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HIV-1 and coinfection with hepatitis B and delta viruses: What is the impact of HIV-1 infection on hepatitis B chronic carriage and the sero-prevalence of delta virus in Uganda?

Opio, Alex Achol, Ph.D.

Case Western Reserve University (Health Sciences), 1994
HIV-1 AND COINFECTION WITH HEPATITIS B AND DELTA VIRUSES: What is the impact of HIV-1 infection on hepatitis B chronic carriage and the sero-prevalence of delta virus in Uganda?

by

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Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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May, 1994
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GRADUATE STUDIES

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HIV-1 AND COINFECTION WITH HEPATITIS B AND DELTA VIRUSES: What is the impact of HIV-1 infection on hepatitis B chronic carriage and the sero-prevalence of delta virus in Uganda?

Abstract

by

DR. ALEX ACHOL OPIO

Since clearance of HBV infection is dependent on normally functioning cell-mediated immunity, it is possible that immunological dysfunction induced by HIV-1 infection leads to poor clearance of HBV. Consequently, HIV infection is expected to promote the spread of HBV infection.

To determine whether HIV infection has the potential to promote the spread of HBV infection, a hospital-based cross-sectional study was conducted in Uganda. Information was collected on respondent's demographic characteristics, risk factors for, and clinical features of HBV infection. In addition, blood was obtained for HIV-1 and hepatitis tests.
The overall prevalence of HBsAg, anti-HBs, anti-HBc and total HBV infection was 15.7%, 54.0%, 42.9% and 66.9% respectively. 64.4% of subjects with detectable levels of anti-HBs had "protective levels" of the antibody.

Of 1392 subjects tested for all the three key markers of HBV infection (HBsAg, anti-HBs and anti-HBc), 463 (33.2%) had no evidence of infection, 51 (3.7%) were in a serologic window period, 210 (15.1%) had evidence of infection that occurred in the distant past, 450 (32.3%) were in convalescence, and 218 (15.5%) had active infection. Of 218 subjects with active infection, 111 (50.9%) were in the early phases of infection, 96 (44.1%) had either acute or chronic hepatitis, and 11 (5.0%) had reinfections. 43 subjects had concurrent circulation of HBsAg and anti-HBs.

The overall prevalence of HBV chronic carriage was 4.9%. HIV positive individuals were more likely than those negative to have chronic carriage (P=0.0007). After controlling for other variables, being HIV positive was associated with a 3.66 fold increase in the likelihood of chronic carriage. Being a male or having both fever and fatigue were independently predictive of HBV chronic carriage.

Of 172 HBsAg positive subjects tested for anti-HD, 4
(2.3%) had the antibody. Of 152 HBsAg positive subjects tested for HBeAg, 23 (15.1%) had the antigen. There was no difference in HBeAg seropositivity by HIV status (P>0.05).

In conclusion, the study shows that HBV infection is highly endemic in Uganda, HDV infection is not a problem in southern Uganda, HIV infection has the potential to promote HBV chronic carriage, and, presence of anti-HBs may not always represent immunity.

Ensuing from above, recommendations are made: a; Institution of HB vaccination programme, b; Prevention of HBV infection through blood screening and sterilization of injection/surgical equipments, c; Continued prevention of HIV infection through counselling, d; Further research to find out how maternal transmission of HBV is impacted by HIV infection, and to elucidate the subject of co-occurrence of HBsAg and anti-HBs.
This work is dedicated to my wife, Irene, and children, Mark and Ann.
ACKNOWLEDGEMENTS

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**Abbreviations used**

AIDS  Acquired Immunodeficiency Syndrome  
Anti-HBc  Antibody to hepatitis B core antigen  
Anti-HBs  Antibody to hepatitis B surface antigen  
Anti-HD  Antibody to hepatitis delta agent  
B  Beta  
BCG  Bacille Calmette Guerine  
C  Centigrade  
CAH  Chronic active hepatitis  
CI  Confidence Interval  
CMI  Cell mediated immunity  
CPH  Chronic persistent hepatitis  
CPM  Counts per minute  
DNA  Deoxyribonucleic Acid  
DPT-1  Diphtheria Pertussis Tetanus Vaccine Dose 1  
EIA  Enzyme immunoassay  
EXP(B)  Exponential of Beta  
HB  Hepatitis B  
HBCAg  Hepatitis B core antigen  
HBeAg  Hepatitis B e antigen  
HBsAg  Hepatitis B surface antigen  
HBV  Hepatitis B Virus  
HCC  Hepatocellular Carcinoma  
HD  Hepatitis Delta
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>HDV</td>
<td>Hepatitis Delta Virus</td>
</tr>
<tr>
<td>HIV-1</td>
<td>Human Immunodeficiency Virus Type 1</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>Radioactive Iodine</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin type G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin type M</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>mIU</td>
<td>Milli International Units</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>OPV-1</td>
<td>Oral Polio Vaccine Dose 1</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>STD</td>
<td>Sexually transmitted disease</td>
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<tr>
<td>WB</td>
<td>Western blot</td>
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CHAPTER 1

1.0; INTRODUCTION.

1.1; Magnitude of the problem.

Hepatitis B virus (HBV) infection is a worldwide problem [1]. This infection can be acute or chronic in nature. The acute form follows a self-limiting course most of the time. For instance, 90 to 95 percent of adults with acute infection eliminate the virus from their body and have no long term effects. The remainder (5 to 10 percent) become chronically infected with the virus [1]. These people who remain chronically infected constitute a reservoir of virus placing the non-infected people at risk of infection. In addition, these carriers may die from chronic active hepatitis, liver cirrhosis and primary liver cancer [2,3,4].

Worldwide, it is estimated that over 2 billion individuals have been infected with HBV resulting in about 300 million chronic carriers [1]. These individuals represent a significant proportion of the current world population (about 5.5 billion). Furthermore, it is estimated that one to two million deaths a year are directly related to HBV infection [1]. All these indicate that HBV infection is of great public health importance.

The severity of the liver problem that results from chronic HBV infection is said to be influenced by
concurrent delta hepatitis \cite{5,6,7,8}. It is generally believed that concurrent hepatitis delta virus (HDV) infection in chronic HBV carriers is usually associated with more liver damage, accelerated progression to cirrhosis, and increased mortality \cite{5,9,10,11,12}. Indeed, clinical evidence consistently points to an important role of HDV in the pathogenesis of chronic hepatitis. Hepatitis delta virus is a "defective" RNA virus that can replicate only in the presence of HBsAg, the "helper function" provided by HBV \cite{13,14}. For this reason, delta hepatitis occurs only in patients who have HBsAg in serum. In a patient who is already a chronic carrier before the delta infection, HBsAg will persist and therefore, permit persistence of HDV. Prevention of HBV infection is therefore crucial for stopping the development of HBV chronic carriage state and possible persistence of HDV infection.

At the moment, HB vaccine is the most effective tool for preventing HBV infection and is recommended for countries or areas where HBV infection is a significant public health problem. The World Health Organization recommends that use of the vaccine should be considered in areas with hepatitis B surface antigen (HBsAg) sero-prevalence rate of 2 to 5\% and that its use should become a priority when the sero-prevalence rate in the population
is 8 to 10% [1].

HBV and human immunodeficiency virus type 1 (HIV-1) infections have some similarities [15,16,17,18]. Both infections can be acquired through sexual means, blood or blood products and transplacental or perinatal mechanism. Furthermore, to a variable extent, T-lymphocytes play some role in the clearance or control of infection by both HBV and HIV-1 [19,20,21]. In general terms, the main function of effector T-cells is to clear an infection. Though antibodies can contribute to the recovery from viral infection, T cells are the principal mechanism for achieving this. Infectious agents causing chronic persistent infections have generally found a way to escape cell mediated immunity through the following mechanisms: a; generation of cells that suppress T-cells. b; down regulation of the major histocompatibility complex (MHC) production in infected cells so they are not recognized and destroyed by T-cells. c; establishment of infection in immunoprivileged sites such as the brain.

In view of the foregoing, it seems reasonable to say that if cellular immunity were impaired, both uncontrolled replication of HBV and more frequent development of infection could be expected as a result of failure of the host to clear HBV infection. Hence, the immunological dysfunction induced by HIV-1 [22,23], predominantly a
defect of cellular immunity, could be expected to modify the course of HBV infection since the clearance of HBV is dependent on normal functioning of cell-mediated immunity [24]. Cell-mediated immunity is important for resolution of HBV infection and inflammation of HBV chronic liver disease [19,21]. Chronic HBV carriers are said to produce subnormal amount of alpha interferon. Since interferons are the first line of resistance against viral infections, its subnormal production increases the potential for development of HBV chronic carriage. Chronic HBV carriers also experience abnormal activation of their hepatocytes by interferon. As a result, it is hypothesized that T-cell lysis of infected cells fails during HBV replication. It is also reported that T cells of the majority of chronic HBV carriers produce a secreted soluble factor that selectively suppresses the production of antibodies to hepatitis B surface antigen (anti-HBs).

1.2; Rationale of the study.

Currently, there is an ongoing worldwide epidemic of HIV-1 infection [25,26]. Countries where HBV infection is highly endemic are no exceptions to this [26,27,28,29]. Thus, the potential for coinfection of HIV-1 and hepatitis B and delta viruses is very high. Understanding the outcome of this coinfection especially in terms of its impact on HBV chronic carriage is of great public health importance.
Since HIV-1 causes aberration of cellular immune response, one would expect that if HBV infection sets in an already HIV-1 infected individual, poor clearance of HBV infection could arise and consequently the proportion of the newly HBV infected individuals who remain chronically infected will rise. It is also possible that an HIV-1-infected individual may reactivate HBV and more likely become a carrier, just as in tuberculosis or other opportunistic infections [19,30]. Thus, I am hypothesising that there has been an increase in the prevalence of HBV infection in populations with high prevalence of both HIV infection/AIDS and HBV infection. This is the rationale of the proposed study.

This hypothesis regarding the role of HIV-1 infection in promoting HBV chronic carriage state and the possibility of consequent increased sero-prevalence of HDV markers derives support from observations made in a number of studies [24,31,32]. Studies have been conducted to test the hypothesis that prior HIV-1 infection leads to poor clearance of HBV infection. One study [24], showed that persons with HIV infection preceding HBV infection had a significantly higher risk of developing HBV chronic carriage. This study examined the effect of prior HIV-1 infection on the course of HBV infection in homosexual men who participated in a Centers for Disease Control
multicenter HB vaccine trial. The incidence of HBV infection by HIV-1 status in the unvaccinated group was compared with that in the vaccinated group. HBV chronic carriage was defined as persistence of HBsAg for 7 or more months. A similar result has been observed by Bodsworth and others [31] during a retrospective study of homosexual men. They reported that the HIV-1 infected individuals were more likely to develop chronic HBV infection. They further reported that the HIV-1 positive subjects who cleared HBsAg had significantly more circulating CD4+ lymphocytes than those who never cleared HBsAg. Another study, by Einzensderger et al [32], involved evaluation of the prevalence of antibodies to HBV in HIV-positive patients at different stages of infection and comparison with that in HIV-negative patients. The results showed that the prevalence of HBsAg and anti-HBc varied with stages of HIV infection. The more advanced stages of HIV infection (II and III) were associated with higher prevalences of these markers. This finding may suggest that HIV infection promotes chronic carriage of HBsAg.

There have also been reports of occurrence of reinfection or reactivation of latent HBV in certain groups of immunocompromized patients including those with HIV-1 infection [33,34,35]. Another interesting observation is based on evidence which indicates that though HIV infection
may promote replication and therefore infectivity of HBV, it is possible that concurrent HIV infection may lead to a milder course of chronic hepatitis B infection as a result of associated improvement in the biochemical and histological features of chronic hepatitis, thus increasing the potential for the spread of HBV infection [36,37]. In some studies involving AIDS patients, it was found that despite evidence of HBV replication exhibited by the subjects, there was little evidence of an accompanying hepatitis as defined by low serum alanine aminotransferase levels.

All the above observations support the hypothesis put forward. To my knowledge, at the moment there is nothing concrete in literature on this subject. Most related studies have mainly focused on demonstrating whether or not HBV infection as a cofactor is a good predictor for HIV-1 infection and vice versa [38,39,40,41,42]. Furthermore, most studies that attempted to contrast the prevalence of HBV markers in HIV positive and HIV negative individuals involved highly selected groups such as intravenous drug addicts [21,43], homosexuals [24,31], soldiers [33] and patients with sexually transmitted diseases. Clearly, homosexuals and injection drug abusers form the very high risk groups for HBV infection and therefore do not constitute the best population for testing the given
hypothesis, especially if inappropriate denominators are used in the estimation of the rate of HBsAg chronic carriage. The results would have serious biases. Populations that do not have homosexuality and IV drug use as the main risk factors for HBV infection would be preferred. Such types of populations exist in Uganda. In Uganda, HBV infection is highly endemic [1]. According to the World Health Organization, in areas of high endemicity, most HBV infections occur during childhood [1]. They may occur around the time of birth (perinatal transmission), or from one infected child to another (child to child transmission). For instance, a study conducted in Nigeria found that one third of the infants born to HBsAg carrier mothers had persistent antigenemia during an eight month follow-up period [44], hence suggesting that vertical transmission is a very significant route of transmission in areas where HBV infection is highly endemic. The most important time of transmitting HBV transplacentally is when the mother has acute HBV infection during the third trimester of pregnancy [45]. Furthermore, a study by Hudson et al has suggested that, although in North America and Europe the epidemiology of AIDS and hepatitis B virus are very similar, this is not the case in Uganda [38]. For example, there was no overlap of the age-specific distribution of both HIV and HBV infection. 40% of the 10-
14 year old were Anti-HBc positive whereas all the HIV postives were aged 20 years or over.

1.3; Objectives of the study.

The main aim of the study is to document the sero-prevalence of HBV and HDV markers being experienced in Uganda during the current AIDS epidemic as an attempt to determine whether HIV infection/AIDS has the potential to promote the spread of HBV in populations with high prevalence of both HBV and HIV infections.

More specifically,

1.3.1; Determine the overall prevalence of HBV markers in the study population.

1.3.2; Determine the prevalence of HDV markers in those individuals having HBsAg in their sera.

1.3.3; Determine whether there is an association between HIV-1 infection and the prevalence of chronic HBV infection. That is, determine whether the HIV infected individuals have a higher prevalence of HBV chronic carriage state than their non-HIV infected counterparts.

1.3.4; Determine the effect of HIV-1 infection on the sero-prevalence of HDV markers. That is, determine whether the HIV-1 infected individuals having HBsAg have a higher prevalence of HDV infection when compared with the non-HIV infected individuals.
with HBsAg.

1.4; Literature review: Overview of hepatitis B virus infection.

This section is a review of the subject of HBV infection. It starts with a description of the causative agent (hepatitis B virus). This is followed by highlights on the global distribution of HBV infection, a description of the routes of transmission of HBV and then a discussion of the outcomes of HBV infection and factors that promote persistence of infection. At a later stage, highlights of the sequelae of HBV infection are made including the roles of HIV and delta virus. Identification of assays that can be used to detect hepatitis B antigens and antibodies is then done. Finally, a discussion of the measures that can be employed in the prevention of HBV infection is presented. Throughout the section, points that are of direct or indirect relevance to hepatitis B virus carrier state are re-emphasized as an attempt to shed more light on the subject.

1.4.1; The causative agent of hepatitis B infection.

Hepatitis B virus is a DNA-virus that belongs to the hepadnaviridae family [12]. Originally termed the DANE particle, the virus is composed of an inner core which is about 27 nm in diameter surrounded by a 7 nm thick envelope [46].
Present within the envelope and the viral core are a number of antigen/antibody systems. These are: a; Hepatitis B surface antigen (HBsAg) located in the outer envelope, b; Hepatitis c antigen (HBeAg) located in the viral core, and c; Hepatitis e antigen (HBeAg) located in the inner core. These antigens induce specific antibodies. HBsAg induces antibody to HBsAg (Anti-HBs), HBeAg induces antibody to HBeAg (Anti-HBe). These antibodies are specific in the sense that there is no cross-reactivity between them. Within the core are also found a double-stranded DNA and a DNA-dependent DNA polymerase. The antigen/antibody systems, the DNA and the DNA-dependent DNA polymerase form the markers of HBV infection. These markers can be used in the diagnosis and follow up of HBV infection and in vaccine development. They can be detected by serologic tests and other methods.

Among the notable biologic features of HBV is its striking tropism of liver cells and its propensity to cause persistent infection (chronic carrier state). Persistent infection is characterized by high concentrations of viral antigen consisting of incomplete or complete viral forms. These may remain in blood for many months or years.

Noteworthy also is the fact that the virus only has a limited "host range". It appears that the only reservoir or
source of virus for human infection is humanity itself [12]. No important animal reservoir is known. Although some higher primates other than man may be infected in nature, there is no evidence that they are important sources of human infections.

