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TRANSMISSION OF HEPATITIS B VIRUS AMONG PRISON INMATES IN GHANA

BY

ANGELA NAA AMERLEY AYIKU

(10220886)

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Angela Naa Amerley Ayiku towards the award of the Master of Philosophy in the Department of Microbiology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana.

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Candidate: **Angela Naa Amerley Ayiku** Date

…… ……

Principal Supervisor: **Dr. Kwamena W. C. Sagoe** Date

…… ……

Co-Supervisor: **Dr. Augustine Ankomah** Date

ABSTRACT

Background: Data on the transmission of hepatitis B virus (HBV) within correctional facilities is limited in Ghana, even though the prevalence suggests intra-prison transmission.

Aim: This study determined the level of transmissibility, new infections, and intra-prison transmission of HBV among inmates in Ghana

Methodology: Immunoglobulin M antibodies to hepatitis B core antigen (anti-HBc IgM) and Hepatitis B e antigen (HBeAg) were determined for 323 archived plasma samples obtained from a nationwide prison survey. Risk factors for high HBV transmissibility and recent infections were determined using Pearson's chi square test, t test and a binary logistic regression analysis. The Surface (S) gene was sequenced for 17 plasma samples, 9 of which were from one prison and 8 with recent infections from other prisons. Phylogenetic analysis and sequence identity matrix were used to establish possible transmission within a selected prison.

Results: Out of the 323 HBsAg positive samples screened, 91(28.2%) were HBeAg positive and 16 (5.0%) were anti-HBc IgM positive. HBeAg was found to be strongly associated with the type of prison ($p< 0.001$) and the length of stay within the prison ($p= 0.033$). Fever, genital pain and discharge were also found to be significantly associated with recent infections ($p=0.040$) and transmissibility ($p= 0.005$, $p=0.010$), respectively. Majority (62.5%) of the anti-HBc IgM positive inmates had been

incarcerated for more than one year. Although there was no specific intra-prison clustering of sequences, an identity matrix revealed a narrower similarity range for intra-prison sequences than for inter-prison sequences. All sequences clustered with HBV genotype E with the exception of two which clustered with subgenotypeA3/genotype E recombinant.

Conclusion: Recent infections suggest that there is on-going transmission within the prisons. The high HBeAg prevalence may be attributed to lack of adequate nutrition and poor healthcare due to long duration of stay.

DEDICATION

This Thesis is dedicated to Mr. David Amar Ayiku (deceased), Mrs. Dinah Ayiku and my entire family.

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LIST OF ABBREVIATIONS

ALT/AST- Alanine aminotransferase/ Aspartate transaminase

Anti-HBc IgM- Immunoglobulin M antibodies to hepatitis B core antigen

Anti-HBc IgG- Immunoglobulin G antibodies to hepatitis B core antigen

Anti-HBe- Hepatitis B e antibodies

Anti-HBs- Antibodies to Hepatitis B surface Antigen

bp- Base pair(s)

BBV- blood borne virus

cccDNA- Covalently closed circular DNA

CDP- contagious disease prison

DNA- Deoxyribonucleic acid

dNTP- Deoxyribonucleotide triphosphate

ELISA- Enzyme Linked immunosorbent Assay

Enh- Enhancer

GPS- Ghana Prison Service

HBcAg- Hepatitis B core antigen

HBeAg- Hepatitis B e antigen

HBsAg- Hepatitis B surface antigen

HBV- Hepatitis B virus

HBx- Hepatitis B x antigen

HIV- Human immunodeficiency virus

IDU- Injecting Drug Users

IFN- Interferon

kb- Kilo base(s)

mg- milligrams

min- Minute(s)

ml- milliliter

mRNA- Messenger ribonucleic acid

MSM- Men who have sex with Men

MEGA- Molecular Evolutionary Genetics Analysis

NR- Non-Reactive

nt- Nucleotide(s)

-ve - Negative

OD- Optical Density

ORF- Open reading frame

PCR- Polymerase chain reaction

PCP- Phencyclidine

POTS- Prison Officer's Training School

pgRNA- Pregenomic RNA

+ve - Positive

R- Reactive

RNA Ribonucleic acid

RNase- Ribonuclease

rpm- Revolutions per minute

RT-PCR- reverse trancriptase polymerase chain reaction

sec- Second(s)

S- Surface

Surface proteins

L- Large

M- Medium

S-Small

STD- Sexually Transmitted Disease

µl- microliter

WP- Within Prison

CHAPTER ONE

3.1 INTRODUCTION

Hepatitis B is a blood borne virus (BBV) which causes acute and chronic necroinflammatory liver diseases and spreads basically through blood and other body fluids (Gyawali, Rice, & Tilzey, 1998; Liang, 2010). There may be no signs or symptoms of the infection but early signs and symptoms may include generally feeling unwell, loss of appetite, weight loss, vomiting, tiredness, dark urine, yellowing of skin (jaundice) and right upper abdominal pain (Connor, Jake Jacobs, & Meyerhoff, 2006). As the disease advances some symptoms such as fluid retention, bruising and prolonged bleeding may be experienced (Parole Officers, 2013; Jack 2011).

Chronic hepatitis B virus (HBV) infection is the leading cause of cirrhosis and hepatocellular carcinoma and the highest prevalence areas for HBV infection (48%) are in African, Western Pacific and Asian countries (Lavanchy, 2004; D'Souza & Foster, 2004). In Accra, Ghana, the risk of developing cirrhosis was found to increase 8-fold in patients with HBV infections than those without (Blankson, Wiredu, Gyasi, Adjei, & Tettey, 2005). Therefore, HBV infection was labeled a major risk factor for developing liver cirrhosis in Accra (Blankson *et al.*, 2005). Globally two billion people are currently infected with HBV; more than 370 million people have developed chronic HBV infection, causing over one million HBV-related deaths each year due to the complications of HBV-related chronic liver diseases (Ishikawa, 2012; Lavanchy, 2004; Nguyen & Dore, 2008).

Diagnosis of HBV infection involves the use of serological assays such as ELISA (Enzyme Linked immunosorbent assay) for the detection of HBV antigens (HBsAg and HBeAg) and antibodies in a patient's blood (D'Souza & Foster, 2004). There are some timely serological markers which upon screening can help determine susceptibility to infection, immunity as a result of resolved infection or vaccination, acute infection, or chronic infection.

Infection with HBV can be prevented by vaccination and also 95% of adults newly infected with hepatitis B naturally clear the virus and become immune for life (Ishikawa, 2012). There is long-term antiviral treatment for chronic hepatitis B to prevent further liver damage; recombinant subcutaneous interferon-ɣ, oral lamivudine and also oral adefovir (Lavanchy, 2004; D'Souza & Foster, 2004; Desombere, 1998, unpublished). Undetectable HBV DNA (5105 copies/mL) in serum, continued loss of HBeAg either with or without detection of anti-HBe (HBeAg seroconversion), improvement in liver disease, normalization of aminotransferases and decrease in necroinflammation are considered signs of response to treatment (D'Souza & Foster, 2004). Although there is treatment available for this infection it does not cure (Parole Officers, 2013). Therefore the main purpose of treatment of chronic hepatitis B is to suppress HBV replication and retard the progression of liver disease before the development of cirrhosis and hepatocellular carcinoma (D'Souza & Foster, 2004).

HBV prevalence has stabilized and gradually reducing globally due to the general scaling up of programmes to prevent infection and provision of care and treatment to infected individuals (Ishikawa, 2012). Unfortunately the disease burden and number

of new infections remains high implying that there may still be HBV prevention interventions which must be addressed (Kilmarx, 2009). Penal institutions all over the world are considered environments for fast and uncontrolled spreading of BBVs due to specific risk practices, poor health care and living conditions (Hellard, Crofts, & Hocking, 2002).

3.2 PROBLEM STATEMENT

Studies on transmission of BBVs such as HBV within correctional facilities are limited worldwide (Adjei *et al.*, 2006; Votano, Parham, Hall, Kier, & Hall, 2004). Yet there is compounding evidence of on-going HBV transmission among prison inmates (Adjei *et al.*, 2006; Haber *et al.*, 1999; Hutchinson *et al.*, 1998; Mutter *et al.*, 1994; Stark *et al.*, 1997; Taylor *et al.*, 2002). In Ghana, the prevalence of HBV within prisons (13-17%) seems to be higher than the national prevalence (9-12%) reported by various studies from different populations within the country which may be as a result of inmates engaging in high-risk behaviours leading to increased transmission within prisons (Adjei *et al.*, 2008; Amidu *et al.*, 2012; Walana *et al.*, 2014). Nonetheless, intra-prison spread has been suggested but not confirmed (Adjei *et al.*, 2006). Scanty information is also available on the transmissibility and recent infections in prisons.

3.3 JUSTIFICATION

In Ghana the monthly population of prison inmates was estimated at 13,908 by the Ghana Prisons Service Annual Report in the year 2013 (GPS, 2013). Three hundred and thirty (330) of this inmate population are foreigners from Togo, Burkina Faso, Nigeria, Niger, Benin, Cameroon, Mali, Liberia, Ivory Coast, Kenya, Morocco, India, Gabon, Guinea Bissau, Thailand and China (GPS, 2013). Continuation of risk behaviours such as unsafe and unprotected sex and injecting drug use, shortly after release from prison is common and expected making the fight against HBV difficult (Dolan & Larney, 2010).

As prevalence of HBV in the prisons remains higher than that in the general population, it poses a threat to the prisoners, their families and even the community at large (Jack, 2011). The prevalence alone may not be enough to completely explain transmission within the prisons. Although incarcerated individuals are considered as isolated populations, most of them eventually return to the general population. For effective prevention of transmission of BBVs in correctional facilities, there is a need to have a clear understanding of transmission patterns within in order to identify new infections and those who have high transmissibility.

3.4 AIM

The aim of this study was to determine the transmissibility and recent HBV infections in prisons in Ghana.

3.5 SPECIFIC OBJECTIVES

- To determine HBeAg and anti-HBc IgM prevalence in HBsAg carriers in prisons and associated risk factors.
- To determine phylogenetic relatedness of HBV strains within a selected prison

CHAPTER TWO

2.1 LITERATURE REVIEW

2.1.1 THE PRISON ENVIRONMENT

Correctional institutions serve as a measure of a society's humanity and are important components of the safety and stability of its environment (UN, 2012). The Prison environment comprises of the interactions between physical, psychological, and social factors therefore measuring the properties of the prison environment requires the psychological and structural approach (Saylor, 1984). One way of attaining such information as suggested by many researchers may be through the perceptions of the various factions of the organization such as prison officers, inmates, health care personnel and all other prison staff (Herbert, 2001; Awolutugu, 2013). Prison environments vary from facility to facility and most of these facilities are designed from a security and functional angle rather than care and reform although studies over the years to date have shown that a hospitable physical environment is an important factor that can promote health and well-being of inmates (WHO, 2007).

2.1.2 GHANAIAN PRISON ENVIRONMENT

The Prisons Service in Ghana comprises 45 establishments; Prisons Headquarters, Prison Officers' Training School (POTS), Senior Correctional Centre, (formerly Ghana Borstal Institute), Seven (7) Central Prisons, Thirteen (13) Local Prisons, Seven (7) Female Prisons, Three (3) Open Camp Prisons, Nine (9) Agricultural

Settlement Camp Prisons, One (1) Medium Security Prison, One (1) Maximum Security Prison and One (1) Contagious Disease Prison (CDP)(GPS, 2013) (figure 2.1). The prisons are classified based on level of security and activities undertaken at the various establishments (GPS, 2013). The Central prisons equip prisoners with employable skills for their effective reintegration into society. Therefore they take custody of prisoners with long-sentences. It admits all categories of prisoners except condemned prisoners. The Local Prisons usually take custody of short-sentenced prisoners due to the lack of space for trade training activities. The Open Camp Prisons undertake agricultural activities and train inmates in agricultural practices. Prisoners nearing the end of their sentence are often moved to these facilities as transit to prepare them for their final discharge into society. The level of security is quite relaxed; there is usually no fencing (GPS, 2013).

Ghana's current prison system based on the account of the Ghana Prisons Service (GPS) was modeled after the informal method of imprisonment used during the colonial era by the British and formalized by the year 1841. By 1920 the focus of the system had changed from retributive to reformative (GPS, 2011). Ever since this formalization, the prison service has diligently embraced modern concepts of imprisonment aimed at reform and reintegration of inmates into society (GPS, 2011). As a matter of fact, section 1 of the Prisons Service Decree mandates the prison service to maintain "the safe custody and welfare of prisoners and, whenever practicable, to undertake the reformation and rehabilitation of prisoners" (Prisons Service Decree, 1972, NRCD 46). However, it seems the service has failed in this regard. Prisons in Ghana today are overcrowded and ridden with very poor sanitary

conditions and healthcare. As at 2005, the Nsawam Medium Security Prison, which was designed for a maximum of 600 prisoners, had an inmate population over 2000 with an average of 65 occupants in the dormitory-type rooms which have a maximum capacity of 20 individuals (Adjei *et al*., 2006). Also the James Fort and James Camp prisons, which were each estimated to accommodate a maximum of 300 prisoners, had over 900 and this number is only increasing with time (Adjei *et al*., 2006). The annual Ghana Prison service report recorded an increase in prisoner population at the rate of 3.12 % during the reporting year (GPS, 2013). The average daily prisoner population in the year 2010 was 13,507 which increased to13,396 in the year 2011. This value only increased to 13,482 in 2012 and then to 13,908 the following year (GPS, 2013). Prisoners interviewed in the Kumasi central prison in Ghana attributed the causes of morbidity in that prison to poor diet, overcrowding, lack of logistic support and poor sanitary conditions in the prison (Herbert, 2001). Indeed health care providers in the same prison agreed that most of the diseases that incapacitate prisoners and caused deaths were preventable and also that access to the infirmary was unrestricted but the standard of care was poor due to shortage of essential medical logistics (Herbert, 2001). Therefore the extent to which these prison facilities have been modified and how much modernization has influenced the wellbeing of the inmates and the population at large is not quite clear.

