A COMPARATIVE STUDY OF HEPATITIS B VIRUS AMONG IN-MATES AND LOW RISK VOLUNTARY BLOOD DONORS IN GARISSA COUNTY, KENYA

VINCENT BAHATI ODALLO (B.Sc., MLS)
P150/27265/2018

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INFECTIOUS DISEASES (MEDICAL VIROLOGY) IN THE SCHOOL OF HEALTH SCIENCES, KENYATTA UNIVERSITY

JULY, 2023

DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other University.

Vincent Bahati Odallo

P150/27265/2018

Department of Medical Laboratory Sciences

Signature:..... Date:.....

Supervisors:

We confirm that the work reported in this thesis was carried out by the student under our approval as university supervisors.

1. Dr. George Gachara

Department of Medical Laboratory Sciences

School of Medicine, Kenyatta University.

Signature:..... Date:.....

2. Prof. Wallace Bulimo

Department of Biochemistry

School of Medicine, University of Nairobi

Signature:..... Date:.....

DEDICATION

I dedicate this work to my lovely family; my wife Caroline Kanuna and my children Blessy and Cheryl. The relentless sacrifice, encouragement and continuous support I received from my wife cannot be wished away. Thank you so much dear ones, and may God bless you abundantly.

ACKNOWLEDGEMENT

I thank God this far. He enabled me to start and complete this work. May I acknowledge all the participants who played a role in making this study a reality. Just to mention a few, I would like to sincerely thank Dr. George Gachara of Kenyatta University, Department of Medical Laboratory Sciences for his technical guidance, encouragement and continuous support throughout the course of this work. May I also thank the Kenya National Blood Transfusion Services (KNBTS), Kenyatta University Ethical Committee, Graduate school and The Kenya Prisons Directorate of Trainings mandated by the Commissioner General for Prisons for the approval of this study? My gratitude also goes to Rithwan Yusuf, for the support she offered during the write up of this thesis. Last but not least, completion of this work could not have been possible without the moral support of my wife Caroline Kanuna and above all your prayers. God bless you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

HBV	Hepatitis B virus
CDC	Center for Disease Control and Prevention
CHBI	Chronic Hepatitis B Infection
DNA	Deoxyribonucleic acid
GCRH	Garissa County Referral Hospital
GMP	Garissa Main Prison
KEMRI	Kenya Medical Research Institute
KEPI	Kenya Expanded Programme on Immunization
KNBTS	Kenya National Blood Transfusion Services
MOH	Ministry of Health
NTCP	Sodium Taurocolate Co-transporting polypeptide
OBI	Occult Hepatitis B infection
ORF	Open reading frame
PCR	Polymerase chain Reaction
WHO	World Health Organization

ABSTRACT

Hepatitis B virus (HBV) infection is a life-threatening infection that attacks the liver and can cause both acute and chronic disease. This creates a high risk of death from cirrhosis and liver cancer. Hepatitis B infection poses a major health concern globally. It is estimated that in 2019, about 296 million people had been chronically infected with HBV globally and about 820, 000 deaths from hepatocellular carcinoma and cirrhosis were reported. In Kenya, HBV prevalence stands at chronic states of intermediate range (5-7%) and high (≥8%) with regional variations. Garissa County carries a high HBV infection risk with a reported prevalence of 14.1% in pregnant women attending antenatal care (ANC) clinics. This study sought to determine the seroprevalence and circulating Hepatitis B virus genotypes among the prisoners and compare it with that of the surrounding general population at Garissa Main Prison and Garissa County Referral Hospital respectively in Garissa, Kenya. A total of 130 in-mates and 130 voluntary blood donors were sampled in this study and a questionnaire used to collect data on their socio-demographic characteristics. Blood was then collected in plain vacutainers and the resulting serum tested for Hepatitis B surface antigen (HBsAg) using a rapid test strip. HBV DNA was then extracted from the serum and a 930bp region of the overlapping P/S gene amplified and sequenced. Logistic regression was used to evaluate socio-demographic factors associated with HBV seropositivity. Resulting HBV DNA sequences were manually edited, assembled into contigs and then aligned. Genotype identity of the aligned sequences was initially made using the Geno2pheno [hbv] 2.0 online data base. The aligned sequences from this study and genotype specific sequences in Genbank were then used to infer phylogenetic relationships of the local strains. The Geno2pheno [hbv] 2.0 online data base was further used to identify mutations in the samples associated with diagnostic failure and vaccine failure (in the S gene) and antiviral resistance (in the P gene). Majority of the study participants were males (86.9% among inmates and 95.4% among blood donors. Majority (76.2%) of the in-mates and of the donors (83.1%) were aged between 20-40 years while majority (51.4 % of the donors and 81.5% of in mates) had only a primary school level of education. HBV seroprevalence was significantly higher among in-mates compared to blood donors. Out of the total number of in-mates tested, 7 (5.4%) were HBV seropositive. Conversely, among blood donors 4 (3.1 %) were seropositive. There was a significant association between HBV seropositivity and gender among both the blood donors and in-mates. Out of the 22 HBV-DNA positive samples, genotype D was the most prevalent among both the in-mates and blood donors (81.8%). Genotype A sub-genotype A1 was only detected among the in-mate population (18.2%). All the genotype D sequences were found to be either D/A or D/E recombinants. HBsAg escape mutations associated with diagnostic failure were noted among both study populations. As a pioneer study on HBV in Kenyan prisons, the current study confirms the categorization of in-mates as HBV high risk populations. The dominance of HBV genotype D is a novel finding as studies in other regions of the country have identified genotype A as the most prevalent. Further studies should be carried out in other regions to determine circulating HBV genotypes and HBsAg escape mutations that may influence HBV infection diagnosis and treatment outcome in those regions.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Inflammation of the liver is referred to as hepatitis. This is majorly caused by a viral infection but may also be caused by other etiologies. These include; toxins, heavy alcohol use, some medications and autoimmune hepatitis (US-CDC, 2021). Autoimmune cases occur when the body produces antibodies against the liver tissue. Apart from acute infection, hepatitis may also lead to chronic infection associated with both liver cancer and cirrhosis (WHO 2021).

Hepatitis viruses (A-E) are the major causes of a viral hepatitis infection. Hepatitis B virus (HBV) is classified under a steadily growing family of viruses called *Hepadnaviridae* (Caligiuri *et al*., 2016). The HBV genome is quite diverse and is categorized into ten different genotypes ranging from A-J (Kramvis, 2014) and 35 sub-genotypes (Croagh *et al*., 2015). HBV causes Hepatitis B virus infection affecting many people globally. In the year 2019, approximately 296 million people globally, had been diagnosed with chronic HBV infection and about 820,000 deaths from hepatocellular carcinoma and cirrhosis reported (WHO, 2021). Majority of those chronically infected reside in Africa (Breakwell *et al.,* 2017). Several studies on HBV in Kenya have reported significant variations in HBV prevalence amongst various study populations. Turkana County showed a 15.3% seroprevalence among pregnant women (Mutuma *et al*., 2011). Previous studies related to this study among blood donors, Nairobi had a seroprevalence of 2.3% (Aluora *et al.*, 2020) while Homabay, Siaya and Kisumu Counties had a seroprevalence of 3.46% (Onyango *et al*., 2018). There also exists a variation within different target groups with a prevalence of 2.1% in both Kenyan adults and adolescents who are Human Immunodeficiency Virus (HIV) negative (Ly *et al.,* 2016).

A high HBV seroprevalence of 14.1% was previously reported in Garissa amongst antenatal mothers (Mohammed, 2012). This poses a significant risk associated with the spread of HBV infection since these mothers are a representative sample of the general community.

HBV transmission is greatly influenced by some practices that pose as risks for the spread of HBV infection. They include; tattooing, acupuncture and intravenous drug use (IDUs). Equally, some categories of people have been identified to have a higher risk of transmission. They include; health care workers, prisoners, commercial sex workers, blood recipients, infants during birth and relatives caring for HBV infected patients (Jafari *et al*., 2010). Exposure to infectious blood and body fluids also serves as a risk to HBV transmission (Miri *et al*., 2011). Incarcerated individuals have a disproportionate burden of HBV infection (Smith *et al.,* 2017). Prisoners stand out in this category with the highest risk of HBV infection (Ramamoorthy *et al.,* 2016). HBV being a blood borne infection, it poses a threat of higher rates of infection to prisoners, since they indulge in unprotected sex and sharing of sharp objects such as razor blades ,needles and syringes during drug use (Smith *et al.,* 2017). While studies on most of these risk groups have been conducted in Kenya, no data currently exists on HBV among prisoners in the country. It is based on this observation that, this study sought to determine the seroprevalence and circulating Hepatitis B viruses among the prisoners and compare it with that of the surrounding general population.

1.2 Statement of the Problem

Prisoners are among the high risk groups in respect to HBV infection (Ramamoorthy *et al.,* 2016). The higher HBV infection rates among the prisoners is because they indulge in unprotected sex and sharing of needles and syringes during injection drug use (Smith *et al.,* 2017). When prisoners

leave prison, they later reunite with their families in the community after serving their sentence posing the risk of HBV infection transmission within the newly joined population. Currently no data exists on the burden of HBV in Kenyan prisons. Besides Prison, other factors that triggered the need for this study, were social-cultural practices and increased HBV positivity rates reported at Garissa County Referral Hospital (GCRH) and other health facilities in the neighborhood. On social-cultural factors, the Somali community which forms the largest group within the study populations, some of the members still practice Female Genital Mutilation (FGM). This poses a high risk for HBV infection transmission as normally only one knife may be used on all the victims. Also, mostly within the rural areas, the community still strongly believe in spiritual healing for the sick and use of herbal medicines. This acts as an obstacle to medical care for the sick posing a high risk for the transmission of HBV infection. This has consequently impacted negatively on the rate of HBV infection in the region. A high HBV seroprevalence rate of 14.1% was previously reported in Garissa among antenatal mothers (Mohammed, 2012). This poses a significant risk associated with the spread of HBV infection since these mothers are a representative sample of the general community. Additionally, donor blood from Garissa County Referral Hospital (GCRH) is screened for HBV at the Nairobi regional blood transfusion center (NrBTC) using Chemiluminescent Microparticle Immunoassay (CMIA) with enzyme linked immunosorbent assay (ELISA) used as a backup. Comprehensive HBV screening with this method has been shown to be infective with a recent report of positive HBV DNA of 2.4% from HBsAg negative samples screened using the same method (Aluora *et al.,* 2020). This poses a significant risk associated with HBV infection through blood transfusion due to low sensitivity of serological methods.

1.3 Justification

There is need for clean and safe blood for transfusion. However, HBV is one of the major threats to safety of blood and related products. This study aimed at determining and comparing the HBV seroprevalence and genotypes circulating among the low-risk voluntary blood donors and high-risk prisoner populations in Garissa County, Kenya. The findings reported in this study will inform public health interventions aimed at reducing the spread of HBV and improving our understanding of the epidemiological patterns of hepatitis B virus infection within the general population.

1.4 Research Questions

i. What is the seroprevalence of HBV infection among the inmates and the blood donor populations at Garissa Main Prison and GCRH respectively?
ii. What are the genotypes of HBV circulating at GCRH and Garissa Main Prison?
iii. What are the genetic mutations of HBV associated with antiviral susceptibility and diagnostic failure in the study region?

1.5 Objectives

1.5.1 General objective

To compare seroprevalence and circulating genotypes of HBV among the blood donor and prisoner populations in Garissa, Kenya.

1.5.2 Specific objectives

1. To determine and compare the seroprevalence of HBV among the blood donor and prisoners populations.

2. To identify and compare the circulating HBV genotypes among the blood donor and prisoners populations.
3. To determine genetic mutations associated with antiviral resistance and diagnostic failure in the circulating HBV genotypes.

1.6 Significance of the Study

The findings that have been produced from this study, will be shared at the Ministry of Health and the Prison department to help review and develop policy guidelines for the screening of donor blood and control of HBV infection among in-mate population in the respective set ups. It will further advise on measures to curb HBV infection spread and management among other prisons and regions in the country.

1.7 Study limitations

This study did not investigate data on liver function markers for the affected individuals and the HBV viral loads. This therefore makes it impossible to link the clinical outcomes of the affected individuals to the study findings. Equally, the study failed to determine the patterns of disease progression since no follow up was conducted after diagnosis.

CHAPTER TWO: LITERATURE REVIEW

2.1 Classification and Nomenclature

Hepatitis B virus (HBV) is classified under a steadily growing family of viruses called *Hepadnaviridae*, and genus *Orthohepadnavirus* (Grewal *et al.*, 2017). The HBV genome is prone to replication errors and is further categorized into 10 different genotypes (A-J) (Kramvis, 2014). Moreover, using intergroup nucleotide difference of about 4%, HBV genotypes have been sub-divided into 35 sub-genotypes (Kramvis, 2014). Reports indicate that geographic distribution greatly influences HBV genotypic variation and plays a role in determining the transmission and evolution of the virus (Assih *et al*., 2018). The identified variations, affect the effectiveness of treatment, vaccine efficacy and severity of the disease (Gao *et al.*, 2015).

2.2 Epidemiology

The global HBV infection prevalence rate is alarming. By the year 2019, an estimate showed that 296 million people had been infected globally with chronic HBV infection and the new infections stood at 1.5 million people annually (WHO, 2021). During the same period, about 820,000 deaths were reported annually from hepatocellular carcinoma and cirrhosis (WHO, 2021). The World Health Organization (WHO) categorizes regions in terms of HBV infections. Western Pacific Region carries the highest burden of infection where 116 million people are chronically infected. This is followed by the African Region, with about 81 million people estimated to have chronic infections. Other regions are also affected with about 60 million, 18 million and 14 million people infected in the Eastern Mediterranean Region, South-East Asia Region and European Region respectively. America region carries the least chronic infections at about 5 million people (WHO, 2021).

In terms of endemicity, reports indicate that developing regions such as, China, Africa, Amazon basin and South-East Asia, have >8% HBV endemicity with a carrier rate of about 8%-20% (Zampino *et al.,* 2015). These regions are largely affected by asymptomatic chronic HBV infections with a majority of them occurring during infancy. This is because the risk of progression from acute to chronic state is largely influenced by age. HBV infection affects more males than females with variations in age. A study carried out in Pakistan showed 68.2% males were more affected compared to 31.9% of females. This is because the males are more exposed to risk factors than females and the younger ages social activities expose them to more risk factors compared to children and the old people (Khan *et al.,* 2011).

The (WHO) categorizes Kenya as an HBV endemic country with >8% HBsAg seroprevalence (Ochwoto *et al.,* 2016). However, the actual seroprevalence shows great disparities across different regions and study groups. Among pregnant women, in Turkana County, a seroprevalence of 15.3% was reported (Mutuma *et al*., 2011) while among the same study group in Garissa, a seroprevalence of 14.1% was reported (Mohammed, 2012). Studies among blood donors have also shown regional disparities, Nairobi at 2.3% (Aluora *et al.,* 2020) while Homabay, Siaya and Kisumu Counties had a prevalence of 3.46% (Onyango *et al*., 2018).

The high-risk groups have a higher prevalence of HBV infection as compared to the general population. The global HBV infection prevalence among prisoners and intravenous drug users (IDUs) stands at 4.8% and 9.1% respectively (Moradi *et al.,* 2020). While there have been sporadic reports of outbreaks of HBV in Kenyan prisons in the local dailies, there has been no studies of the same.