Outside the human host, the virus appears to maintain its stability for quite a long period. For instance, it is said that it is able to retain infectivity of humans for six months when stored in serum at 30-35 degrees centigrade and for 15 years when frozen at -20 degrees centigrade [47].

1.4.2; Global distribution of hepatitis B infection.

Hepatitis B is a disease of global distribution [1,48]. Based on prevalence studies, the world can be divided into three regions that correspond to three distinct geographic patterns of the prevalence of HBV infection [48,49,50]. These are: a; Low endemicity areas, b; Intermediate endemicity areas, and c; High endemicity areas. This distinction has significance in the control of HBV infection. Any strategy for control of HBV infection must therefore take it into consideration.

In high endemicity areas, the prevalence of chronic hepatitis B infection is 8 to 15 percent and the prevalence of total hepatitis B infection is over 60%. These areas are further subdivided into two patterns; one is characterized
by the significant proportions of infections that occur during the perinatal period and is found primarily in Asia, while the other is characterized by early childhood (horizontal) transmission of infections as is primarily seen in Africa. In Asia, mother to child transmission of HBV during perinatal period is a very important route of transmission. 8 to 15 percent of women of child bearing age have persistent infection with HBV [48], and 30-50% of these are HBeAg positive. Since HBeAg is highly correlated with infectivity, this high rate of HBeAg positivity increases the efficiency and the rate of transmission of HBV from the mothers to their newborn infants. In Africa, some parts of South America, the Middle East and the Pacific Islands, the rate of HBV chronic carriage is equally high among women of child bearing age (10% or more), but fewer than 20% are HBeAg positive. This makes perinatal transmission of HBV relatively less efficient in these parts of the world.

In intermediate endemicity areas, the prevalence of chronic HBV infection is 2-7% whereas that of total infection is 20-60%. These areas include Eastern Europe, Southern Europe, the former Soviet Union, Central Asia, Japan and Northern parts of South America. In these areas, the patterns of transmission generally represent a combination of perinatal, childhood and adult transmission.
Although early childhood infection is probably responsible for the maintenance of high rates of chronic infection, the highest rates of infection are probably among the older children, adolescents, and young adults [49].

North America, Western Europe, Australia, New Zealand and some parts of South America exhibit low endemicity of HBV infection. In these areas, the prevalence of chronic HBV infection is less than 2% and that of total infection is less than 20%. Transmission of HBV in these areas occurs primarily among adults with life-styles or behaviors that place them at high risk of infection [49]. Within these areas reside ethnic groups with HBV infection rates that are significantly higher than those of the general population and in whom patterns of infection are similar to those found in the high or intermediate endemicity populations. For example, In United States, perinatal transmission, which accounts for 15-20% of new HBV carriers, occurs primarily in minority groups, with Asians and blacks accounting for 60 and 15% of perinatal transmission, respectively [50].

1.4.3; Routes of transmission of hepatitis B virus.

Those people with persistent HBV infection form the primary reservoir for the virus whereas the acutely infected persons serve as temporary sources of infection since they have high levels of HBV in their blood. The
acutely infected persons are especially important sources of transmission in areas of intermediate and low endemicity of infection [50].

Although blood and blood products are the best documented sources of infectious virus, HBsAg has also been detected in feces, urine, bile, sweat, tears, saliva, semen, breast milk, vaginal secretions, cerebrospinal fluid, synovial fluid and cord blood. Thus, exposure to any of these materials through an appropriate route should put a person at risk of infection, at least on theoretical grounds.

Because of the aforementioned diversity of body fluids that contain HBsAg, HBV transmission may occur through a wide variety of exposures and routes. These include transmission through parenteral route, sexual transmission, transmission to infants or young children from contact with an infected person, and transmission from mother to infant at birth [51,52,53,54,55,56,57,58,59]. How much any of these routes contributes to the volume of HBV carriers varies with place. Some of these routes of transmission are further discussed below.

The primary vehicle for transmission is blood or serous fluids from HBV infected individuals. This may happen through blood transfusion, use of improperly or unsterilized injection or other skin piercing equipments,
direct transfer of infected materials on raw skin or mucus membrane, mother to child transmission via placenta, and other means.

Yet another important route of transmission is sexual means. This is a well-documented route of transmission, especially in developed countries. It may occur through homosexual or heterosexual route. In this regard, an increasing number of sexual partners and history of prior infection with syphilis have been associated with increased risk of HBV infection. Syphilis causes ulcerative lesions that would increase the efficiency of HBV infection. Syphilis could also be a surrogate marker for other chronic ulcerative genital diseases or for sexual contact with high risk persons.

Another important route is perinatal and early childhood transmission. Perinatal transmission may occur in two distinct settings: a; When the mother has acute hepatitis B in the third trimester or shortly thereafter, or b; When the mother is asymptomatic HBsAg carrier [55]. In the former situation, a very high proportion of infants become chronic HBsAg carriers [48,60], the rate of persistence of infection being indirectly related to age at primary infection. Early childhood transmission is due to a multiple of factors. This could be through person-to-person exposure or parenteral exposure to an infected body
fluid [61,62]. The presence of a household member who is a chronic HBV carrier is an important factor in childhood transmission of HBV.

1.4.4; Outcomes of acute hepatitis B virus infection:

A number of outcomes have been defined on the basis of serologic responses to primary HBV infection. In general, three main patterns of serologic response are known [12,20,63]. These are; a; A self-limited transient surface antigenemia, the most common of all classes of response, b; A self-limited primary antibody response without surface antigenemia, and c; Persistent HBV infection, the most important class of response to primary infection with HBV.

1.4.4.1; A self-limited transient surface antigenemia.

It is most commonly associated with symptomatic acute HBV infection. One to three months after exposure, HBsAg, the major viral capsid protein, becomes detectable. This antigen is the first marker of HBV infection to appear. The next marker to appear is HBeAg, being detectable in serum shortly after the appearance of HBsAg [12,63]. Presence of HBeAg is indicative of circulating DANE particles and is associated with high levels of infectivity [46,64,65,66]. The third marker to appear is the DNA polymerase, and can be detected in serum. This is also a marker for circulating virion. Anti-HBc is the fourth marker to appear following infection [20,63]. It appears in the serum of all infected
individuals, usually about a month after the appearance of HBsAg. The rise in aminotransferase levels is coincident with the appearance of IgM anti-HBc [63,67]. Anti-HBc typically remains detectable for years after uncomplicated infection though the titer is usually low [20,63]. In contrast, the titer of anti-HBc is usually high in persistent HBV infection [20,63]. The last marker to appear following a self-limited HBV infection is anti-HBs. This can be demonstrated in the serum of more than 90% of patients with primary infection with HBV [20,63] after the disappearance of HBsAg. Its appearance can however be quite delayed; Hoofnagle et al reported that based on the result of their follow up study, four months after the onset of symptoms, only 60% of patients will have detectable levels of anti-HBs [63]. This delay leads to a "serologic window", that is, a period between the disappearance of HBsAg and the appearance of anti-HBs during which anti-HBc may serve as the only marker of primary infection [20,63]. Once present, anti-HBs usually persists indefinitely and protects against subsequent attacks of acute hepatitis.

1.4.4.2; A self-limited primary antibody response without detectable HBsAg.

This pattern of serologic response is experienced by a significant fraction of patients with acute, self-limited primary HBV infection. In these patients, anti-HBs usually
appears 4-12 weeks after exposure (at about the time when HBsAg appears in patients with detectable HBsAg), and the titer typically rises rapidly to high levels and is sustained. Anti-HBc response is also detected though it usually appears only in low titer and may not persist. Mild elevations of serum transaminase levels are frequently documented. Patients are generally asymptomatic. Although this type of infection could lead to persistent HBV infection, available evidence indicates that it is self-limited because of seroconversion to anti-HBs, the transient nature of the liver damage, and the failure of subjects to maintain high titer of anti-HBc.

1.4.4.3; Persistent infection with HBV.

This is the third and most important type of response to primary infection with HBV. In this response, the early serologic events are similar to those in transient antigenemia. However, HBsAg is not cleared and anti-HBs is not detectable. There is also an Anti-HBc response, this antibody is found in virtually all persistent carriers, generally in high titer [63,68,69,70,71]. The Dane particle markers, HBeAg and DNA polymerase, remain detectable in a variable proportion of patients. The chronic carrier state is somewhat arbitrarily defined as sustained surface antigenemia persisting for longer than six months [12,20]. However, while most HBV infected persons clear the HBsAg by
six months, there is evidence which shows that some chronic carriers may experience a delayed clearance of HBsAg beyond seven months of follow up [72]. This has implications on the magnitude of the problem of HBV chronic carriage in the population being studied.

Table 1: Serologic Markers in Different Stages of hepatitis B Infection and Convalescence.

<table>
<thead>
<tr>
<th>Stage of HBV Infection</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HBc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late incubation period of hepatitis B</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acute hepatitis B</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HBsAg-negative acute hepatitis B</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Healthy HBsAg carrier</td>
<td>+</td>
<td>-</td>
<td>++++ +or-</td>
</tr>
<tr>
<td>Chronic hepatitis B</td>
<td>+</td>
<td>-</td>
<td>+++ +or-</td>
</tr>
<tr>
<td>HBV infection in the recent past</td>
<td>-</td>
<td>+</td>
<td>++ +or-</td>
</tr>
<tr>
<td>HBV infection in the distant past</td>
<td>-</td>
<td>+ or -</td>
<td>+or- -</td>
</tr>
<tr>
<td>Recent HB vaccination</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: An abstract from reference 12.

The definition just referred to requires serial testing of serum for HBsAg. While testing of sequential serum specimens is most helpful in diagnosing serologic status, frequently only a single specimen is available and a set of results requires interpretation. A summary of various combinations of serologic results along with possible interpretations is shown on table 1. It is however
important to note that the serologic status of an HBV infected person is dynamic in nature; individual markers appear and/or disappear during the evolution of infection, a picture not conveyed by the table.

Relevant to the present study is the suggestion made by Hoofnagle and others \([63,68]\). They proposed that differentiation of acute HB from the chronic carrier state can be done by tittering anti-HBc. That this antibody should yield a relatively low or moderate titer in early HB, but will be present in high titer in patients who are chronic carriers \([69,70]\). In addition, anti-HBs is not detectable in the sera of chronic carriers.

1.4.5; Factors that influence viral persistence.

Underlying the development of chronic carrier state are a multiple of factors. Overall, two classes of factors have been identified: those that affect the probability of exposure to HBV, and those that, given an exposure, affect the probability that a persistent infection will result. These factors are mainly host-dependent rather than virus-dependent.

Factors that affect the probability of exposure include: exposure to blood products, sexual behavior, socioeconomic level, and HBV status of household contacts \([56]\).

Factors that affect the probability of viral
persistence include age of the host at the time of primary HBV infection, the immunologic status of the individual, the severity of the primary infection, the size of the inoculum, the sex of the person, and probably genetic factors [20,73]. Some of these factors will be discussed further.

Age at primary infection is perhaps the single most important factor in the persistence of HBV. It is inversely related to the probability of development of carrier state [20,74]. The younger the individual at the time of primary infection, the higher the risk of becoming a chronic carrier. For instance, 70-90% of those individuals with acute infection in infancy develop persistent state of viral carriage. This declines to 6-10% in individuals with infection acquired after the fifth or sixth year of life [48]. Neonatal infection is therefore associated with the highest rates of persistence.

Immunologic status is also associated with viral persistence. It has been suggested that deficiency in immune status promotes development of HBV carriage [20,74]. This may take the form of either a failure to produce specific antibodies (B cell function) or a failure to develop sufficient cell-mediated immune response (T cell response). Indeed, normally functioning T cells are required for the clearance of HBV infection. The notion
that impaired immunity inhibits the elimination of HBV and thus promotes viral persistence is derived from several sets of observations. Most of the evidence however derives from the observations made on conditions characterized by immunologic deficits. It has been observed that these conditions are associated with high rates of HBsAg carriage. These are: chronic hemodialysis, Down’s syndrome, chronic lymphocytic leukaemia, and lepromatous leprosy. It is reported that the incidence of carriage is threefold higher among patients with lepromatous leprosy than among those with tuberculoid leprosy. Lepromatous leprosy is associated with impaired cell-mediated response.

Severity of a primary infection also has a bearing on persistence of HBV infection. Persistent infection occurs more frequently after initial anicteric hepatitis compared with initial icteric disease. Survivors of fulminant hepatitis rarely become persistently infected, and HBsAg carriers frequently have no history of recognized hepatitis [20].

Sex of a person: there seems to be sex predisposition to HBV chronic carriage. Virtually all studies of prevalence of HBsAg in populations reveal greater numbers of males than females among carriers; usually the male-to-female ratio is more than 2:1. However, the prevalence of HBsAg is affected by a variety of factors - including the
risk of exposure to HBV that could be related to sex. For example, in the United States, sexual transmission or person-to-person contact accounts for an almost equal number of infections among adolescent females and males, but in adults this risk factor accounts for twice as many infections in females as in males [50]. Thus, in contrast to adults in whom the male to female ratio is 2:1, in adolescents, females outnumber males 1.4 to 1.

The risk of HBV infection is also influenced by sexual activity. It increases with the number of sexual partners, number of years of sexual activity and history of sexually transmitted diseases [76].

Another factor of great importance is the well recognized risk of occupationally acquired HBV infection. This risk is associated with exposure to blood or potentially infected body fluids that are contaminated with blood. This is a special risk factor among health workers and those who work at blood banks and laboratories.

1.4.6; Sequelae of hepatitis B infection.

Studies of natural HBV infections in humans have shown that most primary infections in adults are self-limited and resolve completely within six months. Most infections also appear to be subclinical and are detected only by serologic testing and other methods. However, a fraction of infections fail to resolve, become persistent, and may
continue for many years with a possibility of consequences such as healthy carrier state, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [20,77,78,79,80].

The so-called "healthy" carrier state is usually associated with normal liver function, no detectable markers of HBV replication, and no detectable circulating virion. Healthy carriers also have no symptoms of hepatitis. On the other hand, patients with chronic HBV infection usually have elevated serum aminotransferase activities and liver biopsies reveal chronic inflammatory changes as well as liver cell necrosis. They may or may not be symptomatic, but most are seropositive for HBeAg, DNA polymerase, and HBV DNA [77]. Chronic hepatitis can further be differentiated on morphological grounds into chronic active hepatitis (CAH) and chronic persistent hepatitis (CPH).

CAH is a syndrome characterized by progressive hepatocellular dysfunction and destruction of liver tissue eventually leading to the development of cirrhosis and hepatic insufficiency. Although the onset of clinical manifestations may be insidious, some patients may experience gradual development of fatigue and anorexia followed by jaundice. Physical examination is likely to reveal positive findings such as jaundice, moderate to marked hepatomegaly, splenomegaly, and spider angiomata.
Ascites, edema, and hepatic encephalopathy are late features. The diagnosis of CAH is most firmly based on evidence of ongoing hepatic injury which has been present for at least six months combined with the finding on liver biopsy of characteristic histologic pattern.

CPH is a continuing inflammatory reaction in the liver characterized by mild hepatic enzyme abnormalities which persist for many months or years. These findings are not associated with irreversible destruction of liver tissue, cirrhosis, or other serious sequelae. Krugman and Gocke [78] suggested that since the syndrome of CPH often follows a recognized episode of acute hepatitis, it may simply represent a delayed recovery phenomenon or alternatively it could represent an inactive phase of the chronic active syndrome. Histological findings include minimal or absent hepatocellular damage, preserved lobular architecture of the liver, and only slight or absent fibrosis. Most notably, the piecemeal necrosis characteristic of CAH is lacking. Patients have persistent or recurrent SGOT and SGPT without jaundice. Physical examination findings often are completely normal, or there may be slight hepatomegaly with mild liver tenderness. Stigmata of advanced liver injury such as spider angiomata, splenomegaly, ascites, edema, and the like are absent.

The delta agent may be of importance in the
progression of the "healthy" carrier state. Chronic delta hepatitis usually occurs as superimposed hepatitis presenting commonly as unexplained exacerbation of previously stable or inactive chronic type B hepatitis.

Several immunological mechanisms are probably involved in the destruction of hepatocytes during acute and chronic HBV infection [67]. Most of the liver damage is probably caused by clearance of hepatocytes supporting replicating HBV. In some patients the clearance of hepatocytes may be protracted and these patients run the risk of developing liver cirrhosis. Serious or progressive hepatitis due to HBV is said to be rare in HIV infected individuals [81] as a result of immunological deficiency associated with AIDS. It is suggested that these immunological deficiencies may well lead to an improvement in the biochemical and histologic features of chronic type B hepatitis.