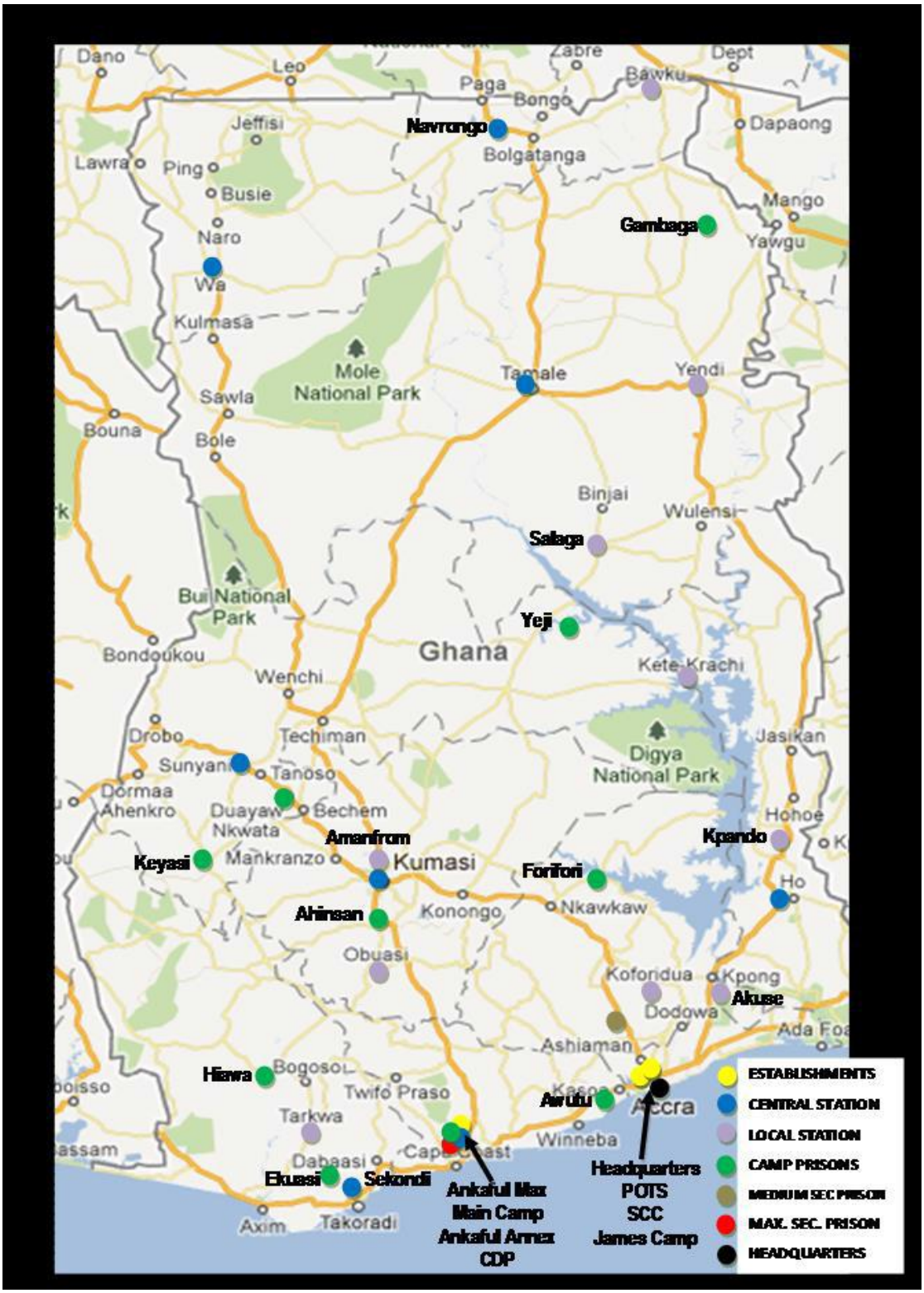


FIGURE 2.1: A map showing the location of the various prison establishments (Source: GPS, 2013).

2.1.3 PREVALENCE OF HBV IN CORRECTIONAL FACILITIES

Many studies have shown that the prevalence of BBVs such as HBV in the general population is lower than that among prisoners (Adjei *et al*., 2006). Among these high risk groups Injecting drug users (IDU) and Men who have sex with Men (MSM) are said to be more common among incarcerated populations resulting in HBV being more prevalent in prison populations than in the general population (Dolan & Larney, 2010; Weinbaum *et al*, 2008; Viswanathan *et al*, 2010). As such prisoners represent a high-risk group for blood borne diseases and STDs (Adjei *et al*., 2006). For several years, the high transmission rates of HBV within correctional facilities have been well known globally and alleged in Ghanaian prisons (Dolan & Larney, 2010; Mutter *et al*., 1994; Adjei *et al*., 2006). However there is limited information about the virus in correctional facilities across the country and in different types of such facilities (Adjei *et al*., 2008; Adjei *et al.*, 2006). There have been many suggestions as to what could account for the higher prevalence in the correctional facilities in many studies (Dolan and Larney, 2010; Mutter *et al*., 1994; Adjei *et al*., 2006; Butler *et al.*, 2005; Parole Officers, 2013). Some other factors that have been said to increase the risk of HBV infection and also account for this higher prevalence in these facilities is previous imprisonment, tattooing, body piercing and some high-risk sexual behaviours in addition to poor conditions, overcrowding, poor nutrition and inadequate healthcare (Adjei *et al*., 2006; Butler *et al.*, 2005; Parole Officers, 2013). There has been unconfirmed reports over the years in some parts of the world of a great deal of unsafe sexual activity such as sodomy, homosexual and lesbian activities, paid sex and sexual favours to prison officers, drug injection and needle sharing within Ghanaian

prisons and most prisons all over the world (Adjei *et al*., 2006; Butler *et al*., 2005; Hellard *et al*., 2002; Mahfoud *et al*., 2010). It is therefore apparent that prison environment is a place where practices which increase the risk of acquiring BBV continue to take place (Hellard *et al*., 2002).

In a related study conducted in Ghana, a greater proportion of the inmates admitted they were using drugs like marijuana, cocaine, heroin and phencyclidine (PCP) and 35/ 281 inmates had themselves tattooed using shared needles and ink. As many as 30.8% (73/237) of the male inmates admitted to engaging in homosexuality, with 11 of them reporting initiation of this behaviour and 22.7% (10/44) of the female inmates admitted to engaging in lesbian acts, with 5 of them reporting initiation of this behavior while in prison. There were no reports of sexual contact between officers with female or with male inmates (Adjei *et al*., 2006). This study was not a nationwide study but shed some light on what may be going on in most prisons in the country.

Owing to the reports above, it is apparent that there is compounding evidence that HBV infections have either been transmitted to individuals while they were in prison or inmates had been infected before they were incarcerated. Unfortunately prevalence can only go as far as providing the number of HBV infected cases in the prisons but not how and when they were infected.

2.1.4 THE HEPATITS B VIRUS

The virus is a hepadnavirus from a family of enveloped DNA viruses, 42 nm in diameter and its genome is composed of a circular partially double stranded DNA (Scaglioni *et al*., 1996, Mohammed & Eldaif, 2014). Structurally it consists of a nucleocapsid core (HBcAg), and surrounded by an outer lipoprotein coat (envelope) which contains the surface antigen (HBsAg) (Desombere, 1998, unpublished). The hepatitis B e Antigen (HBeAg) is an immunologically distinct soluble protein manufactured by the viral particle, which is secreted into circulation by binding to smooth endoplasmic reticulum within cells. Since HBeAg is secreted into the serum of infected individuals, it then becomes an easily measured marker for active HBV replication in chronic infections (Gumaste, 1995).

The initial stage of its life cycle involves the binding of the HBV virion to a receptor at the surface of the liver cells known as hepatocyte which forms 70% of the liver (Seeger & Mason, 2000). The viral nucleocapsid enters the cell by a receptor mediated endocytosis which is followed by uncoating of the envelope (Scaglioni *et al*, 1996). The nucleocapsid then delivers the viral genome into the nucleus (Scaglioni *et al*, 1996). Here the second-strand DNA is fully synthesized by an endogenous polymerase to complete the double strand and this serves as a template for transcribing four viral RNAs (Desombere, 1998, unpublished). These RNAs are polyadenylated, conveyed to the cytoplasm and then translated into the envelope (S-small, M- medium, L- large), the nucleocapsid and pre core antigen (C, pre-C),

polymerase (P), and transcriptional transactivating proteins (X) and all form the various structural components of the virion (Beck & Nassal, 2007).

Hepatitis B viral DNA has four partially overlapping open reading frames (ORFs: (*S*, *C*, *P*, and *X*)). The *S* ORF codes for the viral surface envelope proteins (HBsAg), the *C* ORF encodes the HBcAg or HBeAg, the *P* ORF encodes the polymerase (pol) which is functionally divided into three domains (the terminal protein domain, the reverse transcriptase (RT) domain and H domain) and the HBV *X* ORF encodes the HBxAg (Liang, 2010). The *S* ORF can be structurally and functionally divided into the pre-S1, pre-S2, and S regions (figure 2.2).

All three envelope proteins mentioned earlier contain HBsAg, which is heterogeneous antigenically resulting in 4 major subtypes (adw, ayw, adr and ayr) and their distribution varies geographically (Mohammed & Eldaif, 2014; Desombere, 1998, unpublished). The S protein is quantitatively the most essential component in the empty viral particles as well as in the complete virion because its hydrophilic region may be involved in attachment and development of the core particle during maturation of the virion (Gous, 2006, Unpublished). The S gene is coded for by a highly conserved region and from many related studies the S gene is the subgenomic region that has been found to be commonly used for HBV genotyping (Lin *et al*., 2005; Martin *et al.*, 2012; Uy *et al*., 1992; Desombere, 1998, unpublished). The organization of the HBV genome has been shown in figure 2.2.

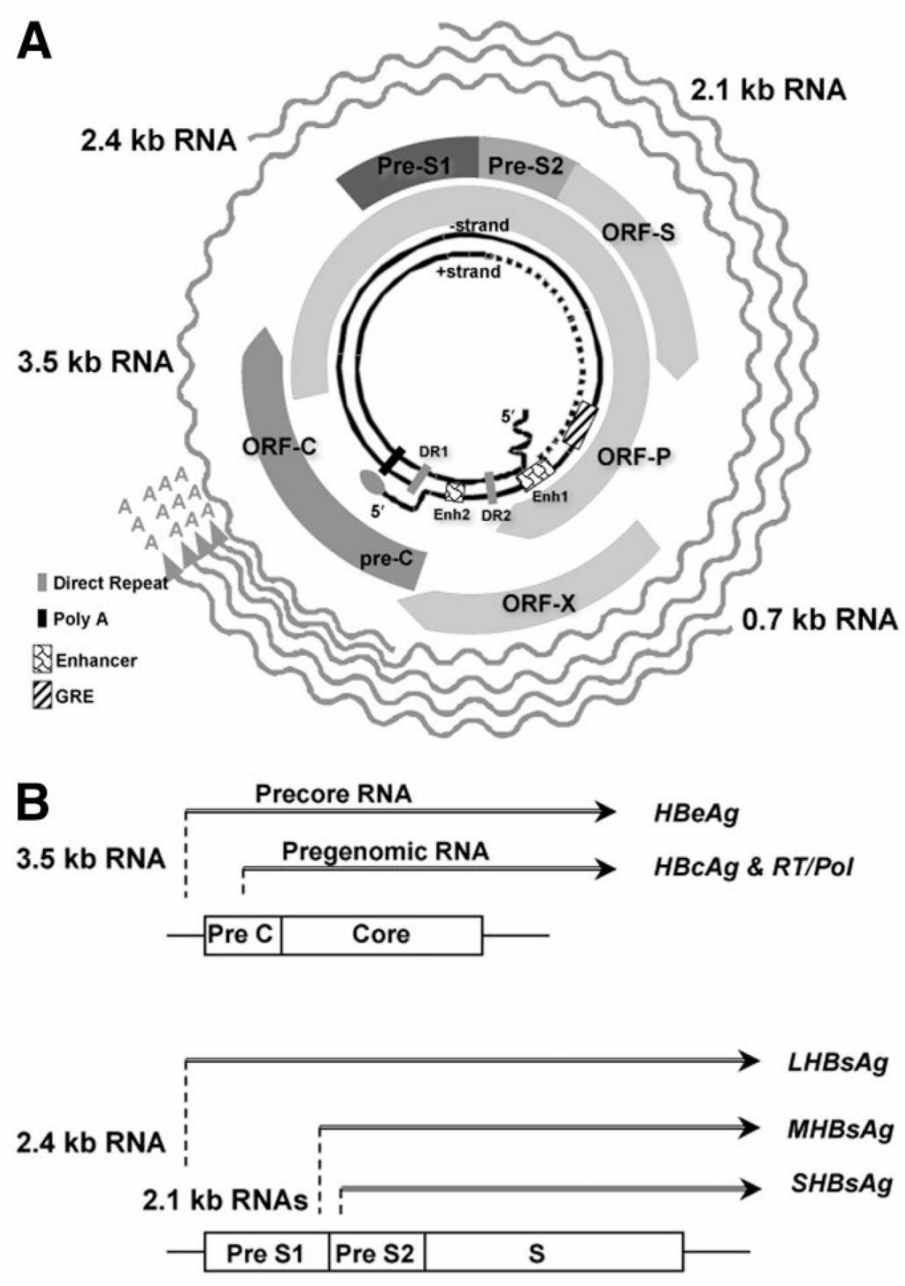


FIGURE 2.2: The figure above includes; A-The genomic organization of Hepatitis B virus, RNA transcripts and gene products with several main regulatory elements. B-The transcription start sites of various HBV transcripts and the proteins they encode.

(Source: Liang, 2010)

2.1.5 SEROLOGICAL MARKERS

Hepatitis B virus diagnosis is established by a specific serologic profile and this involves measurement of a number of HBV-specific antigens and host antibodies that react to these antigens (Mahoney, 1999). The interpretation of the results is often complex, and many possible outcomes based on the individual responses may exist. The outcomes of the serologic tests can determine whether a patient is prone to infection, has developed immunity due to recovery from a previous infection or vaccination, acutely infected, or chronically infected (Mast *et al*, 2006).

HBV incubation period may last for an average of 12 weeks (Mizukoshi *et al.*, 2015). Screening for hepatitis B virus (HBV) infection involves measurement of distinct HBV-specific antigens and host antibodies that react to these antigens and these appear in a typical pattern (Weinbaum *et al*, 2008). The first serologic marker to be detected is hepatitis B surface antigen (HBsAg). It can be detected in serum as early as 1 to 12 weeks after infection and HBeAg becomes evident shortly after (Liang, 2010). The presence of HBV DNA can also be detected by a serum assay for HBV DNA prior to the detection of HBsAg or HBeAg but it is generally not performed when it comes to diagnosing acute infection (Liang, 2010; Krogsgaard *et al*, 1985). The presence of HBeAg is an indication of infectivity or transmissibility unlike HBsAg which neither represents viral replication or infectivity (www.cdc.gov/hepatitis). Nonetheless very limited data are available on the seroprevalence of HBeAg among Ghanaians.

Antibodies to hepatitis B core antigen (anti-HBc IgM) appear just about the time that clinical symptoms develop (Prevention, 2005; Tedder & Wilson-Croome, 1981; Towell & Cowie, 2012). Anti-HBc IgM in the serum decline to undetectable levels within 6 months, but antibodies to hepatitis B core antigen IgG class (anti-HBc IgG) persists indefinitely as a marker of previous HBV infection (Jellinek, 1954). For patients with resolved HBV infection, the HBsAg disappears at about 3 to 6 months prior to the detection of antibodies to hepatitis B surface antigen (anti-HBs) (Gitlin, 1997). The presence of anti-HBs after an acute infection basically indicates recovery and protective immunity against re-infection (Center for Disease Control and Prevention (CDC), 2014). Also HBeAg disappears, antibodies to hepatitis B e antigen (anti-HBe) develops and these patients have persistence of anti-HBc (predominantly consisting of IgG) for life (Lee, 1997). Therefore presence of HBsAg beyond 6 months after infection and also the detection of HBsAg along with the absence of IgM anti-HBc in a single serum specimen is also indicative of chronic HBV infection (Towell & Cowie, 2012). Therefore chronic or persistent HBV infection with HBsAg and anti-HBc IgG generally persist for life.

While the detection of anti-HBc IgM indicates recent, acute infection of less than 6 month's duration, non-reactive anti-HBc IgM does not rule out chronic infection (Curry & Choprah, 2010). The use of anti-HBc IgM to determine transmission may not be completely efficient in that prisoners who were infected within the facility but have been infected for more than 6 months will go undetected and also recently infected inmates who have been in imprisoned not more than 6 months who got

infected just before incarceration will give a false picture. Therefore transmission within the prison facilities will also depend on the duration of incarceration.

