PREVALENCE AND GENOTYPIC CHARACTERISATION OF OCCULT HEPATITIS B INFECTION IN BLOOD DONORS AT GULU REGIONAL BLOOD BANK, NORTHERN UGANDA.

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OCTOBER, 2026.

Declaration

This work is entirely my own and contains no materials submitted for any degree or publication, except where I have stated the materials and sources used in the preparation of this report. I also declare that the work is prepared specifically in partial fulfilment for academic award.

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Dedication

I devote this amazing project to my family, especially to my cherished wife and children, who have supported me throughout the development of the proposal and this report

I appreciate my mother, Aceng Judith, for her unwavering support and encouragement as a dedicated parent

Acknowledgement

I want to sincerely thank the many individuals that helped in completing this report document. I am particularly thankful to my supervisors, Dr. Simon Peter Alarakol (PhD) and Dr. Alex Olia (PhD), for their guidance and assistance throughout the completion of this research project. More special thanks goes to the staffs of Gulu University Multifunctional Laboratories, headed by the Chief, Associate Professor Richard Echodu, for their support during data collection and analysis.

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Abbreviations and acronyms

UBTS	Uganda Blood Transfusion Service
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface antigen
Anti-HBc	Antibody to Hepatitis B core antigen
Anti-HBs	Antibody to Hepatitis B Surface antigen
CHB	Chronic Hepatitis B infection
HCC	Hepatocellular carcinoma
OBI	Occult Hepatitis B infection
HCV	Hepatitis C virus
HIV	Human immune deficiency Virus
DNA	Deoxyribonucleic Acid
rcDNA	Relaxed circular DNA
cccDNA	Covalently closed circular DNA
NAT	Nucleic acid amplification Test
ORF	Open reading frame
mRNA	Messenger ribonucleic acid
PCR	Polymerized chain reaction
IgG	Immunoglobulin Gama
IgM	immunoglobulin meu
HBeAg	Hepatitis B e antigen
HBcAg	Hepatitis B core Antigen
VNRBDs	Voluntary non-remunerated blood donors
GRBB	Gulu regional blood bank
WHO	World Health Organisation

Operational definitions

Occult hepatitis B infection (OBI): This is described as availability of HBV DNA in the liver, or both in liver and serum of persons with undetected HBsAg.

Chronic hepatitis B (CHB) disease: This is described as continuous presence of HBsAg in an individual for a period not less than six (6) months following infection with hepatitis B virus, and its diagnosis is based on detection of HBsAg and IgG anti-HBc, and lack of IgM anti-HBc in serum of patients

Acute hepatitis B (AHB): This is a brief sickness that happens within the first six (6) months following exposure to hepatitis B virus. It is diagnosed by finding IgM anti-HBc and HBsAg in blood.

Transcription: This is the process of making an RNA (mRNA) replica from the genetic sequence of a gene

Reverse transcription: This is the process through which RNA is converted to DNA by the help reverse transcriptase enzyme

Translation: This is the process through which information encoded in messenger RNA (mRNA) is converted to amino acids sequences during protein synthesis.

Polymerase chain reactions (PCR): Is a molecular biology technique that quickly generates numerous copies of a segment of DNA by amplification.

Sensitivity of a test: This is ability of a test to detect individuals with a particular disease or a measure of true positivity rate

Specificity of a test: This is the relative ability to correctly detect individual without a specific disease, or a measure of true negative rate.

Likelihood ratio: This is likelihood that a given result will occur in an individual with a disease than in an individual without the disease

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Abstract

Occult hepatitis B infection is defined as presence of HBV DNA in blood or liver cells of individuals who test negative for hepatitis B surface antigen (HBsAg). HBV remains one of the most prevalent viral infections spread through blood transfusion in Northern Uganda.

This study aimed to ascertain the prevalence of OBI in blood donors from northern Uganda to provide evidenced data to improve blood safety program in the region, and consequently reduce HBV infection through blood transfusion.

A cross-sectional study was used to determine the prevalence of OBI among individuals who donate blood at Gulu regional blood bank. A total of 1,067 donated blood sampled using systematic random sampling technique were screened for HBsAg and HBV nucleic acid (DNA). Samples that tested HBsAg negative and HBV DNA positive were further tested for anti-HBc as marker for seropositive OBI, and OBI DNA sequences were genotyped by phylogenetic analysis using MEGAv.12 software to ascertain HBV genotype. Data were entered in MS Excel software and analysed using IBM SPSS Software V.20.

In a total of 1,067 blood samples collected from voluntary blood donors, 35.2% (n=376) and 64.8% (n=691) were females and males respectively. Male and female blood donors had statistically significant HBV infection rates of 2.8% and 0.5%, respectively (X^2 *(1, N=1067) = 6.962, p=0.008*). While the frequency of HBV infection in new and repeat blood donors was 1.8% and 1.5% respectively; however, there was no significant difference (X^2 *(2, N=1067) = 4.190, p= 0.123*). The prevalence of OBI was 0.9% (10/1067), hepatitis B recombinant genotype D/E was (83.3%) and genotype D was (16.7%) respectively. Sensitivity and specificity for HBsAg was 71.4% and 99.9% respectively. There was significant difference between use of HBsAg and HBV DNA NAT for screening of hepatitis B infection among blood donors in northern Uganda (McNemar test X^2, P=.012).

The study provides evidence of OBI infection and its circulating genotypes in northern Uganda. HBsAg test showed low analytical sensitivity compared to NAT test. Therefore, use of HBsAg alone for screening of blood donation is not adequate to eliminate the risk of transmission of HBV through blood transfusion. Introducing parallel testing with both HBsAg and HBV DNA could reduce on the risk of OBI transmission and better confirm HBV infection in blood donors in Northern Uganda. The study further recommends studies using larger number of HBV sequences to comprehensively explore the OBI strains in the region.

CHAPTER ONE: INTRODUCTION

1.1 Back ground of the study

Hepatitis B virus (HBV) is an enveloped DNA virus belonging to the family *Hepadnaviridae* (Dias et al., 2021). Hepatitis B is a disease which occurs following infection with Hepatitis B virus (HBV), a virus with predilection for liver cells and the potential to cause cancer (Aber *et al*., 2022).

HBV infection is universally a main health concern and it causes the most severe form of hepatitis characterized by more cases of liver cancer and death than from hepatitis C virus (HCV*)* (Falade-nwulia *et al.*, 2012). About 297 million individuals worldwide have been infected with HBV and an estimated 820,000 deaths from HBV related causes in 2019 (WHO, 2021). According to the most recent WHO report, HBV prevalence is highest in African (7.5%) and western pacific regions (5.9%). In Africa, an estimated 82.3 million people have HBV infection (WHO, 2021). In Uganda, the seroprevalence of HBV is estimated at 4.1% and 0.6% among adults and children respectively (Ministry of Health, 2019). This prevalence varies in the different regions of the country with the lowest (0.8%) in south-western and the highest (4.6%) in mid-northern (Ministry of Health, 2019). In Northern Uganda, HBsAg prevalence is estimated at (7.9%) with a variation among gender of (6.8%) and (11.7%) between female and males respectively (Chiesa *et al.*, 2020).

HBV is still one of the most frequent viruses transmitted through blood transfusion. In recent years, the possibility that blood transfusion might transmit HBV has steadily declined due to recruitment of volunteer unpaid blood donors, pre-donation selection based on behavioral assessment, and development of more sensitive blood screening assays (Fopa *et al*., 2019). In northern Uganda, preventing the spread of HBV by blood transfusion is dependent on serological test for hepatitis B surface antigen (HBsAg) in blood donors. However, in the absence of Nucleic acid amplification test (NAT), blood transfusion has

potential to spread HBV especially from individuals with occult hepatitis infection (Fopa *et al.*, 2019).

Occult HBV (OBI) means detectable small amount of HBV nucleic acid (less than 200 IU/ml) in hepatocytes or serum, but HBsAg is not detectable, and there may or may not be anti-HBc or anti-HBs present, excluding the period prior to seroconversion (Kwak & Kim, 2014). OBI remains a possible threat of hepatitis B infection during blood transfusion hence a possible concern to blood safety (Fopa *et al*., 2019). Previous studies in western Africa among blood donors reported OBI prevalence of (0.56%) and (17%) respectively (Fopa *et al.*, 2019; Oluyinka *et al*., 2015). Moreover, frequency of OBI was found to be higher in communities with increased possibility of infection and in endemic parts of the globe (greater than 8% prevalence) (Jepkemei *et al*., 2020). The risks presented by OBI includes; ongoing transmission due to false negative test caused by lack of HBsAg, progression to liver disease and reactivation during immunosuppression (Fopa et al., 2019; Jepkemei *et al.*, 2020; Oluyinka *et al*., 2015; Ondigui *et al.*, 2022). In Northern Uganda, there is scarcity of facts on the frequency of OBI in the region making little effort in the prevention of possibility of HBV spread to blood recipients from blood donors with OBI. Therefore, the aim of this study was to examine and characterize OBI in blood donors from Northern Uganda to suggest evidence-supported recommendations to enhance HBV blood safety program.

1.2 Statement of the problem

HBV remains among the most prevalent viral infections transmitted through blood transfusion. According to estimates, 4.1% of Uganda's adult population have HBV infection (Ministry of Health, 2019). Besides, about 6% of Ugandans had CHB infection, and approximately 1,250 HBV-related deaths were recorded in 2022. (*Uganda-Elevates-*

Health-for-All-as-the-Population-Accesses-Free-Hepatitis-Testing--Treatment-and-Vaccines, 28.07.2023).

Over the previous decennaries, the possibility of transfusion-related HBV infection has been reduced by recruitment of VNRBD based on evaluation of behavioral risk and by the acquisition of more sensitive HBsAg test assays. In some other countries the use of anti-HBc screening and HBV nucleic amplification test (NAT) have been reported; and that the risk of HBV transmission still exists where there is no Anti-HBc test and nucleic amplification test (Fopa *et al*., 2019).