A late result of chronic HBV infection appears to be hepatocellular carcinoma [77,82,83]. HCC is a major fatal disease of man in many parts of the world, the majority of cases can be attributed to chronic hepatitis B infections. The causal link between HBV and HCC include: a, the distribution of HCC parallels that of chronic HBV infection; it is especially prevalent in Asia and Africa (areas of high endemicity of HBV infection); b, Case-control studies in most parts of the world have
consistently shown a higher prevalence of HB infection in cases than in matched or population controls; c, Evidence of HB infection is demonstrable in about 80% of HCC worldwide; d, Infection with HBV has been shown to precede the tumor by many years; e, HB viral DNA has been identified in many tumor tissues; and f, In animal models, HBV leads to HCC.

1.4.7; Infection with hepatitis Delta virus.

The delta virus is a unique agent that was first described by Rizzetto and coworkers in 1977 [84]. It belongs to the hepadnaviridae family [12]. Like other members of the hepadnaviridae family, HDV is hepatotropic [74,77] and may be associated with acute or chronic hepatitis.

Delta infection is most prevalent in the Mediterranean region, being common in Southern Italy [85], the Middle East, Northern parts of Africa, and parts of South America. It is rare in the United States and much part of Western Europe. In industrialized countries, delta infection is most common in HBsAg-positive persons who have had multiple parenteral exposures such as hemophiliacs and injection drug abusers [86]. Studies conducted in Sub-Saharan Africa have also revealed the existence of delta infection in this region [87].

Delta infection appears to represent a final mechanism
to liver damage in some patients during HBV infection. Simultaneous infection with HBV and HDV is said to lead to severe or fulminant hepatitis more often than infection with HBV alone. Due to a marked geographic variation in the prevalence of HDV, the role the agent plays in chronic liver disease is bound to vary accordingly. The agent plays a more prominent role in chronic liver disease in places where it is most common, for instance, in Italy. Due to the helper function provided to HDV by HBV, the damage done to the liver is dependent on HBV replication. If HBV replication is transient, the delta agent does not outlive HBV, and the harm done is limited. However, in the event of superinfection of an HBV carrier, the continued HBV replication provides conditions appropriate for the full expression of the delta agent in terms of liver damage.

The diagnosis of delta-associated chronic hepatitis can be done using serology and other methods [77]. In this condition, the agent may be demonstrated in the liver and/or its antibody (anti-HD) may be found in blood. Although separation of acute from chronic delta infections usually requires time, as a rule, anti-HD persists in high titer in chronic delta-associated HBV infections and finding delta agent in serum suggests acute infection, at least in chimpanzees [88].

1.4.8; Assays to detect HBV antigens and antibodies.
Methods to detect HB antigens and antibodies are very important because of their applications. The primary use of these tests is detection of HB antigens and antibodies for diagnosing human infections, to assess the prognosis or track convalescence, as well as to aid in HBV vaccine development [89].

In the case of vaccine development, it has been shown that anti-HBs levels correlate with immunity, so that assays to detect anti-HBs are among the most important assays. Quantitative evaluation of the immune response after immunization, by measurement of anti-HBs, is desirable to identify inadequate responders and non-responders [90]. Though an anti-HBs level of equal or more than 10 IU/L is considered protective, a good response is regarded as anti-HBs levels equal or more than 100 IU/L. This is usually followed by long term immunity. Persistence of anti-HBs is related to the maximum antibody response. Assays for HBsAg are important to quantitation of the actual vaccine antigen during manufacture and formulation. However, finding HBsAg in sera of vaccinees more than one month after vaccine administration is a marker of infection and probable vaccine failure. Measurement of anti-HBc is important in the conduct of successful vaccine trials. Since HBCAg is absent in all commercially available vaccines, the appearance of anti-HBc is considered as
concurrent HBV infection which is confirmable by detection of IgM isotype of anti-HBc.

A number of assays are now in use to detect HB antigens and antibodies. They have varying sensitivities. The present state-of-the-art radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA or EIA) methods remain the most sensitive, able to detect antibody and antigen concentrations as low as 0.5 ng/ml. Furthermore, the RIA and EIA are also specific and rapid in nature.

Assays to measure antibodies include: Immunodiffusion (ID), Counter electrophoresis (CIEF), Complement fixation (CF), Immune adherence hemagglutination (IAHA), Passive hemagglutination assay (PHA), Competitive radioimmunoassay, EIA and RIA mentioned in ascending order of sensitivity. Most of these methods are solid-phase based, the detection of antibody being highly dependent upon the affinity or avidity being determined. The affinity is the strength of binding an antibody to a single distinct antigen, while avidity is the net overall binding of a plurality of antibodies to a complex antigen with more than one antibody binding domain.

Tests for detection of HBsAg include: Agar gel diffusion, Counter electrophoresis, CF, Immunofluorescence, Platelet agglutination, Immune electron microscopy, Hemagglutination inhibition, Immune adherence, Latex
agglutination, Reverse passive hemagglutination, EIA, and RIA in ascending order of sensitivity [91]. The tests have been described in detail by others [86,89,91,92,93].

The improvement and commercialization of hepatitis tests during the last decade constitutes one of the most important advances in the field of hepatitis. As a result, numerous publications have come up based on utilization of HB antigens and antibodies as markers of HBV infection or immunity in case of the latter. For instance, finding of HBsAg in serum of an individual indicates that the person has HBV infection and potentially can transmit it to others. Donor blood that is HBsAg positive can transmit HBV to susceptible recipients. The finding of HBeAg in serum along with HBsAg shows that the individual is highly infectious. Similarly, all HBsAg-positive materials are potentially infectious for HB unless treated or inactivated by some means. Again, when HBeAg is present along with HBsAg, the material should be considered highly infectious.

As mentioned earlier, due to the significance of anti-HBs, tests to detect this antibody are among the most important assays. Anti-HBs is a specific antibody directed against HBsAg. It is generally an IgG antibody, and appears after exposure to HBsAg in the form of vaccine or following recovery from HBV. The presence of anti-HBs in an individual without associated history of viral hepatitis or
receipt of vaccine means that the person has had an inapparent infection with HBV at some point in life. Further, anti-HBs almost always indicates that an individual is immune to infection by HBV.

Anti-HBe is another antibody of clinical and immunological significance. In virtually all cases of acute HBV infection, anti-HBe is present just after the appearance of HBsAg, during the period when HBsAg persists, and when HBsAg clears. It can also be demonstrated in virtually all chronic carriers, usually in high titer.

1.4.9; Prevention of hepatitis B infection.

Several preventive measures are available to hinder the spread of HBV infection, and hence stop further development of chronic carrier state. These include the application of environmental measures to contain the virus and prevent exposure, and the use of passive and active immunization to render people resistant [12]. Of all these measures, immunization seems to be the most effective.

Two types of HB vaccines are currently in use, the plasma derived vaccine and the recombinant vaccine [1,82,89,94,95,96,97,98,99]. Both vaccines are equally effective, their efficacy being in excess of 90% after a three dose schedule and when given to healthy individuals [1,82,100,101,102]. Non-healthy individuals such as those immunocompromized may have poor responsiveness [103] and
may require more frequent boosters. The two vaccines are also equally safe [82,104]. They can be administered by themselves or in combination with HB immunoglobulin [82,94, 101,105,106].

HB vaccines that are currently in market are all based on immune response to HBsAg. Thus, following a successful vaccination, there is seroconversion to anti-HBs. It is important that a vaccine recipient gets an optimal seroconversion since anti-HBs levels correlate with immunity [89,90], and protection can only occur if the serum anti-HBs level is 10 mIU/mL or more, a level that is equivalent to 10 SRU by RIA or a positive test by ELISA [107]. In this regard, it is recommended that one might consider revaccinating an individual if the anti-HBS level falls below 10 mIU/mL or once 5 to 7 years after the initial course of infection [90].

Although an anti-HBs level of 10mIU/mL or more is considered protective, reinfections with HBV have been reported. Serologic findings in these reinfections are characterized by concurrent circulation of both HBsAg and anti-HBs, the antigen and antibody being of different subtypes.

While all HB vaccines are derived from HBsAg, it is also worthy to point out that HBsAg is a complex antigen [12]. At least five antigen specificities may be found on
HBsAg particles. A group-specific determinant "a" is shared by all HBsAg preparations. In addition, two pairs of subtype determinants (d,y and w,r) have been identified. This has led to identification of at least eight HBsAg subtypes (ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, and adr), an indication of the complexity of the antigen.

An antibody response to HBsAg represents the aforementioned complexity. The response may be group-specific or sub-type specific. An antibody response to the group determinant "a" usually protects against infection by all subtypes of HBV. Protection provided by a sub-type antibody is however limited to a specific HBsAg sub-type. Hence, a person having a sub-type antibody may still be infected by another HBsAg sub-type leading to concurrent circulation of both HBsAg and anti-HBs.

Two strategies for the control of HBV infection through immunization have been proposed. One is the high-risk group immunization and the other is universal child immunization [100]. There is however considerable evidence to suggest that high-risk group immunization, although medically indicated for many individuals, will not lead to control of HBV infection on a population basis [108], and that universal immunization is the only strategy that will lead to control of HBV infection in all regions of the world [49]. The immunization strategy that aims at target
groups such as pregnant women has associated problems especially in developing countries. First, it requires universal HBsAg screening of pregnant women, an exercise that can be very expensive. Second, it can only work if all cases of HBV infection have identifiable risk factors. This may not be true all the time [49]. Surveillance reports in United States have revealed that up to 30% of HBV cases in this country may not have identifiable risk factors, hence would be excluded by this strategy.

Besides control of HBV infection through immunization, there are other important and effective approaches. Among these are the use of environmental barriers and personal hygiene to prevent virus transmission. The principal modes of transmission that must be interrupted, at least in the United States appear to be: a; direct percutaneous inoculation of infective blood or serum (for example, by administration of contaminated blood or blood products, tattooing, ear piercing, acupuncture, drug abuse, accidental needle sticks in health personnel), b; contamination of mucosal surfaces by infective blood or serum (for example, mouth by pipettes, splashes in the eyes or transfer from the hands to the eyes or mouth) or by other infective secretions (by sexual or other activity involving semen or saliva), and c; transfer of such materials via vectors or environmental surfaces into skin.
breaks or mucus membranes (by contaminated objects such as toothbrushes, toys, razors, cups or glasses, towels or hospital equipments).

Finally, since HDV is dependent on the replication of HBV for its survival, prevention of HBV leads to prevention of HDV [14]. Patients immune to HBV, as a result of previous infection or HB vaccination, should be considered immune to delta hepatitis. On the other hand, patients who are susceptible to HBV should be considered susceptible to delta hepatitis as well.
2.0; METHODOLOGY.

2.1; Methods available to study the subject.

The main aim of the study is to document the sero-prevalence of HBV and HDV markers being experienced in Uganda during the current AIDS epidemic as an attempt to determine whether HIV infection / AIDS has the potential to promote the spread of HBV in populations with high prevalence of both HBV and HIV infections. My hypothesis is that HIV infection / AIDS promotes the development of hepatitis B chronic carrier state, and consequently an increase in the prevalence of delta virus infection. An increase in the number of chronic hepatitis B carriers in the society will put the non-infected persons at more risk for HBV infection because of more contact between the non-infected people and the carriers. The end result of this is a promotion of the spread of HBV infection in the society. This promotion of the spread of HBV infection is expected to be substantial especially in societies where both HBV and HIV infections have high prevalence. An increase in the number of carriers in the society ensures a continued replication of HBV in the society, a condition which is conducive for survival of delta virus. HIV infection / AIDS is therefore expected to promote the spread of delta virus especially in societies with high prevalence of both HBV
and HIV infections. The scenario described above is more or less a natural history event that is best studied using a prospective study design. However, cross sectional or prevalence studies can also be beneficial in this area.

2.1.1; Prospective studies.

The conventional definition of HBV chronic carriage state has been based on demonstrable persistence of hepatitis B surface antigen in serum for a period of 6 months or more [1,20,74]. This definition requires serial testing of sera for hepatitis B markers, an exercise that can only be accomplished through a follow-up study that extends over six months. While a prospective study design would make the best choice, it is not free from disadvantages. Those disadvantages have very important practical implications. For instance, serial testing of sera requires additional resources in form of seeing subjects at some intervals of time in order to draw fresh blood samples, and the costs of the laboratory tests to be performed on the samples. If the sample size is large and the follow up period is long, the amount of additional resources can be enormous.

Another problem that is commonly associated with prospective studies is loss to follow up of subjects. If substantial, can undermine the validity of the study results.
2.1.2; Cross sectional studies.

The present study encompasses doing serologic tests to determine the presence or absence of the serologic markers of HBV and HDV in the sera at the time the blood samples were taken. Cross sectional studies have long been used when serologic techniques are employed to measure various components in samples of blood drawn from individuals [109]. The end result is a measure of the prevalence of the serologic markers in consideration [109]. In this context, prevalence represents the presence of the serologic marker while the prevalence rate is the number of sera with that marker divided by the total number of persons whose blood was tested. For viral infections, the presence of antibody represents the cumulative infection rate over recent and past years depending on the duration of the antibody. If the antibody is of IgG type, which often lasts a lifetime, then the prevalence pattern will reflect infections from birth to the time of sampling. If it is of the IgM type, then the presence of the antibody indicates that the person was infected within a recent period.

Ensuing from above, it can be seen that using a cross sectional study design to distinguish between recent and chronic HBV infections may be coupled with some problems. One can however circumvent those problems by carrying out multiple tests on the same sample. Instead of relying only
on the presence of HBsAg to distinguish between recent and chronic HBV infections, measurements are also carried out of the exact titers of anti-HBs and anti-HBc. A distinction between recent and chronic HBV infection can then be made by defining HBV chronic carriage as a composite variable that takes into account the presence of HBsAg, anti-HBs and anti-HBc as well as the titer of anti-HBc. This method was proposed and has been used by Hoofnagle and others.

Cross sectional studies have advantages over follow up studies. First, they are associated with reduction in the high cost of repeated collection and testing of large numbers of blood samples that is done during the conduct of a prospective study. Secondly, since only one blood sample is taken, the study can be performed over a short period of time.

2.2: Method used and its limitations.

Based on practical reasons, a cross sectional study design was used. Two main points were against the use of a prospective study design: a; only a limited amount of funds was available to be spent on the study, an amount that was far less than that required for a prospective study. b; in accordance with the condition stipulated by the funding agency (Forgarty International), I had to complete my studies in no later than three years. The time period allowed for in-country field work is only one semester.
This therefore meant that any planned study had to be completed within a period of five months maximum. Since at least a six-months period would be required for the conduct of a prospective study, a decision was taken in favor of a cross sectional study.

As pointed out earlier, the use of a cross sectional study design to study the subject of HBV chronic carriage has some limitations. For instance, it is likely that the prevalence of HBV chronic carriage would be slightly over-estimated since some short term HBV infections may also be associated with high titers of anti-HBc. The results are therefore not as definitive as that obtainable from a prospective study design. Nevertheless, results from cross-sectional studies can still give very important insights into the subject under consideration.

2.3; Variables that were considered.

Variables refer to the characteristics of the subjects that were measured during the study. They were measured on either continuous or categorical scales.

The response or dependent variables consisted of markers of HBV and HDV infections. These are; HBsAg seropositivity, anti-HBs seropositivity, anti-HBc seropositivity, anti-HD seropositivity and HBeAg seropositivity. Using a combination of these markers, one composite dependent variable was defined - HBV chronic
carrier state. A subject was considered to be an HBV chronic carrier if the serum was positive for both HBsAg and anti-HBc but negative for anti-HBs. In addition, the anti-HBc had to have a high titer (a positive test at a dilution of 1:160 or higher).

The main independent variables were HIV status as defined by seropositivity of serum for HIV-1 using ELISA technique and CD4+ T-cell counts. The other independent variables fall into three major categories; the demographic characteristics, risk factors for HBV infection and clinical features of hepatitis B. These latter groups of independent variables are potential confounders [13,18,41, 45,110,111,112,113,114,115,116,117]. It is therefore important that they be controlled for during the analysis in order to avoid confounding bias.

The demographic characteristics were: age at last birthday, gender, ethnic group, religious affiliation, type of employment, and number of years spent in school.

The risk factors for HBV infection that were considered were: history of blood transfusion and number of blood units received, history of injection from outside a recognized health facility and number of injections received from there, history of traditional marks and the number of marks on the body, history of family member with hepatitis, history of sexually-transmitted diseases and its
timing. History of past recognized episode of HBV infection was also elicited.

Variables related to the clinical features of hepatitis B were: fever, loss of appetite, a feeling of general fatigue, pain in the right upper quadrant of abdomen and history of dark urine around the time of interview.

2.4: Operational definitions of terms used.

Exposure to HBV: A person is considered to have been exposed to HBV if the serum specimen is positive for HBsAg or anti-HBc or anti-HBs. Subjects falling under this category constitute the denominator for determination of the proportion of subjects with HBV chronic carrier state.

HBV chronic carrier state: When the serum specimen is positive for HBsAg, has high titer for anti-HBc, and is negative for anti-HBs. Anti-HBc positivity at a dilution level of 1:160 or higher is considered high titer.

HIV infected person: A person who tested positive for HIV-1 antibody using enzyme linked immunosorbent assay (Recombigen HIV-1 EIA, Cambridge Biotech Corporation).