2.1.6 PHYLOGENETIC RELATIONSHIPS

Phylogenetic analysis is the means used to estimate evolutionary relationships and it involves the use of macromolecular sequences to reconstruct the evolutionary relationships between organisms (Brinkman & Leipe, 1998). Although phylogenetic analysis is frequently used for studies on population-based HIV transmission and can provide critical information about HIV epidemics that are otherwise difficult to obtain through traditional study design (transmission of drug-resistant virus, mixing between demographic groups, and rapidity of viral spread within populations), it may also help determine the source of new viral infections in general (Dennis, Burns, Eshleman, Cohen, & Foundation, 2012; Grabowski & Redd, 2014). A direct phylogenetic analysis consists of four steps; Alignment (both building the data model and extracting a phylogenetic dataset), determining the substitution model, tree building and finally tree evaluation (Brinkman and Leipe, 2001). Although full-length HBV sequence analysis is the gold standard for phylogenetic analysis, other specific genomic regions have been found to reliably infer phylogenetic relationships (Datta, Banerjee, Chandra, & Chakravarty, 2007). Phylogenetic analysis of the virus is complicated due to its compact genomic structure and as such the genetic variability of HBV among the study population, their serological profile, and the mode of probable transmission should all be factors to consider before even selecting a genetic

region for investigation of transmission (Datta *et al*., 2007). There are currently eight major HBV genotypes which have been shown to have distinct geographical distribution (Simon, Kundi, & Puchhammer, 2013). HBV genotype E and A are predominantly found in Africa but the genotype E is exclusively found in West Africa (Huy *et al*., 2006; Kidd-Ljunggren, Miyakawa, & Kidd, 2002; Mulders *et al*., 2004; Simon *et al*., 2013). The HBV genotype E has been reported severally to have low genomic diversity over a wide geographical area despite the high endemicity of the genotype in West Africa (Kramvis & Kew, 2007; Mulders *et al*., 2004).

The S gene has been successively used to establish transmission. The transmission of HBV was confirmed by molecular evolutionary analysis in family members in north eastern Egypt using the preS2 and S regions of HBV genome. The HBV isolates were of sub-genotype D in nine index cases and 14 family members (Ragheb *et al*., 2012). Also using the S gene, identical genotyping results between parents and carrier children indicated three patterns of transmission. Pattern I clustering was caused by maternal transmission, whereas pattern II clustering was caused by paternal transmission. In pattern III clustering, a concordant HBV genotype between carrier children and mother or father was found in 3 and 2 families, respectively. The modes of transmission were confirmed by phylogenetic analysis in 1 family of each pattern (Lin *et al*., 2005).

Phylogenetic analysis may have some limitations due to the fact that not the whole genome is being compared but rather sites and the fact that the virus in question is subject to high mutation rates in terms of insertions, deletion or substitutions

(Brinkman & Leipe, 1998). Nonetheless methods of analyzing sequences have been tested against several parameters over the years and have been adjusted to reduce the error margin as much as possible in order to make true inferences from sequences.

CHAPTER THREE

3.1 METHODOLOGY

This study was derived from a national survey performed in prisons all over Ghana in the year 2013 (February- March). The specific objectives of this study were accomplished by the use of the methods below.

3.5.1 STUDY DESIGN

As part of a nationwide study on prevalence of HBV and HIV in prison facilities in Ghana, all samples taken were thoroughly characterized using two screening assays to detect HBsAg seropositive inmates from various correctional facilities in Ghana. Data such as risk behaviours, duration of incarceration and nature of the prison environment were obtained through a standardized questionnaire. These therefore formed the cross-sectional study basis for this investigation.

3.5.2 STUDY POPULATION

During the national survey, a stratified systematic sampling procedure was used to select male prison inmates. The selection was first stratified by type of prison and thereafter twelve (12) out of 42 eligible prison stations were selected randomly. The sampling was done in a way that all the five categories of prisons under the Ghana Prison Service, namely, maximum security, medium security, central, local and Agricultural camps were adequately represented. Female prison inmates are a

population of approximately 260 scattered across seven prison locations. A census was conducted of all the female inmates who consented to be part of the study.

The inmates were randomly selected from a complete list of inmates covering the entire population in each selected station obtained from the local prison authorities. In the few instances where selected individuals refused to come out for interviews, they were replaced by the inmate next to the inmate who declined invitation.

The study population comprised of 323 HBV infected individuals seen during the nationwide study (Table 3.1) which included all types of prisons; the maximum security, minimum security, central, local and camp prisons. These inmates were part of the 2238 inmates who were involved in the study from a total of 12 male and 7 female prisons sampled. Based on results and existing demographic data obtained through the standardized questionnaire (Appendix I), samples were selected for genetic material extraction and amplification. The S region of HBV genome obtained from the PCR products was commercially sequenced and analyzed.

3.5.3 SAMPLE SIZE

All the 323 HBV infected inmates (Table 3.1) were tested for anti-HBc IgM and HBeAg. Since anti-HBc IgM and HBeAg suggest the level of recent infections and transmissibility respectively, a central prison with relatively high prevalence of both markers was selected for genetic analysis to confirm intra-prison transmission. A cross-section of 42 samples was selected for the second phase of the study.

The second phase of the study involved amplification and sequencing of selected samples. Eleven (11) out of 16 HBV DNA from the samples that were found to be anti HBc IgM positive were successfully amplified and submitted for sequencing. In addition, amplified HBV DNA samples from a selected prison were also sequenced

Selection of this prison was done based on a combination of factors. The list of prisons was narrowed down to those with high anti HBc IgM prevalence. Then the list was narrowed down further to a single prison based on high HBeAg prevalence, duration of stay (to rule out infection before incarceration) and the type of prison (open prisons were excluded to reduce the possibility of transmission from sources outside the prison environment). A central prison was chosen based on the nature of prison environment and level of security. Sixteen (16) out of the 26 samples from the selected prison were amplified and submitted for sequencing. A total of 27 samples were submitted for sequencing.

TABLE 3.1: Prevalence of HBsAg within each prison which formed the sampling population for this study

PRISON	TOTAL POPULATION	PROPOSED SAMPLE	ACTUAL SAMPLE	HBsAg +ve	HBsAg +ve (%)
Maximum Security Prison					
1	264	45	49	9	18.4
Medium Security Prison					
2	3,570	611	652	91	14.0
Central Prisons					
3	1,940	456	437	56	12.8
4	852	200	199	26	13.1
5	274	65	67	7	10.4
6	200	47	65	16	24.6
7	370	87	126	20	15.9
Local Prisons					
8	185	172	117	13	11.1
9	161	150	80	13	16.3
10	264	264	208	29	13.9
Camps Prisons					
11	200	85	104	10	9.6
12	330	140	141	20	14.2
TOTAL MALES	**8,610**	**2322**	**2245**	**310**	**13.8**
Female Prisons					
13	133		98	3	3.1
14	15		11	0	0.0
15	26		23	3	13.0
16	38		26	2	7.7
17	20		17	2	11.8
18	18		16	1	6.3
19	9		7	2	28.6
TOTAL FEMALES	**259**		**205**	**13**	**6.3**

The numbers in the prison column of the table above represents the individual prisons.

3.5.4 SAMPLE COLLECTION

Archived plasma samples in a study bank were used. These inmates signed consent and specimen banking forms during enrolment (Appendix II).

3.5.5 LABORATORY ANALYSIS

All HBV positive samples were tested for HBeAg using the EASE BN-96 (TMB) ELISA and anti-HBc IgM using the ANTICORASE MB-96 (TMB) ELISA from General Biologicals, Taiwan.

3.5.5.1 Detection of HBeAg

Prior to determining the presence of HBeAg, all reagents for the EASE BN-96 (TMB) ELISA were brought to room temperature. Each vial of reagent was then swirled gently before use. One hundred microliters (100µl) of controls and specimen were transferred into the appropriate wells leaving one blank. The plate was then sealed with an adhesive slip and incubated at 37°C ± 1 for 1 hour. The adhesive slip was removed after incubation and the plate washed six times using 1x buffer concentration using a PW40 plate washer, (BIO-RAD, Marnes-la-Coquette, France). Next, one hundred Microlitres of anti-HBe peroxidase solution was added to each reaction well except the blank. The plate was tapped gently, sealed and incubated at 37°C ± 1 for 1 hour followed by a cycle of washing.

Equal volumes of TMB solutions A and B was mixed in a clean V-shaped trough, and then 100µl of the mixture was added to each well including the blank. Incubation of

the plate was carried out in the dark for 30 minutes at room temperature. The reaction was stopped after the incubation by adding 100µl of 2N H_2SO_4 to each well including the blank. The plate was read at 450nm wavelength on the MULTISKAN microplate reader (Labsystems, Finland). Samples with absorbance values greater than the calculated Cut-off [(NCx +0.06)*1.1] was considered reactive. Specimen with absorbance value within the Retest Range [(NCx +0.06) ± 10%] was repeated in duplicate and interpreted as above.

NB. NCx is the mean absorbance for the negative control.

3.5.5.2 Detection of anti-HBc IgM

All patients confirmed as having HBsAg were also tested for Hepatitis B core IgM antibodies (anti-HBc IgM). All reagents for determining anti-HBc IgM were brought to room temperature before tests were performed. Five microlitres (5µl) of each patient plasma sample was diluted with 500µl of specimen diluent. Hundred microlitres (100µl) of controls and specimen diluent were added to appropriate wells except one blank. Five microlitres (5µl) of previously diluted specimen was added to appropriate wells containing 100µl specimen diluent. The plate was sealed with an adhesive slip, gently tapped on the sides, and incubated at 37°C ± 1 for 1 hour. Washing of the plate was subsequently done with the PW40 microplate washer as described earlier. Fifty microlitres of HBcAg solution and 50µl of anti-HBc peroxidase solution were then pipetted into each well except one blank. The plate was incubated at 37°C ± 1 for 1 hour followed by washing.

Equal volumes of TMB solutions A and B were mixed and 100 µl of the mixture added to each well including the two blanks. The plate was then incubated in the dark at room temperature for 30 minutes. The reaction was stopped after incubation by adding 100µl of 2N H_2SO_4 to each well including the two blanks. The plate was read at 450nm wavelength on the MULTISKAN microplate reader (Labsystems, Finland). The cut-off was calculated with the formula; Cut-off = NCx + (0.25 X PCx), where NCx and PCx are the mean absorbance for the negative and positive controls respectively. Samples with absorbance/cut-off ratio ≥ 1.1 were considered reactive. Samples with absorbance/cut-off ratio within Retest Range (0.9-1.1) were repeated.

3.5.5.3 Hepatitis B virus DNA extraction

Archived plasma samples were used in the extraction of HBV DNA.

Roche High pure viral nucleic acid kit was used and the extraction procedure was performed according to the manufacturer's instructions. All anti-HBc IgM positive samples and all HBsAg positives from the selected central prison were subjected to HBV DNA extraction. In all a total of 40 samples were used in this section. Two hundred microliters (200µl) of binding buffer supplemented with poly(A) was pipetted into the tube along with 200µl serum and 50µl proteinase K and incubated at 72°C for 10 minutes in a heating block. The samples were transferred into the high pure filter tubes combined with collection tubes and were together centrifuged for 1 minute at 8000xg. The collection tubes were discarded and replaced with fresh ones and 500µl of inhibitor removal buffer was added and the solution was centrifuged for 1minute at 8000xg. The collection tubes were discarded and replaced with fresh ones

and 450µl of wash buffer was added and the solution was centrifuged for 1minute at 8000xg. The collection tubes were discarded and replaced with fresh ones and another 450µl of wash buffer was added and the solution was centrifuged for 1minute at 8000xg. The solution was centrifuged again for 10 seconds at 13000xg. The collection tubes were discarded and replaced with fresh ones and 50µl of elution buffer was added. The solution was centrifuged for 1minute at 8000xg. The supernatant containing the purified viral nucleic acids in the flow through tube or collection tube was transferred into final storage tubes and then stored at -20°C until required for further experiment.

3.5.5.4 PCR amplification

In order to examine the intra-prison transmission, the S gene (541bp) of HBV for all new infections seen in the study (n=11) and from a selected prison (n=16) were amplified. An established and published protocol with slight modifications using the primers in table 3.2 was used (Gos 2006). Briefly, DNA amplification was performed using the QIA amp DNA blood midi kit (QAIGEN, USA) according to manufacturer's instructions. Ten microliters of HBV DNA served as the template. The Master Mix contained the following in their various volumes per reaction; dNTPs (0.5µl each), reaction buffer containing $MgCl_2$ (5.0µl), AmpliTaq DNA polymerase (0.125µl), nuclease free H_2O (6.875µl), HBV-F and HBV-R primers (0.5µl each). Fifteen microliters of the master mix was aliquot into PCR tubes along with the 10µl template. A previously amplified HBV-positive patient serum sample served as a positive control, while HBV DNA-negative serum samples and a reaction without

template served as negative control. PCR was done using the specific primers for HBV S gene sequences. The PCR conditions were as follows; there was an initial denaturation step of 30 sec at 95˚C, followed by 40 cycles of denaturation at 95˚C for 45 sec, annealing at 53˚C for 60 sec and primer extension at 72˚C for 90 sec, followed by a final primer extension at 72˚C for 7 min. DNA amplification was performed using the QIAamp DNA blood midi kit (QAIGEN, USA) according to manufacturer's instructions. Ten microliters of HBV DNA served as the template. The Master Mix contained the following in their various volumes per reaction; dNTPs (0.5µl each), reaction buffer containing $MgCl_2$ (5.0µl), AmpliTaq DNA polymerase (0.125µl), nuclease free H_2O (6.875µl), HBV-F and HBV-R primers (0.5µl each). Fifteen microliters of the Master Mix was aliquot into PCR tubes along with the 10µl template. A previously amplified HBV-positive patient serum sample served as a positive control, while HBV DNA-negative serum samples and a reaction without template served as negative control. PCR products were detected in 2% ethidium bromide-stained agarose gel (Appendix III).

TABLE 3.2: Primer sequences used for HBV genotyping

Name	Nucleotide Position	Primer Sequence 5'-3'	Size of Amplicons (bp)	Reference
HBV-F (sense)	256 – 278	GTGGTGGACTTCTCTCAATTTTC	541	Gous, 2006
HBV- R (anti-sense)	796 – 776	CGGTATAAAGGGACTCACGAT		

3.5.5.5 Nucleotide Sequencing and Phylogenetic analysis

A total of 27 samples were successfully amplified, 11 anti-HBc IgM positive and 16 HBsAg positive only. PCR products were sequenced commercially by INQABA BIOTEC, South Africa. The sequences were aligned with reference sequences from HBV genotypes A–H using the CLUSTAL W implemented in Molecular Evolutionary Genetics Analysis version 6 (MEGA6) software. The evolutionary history was inferred using the Neighbor-Joining method (Saitou N. & Nei M., 1987). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein J., 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Felsenstein J., 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura K., Stecher G., Peterson D., Filipski A., & Kumar S., 2013) and are in the units of the number of base substitutions per site. The analysis involved 53 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 464 positions in the final dataset.

Evolutionary analyses were conducted in MEGA6 (Tamura K., Stecher G., Peterson D., Filipski A., & Kumar S., 2013). However HBV genotypes were confirmed also by Basic Local Alignment Search Tool (BLASTN program, blast.ncbi.nlm.nih.gov)

3.5.5.6 Mutational analysis

The surface reading frame was analyzed for mutations using the internet tool Geno2pheno [HBV] (http://hbv.bioinf.mpi-inf.mpg.de/)(Sayan & Dogan, 2012; Simon *et al.*, 2013).