In Uganda, particularly northern Uganda, screening for HBV among those who donate blood is based on testing for HBsAg in serum. Therefore, there is possibility that blood transfusion from this region carries risk of transmitting HBV from blood donors with OBI (Fopa *et al.*, 2019). Additionally, there is scarcity of statistics about the frequency of OBI within blood donors from Northern Uganda.

1.3 Justification

Occult hepatitis B is a viral disease with serious impact on the individuals. The disease has the possibility of being transmitted through blood transfusion. In immunosuppressed persons, the infection may result into viral reactivation with consequence of progression to chronic liver disease. The disease also has potential of development into hepatocellular carcinoma (HCC) (Saitta *et al*., 2022). Northern Uganda was chosen because of the high prevalence of HBV infection in the area and the fact that donor screening for HBV infection is based only on HBsAg detection.

1.4 Significance of the study

The study generated information on prevalence of OBI in the region since this information was lacking. The findings from this study are useful to adopt new guidelines on HBV testing in Northern Uganda. Previous reports confirm that several countries have adopted

Nucleic Amplification tests (NAT) for the purpose of testing donated blood samples to reduce the possibility of transfusion of blood with OBI (Fopa *et al.*, 2019). Detection of OBI has reduced on transfusions which can lead to HBV infection in blood recipients since blood donations that were found to HBsAg negative but hepatitis B DNA positive was excluded from blood supply and discarded. HBV genotyping has furnished facts on genotypes which may be beneficial for guiding therapy and controlling liver diseases (Ma *et al*., 2011).

1.5 Goals of the study

The goal of this study was to assess the prevalence of OBI among persons who donate blood.

1.6 **Research objectives**

1.6.1 Main objective

The study was to determine the prevalence of occult HBV (OBI) among individuals who donate blood in Northern Uganda and to provide evidence-based recommendations to enhance blood safety.

1.6.2 Specific objectives

1. To ascertain the prevalence of OBI within individuals who donate blood at Gulu Regional Blood Bank, Northern Uganda
2. o determine the sensitivity and specificity of HBsAg for the screening of HBV among individuals who donate blood at Gulu Regional Blood Bank, Northern Uganda using HBV DNA NAT as the gold standard.
3. To ascertain the genotypes of the OBI among individuals who donate blood at Gulu Regional Blood Bank, Northern Uganda.

1.7 Research questions

1. To what extent do blood donors have OBI in Northern Uganda?
2. Is there a difference in the prevalence of HBV between male and female blood donors?
3. Is there a difference in the prevalence of HBV among new and repeat VNRBDs blood donors?
4. What is the Sensitivity and specificity of HBsAg for HBV screening?
5. What are the OBI genotypes among individuals who donate blood in Northern Uganda?

1.8 Scope of the study

The research was confined to only individuals who turned-up to donate blood at Gulu Regional blood bank. The study looked at serological detection of HBsAg, Nucleic Acid Amplification test (NAT) and Hepatitis B Anti-HBc testing on NAT reactive samples to confirm OBI. Study was conducted between September 2024 to August 2025.

CHAPTER TWO: LITERATURE REVIEW

2.1 Hepatitis B virus

Hepatitis B virus (HBV) is a tiny DNA virus from the Hepadnaviridae family, categorized into eight genotypes (A to H) based on sequence comparison. The infective form of HBV virion (Dane particle) is around double-layered structure about 42nm in diameter with an outer lipid envelope consisting of HBsAg, and a nucleocapsid containing HBcAg attached to viral polymerase and viral DNA genome (Liang, 2009)

2.1.1 Structure of Hepatitis B virion

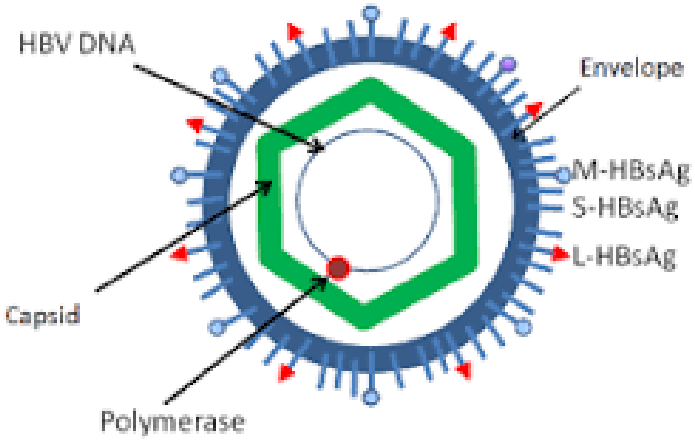


Figure 2.1:Schematic diagram of HBV structure

Adaptedfrom:www.google.com/search?q=hbv+structure+schematic+diagram&rlz=1C1AVUB_enUG915UG915&oq=HBV+structure+sche&aqs=chrome.2.69i57j33i

2.1.2 HBV Genome organization

HBV features 3.2 kilobase (kb) pairs of partly double-stranded circular DNA. Four ORFs that overlap one another—S, C, P, and X—exist in the HBV genome. The S-gene encodes viral surface coat protein (L, M, & S HBsAgs) and further separates into Pre-S1, Pre-S2, and S regions. In the C-gene, the pre-core and core regions encode HBeAg and HBcAg. Polymerase (pol) produced by P-gene has three parts namely; the terminal protein, reverse transcriptase (RT) and the Nuclease H domains: which play important roles in encapsidation and initiation of backward strand synthesis, genome synthesis, and pre-

genomic RNA breakdown respectively. X ORF encodes HBxAg, which participates in signal transduction, transcription activation, DNA repair, and break down of proteins (Liang, 2009).

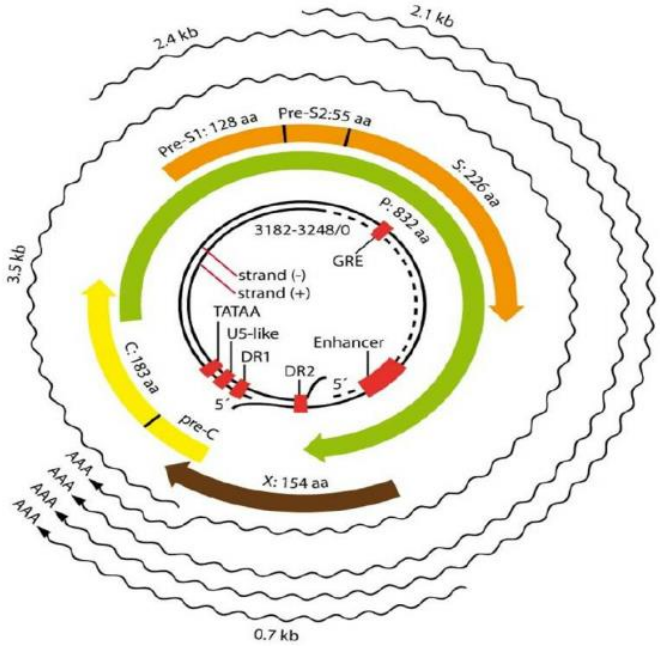


Figure 2.2: Schematic diagram of HBV genome organization

Adapted from; https://www.researchgate.net/profile/Martha-Eugenia-Ruiz-Tachiquin?_tp=eyJjb250ZXh0Ijp7ImZpcnN0UGFnZSI6Il9kaXJlY3QiLCJwYWdlIjoiX2RpcmVjdCJ9fQ

2.1.3 Life cycle of HBV

First, mature virion is fused to the host cell using Pre-S part of the surface proteins, then viral receptor sodium taurocholate co-transporting polypeptide (NTCP) facilitates viral entrance into cytoplasm by endocytosis. After uncoating, nucleocapsid releases viral genome (relaxed circular DNA (rcDNA)) which enters the nucleus. Pol proteins repair the viral genome's SS-gap region to generate cccDNA in the nucleus. Using cellular machinery, this form (cccDNA) is transcribed to create multiple viral RNAs for protein synthesis and viral particle production (Karayiannis, 2017).

RNA polymerase II synthesizes viral mRNA from chromatinized cccDNA during transcription and translation. Four transcripts are produced from chromatinized cccDNA and translated into distinct proteins: (1) Precore mRNA (Pre-C; 3.2kb), an initiation codon and precursor for HBeAg protein. (2) pre-genomic mRNA (Pg-RNA; 2.1kb) encodes HBcAg, and polymerase proteins, which self-assemble into nucleocapsids. Also, during viral genome replication, pg mRNA serves as a model for DNA synthsis. (3) The Pre-S/S ORF (Pre S1&S2 RNA; 2.4kb) encodes large (L), medium (M), and small (S) HBsAgs that produce glycoproteins. The fourth and shortest ORF (HBx RNA; 0.7kb) encodes HBX protein, which modulates host-cell signal transmission (Karayiannis, 2017).

In the cytoplasm, PgRNA is packed in capsid and reverse-transcribed into rcDNA. At Multivesicular Body (MVB), capsids are then enveloped and released into a new virion or returned into the nucleus through nuclear pore to increase covalently closed circular DNA (Tsukuda & Watashi, 2020*)*.