Non-HIV infected person: A person who tested negative for HIV-1 antibody using EIA (Recombigen HIV-1 EIA, Cambridge Biotech Corporation).

HDV infection: A person is considered to have delta virus infection if the serum sample tests positive for
anti-HD by the criteria of Abbott Anti-Delta EIA.

2.5; Data extraction sheet design.

In order to ensure collection of information in a standardized manner, a structured questionnaire was designed and used (appendix I). The questionnaire consisted of thirty two questions. Most of the questions were closed-ended.

The questions were constructed such that the answers given would yield information on demographic characteristics, risk factors for HBV infection and clinical features of HBV infection.

Before the main study began, the questionnaire was subjected to pre-testing in order to determine its suitability. The pre-test involved administering the questionnaire to 20 patients from the same clinics where the main study was eventually conducted.

Through the pre-test, it was possible to test the questionnaire in regard to the logical sequence of the questions and the ease of translation into the local languages. It was also possible to assess the reaction of respondents especially to sensitive questions. Furthermore, the pre-test provided a forum for rehearsal that was necessary before the interviewers embarked on the main study.

Some lessons were learnt from the pre-test. For
instance, the pre-test revealed that answers to questions 31 and 32 were unreliable. These questions had aimed at finding out whether respondents had ever had sex for money, and if yes, the number of times this happened. Due to the unreliability of the answers, the two questions were left out from the main study.

2.6; The procedure for choosing the study populations.

The main aim of the study was to look at the subject of HBV chronic carriage in relationship to concurrent HIV infection. The procedure for choosing the study population was therefore such that it would allow selection of subjects who had developed or have had the opportunity to develop HBV chronic carriage. The study population should also be at risk of HIV infection.

2.6.1; Criteria for selection and exclusion of individuals from the study.

The main criteria for selection of subjects was age. This was to ensure that the subject had a reasonable likelihood of coinfection with both HIV and HBV. Results from prevalence studies conducted in Uganda indicate that those persons in the age groups 14 years and above are the ones most likely to have infection with both HBV and HIV-1. On this basis, a decision was taken to limit the study to those aged 14 years or more. Those aged less than 14 were therefore, to be excluded from the study.
2.6.2; Sampling procedures.

A systematic sampling procedure was used. Individuals were considered for recruitment into the study in order of their arrival at the clinics. All persons who attended Entebbe General Hospital’s outpatients’ and ante-natal clinics during the study period were asked to consent for the study provided they were aged 14 years or more. Subsequently, all persons who consented were interviewed and blood samples drawn.

There is no reason to believe that there was any underlying pattern in the order of arrival of individuals at the clinics. Thus, the study population should be equivalent to a random sample of individuals who usually attend hospital clinics [118].

In addition to the sample drawn from the hospital, about 270 respondents were recruited from the Uganda Virus Research Institute (UVRI) HIV testing clinic using the same sampling procedure. People who presented themselves for testing at the UVRI had three main motivating factors: a, HIV testing as a precondition to finalizing wedding arrangements (self seeking or sent by a religious leader); b, HIV testing being a requirement for applicants wishing to assume scholarships overseas (requested by Ministry of Education); and c, Personal interest to know one’s HIV status. Since those presenting at the UVRI HIV testing
center were already well motivated, the response rate at the center was 100%.

2.6.3; Reasons for non-response.

It was not possible to recruit each and every eligible person because of failure to get consent from some persons. Virtually all those who never consented said they were not interested in knowing their HIV status because of a possible psychological torture that may arise in case the result turns out to be positive. Some patients presented with emergency problems and therefore could not be interviewed.

There were however only a few non-respondents. In addition, non-respondents were not in any way different from those who consented for the study, hence no reason to believe that they could have led to some bias.

2.7; Laboratory tests.

Various laboratory tests were performed to detect the markers of HIV infection, HBV infection and HDV infection. Hepatitis tests were performed on sera that had been collected and stored most of the time at a temperature of -70 degrees C. Just before running the assays, the sera was allowed to attain room temperature.

2.7.1; HIV tests.

All samples were tested by at least one ELISA for HIV-1 antibody. Whenever a respondent sought to be informed of
test results, the WHO’s recommended test strategy II [119] was adopted. In addition, as a quality assurance measure, about 10% of the samples was retested using Immunoblot.

2.7.1.1; EIA for HIV-1.

Samples were tested using Recombigen HIV-1 EIA (Cambridge Biotech Corporation). The principles behind the test is as follows.

Diluted samples or controls (positive and negative) are added to pre-assigned antigen coated microwells. Antibodies to the ENV or GAG antigens, if present, will bind to the antigen coated wells. After removal of unbound antibody by aspiration and washing, enzyme-labelled anti-human antibody conjugate is added to each well and allowed to incubate. Unbound conjugate is removed by aspiration and washing and a substrate solution is added to all wells. A blue color will develop when HIV-1 antibodies are present; addition of a stop solution changes this color to yellow. The intensity of the color, read on a spectrophotometer, is proportional to the amount of antibodies bound to the microwell. A cutoff value is calculated to ease interpretation of results. The cutoff value equals the average of the positive controls times 0.4. Specimens with absorbance values less than the cutoff value are considered not reactive by the criteria of Recombigen HIV-1 EIA and may be interpreted as negative. Specimens with absorbance
values greater than or equal to the cutoff value are considered initially reactive by the criteria of Recombigen HIV-1 EIA.

Specimens that were initially reactive were interpreted as HIV-1 positive. Recombigen HIV-1 EIA is a sensitive and specific method, its sensitivity is 100% and specificity is 99.97%.

2.7.1.2; HIV-1 Immunoblot.

Ten percent of the samples were tested using Novapath TM HIV-1 Immunoblot (Bio-Rad). Novapath TM HIV-1 Immunoblot is an in-vitro qualitative assay for the detection of antibodies to individual proteins of HIV-1 in human serum or plasma.

To perform the assay, Wash/Diluent and the specimen are incubated with nitrocellulose strips; HIV-1 antibody present in the sample will bind to viral antigens located on the strip as discrete bands. Unbound material is aspirated, the strip is washed and alkaline phosphatase (AP) enzyme-linked antibody to human immunoglobulin is added. After aspirating the unbound enzyme-antibody conjugate, the strip is washed and an AP substrate is added. Aspiration followed by another wash terminates the enzyme reaction. If antibodies to any of the major HIV-1 antigens are present in the specimen in sufficient concentrations, bands corresponding to the position of one
or more of the following HIV proteins (p) or glycoproteins (gp) will be seen on the nitrocellulose strip: p18, p24, p32, gp41, p51, p55, p65, gp120 (numbers refer to apparent molecular weight in kilodaltons).

The presence or absence of antibodies to HIV-1 in a sample, and the identity of any antibodies present is determined by comparison of each nitrocellulose strip to the strips used for the NON-REACTIVE and WEAKLY REACTIVE CONTROLS tested with that run, and the strip used for the STRONGLY REACTIVE CONTROL tested once with that kit. The immunoblot is then interpreted as NEGATIVE, INDETERMINATE, or POSITIVE based on the pattern which is present. Positive when there is reaction to at least one major product of each of the structural genes GAG, POL and ENV. The bands must also have the scores of + or greater. Indeterminate when with any pattern of one or more reactive bands that does not meet the positive criteria. Negative when there is no reactive band on the strip.

2.7.2; CD4+ T cell enumeration (Coulter Clone Rosetting/Aggregation Assay).

CD4+ T cell enumeration was performed using a manual method (CD4 Cytospheres) as described below: Two tubes were initially labelled, one as "CD4" and the other with sample number. 100 microliter of blood sample was put into one tube, 10 microliter of monocyte blocking reagent added to
it and the tube shaken to mix the content. 10 microliter of cytosphere reagent was added to the above mixture and the tube further shaken to ensure a thorough mixing. 100 microliter of lysing solution was pipetted into the second tube. 10 microliter of the mixture from the first tube was added to the lysing solution in the second tube and the tube shaken for about 10 to 15 seconds. Both chambers of the Neubauer were then loaded with the solution from tube 2 to allow counting. Cells were allowed to settle for two minutes in the chambers before the counting was finally done under a light microscope (40X Objective). Lymphocytes with three or more cytospheres were counted as CD4+ cells. The results were expressed as total number of positive lymphocytes multiplied by a factor 7.3. The normal range of CD4+ cells is 500 to 1800 per microliter of specimen or 0.5 to 1.8 cells per liter.

2.7.3; Serologic tests for HBV and HDV markers.

Serum samples were tested with commercial RIA or EIA reagents supplied to the US Centers for Disease Control and Prevention by Abbott laboratories: AUSRIA II for HBsAg, AUSAB for Anti-HBs, CORAB for Anti-HBc, ABBOTT HBe for HBeAg and ABBOTT Anti-Delta for Anti-Delta. Procedures for the assays are described below.

2.7.3.1; AUSRIA-II-Radioimmunoassay for detection of HBsAg.

This is a qualitative third generation RIA for the
detection of HBsAg in serum or plasma. 200 microliter of specimens and controls were pipetted into appropriate wells of reaction trays. There were 7 negative controls, 3 positive controls and 1 in-house control. One anti-HBs coated bead was added to each well containing a sample or control and the reaction trays sealed and incubated at room temperature (10 to 15 degrees centigrade) for 16 hours. After the 16 hours, the beads were washed in distilled water and further incubated in 200 microliter of $^{125}$I-anti-HBs for 1 hour at 45 degrees C. Thereafter, the beads were washed and transferred to appropriate counting tubes. The results were then read on a gamma counter. The presence or absence of HBsAg was determined by relating net counts per minute (cpm) of the unknown to the cutoff value. The cutoff value was the net cpm of the negative control mean times a factor 2.1. Specimens with cpm equal to or greater than the cutoff value were considered reactive for HBsAg.

2.7.3.2; AUSAB Enzyme Immuno Assay for Quantitation of Antibody to HBsAg.

Quantitation of anti-HBs was done using AUSAB-EIA (Abbott laboratories). The procedure was as follows. 200 microliter of samples or controls (3 negative and 2 positive) were pipetted into assigned wells followed by addition of HBsAg coated beads to all wells containing either the sample or control. The reaction tray was sealed
and then incubated at room temperature for 18 hours. After the initial incubation, the beads were washed with distilled water and further incubated in 200 microliter of a solution containing 0.11 mL of Biotin (B-HBsAg) and 0.11 mL of Peroxidase Conjugate (Anti-B-HRPO) at 40 degrees C for two hours. Thereafter, the beads were washed and transferred to appropriate assay tubes and then incubated in 300 microliter of freshly prepared OPD substrate solution at room temperature for 30 minutes. 1 mL of 1 N Sulfuric acid was then added to each tube, the tubes placed on the Abbott QUANTAMATIC Spectrophotometer and results read. The results were expressed in mIU/mL.

One drawback of this assay is its inability to give specific values of anti-HBs if it is in excess of 150mIU/mL. Any results in excess of 150mIU/mL is reported as >150mIU per mL.

Anti-HBs quantitation is a useful tool to monitor vaccinees [82,107] and to establish the approximate time for revaccination [120].

2.7.3.3; CORAB Radioimmunoassay for detection of Anti-HBc.

CORAB is a radioimmunoassay for the qualitative determination of total antibody to HBCAg in human serum or plasma. The method was used in the following manner. 100 microliter of 125I antibody was put into every marked well of reaction trays and then mixed with 100 microliter of
specimen or control (3 negative, 2 positive and 1 in-house). HBCAg coated beads were added to the wells and the trays incubated at room temperature for 20 hours. After the incubation, the beads were washed, transferred to appropriate counting tubes and the results read on the Abbott ANSR gamma counter. The results were expressed by cpm. The presence or absence of anti-HBc was determined by comparing the net cpm to a cutoff value. Specimens with cpm greater than the cutoff value were considered negative by the criteria of CORAB.

HBsAg positive samples that were also positive for anti-HBc were subjected to further testing (titration of anti-HBc). A two-fold serial dilution of the samples was performed followed by CORAB RIA as described above. The titer of anti-HBc was expressed by the highest dilution of test serum that exhibited reactivity.

2.7.3.4; Abbott HBe (rDNA).

This RIA is indicated for qualitative or semi-quantitative detection of HBeAg and/or anti-HBe in serum or plasma. The method was used to detect HBeAg in sera that were positive for HBsAg by the criteria of AUSRRIA II.

300 microliter of each specimen and control (3 negative and 2 positive) was put into appropriate wells. One anti-HBe coated bead was added to each well, the reaction trays sealed and then incubated at room
temperature for 20 hours. Thereafter, the beads were washed and further incubated in 200 microliter of anti-HBe $^{125}$I for three hours at 45 degrees C. The beads were then washed again, transferred to assay tubes and the results read on a gamma counter. The presence or absence of HBeAg was determined by comparing net cpm of the unknown specimen to a cutoff value (negative control mean times 2.1). Unknown specimens whose net count rates were equal to or greater than the cutoff value were considered reactive for HBeAg.

2.7.3.5; Abbott Anti-Delta Enzyme Immunoassay.

This is an in vitro EIA for the qualitative determination of total anti-HD in human serum or plasma. The procedure was as follows. 100 microliter of each specimen or control (3 negative and 2 positive) was put into appropriate wells of reaction trays. One hepatitis delta antigen coated bead was added to each well containing specimen or control, the reaction trays sealed and then incubated for 20 hours at room temperature. The initial incubation period was followed by a wash of all beads and a further incubation of the beads in 300 microliter of OPD substrate solution at room temperature for 30 minutes. Finally, 1 mL of 1 N Sulfuric acid was added to each tube and the results read on an EIA Spectrophotometer.

The presence or absence of anti-delta was determined by comparing the absorbance of the specimen to a cutoff
value. This cutoff value was calculated from the negative control mean and positive control mean absorbance. Specimens with absorbance values less than or equal to the cutoff value were considered reactive for anti-HD by the criteria of Abbott Anti-Delta EIA. Those specimens with absorbance values higher than the cutoff value were considered negative.

Determination of anti-HD in serum or plasma was the assay of choice for the diagnosis of HDV infection since hepatitis D antigen is generally detectable only in the liver, and is transient or non-detectable in serum [121].

2.7.4; Quality assurance for laboratory tests.

For the results to be valid, it is desirable that the laboratory tests used be reproducible, specific (with few false positives) and sensitive (with few false negatives). Specificity and sensitivity are indices of the accuracy of measurements.

To get some sense of the quality of the tests that were performed, a test-retest procedure was adopted. 10% of the samples were retested for the serologic markers of HIV and HBV.

The retests showed that the test procedures were very reliable. The initial HIV test results were confirmed with 100% sensitivity and 100% specificity. Similar results were obtained with repeat hepatitis tests.
2.8; Data processing and analysis.

EPIINFO version 5 was the main database. After coding, data was entered into an Epiinfo REC file on an IBM compatible personal computer. This was followed by data cleaning. Thereafter, statistical analysis was performed.

Statistical evaluation of the difference in proportions of various groups of the categorical variables was performed using chi square test or Fischer’s exact test. Subsequently, logistic regression analysis was performed to assess the simultaneous effects of the independent variables, using SPSS/PC+ version 3.1. For continuous variables, statistical evaluation of the difference in the means of various groups was performed using the Student’s t-test, and where normality assumption was violated, an equivalent non-parametric test was used.
3.0; RESULTS.

3.1.1; Characteristics of the study population.

A total of 1506 respondents were recruited for the

Table 2; Demographic Characteristics of 1500 Respondents.

<table>
<thead>
<tr>
<th>Characteristic of the respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: Females</td>
<td>1142</td>
<td>76.1</td>
</tr>
<tr>
<td>Males</td>
<td>358</td>
<td>23.9</td>
</tr>
<tr>
<td>Age: 0 to 9 years</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>10 to 19</td>
<td>334</td>
<td>22.3</td>
</tr>
<tr>
<td>20 to 29</td>
<td>807</td>
<td>53.8</td>
</tr>
<tr>
<td>30 to 39</td>
<td>272</td>
<td>18.1</td>
</tr>
<tr>
<td>40 to 49</td>
<td>60</td>
<td>4.0</td>
</tr>
<tr>
<td>50 to 59</td>
<td>9</td>
<td>0.6</td>
</tr>
<tr>
<td>60 to 69</td>
<td>9</td>
<td>0.6</td>
</tr>
<tr>
<td>Did not know their ages</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td>Religious Affiliation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catholic</td>
<td>667</td>
<td>44.4</td>
</tr>
<tr>
<td>Church of Uganda</td>
<td>550</td>
<td>36.7</td>
</tr>
<tr>
<td>Islam</td>
<td>204</td>
<td>13.6</td>
</tr>
<tr>
<td>Orthodox Church</td>
<td>13</td>
<td>0.9</td>
</tr>
<tr>
<td>Others</td>
<td>66</td>
<td>4.4</td>
</tr>
<tr>
<td>Employment for Money:</td>
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<td></td>
</tr>
<tr>
<td>Employed</td>
<td>577</td>
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</tr>
<tr>
<td>Unemployed</td>
<td>923</td>
<td>61.5</td>
</tr>
<tr>
<td>Occupation:</td>
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<td></td>
</tr>
<tr>
<td>Medical</td>
<td>32</td>
<td>2.1</td>
</tr>
<tr>
<td>Policeman or Soldier</td>
<td>41</td>
<td>2.8</td>
</tr>
<tr>
<td>Teachers, Self employed etc.</td>
<td>512</td>
<td>34.1</td>
</tr>
<tr>
<td>None</td>
<td>915</td>
<td>61.0</td>
</tr>
<tr>
<td>Ever been to school:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1365</td>
<td>91.0</td>
</tr>
<tr>
<td>No</td>
<td>135</td>
<td>9.0</td>
</tr>
<tr>
<td>Ethnic Group:</td>
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<td></td>
</tr>
<tr>
<td>Bantu</td>
<td>1321</td>
<td>88.1</td>
</tr>
<tr>
<td>Nilo-hamite</td>
<td>156</td>
<td>10.4</td>
</tr>
<tr>
<td>Others</td>
<td>23</td>
<td>1.5</td>
</tr>
</tbody>
</table>
study. A summary of the demographic characteristics of 1500 of these respondents is presented on table 2. Six respondents do not appear on this table because of missing data collection forms.