2.3 Genome Structure

HBV has a circular partially double-stranded DNA genome measuring about 3.2 Kbp with variations among the different genotypes. The viral polymerase is covalently bound to the 5' end of the minus strand (Datta *et al.,* 2012). The HBV genome encodes four overlapping open reading frames (ORFs) which include Pre S/S ORF, P ORF, C ORF and the X ORF (Slagle *et al.,* 2016). HBsAg, PreS1 and PreS2 envelope glycoproteins are encoded by the S ORF. The C ORF codes for two proteins with the help of at least two in-frame start codons. The C and S genes provide for in-frame multiple translations made possible by exploitation of more than one in-frame initiation codons in these ORFs. For the C ORF to encode for either nucleocapsid HBeAg or HBcAg, translation has to be initiated from the pre core or core regions, causing the core protein to be assembled into a capsid-like structure (Tan *et al.,* 2015). The C terminus of the capsid structure has amino acids characterized by RNA binding activity (Tan *et al.,* 2015). The signal peptide is coded for by the pre core ORF which will dictate translation that will further lead to secretion of HBeAg which is considered an immune tolerogen (Li *et al.,* 2017).

There are (3) three entities that result from the encoding of polymerase by the P ORF namely Ribonuclease H, reverse transcriptase and terminal protein. These perform different functions. Ribonuclease H degrades pregenomic RNA during synthesis of minus strand DNA synthesis, reverse transcriptase (RT) production enhances catalysis of genome synthesis and the terminal protein which binds pregenome RNA serves as a primer for (-) strand DNA synthesis. The HBxAg is encoded by the HBV X ORF. This serves in transcription activation, inhibition of protein degradation and DNA repair (Milich *et al.,* 2003). However, there is evidence of non-encoding RNA elements in the HBV genome (Qiu *et al.,* 2017). The genome contains two direct repeats (DR1 and DR2) at the 5' end which helps in the DNA synthesis during replication.

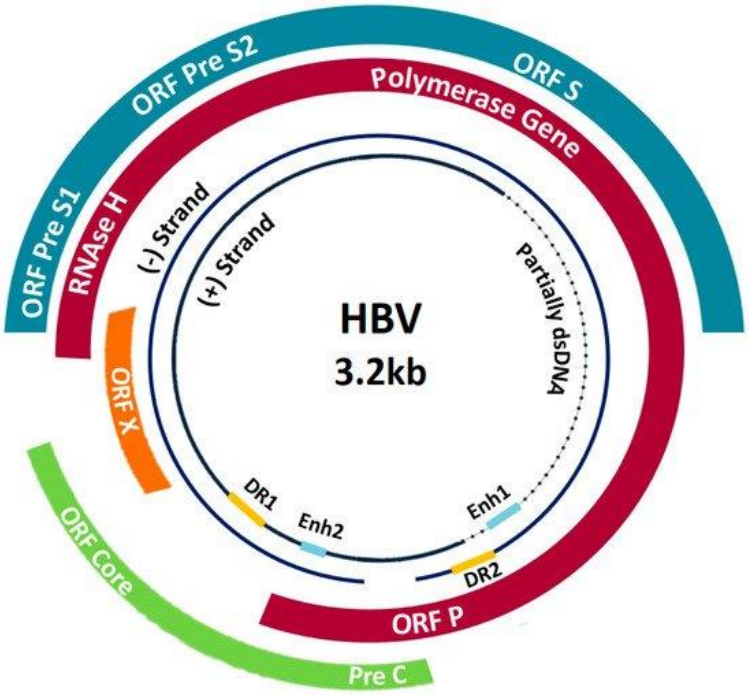


Figure 2.1: Hepatitis B genome organization

HBV Genome Organization (Al-Sadeq *et al.*, 2019).

2.4 Replication

HBV replicates through a process called reverse transcription. To facilitate entry into the cell, the virus binds to a tissue specific receptor sodium taurocolate co-transporting polypeptide (NTCP) in the hepatocytes cell membranes (Yan *et al.,* 2012). The cellular viral entry happens through endocytosis, where the viral envelope fuses with the cell membrane enabling release of the nucleocapsid and subsequently deposits it into the cytoplasm (Watashi *et al*, 2015). HBV genomic DNA is then trafficked to the cellular nucleus via host micro tubular network. Though viral disassembly and transfer of DNA into the nucleus is not well known, viral DNA is still transported to the nucleus (Gallucci & Kann, 2017). Once into the nucleus, the host DNA polymerase converts the viral partially double stranded DNA into a double stranded DNA. For transcription to occur, covalently closed circular DNA (cccDNA) is made from a stable double stranded DNA (Yang *et*

al, 2014) The cccDNA transcripts possess a 5'cap structure and are polyadenylated and unspliced. The 3.5 Kb genomic transcripts produce pre-core and pre-genomic RNAs which serve specific functions. The translation of pre-core product, is directed by the pre-core RNA while pre-genomic RNA acts as mRNA for core and polymerase as well as a template for reverse transcription. The polymerase translation is initiated through start codon of the pregenomic RNA (pgRNA) (Zajakina *et al.*, 2004). Replication is aided by the largest mRNA enabling production of more genome copies, viral RNA-dependent–DNA- polymerase and capsid core proteins.

The assembly process is started by the encapsidation of the genome. Epsilon signals the packaging (Pollack *et al.,* 1993). The nucleocapsid is formed from the interaction of Pol terminal protein epsilon and the core protein (Lott *et al.,* 2000). Assembly of mature virions take place in the endoplasmic reticulum where the formed nucleocapsid interacts with the endoplasmic envelope proteins. These mature virions are passed into the extra-cellular environment.

2.5 Hepatitis B Virus proteins

HBV consists of various antigens. These antigens are key in serological studies and are used as diagnostic markers.

2.5.1 Hepatitis B surface antigen (HBsAg)

This is the first antigen produced during HBV infection making it a good diagnostic marker because of its early appearance. However, it can also be cleared by the host immunity early enough (Parry *et al.,* 2017). HBsAg is spherical and protects the viral nucleocapsid by enveloping it. The envelope contains three proteins (large, middle and small) which are encoded for by the pre-S1/S2/S ORFs. L protein (pre-S1 domain) serves as the virus attachment protein for HBV receptor

attachment; M protein (pre-S2 domain) function has not yet been understood and, finally, S protein (S domain) represents HBsAg (Caligiuri *et al.,* 2016). All the three proteins; small, middle and the large are detected as HBsAg. The detection of HBsAg helps with prediction of treatment outcome and in monitoring the natural history of infection. When quantified, HBsAg helps in differentiating between chronic HBeAg-negative HBV patients and inactive carriers, who pose the risk of reactivating HBV infection (Churin *et al.,* 2015).

2.5.2 Hepatitis B core antigen (HBcAg)

HBcAg is a helical homodimer made of 183 amino acid residues, performing multiple functions. The core antigen (HBcAg) mainly protects the HBV genome and DNA polymerase by enclosing them. HBcAg also regulates the transcription of viral genes in the nucleus by binding to nuclear HBV DNA to regulate its epigenetics (Zhao *et al.,* 2021).

2.5.3 Hepatitis B e antigen (HBeAg)

HBeAg is an HBV accessory protein translated from the preCore mRNA. HBeAg is diagnostically used to evaluate active viral replication and is linked to the development of hepatic inflammation and HBV chronic infection (Zhao *et al.,* 2021).

HBeAg has been shown to impact on immune regulation by activating macrophages to enhance production of multiple inflammatory factors leading to liver injury (Wang *et al.,* 2018). Other than immune regulation, the HBeAg is associated with tumor growth and hepatocarcinogenesis (Yang *et al*., 2002). This is achieved by HBeAg and its precursor preCore/p22 interacting with NUMB-

cell fate determinant. This leads to degradation of p53 that further inhibits its translocation from cytosol to the nucleus, hence contributing to HCC development (Liu *et al.,* 2016).

2.5.4 HBx

HBx is a viral regulatory protein which promotes hepatocellular carcinogenesis by influencing cellular events. These include transactivation of the viral and cellular promoters, epigenetic modifications, regulation of HBV transcription and ubiquitination (Slagle & Bouchard, 2016).

2.6 Transmission and Pathogenesis

Viral transmission is greatly influenced by some practices that pose as risks for the spread of HBV infection. They include; tattooing, acupuncture and intravenous drug use (IDUs). Equally, there is an increased risk of transmission among curtain groups some. They include; health care workers, prisoners, commercial sex workers, blood recipients, infants during birth and relatives caring for HBV infected patients (Jafari *et al*., 2010). Exposure to infectious blood and body fluids also serves as a risk to HBV transmission (Miri *et al*., 2011).

Pathogenesis of HBV infection is initiated when the virus specifically binds to sodium taurocolate co-transporting polypeptide (NTCP) receptor located on the hepatocytes (Yan *et al.,* 2012). The viral virion uses the pre-S domain on its surface to bind to host receptor triggering endocytosis of the virion into the host cell. Hepatitis B virus infection will trigger immunological response from the host aimed at viral clearance. Though at the same time, inflammatory conditions attract activated platelets to the infection site which consequently increase cytotoxic T lymphocytes (CTLs) at the same site rendering damage to the liver (Iannacone *et al.*, 2007). cytotoxic T

lymphocytes (CTLs) generated through host immune response, will kill infected hepatocytes thereby eliminating infection (Long *et al.,* 2008). Hepatocellular carcinoma (HCC) is attributed to HBV when it integrates into the host genome during early steps of clonal tumor expansion. This causes both direct insertional mutagenesis and genomic instability. Cell transcription and proliferation regulation is altered by continuous expression of the viral regulatory protein HBx. HBx alters signal pathways such as inhibition of P53 which leads to cell apoptosis thus directly contributing to HCC development (Lin & Kao, 2021).This leads to sensitization of liver cells to carcinogenic factors (Levrero *et al.,* 2016). Accumulation of preS2/S mutant proteins and preS1 large envelope proteins can also induce transformation of the hepatocytes (Levrero *et al.*, 2016).

2.7 Clinical features of HBV infection

Most clinical symptoms show during acute infection while chronic infection is asymptomatic. Infected individuals, mostly present with vomiting, jaundice, fatigue, dark urine and abdominal pains. Individuals who get infected with HBV at birth are likely to develop chronicity later in life (Kumar *et al,* 2012), thus enhancing chances of liver cancer occurring (He *et al,* 2010).

2.8 Laboratory diagnosis of HBV infection

Laboratory diagnosis is achieved using either serological or molecular methods.

2.8.1 Serological diagnosis

Serological testing of HBV is based on HBsAg and HBeAg. The first detectable HBV antigen is HBsAg. It is mostly used as a serological marker though it may be absent at the onset of the

infection (Karra *et al.,* 2016). Detection of HBeAg correlates to active replication of the virus (Hadziyannis *et al.,* 2018) and acute HBV infection (Elghannam *et al.,* 2009).

2.8.2 Molecular methods

These are useful in detection of HBV genome, determination of viral load, monitoring of treatment and detection of mutations (Zoulim, 2006). Polymerase chain reaction (PCR) is the most common molecular method used in diagnosis. Several PCR formats exist, each having advantages and limitations.

The standard PCR is also called conventional PCR. The reaction flows through some steps. The first step involves mixing the HBV target DNA template primers, DNA polymerase, Dnase free water and a reaction buffer in a master mix, then transferring the contents into the PCR tubes. This is followed by HBV DNA amplification based on primer parameters. Amplified HBV DNA is then visualized on agarose gel electrophoresis. The DNA purification and sequencing help in determining the specificity of the PCR reaction. (Walker-Daniels, 2012).

Nested PCR is a modified method designed to enhance the sensitivity and specificity of PCR reaction. Nested PCR protocol involves two successive amplification reactions. Each set of reaction utilizes a specific pair of primers. The first round PCR HBV amplicons are used as the template for the second-round reaction where the second set of primers is involved. However, this kind of PCR has the disadvantage of contamination due to carryover effect arising from enhanced manipulation during amplification (Jeanne Carr *et al*., 2010). Specificity is achieved by two different sets of primers binding to the same target template (Green & Sambrook, 2019).

Real-time PCR is a quantitative PCR method where one step completes both amplification and detection at the same time. Data is continually collected throughout as the PCR reaction is happening. Compared to other PCR methods, real time PCR produces both specific and quantitative data. Specificity is increased through melt curve analysis (Walker-Daniels, 2012).

2.9 Diagnostic failure

Conformational changes within the "a" determinant region in the HBsAg epitope due to mutations will lead to failure of HBsAg inducing specific antibodies. This prevents serological diagnostic assays from detecting HBsAg hence diagnostic failure (Horvat, 2011). Of importance, is also occult HBV infection (OBI) which is defined as the presence of detectable hepatitis B virus (HBV) DNA in HBsAg negative individuals (Pak, Ordway, & Torres, 2020).

Pathogenesis of OBI is influenced by both viral and host factors which negatively impact on the HBV infection by suppressing viral replication. This helps keep the infection under control (Hollinger *et al.,* 2010). Different hypotheses have tried to explain OBI. OBI can be caused by escape mutations within HBsAg that are not detectable by commercial assays, mutations related to secretion of HBsAg or immune response by the host to chronic HBV infection where HBsAg production is low (Squadrito, Spinella, & Raimondo, 2014).

OBI can also develop during the window period of an acute HBV infection (Makvandi, 2016). OBI can be either seropositive which is defined as OBI with the presence of anti-HBc and /anti-HBs or seronegative OBI which is without either antibody (Pak *et al.,* 2020). OBI cases lack active

liver disease but show certain degree of histological damage with variations in residual fibrosis. However during immunosuppression ,HBV may be reactivated causing infection (de la Fuente *et al.*, 2011). HBsAg detection assays performance decreases due to escape mutations. Low viremia levels are detected in majority of OBI cases. These are secondary to overt HBV infection and represent a residual low viremia level. During acute and chronic HBV infections, histological derangements occur which together with strong immune response lead to low viremia (Ozaslan *et al.*, 2009). Mutations in the polymerase domain may also lead to decrease in HBsAg expression and replication of HBV DNA (Said, 2011). Blood transfusion carries the risk of transmission of OBI because transfusions that only rely on negative HBsAg can result to transfusions of blood collected during the late stages of infection or Window period (Candotti *et al.*, 2009).

2.10 Treatment

Acutely infected HBV individuals may not be subjected to treatment since their immune system is highly active and can clear the infection unless compromised by other health factors. However, progression to chronic stage calls for treatment with -anti-HBV drugs; L-nucleoside analogues, nucleoside phosphonates and cyclopentane ring group. Nucleoside/nucleotide analogs (NAs) have a similarity in structure with the natural nucleotides though modified in the sugar ring or base group, to aid them compete effectively with the natural nucleotides in binding to the polymerase. The mode of action of all currently available nucleotide drugs is by targeting at either one or more of these steps: the priming reaction which involves synthesis of the short negative DNA strand, conversion of pgRNA to negative DNA strand by reverse transcription, or DNA-dependent DNA replication (Lazarevic, 2014). These drugs include:

2.10.1 Lamivudine

Lamivudine was approved in 1998 for the treatment of Chronic Hepatitis B Infection (CHB). Lamivudine is first activated to its active form through phosphorylation before acting as a chain terminator by competing for incorporation into viral DNA. It also enhances HBeAg seroconversion aimed at suppressing HBV DNA and reversing fibrosis. The major disadvantage of lamivudine is the high rate of genotypic drug resistance mutations. Lamivudine resistance is mainly caused by rtM204V and rtM204I mutations (James Fung, 2011).

2.10.2 Adefovir dipivoxil

Adefovir was approved in 2002 for treating CHB. It is effective against lamivudine-resistant mutants and helps improve histological, virological and biochemical parameters. However, Adefovir has also faced resistance from two major mutations rtA181V/T and rtN236T. Adefovir is also responsible for dose-dependent nephrotoxicity (James Fung, 2011).

