virale B occulte chez les donneurs de sang au CHU de Yaoundé

I. Caractéristiques sociodémographiques des donneurs

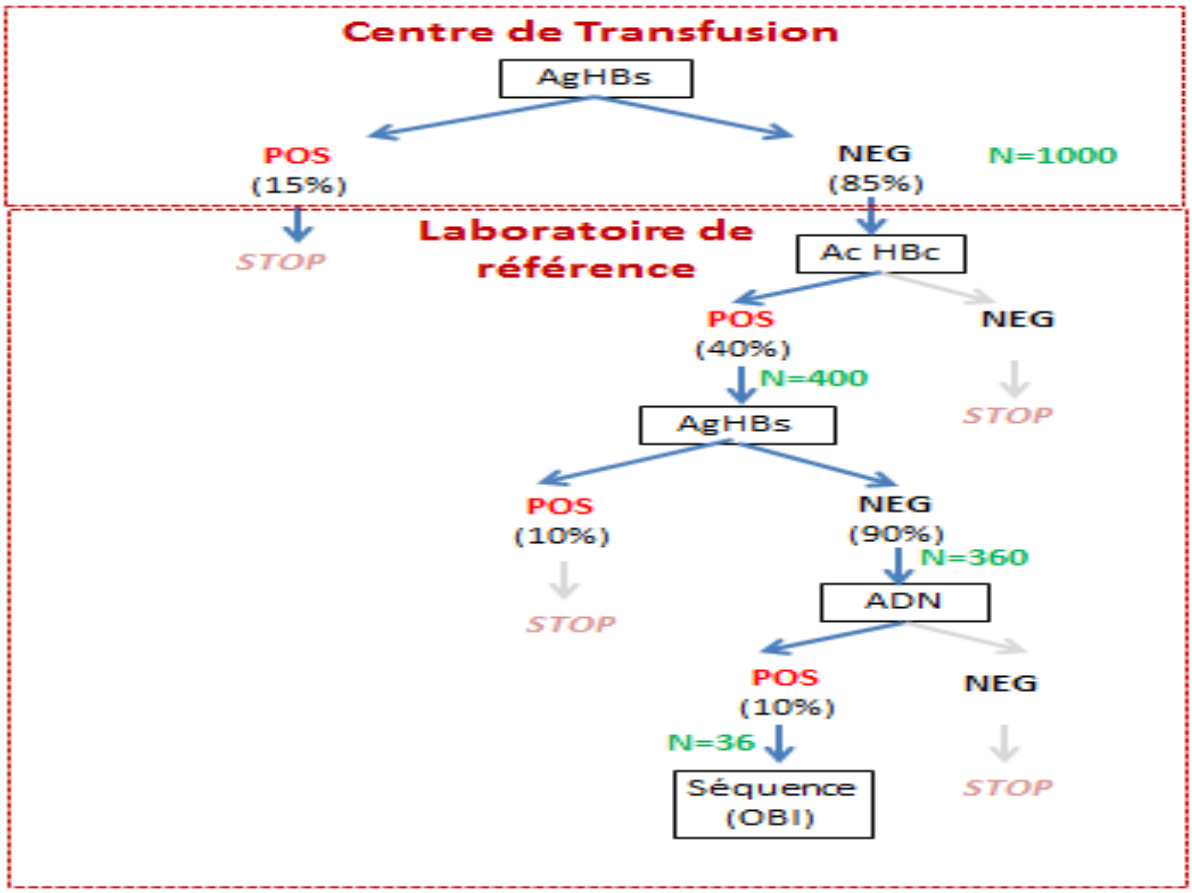
A. Identité du donneur et caractéristiques du don

1. Code du donneur :	
2. Age : ____ Ans	3. Sexe : Masculin □ Féminin □
4. Type du don	a) Familial ou de remplacement □ b) Bénévole et volontaire □
5. Téléphone /email	
6. Résidence :	a) Yaoundé □ b) Hors de Yaoundé □