The results show that most of the subjects were females, over 90% belonged to the age group 10 to 39, 81% were christians, 61.5% were employed, 91% had spent at least a year schooling and 88.8% were of bantu origin. The age range was 4 to 66 years with a mean of 25.27 and standard deviation of 7.55. The range for years spent in school was 1 to 24 with a mean of 8.48 and standard deviation of 3.93.

The final sample consists of 1429 respondents. Of the 1506 blood specimens drawn earlier, 77 (5%) never came from Uganda and therefore never underwent hepatitis tests. For this reason, they have been excluded from the final sample.

The demographic characteristics of the 1429 and 77 respondents are presented on tables 3 and 4 respectively. Comparison of the two tables indicate that both groups are more or less similar in their demographic characteristics.

3.1.2; HIV status and level of immune depression.

Out of the original 1506 respondents, 429 (28.5%) were HIV-1 positive by the criteria of Bio-Rad Recombigen HIV-1 EIA. There was no significant statistical difference in HIV seropositivity rate by sex. There was however a correlation
between HIV status and past history of sexually transmitted diseases. Those with a positive history of STD were more likely to be HIV positive (odds ratio of 2.40 with a confidence interval of 1.89, 3.05).

Table 3: Demographic Characteristics of 1423 Respondents Whose Blood Samples Were Analyzed.

<table>
<thead>
<tr>
<th>Characteristic of the respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
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<td></td>
</tr>
<tr>
<td>Females</td>
<td>1081</td>
<td>75.0</td>
</tr>
<tr>
<td>Males</td>
<td>342</td>
<td>24.0</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 9 years</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>10 to 19</td>
<td>320</td>
<td>22.5</td>
</tr>
<tr>
<td>20 to 29</td>
<td>771</td>
<td>54.2</td>
</tr>
<tr>
<td>30 to 39</td>
<td>251</td>
<td>17.6</td>
</tr>
<tr>
<td>40 to 49</td>
<td>58</td>
<td>4.1</td>
</tr>
<tr>
<td>50 to 59</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>60 to 69</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>Did not know their ages</td>
<td>6</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Religious Affiliation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catholic</td>
<td>636</td>
<td>44.7</td>
</tr>
<tr>
<td>Church of Uganda</td>
<td>521</td>
<td>36.6</td>
</tr>
<tr>
<td>Islam</td>
<td>190</td>
<td>13.4</td>
</tr>
<tr>
<td>Orthodox Church</td>
<td>13</td>
<td>0.9</td>
</tr>
<tr>
<td>Others</td>
<td>63</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>Employment for Money:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>546</td>
<td>33.4</td>
</tr>
<tr>
<td>Unemployed</td>
<td>877</td>
<td>61.6</td>
</tr>
<tr>
<td><strong>Occupation:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>29</td>
<td>2.0</td>
</tr>
<tr>
<td>Policeman or Soldier</td>
<td>40</td>
<td>2.8</td>
</tr>
<tr>
<td>Teachers, Self employed etc.</td>
<td>486</td>
<td>34.2</td>
</tr>
<tr>
<td>None</td>
<td>868</td>
<td>61.0</td>
</tr>
<tr>
<td><strong>Ever been to school:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1298</td>
<td>91.2</td>
</tr>
<tr>
<td>No</td>
<td>125</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>Ethnic Group:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bantu</td>
<td>1261</td>
<td>83.6</td>
</tr>
<tr>
<td>Nilo-hamite</td>
<td>147</td>
<td>10.3</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>1.1</td>
</tr>
</tbody>
</table>
The range for CD4+ T cell count was 7 to 1132 with a mean of 273.87 and standard deviation of 189.10. Most subjects had counts below the normal range, about 77% of

Table 4: Demographic Characteristics of 77 Respondents Whose Blood Samples Never Came From Uganda.

<table>
<thead>
<tr>
<th>Characteristic of the respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>61</td>
<td>79.2</td>
</tr>
<tr>
<td>Males</td>
<td>16</td>
<td>20.8</td>
</tr>
<tr>
<td>Age: 0 to 9 years</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>10 to 19</td>
<td>14</td>
<td>18.2</td>
</tr>
<tr>
<td>20 to 29</td>
<td>36</td>
<td>46.7</td>
</tr>
<tr>
<td>30 to 39</td>
<td>21</td>
<td>27.3</td>
</tr>
<tr>
<td>40 to 49</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>50 to 59</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>60 to 69</td>
<td>2</td>
<td>2.6</td>
</tr>
<tr>
<td>Did not know their ages</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Religious Affiliation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catholic</td>
<td>31</td>
<td>40.3</td>
</tr>
<tr>
<td>Church of Uganda</td>
<td>29</td>
<td>37.7</td>
</tr>
<tr>
<td>Islam</td>
<td>14</td>
<td>18.2</td>
</tr>
<tr>
<td>Orthodox Church</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>Employment for Money:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>31</td>
<td>40.3</td>
</tr>
<tr>
<td>Unemployed</td>
<td>46</td>
<td>59.7</td>
</tr>
<tr>
<td>Occupation:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>3</td>
<td>3.9</td>
</tr>
<tr>
<td>Policeman or Soldier</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Teachers, Self employed etc.</td>
<td>26</td>
<td>33.8</td>
</tr>
<tr>
<td>None</td>
<td>47</td>
<td>61.0</td>
</tr>
<tr>
<td>Ever been to school:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>67</td>
<td>87.0</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>13.0</td>
</tr>
<tr>
<td>Ethnic Group:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bantu</td>
<td>71</td>
<td>92.2</td>
</tr>
<tr>
<td>Nilo-hamite</td>
<td>4</td>
<td>5.2</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Note: 26% or 20 out 77 of these respondents were HIV positive.
samples that underwent CD4 count had counts of 400 per microliter or less. Only 12.9% of subjects had CD4+ T cell counts above 500/microliter.

3.1.3; Risk factors for hepatitis B virus infection.

Table 5 depicts risk factors for HBV infection. It shows that 29.5% of respondents reported that they have

Table 5; Risk Factors for Hepatitis B Virus Infection.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has had blood transfusion:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57</td>
<td>3.8</td>
</tr>
<tr>
<td>No</td>
<td>1443</td>
<td>96.2</td>
</tr>
<tr>
<td>Past history of hepatitis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>442</td>
<td>29.5</td>
</tr>
<tr>
<td>No</td>
<td>1051</td>
<td>70.1</td>
</tr>
<tr>
<td>No idea</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Had sexually transmitted disease:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>523</td>
<td>34.9</td>
</tr>
<tr>
<td>No</td>
<td>977</td>
<td>65.1</td>
</tr>
<tr>
<td>History of hepatitis in family:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>409</td>
<td>27.3</td>
</tr>
<tr>
<td>No</td>
<td>1090</td>
<td>72.6</td>
</tr>
<tr>
<td>No idea</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>Had traditional marks (tattoos):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>72</td>
<td>4.8</td>
</tr>
<tr>
<td>No</td>
<td>1425</td>
<td>95.0</td>
</tr>
<tr>
<td>No idea</td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>Has had injection outside hospital, health center or dispensary:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>864</td>
<td>57.6</td>
</tr>
<tr>
<td>No</td>
<td>636</td>
<td>42.4</td>
</tr>
</tbody>
</table>

ever experienced hepatitis, 3.8% have had blood transfusion, 34.9% have had sexually transmitted diseases, 27.3% reported occurrence of hepatitis in a family member,
4.8% had traditional marks on their bodies and 57.6% admitted receiving injections from a place other than a hospital, health center or dispensary.

3.1.4: Clinical manifestations of HBV infection.

An attempt was made to elicit signs and symptoms that could be related to acute or chronic active HBV infection. Subjects were asked whether they were experiencing fever, fatigue, passage of dark urine, pain at the right hypochondrium or poor appetite, at the time of interview. In response, 22.7% said they had fever, 28.9% reported a feeling of fatigue, 18.1% reported passage of yellow or Table 6; Clinical features that could be related to HBV infection (acute or chronic active hepatitis).

<table>
<thead>
<tr>
<th>Characteristic of the subject</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>With fever at interview time:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>340</td>
<td>22.7</td>
</tr>
<tr>
<td>No</td>
<td>1160</td>
<td>77.3</td>
</tr>
<tr>
<td>With general malaise:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>433</td>
<td>28.9</td>
</tr>
<tr>
<td>No</td>
<td>1067</td>
<td>71.1</td>
</tr>
<tr>
<td>With yellow or dark urine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>272</td>
<td>18.1</td>
</tr>
<tr>
<td>No</td>
<td>1169</td>
<td>77.9</td>
</tr>
<tr>
<td>No idea</td>
<td>59</td>
<td>4.0</td>
</tr>
<tr>
<td>Pain at the right hypochondrium:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>202</td>
<td>13.5</td>
</tr>
<tr>
<td>No</td>
<td>1298</td>
<td>86.5</td>
</tr>
<tr>
<td>With poor appetite:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>333</td>
<td>22.2</td>
</tr>
<tr>
<td>No</td>
<td>1167</td>
<td>77.8</td>
</tr>
</tbody>
</table>

dark urine, 13.5% said they had pain at the right
hypochondrium and 22.2% had poor appetite.

3.2.0; Prevalence of markers of hepatitis B infection.

Sera was tested for various markers of hepatitis B infection. The results of this is presented on table 7. The table shows that 1429 samples were tested for HBsAg. Of these, 225 (15.7%) were positive for HBsAg. Of the 1416

Table 7: Prevalence of Hepatitis B Markers in the Study Population.

<table>
<thead>
<tr>
<th>Marker of HBV</th>
<th>No. positive/No. tested</th>
<th>Prevalence of HBV Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>225/1429</td>
<td>15.7%</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>765/1416</td>
<td>54.0%</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>603/1404</td>
<td>42.9%</td>
</tr>
<tr>
<td>Total Infection</td>
<td>956/1429</td>
<td>66.9%</td>
</tr>
</tbody>
</table>

samples tested for anti-HBs, 765 (54.0%) had positive test results. Of 1404 subjects on whom determination of anti-HBc was performed, 603 (42.9%) tested positive for the marker. The results further show that, of 1429 subjects on whom hepatitis tests were performed, 956 (66.9%) tested positive for at least one marker of HBV infection. This group represents total HBV infection in the study population.

3.2.1; Prevalence of a protective level of anti-HBs among those subjects with detectable levels of anti-HBs.

Subjects who had detectable levels of anti-HBs were further subdivided according to whether or not they had a "protective level" of anti-HBs (figure 1). A protective
level of anti-HBs is defined as a level of anti-HBs equal to or more than 10 mIU/mL of specimen [82,107,122,123, 124]. Of the 765 subjects with detectable levels of anti-HBs, 493 (64.4%) had protective levels of the antibody. Of these 493 subjects with protective levels of anti-HBs, 312 (63.3%) had levels of more than 150mIU/mL of specimen.

Figure 1; Protective Level of Anti-HBs (Prevalence in those with detectable levels of Anti-HBs).
3.2.2; Sex-specific prevalence of markers of HB infection.

Table 8 represents a breakdown of the prevalence of hepatitis B markers by gender. The result shows that males had a higher prevalence of HBsAg (19% compared to 14.8% in females). A similar result was observed for anti-HBc, 46.2% of males and 41.8% of females were positive for anti-HBc respectively. The reverse was true in case of anti-HBs,

**Table 8: Sex-specific Prevalence of Hepatitis B Markers.**

<table>
<thead>
<tr>
<th>Marker of HBV</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>65/342 (19.0%)</td>
<td>160/1081 (14.8%)</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>172/335 (51.3%)</td>
<td>587/1075 (54.6%)</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>154/333 (46.2%)</td>
<td>445/1065 (41.8%)</td>
</tr>
<tr>
<td>Total Infection</td>
<td>231/342 (67.5%)</td>
<td>719/1081 (66.5%)</td>
</tr>
</tbody>
</table>

*Note: For HBsAg $x^2=3.45, P=0.06$; For Anti-HBs $x^2=1.09, P=0.30$; For Anti-HBc $x^2=2.06, P=0.15$; and For Total HBV infection $x^2=0.12, P=0.72$;*

the prevalence was lower in males (51.3% compared to 54.6% in females). In case of total HBV infection, males had a slightly higher prevalence, 67.5% compared to 66.55 in females. None of the gender differences was statistically significant. When the rates were adjusted for differences in the age compositions of the two sexes, using direct standardization, similar results were obtained. Comparison of sex/age-specific prevalences had however revealed that, females had higher rates in the younger age categories.
3.2.3; Prevalence of markers of hepatitis B by HIV status.

Analysis was performed to see if there is a correlation between HIV sero-status and prevalence of HBV markers. The results (table 9) show that those sero-positive for HIV-1 were more likely to test positive for HBsAg, anti-HBs and anti-HBc. Similarly, presence of at least a marker of HBV was more likely in those positive for

Table 9: Prevalence of Hepatitis B Markers by HIV Status.

<table>
<thead>
<tr>
<th>Marker of HBV</th>
<th>Positive for HIV</th>
<th>Negative for HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>76/409(18.6%)</td>
<td>149/1020(14.6%)</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>233/408(57.1%)</td>
<td>532/1008(52.8%)</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>224/404(55.4%)</td>
<td>379/1000(37.5%)</td>
</tr>
<tr>
<td>Total Infection</td>
<td>299/409(73.1%)</td>
<td>657/1020(64.4%)</td>
</tr>
</tbody>
</table>

Note: For HBsAg $x^2=3.48, P=0.06$; For Anti-HBs $x^2=2.19, P=0.14$; For Anti-HBc $x^2=36.15, P<0.00001$; For Total HBV infection $x^2=9.96, P=0.002$.

HIV-1. The differences in prevalence rates between HIV positive and HIV negative individuals were however only statistically significant for anti-HBc and total HBV infection. Adjustment of above rates to take into account the variation in age compositions of the HIV negative and HIV positive individuals produced similar results.

3.2.4; Age-specific prevalence of markers of hepatitis B infection.

Previous results have highlighted the importance of
age as a determinant of prevalence of HBV infection. In order to determine whether age is an important factor in the present study, the prevalence of HBV markers was stratified by age (table 10). Age was categorized into three groups, 20 years or less, 21 to 40 years and 41 years and above. Using a $x^2$ test for linear trend, the results show that there is a dose-response relationship between age and prevalence of HBV markers. For instance, as age increases, the prevalences of anti-HBs, anti-HBc and total HBV infection also increase. An opposite trend was however observed in the case of the prevalence of HBsAg, the lower the age, the higher the prevalence of HBsAg and vice versa. The differences seen were statistically significant for the

<table>
<thead>
<tr>
<th>HBV marker</th>
<th>20 yrs or less</th>
<th>21 to 40 yrs</th>
<th>41 years and above</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>76/456 (16.7%)</td>
<td>145/909 (16.0%)</td>
<td>4/52 (7.7%)</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>226/455 (49.7%)</td>
<td>499/897 (55.6%)</td>
<td>33/52 (63.5%) *</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>163/450 (36.2%)</td>
<td>400/890 (45.0%)</td>
<td>34/52 (65.4%) *</td>
</tr>
<tr>
<td>Total HBV Infection</td>
<td>290/456 (63.6%)</td>
<td>626/909 (68.9%)</td>
<td>37/52 (71.2%) *</td>
</tr>
</tbody>
</table>

Note: For HBsAg $x^2=1.17, P>0.05$; For Anti-HBs $x^2=6.21, P<0.05$; For Anti-HBc $x^2=18.28, P<0.001$; For Total HBV infection $x^2=4.05, P<0.05$. |
prevalences of anti-HBs, anti-HBc and total HBV infection.