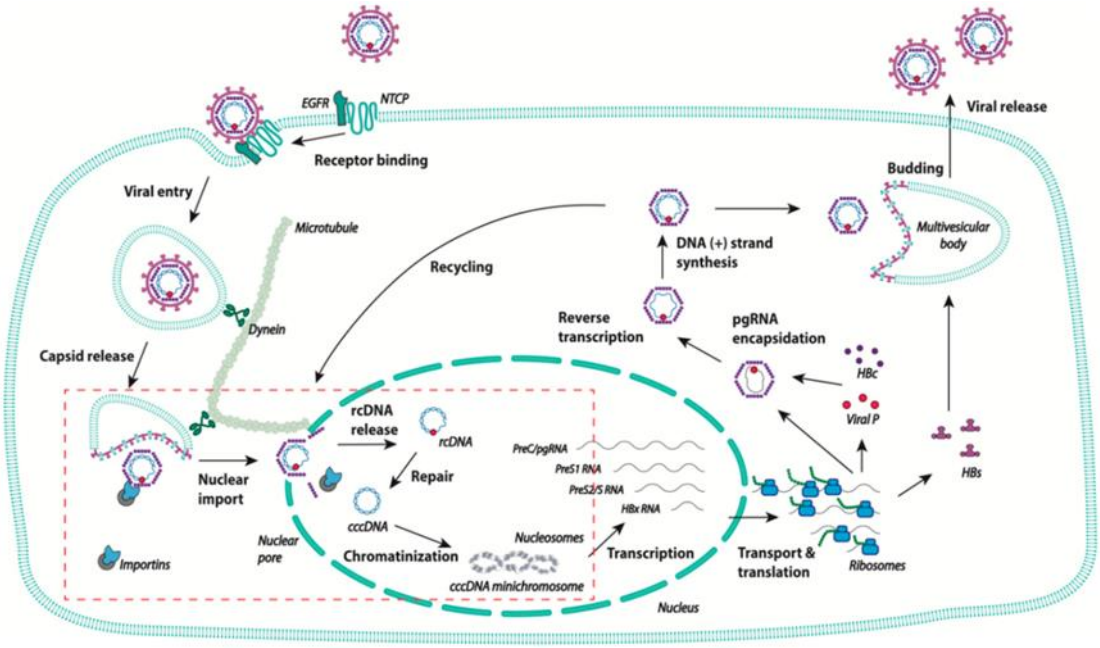


Figure 2.3 schematic representation of human HBV life cycle

Adapted from: https://www.mdpi.com/viruses/viruses-13 0757/article_deploy/html/images/viruses-13-00757-g001.png

2.2 HBV distribution

According to the WHO estimates from 2015, 3.5% of the world's population was infected with HBV; with the highest prevalence in the west pacific (6.2%), followed by Africa (6.1%), the eastern Mediterranean (3.3%), Europe (1.6%), South-east Asia (2.2%), and America (0.7%). WHO also reports 257 million chronic HBV patients (Global Hepatitis Report, 2017). In 2021, WHO reported that high-income countries had 0.005-0.12% HBV-positive blood donations, middle-income countries 0.13-4.74%, and low-income countries 2-6% (World Health Organization, 2021).

WHO reported a 6.1% HBV prevalence in Africa, with regional variation of (2-4%) in Northern Africa, (5-7%) in central, eastern, and southern Africa, and over 8% in western Africa. A systematic analysis found HBV prevalence in Ghanaian and Nigerian blood donors at 7.23–14.6% and 7.5–19.9%, respectively *(*Donkor & Maimaiti, 2018*).*

A 2017 Ugandan study found HBV prevalence of 4.1% among adults and 0.6% among children. HBV prevalence ranged from 0.8% in the southwest to 4.6% in the mid-northern. According to the Ministry of Health (2019), the Acholi tribe (9.2%) have the highest rate, while the Banyankole and Bakiga tribes (1.5%) have the lowest. According to Uganda Blood Transfusion Service (UBTS), 2.3% of blood donors had HBV in FY 2016/2017, with Arua (4.98%) and Mbale (4.21%) having the highest rates (Ministry of Health, 2018). An unpublished activity report from Gulu regional blood Bank (GRBB) shows that 4.3% of blood donations in FY 2022/2023 were HBsAg-positive

2.3 Transmission of HBV

The infection can be contracted when the skin and mucous membranes come into contact with infected substances such as saliva, semen, vaginal secretions, blood, and menstrual fluids. Unvaccinated individuals who engage in sexual activity with several partners, regardless of their sexual orientation, can transmit infections through sexual contact.

Furthermore, transmission of HBV also takes place via the utilization of contaminated surgical instruments in healthcare facilities and among individuals who engage in drug injection. In addition, infection can arise from medical procedures such as surgical operations, blood transfusion with unproperly screened blood, dental and tooth extractions, as well as from beauty therapies like tattooing, body piercing, and acupuncture. Furthermore, sharing sharp objects like razor blades, improperly sterilized syringes, and needles contaminated with body fluids carrying HBV infection can also lead to infection. HBV can also be transmitted by the perinatal pathway, when mothers who are seropositive for HBeAg pass the infection to their infants either during or immediately after birth, if no preventive measures are taken. Horizontal transmission, particularly within households and between family members, such as from child to child, is also important (World Health Organization, 2015).

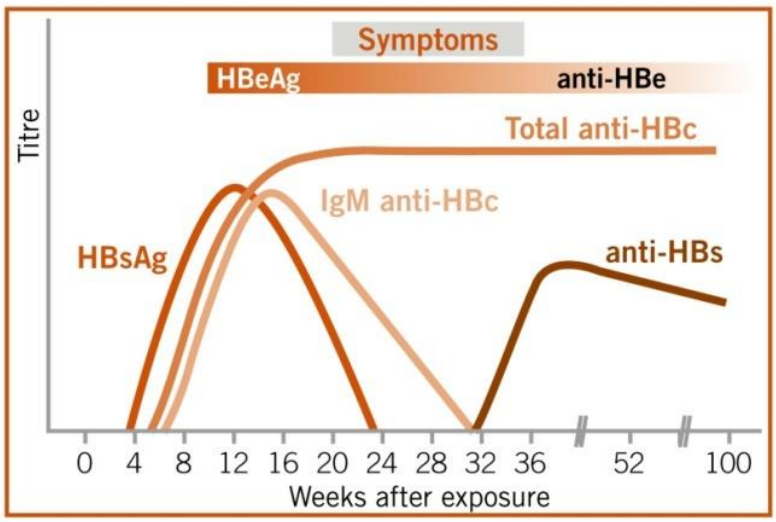
2.4 Hepatitis B infection

Hepatitis B infection results from being infected by hepatitis B virus (HBV), an enveloped DNA virus. This virus specifically targets the liver and causes hepatocellular inflammation. The infection can manifest as either acute or chronic, with different levels of disease, ranging from asymptomatic to symptomatic to progressing liver disease. (World Health Organisation, 2015). Chronic hepatitis B (CHB) is a big health concern with 257 million chronic cases reported. Between 20% and 30% of people with CHB progress to liver cirrhosis and hepatocellular carcinoma (HCC), and annually 650,000 die of CHB (World Health Organisation, 2015). Overall, CHB accounts for 45% and 30% of HCC and liver cirrhosis cases respectively (World Health Organisation, 2015). The average period it takes for HBV to show symptoms is 75 days but it can range from 30 to 180 days. The implementation of a universal program for immunization against hepatitis B, which commences with the administration of the first dose at birth, has shown to be incredibly successful in lowering the occurrence of the infection in regions where the disease is

prevalent. There are antiviral medications, such as pegylated interferon, that can effectively inhibit making more copies of the hepatitis B virus (HBV). These medications not only prevent the advancement of liver cirrhosis but also reduce the chance of acquiring hepatocellular carcinoma (HCC) and hepatitis-related fatalities. (World Health Organisation, 2015)

2.5 Hepatitis B disease

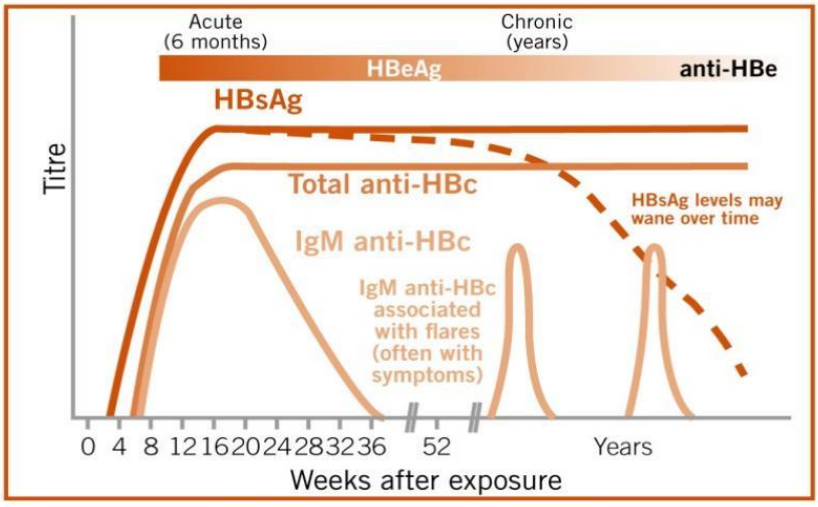
In acute hepatitis, two-thirds of individuals have mild, asymptomatic, and subclinical sickness, whereas one-third develop clinical symptoms like fatigue, nausea, jaundice, and severe liver failure. Liver failure affects 1% of acute hepatitis B patients, associated with lowering HBsAg and HBV DNA levels (Liang, 2009). During the initial stage of the disease, HBcAg becomes detectable just before the development of clinical symptoms, followed by the formation of Anti-HBc IgM antibody. As the levels of IgM titre in the blood decrease, the levels of IgG anti-HBc increase. Moreover, anti-HBe existence often follows the elimination of HBeAg, indicating the onset of the recovery process. In summary, the key markers for diagnosis of acute infection depends on finding of IgM anti-HBC and HBsAg in serum (Liang, 2009).