2.10.3 Entecavir

Entecavir is a carboxylic analogue of guanosine that is activated to its active form 5′ triphosphate metabolite through intracellular phosphorylation. It was approved for the treatment of naive and lamivudine-resistant CHB in 2005. Entecavir acts by inhibiting HBV DNA polymerase priming. Unlike other NAs, Entecavir is a non-obligate chain terminator because it contains a 3′-hydroxyl group that allows incorporation of additional nucleotides prior to chain termination. Entecavir has a reduced drug resistance rate. This is because it requires a combination of three different mutations before resistance develops (James Fung, 2011).

2.10.4 Tenofovir

Tenofovir is used in the treatment of CHB. With the help of HBV DNA polymerase, it acts as a chain terminator. Compared with Adefovir, Tenofovir is more superior in enhancing suppression of HBV DNA, HBeAg seroconversion and normalization of ALT. (James Fung, 2011).

2.10.4 Telbivudine

Telbivudine is an L-nucleoside analogue of thymidine. It was approved for the treatment of CHB infection in 2006. L-Nucleosides differ in their structural configuration compared with natural nucleosides. This is because their sugars and base moieties are arranged in the L configuration rather than the D configuration. Active form triphosphate is formed when Telbivudine undergoes phosphorylation by cellular kinases. The triphosphate inhibits HBV DNA polymerase by competing with thymidine 5′-triphosphate (James Fung, 2011).

2.10.5 Treatment in Kenya

In Kenya Telbivudine and Adefovir are not recommended due to resistance. Entecavir and Tenofovir are the preferred choices for nucleoside analogues. In addition, all cases where contraindication exists, Pegylated interferon has been recommended. This is according to HBV treatment guidelines by the gastroenterology society of Kenya in conjunction with Ministry of Health (MoH), 2016.

2.11 Vaccination

HBV vaccination prevents the vaccinated persons from getting infected with HBV infection. WHO and CDC recommends vaccination to be done from birth up to the age of 18years. Persons (adults) at high risk should also be considered for HBV vaccination (Hepatitis B Foundation, 2019).

For children, the vaccine should be given at birth followed by 2 or 3 doses. The first dose requires monovalent (protecting HBV alone) but the 2nd and 3rd dose may either require monovalent or combined vaccine protecting against HBV and other diseases (Young, 2021). Hepatitis B vaccine is considered one of the safest and effective vaccine by the WHO and CDC. Effective and sustained use of HBV vaccine in <5 years children has led to low incidence of chronic HBV infection. Vaccine use offers 95% antibody protection to the vaccinated persons. Protection can also be life-long (WHO, 2019).

2.11.1 Vaccination in Kenya

HBV vaccination was initiated in Kenya in the year 2001 through the expanded program on immunization (EPI) (François *et al.*, 2008). Kenya expanded programme on immunization (KEPI), manages the vaccines offered in the hospitals for immunization purposes. The HBV vaccine given is a pentavalent with inclusion of tetanus, diphtheria, pertussis and *H.influenza* type B. A child born will be given as per this schedule; at 6 weeks, 10 weeks and then at 14 weeks (Mother and child health handbook-Ministry of health-MOH 216).

2.11.2 Vaccine escape for HBV infection

The protection levels offered stand at ≥94% of the recipients of the HBV vaccine (Bruce *et al*., 2016). However, the effectiveness of HBV vaccine, largely depends on the major antigenic alpha

"a" determinant region of the HBsAg (Nyairo et al., 2016). This is a region located from amino acid position 124-147 in the major hydrophilic region (MHR), spans from position 99-169 of HBsAg and contains neutralizing B cell epitopes. Continuous administration of hepatitis B vaccine can lead to mutations in the epitope regions of the Pre-S,S and polymerase of HBV (Romanò *et al.*, 2015).

The vaccine triggers production of specific antibodies that react with the "a" determinant region of the surface antigen. Emergence of mutations around "a" determinant will trigger antigenicity disruption of the surface antigen protein. This will lead to failure of antibodies directed against the surface antigen protein being unable to neutralize the virus (Romanò *et al*., 2015).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Areas of Study

The study was conducted in Garissa County in two study sites namely GCRH and Garissa Main Prison both located in Garissa County within the Township area. This County is found at the North Eastern part of Kenya. Other bordering Counties include; Tana River, Wajir, Isiolo and Lamu. (**Appendix 1)**. GCRH receives patients from Garissa County and beyond, some of whom are referrals from bordering counties. Equally, Garissa Main Prison is a host to in-mates from both Garissa County and other parts of the country.

3.2 Study Design

This study used a descriptive cross-sectional study design which allows collection of data from different individuals at a single point in time without influencing them.

3.3 Study Population

Voluntary healthy blood donors and consenting inmates who consented were the targeted study populations. The blood donors presented at Garissa County Referral Hospital (GCRH) while the inmates were incarcerated at Garissa Main Prison.

3.3.1 Inclusion Criteria

Irrespective of gender, all voluntary blood donors were selected according to Kenya National Blood Transfusion Services (KNBTS) criteria. Each donor was aged between 18 – 65 years and weighed at least 50 Kgs and above. Every consenting inmate aged 18 years and above was also included.

3.3.2 Exclusion Criteria

Any voluntary blood donor weighing < 50 kg and aged >65 years or < 18 years was excluded. Inmates were excluded from the study for failing to give consent and having not attained the age of 18 years.

3.4 Determination of Sample Size

The minimum sample size for each study group was calculated using a formula for equal sample sizes with a specific significance and power (Casagrande *et al*., 1978).

$$n = \frac{\left\{Z_{1-\alpha/2}\sqrt{[2P(1-P)]} + Z_{1-\beta}\sqrt{[P_1(1-P_1) + P_2(1-P_2)]}\right\}^2}{(P_1 - P_2)^2}$$

Where:

α =Type I error (0.05)

β= Type II error ($0.20^a or\ 0.10^b$)

At 95% confidence, $Z_{1-\alpha/2} = 1.96$

At 80% power, $Z_{1-\beta} = 0.842^a$

At 90% power, $Z_{1-\beta} = 1.28^b$

P_1 =Assumed/Estimated Proportion of outcome 1

(Prevalence of HBV in prisoners) = 0.50

P_2= Estimated proportion outcome 2 (prevalence of HBV in general population) = 0.30

n= $\frac{\{1.36+0.87\}^2}{(0.50-0.30)^2}$, n=124

Therefore, the 2 populations each with 124, rounding up to 130. For both populations: 130x2= 260.

3.5 The sampling technique

Considering the objectives of the study, simple random sampling was adopted since it provided an equal opportunity for the study subjects to participate. This method of sampling minimizes bias and requires little knowledge to execute. A list of the visiting voluntary blood donors was created and study subjects randomly picked and blood collected on a daily basis until the target sample size was achieved. Equally, a list of all the inmates was prepared and study subjects randomly picked to participate in the study. A total of 130 samples were collected from each inmate and donor populations. Serological and PCR analysis utilized a total of 260 serum samples from both study populations. All the 29 HBV DNA positive samples were considered for sequence analysis.

3.6 Sample Collection and Processing

Each voluntary blood donor and consenting adult inmate offered themselves for blood collection. Before blood collection, the researcher explained the significance of the study to all the participating voluntary blood donors and inmates. Consent forms were then administered by the researcher to each participating voluntary blood donor and inmate to be filled and signed. A puncture site for blood collection was thoroughly sterilized with 70% alcohol swab. Approximately 4ml of blood was collected from selected vein of each individual into a plain vacutainer.

Serum for serological work was prepared by spinning whole blood at 3000xg for five minutes using a centrifuge. Serum was aseptically aspirated and dispensed into already prepared cryotubes.

These were later refrigerated at −80 °C awaiting further research work. Initial blood processing was done at GCRH laboratories.

3.7 Laboratory Procedures

3.7.1 Detection of HBV using serology

One Step HBsAg kit (Qingdao Hightop Biotech Co., Ltd, Qingdao, China) was used to test for HBsAg in serum according to a Manufacturer's test procedure below. This is a rapid one step test for the qualitative detection of HBsAg in human serum, plasma and whole blood. The test strip uses gold immunochromatography based on antibody sandwich method where mixed colloidal-gold monoclonal antibody moves along the membrane to the T line and form a visible colored line with plasma, serum or whole blood when HBsAg is present. The kit's relative sensitivity is pegged at 100%, specificity at 99.84% and accuracy at 99.90% of HBsAg in the serum concentration.

Test procedure

The test strip was removed from the sealed pouch. The end of the strip which was printed with arrows was inserted into the serum in a test tube taking precaution not to exceed the maximum line. The strip was then removed after 15s and placed on a clean level surface. Test results were observed immediately within 15-20 mins. The test results were considered invalid over 20 mins. A reactive/positive test was indicated as two-colored lines (irrespective of the intensity), one in the test area (P = Positive) and the other in the control area (C = Control). A non-reactive/negative test was indicated by only one colored line in the control area. If the control line was not visible, the test was considered invalid and repeated.

3.7.2 HBV DNA Extraction

Isolate II Genomic DNA Extraction kit (Bioline, Germany), was used to extract viral DNA from 200µL of serum. Key components of the kit included; Proteinase K (lyophilized), Proteinase K Buffer PR, Wash Buffer GW1, Wash Buffer GW2 (concentrate), Lysis Buffer GL, Buffer G3, Elution Buffer G, Spin columns and collection tubes (2 ml). Before extraction, Elution Buffer G was preheated to 70°C and 50 ml Wash Buffer GW2 (concentrate) was prepared by adding 200 ml of 96% ethanol, labeled and stored at room temperature. Proteinase K solution was prepared by adding 6.70 ml of Proteinase K Buffer PR to lyophilized Proteinase K and stored at -20°C. To lyse serum samples, 25µL of Proteinase K was mixed with 200µL of serum in a 1.5 ml DNase free microcentrifuge tube for each sample processed. Some 200µL of Lysis Buffer G3 was added and vortexed vigorously for 10s. The samples were then incubated at 70°C for 10 min. DNA binding conditions were adjusted by adding 210µL of 96% ethanol in each sample and vortexed vigorously for 10s. DNA was bound by placing one Isolate II Genomic DNA Spin Column into a Collection tube allowing for the loading of the sample onto the column. To ensure complete filtration of the loaded lysate, centrifugation was done at 11,000 x g for 1 min in an Eppendorf 5414 microcentrifuge. Silica membrane was washed by wash Buffers GW1 and GW2 respectively and dried to remove residual ethanol. DNA was eluted by addition of 100µL of preheated Elution Buffer G onto silica membrane for each preparation. The eluted DNA was then stored at -20°C awaiting amplification process.

3.7.3 Amplification of overlapping HBV P and S gene

A heminested PCR protocol was used to amplify an overlapping portion of the HBV P and S genes from extracted viral DNA. To detect HBV DNA, sets of primers and known protocols were used to amplify the region 251-1190 nucleotides from the EcoR1 site of the viral genome (Chook et al.,

2015). To perform the first round of PCR reaction, a 25 µl My Taq master mix (Bioline Meridian Bioscience, Country) was used. This contained 0.5µl My Taq DNA polymerase, 10 µl 5X My Taq Reaction buffer, 0.5µl each of 20µM first round primers (251f and 1797r), 7.5µl of nuclease free water and 6µl of the DNA template. Both initial denaturation and denaturation were performed at 95 °C for all the samples. An initial denaturation for 2 minutes was followed by 35 cycles of denaturation for 15s, annealing at 58°C for 30s and extension at 72 °C for 30s. A final extension was performed at 72 °C for 2 mins while a final hold was set at 4 °C for an infinite period to terminate the reaction. The second round PCR reaction was performed using five 5 µl of the template which was a product of the first round PCR reaction. This procedure used second set of primers (251f and 1190r) under the same reaction conditions. Each run utilized negative and positive controls.

Table 3.1: Primers used for amplification of P and S genes

First round primers - 5'-3'	**Second round primers - 5'-3'**
251f: GACTYGTGGTGGACTTCTC	251f: GACTYGTGGTGGACTTCTC
1797r: CCAATTTMTGCYTACAGCCTC	1190r: TCAGCAAAYACTYGGCA

Agarose gel electrophoresis was used to visualize and resolve the PCR products. A 1.5% agarose gel stained with 5µl ethidium bromide (EtBr) was prepared. 1.0g of DNA agarose gel (1.5% w/v) was weighed and added into a conical flask containing 100ml of 1X Tris-borate-EDTA (TBE) buffer. The mixture was heat in a microwave and completely melted until clear. Some relative

cooling for about 5 mins was allowed to the resultant solution before adding 5µl of EtBr. After well mixing, the liquid was poured into the gel casting tray and allowed to solidify for about 45 mins. To run the gel, the comb was removed from the gel and gel placed into the electrophoretic tank. 1X TBE buffer was added until it covered the top of the gel. 2µl of each sample was mixed with 2µl of DNA loading dye and introduced into the gel alongside 2µl of 1 Kb and 100 bp ladders each. The gel was allowed to run automatically at 110V, 108 mA for 45mins. The interpretation was done under the UV light using a transilluminator. Purification of the HBV PCR positive amplicons before sequencing was done by treating the amplicons with ExoSAP-IT™ (ThermoFisher Scientific, CA, USA) to remove remaining dNTPs and left over primers protocol as previously described (Dawkins-hall, 2017). Briefly, 5µl of the PCR product was mixed with 2µl of ExoSAP-IT™ in a PCR tube. This mixture was then incubated at 37^0C for 15 minutes followed by an incubation at 80^0C for 15 minutes. The purified PCR products were then shipped to Inqaba diagnostic laboratories for Sanger sequencing on an ABI 3500xL (Applied Biosystems, CA, USA) sequencer.

3.7.4 Sequence analysis

The resulting forward and reverse reactions were assembled into contiguous nucleotide sequences and manually edited in BioEdit version 7.25 (Hall, 1999) sequence editor. The Clustal W program implemented in BioEdit was used in alignment of the resulting nucleotide sequences. To determine the genotype of the aligned sequences, an online tool Geno2Pheno HBV (Max Planck Institute Informatik, Germany; http://hbv.geno2pheno.org) was used. Each sequence was pasted into the online tool and the tool identified the genotype of the sequence. It also provided the mutations in the immunogenic small hepatitis B surface protein and also within the reverse transcriptase domain

of the P protein. To achieve phylogenetic reconstruction, MrBayes program version 3.1.2 was used (Ronquist *et al.,* 2005). First, genotype specific sequences were downloaded from Genbank and the resulting fasta file which included the study sequences was converted into the nexus format using concatenator. The nexus file was then run for 1 million generations aimed at achieving inference of the Bayesian tree.

Visualization of the resulting phylogenetic tree and determination of the HBV genotypes was done using Fig Tree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/) after incorporation of known HBV genotype prototype strains.