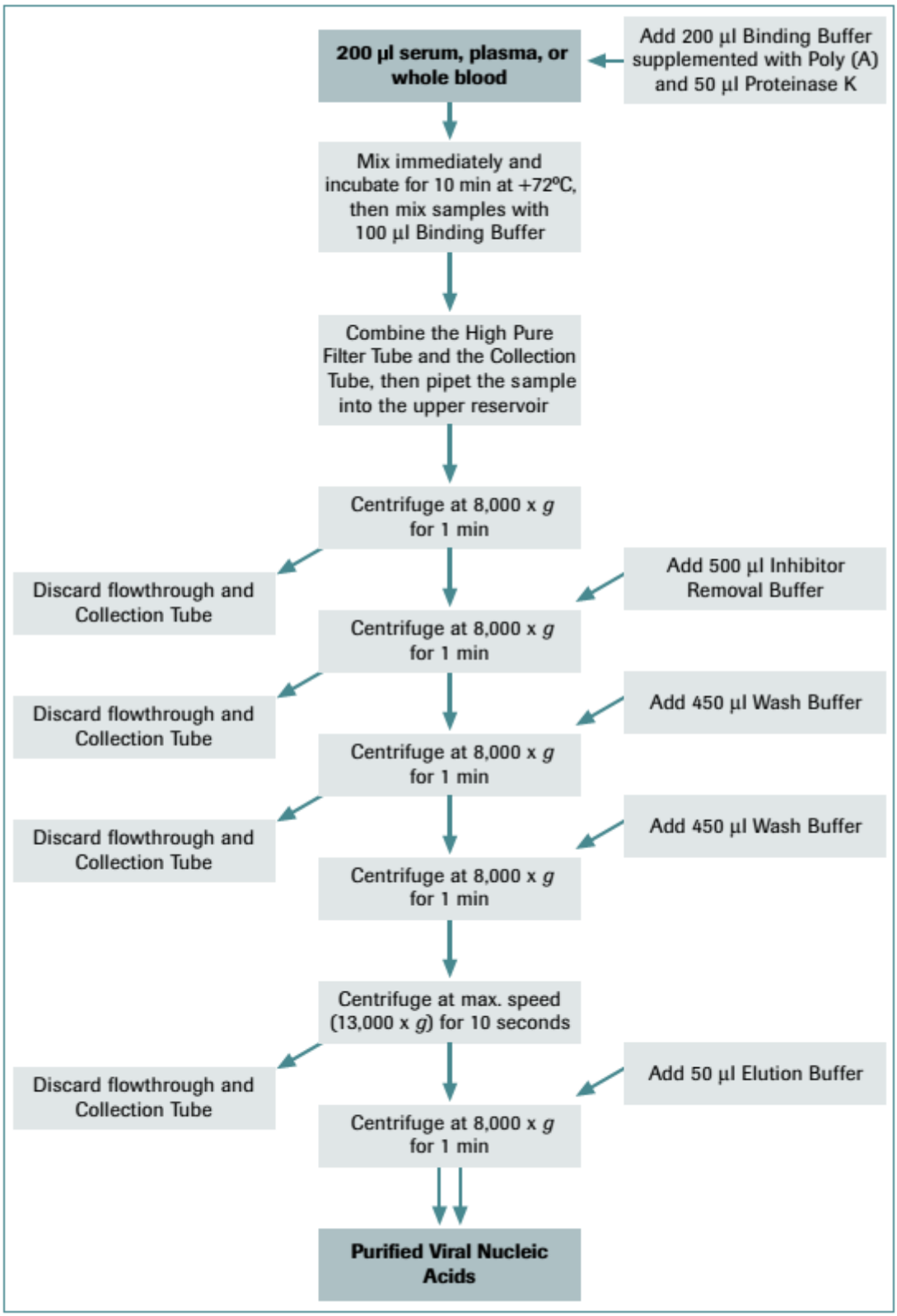
II. Résultats d'analyses du laboratoire

Tests réalisés	Résultats
16. Dépistage de l'antigène HBs:	Positif □ Négatif □
17. Dépistage de l'infection à VIH :	Positif □ Négatif □
18. Dépistage de l'infection à VHC :	Positif □ Négatif □
19. Dépistage de l'anticorps anti-HBc :	Positif □ Négatif □
20. Dépistage de l'anticorps anti-HBs	Positif □ Négatif □
21. Dépistage génomique virale du VHB	a. Positif □ Négatif □
b. Charge virale du VHB	_____UL/µL
c. Résultats de séquençage	