3.5.6 STATISTICAL ANALYSIS

Data was entered into a Microsoft Excel (2010) spread sheet, coded and transferred into an SPSS v.16.0 for statistical analysis. The t test as well as the chi-square test with odds ratios was used to estimate the risk of being HBeAg or anti-HBC IgM positive among other variables including demographics, details of incarceration, history of incarceration and clinical manifestations (Table 4.2). The categorical variables were compared using chi-squared test and the continuous variables, were compared using the t-test. A $P<0.05$ was considered statistically significant.

The questionnaire included questions about the health of inmates where they were asked to select from a list, the illnesses. The illnesses were compared to the HBeAg and anti HBc IgM results to determine if there was any significant difference between the variables. The Pearson's chi-square test was used and each illness was compared individually with the two variables of interest (HBeAg and anti HBc IgM).

A binary logistic regression was also conducted to identify the binary dependent variables using HBeAg as the dependent variable.

3.5.7 ETHICAL ISSUES

Study participants had agreed to participate in specimen banking during the parent study and signed a document pertaining to that to allow further studies be done with the specimen (Appendix II). Approval was obtained from University of Ghana Medical School Ethical and Protocol Review Committee.

CHAPTER FOUR

4.1 RESULTS

Overall, all samples were successfully tested for HBeAg and anti-HBc IgM (n=323). The prevalence of HBeAg and anti-HBc IgM were 28.1% and 5.2% respectively. Seventeen (17) samples were successfully sequenced.

4.1.1 SEROLOGICAL MARKERS

4.1.1.1 Prevalence of HBeAg and anti-HBc IgM

Out of the 310 HBsAg positive samples from the male prisons, 87(28.1%) and 16(5.2%) were positive for HBeAg and anti HBc IgM respectively. For the female prisoners enrolled, it appeared that none of them were anti HBc IgM positive but 4 (30.8%) were HBeAg positive. The Local prison (9) recorded the highest prevalence of HBeAg positives for the male prisons. Although the sample size for prison 17 (female prison) was relatively low it recorded two HBsAg positive samples of which both were also HBeAg positive representing 100% as compared to prison 13 (female prison) which had the highest sample size for that category yet only 3 samples were found to be HBsAg positive of which none was HBeAg positive (Table 4.1).

TABLE 4.1: Prevalence of HBeAg and anti HBc IgM positives within each prison

PRISON	SAMPLE POPULATION	HBeAg Positive	HBeAg Positive (%)	Anti-HBc IgM Positive	Anti-HBc IgM Positive (%)
Maximum Security Prison					
1	9	2.0	22.2	0.0	0.0
Medium Security Prison					
2	91	10.0	11.0	4.0	4.4
Central Prisons					
3	56	21.0	37.5	1.0	1.8
4	26	12.0	46.2	2.0	7.7
5	7	2.0	28.6	0.0	0.0
6	16	4.0	25.0	2.0	12.5
7	20	8.0	40.0	1.0	5.0
Local Prisons					
8	13	3.0	23.1	1.0	7.7
9	13	7.0	53.8	1.0	7.7
10	29	9.0	31.0	2.0	6.9
Camps Prisons					
11	10	3.0	30.0	0.0	0.0
12	20	6.0	30.0	2.0	10.0
TOTAL MALES	**310**	**87.0**	**28.1**	**16.0**	**5.2**
Female Prisons					
13	3	0.0	0.0	0.0	0.0
14	0	0.0	0.0	0.0	0.0
15	3	1.0	33.3	0.0	0.0
16	2	1.0	50.0	0.0	0.0
17	2	2.0	100.0	0.0	0.0
18	1	0.0	0.0	0.0	0.0
19	2	0.0	0.0	0.0	0.0
TOTAL FEMALES	**13**	**4.0**	**30.8**	**0.0**	**0.0**

The numbers in the prison column of the table above represents the individual prisons.

4.1.1.2 Binary Logistic Regression Analysis

Factors such as sentence years spent (p= 0.033), genital discharge (p= 010) and persistence of anti HBc IgM (p= 0.004) were found to be significantly associated with HBeAg positive inmates as compared to all other factors listed in the table 4.2 using the binary logistic regression analysis.

TABLE 4.2: Results of a binary logistic regression analysis between HBeAg and other variables

COVARIATES	Df	Sig.
Educational Level	3	.813
Educational Level (1)	1	.665
Educational Level (2)	1	.524
Educational Level (3)	1	.397
Marital Status Recoded	2	.754
Marital Status Recoded(1)	1	.870
Marital Status Recoded(2)	1	.497
Sentence Years	1	.189
Sentence Years Spent	**1**	**.033**
Previous Incarceration(1)	1	.811
Fever(1)	1	.999
Jaundice(1)	1	.999
Body/ Joint pain(1)	1	.999
Skin Disease(1)	1	.999
High Blood Pressure(1)	1	.999
Night Sweat(1)	1	.999
Appetite Loss(1)	1	.999
Weight Loss(1)	1	.999
Number of HBV Symptoms	1	.999
STD WP(1)	1	.504
Genital Pain WP(1)	1	.229
Urination Pain WP(1)	1	.324
Discharge WP(1)	**1**	**.010**
Blades Access(1)	1	.999
Shared Blades(1)	1	.172
Pierced/ Tattoo(1)	1	.538
Blood Covenant WP(1)	1	.371
Self-Drug Use WP(1)	1	.671
Anti HBc IgM	**1**	**.004**

4.1.1.3 Correlates for HBeAg and anti-HBc IgM

The statistical significance between demographic data and HBeAg or anti-HBc IgM was determined using the Pearson's chi-square test and t-test where appropriate. The P-values are included in Table 4.3. This test of significance was to find out the factors that were independently associated with the serological markers and risk factors.

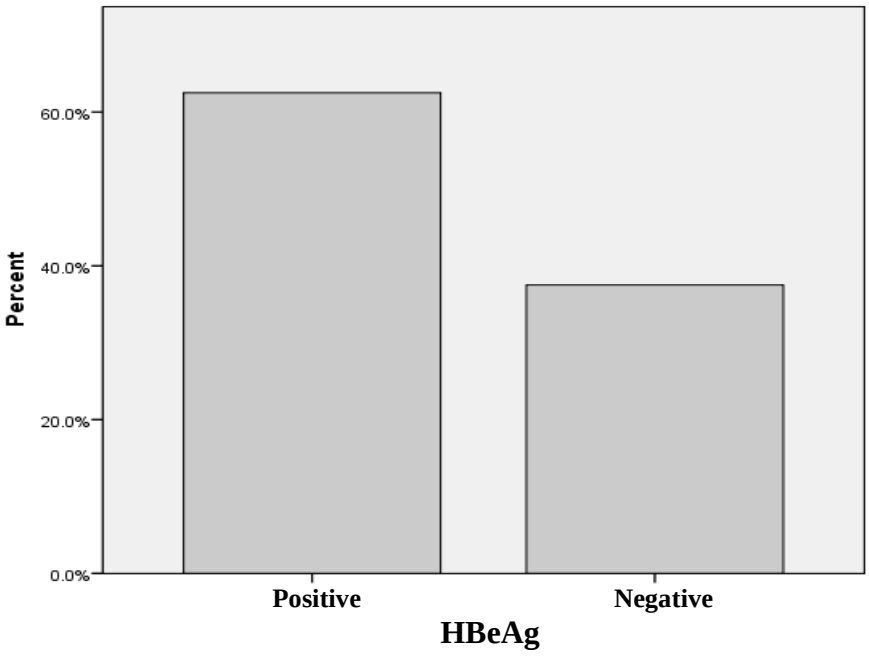
TABLE 4.3: The test of significance of the relationship between demographic data and anti HBc IgM or HBeAg

Variables	**P-Value**
IgM*HBeAg	0.002
HBeAg*Type of Prison	<0.001
IgM*Previous incarceration	0.013
Type of prison*Shared Blades	0.037
Type of prison*Piercing and Tattooing	0.001
HBeAg*Years in Prison	<0.001
HBeAg*Age	<0.001
IgM*Years in Prison	0.001
IgM*Age	<0.001

The percentage of inmates that were anti HBc IgM positive as well as HBeAg positive (62.5%=10 inmates) is represented in figure 4.1. Figure 4.2 shows the percentage distribution of the inmates who tested positive for HBeAg per prison type. From the graph the Central prisons recorded the highest percentage which was significantly different from the lowest percentage recorded according to the P-value calculated. Figure 4.3 gives the breakdown of the specific prisons and the relative HBeAg positive percentage distribution. Based on the graph, Central prisons (3 and 4) recorded the highest percentages. Inmates who had been previously incarcerated had a lower anti HBc IgM prevalence as compared to those that had never been imprisoned (figure 4.4). The prevalence of the two serological markers tested was also compared per age ranges (figure 4.5). The highest prevalence for both markers was between the ages of 18 to 45, with inmates older than 45 recording extremely low prevalence.

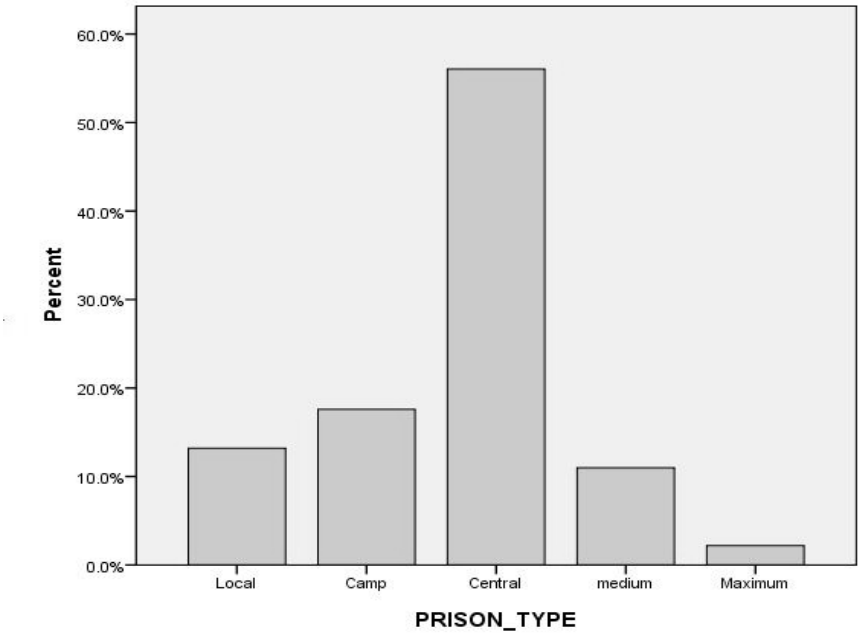
Inmates reported on illnesses they had suffered during their incarceration, both clinically confirmed and not. Figure 4.6 summarizes the percentage of HBV positive inmates who complained of particular illnesses. Among these illnesses, night sweat, skin diseases, weight loss and appetite loss were the most commonly stated. The estimated risk and level of association of these symptoms with HBeAg and anti HBc IgM are shown below in table 4.4.

With respect to table 4.4, inmates who tested positive for anti HBc IgM were 3 times more likely to experience fever. In totality, fever was the most significant and most common illness complained of by HBsAg positive inmates who tested positive for anti HBc IgM. Also there is a 2 to 3 time's likelihood of an inmate who is HBeAg and anti HBc IgM positive to have genital pains.



P-Value=0.002

FIGURE 4.1: Bar graph showing percentage of anti HBc IgM positives (n=16) that were either HBeAg positive or negative.



[*P*-Value < 0.001]

FIGURE 4.2: Bar graph showing HBeAg prevalence (n=91) for each prison type.

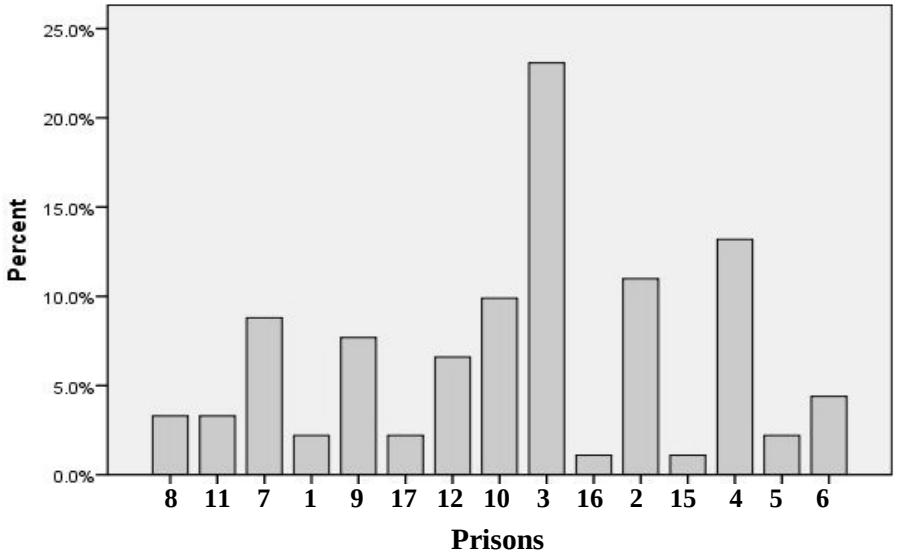


FIGURE 4.3: Bar graph showing HBeAg prevalence (n=91) for the individual prisons (from 1 to 19).

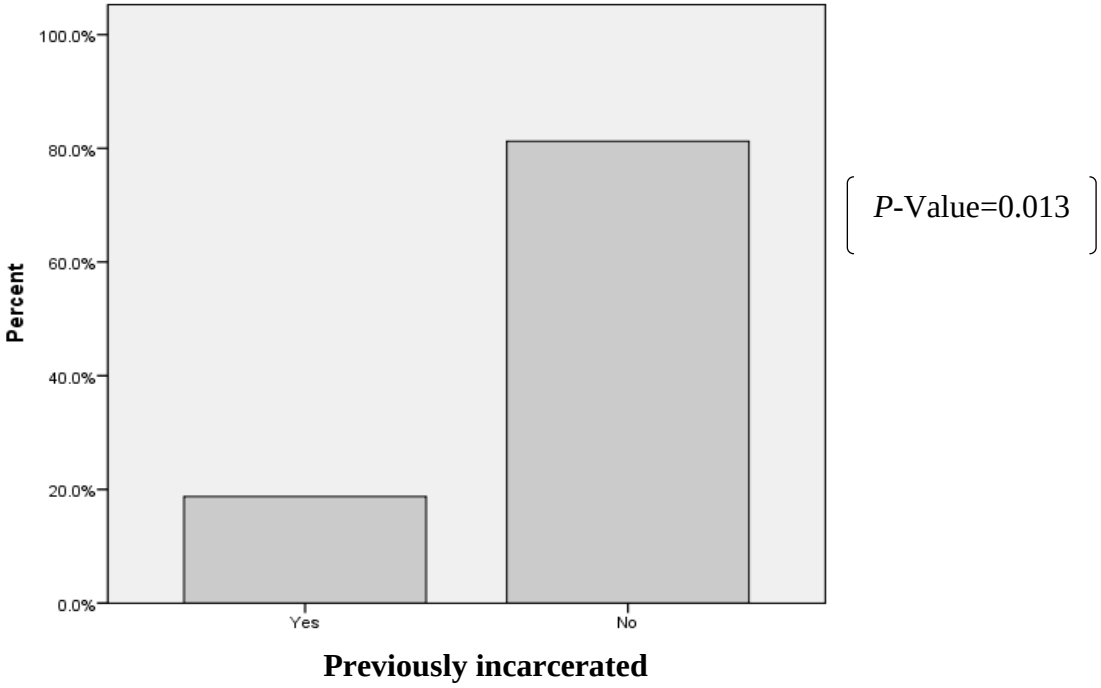


FIGURE 4.4: Bar graph showing anti HBc-IgM prevalence (n=16) of inmates who were previously incarcerated and those that were not.