3.8 Data Analysis

SPSS program version 20 (IBM, Chicago, IL, USA) was used to statistically analyze the data. Descriptive statistics were used to present study participants socio-demographic characteristics. The association between HBV seropositivity and socio-demographic factors was determined by bivariate logistic regression. A p value of ≤ 0.05 was considered statistically significant. The geno2Pheno database (https://hbv.geno2pheno.org) and phylogenetic trees were used for HBV genotypic determination while GeneDoc and the geno2Pheno database were used for mutational analysis to assess for mutations associated with diagnostic failure and vaccine escape. Recombination was analyzed using the HBV NCBI genotyping tool (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

3.9 Ethical Consideration and Confidentiality

Ethical approval was granted from Kenyatta University Ethics and Review Committee (KU-ERC, PKU/2043/I1190). A research permit from National Commission for Science, Technology & Innovation (NACOSTI) to conduct the research was obtained under License No. NACOSTI/P/20/4150. To further facilitate the study, clearance was sought from the Kenya National Blood Transfusion Services (KNBTS) trainings board and the Commissioner General and the Trainings Board of the Kenya Prisons Service (**Appendices** II, III, IV and V). Each one of the study participants consented in writing and participated in the study without any one of them being coerced or remunerated (**Appendix** VIII). To attain confidentiality, the study samples were coded using special numbers and not the study participant's names.

CHAPTER FOUR: RESULTS

4.1 Socio-Demographic Characteristics of Study Participants

4.1.1 Gender

In the two study populations, it was observed that there were more males than females; 95.4% among the blood donor population and 86.9% among the inmate population as shown in **Table 4.1**.

Table 4.1: Gender Distribution among the blood donor and inmate study populations

	Donors		**Inmates**	
	Frequency	(%)	Frequency	(%)
Male	124	95.4	113	86.9
Female	6	4.6	17	13.1
Total	**130**	**100.0**	**130**	**100.0**

The participant's age was categorized into three sets; <20, 20-40 and >40 years. In both the donor and inmate study populations, majority were aged between 20- 40 years. Among the blood donor population, this age group comprised 83.1% of the study population while in the inmate population it comprised 76.2% of study population. Those aged less than 20 years were only 9.2 % among the donor population and only 3.1 % among the inmate population. However, those aged above 40 years were more among the inmate population (20.8%) than in the donor population with 7.7 % (**Table 4.2).**

Table 4.2: Distribution of age groups among the donor and inmate populations

			Inmates	
Age Group	Frequency	(%)	Frequency	(%)
<20	12	9.2	4	3.1
20-40	108	83.1	99	76.2
>40	10	7.7	27	20.8
Total	**130**	**100.0**	**130**	**100.01**

4.1.2 Level of Education

Based on the results few individuals had not attained any form of education in the donor (8.5 %) and inmate population (4.6 %). However, there were more inmates (81.5 %) than donors (51.5 %) who had primary school level of education qualifications. Only (3.1 %) of inmates had a tertiary level of education qualifications as compared to (10 %) of the donor population (**Table 4.3)**

Table 4.3: Distribution of Level of education among the donor and inmate populations

	Donors		Inmates	
Level of Education	Frequency	(%)	Frequency	(%)
None	11	8.5	6	4.6
Primary	67	51.5	106	81.5
Secondary	39	30.0	14	10.8
Tertiary	13	10.0	4	3.1
Total	**130**	**100.0**	**130**	**100.0**

4.2 The Seroprevalence of HBV among study participants

The donor and inmate populations had different seroprevalence rates of HBV infection. Compared to inmates, the blood donors had a lower HBV seropositivity of 4/130 while among the inmates 7/130 was HBV seropositive as shown in Table 4.4. This gave a seroprevalence of 3.1% and 5.4% among blood donors and inmates respectively.

Table 4.4: The Seroprevalence of HBV among study participants

HBV Seropositivity	**Donors**		**Inmates**	
	Frequency	**(%)**	**Frequency**	**(%)**
Negative	126	96.9	123	94.6
Positive	4	3.1	7	5.4
Total	**130**	**100**	**130**	**100**

4.3 The Association of HBV seropositivity with Socio-Demographic Factors

The relationship between HBV seropositivity and different socio-demographic characteristics of the study population was determined. In both study groups, HBV seropositivity was lowest in ≥40 years' age group compared to the 20-40 years age group that showed a higher rate of seropositivity. In the ≤ 20 years age group, no HBV was detected. However, there was no significant association between HBV seropositivity and age. Gender was the only sociodemographic variable that was significantly associated with HBV seropositivity in both study groups, p value of 0.04 among donors and 0.01 among inmates. Males reported a higher HBV seropositivity compared to females in both study populations. Considering the level of education of the study participants, the seropositivity rates differed with the primary level of education reporting a higher rate than secondary and tertiary levels of education in both the inmates and blood donors. However, there was no significant association between the level of education and HBV seropositivity in both donor population (P=0.32) and inmate population (P=0.67) (**Table 4.5).**

Table 4.5: Distribution of Seropositivity rates based on variables among the study participants.

		DONORS					INMATES				
Variables	**Categories**	**Negative**		**Positive**		**P-Value**	**Negative**		**Positive**		**P-Value**
		n=126	%	n=4	%		n=123	%	n=7	%	
Age Group	<20	12	9	0	0	0.36	4	3.3	0	0	0.46
	20-40	105	83	3	75		94	77.2	5	71.4	
	>40	9	7	1	25		25	20.3	2	28.6	
Sex	Male	120	95	4	100	0.04	107	86.9	6	85.7	0.01
	Female	6	4.7	0	0		16	13	1	14.3	
Level of Education	None	10	7.9	1	25	0.32	6	4.9	0	0	0.67
	Primary	65	51	2	50		100	81.3	6	85.7	
	Secondary	39	30	0	0		13	10.5	1	14.3	
	Tertiary	12	9.5	1	25		4	3.3	0	0	

Significance P< 0.05

4.4 Circulating HBV genotypes

In twenty-nine (29) samples, HBV DNA was successfully amplified using PCR. These included 4 samples that were HBsAg positive and 25 samples that were HBsAg negative.

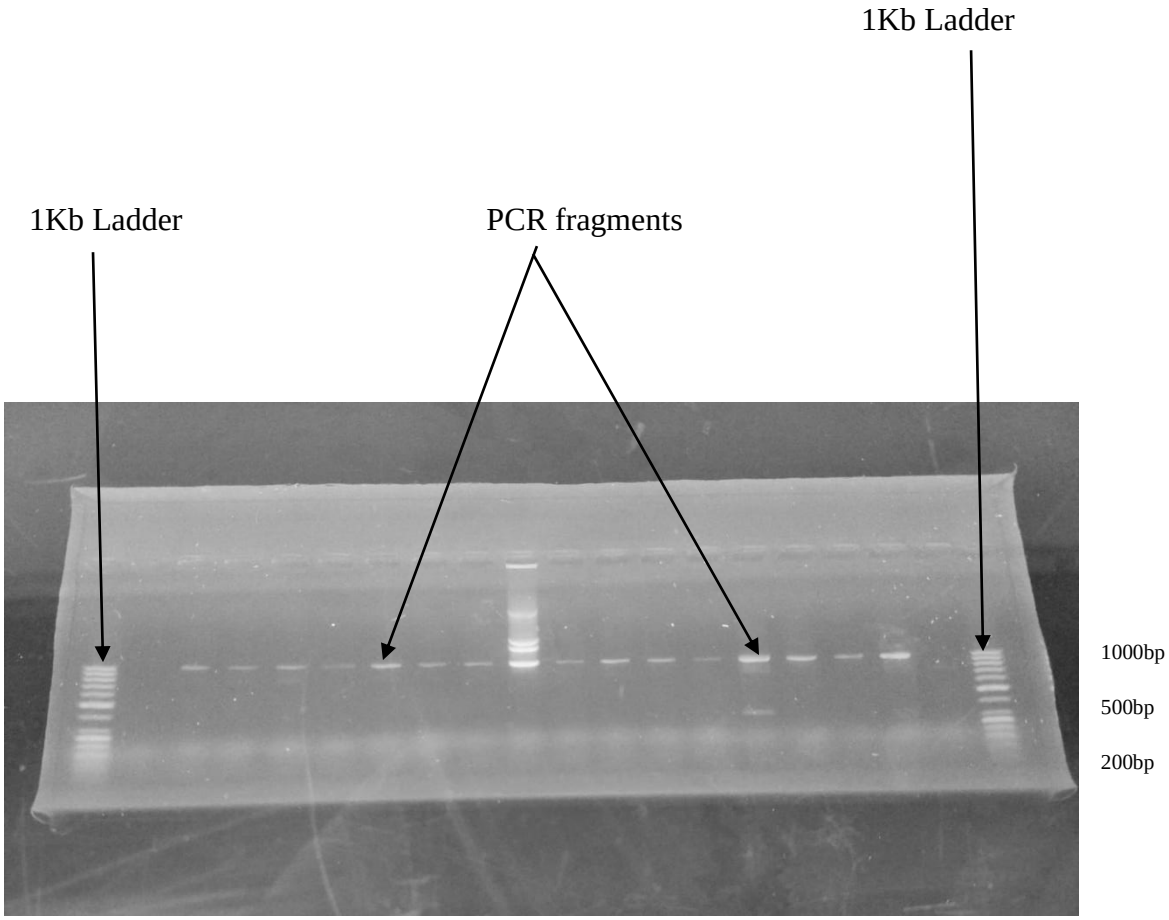


Figure 4.1: Representative Agarose gel showing PCR products from the study samples. The first and the last wells contain the ladder, well 2 and 3 the negative and positive controls respectively. Expected DNA band sizes of 940bp was detected in the next 14 wells.

Out of the 29 HBV DNA samples, twenty-two (22) samples were successfully sequenced. The geno2Pheno database showed that out of the 22 HBV sequences, 18 (81.8%) were identified as genotype D sub-genotype D4 circulating among both blood donor and inmate populations.

Genotype A sub genotype A1 was identified only among the inmate population with 18.2% **(Table 4.6).**

Table 4.6: Circulating HBV Genotypes among the study participants

Genotype	Study Population	No. of positives	% of positives
D	Inmate and donor	18	81.8
A	Inmate	4	18.2
Total		22	100

Table 4.7: Sequenced HBV DNA samples with characteristic genotypes

Sample Identity	Genotype
GARBD004, GARBD006, GARBD007 GARBD008, GARBD005, GARBD0A8 GARBD009, GARP025, GARP072, GARP073, GARP074, GARP118, GARP093, GARP077, GARP078, GARP079, GARP092, GARP123,	D
GARP098, GARP103, GARP016, GARP019	A

The phylogenetic analysis confirmed the identities of the circulating HBV genotypes and also the A1 subgenotype. However, identity of the genotype D subgenotype could not be resolved by phylogeny. The genotype D sequences from this study did not cluster with published subgenotype

D4 sequences but instead distinctly clustered away from the other subgenotypes. This cluster of local genotype D sequences from the study was well supported with a posterior probability of 91% **(Figure 4.2).**

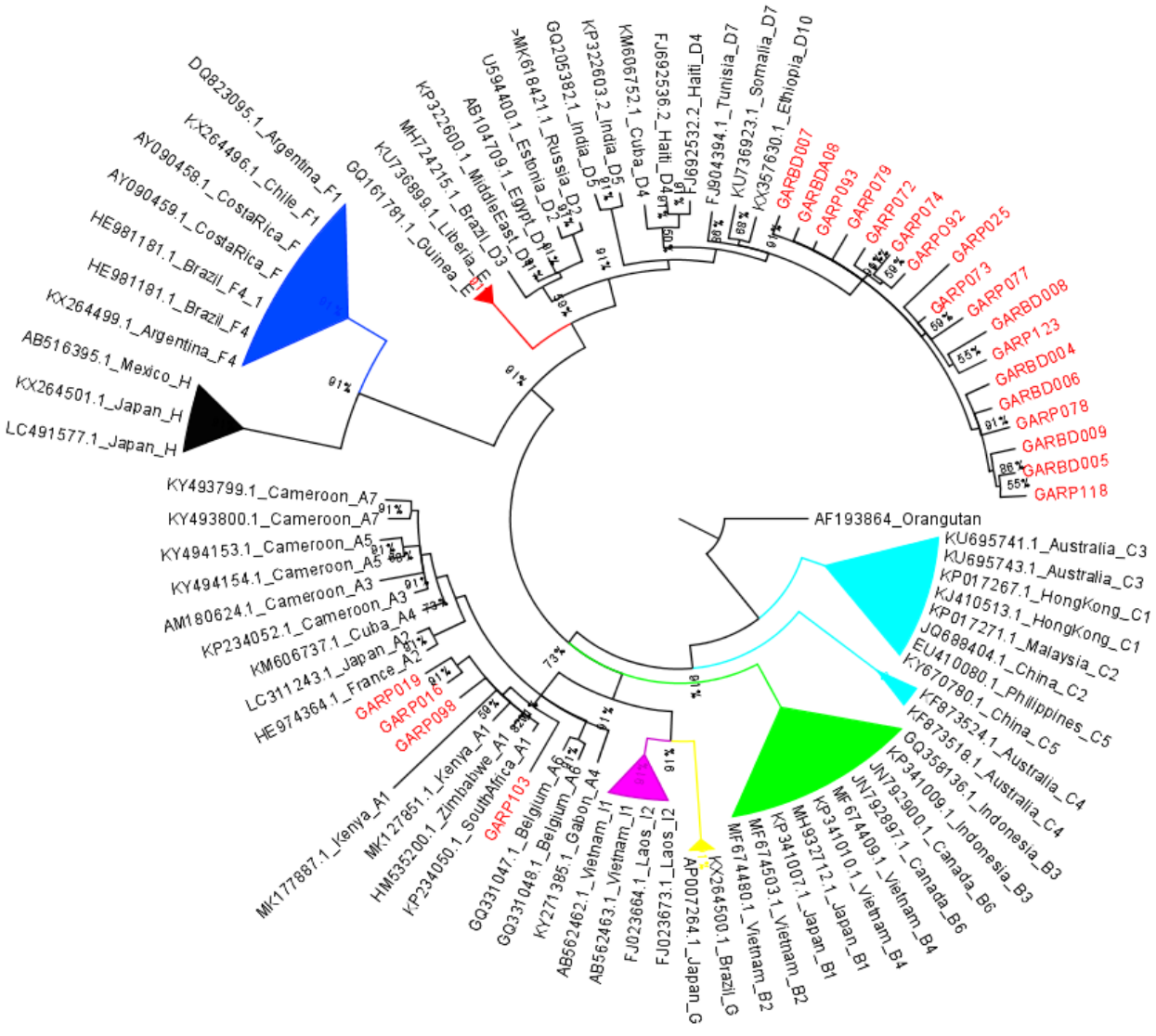


Figure 4.2: Phylogenetic tree of HBV S gene sequences from study participants

Phylogenetic tree of HBV S gene sequences from GCRH and Garissa Main prison. HBV sequences from the current study are shown in red color at the taxa while the percent posterior probabilities are indicated at the branch.