Taken from: *Guidelines On Hepatitis B And C Testing (Who 2017) P22* (Karayiannis, 2017)

Figure 2.4: The serology profile of Acute hepatitis B infection

In early CHB infection HBeAg, HBsAg and HBV DNA levels are always found in high concentration with mild to moderate levels of aminotransferase enzyme constituting the "immune tolerance phase". The infection can evolve into an "inactive carrier state" defined by disappearance of HBeAg, emergence of anti-HBe and drop in HBV DNA quantity to low or undetectable levels. CHB patients may show no symptoms of disease or have non-specific symptoms such as exhaustion, mild pain in right upper quadrant, splenomegaly among others. In severe chronic hepatitis B and late cirrhosis; survival rate of patients is about 50% with symptoms of ascitis, peripheral edema, encephalopathy and abdominal bleeding among others (Liang, 2009). In CHB, initial serological pattern shows presence HBsAg, HBeAg, HBV DNA, anti-HBc and later, serum samples continue to reveal HBsAg, HBeAg, and HBV DNA. In short, diagnosis of CHB is premised on detection for availability of HBsAg and IgG anti-HBc, as well as lack of IgM anti-HBc in serum of patients (Liang, 2009).



Taken from: GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22 (Karayiannis, 2017)

Figure 2.5: Serology profile of chronic hepatitis B infection

2.6 OBI infection

Occult hepatitis B infection (OBI) can be described as availability of HBV DNA in the liver, or both in liver and serum of persons with undetected HBsAg. OBI is categorized into seropositive and seronegative forms. Seronegative OBI occurs in persons who test negative for all antibody and antigen parameters, like anti-HBc and anti-HBs. The one parameter that is positive in these individuals is HBV DNA. On the other hand, seropositive individuals have measurable anti-HBc and/or Anti-HBs in their serum (Saitta *et al*., 2022).

The inability to detect HBsAg regardless of the continued existence of HBV DNA in serum is assumed to be owing to inhibition of replication and gene expression imposed by epigenetic and host immunological regulatory systems. This is evidenced in a study that showed hypermethylation

in CpG sites of OBI than CHB, indicating distinct epigenetic regulation in OBI and CHB (Vivekanandan *et al*., 2008). Another study demonstrated that transcription and replication is controlled by post transcriptional modification (acetylation) of cccDNA-bound histones. Particularly, low acetylation of the cccDNA-associated H_3 and H_4 histones were related to low HBV replication (Hong *et al*., 2017). Furthermore, some studies also report mutation in pre-S/S gene region in OBI individuals than in CHB as another explanation for undetectable HBsAg caused by reduction in antigenicity or modification of HBsAg synthesis and secretion. However, the studies continue to suggest that HBV DNA in OBI is replication competent and may be transferred by hemotransfusion and organ transplant, resulting in hepatitis B infection of the recipient (Enriquez-Navarro et al., 2020; Lin et al., 2022; Wang *et al*., 2022).

The incidence of OBI relies on the sensitivity of both HBsAg and HBV DNA test assays, as well as the widespread presence of the infection in the community (Kwak & Kim, 2014). A meta-analysis using electronic literature search reports OBI prevalence of 14.8% in Africa and OBI prevalence of 7.4% and 20% for seronegative and seropostive OBI

respectively (Ondigui *et al.*, 2022). Studies conducted in Kenya among men mainly having sex with men (msm-sw) and in Egypt among blood donors reports a high OBI prevalence of 18.7% and 4.1% respectively (El-Ghitany *et al*., 2013; Jepkemei et al., 2020). Another study among blood donors in cameroon reported a lower OBI prevalence of 0.56% (Fopa *et al*., 2019).

Over the years, the possibility of HBV spread through hemotransfusion has reduced due to the use more sensitive blood screening assays. However, the risk still exist where there is no Anti-HBc test and Nucleic amplification test (NAT) with detrimental effects of reactivation of latency, advancement to chronic liver disease (CHB) and hepatocellular carcinoma (HCC) (Saitta *et al*., 2022). A study reports all forms of OBI as infectious in immunocompromised patients, but in immunocompetent recipients, only the anti-HBc containing components are infectious (Kwak & Kim, 2014).

2.6.1 Diagnosis of OBI

The identification of OBI is based upon finding HBV DNA in the liver or blood of persons with undetected HBsAg. The most reliable method for detecting OBI is by testing for HBV genome from DNA extracts taken from the liver, although, testing for HBV genome in blood is more commonly conducted. Additionally, Anti-HBC testing can serve as a substitute indicator for detecting individuals who are seropositive for OBI. Moreover, it is recommended to employ anti-HBs probes targeting different epitopes of HBsAg in all HBsAg assays to complement the identification of S-escape variants. Additional suggested methods for cases with a limited amount of viral particles include nested PCR that focuses on at least three specific areas of the genome, real-time PCR, and droplet digital PCR (Saitta *et al*., 2022).

2.7 HBV genotype

Currently, 10 genotypes (A-J) and 40 sub-genotypes of HBV have been identified, with ≥8% and 4-8% nucleic acid variance in the viral genome. Some studies show that genotype

A (sub-genotypes A1-A8) has pluralistic distribution, genotype B (sub-type B1-B10) and genotype C (C1-C17) are more common in Asia, genotype D (sub-type D1-D12) is more common in southern Europe, India, and Russia, and genotype F has worldwide distribution. Genotypes E, G, H, I, and J sub-types are poorly described, although the studies list genotype E as frequent in Africa, genotype H in North and Central America, and genotype J in Japan. (Kafeero *et al*., 2023; Liu *et al.*, 2021).

According to a study, genotype A is most common in Africa, and there is regional diversity in the distribution of different genotypes. The main genotypes found in south-eastern Africa, North Africa, and west Africa are genotypes A, D, and E, respectively (Liu *et al*., 2021). Research undertaken in Bukina Faso, Egypt (specifically among hemodialysis patients), and Kenya (specifically among high-risk groups) found that the most common HBV genotypes among OBI cases are genotype E, genotype B, and genotype A, respectively (Diarra *et al*., 2018; Esmail *et al*., 2016; Jepkemei *et al*., 2020).

A Ugandan study reports the distribution of HBV Genotype D, genotype A and genotype E at (50.5%), (17.2%) and (1.1%) respectively. The study further reports prevalence of mixed infections with genotype A/E at 9.7% and with genotype A/D at 6.5% (Butt *et al*., 2014). Similarly, another study reports Genotype A and D to be predominant in Uganda at (46%) and (38.2%) respectively. There is scarcity of data on the frequency of OBI genotypes in the study area and Uganda as a whole.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Research site

The research site was Gulu Regional Blood Bank (GRBB), located in Laroo-Pece division, about 1km north of the central business area of Gulu city. Gulu Regional blood bank was selected because it is the regional collection Centre for blood donations in northern Uganda. Serological testing of blood samples for HBsAg was carried out at GRBB laboratory while samples for HBV DNA testing were tested at Nakasero laboratories (Kampala) where equipment are available. Extraction of HBV DNA from OBI reactive samples and quality control of HBV DNA isolates was carried out at Gulu University Multifunctional Laboratory. HBV DNA extracts were shipped to Inqaba Biotechnical Industries (Pty) Ltd (South Africa) for DNA sequencing.

3.2 Study population

This consisted of adult individuals (18-65yrs) who came to donate their blood at Gulu Regional Blood Bank facility and blood donation sites within Gulu city, and neighboring districts for convenience of sample handling.

3.3 Selection criteria

3.3.1 Inclusion criteria

All blood donors aged 18-65 years who accepted to participate in the study were included. Blood donations that test reactive for HBV DNA and non-reactive for HBsAg was selected for anti-HBc total antibody testing and HBV genotyping.

3.3.2 Exclusion criteria

The study excluded all potential blood donors who did not meet the requirement of the UBTS donor selection criteria and those under 18years since parental permission may be required.

3.4 Sample size determination

The following formula by Cochran (1963) was used to compute the sample size

$$n_o = \frac{Z^2 * p * q}{e^2}$$

Where: n_o = refers to sample size,

z = Refers to normal standard deviation set at 1.96 equivalent to 95% CI

P= Proportion of OBI in the general population is not known. Assum. P= 0.5,

e= Standard error allowed in the study set at, 3% (0.03)

q=1-P 0.5

$$n_o = \frac{1.96^2 * 0.5 * 0.5}{0.03^2}$$

By substitution therefore, the samples size was 1,067 blood donors.

3.5 Sampling procedure

Gulu Regional blood bank was purposively selected because it is the regional collection Centre for blood donations in northern Uganda. The participants who came to donate blood were selected randomly using systematic random sampling. Briefly, systematic random sampling was conducted as follows; a sampling frame consisting of 2,144 donors which corresponds to the average monthly blood units donated at Gulu Regional Blood Bank was used. The sampling frame of 2,144 was then divided by the sample size (1,067) to obtain the sampling interval of two (02). Therefore, participants were registered consecutively as they arrived at the donation site. The first sample was randomly selected and then every 2nd blood donor was selected until the sample size was realized. In the case of selected blood donors who refused to consent to the study, the next blood donor in line was selected.

3.6 Study design

The study was a cross-sectional laboratory-based study where blood donations were serologically screened for HBsAg using Architect chemiluminescent microparticle

immune assay (CMIA) and HBV DNA testing conducted. Donated blood samples that test reactive for HBV DNA were further tested for anti-HBc antibodies by MBN laboratories Gulu, and sequenced to determine the genotypes of the OBI infection at Inqaba Biotechnical Industries (Pty) Ltd (South Africa).

3.6.1 Specimen collection and handling

Blood samples (6mls) from blood donors were collected in plain tubes (red top) and plasma separation tubes containing gel (lithium heparin), and immediately centrifuged at 4500 rpm for 10mins to obtain clear serum and plasma respectively. The serum samples were tested for HBsAg while the plasma samples were aliquoted and frozen at -20^{O}C. One part of the frozen plasma aliquot was packed following recommended standards for shipment and transportation for HBV DNA testing. HBV DNA was extracted from aliquot of Plasma samples that tested non-reactive for HBsAg and reactive for HBV DNA and stored at -20^{o}C until further analysis.