Figure 2 is a diagramatic representation of the age-specific prevalence of HBV markers. Instead of the three age groups used in the earlier analysis, five age categories were adopted. If the age-group 50 or more is ignored, the results show that prevalences of anti-HBs and anti-HBc increase with age. The prevalence of HBsAg however remains more or less constant with increasing age until after the age of 39 when it dips down. The number of respondents in the age group ignored is so small. The small number may lead to some distortion of the true picture of the effect of age on the prevalence of HBV markers.

3.2.4.1; Association between HBsAg sero-positivity and the demographic characteristics.

Since prevalence of HBsAg sero-positivity is the key outcome variable in the present study, it was subjected to a much more detail analysis. In this analysis, the effects of respondent's occupation, ethnic background, known risk factors of HBV infection and presence of clinical features of HBV infection were investigated. This involved both univariate and multivariate analyses.

Table 11 highlights association between the seroprevalence of HBsAg and respondent's occupation and
Figure 2: Age-Specific Prevalence of Hepatitis B Markers Among the Study Population.

% OF AGE-GROUP WITH HBV MARKER

AGE-GROUPS IN YEARS

- HBsAG
- ANTI-HBs
- ANTI-HBc
ethnic grouping. The result indicates that those with high risk jobs (medical worker, police or soldier) were 1.52 times more likely to test positive for HBsAg than those with low risk jobs. The difference was however not statistically significant. Table 11 further shows that people of bantu ethnic origin were 1.16 times more likely to test positive for HBsAg when compared with people of nilotic origin. This result was also not statistically significant.

Subsequently, the simultaneous effects of some selected demographic characteristics on HBsAg seropositivity were examined by logistic regression analysis.

**Table 11: Association Between Seroprevalence of HBsAg and Respondent's Occupation and Ethnic Background (A Univariate Analysis).**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Test for HBsAg P-value</th>
<th>OR</th>
<th>95% CI</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve No. (%)</td>
<td>-ve No. (%)</td>
<td>x²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation (N=1423)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Risk</td>
<td>9(22.0)</td>
<td>32(88.0)</td>
<td>0.27</td>
<td>1.52</td>
<td>(0.66,3.37)</td>
</tr>
<tr>
<td>Low Risk</td>
<td>216(15.6)</td>
<td>1166(84.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnic group (N=1408)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bantu</td>
<td>204(16.2)</td>
<td>1057(83.8)</td>
<td>0.55</td>
<td>1.16</td>
<td>(0.70,1.94)</td>
</tr>
<tr>
<td>Nilotic</td>
<td>21(14.3)</td>
<td>126(85.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** High risk group includes medical workers, police and soldiers. The ethnic group category "others"
was excluded from this analysis.

A model that consisted of gender, age grouping, ethnic background and occupation was constructed (table 12). The results show that male gender is significantly associated with increased risk of testing positive for HBsAg ($p<0.05$). Age group 20 years or less was also associated with an increased likelihood of testing positive for HBsAg, the results being marginally significant ($p=0.07$). Although age group 21 to 41 years was associated with increased likelihood of testing positive for HBsAg, the result was not statistically significant. In regard to ethnic background, being a bantu was associated with a 1.20 fold increase in the risk of testing positive for HBsAg. With occupation, engagement in high risk jobs was associated with a 6% increase in the risk of testing positive for HBsAg, though the result was not statistically significant.

3.2.4.2; Association between seroprevalence of HBsAg and known risk factors for hepatitis B infection.

Tattooing, HBV infection in a family member, blood transfusion, unsterilized injection equipments, unsterilized cutting instruments and sexually transmitted diseases have been shown to be associated with increased risk of HBV infection [52,53,54,55]. Tables 13 and 14 show
the results of analyses conducted in order to find out how

Table 12: A Logistic regression Model Depicting the Relationship Between HBsAg Sero-Positivity and Respondent’s Gender, Age, Ethnic Background and Occupation.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE GENDER</td>
<td>.3916</td>
<td>0.03</td>
<td>1.48</td>
<td>(1.05 ,  2.08)*</td>
<td></td>
</tr>
<tr>
<td>AGE GROUP 20 YEARS OR LESS</td>
<td>1.0012</td>
<td>0.07</td>
<td>2.72</td>
<td>(0.93 ,  7.92)</td>
<td></td>
</tr>
<tr>
<td>AGE GROUP 21 TO 40 YEARS</td>
<td>.8797</td>
<td>0.10</td>
<td>2.41</td>
<td>(0.84 ,  6.86)</td>
<td></td>
</tr>
<tr>
<td>BANTU ETHNIC GROUP</td>
<td>.1856</td>
<td>0.46</td>
<td>1.20</td>
<td>(0.74 ,  1.97)</td>
<td></td>
</tr>
<tr>
<td>HEALTH WORKER, POLICE, SOLDIER</td>
<td>.0594</td>
<td>0.86</td>
<td>1.06</td>
<td>(0.56 ,  2.03)</td>
<td></td>
</tr>
</tbody>
</table>

Note: For age, the age category 41 years and above is the reference group.

important these factors are in the present study. A univariate analysis revealed that there was no significant effect of any of these variables on the likelihood of testing positive for HBsAg. Similar results were produced by a logistic regression analysis. No significant effect was seen.

The logistic regression model also included HIV sero-positivity. Being HIV positive was associated with a 1.36 fold increase in the likelihood of testing positive for HBsAg (p=0.06).
Table 13: A Univariate Analysis of Association Between the Seroprevalence of HBsAg and Known Risk Factors for Hepatitis B Infection.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Test for HBsAg</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tattoos on skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1420) Yes</td>
<td>11 (16.4)</td>
<td>56 (83.6)</td>
<td>0.88</td>
<td>1.05 (0.51, 2.11)</td>
</tr>
<tr>
<td>No</td>
<td>213 (15.7)</td>
<td>1140 (84.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever suffered from hepatitis (history)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1416) Yes</td>
<td>69 (16.5)</td>
<td>350 (83.5)</td>
<td>0.66</td>
<td>1.07 (0.78, 1.48)</td>
</tr>
<tr>
<td>No</td>
<td>155 (15.5)</td>
<td>843 (84.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of hepatitis in a family member</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1422) Yes</td>
<td>64 (16.8)</td>
<td>316 (83.2)</td>
<td>0.52</td>
<td>1.11 (0.80, 1.54)</td>
</tr>
<tr>
<td>No</td>
<td>161 (15.5)</td>
<td>881 (84.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever got blood Transfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1423) Yes</td>
<td>5 (9.3)</td>
<td>49 (90.7)</td>
<td>0.18</td>
<td>0.53 (0.16, 1.35)</td>
</tr>
<tr>
<td>No</td>
<td>220 (16.0)</td>
<td>1149 (84.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of injections from outside hospital, health center/dispensary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1423) Yes</td>
<td>127 (15.7)</td>
<td>689 (84.3)</td>
<td>0.77</td>
<td>0.96 (0.71, 1.29)</td>
</tr>
<tr>
<td>No</td>
<td>98 (16.1)</td>
<td>509 (83.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever received treatment through skin cut</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1423) Yes</td>
<td>92 (17.5)</td>
<td>435 (82.5)</td>
<td>0.19</td>
<td>1.21 (0.90, 1.64)</td>
</tr>
<tr>
<td>No</td>
<td>133 (14.8)</td>
<td>763 (85.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has ever suffered from STD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=1423) Yes</td>
<td>80 (16.1)</td>
<td>417 (83.9)</td>
<td>0.83</td>
<td>1.03 (0.76, 1.41)</td>
</tr>
<tr>
<td>No</td>
<td>145 (15.7)</td>
<td>781 (84.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 14: A Logistic Regression Model Showing the Effects of Known Risk Factors for Hepatitis B Infection on the Sero-positivity for HBsAg.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE FOR HIV</td>
<td>.3051</td>
<td>0.06</td>
<td>1.36</td>
<td>(0.99 , 1.86)*</td>
<td></td>
</tr>
<tr>
<td>HAVING HAD STD</td>
<td>-.0564</td>
<td>0.72</td>
<td>0.94</td>
<td>(0.69 , 1.29)</td>
<td></td>
</tr>
<tr>
<td>A FAMILY MEMBER WITH HBV INFECTION</td>
<td>.1045</td>
<td>0.53</td>
<td>1.11</td>
<td>(0.80 , 1.54)</td>
<td></td>
</tr>
<tr>
<td>RECEIPT OF BLOOD TRANSFUSION</td>
<td>-.6315</td>
<td>0.19</td>
<td>0.53</td>
<td>(0.21 , 1.35)</td>
<td></td>
</tr>
<tr>
<td>GETTING INJECTION OUTSIDE HOSPITAL</td>
<td>-.0876</td>
<td>0.56</td>
<td>0.91</td>
<td>(0.68 , 1.23)</td>
<td></td>
</tr>
<tr>
<td>GETTING MEDICINE THROUGH SKIN CUT</td>
<td>-.1867</td>
<td>0.21</td>
<td>0.83</td>
<td>(0.62 , 1.11)</td>
<td></td>
</tr>
<tr>
<td>SELF REPORTED HISTORY OF HBV</td>
<td>.0759</td>
<td>0.64</td>
<td>1.08</td>
<td>(0.78 , 1.49)</td>
<td></td>
</tr>
</tbody>
</table>

3.2.4.3; Association between seroprevalence of HBsAg and clinical features of hepatitis B infection.

Passage of unusually yellow or dark urine, pain at the right upper quadrant of abdomen, fatigue, poor appetite and fever are some of the signs and symptoms of acute or chronic active hepatitis. In order to find out if any of these variables is correlated with HBsAg sero-positivity, both univariate and multivariate analyses were performed. Table 15 shows that passage of yellow or dark urine, poor appetite and fever are significantly associated with
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Test for HBsAg</th>
<th>P-value OR</th>
<th>95% CI OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve No. (%)</td>
<td>-ve No. (%)</td>
<td>$x^2$</td>
</tr>
<tr>
<td>With yellow urine (N=1364)</td>
<td>55(21.2)</td>
<td>205(78.8)</td>
<td>0.02 1.52 (1.06,2.16)*</td>
</tr>
<tr>
<td>With poor appetite (N=1423)</td>
<td>62(19.9)</td>
<td>250(80.1)</td>
<td>0.03 1.44 (1.03,2.02)*</td>
</tr>
<tr>
<td>Pain at right upper quadrant of abdomen (N=1423)</td>
<td>38(20.4)</td>
<td>149(79.6)</td>
<td>0.07 1.43 (0.95,2.14)</td>
</tr>
<tr>
<td>Fever at time of interview (N=1423)</td>
<td>63(19.8)</td>
<td>255(80.2)</td>
<td>0.03 1.44 (1.03,2.10)*</td>
</tr>
<tr>
<td>History of fatigue (N=1423)</td>
<td>69(16.8)</td>
<td>341(84.2)</td>
<td>0.50 1.11 (0.81,1.53)</td>
</tr>
</tbody>
</table>

Note: Six respondents who had missing information on demographic characteristics were left out. In addition, those who gave "don’t know" answers on any variable were left out.

testing positive for HBsAg. A person having any of these signs or symptoms has an increased risk of testing positive
for HBsAg. Pain at the right upper quadrant of abdomen is associated with a 1.43 fold increase in the risk of testing positive for HBsAg (p=0.07). A person with fever is 1.11 times more likely to test positive for HBsAg than a person without fever, though the result is not statistically significant.

Table 16 shows the simultaneous effects of the above variables. A logistic regression model was constructed to find out if the results obtained from univariate analysis still hold. Although the results show that none of the variables have a significant effect on the risk of testing positive for HBsAg, having fever and poor appetite almost had significant effects (p<0.10).

A model that includes a combination of demographic characteristics, some risk factors of HBV infection and possible clinical features of HBV infection was built (table 16b). The results show that out of all variables considered, male gender and a newly created variable called "risk" were the only good independent predictors of HBsAg sero-status. The variable "risk" is defined as: 1 if a subject has history of both fever and unusual coloration of urine, 0 if otherwise. Among these two variables, "risk" was the most powerfull. Being HIV positive and age
categories 20 years or less and 21 to 40 years came out as poor predictors.

Table 16: Symptoms of Hepatitis B Infection as Predictors For Sero-Positivity For HBsAg (A Logistic Regression Analysis).

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAVING YELLOW URINE</td>
<td>-.0832</td>
<td>0.18</td>
<td>0.92</td>
<td>(0.81, 1.04)</td>
</tr>
<tr>
<td>PAIN AT THE RIGHT UPPER QUADRANT OF ABDOMEN</td>
<td>.2906</td>
<td>0.15</td>
<td>1.33</td>
<td>(0.90, 2.00)</td>
</tr>
<tr>
<td>HAVING FEVER</td>
<td>.3002</td>
<td>0.09</td>
<td>1.35</td>
<td>(0.95, 1.91)</td>
</tr>
<tr>
<td>HAVING FATIGUE</td>
<td>-.1208</td>
<td>0.50</td>
<td>0.89</td>
<td>(0.62, 1.81)</td>
</tr>
<tr>
<td>HAVING POOR APPETITE</td>
<td>.3028</td>
<td>0.10</td>
<td>1.35</td>
<td>(0.94, 1.95)</td>
</tr>
</tbody>
</table>

3.3.0; Prevalence of hepatitis Delta virus infection.

Correlation studies have established that the presence of antibodies to Delta agent (anti-HD) in serum reflects ongoing delta virus replication in the liver. Anti-HD is therefore a marker of infection with HDV.

Determination of anti-HD in serum specimens was performed to estimate the prevalence of HDV infection. The test was limited to only those specimens that tested positive for HBsAg. Of the 173 respondents who still had enough sera remaining for the test, 4 (2.3%) turned out to be anti-HD positive (figure 3). Of the 4 subjects who were
anti-HD positive, 2 (50%) were females. All the 4 subjects

Table 16b: A Logistic Regression Analysis of the Relationship Between HBsAg Sero-status and a Combination of Demographic Characteristics, Risk Factors for and Clinical Features of HBV Infection.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV POSITIVE</td>
<td>0.2927</td>
<td>0.07</td>
<td>1.34</td>
<td>(0.97, 1.85)*</td>
<td></td>
</tr>
<tr>
<td>AGE 20 YRS OR LESS</td>
<td>1.0499</td>
<td>0.06</td>
<td>2.86</td>
<td>(0.97, 8.44)*</td>
<td></td>
</tr>
<tr>
<td>AGE 21 TO 40 YEARS</td>
<td>0.8909</td>
<td>0.10</td>
<td>2.44</td>
<td>(0.85, 7.00)</td>
<td></td>
</tr>
<tr>
<td>MALE GENDER</td>
<td>0.3761</td>
<td>0.04</td>
<td>1.46</td>
<td>(1.03, 2.07)*</td>
<td></td>
</tr>
<tr>
<td>BANTU ETHNIC GROUP</td>
<td>0.4211</td>
<td>0.34</td>
<td>1.27</td>
<td>(0.78, 2.08)</td>
<td></td>
</tr>
<tr>
<td>HIGH RISK JOB</td>
<td>0.0213</td>
<td>0.95</td>
<td>1.02</td>
<td>(0.53, 1.97)</td>
<td></td>
</tr>
<tr>
<td>PAST H/O HEPATITIS</td>
<td>-0.0393</td>
<td>0.73</td>
<td>0.96</td>
<td>(0.77, 1.20)</td>
<td></td>
</tr>
<tr>
<td>HISTORY OF STD</td>
<td>-0.0813</td>
<td>0.62</td>
<td>0.92</td>
<td>(0.67, 1.27)</td>
<td></td>
</tr>
<tr>
<td>HBV IN A FAMILY MEMBER</td>
<td>0.0430</td>
<td>0.78</td>
<td>1.04</td>
<td>(0.78, 1.40)</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL YELLOW COLORATION</td>
<td>-0.0953</td>
<td>0.17</td>
<td>0.91</td>
<td>(0.79, 1.04)</td>
<td></td>
</tr>
<tr>
<td>RISK</td>
<td>0.7708</td>
<td>0.001</td>
<td>2.16</td>
<td>(1.34, 3.48)*</td>
<td></td>
</tr>
</tbody>
</table>

Note: For age, the age category 41 years and above is the reference category.

were HIV negative with ages 24, 25, 26 and 32 years. Of the 4, one was a soldier and the other three were unemployed. Three of the four respondents were of nilotic origin (1 Acholi, 1 Lugwara and 1 Kumam) while the other was of bantu origin (Muganda). Thus, three are from the North and one is
3.4.0; Chronic Carriage of Hepatitis B s Antigen.