Annexe 4. Algorithmes



Annexe 5. *Flow chart for Viral nucleic acid extraction using roche kit*



Annexe 6. PCR procedures

HBV whole genome nested PCR				
BioTaq				
First PCR				
Component	**volume/tube**	**final conc**		
DEPC water	8,5			
10 X NH4 buffer	5	1x		
50 mM MgCl2	2,25	2.25mM		
40 mM dNTPs	0,25	0.2mM		
10µM PW1	1,5	0.3µM		
10µM PW5	1,5	0.3µM		
BioTaq 5U/µl	1 /20	0.1U/µl		
DNA	30			
Second PCR				
Component	**volume/tube**	**final conc**		
DEPC water	33			
10 X NH4 buffer	5	1x		
50 mM MgCl2	2,25	2,25mM		
40 mM dNTPs	0,25	0.2mM		
10µM P3W	1,5	0.3µM		
10µM P4W	1,5	0.3µM		
BioTaq 5U/µl	0,5/44	0.05U/µl		
DNA	6			
1st-PCR Program			**2nd PCR Program**	
95°C	3 min		95°C	3 min
94°C	40 sec		94°C	40 sec
50°C	35 sec		55°C	35 sec
68°C	3.5 min	1st 10 cycles	68°C	3.5 min
every 10cycles plus 1.5min	40 Cycle			40 Cycle
68°C	10 min		68°C	10 min