3.6.2 Serodiagnosis of HBsAg

Centrifuged blood samples in plain tubes were tested serologically by routine UBTS screening protocols for HIV1/2, HBsAg, anti-HCV and syphilis using the Architect chemiluminescence micro particle immune assay (CMIA, Abbot Laboratories Diagnostics Division, Abbott Park, IL, USA) method while following assay protocols. Briefly, the Architect system inventory was checked and updated with supplies of solid/liquid wastes; wash buffer, trigger and pre-trigger solutions, and reaction vessels. Samples were then loaded into the sample carrier and test order were created by entering carrier ID, sample position and sample ID in the C, P, and SID data entry boxes respectively. Test assays were then selected from the assay list and F3 was selected to add orders. Carriers containing samples was placed in the load queue for the processor to automatically test the samples.

3.6.3 Molecular diagnosis of HBV

Plasma samples collected in plasma separator tubes were tested for HBV DNA using cobas 6800 (Roche) qualitative nucleic amplification test (NAT) while following assay protocols. Briefly, the Cobas MPX 6800 utilized real-time PCR technique on a completely automated DNA extraction and ablution, which was then superseded by PCR amplification and detection. Automated data management system classifies all test results as non-reactive, reactive and invalid. Details of the procedure is in appendix IV

3.6.4 Serodiagnosis of Anti-HBc

Plasma samples that tested non-reactive for HBsAg but reactive for HBV DNA was packed and transported to MBN laboratories, Gulu and assayed for hepatitis B core antibodies using commercial ELISA kit LIAISON XL MUREX Anti-HBc (DiaSorin Inc, S.A). The cut-off value was calculated by formular = negative control (Nc) + 0.05

3.6.5 DNA extraction

Occult hepatitis B genome was extracted from 100µl of plasma using Quick-DNA™ Viral Kit/ Zymo-Spin™ IC Columns (Zymogen research international, Cambridge Bioscience Limited, UK). The extracted DNA was stored at -20^{O}C until use. Briefly in the Quick-DNA™ Viral Kit assay protocol, 300µl of DNA/RNA shield was added to 100 µl of plasma (immediately taken out of the freezer) in 1.5 ml microcentrifuge tubes, mixed and allowed to equilibrate to room temperature. 20 µl of proteinase K was added to the mixture, mixed and incubated at 55ºC for 45minutes. 400 µl of viral DNA buffer was then added to the mixture, briefly vortexed, and left to stand at room temperature for 10minutes. The mixture was then centrifuged at 16000xg for 5mins to pellet debris. The supernatant fluid was then transferred to Zymo-spin column in a collection tube and re-centrifuged at 16000xg for 1minute and discarded the flow through in the collection tube. 300 µl of wash buffer was added to the column, followed by centrifugation at 16000xg for 1minute and discarded the

flow through in the collection tube. The spin column was placed on microcentrifuge tube, then 20 µl of elution buffer was added directly to the column matrix and left to stand for 1 minute at room temperature, followed by centrifugation for 1minute to elute the DNA. Eluted DNA was then stored at -20ºC until further analysis.

3.6.6 Primer Design and DNA Amplification

A set of primers OP-F2 (5'-ATGTTGCCCGTTTGTCCTCT-3') and OP-R2 (3'-GCCCTACGAACCACTGAACA-5') to the S-gene region (nt 463-712), expected to give amplicon size of 250bps was designed using Primer-BLAST tool from HBV reference nucleotide sequence (Accession number NC_003977.2) obtained from NCBI data Bank. The primer pair was then validated for PCR primer properties using Primer STAT tool. The primer pair was synthesised by Macrogen Inc. (Europe)

DNA fragments of the S gene was amplified using Taq polymerase (New England BioLabs) by conventional PCR. 2µl of HBV DNA isolated from each donated plasma sample was added to 23µl of PCR mixture. The PCR mixture contained; 12.5µl of Master-Mix, 0.5µl each of OP-F2 and OP-R2 primers to make final concentration of 0.2µM, and 9.5µl of nuclease free water to make a total volume of 25µl. Amplification was realized using thermal cycler (Applied biosystems) as follows; an initial 5minutes denaturation step at 94ºC, followed by 30 cycles of denaturing at 94ºC for 45seconds, annealing step at 55ºC for 30seconds, and an elongation step at 72ºC for 60seconds, with a final extension duration of 10minutes at 72ºc. Five microliters (5µl) of each PCR product mixed with loading dye in 1% Agarose gel stained with 5µl of ethidium bromide was electrophoresed at 120voltage for 40 minutes and the DNA bands were visualized by gel documentation system (Bio-Rad ChemiDoc Image systems).

3.6.7 Sequencing and sequence analysis

A total of six PCR products that showed positive bands on 1% Agarose gel were packed and shipped to Inqaba Biotechnical Industries (Pty) Ltd (South Africa) for DNA sequencing. All six (100%) of conventional PCR product were successfully sequenced. The primary analysis of the PCR product was carried out using the BLAST algorithm (http://www.ncbi.nlm.nih.qov/BLAST) on the nucleotide sequences provided in the GenBank database.

Phylogenetic analysis using Bioinformatics system (MEGAv.12 software) was used to determine OBI genotypes. Sequences obtained from the nucleotide BLAST, reference sequences of HBV genotype (A-H) acquired from NCBI-GenBank and PCR product sequences in FASTA format were uploaded on MEGA version.12 software, and subjected to multiple sequence alignment using ClustalW alignment explorer. The alignment data was then trimmed to remove overlapping sequences and saved in MEGA format file. Phylogenetic tree of the MEGA file was constructed using Maximum likelihood method, reliability of the tree was tested using bootstrap value of 1000 replicates, Jukes-Cantor model. The Jukes-Cantor model was used since it's a standard model for correcting multiple Hits in the nucleotide sequences. OBI genotypes were inferred by comparing evolutionary closeness between DNA fragment sequences and the reference HBV genotypes

3.7 Study variables

3.7.1 Independent variable

The independent variables were, age, sex, type of blood donor, and type of HBV test assay

3.7.2 Dependent variables

The dependent variable was undetected occult hepatitis B infection among blood donor

3.8 Data analysis

3.8.1 Statistical analysis

HBsAg and HBV DNA results were input into Microsoft excel computer program and exported to IBM SPSS V. 20 software for analysis. The prevalence of OBI was calculated by dividing the number of OBI positive samples (nominator) by the total number of samples tested (denominator) and expressed as a percentage. McNemar's and Pearsons chi-square tests were used to detect significance difference between the different HBV markers (HBsAg and HBV DNA), prevalence of HBV infection between male and female, new and repeat blood donors. A $P< 0.05$ was considered statistically significant. Additionally, sensitivity, specificity and likelihood ratio were used to compare the HBsAg to HBV DNA tests for screening of donated blood. Sensitivity (true positive/true positive + False negative) and specificity (true negative/true negative + false positive) is a measure of true positive and true negative rates respectively. Positive likelihood ratio (LR+) and Negative likelihood ratio (LR-) was calculated using the formulas Sensitivity/1- Specificity and 1-Sensitivity/Specificity respectively. A LR+ value greater than 10 indicated good specificity while LR- value less than 0.1 indicated good sensitivity. Furthermore, the data was also presented using frequency tables.

3.8.2 Data management and Quality control

Electronic data was password-protected and all paper-based documents remained secured with a lock to avoid any loss and unauthorized access. In order to guarantee appropriate data collection, research assistants were trained on research protocol. The researcher and research assistants adhered to standard operating protocols as set by UBTS, and manufacturer's instructions during laboratory testing. Data collection tools included; data collection forms, test results, consent forms, and pens. Commercial negative and positive control samples were tested daily to evaluate the performance of the testing equipment and

reagents before testing of research samples. Extracted DNA was visualized on 1% agarose gel electrophoresis to ascertain DNA quality.

3.9 Limitation of the study

The study was limited by the small OBI sample size (06) from which sequences could be obtained for genotyping. In addition, the study used short S-gene fragment sequence of 250bps which could have affected the detection of other HBV genotypes because of the high cost of whole genome sequencing. Furthermore, the study only performed serological testing for HBsAg without performing HBsAg confirmatory test. Instead, anti-HBc was tested as a surrogate marker for occult hepatitis B infection.

3.10 Ethical consideration

The research protocol was approved by Gulu University Research Ethics Committee (GUREC) on May 06th, 2024 (number: GUREC-2024-815). Permission to conduct research on donated blood samples was obtained from management of Uganda Blood Transfusion service, Gulu. In addition, consent was also obtained from all blood donor participants. Study participants with abnormal results were referred to the hepatitis B clinic at Gulu Regional Referral Hospital for care and treatment using the available UBTS referral system.

3.11 Dissemination Plan

Final report of this study was submitted to Gulu University Directorate of graduated studies in partial fulfillment of the requirements for the award of master of science of Molecular Biology and Biotechnology. In addition, a copy of the final report was disseminated to Uganda Blood Transfusion Service. Furthermore, the finding of the research will be presented on conferences and also published in an international journal.

CHAPTER FOUR: PRESENTATION OF RESULTS

4.1 Research participant demographics

A Total of 1,067 Voluntary non-remunerated blood donors (VNRBDs) were recruited in the study, 35.2% (n=376) and 64.8% (n=691) were females and males respectively; the average age of the study participants was approximately 22 years as shown in table 1 and 2 below.