The 18 genotype D sequences were checked for recombination to resolve the subgenotype identity. All the sequences were found to be recombinants with genotype A and E. There were 11/18 genotype D/A recombinants and 7/18 genotype D/E recombinants (**Figure 4.3**)

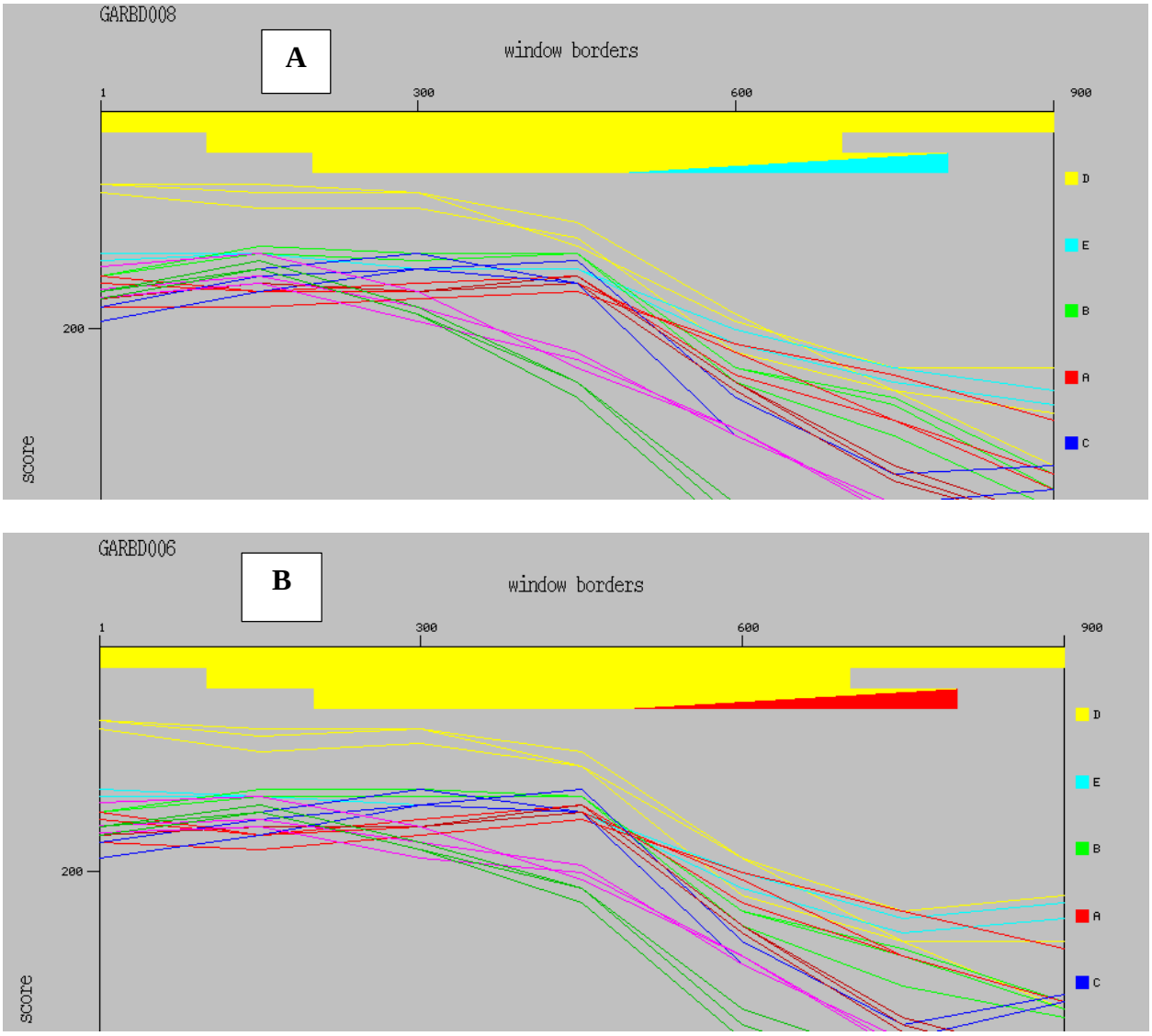


Figure 4.3: Recombination analysis of the HBV genotype D sequences showing D/A (A) and D/E (B) recombinants.

4.5 Escape mutations in the HBsAg

The major hydrophilic region (MHR) of the S gene had escape mutations associated with diagnostic failure in serological assays. These mutations were detected in 21 out of the 22 samples that were sequenced as shown in **Tables 4.8 and 4.10.** In total, this region showed 12 escape mutations that were described. Out of these, 2 mutations, were located within the "a" determinant region of the S gene (position 124-147) i.e., C124W affected 1 sequence, while T127P affected 18 sequences.

Table 4.8: HBsAg escape Mutations within the 'a' determinant region position (124-147)

Mutation	Number of sample affected	Identity of sample
T127P	18	GARBD004, GARBD006, GARBD007 GARBD008, GARBD005, GARBD0A8 GARBD009, GARP025, GARP072 GARP073, GARP074, GARP118 GARP093, GARP077, GARP078 GARP079, GARP092, GARP123
C124W	1	GARP098

Additionally, 10 other mutations were detected outside the "a" region of MHR within positions (99-123) and (148-169) i.e., S155Y, K160R, A166V, R122I, T123N, T123A, L104M, K122R, G102D and T123S, each affecting 1 sequence. All these mutations, were observed in HBsAg negative samples except K122R that affected a HBsAg positive sample.

Table 4.9: Relationship between HBV escape mutations and HBV testing.

Mutation	Sample affected	HBsAg serology status	HBV DNA status
S155Y K160R A166V	GARBD004	Negative	Positive
R122I	GARBD007	Negative	Positive
T123N	GARBD008	Negative	Positive
T123A	GARBD005	Negative	Positive
L104M	GARP077	Negative	Positive
K122R	GARP016	Positive	Positive
G102D T123S	GARP019	Negative	Positive

Table 4.10: HBsAg escape Mutations outside 'a' region (99-123) & (148-169)

Mutation	Number of samples affected	Identity of sample
S155Y K160R A166V	1	GARBD004
R122I	1	GARBD007
T123N	1	GARBD008
T123A	1	GARBD005
L104M	1	GARP077
K122R	1	GARP016
G102D T123S	1 1	GARP019

CHAPTER FIVE: DISCUSSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Blood is regarded as one of the most vital fluids in the body. Humans are exposed to factors such as leukemia, accidents and injuries that can contribute to low blood levels. When this happens, it necessitates the need for blood transfusion. Though, there is need for clean and safe blood for transfusion. The safety of blood and related products is compromised by the risk associated with

the transmission of HBV infection among the blood donors and the accuracy of the HBV screening tests. This calls for control measures towards transmission and spread of HBV infection among the population. This study aimed at determining and comparing seroprevalence and circulating HBV genotypes among the low-risk voluntary blood donors and high-risk prisoner populations in Garissa County, Kenya. In relation to this, this study employed PCR technique to determine circulating HBV genotypes from all serologically negative and positive samples from both study populations. The results generated will be important in controlling and managing of HBV infection among the categories of people affected.

5.1.1 Seroprevalence

The two study populations in the current study were the high-risk inmates and low risk voluntary blood donors. Comparing the seroprevalence of HBV infection in both populations, the inmates had a higher seroprevalence of 5.4% than the voluntary blood donors who had a seroprevalence of 3.1% as shown in **Table 4.4**. This observation could be attributed to a knowledge gap about HBV transmission modes in prisons leading to a higher risk of HBV transmission (Gétaz *et al.*, 2018). Other risk factors associated with HBV transmission among the prisoners include injection of intravenous drug use (IDU), skin piercing including tattooing and frequently engaging in unprotected sexual intercourse (Jafari *et al.*, 2010).

Comparing the findings of this study to other related studies, a study carried out among the voluntary blood donors in Nairobi, Kenya using the chemiluminescent microparticle immunoassay (CMIA) (Aluora *et al.*, 2020) which had a HBV seroprevalence of 2.3%, this study recorded a marginally higher HBV seroprevalence of 3.1% in the same group. However, this was similar to

3.46% seroprevalence from a study carried out using ELISA and confirmed by CMIA (Onyango *et al.,* 2018) in Siaya, Kisumu and Homabay Counties, Kenya. The differences in the HBV seroprevalence, could be linked to variations in the distinct geographical regions within the country as noted previously (Mutuma *et al.,* 2011) and due to disparities in sensitivity of the screening methods used in the detection of HBsAg.

Furthermore, the HBV seroprevalence of 5.4% among the inmates in this study, was higher compared to the seroprevalence of 3.3% in the same group in a study carried out in Iran (Zary Nokhodian, 2014). However, the current study findings are similar with those from a study carried out in Pakistan in the same population which showed a HBV seroprevalence of 5.6% and 3.1% amongst the blood donors who were regarded as a control group (Fayyaz, 2006) This could be attributed to differences in the sensitivity of the testing kits used and variations in geographical locations.

In the current study, there were statistically more HBV seropositive males than females in both study groups. The inmate population had an 85.7% seropositivity for males while among the blood donors all the seropositive samples were from males as shown in **Table 4.5.** A study carried out in Pakistan on HBV infection among different sex and age groups, had similar findings of more males infected than females (Khan *et al.,* 2011). This could be due to the fact that men's activities generally expose them to risk factors associated with HBV infection such as use of intra-venous drugs, alcoholism, barber risk, and unprotected sex than women (Khan *et al.,* 2011). However, these results should be cautiously interpreted since the study population was >90% males.

The other two socio-demographic factors in this study, did not depict any association with the HBV infection. While those in the 20-40 years age group and those with a primary school education showed higher HBV seroprevalence, this was not statistically significant. This could be attributed to a youthful population (20-40) years harboring experimental behavior and engaging in much social activities that expose them to HBV risk factors. Primary school level of education, acts as a hinderance to the understanding of the risk factors associated HBV infection transmission due to low knowledge. This will in turn contribute to the transmission of HBV infection within the affected group. This is in agreement with (Lungle *et al*., 2021) who reported that low level of education in rural population was a risk factor HBV infection.

5.1.2 HBV Genotypes

The genotypic analysis using geno2pheno database showed genotype D sub genotype D4 predominantly (81.8%) circulating among both the blood donor and inmate populations with some subjects among the inmates revealing genotype A sub genotype A1 as shown in **table 4.6.** Though the inmate population recorded a higher prevalence of circulating genotypes as compared to donor population with 15/22 successfully sequenced samples being from inmates. While previous studies have shown the presence of genotypes A ,D and E circulating in Kenya (Mwangi *et al*.,2009,Ochwoto *et al.,* 2013), none has shown a dominance of genotype D. Most studies have shown genotype A sub genotype A1 as the most prevalent (Ochwoto *et al*.,2013 , Aluora *et al.,* 2020). The dominance of genotype D in this study is therefore a novel finding.

Most studies have demonstrated that HBV genotypes show distinct geographic patterns. This genotypic variation attributed to geographical distribution can be affected by both ethnicity and

migration (Kramvis, 2014). This being the first study that has determined HBV genotypes in the North Eastern part of the country, it is possible that genotype D is more prevalent in this region. This could be due to immigration of non-local persons to the region influenced by trade among other factors and also that genotype D could be the most prevalent among the Somali ethnic group since they are the majority of the local population. Phylogenetic analysis of the HBV genotype A subgenotype A1 in this study concurred with the geno2pheno results. However, despite using published genotype D subgenotype sequences, the subgenotype identity of the genotype D sequences could not be determined. The genotype D sequences clustered in separate cluster which was well supported (91% posterior probability) away from the recognized subgenotypes. While subgenotype D1 and D4 have been detected locally (Ochwoto *et al.,* 2013; Kwange *et al.,* 2013), an outlier that could not have been assigned a HBV genotype has also been previously detected (Ochwoto *et al.,* 2013). There are two possibilities that could account for the no clustering of the local genotype D sequences to known HBV genotype D subgenotypes, first, it may be that the HBV sequences belong to a new genotype D subgenotype with a 4% genome divergence or secondly it could be that the sequences are genotype D recombinants.

To test the second likelihood, the 18 genotype D sequences were analyzed for recombination. Majority (61%) of these sequences were found to be D/A recombinants while the rest were found to be D/E recombinants as shown in **Figure 4.3**. Recombination involving genotypes A, D and E are not unexpected considering that these three genotypes have shown the highest prevalence in Africa (Ochwoto *et al.,* 2016). This could be due to the fact that HBV intergenotypic recombinants share similarities in circulation patterns with their parental genotypes (Matlou *et al*., 2019). HBV intergenotypic recombination, impact on the evolution history of the virus (Zhang *et al*., 2016). A D/E recombinant strain was determined in Kenya (Ochwoto *et al.,* 2016). Circulation of hepatitis

B virus recombinant strains in Kenya, could be that the virus is still evolving causing variations which may impact negatively on the diagnosis, treatment and vaccination of individuals for HBV in the country (Gao *et al.*, 2015).

5.1.3 Antiviral resistance and diagnostic failure

Despite HBV being a DNA virus, it's error prone replicative strategy yields numerous non-identical variants occasioned by a higher rate of mutation (Caligiuri *et al.,* 2016). Some of these variants may have mutations conferring escape from serological diagnostic assays and treatment failure. The region between amino acid 100 to 160 of the HBsAg is referred to as the major hydrophilic region (MHR) and the antibody response following natural infection or immunization recognize this region. Within the MHR in positions 124-147, is the 'a' determinant region in which mutations lead to poor detection in serological assays. Escape mutations in the 'a' determinant region were noted in 21 of the 22 samples sequenced.

It was noted that majority of the samples in the current study 81.8%, were HBsAg sero-negative but HBV-DNA positive as shown **Table 5.1.**This could be attributed to neutralizing antibodies used in diagnostic immunoassays , failing to recognize and bind to B-cells when directed against HBsAg (Lazarevic, 2014). The failure by these antibodies to bind to B-cells is due mutations occurring in the 'a' determinant region of the MHR. However, these mutations as much as they interfere with serological assays, they do not inhibit the detection of HBV-DNA through molecular methods like PCR.

HBV escape mutations within the MHR but outside the 'a' determinant region of HBsAg were also noted. A relationship between these mutations and HBV testing was also established as shown in **Table 4.9**. These mutations altered the antigenicity of HBsAg and hindered specific binding by monoclonal anti-HBs antibodies in diagnostic immunoassays hence causing diagnostic failure. This could be due to the fact that these mutations are nearby the "a" determinant region and they can alter the secondary structure of the two loops of amino acids (Lazarevic et al., 2019). However, the detection of HBV-DNA through PCR from the serologically affected samples could not be interfered with.

Table 5.1: HBV HBsAg and HBV DNA positivity status

Number of samples	HBsAg serology status	(%)	HBV DNA status
4	Positive	18.2	Positive
18	Negative	81.8	Positive
Total 22		**100**	

5.1.4 Mutations associated with antiviral resistance

HBV antiviral therapy benefits the host by clearing the virus and preventing the disease in the liver from progressing to cirrhosis and hepacellular carcinoma (HCC). However complete eradication of chronic HBV infection is challenging due to the persistence of cccDNA in the nucleus of infected hepatocytes. HBV mutations have been noted as the major cause to antiviral resistance. The most common form of viral resistance and persistence has been witnessed in the current Nucleoside analogues (NAs) drugs. This happens, when the binding sites of HBV polymerase are altered by drug resistance mutations making it impossible for the interaction with antiviral agents

(James Fung, 2011) . Resistance to NAs has been linked to two types of mutations. These are; primary resistance mutations, which are directly associated with drug resistance, and secondary (compensatory) mutations, which enhance the competence of a resistant strain during replication (Lazarevic, 2014). In the current study, two samples GARBD004 and GARP025 conferred mutations S202N and N236D respectively as shown in **Table 5.2**. These mutations were associated with antiviral resistance to Entecavir for GARBD004 and Adefovir for GARP025 which translated to a 10% of the 22 HBV positive samples. This could be attributed to differences between HBV genotypes and their response to antiviral treatment. Since the dominant HBV genotype in the region is D compared to other regions in country that have recorded higher prevalence of genotype A (Ochwoto *et al*.,2013 , Aluora *et al.,* 2020), then this variation could impact on the antiviral resistance patterns hence affecting the response to HBV treatment in the region as demonstrated by (Sunbul, 2014).