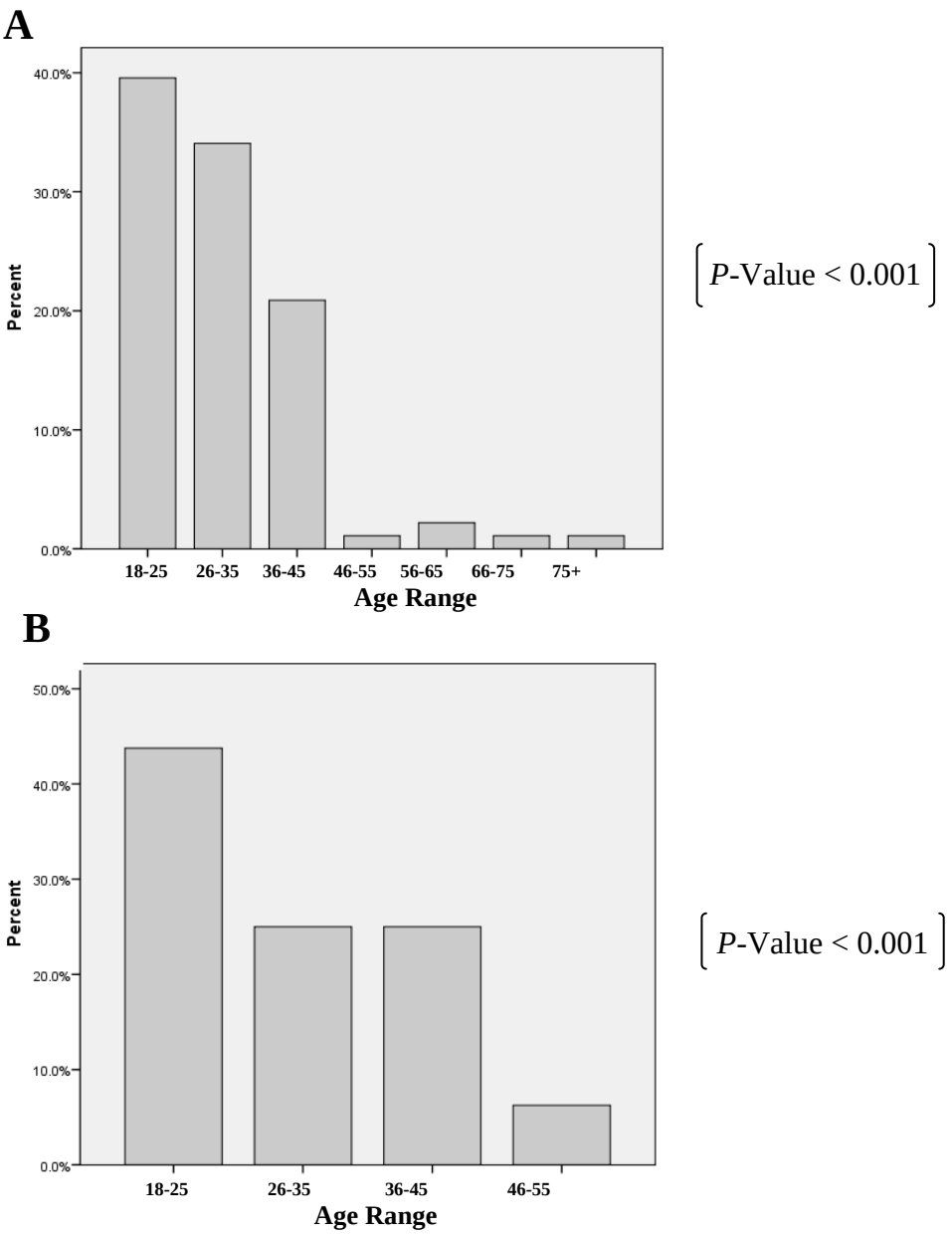


FIGURE 4.5: Graph A and B shows the prevalence of HBeAg (A, n=91) and anti HBc-IgM (B, n=16) according to age ranges.

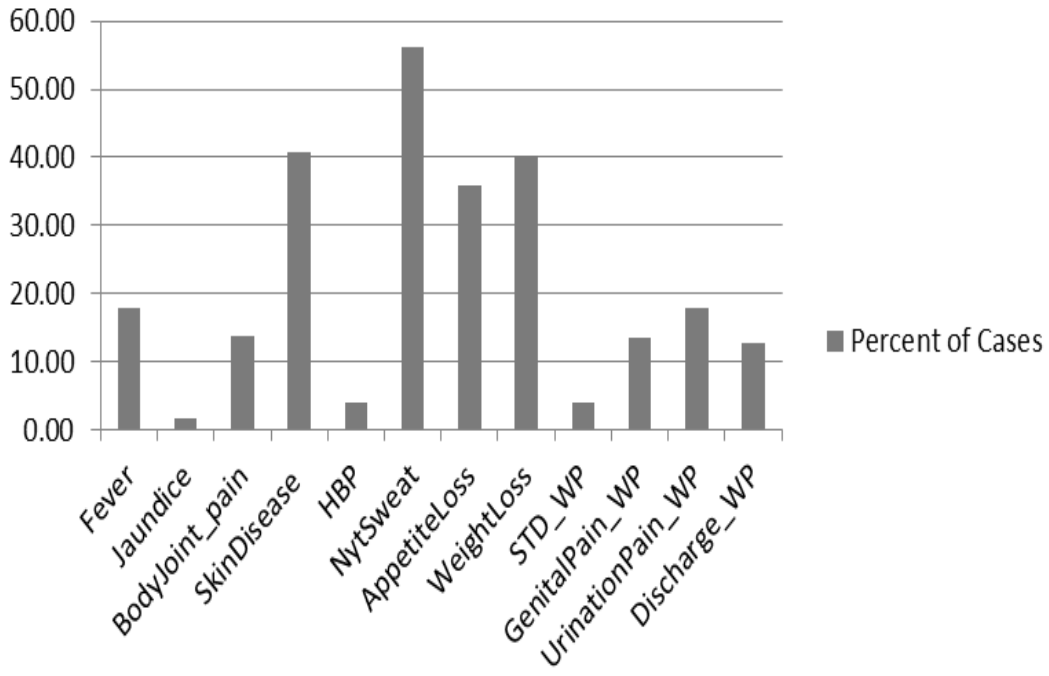


FIGURE 4.6: Bar graph showing frequencies of illnesses suffered by inmates in their current prisons (n= 323).

TABLE 4.4: Test of significance between anti HBc IgM and HBeAg and illnesses suffered

	Anti HBc IgM			**HBeAg**		
ILLNESSES	**NO. (%)**	**OR (95% CI)**	**P- value**	**NO. (%)**	**OR (95% CI)**	**P- value**
Fever	**5 (31.3)**	**3.0 (1.0-9.2)**	**0.040**	14 (15.4)	1.179 (0.6-2.3)	0.721
Jaundice	0 (0)	None	0.646	2 (2.2)	2.584 (0.4-18.7)	0.329
Body/Joint Pain	1 (6.2)	0.535 (0.1-4.2)	0.545	11 (12.1)	1.192 (0.6-2.6)	0.692
Skin Disease	8 (50)	2.232 (0.8-6.1)	0.166	30 (33)	1.071 (0.6-1.8)	0.792
High Blood Pressure	0 (0)	None	0.463	4 (4.4)	1.732 (0.5-6.3)	0.398
Night Sweat	9 (60)	1.940 (0.7-5.6)	0.288	43 (47.8)	1.211 (0.7-2.0)	0.455
Appetite Loss	6 (37.5)	1.546 (0.5-4.4)	0.410	21 (23.6)	0.710 (0.4-1.2)	0.270
Weight Loss	7 (43.8)	1.687 (0.6-4.7)	0.410	28 (31.1)	0.934 (0.6-1.6)	0.894
Genital Pain	**4 (25)**	**3.022 (0.9-10.0)**	**0.057**	**17 (18.9)**	**2.890 (1.4-6.0)**	**0.005**
Urination Pain	3 (18.8)	1.429 (0.4-5.0)	0.588	15 (16.7)	1.320 (0.7-2.6)	0.475
Discharge	3 (18.8)	2.156 (0.6-8.0)	0.241	13 (14.4)	1.839 (0.9-3.9)	0.146

4.1.2 NUCLEOTIDE SEQUENCE ANALYSIS

Phylogenetic relatedness using sequence identity matrix and mutation analysis was determined for sequences from one prison and also for sequences from various prisons. Sequences from the selected prison were labeled with a code starting with PSS. Other sequences from various prisons had different codes starting with NSNM, PSJ, PSA, PSE, PSK and PSW.

The DNA products after PCR were separated by Gel electrophoresis per their sizes on a 2% ethidium bromide stained agarose gel (Appendix III). Sizes of PCR products were assessed relative to the migration patterns of a 100 bp DNA ladder. The products were visualized under ultraviolet light and the images captured as shown in figure 4.7. Table 4.5 is an identity matrix implemented in the Bioedit software which shows how identical the nucleotide sequences are to each other based on the colour code and value. A value of 1 indicates that samples are identical. The level of identity reduces with reduction in the value, i.e. less than 1. The demarcated area represents a comparison between inmates within one prison. The more the colour code varies the higher the divergence. Figure 4.8 represents Neighbor-joining phylogenetic tree using the 17 sequences.

Tables 4.6 and 4.7 also show the mutations in the RT domain and the SHB protein of the surface gene respectively which were determined by using the online mutational analysis tool Geno2pheno (http://hbv.geno2pheno.org/index.php). Escape mutations were found in PSS 0498 (122K, 131N)

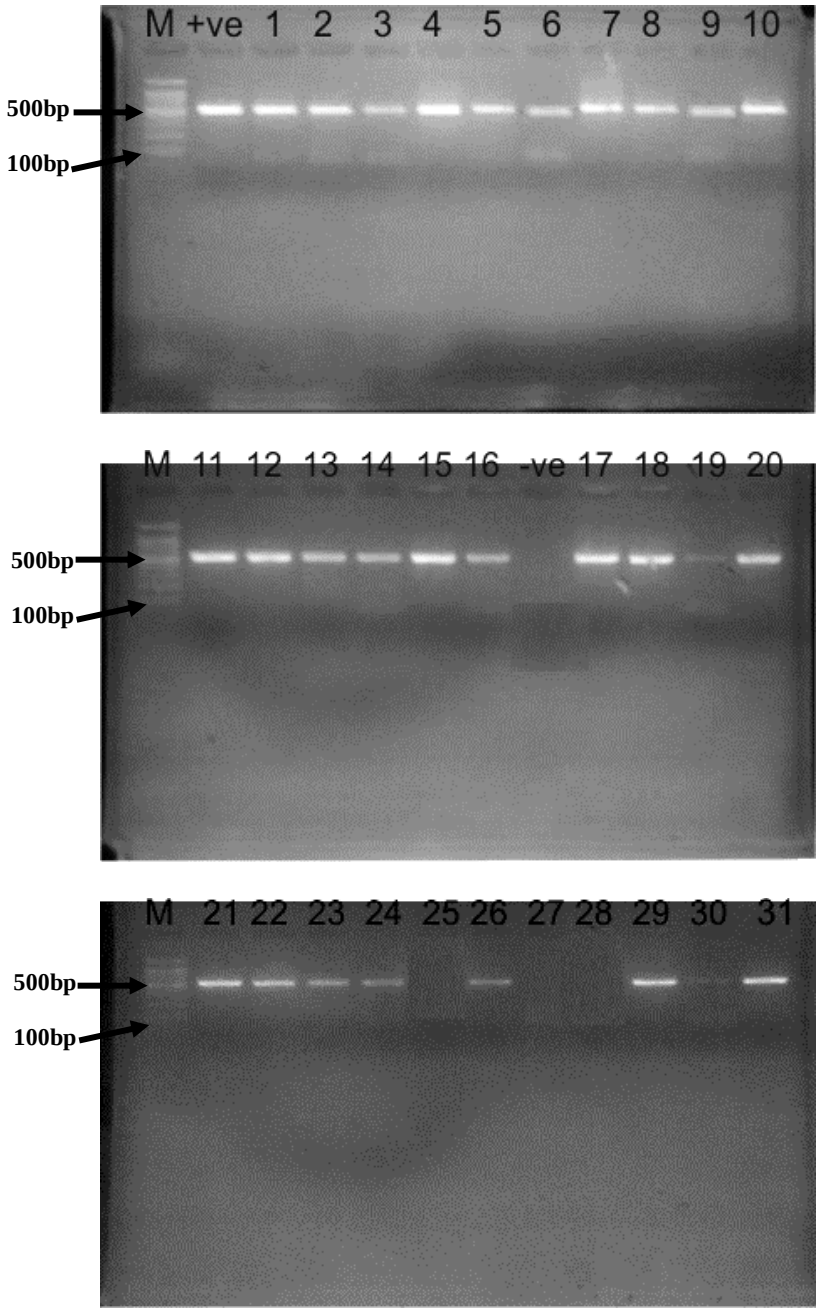


FIGURE 4.7: Identification of the S gene as seen by a 500bp band on 2% ethidium bromide stained agarose gel (M= ladder 100bp)

TABLE 4.5: Sequence Identity matrix (samples with the value 1 are completely identical and values below 1 are less identical)

Seq->	NSNM 2098	PSA 2627	PSE 2794	PSJ 1778	PSJ 1740	PSK 1091	PSW 0309	PSS 0526	PSS 0551	PSS 0432	PSS 0401	PSS 0524	PSS 0361	PSS 0494	PSS 0498	PSS 0475	PSS 0484
NSNM 2098	ID																
PSA 2627	0.948	ID															
PSE 2794	0.997	0.95	ID														
PSJ 1778	0.948	0.991	0.95	ID													
PSJ 1740	1	0.948	0.997	0.948	ID												
PSK 1091	0.997	0.948	0.995	0.946	0.997	ID											
PSW 0309	0.997	0.946	0.995	0.946	0.997	0.995	ID										
PSS 0526	1	0.948	0.997	0.948	1	0.997	0.997	ID									
PSS 0551	0.997	0.946	0.995	0.946	0.997	0.995	0.995	0.997	ID								
PSS 0432	1	0.948	0.997	0.948	1	0.997	0.997	1	0.997	ID							
PSS 0401	1	0.948	0.997	0.948	1	0.997	0.997	1	0.997	1	ID						
PSS 0524	0.997	0.948	0.995	0.946	0.997	0.995	0.995	0.997	0.995	0.997	0.997	ID					
PSS 0361	0.995	0.952	0.993	0.952	0.995	0.993	0.993	0.995	0.993	0.995	0.995	0.993	ID				
PSS 0494	0.995	0.946	0.993	0.946	0.995	0.993	0.993	0.995	0.993	0.995	0.995	0.993	0.991	ID			
PSS 0498	0.967	0.944	0.965	0.944	0.967	0.965	0.97	0.967	0.965	0.967	0.967	0.965	0.967	0.963	ID		
PSS 0475	0.982	0.933	0.98	0.931	0.982	0.98	0.98	0.982	0.98	0.982	0.982	0.985	0.978	0.978	0.95	ID	
PSS 0484	1	0.948	0.997	0.948	1	0.997	0.997	1	0.997	1	1	0.997	0.995	0.995	0.967	0.982	ID