Table 4.1: *Sex distribution of blood donors*

Variable	Category	Frequency	Percent
Gender	F	376	35.2
	M	691	64.8
	Total	1067	100.0

Table 4.2: *Showing total population, Mean, range, standard deviation and variance in age of study participants*

Variable	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Age	1067	44	18	62	21.96	6.353	40.360
Valid N (listwise)	1067						

4.2 Prevalence of occult hepatitis B infection (OBI)

Prevalence of HBV DNA among blood donor in Gulu Regional Blood Bank was approximately 3.3% (35/1067; 95% CI: 2.3-4.5) as detected by Cobas MPX 6800 NAT tests, out of which, about 0.9% (10/1067; 95% CI: 0.5 - 1.7) was Occult hepatitis B infection. While, the prevalence of HBsAg was at 2.4% (26/1067) among blood donors by

Architect HBsAg Qualitative II as presented on tables 4.3, 4.4 and 4.5 below. There was significant statistical difference in the test outcomes between the two test methods for testing for hepatitis B infection, McNemar test X^2, $p=0.012$.

Table 4.3: Prevalence of Occult Hepatitis B infection in blood donors

Variable	Category of results	Frequency	Percent
HBV	Negative	1057	99.1
	OBI Positive	10	.9
	Total	1067	100.0

Table 4.4: Prevalence of HBV DNA (NAT) test in blood donors

Variable	Category of results	Frequency	Percent
NAT	Non-reactive	1032	96.7
	Reactive	35	3.3
	Total	1067	100.0

Table 4.5: Prevalence of HBsAg in blood donors

Variable	Category of results	Frequency	Percent
HBsAg	Non-reactive	1041	97.6
	Reactive	26	2.4
	Total	1067	100.0

The frequency of Hepatitis B infection among male and female blood donors was estimated at 2.8% and .5% respectively as shown on table 4.6 below. The frequency variation was statistically significant, X^2 $(1, N=1067) = 6.962, p=0.008$; implying that the males are more likely to test positive compared to their female counterparts.

Table 4.6: Frequency distribution of HBV by Gender among blood donors

Variable	Category	HBV results		Total
		Non-reactive	Reactive	
Gender	Male	661(61.9%)	30(2.8%)	691(64.8%)
	Female	371(34.8%)	5(0.5%)	376(35.2%)
	Total	1032(96.7%)	35(3.3%)	1067(100.0%)

Approximately 38.1% (n=407) and 61.9% (n= 660) of study participants were new and repeat VNRBDs respectively. The frequency of hepatitis B infection among new and repeat Voluntary non-remunerated blood donors was 1.8% and 1.5% respectively. However, the difference was not significant, X^2 *(2, N=1067) = 4.190, p= 0.123* as shown on table 4.7, below.

Table 4.7: Prevalence of hepatitis B between new and repeat blood donors

Variable	Category of results	Donor type		Total
		New donor	Repeat donor	
HBV	Negative	388_a (36.4%)	644_b (60.4%)	1032(96.7%)
	Positive	13_a (1.2%)	12_a (1.1%)	25(2.3%)
	Positive/OBI	6_a (0.6%)	4_a (0.4%)	10 (0.9%)
	Total	407 (38.1%)	660 (61.9%)	1067(100.0%)

Each subscript letter denotes a subset of Donor type categories whose column proportions do not differ significantly from each other at the .05 level.

4.2.1 Hepatitis B core antibody test

A total of ten (10) samples that tested non-reactive for HBsAg but reactive for HBV DNA were further tested for anti-HBc total IgG/IgM. Approximately 80% (8/10) and 20% (2/10) was seropositive and seronegative OBI respectively.

4.3 Sensitivity and specificity of HBsAg test for screening of hepatitis B infection in blood donors

The sensitivity and specificity for the Architect HBsAg qualitative II test (Abbott diagnostics, Ireland) was calculated at 71.4% and 99.9% respectively against the Cobas MPX 6800 NAT (Roche Diagnostics GmbH, Germany) as shown in table 4.8, below.

Table 4.8: Showing results for sensitivity and specificity of the Architect HBsAg qualitative II test (Abbott diagnostics) for the screening of blood donors

Variable	Category of results	HBsAg		Total
		Non-Reactive	Reactive	
NAT	Non-reactive	1031(99.9%)	1(0.1%)	1032(100.0%)
	Reactive	10 (28.6%)	25(71.4%)	35(100.0%)
	Total	1041(97.6%)	26(2.4%)	1067(100.0%)

4.3.1 Likelihood ratio (LR)

Positive likelihood ratio (LR+) and Negative likelihood ratio (LR-) for the Architect HBsAg Qualitative II (Abbott diagnostics, Ireland) test was Calculated at 714 and 0.286 respectively. indicating that the Architect HBsAg Qualitative II (Abbott diagnostics, Ireland) test was more specific (LR+ = 714; > 10) but less sensitive (LR- =0.289; > 0.1) test for the detection of hepatitis B infection among blood Donors.

4.4 Extraction of Occult Hepatitis B DNA

HBV DNA was successfully extracted from six samples out of ten plasma samples (6/10) that were reactive on Cobas MPX 6800 NAT tests (Roch Diagnostics GmbH, Germany) but Non-reactive on Architect HBsAg qualitative II test (Abbott diagnostics, Ireland). The S gene region was then amplified by conventional PCR with expected reaction products size of 250bps as shown in Figure 7, below.

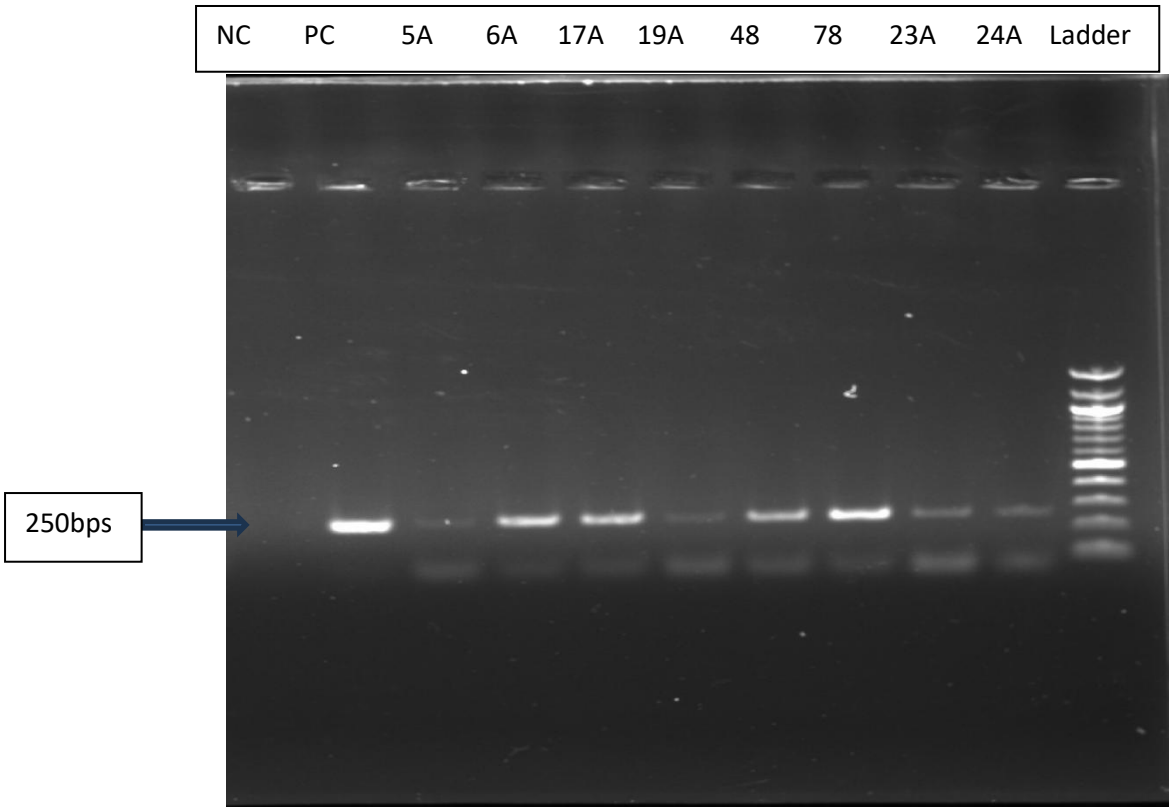


Figure 4.1: Agarose gel electrophoresis of PCR reaction product of the S gene fragment.

Samples 5A, 6A, 17A, 19A, 23A and 24A were reactive for HBV DNA but non-reactive for HBsAg; while samples 48 and 78 were reactive for both HBV DNA and HBsAg. Negative control (NC) and positive control (PC).

4.5 HBV DNA sequencing results

All six HBV DNA amplicons were successfully sequenced by Inqaba Biotechnical Industries (Pty) Ltd (South Africa) and the sequences are shown in appendix V.

4.6 Genotypes of Occult Hepatitis B infections

Five (05) of the S gene fragment nucleotide sequences clustered around recombinant genotype D/E while one (01) sequence clustered around genotype D as shown in the phylogenetic tree below; implying the study identified occult hepatitis B recombinant genotype D/E and genotype D at (83.3%) and (16.7%) respectively in blood donor samples.