Table 5.2: HBV antiviral resistance mutations

Sample Identity	**Mutation**	**Resisted Anti-HBV drug**
GARBD004	S202N	Entecavir, Baraclude
GARP025	N236D	Adefovir, Hepsera

5.2 Conclusions

1. A statistically higher HBV seroprevalence (5.4%) was found among inmates compared to blood donors (3.6%).
2. HBV Seropositivity was statistically higher in males than females in both the inmates and blood donors.
3. The dominance of HBV genotype D is a novel finding as studies in other regions of the country have identified genotype A as the most prevalent.
4. The study findings also showed mutations within MHR 'a' determinant region (124-147) and outside the 'a' determinant region. These mutations are suspected to have caused antigenicity alteration within HBsAg leading to diagnostic failure.
5. The binding sites of HBV polymerase are altered by drug resistance mutations making it impossible for the interaction with antiviral agents hence impacting negatively on antiviral treatment.
6. HBV D/E and D/A recombinants were determined from genotype D sequences. This helped in partially resolving the subgenotype identity.

5.3 Recommendations

1. The government through the Ministry of Health in collaboration with the Prisons Service of Kenya, should design and implement a policy on prevention, diagnosis and management of HBV infection in all prisons and the general population as a whole.
2. Studies should be carried out in other parts of this region and the country to further determine the distribution of the genotype D and the extent of recombination with the other circulating genotypes.

3. For complete, accurate and reliable HBV diagnosis, serological testing should be followed up with molecular PCR testing methods to avoid diagnostic failure. Further, before initiating treatment mutation analysis should be carried out to curb antiviral resistance.

5.3.1 Further studies

Further studies should be carried out to determine unclassified genotype D subgenotypes. Besides, other complex methods of analysis should be employed.

REFERENCES

Al-Sadeq, D. W., Taleb, S. A., Zaied, R. E., Fahad, S. M., Smatti, M. K., Rizeq, B. R., … Nasrallah, G. K. (2019). Hepatitis B virus molecular epidemiology, host-virus interaction, coinfection, and laboratory diagnosis in the MENA region: An update. *Pathogens*, Vol. 8. https://doi.org/10.3390/pathogens8020063

Aluora, P. O., Muturi, M. W., & Gachara, G. (2020). Seroprevalence and genotypic characterization of HBV among low risk voluntary blood donors in Nairobi, Kenya. *Virology Journal*, *17*(1), 1–9. https://doi.org/10.1186/s12985-020-01447-2

Assih, M., Ouattara, A. K., Diarra, B., Yonli, A. T., Compaore, T. R., Obiri-Yeboah, D., … Simpore, J. (2018). Genetic diversity of hepatitis viruses in West-African countries from 1996 to 2018. *World Journal of Hepatology*, Vol. 10, pp. 807–821. https://doi.org/10.4254/wjh.v10.i11.807

Breakwell, L., Tevi-Benissan, C., Childs, L., Mihigo, R., & Tohme, R. (2017). The status of hepatitis B control in the African region. *The Pan African Medical Journal*, Vol. 27, p. 17.

https://doi.org/10.11604/pamj.supp.2017.27.3.11981

Bruce, M. G., Bruden, D., Hurlburt, D., Zanis, C., Thompson, G., Rea, L., … McMahon, B. J. (2016). Antibody Levels and Protection after Hepatitis B Vaccine: Results of a 30-Year Follow-up Study and Response to a Booster Dose. *Journal of Infectious Diseases*, Vol. 214, pp. 16–22. https://doi.org/10.1093/infdis/jiv748

Caligiuri, P., Cerruti, R., Icardi, G., & Bruzzone, B. (2016). Overview of hepatitis B virus mutations and their implications in the management of infection. *World Journal of Gastroenterology*, Vol. 22, pp. 145–154. https://doi.org/10.3748/wjg.v22.i1.145

Candotti, D., & Allain, J. P. (2009). Transfusion-transmitted hepatitis B virus infection. *Journal of Hepatology*. https://doi.org/10.1016/j.jhep.2009.05.020

Casagrande, J. T., Pike, M. C., & Smith, P. G. (1978). An Improved Approximate Formula for Calculating Sample Sizes for Comparing Two Binomial Distributions. *Biometrics*, Vol. 34, p. 483. https://doi.org/10.2307/2530613

Chook, J. B., Teo, W. L., Ngeow, Y. F., Tee, K. K., Ng, K. P., & Mohamed, R. (2015). Universal primers for detection and sequencing of hepatitis B virus genomes across genotypes A to G. *Journal of Clinical Microbiology*, Vol. 53, pp. 1831–1835. https://doi.org/10.1128/JCM.03449-14

Churin, Y., Roderfeld, M., & Roeb, E. (2015). Hepatitis B virus large surface protein: function and fame. *Hepatobiliary Surgery and Nutrition*, Vol. 4, pp. 1–10. https://doi.org/10.3978/j.issn.2304-3881.2014.12.08

Croagh, C. M. N., Desmond, P. V., & Bell, S. J. (2015). Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. *World Journal of Hepatology*, Vol. 7, pp. 289–303. https://doi.org/10.4254/wjh.v7.i3.289.

Datta, S., Chatterjee, S., Veer, V., & Chakravarty, R. (2012). Molecular Biology of the Hepatitis B Virus for Clinicians. *Journal of Clinical and Experimental Hepatology*, Vol. 2, pp. 353–365. https://doi.org/10.1016/j.jceh.2012.10.003

Dawkins-hall, L. S. (2017). *Treatment of PCR products with Shrimp Alkaline phosphatase (SAP) & Exonuclease III (Exo) as a proxy for physical purification* (Vol. 1). Vol. 1.

de la Fuente, R. A., Gutiérrez, M. L., Garcia-Samaniego, J., Fernández-Rodriguez, C., Lledó, J. L., & Castellano, G. (2011). Pathogenesis of occult chronic hepatitis B virus infection. *World Journal of Gastroenterology*, Vol. 17, pp. 1543–1548. https://doi.org/10.3748/wjg.v17.i12.1543

Elghannam, D., Aly, R., Goda, E., Eltoraby, E., & Farag, R. (2009). Clinical significance of antibody to hepatitis B core antigen in multitransfused hemodialysis patients. *Asian Journal of Transfusion Science*, Vol. 3, pp. 14–17. https://doi.org/10.4103/0973-6247.45256

Fayyaz. (2006). *Frequency of hepatitis B and C seropositivity in prisoners _ Virtual Health Sciences Library*.

François, G., Dochez, C., Jeffrey Mphahlele, M., Burnett, R., Van Hal, G., & Meheus, A. (2008). Hepatitis B vaccination in Africa: mission accomplished? *Southern African Journal of Epidemiology and Infection*, *23*(1), 24–28. https://doi.org/10.1080/10158782.2008.11441296

Gallucci, L., & Kann, M. (2017). Nuclear import of hepatitis B virus capsids and genome. *Viruses*, Vol. 9. https://doi.org/10.3390/v9010021

Gao, S., Duan, Z. P., & Coffin, C. S. (2015). Clinical relevance of hepatitis B virus variants. *World Journal of Hepatology*, Vol. 7, pp. 1086–1096. https://doi.org/10.4254/wjh.v7.i8.1086

Gétaz, L., Casillas, A., Siegrist, C. A., Chappuis, F., Togni, G., Tran, N. T., … Wolff, H. (2018). Hepatitis B prevalence, risk factors, infection awareness and disease knowledge among inmates: A cross-sectional study in Switzerland's largest pre-trial prison. *Journal of Global Health*, Vol. 8. https://doi.org/10.7189/jogh.08.020407

Green, M. R., & Sambrook, J. (2019). Nested polymerase chain reaction (PCR). *Cold Spring Harbor Protocols*, Vol. 2019. https://doi.org/10.1101/pdb.prot095182

Grewal, P., & Sanghera, R. (2017). Hepatitis B and C Viruses and Biologics. *Skin Therapy Letter*, Vol. 22, pp. 7–9.

Hadziyannis, E., & Laras, A. (2018). Viral biomarkers in chronic hbeag negative hbv infection. *Genes*, Vol. 9, pp. 1–20. https://doi.org/10.3390/genes9100469

He, Q., Zou, Q., & Guan, Y. S. (2010). Viral hepatitis and liver cancer. *Nose and Viral Cancer: Etiology, Pathogenesis and Treatment*, pp. 291–307.

Hepatitis B- WHO 2019. (n.d.).

Hepatitis B Foundation. (2019). *Hepatitis B Foundation_ Vaccination Guidelines* (p. 1). p. 1. Retrieved from http://www.hepb.org/prevention-and-diagnosis/vaccination/

Hollinger, F. B., & Sood, G. (2010). Occult hepatitis B virus infection: A covert operation. *Journal of Viral Hepatitis*, Vol. 17, pp. 1–15. https://doi.org/10.1111/j.1365-2893.2009.01245.x

Horvat, R. T. (2011). Diagnostic and Clinical Relevance of HBV Mutations. *Laboratory Medicine*, Vol. 42, pp. 488–496. https://doi.org/10.1309/lm7sf4qzmeg5lvpn

Iannacone, M., Sitia, G., Ruggeri, Z. M., & Guidotti, L. G. (2007). HBV pathogenesis in animal models: Recent advances on the role of platelets. *Journal of Hepatology*, *46*(4), 719–726. https://doi.org/10.1016/j.jhep.2007.01.007

Jafari, S., Copes, R., Baharlou, S., Etminan, M., & Buxton, J. (2010). Tattooing and the risk of transmission of hepatitis C: A systematic review and meta-analysis. *International Journal of Infectious Diseases*, Vol. 14. https://doi.org/10.1016/j.ijid.2010.03.019

James Fung, C.-L. L. (2011). *Nucleoside_nucleotide analogues in the treatment of chronic hepatitis B _ Journal of Antimicrobial Chemotherapy _ Oxford Academic*.

JeanneCarrDonna G.WilliamsRandall T.Hayden. (2010). *Molecular Detection of Multiple Respiratory Viruses - ScienceDirect*. Retrieved from https://www.sciencedirect.com/science/article/pii/B9780123694287000240

Karra, V. K., Chowdhury, S. J., Ruttala, R., Polipalli, S. K., & Kar, P. (2016). Clinical Significance of Quantitative HBsAg Titres and its Correlation With HBV DNA Levels in the Natural History of Hepatitis B Virus Infection. *Journal of Clinical and Experimental Hepatology*, Vol. 6, pp. 209–215. https://doi.org/10.1016/j.jceh.2016.07.002

Khan, F., Shams, S., Qureshi, I. D., Israr, M., Khan, H., Sarwar, M. T., & Ilyas, M. (2011). Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. *Virology Journal*, Vol. 8. https://doi.org/10.1186/1743-422X-8-225

Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. *Intervirology*, Vol. 57, pp. 141–150. https://doi.org/10.1159/000360947

Kumar, M., Singh, T., & Sinha, S. (2012). Chronic Hepatitis B Virus Infection and Pregnancy. *Journal of Clinical and Experimental Hepatology*, Vol. 2, pp. 366–381. https://doi.org/10.1016/j.jceh.2012.09.001

Lazarevic et al. (2019). *Immune-Escape Hepatitis B Virus Mutations Associated with Viral Reactivation upon Immunosuppression*.

Lazarevic, I. (2014). Clinical implications of hepatitis B virus mutations: Recent advances. *World Journal of Gastroenterology*, Vol. 20, pp. 7653–7664. https://doi.org/10.3748/wjg.v20.i24.7653.

Levrero, M., & Zucman-Rossi, J. (2016). Mechanisms of HBV-induced hepatocellular carcinoma. *Journal of Hepatology*, Vol. 64, pp. S84–S101. https://doi.org/10.1016/j.jhep.2016.02.021

Li, Y., Cai, Q., Xie, Q., Zhang, Y., Meng, X., & Zhang, Z. (2017). Different mechanisms may exist for HBsAG synthesis and secretion during various phases of chronic hepatitis B virus infection. *Medical Science Monitor*, Vol. 23, pp. 1385–1393. https://doi.org/10.12659/MSM.902889

Lin, C. L., & Kao, J. H. (2021). Prevention of hepatitis b virus-related hepatocellular carcinoma. *Hepatoma Research*, Vol. 7. https://doi.org/10.20517/2394-5079.2020.125

Liu, D., Cui, L., Wang, Y., Yang, G., He, J., Hao, R., … Guo, D. (2016). HBV E Antigen and its Precursors Promote the Progress of HCC by Interacting with Numb and Decreasing P53 Activity. *Journal of Hepatology*, Vol. 64, pp. S386–S387. https://doi.org/10.1016/s0168-8278(16)00595-x

Long, C., Qi, H., & Huang, S. H. (2008). Mathematical modeling of cytotoxic lymphocyte-mediated immune response to hepatitis B virus infection. *Journal of Biomedicine and Biotechnology*, Vol. 2008. https://doi.org/10.1155/2008/743690

Lott, L., Beames, B., Notvall, L., & Lanford, R. E. (2000). Interaction between Hepatitis B Virus Core Protein and Reverse Transcriptase. *Journal of Virology*, Vol. 74, pp. 11479–11489. https://doi.org/10.1128/jvi.74.24.11479-11489.2000

Lungle et al., 2021. (2021). Risk factors associated with hepatitis B virus infection among pregnant women attending the antenatal care unit of the Bamenda Regional Hospital. *Public Health in Practice*, Vol. 2. https://doi.org/10.1016/j.puhip.2021.100160

Ly, K. N., Kim, A. A., Umuro, M., Drobenuic, J., Williamson, J. M., Montgomery, J. M., … Teshale, E. H. (2016). Prevalence of hepatitis B Virus infection in Kenya, 2007. *American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.16-0059

Makvandi, M. (2016). Update on occult hepatitis B virus infection. *World Journal of Gastroenterology*, Vol. 22, pp. 8720–8734. https://doi.org/10.3748/wjg.v22.i39.8720

Matlou, M. K., Gaelejwe, L. R., Musyoki, A. M., Rakgole, J. N., Selabe, S. G., & Amponsah-Dacosta, E. (2019). A novel hepatitis B virus recombinant genotype D4/E identified in a South African population. *Heliyon*, Vol. 5. https://doi.org/10.1016/j.heliyon.2019.e01477

Milich, D., & Liang, T. J. (2003). Exploring the Biological Basis of Hepatitis B e Antigen in Hepatitis B Virus Infection. *Hepatology*, *38*(5), 1075–1086. https://doi.org/10.1053/jhep.2003.50453

Miri, S. M., & Alavian, S. M. (2011). Risk factors of hepatitis B infection: Health policy makers should be aware of their importance in each community. *Hepatitis Monthly*, Vol. 11, pp. 238–239.

Mohammed, A. (2012). *Prevalence and Risk Factors for Hepatitis B Infection Among Pregnant Women Attending Antenatal Clinics in Garissa District.* (pp. 1–79). pp. 1–79.

Moradi, G., Mohamadi, P., Zareie, B., Rasouli, M. A., Gouya, M. M., & Jafari, S. (2020). Prevalence of and risk factors for HBV and HCV among incarcerated people who inject drugs in Iran: A cross sectional study. *BMC Infectious Diseases*, Vol. 20. https://doi.org/10.1186/s12879-020-05541-2

Mutuma, G., Mbuchi, M., Zeyhle, E., Fasana, R., Okoth, F., Kabanga, J., … Kaiguri, P. (2011). Prevalence of Hepatitis B Virus (HBV) surface antigen and HBV-associated hepatocellular carcinoma in Kenyans of various ages. *Afr J Health Sci*, *18*(1), 53–61.

Nyairo, E. K., Ng'ang'a, Z., Kimotho, J., Khamadi, S. A., Raphael, L., Ochwoto, M., … Lwembe, R. (2016). Mutations in the "a" Determinant Region of Hepatitis B Virus Genotype A among Voluntary Kenyan Blood Donors. *Journal of Biology, Agriculture and Healthcare*, *6*(14), 1–8. Retrieved from www.iiste.org

Ochwoto et al. (2016). *Hepatitis B infection is highly prevalent among patients presenting with jaundice in Kenya _ BMC Infectious Diseases*.