Colour Code:	Value
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	0.99-1
	0.98-0.99
	0.97-0.98
	0.96-0.97
	≤ 0.95

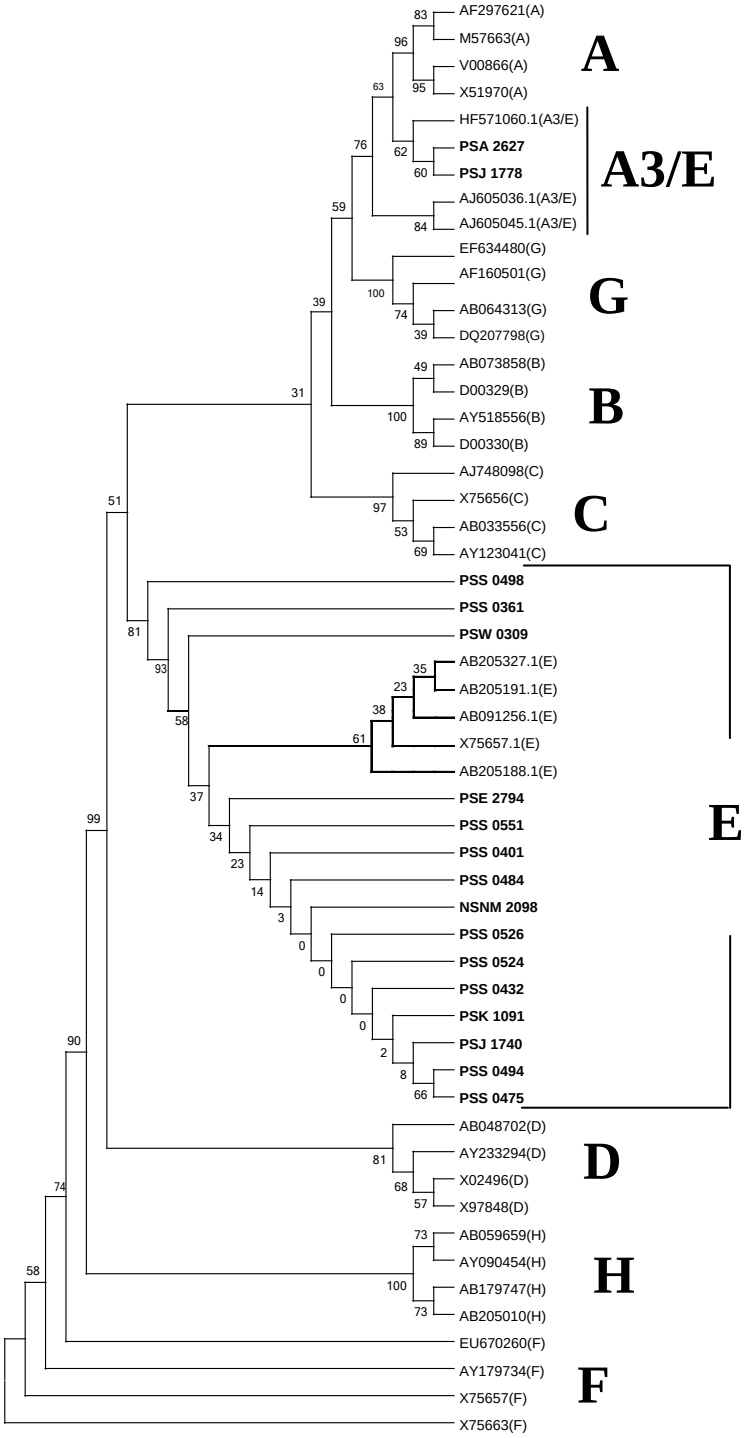

FIGURE 4.8: Neighbor-joining phylogenetic tree of the S gene sequences from the 17 Ghanaian prison samples (in bold font) and 36 genotype A–H strains from the GenBank database.

TABLE 4.6: Mutations in the RT (Reverse Transcriptase) domain generated by an online bioinformatics tool (Geno2pheno [HBV])

SEQUENCES:	NSNM 2098	PSA 2627	PSE 2794	PSJ 1778	PSJ 1740	PSK 1091	PSW 0309	PSS 0526	PSS 0551	PSS 0432	PSS 0401	PSS 0524	PSS 0361	PSS 0494	PSS 0498	PSS 0475	PSS 0484
Mutations in *RT* domain																	
L217R	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
N122H		X		X													
M129L		X		X													
W153R		X		X													
V163I		X		X													
L164M		X		X													
M145L																	
Y126H															X		
P130Q													X		X		
N188D																	
I122Y																	
T128A																	
L129M															X		
N131D															X		
N139Q															X		
F148Y															X		
F151Y															X		
K154Q															X		
Q67R																X	
S68*																X	
T70Y																X	
W79*																X	
V84G																X	
H90R																X	

TABLE 4.7: Mutations in the SHB protein of the surface gene generated by an online bioinformatics tool (Geno2pheno[HBV])

SEQUENCES:	NSNM 2098	PSA 2627	PSE 2794	PSJ 1778	PSJ 1740	PSK 1091	PSW 0309	PSS 0526	PSS 0551	PSS 0432	PSS 0401	PSS 0524	PSS 0361	PSS 0494	PSS 0498	PSS 0475	PSS 0484
Mutations in the SHB protein of the Surface gene																	
L209V	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
K122R		X		X													
T189I						X											
T189IT		X															
A194V		X		X													
P203R												X				X	
P203PR		X															
S207N		X		X													
T57I									X								
S114T															X		
R122K															X		
T131N															X		
S140T															X		
S143T															X		
H60N																X	
N59D																X	
P62T																X	
G71D																X	
C76G																X	
I82V																X	

CHAPTER FIVE

5.1 DISCUSSION

5.1.1 TRANSMISSIBILITY AND RECENT INFECTIONS

The detection of HBeAg in the serum coupled with hepatitis B virus infection may have several implications. First of all it is a reflection of active viral replication in hepatocytes and therefore considered a surrogate marker for the presence of hepatitis B viral DNA (Fang, Zhuang, Wang, Ge, & Harrison, 2004). This antigen is a non-particulate secretory protein, which is not crucial for assembly or replication of the viral particle but is key in the detection of persistent or chronic infection (Lau & Wang, 2012).

During acute infection, HBeAg briefly appears and then after quickly cleared by the host's immune response. However HBeAg may still persist in chronic viral infection. Continued replication may lead to greater hepatic inflammation and subsequent necrosis and fibrosis and ultimately resulting in cirrhosis and end-stage liver disease (Dahiru & Yayo, 2012; Hwang & Cheung, 2011). This implies that HBeAg presence may mean a much greater risk for the development of hepatocellular carcinoma in the case of chronically infected individuals and also risk of transmission of the infection. Hepatocellular carcinoma is the sixth most common cancer and the third most common cause of cancer caused death in the world (Dahiru & Yayo, 2012; Hwang & Cheung, 2011). In Accra, the capital city of Ghana, the risk of developing cirrhosis was found to increase 8-fold in patients with HBV infections than those without

(Blankson *et al.*, 2005). The prisons in Ghana recorded unusually high HBeAg (~28% = 91inmates) prevalence as compared to that in the general population during this study. This result indicates the high risk of HBV related liver disease within prisons in the country and this will have significant public health implications. Also high transmissibility or infectivity basically could imply higher HBV prevalence as the possibility of infection is greatly increased within the prisons. This may be one specific area attributing to the high prevalence of HBV especially within prisons in developing countries (Hou, Liu, & Gu, 2005; Hwang & Cheung, 2011; Kramvis & Kew, 2007).

Even though presence of HBeAg has already been documented to down-regulate the innate immune response, so many other prevailing factors in the prison environment can further contribute to this immune suppression which may consequently lead to the high HBeAg prevalence (Kumar, 1997; Lau & Wang, 2012). Amongst these are poor health and healthcare, poor diet and stressful conditions in the prisons. The prison environment as described earlier by the various factions in the prison is that which fuels the spread of communicable diseases in the absent of quality health care and this could greatly suppress immune response to all other diseases (Awolutugu, 2013; Herbert, 2001). Tuberculosis, STDs such as HIV and syphilis, malaria, among many other diseases which were reported by inmates has also been associated with immune suppressed state and may reduce host immune defense against HBV (Center for Disease Control and Prevention (CDC), 2014; Huang *et al*., 2014; Peters *et al*., 2014). Unfortunately reports from the prisons imply poor management and treatment of such infections as well. Nutrition, a critical determinant of immune responses is one of the

most common causes of immunodeficiency worldwide (Kumar, 1997). As a matter of fact, it was established by Kumar (1997) that, "impaired immunity could be enhanced by modest amounts of a combination of micronutrients." Nonetheless the inmates in Ghanaian prisons lamented a great deal about the inadequate nutrition in the facility and a longer stay in such an environment may lead to malnourishment and compromised immune systems (Herbert, 2001). Coincidentally based on the results, prisons that are fashioned for long stay such as the Central prisons recorded higher HBeAg prevalence as compared to those in the other prisons. Also the length of stay of prisoners (sentence years spent) was found to be significantly (0.033) associated with HBeAg. Implying that a greater number of the HBeAg positive inmates had been in the prison environment longest. The length of stay in the prison environment is therefore a contributing factor to the high HBeAg prevalence in the prison. Evidently, the prison environment is that which suppresses the immune system and may attribute to the high HBeAg prevalence within penal institutions.

The presence of the anti-HBc IgM could be used to distinguish acute or recently acquired infection (Eksteen, Walker, & Adams, 2005). It is detected at the onset of acute hepatitis B and persists for up to 6 months if the disease resolves but can persist at undetectably low levels during viral replication (documented for assays in the United States) in patients who develop chronic hepatitis B (Center for Disease Control and Prevention (CDC), 2014). The anti-HBc IgM positives detected in this study recorded high levels of the antibody in 16 HBsAg positive inmates at the time of sampling who had been incarcerated from a period less than 1 month to 5 years. Ten (62.5%) of these inmates from 7 different prisons (Nsawam MS, Kumasi Central,

Sunyani Central, Wa Central, Kpando Local, Amanfrom Camp and James Camp prisons) classified under 4 different types of prison (Medium Security, Central, Local and Camp prisons), had been incarcerated for a year or more which implies possible infection within the prison.

The questionnaire administered in the nationwide survey from which the samples were derived recorded details about illnesses suffered by inmates within their current incarceration. Night sweat, appetite loss, weight loss, pain during urination and fever were the most frequently mentioned. Inmates reported illnesses related to symptoms of both acute and chronic HBV infection. Of all the illnesses recorded, fever was significantly high (*p-value*=0.04) among anti-HBc IgM positive inmates. Inmates with persistent anti-HBc IgM were three (3) times more likely to have or have had fever. Unfortunately the overcrowded nature of the prison and the poor bedding arrangement may also account for the generally high records of fever in the prison. Also, fever is not a symptom related to only HBV infection but to many other infections, as such, it would be inaccurate to finger HBV infection as the only contributor to fever reported by the inmates. Although genital pain was not that frequently reported by all HBV positive inmates, a significant number of HBeAg (*p-value*=0.005) positive inmates complained of genital pain. Also, genital discharge from the binary logistic regression analysis was significantly high among HBeAg positive inmates. Genital pain and discharge even though unspecific is suggestive of certain unsafe sexual practice as well as sexually transmitted infections reported by inmates in this study.

The study recorded a significantly higher HBeAg and anti HBc-IgM prevalence among inmates within the ages of 18-45years with the highest prevalence being recorded for inmates within the age range of 18-25years (*p-value* < 0.001). Similar reports in studies conducted over the years in Ghana and in West Africa reported similar patterns (Dahiru & Yayo, 2012; Forbi, Iperepolu, Zungwe, & Agwale, 2012; Rufai *et al*., 2014; Simidele, Ihinacho, & Prince, 2013). Rufai *et al*. (2014) reported higher HBeAg prevalence among the ages of 20-10 years, in blood donors at the Komfo Anokye Teaching Hospital in Kumasi (Ghana), Simidele *et al.* (2013) reported highest HBeAg prevalence in HBV positive Nigerians less than 22years of age and subsequently this trend was repeated in many other studies with a decrease in HBeAg as age increased.

Also the significantly higher prevalence of HBeAg and anti HBc-IgM were recorded for inmate who had been incarcerated for less than 2years (*p-value* < 0.001 and 0.001 respectively). This indicates the likelihood of inmates contracting HBV within the first two years of their stay in the prison as compared to those who had been incarcerated for more than two years.

The inmates enrolled in this study reported risk behaviours such as blade sharing, MSM, tattooing and piercing was reported. One (1) inmate reported of having been forced to have sex with another inmate who incidentally happen to have been in the prison facility for approximately 17 months and was both HBeAg and anti-HBc IgM positive. This incident is most likely to be repeated as majority of the inmates admitted to knowledge of such acts in the facility which poses a high risk to the

perpetrator and other victims of this same perpetrator. Three (3) inmates admitted to forcing other inmates into having unsafe sexual practices and 1 of these three inmates tested positive for HBeAg. Although just a few inmates personally made such reports the possibility of such acts in the prisons going unnoticed may pose a big threat to other inmates.

5.1.2 NUCLEOTIDE SEQUENCE ANALYSIS

Twenty (20) sequences were successfully sequenced of which 17 were analysed. A phylogenetic tree using the Neighbour-Joining method, MEGA6 software including four reference sequences from genotype A-H each from the GenBank along with the 17 sample sequences was generated (Figure 4.8). These nucleotide sequences from the pre S and S region closely clustered with genotype E strains from Ghana, Ivory Coast and Senegal (Huy et al., 2006) with the exception of two samples from two different prisons which clustered with the recombinant sequence subgenotype A3/genotype E. Genotype E has been reported to be the predominant genotype in Ghana and the subgenotype A/genotype E has been reported in the population (Candotti, Danso, & Allain, 2007; Huy *et al.*, 2006; Rufai *et al*., 2014). Even though the sequences were short, the S region is a highly conserved region due to its role in attachment to host cells and this makes it a more accurate region for genotyping. This was apparent in a study that characterized the hepatitis B virus in Ghana using full Length Genome Sequences (Huy *et al*., 2006). This study compared phylogenetic

analysis using both the full genome and the S region. There was no difference in the genotypes identified using the full genome as well as the S region.

With respect to the identity matrix a value of 1 represents completely identical sequences while values less than one 1 are less identical. Values at 1 or closer to 1 are represented with lighter shades of colour and those farthest from one or closer to the lower limit have darker shades of colour. From the matrix (Table 4.5), even though the West African sub-region has been reported to have very low genetic diversity or variability, it was apparent that samples within one prison were more closely related (1-0.963 with one outlier- 0.95) and varied less as compared to the relationship between nucleotide sequences from other prisons (1-0.931). Evidently the only two recombinant sequences from the phylogenetic tree recorded an identity value of 1. Nonetheless some sequences from different prisons expressed very close relationship. Identical sequences from unrelated samples could be due to the high degree of conservation of surface (S) gene sequence but further investigations revealed that the individuals from which these samples were taken had been in the same prison at some point of their incarceration. Unfortunately the demographic data does not provide information of the time line for each prison transited during the incarceration period. Sequences with the labels NSMN 2098 and PSJ 1740 for instance recorded an identity of 1. Further investigation on these samples based on demographic data revealed that inmate with the label PSJ 1740 had been previously incarcerated in the NSNM prison during this current sentence. PSJ is a camp prison and transit of prisoners from all other prisons getting to the end of their sentence is common and is a functional attribute of that type of prison. The prison system is one which allows the transit of

inmate to and fro from one prison type to the next at any given time of a sentence. And this could greatly contribute to the sequences being closely related even in the case of little evidence of contact.