Annexe 7: Screening results

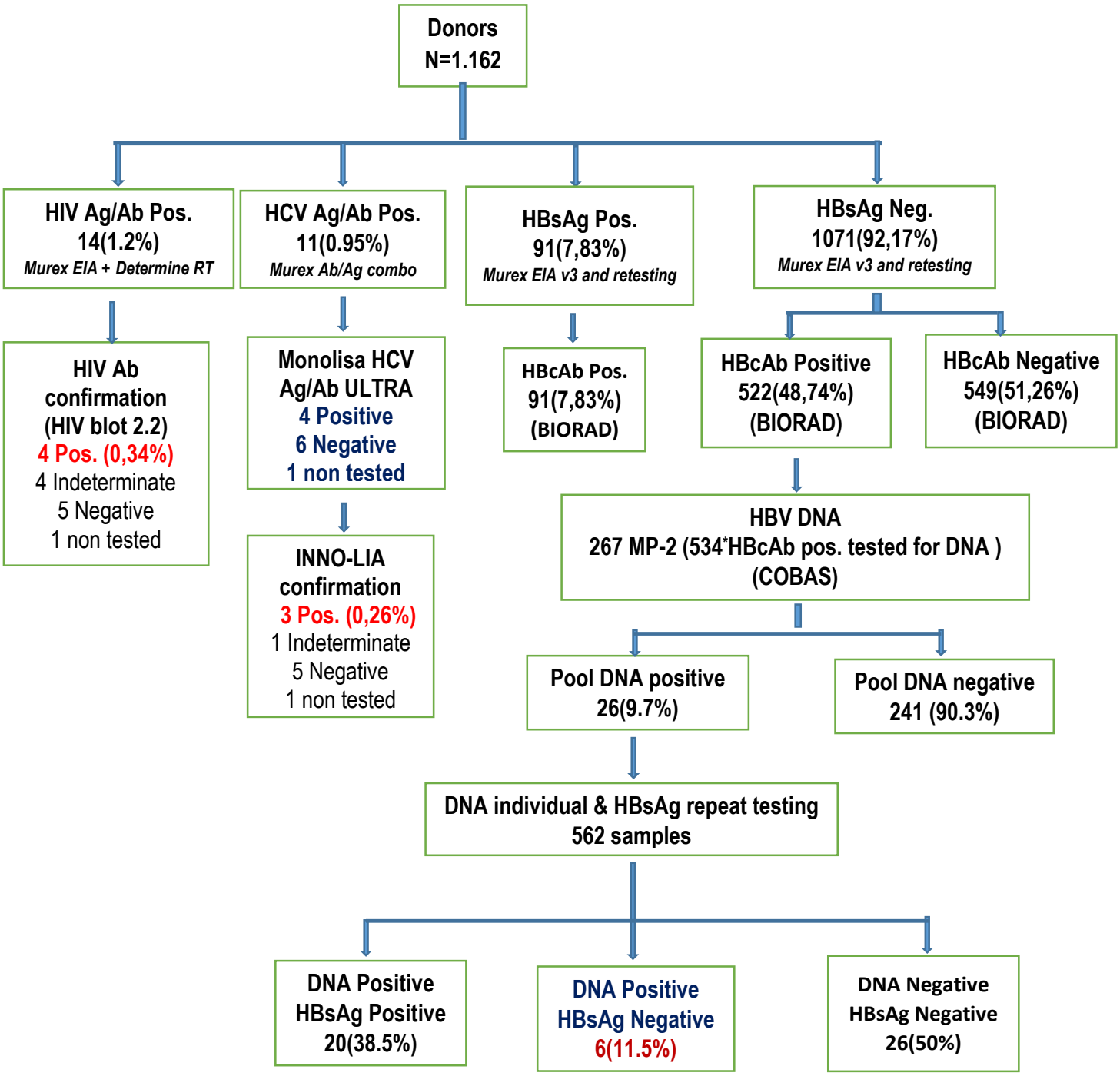


Figure 3. Blood donor serological screening and HBV molecular testing

Table XV. Demographic and viral characteristics of the studied population

Characteristics		HBsAg n(%)				HBcAb in HBsAg Negative n(%)				HBV DNA in HBsAg negative/HBcAb positive n(%)			
		Positive 91(7,83)	**Positive 1071(7,83)**	**Total 1162**	**P value**	**Positive 522 (48,74)**	**Negative 549 (51,26)**	**Total 1071**	**P value**	**Positive 6(1,15)**	**Negative 516(98,85)**	**Total 522**	**P value**
Gender	Male	81(8,08)	921(91,92)	1002	0.630	455(49,40)	466(50,60)	921	**0,**282	5(1,10)	450(98,90)	455	**0,**77
	Female	10(6,25)	150(93,75)	160		67(44,67)	83(55,33)	150		1(1,49)	66(98,51)	67	
Type of donor	Benevolent	25(7,62)	303(92,38)	328	0.686	133(43,89)	170(56,11)	303	0,046	0(,00)	133(100,00)	133	0,15
	Family	66(7,91)	768(92,09)	834		389(50,65)	379(49,35)	768		6(1,54)	383(98,46)	389	
Age groups (years)	[18;20]	9(9,18)	89(90,82)	98	0.243	34(38,20)	55(61,80)	89	**0,0001**	0(,00)	34(100,00)	34	**0,049**
	[21;30]	51(7,70)	611(92,30)	662		275(45,01)	336(54,99)	611		1(,36)	274(99,64)	275	
	[31;40]	21(7,66)	253(92,34)	274		144(56,92)	109(43,08)	253		5(3,47)	139(96,53)	144	
	[41;50]	10(10,20)	88(89,80)	98		48(54,55)	40(45,45)	88		0(,00)	48(100,00)	48	
	[51;60]	0(0,00)	28(100,00)	28		21(75,00)	7(25,00)	28		0(,00)	21(100,00)	21	
	[60;65]	0(0,00)	2(100,00)	2		0(0,00)	2(100,00)	2		0(,00)	0(,00)	0	