Ochwoto, M., Chauhan, R., Gopalakrishnan, D., Chen, C. Y., Ng'ang'a, Z., Okoth, F., … Kramvis, A. (2013). Genotyping and molecular characterization of hepatitis B virus in liver disease patients in Kenya. *Infection, Genetics and Evolution*, Vol. 20, pp. 103–110. https://doi.org/10.1016/j.meegid.2013.08.013

Onyango, C. G., Ogonda, L., Guyah, B., Okoth, P., Shiluli, C., Humwa, F., & Opollo, V. (2018). Seroprevalence and determinants of transfusion transmissible infections among voluntary blood donors in Homabay, Kisumu and Siaya counties in western Kenya. *BMC Research Notes*, Vol. 11. https://doi.org/10.1186/s13104-018-3276-y

Ozaslan, E., & Purnak, T. (2009). Controversies about occult hepatitis B virus infection. *World Journal of Gastroenterology*, Vol. 15, pp. 4986–4987. https://doi.org/10.3748/wjg.15.4986

Pak, K., Ordway, S., & Torres, D. (2020). Occult HBV Infection: A Case Series at a Military Treatment Facility. *Military Medicine*, Vol. 185, pp. E795–E798. https://doi.org/10.1093/milmed/usz379

Parry, J. V., Easterbrook, P., & Sands, A. R. (2017). One or two serological assay testing strategy for diagnosis of HBV and HCV infection? The use of predictive modelling. *BMC Infectious Diseases*, Vol. 17. https://doi.org/10.1186/s12879-017-2774-1

Pollack, J. R., & Ganem, D. (1993). An RNA stem-loop structure directs hepatitis B virus genomic RNA encapsidation. *Journal of Virology*, Vol. 67, pp. 3254–3263. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7684464%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC237666

Qiu, L., Wang, T., Xu, X., Wu, Y., Tang, Q., & Chen, K. (2017). Long non-coding rnas in hepatitis b virus-related hepatocellular carcinoma: Regulation, functions, and underlying mechanisms. *International Journal of Molecular Sciences*, Vol. 18. https://doi.org/10.3390/ijms18122505

Ramamoorthy, M., Venketeswaran, A., Seenivasan, P., Revathy, M., Manimaran, M., Chitra, S., … Frederick, T. (2016). Risk factors and prevalence, hepatitis B virus and hepatitis C virus among prison inmates, Chennai, India, 2015. *International Journal of Infectious Diseases*, Vol. 53, p. 90. https://doi.org/10.1016/j.ijid.2016.11.227

Romanò, L., Paladini, S., Galli, C., Raimondo, G., Pollicino, T., & Zanetti, A. R. (2015). Hepatitis B vaccination: Are escape mutant viruses a matter of concern? *Human Vaccines and Immunotherapeutics*, Vol. 11, pp. 53–57. https://doi.org/10.4161/hv.34306

Ronquist, F., Huelsenbeck, J. P., & Mark, P. van der. (2005). MrBayes 3.1 Manual; Draft 5/26/2005. *School of Computational Science, Florida State University*, 1–69. Retrieved from papers2://publication/uuid/F1F34A7E-D541-47E3-BB3C-A4B2959E338C

Said, Z. N. A. (2011). An overview of occult hepatitis B virus infection. *World Journal of Gastroenterology*, Vol. 17, pp. 1927–1938. https://doi.org/10.3748/wjg.v17.i15.1927

Slagle, B. L., & Bouchard, M. J. (2016). Hepatitis B virus X and regulation of viral gene expression. *Cold Spring Harbor Perspectives in Medicine*, Vol. 6. https://doi.org/10.1101/cshperspect.a021402

Smith, J. M., Uvin, A. Z., Macmadu, A., & Rich, J. D. (2017). Epidemiology and Treatment of Hepatitis B in Prisoners. *Current Hepatology Reports*, Vol. 16, pp. 178–183.

https://doi.org/10.1007/s11901-017-0364-8

Squadrito, G., Spinella, R., & Raimondo, G. (2014). The clinical significance of occult HBV infection. *Annals of Gastroenterology*, *27*(1), 15–19.

Sunbul, M. (2014). Hepatitis B virus genotypes: Global distribution and clinical importance. *World Journal of Gastroenterology*, Vol. 20, pp. 5427–5434. https://doi.org/10.3748/wjg.v20.i18.5427

Tan, Z., Pionek, K., Unchwaniwala, N., Maguire, M. L., Loeb, D. D., & Zlotnick, A. (2015). The Interface between Hepatitis B Virus Capsid Proteins Affects Self-Assembly, Pregenomic RNA Packaging, and Reverse Transcription. *Journal of Virology*, Vol. 89, pp. 3275–3284. https://doi.org/10.1128/jvi.03545-14

US-CDC. (2021). *What is Viral Hepatitis? | CDC*. Retrieved from https://www.cdc.gov/hepatitis/abc/index.htm

Walker-Daniels, J. (2012). Current PCR Methods. *Materials and Methods*, Vol. 2. https://doi.org/10.13070/mm.en.2.119

Wang, W., Bian, H., Li, F., Li, X., Zhang, D., Sun, S., … Qi, J. (2018). HBeAg induces the expression of macrophage miR-155 to accelerate liver injury via promoting production of inflammatory cytokines. *Cellular and Molecular Life Sciences*, Vol. 75, pp. 2627–2641. https://doi.org/10.1007/s00018-018-2753-8

Watashi, K., & Wakita, T. (2015). Hepatitis B virus and hepatitis D virus entry, species specificity, and tissue tropism. *Cold Spring Harbor Perspectives in Medicine*, Vol. 5, pp. 1–10. https://doi.org/10.1101/cshperspect.a021378

WHO. (2018). *What is hepatitis*.

WHO. (2021). *Hepatitis B_WHO*.

Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., … Li, W. (2012). Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *ELife*, Vol. 2012. https://doi.org/10.7554/eLife.00049

Yang et al. (2014). Persistence of hepatitis B virus covalently closed circular DNA in hepatocytes: Molecular mechanisms and clinical significance. *Emerging Microbes and Infections*, Vol. 3. https://doi.org/10.1038/emi.2014.64

Yang, H.-I., Lu, S.-N., Liaw, Y.-F., You, S.-L., Sun, C.-A., Wang, L.-Y., … Chen, C.-J. (2002). Hepatitis B e Antigen and the Risk of Hepatocellular Carcinoma. *New England Journal of Medicine*, Vol. 347, pp. 168–174. https://doi.org/10.1056/nejmoa013215

Young, J. H. P. van D. (2021). Hepatitis B vaccines. *Vaccine*, Vol. 2, p. 167. https://doi.org/10.1016/0264-410X(84)90032-X

Zajakina, A., Kozlovska, T., Bruvere, R., Aleksejeva, J., Pumpens, P., & Garoff, H. (2004). Translation of hepatitis B virus (HBV) surface proteins from the HBV pregenome and precore RNAs in Semliki Forest virus-driven expression. *Journal of General Virology*, Vol. 85, pp.

3343–3351. https://doi.org/10.1099/vir.0.80388-0

Zampino, R., Boemio, A., Sagnelli, C., Alessio, L., Adinolfi, L. E., Sagnelli, E., & Coppola, N. (2015). Hepatitis B virus burden in developing countries. *World Journal of Gastroenterology*, Vol. 21, pp. 11941–11953. https://doi.org/10.3748/wjg.v21.i42.11941

Zary Nokhodian, M. Y. (2014). *Seroprevalence of hepatitis B markers among incarcerated intravenous drug users*.

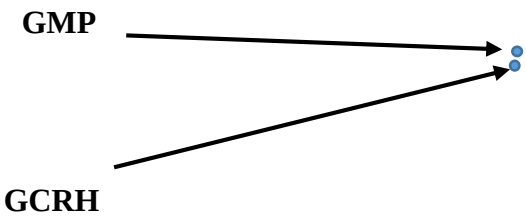
Zhang, Z. H., Wu, C. C., Chen, X. W., Li, X., Li, J., & Lu, M. J. (2016). Genetic variation of hepatitis B virus and its significance for pathogenesis. *World Journal of Gastroenterology*, Vol. 22, pp. 126–144. https://doi.org/10.3748/wjg.v22.i1.126

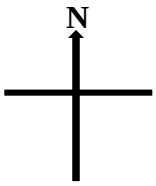
Zhao, F., Xie, X., Tan, X., Yu, H., Tian, M., Lv, H., … Zhu, Q. (2021). The Functions of Hepatitis B Virus Encoding Proteins: Viral Persistence and Liver Pathogenesis. *Frontiers in Immunology*, Vol. 12. https://doi.org/10.3389/fimmu.2021.691766

Zoulim, F. (2006). Hepatitis B virus resistance to entecavir in nucleotide naïve patients: Does it exist? *Hepatology*, Vol. 44, pp. 1404–1407. https://doi.org/10.1002/hep.21451

APPENDICES

Appendix I: A study sites map





KEY

GMP-Garissa Main Prison

GCRH-Garissa County Referral Hospital

Appendix II: KNBTS Research Authorization Letter

MINISTRY OF HEALTH

Telephone: 020-2012867
Hotline: +254 716775245
Email: info@nbtskenya.or.ke
Website: www.nbtskenya.or.ke
When replying please quote:

NATIONAL BLOOD TRANSFUSION, TISSUE &HUMAN ORGAN TRANSPLANTATION - HQS
LOCATION: KENYATTA NATIONAL HOSPITAL, NPHLS GROUNDS
P.O.BOX 29804-00202

Ref: No.

19th August 2020

Mr. Vincent Odallo Bahati
Box 256-70100
Nairobi

Re: Letter of no objection- Research Authorization at Garrisa Satellite
We refer to your letter dated 30th June 2020 on the above subject matter.

KNBTS has no objection for you to carry out thestudy at the KNTBS Garrisa Satellite Center on the **A COMPARATIVE STUDY OF HEPATITIS B VIRUS AMONG IN-MATES AND LOW RISK VOLUNTARY BLOOD DONORS IN GARISSA COUNTY, KENYA**

You are authorized to collect the demographic information to include age, gender, occupation and level of education from the donor during the sample collection

You are expected to give feedback of the study findings to the office of the undersigned before publication.

Charles Rombo

HEAD KENYA NATIONAL BLOOD TRANSFUSION TISSUE AND HUMAN ORGAN TRANSPLANTATION

Kenya National Blood Transfusion Service
It's safe and it saves

ISO 9001 : 2015 Certified

Appendix III: Kenya Prisons Research Approval Letter

MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT
STATE DAPARTMENT OF CORRECTIONAL SERVICES
KENYA PRISONS SERVICE

Telegrams: "COMPRISONS", Nairobi
Telephone: +254-20-2722900-6
Fax: +254-2-2714716
Email: commissioner.prisons@gmail.com
When replying please quote
Ref No. PRIS 1/21/VOL V/103

PRISONS HEADQUARTER
P.O. BOX 30175-00100
NAIROBI

Date: 17 November 2020

Vincent Bahati Odallo
P.O. Box 256- 70100
GARISSA

RE: APPLICATION FOR AUTHORISATION TO CONDUCT ACADEMIC RESEARCH.

We acknowledge receipt of your letter requesting approval to conduct an academic research at Garissa Main Prison titled *" A comparative study of Hepatitis B Virus among inmates and lowrisk voluntary blood donors in Garissa County Kenya".*

It is noted that the research will assist the Prisons Department in improving healthcare service delivery. This is therefore to inform you that your request has been approved.

You are expected to adhere to Prison rules and regulations during your research period. You are also required to provide the Prisons Headquarter with a copy of your research findings at the end of your research.

By a copy of this letter, the Officer in Charge Garissa Main Prison is requested to accord you the necessary assistance during your research period.

KENNEDY A. ALUDA, MBS, ndc(K) SACGP/A
FOR: COMMISSIONER GENERAL OF PRISONS

Cc.
Officer in Charge
Garissa Main Prison,

Appendix IV: Ethical Approval Letter from Kenyatta University Ethical Review Committee

Kenyatta University
P.O Box 43844-00100
Nairobi-Kenya

REF: KU/ERC/APPROVAL/VOL1

Date: 25th February, 2020

Vincent Odallo Bahati
P.O Box 43844-00100
NAIROBI

Dear Mr Odallo

RE: A COMPARATIVE STUDY OF HEPATITIS B VIRUS AMONG IN-MATES AND LOW RISK VOLUNTARY BLOOD DONORS IN GARISSA COUNTY, KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2043/I1190.** The approval period is ***25th February, 2020- 25th February, 2021.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.***

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Prof. Judith Kimiywe

CHAIRPERSON- KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.

Appendix V: NACOSTI Research Authorization Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **869317**

Date of Issue: **23/March/2020**

RESEARCH LICENSE

This is to Certify that Mr.. VINCENT BAHATI ODALLO of Kenyatta University, has been licensed to conduct research in Garissa on the topic: A COMPARATIVE STUDY OF HEPATITIS B VIRUS AMONG IN-MATES AND LOW RISK VOLUNTARY BLOOD DONORS IN GARISSA COUNTY,KENYA for the period ending : 23/March/2021.

License No: **NACOSTI/P/20/4150**

869317

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix VI: Garissa County Ministry of Education Research Authorization Letter

MINISTRY OF EDUCATION

STATE DEPARTMENT OF EARLY LEARNING AND BASIC EDUCATION

Telegram: "SCHOOLING" Garissa
Telephone: 046-210-2458, Garissa.
Fax: 046-210-2002
Email:cdegarissacounty@gmail.com
When replying please quote

COUNTY DIRECTOR OF EDUCATION
GARISSA
P. O. Box 8-70100
GARISSA

REF: NEP/ED/6.26/VOL.III (120)

DATE: 25th March, 2020

VINCENT BAHATI ODALLO
KENYATTA UNIVERSITY
P.O.BOX 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION FOR VINCENT BAHATI ODALLO

Refer to your letter **REF: NO NACOSTI/P/20/4150** dated **23RD MARCH, 2020** from Director General/CDE on application for authority to carry out research on "**A comparative study on Hepatitis B virus among inmates and low risk voluntary blood donors in Garissa County, Kenya**", for the period ending **23RD MARCH, 2021**.

I am pleased to inform you that you have been authorized to undertake your research in Garissa County.

COUNTY DIRECTOR OF EDUCATION C.D.E-GARISSA 23 MAR 2020 Sign. P. O. Box 8-70100, GARISSA

HASSAN GURE KORE
FOR: COUNTY DIRECTOR OF EDUCATION
GARISSA.