The conventional definition of HBsAg has been "persistence of HBsAg in serum for more than six months". This definition requires follow up of subjects for over six months. By design, the present study could not allow the use of this definition. An alternative approach was adopted. Multiple laboratory tests were carried out on serum specimens taken at one point in time from a cross section of respondents and definition of chronic carrier
state and other stages of HBV infection done taking into consideration a combination of test results. The results are presented on tables 17a and 17b. Respondents who were only tested for one or two of the three key markers (HBsAg, anti-HBs and anti-HBc) were excluded from this analysis. Altogether, 1392 subjects qualified for this analysis. Of these, 218 are HIV positive and 1174 are HIV negative.

3.4.1; Serologic findings at different stages of HBV infection.

Table 17a shows serologic profiles of 1174 HBsAg negative subjects. The results indicate that 463 subjects are free from HBV infection, 51 are in a "window period" (time between disappearance of HBsAg and appearance of anti-HBs, during which anti-HBc serves as the only marker of HBV infection), 210 subjects have evidence of HBV infection that occurred in the distant past while 450 subjects are in recovery phase of HBV infection.

Table 17b shows four different combinations of hepatitis test results of 218 HBsAg positive subjects. These combinations represent different scenarios of HBV infection. The result shows that 111 subjects have HBsAg in the absence of both anti-HBc and anti-HBs. Of these, 30 (27%) are HIV positive. A second combination is presence
Table 17a: Serologic Markers of HBV in Different Stages of Hepatitis B Infection and Convalescence Among 1174 HBsAg Negative Subjects.

<table>
<thead>
<tr>
<th>Stage of HBV Infection</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HBc</th>
<th>HIV Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>No evidence of HBV Infection</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>108 (33.0)</td>
</tr>
<tr>
<td>Window period of HBV infection</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>24 (7.4)</td>
</tr>
<tr>
<td>HBV infection in the distant past</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>38 (11.6)</td>
</tr>
<tr>
<td>Recovery phase (Convalescence)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>157 (48.0)</td>
</tr>
<tr>
<td>Total Number ( % )</td>
<td></td>
<td></td>
<td></td>
<td>327 (100)</td>
</tr>
</tbody>
</table>

of both HBsAg and anti-HBc in the absence of anti-HBs. 64 subjects fell into this category. Another category is the presence of all the three markers under consideration (HBsAg, anti-HBc and anti-HBs). 32 subjects fell into this category, 15 of whom being HIV positive. The fourth and last category consisted of 11 subjects who had both HBsAg and anti-HBs but no detectable anti-HBc. Of these 11 subjects, 4 (36.4%) were HIV positive.

3.4.2: Prevalence of HBsAg chronic carrier state.

It is documented that virtually all chronic carriers
of HBsAg have circulating anti-HBc, usually in high titers [27,69,70,71]. Thus, in order to find out which subjects had chronic carrier state, 83 HBsAg positive subjects who were also positive for anti-HBc underwent two-fold serial dilutions and were tested again for anti-HBc. The results were reported as titer of anti-HBc. Those subjects with titer of 160 and above were categorized as having high titer whereas those with titers less than 160 were

### Table 17b: Serologic Markers of HBV in Different Stages of Hepatitis B Infection and Convalescence Among 218 HBsAg Positive Subjects.

<table>
<thead>
<tr>
<th>Stage of HBV Infection</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HBc</th>
<th>HIV Status +ve</th>
<th>No. ( % )</th>
<th>HIV Status -ve</th>
<th>No. ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late incubation period or early HBV infection</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>30 (39.5)</td>
<td>81 (57.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute or chronic HBV infection</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>27 (35.5)</td>
<td>37 (26.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute or chronic HBV infection</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>15 (19.7)</td>
<td>17 (12.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinfection in a person who had HBV infection in the distant past</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>4 (5.3)</td>
<td>7 (4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Number</strong></td>
<td><strong>76 (100)</strong></td>
<td><strong>142 (100)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
categorized as having low titer. The two categories were then used for statistical analysis.

Of the 83 serum samples that underwent serial dilutions, 47 (56.6%) had high titers of anti-HBc, 27 of them being HIV positive (table 18). Table 18 further shows that there is a correlation between HIV sero-status and the titer of anti-HBc. HIV positive subjects are 5.5 times

**Table 18: The Relationship Between Titer of Anti-HBc in HBsAg Positive Samples and HIV Status.**

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>High Titer of Anti-HBc</th>
<th>Low Titer of Anti-HBc</th>
<th>Total/Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27(79.4%)</td>
<td>7(20.6%)</td>
<td>34(100%)</td>
</tr>
<tr>
<td>Negative</td>
<td>20(40.8%)</td>
<td>29(59.2%)</td>
<td>49(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>47(56.6%)</td>
<td>36(43.4%)</td>
<td>83(100%)</td>
</tr>
</tbody>
</table>

**Note:** Statistically significant difference (OR=5.5 with CI 1.85 to 17.50) or \(x^2=12.17376, df=1, P<0.0005\).

more likely to have high titers of anti-HBc when compared with those who are HIV negative. The 47 subjects with high titers of anti-HBc were labelled as chronic carriers of HBsAg and thus used to estimate the prevalence of chronic carrier state. The denominator used in the estimation composed of the 956 respondents who had at least one marker of HBV infection. This is so because to become a carrier of HBsAg one must first become infected with the virus. The overall prevalence of chronic carrier state was 4.9%. HIV
positive subjects had a higher prevalence of chronic carrier state, 9.0% compared to 3.0 percent in HIV negative subjects (table 19). HIV positive subjects are 3.16 times more likely to have chronic carrier state than HIV negative subjects, a result that is statistically significant ($x^2=15.7$ and $p=0.0007$). A similar result was obtained from Table 19: Chronic Carriage of Hepatitis B s Antigen.

<table>
<thead>
<tr>
<th>HIV Sero-status</th>
<th># of carriers/# exposed</th>
<th>Carrier rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27/299</td>
<td>9.0%</td>
</tr>
<tr>
<td>Negative</td>
<td>20/657</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

a logistic regression analysis (table 19b). After controlling for a number of covariates, the result shows that HIV positive individuals are 3.66 times more likely to have chronic carrier state than the HIV negative individuals. The result also shows that male gender and having the variable "risk" are both associated with increased likelihood of chronic carrier state. A stepwise logistic regression analysis however revealed that being HIV positive, "risk" and male gender were the only variables independently predictive of HBV chronic carrier state.

An investigation was also carried out to determine whether the stage of HIV infection as defined by CD4+ T cell count is associated with the level of anti-HBc (tables
Table 19b; A Logistic Regression Model Showing the Relationship Between HBsAg Chronic Carrier State and Independent Variables.

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV POSITIVE</td>
<td>1.2975</td>
<td>0.0001</td>
<td>3.66</td>
<td>(4.41, 7.03)*</td>
<td></td>
</tr>
<tr>
<td>RISK</td>
<td>.8738</td>
<td>0.0426</td>
<td>2.40</td>
<td>(1.03, 5.58)*</td>
<td></td>
</tr>
<tr>
<td>MALE GENDER</td>
<td>1.2321</td>
<td>0.0004</td>
<td>3.43</td>
<td>(1.73, 6.80)*</td>
<td></td>
</tr>
<tr>
<td>BANTU</td>
<td>-.1291</td>
<td>0.7566</td>
<td>0.88</td>
<td>(0.39, 1.99)</td>
<td></td>
</tr>
<tr>
<td>HIGH RISK JOB</td>
<td>-.5126</td>
<td>0.4419</td>
<td>0.60</td>
<td>(0.16, 2.21)</td>
<td></td>
</tr>
<tr>
<td>20 YEARS OR LESS</td>
<td>1.0891</td>
<td>0.3215</td>
<td>2.97</td>
<td>(0.35, 25.6)</td>
<td></td>
</tr>
<tr>
<td>21 TO 40 YEARS</td>
<td>.6798</td>
<td>0.5221</td>
<td>1.97</td>
<td>(0.25, 15.8)</td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>-.6002</td>
<td>0.0990</td>
<td>0.55</td>
<td>(0.27, 1.12)</td>
<td></td>
</tr>
<tr>
<td>HBV IN FAMILY</td>
<td>-.2568</td>
<td>0.4931</td>
<td>0.77</td>
<td>(0.37, 1.61)</td>
<td></td>
</tr>
<tr>
<td>HISTORY OF HEPATITIS</td>
<td>.1480</td>
<td>0.2738</td>
<td>1.16</td>
<td>(0.89, 1.30)</td>
<td></td>
</tr>
<tr>
<td>UNUSUAL YELLOW URINE</td>
<td>.1029</td>
<td>0.3416</td>
<td>1.11</td>
<td>(0.90, 1.37)</td>
<td></td>
</tr>
</tbody>
</table>

The results show that there is no significant association between CD4+ T cell counts and titers of anti-HBc. Although table 20 shows that having a CD4+ T cell counts of less than 500 per
Table 20: Association Between CD4 Count and Titer of Anti-HBc Among 34 HIV Positive Subjects Who Underwent Titration of Anti-HBc.

<table>
<thead>
<tr>
<th>CD4 Count</th>
<th>Titer of Anti-HBc &gt; or = 160</th>
<th>Titer of Anti-HBc &lt;160</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500/microL</td>
<td>25 (83.3%)</td>
<td>5 (16.7%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>500 or more</td>
<td>2 (50.0%)</td>
<td>2 (50.0%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (79.4%)</td>
<td>7 (20.6%)</td>
<td>34 (100%)</td>
</tr>
</tbody>
</table>

Note: Fisher's exact test with P-value=0.18 (odds ratio of 5 with CI 0.28, 79.69).

microliter leads to a 5 fold increase in the likelihood of having high titers of anti-HBc, the result was not statistically significant (p=0.18). Similarly, table 21

Table 21: Association Between CD4 Count and Titer of Anti-HBc Among 34 HIV Positive Subjects Who Underwent Titration of Anti-HBc.

<table>
<thead>
<tr>
<th>CD4 Count</th>
<th>Titer of Anti-HBc High</th>
<th>Low</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>209.7</td>
<td>310.0</td>
<td>0.17</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>152.4</td>
<td>230.9</td>
<td></td>
</tr>
</tbody>
</table>

shows that the mean CD4 counts for subjects with high titers of anti-HBc was lower than that of subjects with low titers of anti-HBc (p=0.17).

Further analyses were carried out to determine whether levels of anti-HBs correlate with HIV sero-status (tables 22, 23 and 24). Only those with detectable levels of anti-
HBs were considered. Table 22 shows that being HIV positive leads to a 28% reduction in one’s likelihood of having a non-protective level of anti-HBs, a result which was not statistically significant. Subsequently, to examine the effect of stage of HIV infection as measured by CD4+ T cell counts, analysis was carried out on HIV positive individuals with detectable levels of anti-HBs (table 23). The results show that the mean CD4 counts is slightly higher in those subjects with non-protective levels of anti-HBs (p>0.10). A similar result was obtained when CD4
counts was considered as a dichotomous variable using a figure of 500/microliter as a cutoff point (table 24). The results show that subjects with CD4 counts less than 500

Table 24: Association Between CD4 Count and Titer of Antibody to Hepatitis's Antigen.

<table>
<thead>
<tr>
<th>CD4 Count</th>
<th>Titer of Anti-HBs (&lt;10mIU/mL)</th>
<th>Titer of Anti-HBS (More or = 10mIU/mL)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500/microL</td>
<td>62 (30.1%)</td>
<td>144 (69.9%)</td>
<td>206 (100%)</td>
</tr>
<tr>
<td>500 or more</td>
<td>9 (33.3%)</td>
<td>18 (66.7%)</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>71 (30.5%)</td>
<td>162 (69.5%)</td>
<td>233 (100%)</td>
</tr>
</tbody>
</table>

Note: OR=0.86 with CI 0.34 , 2.21.

were less likely to have non-protective levels of anti-HBs. The result was however not statistically significant. A cutoff point of 500 was chosen based on experience of other researchers \[125,126,127,128\]. CD4+ T cell counts of 500/microliter is used to identify HIV positive individuals who may need Zidovudine therapy.

3.5.0: Prevalence of hepatitis e antigen among HBsAg positive subjects.

Determination of HBeAg was carried out in 152 HBsAg positive subjects who had enough serum specimens left for the test. Since the presence of HBeAg usually correlates with increased numbers of infectious virus (Dane particles) and therefore increased risk of transmitting the virus,
analyses were carried out to determine if HIV positive individuals are more likely to transmit HBV than the HIV negative individuals. This would provide an indirect evidence that supports the hypothesis that HIV infection promotes chronic carrier state. The results of the analyses are presented on figure 4 and the tables that follow.

Figure 4 shows the overall prevalence of HBeAg among HBsAg positive individuals. Of 152 subjects tested, 23 (15.1%) had positive test results. Breaking down this by HIV sero-status indicates that HIV positive subjects are Figure 4; Hepatitis e Antigen (Prevalence among HBsAg positive subjects).
more likely to test positive for the antigen. 17.4% of HIV positive subjects were positive for HBeAg compared to 14.2% of HIV negative subjects. Being HIV positive was associated with a 28% increase in the risk of testing positive for HBeAg. This increase in risk was not statistically significant (table 25). The results also show that an HIV positive individual with a CD4+ T cell count less than 500 is 1.58 times more likely to test positive for HBeAg than an HIV positive individual with CD4 count of 500 or more.

Table 25: The Relationship Between HBeAg seropositivity and HIV sero-status Among 152 HBsAg Positive Subjects Tested for HBeAg.

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>HBeAg Test Positive</th>
<th>HBeAg Test Negative</th>
<th>Total/Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>8 (17.4%)</td>
<td>38 (82.6%)</td>
<td>46 (100%)</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (14.2%)</td>
<td>91 (85.8%)</td>
<td>106 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>23 (15.1%)</td>
<td>129 (84.9%)</td>
<td>152 (100%)</td>
</tr>
</tbody>
</table>

Note: OR=1.28 with CI 0.45 , 3.55.

Table 26: Association Between CD4 Count and HBeAg sero-status Among 46 HBsAg Positive Subjects Who Were Tested for HBeAg.

<table>
<thead>
<tr>
<th>CD4 Count</th>
<th>Positive for HBeAg</th>
<th>Negative for HBeAg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500/microL</td>
<td>7 (18.4%)</td>
<td>31 (81.6%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>500 or more</td>
<td>1 (12.5%)</td>
<td>7 (87.5%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (17.4%)</td>
<td>38 (82.6%)</td>
<td>46 (100%)</td>
</tr>
</tbody>
</table>

Note: Fisher's exact test with P-value=0.5 (odds ratio
of 1.58 with CI 0.15 , 81.28).

(table 26), a result that was not statistically significant. Similar results were obtained when analysis was done with CD4 counts as a continuous variable (table 27). There was no significant difference between the two groups in CD4 counts (Mann-Whitney U - Wilcoxon Rank Sum W Test, Z = -.1160 with P-value = 0.90).

Table 27: Association Between CD4 Count and HBeAg Sero-status Among 46 HBSAg Positive Subjects Tested for HBeAg.

<table>
<thead>
<tr>
<th>CD4 Count</th>
<th>HBeAg Sero-status</th>
<th>Positive</th>
<th>Negative</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (N=8)</td>
<td>Mean Rank</td>
<td>23.00</td>
<td>23.61</td>
</tr>
<tr>
<td></td>
<td>Negative (N=38)</td>
<td>U=148.0</td>
<td></td>
<td>W=184.0</td>
</tr>
</tbody>
</table>

Table 28: Association Between HBeAg and Titer of Anti-HBc Among 54 HBSAg Positive Subjects Who Were Also Positive for both HBeAg and Anti-HBc.

<table>
<thead>
<tr>
<th>Titer of Anti-HBc</th>
<th>Hepatitis e Antigen Test</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>High Titer</td>
<td>10 (33.3%)</td>
<td>20 (66.7%)</td>
</tr>
<tr>
<td>Low Titer</td>
<td>7 (29.2%)</td>
<td>17 (70.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>17 (31.2%)</td>
<td>37 (68.8%)</td>
</tr>
</tbody>
</table>

Note: Odds ratio = 1.21 and 95% CI for the OR is (0.33 , 4.55).

When the level of anti-HBc was considered, the results show
that subjects with high titers of anti-HBc are 1.21 times more likely to be HBeAg positive than subjects with low titers of anti-HBc (table 28). The result is however not statistically significant.

3.5.1; **Association between HBeAg sero-status and respondent’s sex, age and clinical features of hepatitis B infection.**

Univariate and multivariate analyses were carried out to explore the association between HBeAg sero-status and respondent’s sex, age and clinical features of HBV infection. Univariate analysis revealed that though fever, pain at the right upper quadrant of abdomen, passage of yellow urine, poor appetite and fatigue were all associated with increased risk of testing positive for HBeAg, only passage of yellow urine and fatigue had statistically significant associations (table 29). More or less similar results were obtained from a multivariate analysis. All independent variables except yellow urine and fatigue had significant effects (table 31). In regard to gender, although the results were not statistically significant, females were more likely than males to test positive for HBeAg. Age was also associtaed with testing positive for HBeAg, older age groups had a reduced risk of testing
positive for HBeAg (table 30).