Although the S gene has been established to be highly conserved, different specific variability levels for the S gene (genotype, subgenotype, and subtype), in conjunction with mutations, can provide enough confidence to prove transmission events (Datta *et al*., 2007). L217R and L209V mutations in both the RT domain and the SHB protein respectively were found to run across all sequences. Apart from PSA 2627 and PSJ 1778 which had most mutation in common and were found to be of a different genotype, all other shared mutations were found among inmate samples of the same prison and this occurred in two separate events (Tables 4.6 and 4.7). Sequences with the label PSS 0361 and PSS 0498 had one common mutation and strongly clustered in the phylogenetic tree (bootstrap value- 81). Nonetheless the sequences had unique mutations.

5.2 LIMITATIONS

The scope of the study did not include sequencing of all HBV positive samples in the various prisons. This could have given a clearer picture of the level of endemicity of the sequences in the various prisons and resultantly creating a stronger argument of on-going transmission within the prisons. Although all samples were screened for HBeAg and high prevalence of this serological marker was recorded representing high transmissibility within the prisons, an additional serological marker such as anti-

HBe could have been included to detect infectious HBeAg negative individuals as a result of HBeAg seroconversion.

5.3 CONCLUSION

Transmission was established as at the time of sampling within the seven different prisons using the duration of incarceration in conjunction with serological markers.

High HBeAg prevalence is influence by the poor prison environment in Ghanaian prisons.

Fever was found to be associated with recent infection among inmates. Given the prevalence of HBsAg in the prisons, HBeAg status may help identify those at greater mortality risk due to the development of either cirrhosis or hepatocellular carcinoma. A significant number of inmates have high risk of mortality resulting from liver associated diseases based on the high HBeAg prevalence being recorded. Risk behaviours such as blade sharing, piercing, tattooing and sodomization may greatly influence risk of transmission of HBV within the prisons. The prison environment fosters the spread of diseases such as STDs which may have detrimental effects on the society at large as the prison population was not only constituted by Ghanaians.

Nucleotide divergence between all sequences from different prisons was higher than between sequence within one prison. Nonetheless sequences generally expressed low genetic diversity. Sequences were predominantly from genotype E. Two sequences clustered with recombinant subgenotype A3/genotype E recombinant.

Mutational analysis gave a stronger indication of transmission within the prison as sequences of related genotypes located within one prison had mutations in common.

Transmission using molecular analysis may be impaired by the constant transfer of inmates from one prison to the other making the inmates in the various prisons not completely isolated from each other.

5.4 RECOMMENDATION

The alarming results of HBV prevalence and transmission within the prisons may be addressed initially by generally improving sanitation, diet, health care, logistic support and congestion in the prisons. This will go a long way in improving the management, classification and wellbeing of prisoners. Since more individuals are successively being introduced to the prison environment, prisoners should be continually educated about the silent yet damaging effects of HBV infection and the associated risk behaviours that lead to infection. Also they should be equipped with necessary information on prevention methods. Ultimately, HBV immunization should be routinely done for all prisoners including prison staff in all prisons rather than on the basis of an HBV transmission event. Vaccine coverage should be an integral element of a prison's local immunization policy.

Further studies should involve phylogenetic analysis of all isolates using various genomic regions to increase the confidence level of analysis and avoid spurious clustering of unrelated strains and to establish the major source of infection of HBV for each prison setting. Sampling should not be based on only inmates but should

include all prison staff. Also a whole genome sequence of the two recombinant genotypes found in the study is recommended to further confirm the genotype.

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APPENDICES

APPENDIX I: QUESTIONNAIRE

National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana

PRISON INMATES' QUESTIONNAIRE

SCHOOL OF PUBLIC HEALTH

CONSENT FORM

We, the Ghana AIDS Commission (GAC), Ghana Prisons Service, National AIDS/STI Control Programme (NACP) and German International Cooperation (GIZ) [Regional Coordination Unit for HIV & TB], working in partnership with the School of Public Health, University of Ghana are undertaking a national survey of Prison Inmates and Prison Officers to determine the health status of prison inmates. The main aim of this study is to assess the prevalence and situation of HIV, HBV and other key illnesses such as malaria, diabetes, hypertension and underweight amongst inmates.

The study comprises behavioural and biological components. The behavioural component involves one-on-one interview to assess inmates' knowledge, attitude and behaviours on HIV and STIs, and general health situation, while officers will also be interviewed to explore how they perceive inmates' attitude and behaviours regarding HIV and general health situation. The biological part will involve drawing anonymous blood sample from inmates. From the blood sample, rapid tests will be done at the prisons to determine the presence HIV, HBV, Malaria, and Diabetes. Afterwards, plasma for confirmed HIV or HBV infections will be sent to the laboratory for the determination of recent infections and the transmissibility of HBV. Additionally, your blood pressure (BP) will be checked for hypertension, and weight & height measurements taken to estimate Body Mass Index. The study will use very experienced counselors and biomedical personnel who have handled many similar cases before, and so if you test positive to HIV or HBV, you will be counseled and referred for care and treatment and follow up supportive counseling.
This research will give you the chance of knowing, free of charge, your health situation in terms of HIV and Hepatitis B infections, Diabetes, Blood pressure and BMI (whether you are over or under-weight). This will give you the knowledge to enable you to improve your health and get medical support. Additionally, the results of this study will enable the Ghana AIDS Commission and its partners to know the prevalence of HIV and STIs and the general health status of inmates in order to institute appropriate measures to secure the health of prison inmates in Ghana.

We are therefore asking you to participate in this study to contribute to the national effort in preventing HIV and improving the health of inmates through the information you will provide.

We assure you that any information you provide will be strictly confidential; and will be used only for the purposes of this research and never be used against you.

Your participation is voluntary, and you may stop the interview at any time.

Do I have your permission to continue? [___] Yes [___] No

Respondent's signature: _________

[If Yes] I, _____ (interviewer) certify that the respondent has given permission to participate in the study.

Interviewer's signature: _____

If you have any questions concerning this study, please feel free to contact the Principal Investigator:

Dr. Angela El-Adas, MD, MPH
Director-General, Ghana AIDS Commission
4th Floor, Olympic Building, Castle Road, Ridge, Accra, Ghana
Po Box CT 5169, Cantonments, Accra
E-mail : aeladas@ghanaids.gov.gh
Tel : +233 54 0667 251 / +233 30 2218 278

PRISON INMATES' QUESTIONNAIRE

Interview Information

Questionnaire Number : [___|___|___|___]

Name of Prison : _____

Type of Prison : 1 Maximum security (Ankaful New)
2 Medium security (Nsawam)
3 Central Prison
4 Local Prison
5 Agricultural Camp

Region : _____

Date of Interview : Day:_____ Month :_____ Year:_____

Time of Interview : Started _____ Finished _____

Name of Interviewer : _____

Interview Status : 1. FULLY Completed 2 PARTLY Completed 3 Refused

Returned for FBS Test : 1 Yes 2 No

Comments on Interview : _____

Interview Tests

Participant asked & given Verbal Consent to :

1. Questionnaire : 1 Yes 2 No
2. HIV Test : 1 Yes 2 No
3. HPV Test : 1 Yes 2 No
4. Malaria Test : 1 Yes 2 No
5. Diabetes Blood Sugar Test : 1 Yes 2 No
6. Blood Pressure Test : 1 Yes 2 No
7. Weight & Height Measure : 1 Yes 2 No

Signature of Interviewer: _____

Test Results:
HIV= 1 POS. 2 NEG. 3 INDE.
HBV= 1 POS. 2 NEG.
MALARIA = 1 POS. 2 NEG. 3 INDE.
BLOOD PRESSURE = _____
BLOOD SUGAR (RBS)= _____
Blood Sugar (FBS) Test Required? 1 Yes 2 No

If Yes, Blood Sugar (FBS) = _____
HEIGHT (m) = _____
WEIGHT(kg) = _____
BMI = _____ kg/m^2

INSTRUCTION: Please circle the appropriate answer the respondent gives for all closed-ended questions, and write legibly in the spaces provided answers to all open-ended questions.

NO.	QUESTIONS	CODING	SKIP TO
1.0	**SECTION 1** **SOCIO-DEMOGRAPHIC**	**"I am going to start by asking some questions about your background"**	
1.1	What sex is the interviewee? ***[Circle from observation]***	Male 1 Female 2	
1.2	How old are you? ***[Age at last birthday]***	Age in years [___|___] Don't Know 88 No response 99	
1.3	In which country have you lived for most of your adult life?	Ghana 1 Nigeria 2 United Kingdom (UK) 3 Other..... 4 No Response 99	→ 1.5 → 1.5 → 1.5

1.4	In which region did you live for most of your adult life before coming to prison?	Greater Accra 1 Ashanti 2 Western 3 Central 4 Brong Ahafo 5 Northern 6 Upper West 7 Upper East 8 Volta 9 Eastern 10 No response 99	
1.5	What is your nationality?	Ghanaian 1 Non Ghanaian (Foreigner) 2 No response 99	
1.6	Have you ever attended school?	Yes 1 No 2 No Response 99	→ **1.9**
1.7	Up to which level of schooling did you attend?	Primary 1 Junior High 2 Senior High 3 College / University 4 No Response 99	
1.8	What was the highest class you completed?	..... No response 99	
1.9	What is your religion?	Christian 1 Traditionalist 2 Muslim 3 No Religion 4 Other..... 5 No Response 99	
1.10	How often do you attend a religious meeting?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	
2.0	**SECTION 2** **PRISON ENVIRONMENT**	**"I would like to ask a few questions about your time in prison"**	

2.1	Are you on remand or convicted?	On Remand /awaiting trial 1 Convicted 2 No Response 99	→ 2.3
2.2	How long is your prison sentence?	Number of Years [___|___] Number of Months [___|___] Less than one month 66 No response 99	
2.3	How long have you been in prison on this sentence or remand /awaiting trial?	Number of Years [___|___] Number of Months [___|___] Less than one month 66 No response 99	
2.4	How long have you been in this prison?	Number of Years [___|___] Number of Months [___|___] No response 99	
2.5	How many other prisons have you been in for this sentence?	Number of Prisons [___|___] Less than one month 66 No response 99	→ 2.7 if ans=0
2.6	Can you give the names of other prisons you have been in during this sentence & for how many months / years	.....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 No response 99	
2.7	Have you been in prison before this sentence?	Yes 1 No 2 No response 99	→ 3.0
2.8	How many times have you been in prison before?	Number of Times Before [___|___] No response 99	
3.0	**SECTION 3** **HEALTH IN PRISON**	**"Next I will ask you some questions about the general health situation in this prison"**	SKIP TO

3.1	What are the common illnesses in this prison? ***Do not read out answers.*** ***Up to 3 multiple responses possible.*** ***Probe by asking: Any others?***	Malaria 1 Fever 2 Headache 3 Stomach Aches 4 Pneumonia 5 Diarrhoea 6 Dysentery 7 Piles 8 Urethral Discharge 9 HIV/AIDS 10 Jaundice 11 Tuberculosis (TB) 12 High Blood Pressure 13 Skin Diseases 14 Diabetes 15 Other ……16 Other ……16 Other ……16 Don't Know 88 No Response 99	
3.2	What illnesses have you suffered from in this prison? ***Do not read out answers.*** ***Up to 3 multiple responses possible.*** ***Probe by asking: Any others?***	Malaria 1 Fever 2 Headache 3 Stomach Aches 4 Pneumonia 5 Diarrhoea 6 Dysentery 7 Piles 8 Urethral Discharge 9 HIV/AIDS 10 Jaundice 11 Tuberculosis (TB) 12 High Blood Pressure 13 Skin Diseases 14 Diabetes 15 Other ……16 Other ……16 Other ……16 Don't Know 88 No Response 99	
3.3	Have you been coughing frequently (every hour) for the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99	
3.4	Have you been having fever and chills frequently in the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99	→ **3.6**
3.5	Have you had regular headaches with the fever?	Yes 1 No 2 Don't know 88 No response 99	

3.6	Have you been sweating a lot at night for the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99		
3.7	Have you experienced loss of appetite in the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99		
3.8	Have you been waking up to urinate more than 2 times a night in the last week?	Yes 1 No 2 Don't know 88 No response 99		
3.9	Have you noticed yourself losing a lot of weight in the last month?	Yes 1 No 2 Don't know 88 No response 99		
3.10	Have you ever had Sexually Transmitted Infections?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.11	Have you experienced any pains or ulcers in the genital areas?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.12	Have you experienced pain during urination?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.13	Have you ever experienced an unusual discharge?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.14	How did you treat it?	Hospital treatment 1 Herbal treatment 2 Not treated 3 Self-medication 4 Other ..... 5 Don't know 88 No Response 99	Hospital treatment 1 herbal treatment 2 Not treated 3 Self-medication 4 Other..... 5 Don't know 88 No Response 99	
3.15	Does the prison offer health services	Yes 1 No 2 Don't know 88 No response 99		

4.0	**SECTION 4 : HIV RISK - BLOOD CONTACT**	**"I would like to ask you some questions about your experiences in prison"**	
4.1	Is there access to blades / razors for shaving in this prison?	Yes 1 No 2 Don't know 88 No response 99	
4.2	Do you share blade / razor *(for shaving or hair cut)* with other inmates in this prison?	Yes 1 No 2 No response 99	
4.3	Have you ever been tattooed or pierced?	OUTSIDE PRISON Yes 1 No 2 No Response 99 WITHIN PRISON Yes 1 No 2 No response 99	→ **4.5**
4.4	Was the same implement used for other people?	OUTSIDE PRISON Yes 1 No 2 No Response 99 WITHIN PRISON Yes 1 No 2 No response 99	
4.5	Have you ever made a (direct) blood covenant?	OUTSIDE PRISON Yes 1 No 2 No Response 99 WITHIN PRISON Yes 1 No 2 No response 99	
4.6	Do you know other inmates in this prison who have made a blood covenant?	Yes 1 No 2 No Response 99	
5.0	**SECTION 5** **HIV RISK – INJECTING DRUG USE**		
5.1	Do you know inmates in this prison who use drugs?	Yes 1 No 2 Don't know 88 No response 99	→ **5.7**
5.2	How often do they use drugs?	Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99	

5.3	Which drugs do you often see used? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed*** ***Also Specify Combinations***	('Wee') Marijuana 1 Crack / Cocaine 2 Heroin 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99	
5.4	Do you know people in this prison who inject drugs?	Yes 1 No 2 Don't know 88 No response 99	→ **5.7**
5.5	Do these people have to share needles?	Yes 1 No 2 Don't know 88 No response 99	
5.6	Which drugs are usually injected by inmates? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99	
5.7	Have you ever used drugs?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99 → **6.0**
5.8	How often have you used drugs?	OUTSIDE PRISON Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99	WITHIN PRISON Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99