Appendix VII: HBV S gene sequences

>Ref S prot

ATGGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTT
TTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCT
CTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACC
TCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGCTGGATGTGTC
TGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTT
CTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACAA
CCAGCACGGGATCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATC
CCTCCTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCAT
CATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCTTGGCT
CAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTT
TCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCC
TTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD004

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCGAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATACTG

GGCTTTCGGAAGATTCCTATGGGAGTGGGTCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAATTATATG

GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTCTTGTCTTTGGGTATACATTTAA

>GARBD006

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCCCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCTGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD007

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCTACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCATAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD008

GACTTCCCCCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAAACTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGGTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCATTTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD005

GACTTTTTTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATTATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAGCCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGTACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD0A8

GACTTCTTTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARBD009

GACTTCTTTCAATTTTTTAGGGGGAACGATCGTGTGTCTTGGTCAAAATTCGCAGTCCCC

AACCTCCAATCATTCACCAACCTCCTGTCCTCCAACTTGTTCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTGTGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATATGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP025

GGTTTCTCTCAATTTTTTAGGGGGGACTCCCCTGAAATTTGGTATAAATTTGCAGTCCCC

AACTTCCAATCCCTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGGGTTCTATCATCTTCCTCTTCATCCTGCCGGTATGCCTCATCTTCTTGTTGGT

TCTTTTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAG

>GARP072

GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTATGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP073

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP074

GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP098

GACTTCTCTCAATTTTCTAGGGGGCTCACCCGTTTGTGTTGGCCAATATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG

TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT

TCTTCTGGATTACCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC

CAGCACGGGACCCTGCAAAACCTGGACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC

ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACTTGTATTCCCATCCCATCATCTTG

GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP103

GACTTCTTTCAATTTTTTAGGGGAATCACCCGTGTTTCTTGGGCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG

TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT

TCTTCTGGATTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC

CAGCACGGGACCCTGCAAAACCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC

ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG

GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTATATG

GATGATGTGGTACTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTGTACATTTAA

>GARP118

GACTTTTTTCAATTTTTTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTAAACATTTAA

>GARP093

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP077

GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAGATTCGCAGTGGCG

GACTTCCAATCACTCACCAACCTTCTGTCCTCCAAGTTGTTCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGATGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTCCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP078

GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGCCAAAATTCGCAGTCCGC

AATCTTCAGTCACTCACCGACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTACAGTTATATG

GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP079

GACTTCTCTCAATTTTCTAGGGGGAGCTACCGTGTGTCTTGGCCGACATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP092

GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCATTATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP123

GACTTTTTTCAATTTATTAGGGGGAACTACCGTGTGTTTGGGCCAAAATTCGCAGGCCCC

AACCTCCAATCACTCAACAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG

TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGG

TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC

CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC

CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG

GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT

ACCAATTTTCTTTTGTCTTTGGGTATACATTTAA

>GARP016

GACTTCTCTCAATTTCCTAGGGGGCCCACCGGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG

TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT

TCTTCTGGATTACCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC

CAGTACGGGACCCTGCAGAACCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC

ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG

GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTATT

ACCAATTTTCTTTTGTCTCTGGGTATACATTTAA

>GARP019

GACTTCTCTCAATTTTCTAGGGGGCTCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCC

AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG

TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT

TCTTCTGGATTACCAAGATATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC

CAGTACGGGACCCTGCAAATCCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC

ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG

GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT

AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG

GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTATT

ACCAATTTTCTTTTGTCTCTGGGTATACATTTAA

Appendix VIII: HBV P gene sequences

>Ref RT
GAGGATTGGGGACCCTGCGCTGAACATGGAGAACATCACATCAGGATTCCTAGGACCCCT
GCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCT
AGACTCGTGGTGGACTTCTCTCAGTTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAA
TTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTA
TCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCAT
CTTCTTGTTGGGTCTTCTGGACTGTCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGG
ATCTTCAACCACCAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTC
TATGTTTCCCTCCTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCAT
CCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTG
GCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCT
TTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTT
TTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACA
AAAAGATGGGGTTATTCTCTACATTTTATGGGTTATGTCATTGGATGTTGGGGATCATTG
CCGCAAGATCATATCATACACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGG
CCCATTGATTGGAAAGTGTGTCAACGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTT
ACACAATGTGGTTATCCTGCGTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCT
TTTACTTTCTCGCCAACTTACAAGGCCTTTCTGTGTAAACAATACCTGAACCTTTACCCC
GTTGCCCGGCAA
>GARBD004
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCGAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATACTG
GGCTTTCGGAAGATTCCTATGGGAGTGGGTCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAATTATATG
GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTCTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAACGATGGGGA
TGTTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTCCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCGTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARBD006
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCCCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCTGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAACGATGGGGA
TATTCTCTACATTTCATGGGTTATGCCATTGGATGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAGCGTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARBD007
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCTACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCATAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGAAGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTCCCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGGGTTTGCTGA
>GARBD008
GACTTCCCCCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAAACTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGGTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCATTTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAGACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTGCAAGGCCTTTCTGTATAAACTATATCTGAACGCTTACCCCGTGGCCAGGCAA
AGGCCCGGGTTGGACCAAAAGTTTGCTGA
>GARBD005
GACTTTTTTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATTATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAGCCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGTACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACGAAACGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARBDA08
GACTTCTTTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGGCCGGTTTGTGCCAAGTGTTTGCTGA
>GARBD009
GACTTCTTTCAATTTTTTAGGGGGAACGATCGTGTGTCTTGGTCAAAATTCGCAGTCCCC
AACCTCCAATCATTCACCAACCTCCTGTCCTCCAACTTGTTCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTGTGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATATGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAACGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGAAGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARP025
GGTTTCTCTCAATTTTTTAGGGGGGACTCCCCTGAAATTTGGTATAAATTTGCAGTCCCC
AACTTCCAATCCCTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGGGTTCTATCATCTTCCTCTTCATCCTGCCGGTATGCCTCATCTTCTTGTTGGT
TCTTTTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAGACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCTGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATTTGACCCTTTTCCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARP072
GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTATGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGGATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGGCCGGTCTGTGCCAAGTTTTGGCTGA
>GARP073
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCAAAGTGTTTGCTGA
>GARP074
GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATCCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTCTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCGATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTGTGTATAAACAATATCTGAACCTTTGCCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAGTTTTTTGCTGA
>GARP098
GACTTCTCTCAATTTTCTAGGGGGCTCACCCGTTTGTGTTGGCCAATATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG
TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT
TCTTCTGGATTACCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC
CAGCACGGGACCCTGCAAAACCTGGACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC
ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACTTGTATTCCCATCCCATCATCTTG
GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAGCAAAACAAAAAGATGGGGT
TATTCCCTAAACTTCATGGGATATGTAATTGGAAGTTGGGGTACACTGCCACAGGATCAT
ATTGTCCAAAAAATCAAACAATGTTTTAGAAAACTTCCTGTCAATCGACCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTTTGGGCTTTGCCGCTCCATTTACACAATGTGGT
TATCCTGCCTTAATGCCTTTGTATGCATGTATACAAGCGAAACAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTAAGTAAACAGTATATGGACCTTTACCCCGTTGCCCGGCAA
CGGCCTGGTCTGTGCCAAGTGTTTGCTGA
>GARP103
GACTTCTTTCAATTTTTTAGGGGAATCACCCGTGTTTCTTGGGCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG
TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT
TCTTCTGGATTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC
CAGCACGGGACCCTGCAAAACCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC
ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG
GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCTATATG
GATGATGTGGTACTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTGTACATTTAAACCCTAACAAAACAAAGAGGTGGGGG
TATTCCCTAAATTTCATGGGATATGTAATTGGAAGTTGGGGTACTTTGCCACAGGATCAT
ATTGTACAAAAAATCAAACAATGTTTTAGAAAACTTCCTGTCAATCGGCCTATTGATTGG
AAAGTATGTCAGAGAATTGTGGGTCTTTTGGGCTTTGCCGCTCCATTTACACAATGTGGT
TACCCTGCATTAATGCCTTTGTATGCATGTATACAAGCAAAACAGGCTTTTACTTTCTCG
CCAACTTTCAAGGCCTTTGTAAGTAAACAGTATATGAACCTTTACCCCGTTGCCCGGCAA
CGGCCTGGTCTGTGCCAGGTGTTTGCTGA
>GARP118
GACTTTTTTCAATTTTTTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTAAACATTTAAACACTAACAAAACAAAACGATGGGGA
TATTCTCTACATATCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTTTATGCATGTATTCAATCCAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACGATATCTGAACCTTTCCCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGGGTTTGCTGA
>GARP093
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARP077
GACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTGGCCAAGATTCGCAGTGGCG
GACTTCCAATCACTCACCAACCTTCTGTCCTCCAAGTTGTTCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGATGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTCCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTGTCG
CCAACTTACAAGGGCTTTCTGTATAAACAATATCTGGACCTTTACCCCGTTGCCCGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARP078
GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGCCAAAATTCGCAGTCCGC
AATCTTCAGTCACTCACCGACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTACAGTTATATG
GATGATGTGGTATTGGGGGCCAAATCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAACGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAGGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTACGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTGTAAACAATATCTGAACCTTTACCCCGTTGCCCGGCAA
CGGCCCGATTTGTGCCAAGTATTTGCTGA
>GARP079
GACTTCTCTCAATTTTCTAGGGGGAGCTACCGTGTGTCTTGGCCGACATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTTCAAGGCCTTTCTGTGTAACCAATATCTGAGGCTTTACCCCGTTGCGGGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARPO92
GACTTCTCTCAATTTTCTAGGGGGAACTACGGTGTGTCTTGGGCATTATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGT
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTGTGTATAAACAATATGTGAACCTTTACCCCGTTGGCGGGCAA
CGGCCCGGTCTGTGCCAAGTGTTTGCTGA
>GARP123
GACTTTTTTCAATTTATTAGGGGGAACTACCGTGTGTTTGGGCCAAAATTCGCAGGCCCC
AACCTCCAATCACTCAACAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTG
TCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGG
TCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCAC
CAGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTATCCCTC
CTGTTGCTGTACAAAACCTTCGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTG
GGCTTTCGGAAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTTTACCGCTGTT
ACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACACTAACAAAACAAAAAGATGGGGA
TATTCTCTACATTTCATGGGTTATGTCATTGGATGTTGGGGATCATTGCCACAAGATCAT
ATCATCCACAAAATCAAAGAATGTTTCAGAAAACTCCCTGTTAACAGGCCTATTGATTGG

AAAGTATGTCAAAGAATTGTGGGTCTTCTGGGTTTTGCTGCCCCTTTCACGCAATGTGGT
TATCCTGCTTTAATGCCCTTATATGCATGTATTCAATCTAAGCAGGCTTTCACTTTCTCG
CCAACTTACAAGGCCTTTCTGTATAAACAATATCAGAACCTTTACCCAGTTGCCCGGCAA
CGGCCCGGTCTGGGCCAAGTGTTTGCTGA
>GARP016
GACTTCTCTCAATTTCCTAGGGGGCCCACCGGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG
TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT
TCTTCTGGATTACCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC
CAGTACGGGACCCTGCAGAACCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC
ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG
GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTATT
ACCAATTTTCTTTTGTCTCTGGGTATACATTTAAACCCTAACAAAACAAAGAGATGGGGT
TATTCCCTGAACTTCATGGGATATGTAATTGGAAGTTGGGGTACATTGCCACAGGATCAT
ATTGTACAAAAAATCAAACAATGTTTTCGAAAACTTCCTGTCAATCGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTTTGGGCTTTGCCGCTCCATTTACACAATGTGGT
TACCCTGCCTTAATGCCTTTGTATGCATGTATACAGGCGAAACAGGCTTTTACTTTCTCG
CCAACTTACAAGGCCTTTCTAAGTAAACAGTATATGAACCTTTACCCCGTTGCCCGGCAA
CGGCCTGGTCTGTGCCAAGTGTTTGCTGA
>GARP019
GACTTCTCTCAATTTTCTAGGGGGCTCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCC
AACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTG
TCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTATTGGT
TCTTCTGGATTACCAAGATATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCACAACAAC
CAGTACGGGACCCTGCAAATCCTGCACGACTCCTGCTCAAGGCAACTCTATGTTTCCCTC
ATGTTGCTGTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCTTG
GGCTTTCGCAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACT
AGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATG
GATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTATT
ACCAATTTTCTTTTGTCTCTGGGTATACATTTAAACCCTAACAAAACAAAGAGATGGGGG
TATTCCCTGAACTTCATGCGATATGTAATTGGAAGTTGGGGTACATTGCCACAGGATCAT
ATTGTACAAAAAATCAAACAATGTTTTCGAAAACTTCCTGTCAATCGGCCTATTGATTGG
AAAGTATGTCAAAGAATTGTGGGTCTTTTGGGCTTTGCCGCTCCATTTACACAATGTGGT
TACCCTGCCTTAATGCCTTTGTATGCATGTATACAGGCGAAACAGGCTTTTACTTTCTCG
CCAACTTACAAGCGCTTTCTAAGTAAACAGTATAGGAACCTGGACCCCGTTGCACGGCAA
CGGCCTGGTCTGTGCCAAGTGTTTGCTGA

Appendix IX: Consent form

COMPARATIVE STUDY OF HEPATITIS B VIRUS AMONG IN-MATES AND LOW RISK VOLUNTARY BLOOD DONORS IN GARISSA COUNTY, KENYA

My name is Vincent Bahati Odallo. I am a postgraduate student undertaking a Masters program at Kenyatta University. I am conducting a study on "Comparative study of Hepatitis B virus among in-mates and low risk voluntary blood donors in Garissa, Kenya". The information obtained from this study will be used by policy makers in understanding the level of Hepatitis B infection in the general population and among prisoners in Garissa County. The information will also help determine whether current anti Hepatitis B virus drugs are effective.

Followed procedures

For you participate in this study, I will be required to take a copy of the Donor questionnaire form that you have filled for blood donation. From the form I will extract your demographic data, which is gender, age and level of education. From the questionnaire I will also obtain your medical history according to the information you will have filled.

After the blood bag is disconnected from you, I will tap for 5mls (Five) from the blood bag for my study. This therefore means that you will only be injected once. For the high risk inmates, I will explain to you the significance of this study and request you to participate voluntarily. I will then draw 4mls of blood from you.

You will voluntarily participate in this study. You are therefore at liberty to ask any questions as it pertains to this study. For blood donors, refusing to participate in this study does not disqualify you from donating blood. You will still be allowed to donate blood, in case you qualify, regardless of participating in this study or not and given same treatment as other donors. For the high risk inmates, refusing to participate in this study, will not cause you any form of harm in terms of harassment or punishment by the prison authorities.

Benefits

Your participation in this study, will help us understand the levels of Hepatitis B infection in the county. You will also benefit by being screened for overt or occult HBV and if you are found to have the infection you will be advised on the appropriate treatment.

Risks

This study will not seek any extra information from you, apart from that which you will provide in the donor questionnaire. You may fill a slight piercing pain during blood collection. This study will not inflict any other pain on you.

Confidentiality

This study will not use your name or any information that can lead to your personal identification anywhere. Your information will be coded and at no point will the personal information be made public.

Withdrawal privilege

If you decide to withdraw from the study, you can do so without penalty or prejudice at any time. Your withdrawal will not however have any consequences on the services you receive from the Kenya Prison service or the Garissa Regional blood transfusion Centre.

Contact Information

Any concerns or clarifications about this study, should be directed to me through +254722128997 or Dr. George Gachara +254722759578 or Kenyatta university Ethical Review Committee secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

Participant Statement

I confirm that the above information regarding this study is clear to me. I am satisfied by the answers I have been given with regards to all the concerns that I raised. I have voluntarily accepted to take part in this study with expectation of no material or monetary benefit during or at the end of the study. I understand that I am free to withdraw from the study anytime with no consequences for withdrawal.

Code ID for the Participant: …….

Signature/Thumbprint: ……. Date: ……

Investigator's statement

I, the undersigned, have explained to the voluntary donor the above statement and answered all his/her question in a language s/he understands bests. I have explained the procedures to be followed in the study, the risks and the benefits involved.

Name of the Investigator: ……. Phone no.: ……

Investigator's Signature: …… Date: ……