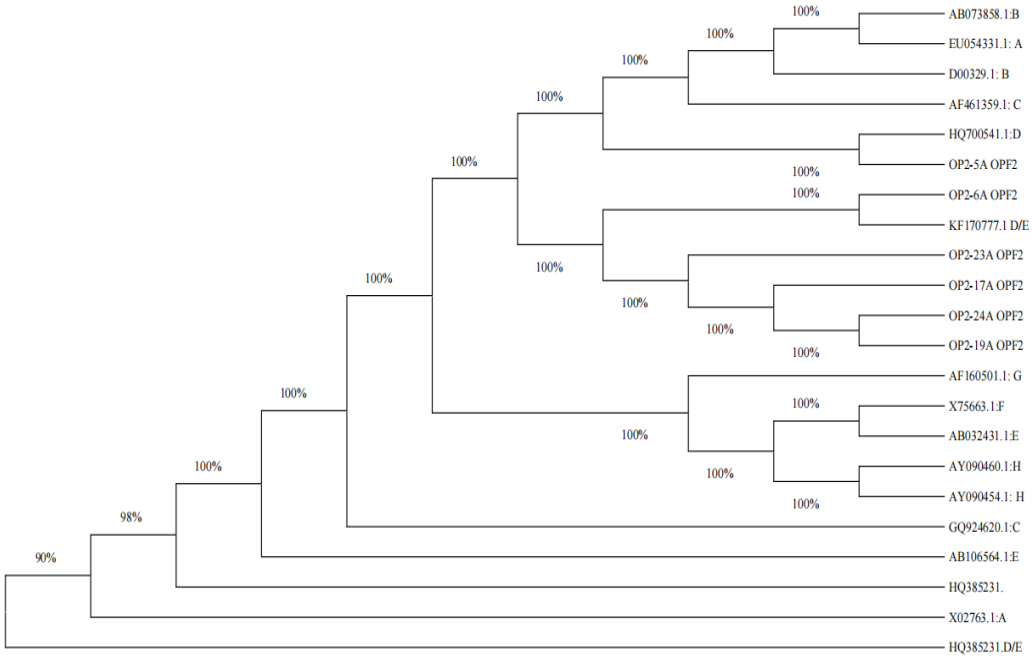


***Figure 4.2**: Phylogenetic tree of Occult hepatitis B (OBI) nucleotide sequences of the fragments of conventional PCR products (OP2-5A_OPF2, OP2-24A_OPF2, OP2-19A_OPF2, OP2-23A_OPF2, OP2-17A_OPF2, OP2-6A_OPF2) and fragments HBV DNA standard genotype sequences of S gene*

CHAPTER FIVE: DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

5.1 Participants demographics

The study participants consisted of 64.8% and 35.2% males and females respectively, with an average age of approximately 22. This can be explained by the fact that most blood donations in the study area are conducted in Secondary Schools and Tertiary Institutions where majority of the learners are in their youthful age. Secondly, the variation between male and female respondents could be explained by the fact that, male donors have more positive attitude to blood donation than their female counterparts.

5.2 Prevalence of Occult hepatitis B infection

OBI is a serious burden in blood donors as it may not be detected when blood donations are screened using serological methods without performing NAT hence posing risk of viral transmission to the blood recipient. This study investigated the prevalence of OBI among blood donors at Gulu Regional Blood Bank using NAT and genotyped the OBI strains.

The study found that the prevalence of OBI among blood donors at Gulu Regional Blood Bank was 0.9% (10/1,067). The finding is consistent with a similar lower rate of infection of 0.56% (6/1,071) reported in Cameroon among blood donors (Fopa *et al*., 2019). Although the finding of 0.9% appears low, the prevalence rate is relatively high since the samples were obtained from healthy blood donors from a Hepatitis B endemic region (Yu Ri Im *et al*, 2022). Higher prevalence of 4.1% (21/508) was reported among Egyptian blood donors (El-Ghitany *et al*., 2013). The variation in the findings in the present study compared to the previous ones could be due to different test methods used in HBV DNA detection. In the current study and the one conducted in Cameroon, Real time PCRs were used compared to semi-nested PCR done in the Egyptian study. Several studies have reported the preferred use of Nested PCR or semi-nested PCR for detection of OBI with

very low viral load (Makvandi, 2016). The finding of the current study, shows that blood donations in the region carry a potential risk of Occult hepatitis B viral transmission to the blood recipients with increased risk of developing liver cirrhosis and hepatocellular carcinoma.

The study also found prevalence of hepatitis B infection in blood donor attending Gulu Regional Blood Bank at approximately 3.3% (35/1067) with a significant frequency distribution between male and female blood donors (2.8% vs 0.5%, P=0.008). The prevalence of HBV infection reported in the current study is lower compared to previous ones of 7.9% and 4.6% from the same study area (Chiesa *et al*., 2020; Ministry of Health, 2019). This difference could be explained by the fact that the current study was conducted among blood donors who were screened using a robust medical donor selection process at GRBB compared to the ones in the previous studies conducted among women attending antenatal services and the general public. Besides, the significant prevalence between male and female blood donors (2.8% vs 0.5%, P=0.008), could be due to the high-risk sexual practices and poor health seeking behavior in males compared to their female peers (Rugaatwa *et al*., 2022).

In addition, the prevalence of HBV infection was at 1.8% and 1.5% between new and repeat donors respectively. The difference in the prevalence could be explained by the fact that repeat donors are considered a low risk for viral hepatitis infection because they are frequently screened for transfusion transmissible infection (TTIs). However, the difference in the prevalence of hepatitis B infection between new and repeat donors was not statistically significant ($p>0.05$); implying that both new and repeat donors in this region pose similar risk of hepatitis B infection to the blood recipients in northern Uganda.

In this study, OBI was classified into two groups, seropositive and seronegative OBI. Seropositive OBI refers to individuals with positive anti-HBc, negative HBsAg and positive HBV DNA tests detectable in plasma. While seronegative OBI refers to individuals with negative serology markers (HBsAg and anti-HBc test) but a positive HBV DNA. The current study found the frequency of seropositive and seronegative OBI at 80% (8/10) and 20% (2/10) respectively. This findings is comparable with previous study conducted on Occult hepatitis B (Saitta *et al*., 2022). Another study conducted in south Korea also reported seronegative OBI prevalence greater than 20% (Kwak & Kim, 2014). The findings from the current study confirms that anti-HBc Ab can be used as a marker for screening of OBI in blood donors especially in resource limited countries where HBV DNA testing is not affordable.

5.3 Sensitivity and specificity of HBsAg test for screening of hepatitis B infection among blood donors

This study evaluated the sensitivity and specificity in analytical performance for HBsAg against HBV DNA for screening of blood donors in the region. Sensitivity is the ability of a test to detect all true positive tests as positive while specificity is ability of a test to detect all true negative tests as negative. In the present study, sensitivity and specificity of the test methods used for blood donor screening was at 71.4% and 99.9% respectively for the Architect HBsAg qualitative II test (Abbott diagnostics, Ireland). The finding from this study on specificity, is consistent with the 99.91% reported by the Abbott Laboratories. However, Abbott Laboratories reports higher sensitivity of 100% (Abbott Laboratories, 2013). This difference in performance sensitivity could be because Abbott laboratories used cadaveric and living donor serum or plasma spiked with human plasma reactive for HBsAg for the study. The results from this study revealed a low sensitivity in analytical performance of the Architect HBsAg qualitative II test (Abbott diagnostics) for the

screening of blood donors; implying that the Architect HBsAg qualitative II test (Abbott diagnostics, Ireland) could miss to detect some HBsAg mutants and Occult hepatitis B infection.

The study revealed the likelihood-ratio for Architect HBsAg qualitative II test (Abbott diagnostics, Ireland) to ascertain that a given result will occur in a blood donor with hepatitis B than in blood donor without hepatitis B. A positive likelihood ratio greater than 10 and negative likelihood ratio less than 0.1 indicates good specificity and good sensitivity respectively. This study, estimated positive and negative likelihood ratio at 714 and 0.286 respectively. The results of likelihood ratios from this study confirms that the Architect HBsAg qualitative II test (Abbott diagnostics, Ireland) exhibits a high specificity in analytical performance but a low sensitivity; implying that it's a better test to confirm presence of hepatitis B infection than for screening of the disease in blood donors and that there is an unmet need for blood screening in the region.

5.4 Occult hepatitis B Genotypes

Genotypes are important for the determination of virus evolution, treatment regimens, and following of transmission patterns. This study reports Occult Hepatitis B Genotype D and recombinant genotype D/E at 16.7% and 83.3% respectively from fragments of S gene in blood donor samples. The finding is not in agreement with earlier studies conducted in Bukina Faso, Egypt and Kenya that report the most common OBI genotypes E, B and A respectively. This difference can be explained by HBV endemicity in the different countries and small sample size in this study which could have affected detection of all the circulating genotypes. However, this study is in agreement with previous studies in Uganda that reported the distribution of HBV Genotype A, recombinant Genotype D/E and Genotype D at 46%, 28% and 26% respectively; and Genotype D, genotype A and genotype E at (50.5%), (17.2%) and (1.1%) respectively ((Kafeero *et al*., 2022; Butt *et al*.,

2014); suggesting that OBI genotypes could have resulted from mutations on S gene of circulating HBV genotypes in the country. Besides, Genotype D is associated with blood transfusion (Kafeero *et al*., 2022); further suggesting that OBI could be transmitted by blood transfusion. Interestingly, the current study reports recombinant OBI Genotype D/E in conformity with a recent study on HBV genotype in Uganda (Kafeero *et al*., 2022). Recombinant Genotype could rise from infections resulting from global and continental travels.

5.5 Conclusion

This study determined the prevalence of Occult hepatitis B infection at 0.9%. The finding revealed that blood donations in the region carry a potential risk of Occult hepatitis B viral transmission to the blood recipients. In addition, the sensitivity and specificity for the Architect HBsAg qualitative II test was at 71.4% and 99.9% respectively, indicating low sensitivity and high specificity in analytical performance. Therefore, use of HBsAg alone for screening of prospective blood donors is not adequate to eliminate the risk of HBV transmission through blood transfusion in northern Uganda. Furthermore, the study reports Occult Hepatitis B Genotype D and recombinant genotype D/E among blood donors in the region. Besides, Genotype D is associated with blood transfusion, further suggesting that OBI strains detected in this region could be transmitted by blood transfusion.

5.6 Recommendations:

- The study revealed that blood donations in the region carry a potential risk of Occult hepatitis B viral transmission to the blood recipients, therefore, the study recommends evaluation of cost of HBV DNA and anti-HBc testing for detection of Occult hepatitis B in all blood donations in the region
- The study also revealed that use of HBsAg alone for screening of blood donation is not adequate to eliminated the risk of transmission of HBV through blood

transfusion, therefore, introducing parallel testing for both HBsAg and HBV DNA on all blood donations could reduce on the risk of HBV transmission and better confirm HBV infection in Northern Uganda.