5.9	Which drugs have you used? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	OUTSIDE PRISON ('Wee') Marijuana 1 Heroin 2 Crack / Cocaine 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99	WITHIN PRISON ('Wee') Marijuana 1 Heroin 2 Crack / Cocaine 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99	
5.10	Have you ever injected drugs?	OUTSIDE PRISON Yes 1 No 2 Don't know 88 No response 99	WITHIN PRISON Yes 1 No 2 Don't know 88 No response 99	→ **6.0**
5.11	How often have you injected the drugs?	OUTSIDE PRISON Every week 1 Every month 2 Few times a year 3 Less than every year 4 Don't know 88 No response 99	WITHIN PRISON Every week 1 Every month 2 Few times a year 3 Less than every year 4 Don't know 88 No response 99	
5.12	Which drugs have you injected? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	OUTSIDE PRISON Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99	WITHIN PRISON Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99	
5.13	Have you ever had to share needles with other drug users?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No response 99	
6.0	**SECTION 6** **HIV RISK – SEXUAL CONTACT**	**"Some of the following questions will be about your personal life and sexual behaviour."**		

6.1	What is your marital status?	Single (never married) 1 Married – One Partner (monogamous) 2 Living together 3 Married – 2+ Partners (polygamous) 4 Divorced 5 Separated 6 Widowed 7 No Response 99	
6.2	Before you came to prison – did you ever have other casual sex partners?	Yes 1 No 2 Don't know 88 No Response 99	→ **6.5**
6.2a	If Yes, in total, with how many different people have you had casual sex with during the last 12 months before you came to prison	ers [___|___] No response 99	
6.3	How often did you have casual sex partners – in the 12 months before you came to prison?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	
6.4	How often was a condom used with a casual partner?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	
6.5	Before you came to prison, did you ever have sex with a commercial sex worker?	Yes 1 No 2 Don't know 88 No Response 99	→ **6.8**

6.6	How often did you have casual partners – in the 12 months before you came to prison?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	
6.7	How often was a condom used?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	
6.8	At this prison – Have you had sex with other inmates?	Yes 1 No 2 No Response 99	→ **6.17**
6.9	Have you had oral sex with other inmates – in this prison?	Yes 1 No 2 No Response 99	→ **6.11**
6.10	How many times have you had oral sex in this prison in the last three months?	..... times 1 Don't Know 88 No response 99	
6.11	Have you had anal sex with other inmates – in this prison?	Yes 1 No 2 No Response 99	→ **6.13**
6.12	How many times have you had anal sex in this prison in the last three months?	..... times 1 Don't Know 88 No response 99	
6.13	How often was a condom used?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	

6.14	Have you ever paid/received money or goods (e.g. cigarettes, drugs, blades) for sex in prison?	Yes 1 No 2 Don't know 88 No Response 99	
6.15	Have you ever engaged in sex with other inmates in this prison who inject drugs?	Yes 1 No 2 Don't know 88 No Response 99	
6.16	Did you use condom during your last sex in this prison?	Yes 1 No 2 Non-Applicable 77 No Response 99	
6.17	Have you heard of inmates being forced to have penetrative sex?	Yes 1 No 2 Don't know 88 No response 99	→ **6.19**
6.18	How often do you think inmates are forced to have penetrative sex within this prison?	every few days 1 every few weeks 2 every few months 3 Once a year 4 Don't know 88 No response 99	
6.19	Have you ever been forced to have anal or oral sex?	**Anal Sex** Yes 1 No 2 No response 99	**Oral Sex** Yes 1 No 2 No response 99 → **6.21**
6.20	How many times have you been forced to have penetrative sex in prison?	..... Times No response 99	
6.21	Have you ever forced anyone to have anal or oral sex?	**Anal Sex** Yes 1 No 2 No response 99	**Oral Sex** Yes 1 No 2 No response 99 → **7.0**
6.22	How many times have you forced anyone to have penetrative sex in prison?	..... Times No response 99	

6.23	How would you rate your chance of getting infected with HIV in this prison?	High risk 1 Medium risk 2 Low risk 3 No risk 4	
7.1.1.1.1	7.1.1.1.2 7.1.1.1.3 SECTION 7: HIV/AIDS KNOWLEDGE AND ATTITUDE	**I am going to ask you some questions about HIV/AIDS. Please do not worry about getting the right answer, just say what you think is true**	
7.1	Have you ever heard of an illness called AIDS?	Yes 1 No 2 No Response 99	
7.2	Can people reduce their chance of getting the HIV by having just one uninfected sex partner who has no other sex partners?	Yes 1 No 2 Don't know 88 No Response 99	
7.3	Can people get HIV from mosquito bites?	Yes 1 No 2 Don't know 88 No Response 99	
7.4	Can people reduce their chance of getting HIV by using a condom every time they have sex?	Yes 1 No 2 Don't know 88 No Response 99	
7.5	Can people get HIV by sharing food with a person who has HIV/AIDS?	Yes 1 No 2 Don't know 88 No Response 99	
7.6	Can people reduce their chances of getting HIV by not having sexual intercourse at all?	Yes 1 No 2 Don't know 88 No Response 99	
7.7	Can people get HIV because of witchcraft or other supernatural means?	Yes 1 No 2 Don't know 88 No Response 99	

7.8	Do you think that HIV can be transmitted by injection with used needle?	Yes 1 No 2 Don't know 88 No Response 99	
7.9	Do you think that HIV can be transmitted by common use of razor blades?	Yes 1 No 2 Don't know 88 No Response 99	
7.10	Do you think that HIV can be transmitted by common use of tooth brushes?	Yes 1 No 2 Don't know 88 No Response 99	
7.11	Do you think that HIV can be transmitted by tattooing?	Yes 1 No 2 Don't know 88 No Response 99	
7.12	Do you think that HIV can be transmitted by sharing blood in brotherhood/ sisterhood rituals?	Yes 1 No 2 Don't know 88 No Response 99	
7.13	Is it possible for a healthy looking person to have HIV?	Yes 1 No 2 Don't know 88 No Response 99	
7.14	If a member of your family became sick with AIDS, would you be willing to care for her or him in your own household?	Yes 1 No 2 Don't know 88 No Response 99	
7.15	If you knew that someone is HIV infected, would you eat with him or her?	Yes 1 No 2 Don't know 88 No Response 99	

7.16	If you knew that someone is HIV infected, would you <u>continue to meet or associate with him/her?</u>	Yes 1 No 2 Don't know 88 No Response 99	
7.17	If you knew that someone is HIV infected, would you <u>share a cell with him/ her</u>?	Yes 1 No 2 Don't know 88 No Response 99	
	Have you ever had an HIV test, before this survey?	Yes 1 No 2 Don't know 88 No Response 99	→ **7.21**
7.19	If yes when was the last time you were tested?	Less than 12 months ago 1 1 to 2 years ago 2 2 or more years ago 3 Don't know 88 No Response 99	
7.20	What was your Test Result? - ***You do not have to share this if you are not comfortable***	Positive 1 Negative 2 Don't know 88 No Response 99	
7.21	**7.1.1.1.4** Do you want to know your HIV status at the end of this Survey?	Yes 1 No 2 No Response 99	
7.22	**7.1.1.1.5** If you were HIV positive do you think that you would feel comfortable disclosing your status in the prison?	Yes 1 No 2 No Response 99	
7.23	**7.1.1.1.6** Are HIV/AIDS services offered in this prison?	Yes 1 No 2 Don't know 88	→ **7.25**
7.24	**7.1.1.1.7** What activities or services have you seen? ***Answer yes/no for each service***	(a) Education - Yes 1 No 2 (b) HIV Testing - Yes 1 No 2 (c) Treatment - Yes 1 No 2 (d) Support Groups - Yes 1 No 2 No Response 99	

7.24a	**7.1.1.1.8** Are these services accessible to every prisoner?	Yes 1 No 2 Don't know 88	
7.25	Do you need information about HIV/AIDS in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.25a	Do you need information about other health issues in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.26	Do you need information about other health issues in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.27	**7.1.1.1.9** Do think condoms should be made accessible in prisons?	Yes 1 No 2 Don't know 88 No Response 99	
7.28	**7.1.1.1.10** **7.1.1.1.11** What would you recommend to help reduce the risk of HIV in this prison? ***Multiple responses possible.***	Better Access to Condoms 1 Access to clean needles 2 Access to Razor Blades so not share 3 Educational Sessions 4 Educational Leaflets 5 Prevent Forced Sex from Happening 6 Other..... 7 Don't know 88 No response 99	
Comments on Interview:			

Time Finished _____

Thank you for your help in answering this questionnaire.

APPENDIX II: SPECIMEN BANKING FORM

AGREEMENT TO PARTICIPATE IN SPECIMEN BANKING

Study Title:
National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana

You have agreed to participate in a study known as **National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana,** sponsored by the Ghana AIDS Commission and partners.

You are being asked to sign this second consent form to indicate if you are willing to allow the samples collected solely for research purposes, which will be referred to as your 'Specimen', to be saved or 'banked' for use in future research studies. At this time, we do not know what future research studies may be done using your Specimen. The specimen bank will be maintained by the Clinical Virology Laboratory, University of Ghana Medical School.

Your signature below will allow your Specimen to be stored in the specimen bank, with the possibility that it will be used in future research studies. It is very unlikely that any future research performed using your Specimen would benefit you directly, but it may provide important medical knowledge that could be helpful in understanding the transmission of viral pathogens in prisons. Information about how your Specimen will be used and protected is as follows.

A. Confidentiality and Privacy of Medical Record
If you sign this form, you give the Clinical Virology Laboratory permission to store your Specimen in a controlled specimen bank, along with portions of your personal health information collected related to the Main Study. This information could be used to link the specimen back to you. Clinical Virology
Laboratory will protect your confidentiality by making sure that no information that could be used to identify you will be used or disclosed by Clinical Virology Laboratory without your authorization or without legally required protections in place. The following rules will apply:

The Clinical Virology Laboratory in charge of the specimen bank will determine for which research studies to release all or part of your Specimen.

1) In most cases, your Specimen will be 'de-identified'; that is, the researcher who is given your
Specimen will not be given enough information to identify you. In these cases, you will not be contacted prior to your Specimen being released to the researcher.

2) Your authorization would not be required for researchers to use partially de-identified (in accordance with legal standards) Specimens for future research studies; however, such researchers would be required to sign a Data Use Agreement, which would protect your privacy by limiting how they could use your Specimen.

3) If an approved researcher wants to use your Specimen and feels that is important that he or she be able to identify you to a) Collect information about you that was not collected as part of your participation in the Main Study; or, b) Collect additional samples of your blood then this would be considered using an identifiable Specimen (meaning the Specimen could be linked back to you). In that case, someone associated with Clinical Virology Laboratory will contact you to provide further information about the proposed study so you can decide whether you will agree to participate. If you decide to participate, you will then be contacted by a researcher for the new study and asked to sign a separate consent form for that study. Clinical Virology Laboratory will apply national legal standards in making decisions about who can review your records in preparing for the study and about who can contact you to provide information about the new study.

4) It is possible that your Specimen could be used for future research purposes without your consent or authorization if a committee of people who know about research, privacy and medical ethics (such as the Noguchi Memorial Institute for Medical Research Institutional
Review Board or the Ghana Health Service Ethical Review Committee) decided that use of your information is necessary and that use of it would be of low risk to you and your privacy.
Clinical Virology Laboratory will ensure that all specimens stored at the Clinical Virology Laboratory will be kept confidential and only shared by the Clinical Virology Laboratory in accordance with the above rules; no other people, including relatives or Ghana Prisons Officials will have access to the stored samples or information about them without your written consent. Appropriate physical and computer security measures will be maintained to limit access to Specimens.
Papers or articles written by Clinical Virology Laboratory researchers which are based on studies involving your Specimen will not identify you by name.

B. Control and Ownership of the Specimen(s)
By consenting to participate in the specimen bank, you authorize the banking of your Specimen for research conducted in accordance with the rules described above. If you should have a need for the
Specimen at some later date for a medical purpose, it usually can be removed from the specimen bank for that purpose unless there is no specimen left.

C. Withdrawal of Your Consent
If you decide at some time in the future that you no longer wish your stored Specimen to be used in future studies, you have the right to request that the Specimen be withdrawn from the specimen bank. However, withdrawal cannot be guaranteed and may be impossible. For example, it is possible that the Specimen might no longer be identifiable as belonging to you, or that it might already have been released for research studies and used up. To request withdrawal of the Specimen from the specimen bank, please write to Dr. Kwamena Sagoe, Clinical Virology Laboratory, Department of Microbiology, University of Ghana Medical School, P. O. Box 4236, Accra (Mobile: 0277408528).

D. Length of Storage
Specimens in the bank will be stored for an indefinite period of time, until research funding is exhausted or the Specimen is no longer usable.

E. Signature
You have the right to refuse to sign this form. Refusing to sign this form will not affect your participation in the Main Study or your access to health services in the prison. If you chose to sign, your signature below indicates that you have read this form and discussed it with researchers associated with the Main Study, and that you wish to participate in the specimen bank in accordance with the terms described in this form.

Participant's Signature	Date
Witness (only if consent presented orally)	Date
Participant's legal representative (If patient unable to sign)	Date

APPENDIX III: SOLUTIONS, CHEMICALS AND REAGENTS

Detection of HBeAg in human plasma/serum

Solution	Content description
Anti-HBe Plate	One microtiter plate coated with antibody to HBeAg (anti-HBe)
Anti-HBe Peroxidase Solution	Containing anti-HBe Peroxidase (horseradish) conjugate dissolved in protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.
HBeAg Positive Control	Containing HBeAg positive serum diluted in buffer with protein stabilizers Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.
HB Negative Control	Containing normal human serum, which is free of HBeAg, anti-HBe and HBsAg. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal
Neutralizing Solution for Anti-HBe	Containing HBeAg positive serum diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal
TMB Substrate Solution A	0.6mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.
TMB Substrate Solution B	Citrate Acid Buffer containing 0.03% H_2O_2
Conc. Washing Solution D (20X)	Concentrated Phosphate buffer with tween-20
2N Sulfuric Acid	$2NH_2SO_4$

Qualitative detection of Anti-HBc IgM in human serum/plasma

Solution	**Content description**
Anti-IgM Microtiter Plate	One microtiter plate (96 wells) coated with anti-human IgM
Anti-HBc Peroxidase Solution	Anti-HBc (Human) Peroxidase (horseradish) conjugated in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.
Anti HBc IgM Positive Control	Anti-HBc (Human) in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.
Anti-HBc IgM Negative Control	Normal (Anti-HBc IgM negetives) human serum containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal
Specimen Diluent	Buffer containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal
HBcAg Reagent	HBcAg in buffer containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal
TMB Substrate Solution A	0.6mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.
TMB Substrate Solution B	Citrate Acid Buffer containing 0.03% H_2O_2
Conc. Washing Solution D (20X)	Phosphate buffer with Tween-20
$2NH_2SO_4$	Sulfuric Acid

2% Agarose Gel (Ethidium Bromide stained)

- 3g Molecular Grade Agarose
- 150 ml 10X TAE Buffer
- Heat in microwave for approximately 3 minutes or until agarose is completely dissolved;
- Cool to ~ 50^{o}C
- Add 8µl ethidium bromide and mix;
- Pour into gel tray with inserted comb and allow to set

Ethidium Bromide Stock (10 mg/ml)

- 0.1 g Ethidium Bromide
- 10 ml Best quality water
- Mix well;
- Store in a dark bottle at 4^{o}C.

TAE Buffer (10X)

- 24,2 g Tris-base
- 5,68 ml Glacial acetic acid
- 10 ml 0.5 M EDTA (pH 8)
- Make up to 1L distilled water;
- Autoclave;
- Store at room temperature.