- In the current study, there was limited HBV sequences analyzed which could have limited detection of various OBI strains; therefore, the study recommends further studies using larger number of HBV sequences to comprehensively explore the OBI strains in the region.

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Appendix I: Consent Form

INFORMED CONSENT DOCUMENT

PREVALENCE AND GENOTYPIC CHARACTERISATION OF OCCULT HEPATITIS B INFECTION AMONG BLOOD DONORS AT GULU REGIONAL BLOOD BANK, NORTHERN UGANDA

Investigator(s): Ojok Polycarp

Institution(s): Gulu University

Introduction
My name is Ojok Polycarp, a student of Masters degree of Science in Molecular Biology and Biotechnology of Gulu University. This informed consent explains the study to you. After the study has been explained, any questions you may have are answered. When you have decided to participate in the study, you will be asked to sign a consent, which you will be given a copy to keep.

A brief description of the sponsors of the research project
This research project will be purely sponsored by myself as the principal investigator and is a requirement for the award of Master degree of science in molecular biology and biotechnology of Gulu University.

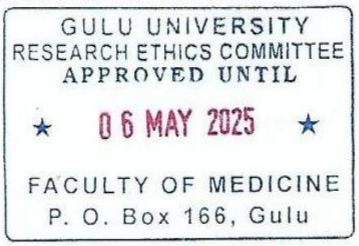
Purpose:
The study seeks to generate information on the prevalence of Occult hepatitis B among blood donors in northern Uganda. The findings of this study may help to adopt new guidelines on Hepatitis B testing among blood donors in the region. Secondly, detection of occult hepatitis B infection will reduce on transfusions which can lead to HBV infection in blood recipients since blood donations that will be found to be HBsAg negative but hepatitis B DNA positive will be excluded from blood supply and discarded.

Procedures:
Your participation in this study will involve consenting to participate in the study and about 6mls of your blood will be drawn in a sample tube during the blood donation process.

Who will participate in the study?
You have been chosen to participate in this study because you are a blood donor in northern Uganda and the blood donation process will last approximately 5-10 minutes, and a total of 1,067 participants will take part in this study.

Risks/discomforts:
There is no foreseeable risk of harm or discomfort that will arise from your participation in this study. The only risk or discomfort will be the inconvenience in terms of time spent during consenting to the study and an extra 6mls of blood drawn on top of the blood donation.

Benefits:



Your participation in this study will help to improve the quality of blood and blood components supplied to the various health facilities for management of patients in the region. You will get feedback on the findings and progress of the study and any new information that affects the study participants (including incidental findings) will be made available to the research participants through Gulu regional Blood Bank.

Confidentiality:
Your identity will not be revealed to any one as we shall only use codes to identify participants. Information obtained will only be accessible by the research team. Soft copies of the data will be protected by password and hard copy files will be kept under lock and key. Confidential information will only be accessed by the principal investigator.

Alternatives:
You do not have to participate in this study if you are not interested. You will not lose any benefit in case of no participation.

Cost:
There will not be any additional cost incurred as a result of participating in this study. However participants will given some refreshment to cater for their time.

Questions:
If you have any questions related to the study as a research participant, you can contact the principal investigator, Ojok Polycarp on telephone number 0770554771 or via email on o.polycarp@yahoo.com

Statement of voluntariness:
Participation in the research study is voluntary and you may join on your own free will. You have a right to withdraw from the study at any time without penalty.
If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson, Gulu University Research Ethics Committee, Dr. Julaina A. Obika Tel: No., 0772964491; email: j.obika@gu.ac.ug ; or the Uganda National Council for Science and Technology, on plot 6 Kimera road, Ntinda, Kampala on Tel 0414705500.

Statement of consent
..... has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw at anytime. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name .....Signature/thumbprint of participant.....Date .....

Name.....Signature of witness.....Date.....

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
★ 06 MAY 2025 ★
FACULTY OF MEDICINE
P. O. Box 166, Gulu

Appendix II: REC approval letter

GULU UNIVERSITY

P.O. Box 166, Gulu (U)
Website: www.gu.ac.ug
Email: gurec@gu.ac.ug

Tel: +256 471 432 096
Fax: +256 471 432 913
Mob: +256 772 305 621
+256 776 812 147

RESEARCH ETHICS COMMITTEE

06/05/2024

To: OJOK POLYCARP

0770554771/ 0758284371

Type: Initial Review

Re: GUREC-2024-815: Prevalence and Genotypic characterization of Occult Hepatitis B infection among blood donors at Gulu Regional Blood Bank, northern Uganda

I am pleased to inform you that at the **111th** convened meeting on **14/03/2024**, the Gulu University REC meeting voted to approve the above referenced application.
Approval of the research is for the period of **06/05/2024** to **06/05/2025.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **06/05/2025** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by Gulu University REC:

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
★ 06 MAY 2025 ★
FACULTY OF MEDICINE
P. O. Box 166, Gulu

No.	Document Title	Language	Version Number	Version Date
1	Protocol	English	2.0	2024-04-17
2	consent form Acholi version	Luo (Acholi)	1	2024-04-17
3	Informed Consent forms	English	2.0	2024-04-17
4	Covid-19 risk management plan	English	2.0	2024-04-16
5	Data collection tools	English	1	--

Yours Sincerely

Julaina A. Obika (PhD)
For: Gulu University REC

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
06 MAY 2025
FACULTY OF MEDICINE
P. O. Box 166, Gulu

Appendix III: Data collection tool

Data collection tool

i) Prevalence of Occult hepatitis B

Sample No.	Sex	Age	Type of donor	HBsAg results	NAT results	Anti-HBc IgM results	Occult Hepatitis B

GULU UNIVERSITY
RESEARCH ETHICS COMMITTEE
APPROVED UNTIL
★ 06 MAY 2025 ★
FACULTY OF MEDICINE
P. O. Box 166, Gulu

II) Genotypic characterization of occult Hepatitis B

Occult Hepatitis B Sample No.	DNA amplification Result	DNA Sequence Result	Occult Hepatitis B Genotype

VERSION 2.0



Appendix IV: permission to conduct study

May 08, 2024.

Principle Medical Officer,

Gulu Regional Blood Bank,

P.O Box 160, Gulu.

Approved the study to conduct

PRINICAL MEDICAL OFFICER
IN-CHARGE
GULU REGIONAL BLOOD BANK
15 SEP 2024
P.O. BOX 160, GULU
TEL: 0471 32 0

Dear Madam,

RE: ASSISTANCE FOR RESEARCH

This is to request for research assistance to conduct research at Gulu regional blood Bank. I am a student of Gulu University **Reg. No. 22/U/1189/MBB/PS.** The research is part of the requirements for the award of a Master Degree of Molecular Biology and Biotechnology of Gulu University.

The research Topic is: **"Prevalence and Genotypic characterisation of Occult Hepatitis B infection among blood donors at Gulu Regional blood Bank".** The research protocol has been approved by Research and Ethics committee of Gulu University (Reg: GUREC-2024-815) and registered with the Uganda National Council for Science and Technology (UNCST). Attached is a copy of the letter of approval.

I am grateful in advance for all the assistance that will be accorded towards the study.

Sincerely Yours,

Ojok Polycarp,

MBB student, Gulu University.

Appendix V: Sequences of OBI DNA amplicons of S gene fragments in FASTA format (250bps)

Samples	HBV DNA sequences of S gene fragment
5A	*>OP2-5A_OPF2* *TCMMRRCACGGGACCATGCAGGAGCTGCACGACTCCTGCT* *CAAGGAACCTCTATGTATCCCTCCTGTTGCTGTACAAAACCT* *TCGGACGGAAACTGCACCTGTATTCCCATCCCATCATCCTGG* *GCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTT* *CTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGT* *AGGGCA*
24A	*> OP2-24A_OPF2* *GMMCMMRRAMMMGGGACCATGCAGAGMCWGCACGACTC* *CTGCTCAAGGAACCTCTATGTATCCCTCCTGTTGYTGTACAA* *AACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCAT* *CCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCC* *CGTTTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGG* *TTCGTAGGGC*
19A	*>OP2-19A_OPF2* *GCMMSMRGACMCCGGGACCATGCAGAGCCWGCACGACTC* *CTGCTCAAGGAACCTCTATGTATCCCTCCTGTTGCTGTACAA* *AACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCAT* *CCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCC* *CGTTTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGG* *TTCGTAGGGCA*
23A	*>OP2-23A_OPF2* *CCCMARWMACKGGGASCATGCASASCCWGYACGACTCCTG* *CTCAAGGAACCTCTATGTATCCCTCCTGTTGYTGTACAAAAC* *CTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCT* *GGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGT* *TTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTC* *GTAGGGC*
17A	*>OP2-17A_OPF2* *CMMCCMARAMMMKRGGACCATGCAGARMCWGCACGACT* *CCTGCTCAAGGAACCTCTATGTATCCCTCCTGTTGCTGTACA* *AAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCA* *TCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGC* *CCGTTTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTG* *GTTCGTAGGGCA*
6A	*>OP2-6A_OPF2* *CMARTAAMSGGGGGACCMTGCAGRACCTGCACGACTCCTG* *CTCAAGGAAMCTCTATGTWTCCCTCMTGTTGCTGTACAAAA* *CCTWCGGATGGAAAYTGCACCTGTATTCCCATCCCATCATCY* *TGGGCTTTCGSAAAATWCCTATGGGAGTGGGCCTCAGYCCG* *TTTCTCTTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTC* *GTAGGGC*

Appendix VI: Map of location of Gulu Regional Blood Bank