3.6.0; Past infection with hepatitis B virus.

Hepatitis B infection that occurred in the distant past may be characterized by the presence of anti-HBs in

Table 29; Correlation Between HBeAg Sero-positivity and Signs and Symptoms of HBV Infection Among 152 HBsAg Positive Subjects Tested for HBeAg (A Univariate Analysis).

<table>
<thead>
<tr>
<th>Sign/Symptom</th>
<th>Test for HBeAg</th>
<th>P-value OR 95% CI for CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>No.(%)</td>
<td>No.(%)</td>
</tr>
<tr>
<td>With yellow urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=149) Yes</td>
<td>11(31.4)</td>
<td>24(68.6)</td>
</tr>
<tr>
<td>No</td>
<td>11(9.6)</td>
<td>103(90.4)</td>
</tr>
<tr>
<td>History of fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=152) Yes</td>
<td>11(23.4)</td>
<td>36(76.6)</td>
</tr>
<tr>
<td>No</td>
<td>12(11.4)</td>
<td>93(88.6)</td>
</tr>
<tr>
<td>With poor appetite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=152) Yes</td>
<td>7(16.7)</td>
<td>35(83.3)</td>
</tr>
<tr>
<td>No</td>
<td>16(14.5)</td>
<td>94(85.5)</td>
</tr>
<tr>
<td>With fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=152) Yes</td>
<td>7(17.5)</td>
<td>33(82.5)</td>
</tr>
<tr>
<td>No</td>
<td>16(14.3)</td>
<td>96(85.7)</td>
</tr>
<tr>
<td>Pain at right upper quadrant of abdomen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=152) Yes</td>
<td>4(15.4)</td>
<td>22(84.6)</td>
</tr>
<tr>
<td>No</td>
<td>19(15.1)</td>
<td>107(84.9)</td>
</tr>
</tbody>
</table>
Note: Three patients who answered don’t know for color of urine were excluded from this analysis.

the absence of HBsAg and anti-HBc. The individual would have lost anti-HBc reactivity, hence accounting for the absence of anti-HBc. This serologic pattern is also consistent with past HBV vaccination.

Table 30; Association Between HBeAg Sero-status and Respondent’s Sex and Age Among 152 HBsAg Positive Subjects Tested for HBeAg (A Univariate Analysis).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Test for HBeAg</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (N=152)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5(13.9)</td>
<td>31(86.1)</td>
<td>0.81</td>
<td>0.88 (0.24, 2.73)</td>
</tr>
<tr>
<td>Female</td>
<td>18(15.5)</td>
<td>98(84.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (N=152)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 or more</td>
<td>13(13.8)</td>
<td>81(86.2)</td>
<td>0.56</td>
<td>0.77 (0.29, 2.07)</td>
</tr>
<tr>
<td>20 or less</td>
<td>10(17.2)</td>
<td>48(82.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis was carried out to determine how common this type of past HBV infection is in the study population. Subsequently, cross-tabulation of HIV sero-status and presence or absence of evidence of HBV infection that occurred in the distant past was done to explore the relationship between the two variables. The results are presented on figure 5 and tables 32 and 33.
Table 31; HIV Sero-status and Clinical Features of HBV As Independent Predictors of HBeAg Sero-status Among 152 HBsAg Positive Subjects Tested for HBeAg (A Logistic Regression Analysis).

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>P-VALUE</th>
<th>EXP(B)</th>
<th>95% CI OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE FOR HIV</td>
<td>.1718</td>
<td>0.75</td>
<td>1.19 (0.41 , 3.42)</td>
<td></td>
</tr>
<tr>
<td>HAVING FEVER</td>
<td>-.1313</td>
<td>0.82</td>
<td>0.88 (0.28 , 2.73)</td>
<td></td>
</tr>
<tr>
<td>PAIN AT THE RIGHT UPPER QUADRANT OF ABDOMEN</td>
<td>-.2014</td>
<td>0.75</td>
<td>0.82 (0.23 , 2.89)</td>
<td></td>
</tr>
<tr>
<td>HAVING YELLOW URINE</td>
<td>.2891</td>
<td>0.09</td>
<td>1.34 (0.95 , 1.87)*</td>
<td></td>
</tr>
<tr>
<td>HAVING POOR APPETITE</td>
<td>-.1859</td>
<td>0.74</td>
<td>0.83 (0.28 , 2.49)</td>
<td></td>
</tr>
<tr>
<td>HAVING FATIGUE</td>
<td>.8843</td>
<td>0.08</td>
<td>2.42 (0.89 , 6.59)*</td>
<td></td>
</tr>
</tbody>
</table>

Note: * indicates that the result is marginally significant.

Figure 5 indicates that 210 (27%) of the 765 respondents who had detectable levels of anti-HBs had test findings consistent with HBV infection that occurred in the distant past. A breakdown of this by HIV sero-status shows that being HIV positive is associated with a significant reduction in the likelihood of having had HBV infection in the distant past. HIV positive subjects have a 59% reduction in the likelihood of having had HBV infection in the distant past (table 32), a highly significant result. When the level of anti-HBs carried by those individuals
with evidence of HBV infection that occurred in the distant past is stratified by HIV sero-status, the result shows

**Figure 5: Evidence of HBV Infection that Occurred in the Distant Past Among 765 Subjects with Detectable Levels of Anti-HBs.**

![Pie chart showing 210 (27%) as present and 555 (73%) as absent.]

**Table 32: Association Between HIV Sero-status and HBV Infection that Occurred in the Distant Past.**

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>Evidence of HBV Infection (Occurred in Distant Past)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Positive</td>
<td>38 (16.3%)</td>
<td>195 (83.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>172 (32.3%)</td>
<td>360 (67.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>210 (27.5%)</td>
<td>555 (72.5%)</td>
</tr>
</tbody>
</table>

**Note:** OR=0.41 with a 95% CI of 0.27, 0.61.

that HIV positive subjects are 1.58 times more likely to have a "non-protective" level of anti-HBs than HIV negative individuals (table 33). This result is however not
statistically significant.

**Table 33: Association Between HIV Sero-status and Titer of Anti-HBs in Subjects with Evidence of Past HBV Infection (N=210).**

<table>
<thead>
<tr>
<th>HIV Status</th>
<th>Titer of Anti-HBs</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10mIU/mL</td>
<td>=or&gt;10mIU/mL</td>
</tr>
<tr>
<td>Positive</td>
<td>34 (89.5%)</td>
<td>4 (10.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>145 (84.3%)</td>
<td>27 (15.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>179 (85.2%)</td>
<td>31 (14.8%)</td>
</tr>
</tbody>
</table>

**Note:** OR=1.58 with a 95% CI of 0.50, 6.62.
CHAPTER 4

4.0; DISCUSSION.

4.1; Hepatitis B infection in the study population.

The population studied composed virtually of people seeking for health care as outpatients in Entebbe General Hospital, a non-specialized government’s hospital located in an urban center, southern part of Uganda. It cannot therefore be taken as fully representative of the entire Ugandan population. In addition, it has been repeatedly shown that the prevalence rates of HBV markers may differ considerably within a country, depending on various local ethnic, socioeconomic, cultural, geographic, religious, and other factors [115]. Thus, the data presented may reflect only the local situation in the area from which the serum specimens were sampled, and situations in communities that are served by government’s hospitals located at any urban center of Uganda. It may not be representative of the whole country. Nevertheless, taking into consideration these limiting factors, the study confirms that hepatitis B infection is highly endemic in Uganda, the prevalence rates of HBsAg and total HBV infection being 15.7% and 66.9% respectively. These figures are indicative of the burden Uganda has as a result of HB infection.
An HBsAg seroprevalence rate of 15.7% found during the present study is higher than those reported in other hospital-based studies conducted in Uganda. For instance, Hudson et al found an HBsAg seroprevalence rate of 5.5% during their study conducted in Kisizi and Kagando hospitals located in western Uganda [38]. Another study, by de Lalla Fausto et al [39], revealed an HBsAg seroprevalence rate of 10%. This latter study was conducted in Kitgum Mission hospital, a health facility located in northern Uganda. Variation in the sensitivities and specificities of the tests used in the studies just referred to may be partly accountable for the differences seen in the seroprevalence rates. The differences seen may also be a reflection of the geographic variation of the seroprevalence rates [115].

Other studies have demonstrated differences in prevalence rates of HBV markers by sex, age, HIV sero-status, geographic region, ethnic grouping and other known risk factors. This study has found no significant differences in prevalence rates of HBsAg by the above factors except for sex and HIV sero-status. The results show that males and those people who are HIV infected have significant higher risks of testing positive for HBsAg. In
fact, being a male or HIV positive are both independently predictive of HBsAg positivity.

Prevalences of anti-HBc and total HBV infection also differ significantly with age and HIV sero-status. That of anti-HBs however differs only with age, a result which indicates a dose response relationship. As age increases, the prevalence of anti-HBs also increases, a finding that may reflect a lifetime or cumulative experience of the study population since anti-HBs is mainly an IgG antibody. In particular, the finding indicates what proportion of the population has had what can be considered as successful recovery from HB infection. In clinical practice, a positive test for anti-HBs can be a useful adjunct for assessing immunity of clinical recovery of the patient.

4.2; Prevalence of protective level of anti-HBs.

The presence of anti-HBs in an individual’s serum indicates that he or she has been exposed to HBsAg either in form of HB vaccine or by infection with HBV; for the latter, it also means that the person has recovered from infection.

A quantitative measurement of anti-HBs is valuable in assessing the level of immune response to HB vaccine. For instance, the finding of an anti-HBs level of 10mIU/mL or
higher following HB vaccination indicates an adequate response. A level of 100mIU/mL or more is predictive of a long-term sustainability of the antibody [123]. In the present study, the AUSAB quantitation panel was used to determine the level of anti-HBs. The result shows that out of the 765 respondents who tested positive for anti-HBs, 493 (64%) had levels equal to or more than 10mIU/mL. Since HB vaccine is currently not in use in Uganda, this result shows that a significant proportion of this Ugandan community has had a successful recovery from HB infection.

64% of respondents with evidence of past infection achieved an adequate natural immune response following HBV infection and is expected to be protected from reinfection, at least on theoretical grounds. Experience has however shown that one can still be reinfected despite presence of high titers of anti-HBs [129,130], indicating that having a protective level of anti-HBs may not guarantee protection all the time.

This study also shows that out of 1429 respondents whose blood samples were analyzed, 450 (31.5%) had both anti-HBs and anti-HBc in the absence of HBsAg. This indicates that about one third of the population was in recovery phase of HBV infection.
51 subjects had anti-HBc as the only marker of HBV infection, indicating that they were in the "serologic window" period, a time between disappearance of HBsAg and appearance of anti-HBs. This result highlights the importance of using anti-HBc as a marker of HBV infection [63]. About 4% of cases of HBV infection would have been missed if anti-HBc was not tested for. Worth mentioning also is a possibility that a few of the 51 subjects may fall under the category of low-level HBsAg chronic carriers. Subjects falling in this category usually have HBsAg circulating at undetectable levels while retaining the capability of transmitting the virus.

The results that have been discussed in this section are rather unique. This study is the only one that has quantitated anti-HBs that has been acquired from natural infection. In addition, to my knowledge, there is nothing in literature regarding the prevalence rates of subjects in recovery phase and serologic window period of HB infection. Thus, there are no figures that can be compared with those obtained from the present study.

4.3; Infection with Hepatitis Delta Virus.

The prevalence of anti-HD among 173 HBsAg positive individuals tested for the marker was 2.3%. Since
correlation studies have established that the presence of anti-HD in serum reflects ongoing delta virus replication in the liver, this result shows that 2.3% of these 173 respondents were having HDV infection. The result also shows that the overall prevalence of HDV infection among the study population was 0.3% suggesting that HDV infection seems not to be a problem in the area surveyed and possibly in Uganda.

This study is the first of its kind in Uganda to examine the problem of HDV infection in a relatively large group of subjects. The only other study which looked into this subject was conducted in Kitgum Mission Hospital, located on northern part of the country. The study revealed that out of the 36 respondents tested, 11 (30.6%) had anti-HD [39], a level which is much higher than the one in the present study. Taken together, the results of the two studies may be a reflection of geographic variation of the prevalence of HDV infection. Similar observations were made by Greenfield and co-workers during their study conducted in Kenya, a neighboring country that borders Uganda throughout its eastern frontier [131]. These workers observed that HDV prevalence varied widely with region, being 2% in the southern Kenya and 30% in northern Kenya.
Furthermore, a study conducted in Ethiopia reported an HDV prevalence of about 10% [132]. Another study [133], conducted in Sudan, reported that of the 166 respondents tested for anti-HD, 16 (10%) tested positive. Sudan is a country that shares a boundary with Uganda with its southern frontier and Ethiopia with its eastern frontier. In central Burundi, another neighbouring country to Uganda, an anti-HD prevalence rate of 0% has been documented [39].

The results of all the studies just referred to seem to suggest that as one moves from Equator northwards, the prevalence of anti-HD increases. They also confirm that HDV prevalence in sub-Saharan Africa is widely variable, from moderate to high in some places to virtually absent in persons from southern parts [134,135].

It is interesting to note that out of the 4 subjects who tested positive for anti-HD during the present study, 3 come from northern/north eastern Uganda and only one comes from the south. Though these numbers are very small, the finding seems to suggest that HDV infection is commoner in people from the north. Moreover, people from the north constitute less than 10% of the study sample. It was also observed that there is no sex difference in the prevalence of HDV infection, 2 (50%) of the respondents being males
and 2 (50%) being females. All the 4 respondents are HIV negative, the number however being too small to warrant making any conclusions about what impact HIV infection may have on the seroprevalence of HDV infection.

HDV infection usually occurs as either coinfection with or superinfection of an HBV carrier. Coinfection usually resolves; superinfection however, frequently causes chronic HDV infection and chronic hepatitis. Both types of infection may cause fulminant hepatitis [122]. It is therefore reassuring to see that the overall prevalence of anti-HD in the study population is less than 1%, a population that has high endemicity of HBV infection. A finding of a higher prevalence of HDV infection would be an indicator of a much more serious problem since HDV infection may cause more severe liver damage than the damage caused by hepatitis B. It has been suggested that development of severe liver complications by HBsAg carriers who were previously asymptomatic or had mild liver disease may occur due to superinfection with HDV.

4.4: Serologic Profiles in HBsAg Positive Subjects.

In an attempt to define the stage of HBV infection at which each of the HBsAg positive subjects was, at the time of blood sampling, a series of analyses was performed. The
analyses led to identification of four main serologic profiles depending on the combination of test results. The sections that follow highlight the most probable interpretations of these serologic profiles.

4.4.1; Late Incubation Period or Early HBV Infection.

Analyses revealed that of the 218 HBsAg positive subjects considered, 111 (50.9%) tested negative for both anti-HBc and anti-HBs. These subjects therefore had a serologic finding that is characterized by the presence of HBsAg without either anti-HBc or anti-HBs. This kind of serologic finding does occur, usually during the first three to five weeks of HBsAg positivity before the appearance of anti-HBc. Most patients with HBsAg alone will be in this early, presymptomatic period of acute HB. 57 percent of the 76 HIV positive individuals who underwent this analysis fell into this classification, compared to 30% of HIV negative individuals, a finding which suggests that HIV negative individuals are more likely than HIV positives to be in this stage of HB infection.

4.4.2; Acute or Chronic Hepatitis B Infection.

It was observed that 64 subjects had both HBsAg and anti-HBc but no anti-HBs. The presence of HBsAg with anti-HBc in the absence of anti-HBs is the usual finding in
patients with acute HB and with chronic HBsAg carrier state [12,63,68,136]. Anti-HBc is usually present by the time that symptoms appear. Hoofnagle and others suggested that titration of anti-HBc can be done in order to distinguish between acute HB and chronic HBsAg carrier state [63,69,70]. That anti-HBc should yield a relatively low or moderate titer in early HB, but will be present in high titer in patients with chronic carrier state.

4.4.3; Co-occurrence of HBsAg and Anti-HBs in the Presence of Anti-HBc.

This study revealed that 32 subjects had co-circulation of HBsAg and anti-HBs in the presence of anti-HBc, a finding that can be explained as follows: a; A chronic carrier of a subtype HBsAg who also has anti-HBc gets a second HBV infection of a different subtype. The second infection leads to an antibody response resulting into formation of a subtype anti-HBs. The subtype anti-HBs is not capable of neutralizing the HBsAg from primary infection. b; The primary infection leads to formation and sustenance of a subtype anti-HBs. Because of the limited protection afforded by the subtype anti-HBs, reinfection occurs following exposure to HBV of a different subtype. At the time of blood sampling, the second infection had
reached the stage of anti-HBc formation, thus the presence of anti-HBc.

Co-circulation of HBsAg and anti-HBs has been documented [91,137,138,139,140]. While the most commonly advanced explanation for this phenomenon is that the HBsAg and anti-HBs are of different subtypes [91,137,138, 139,140], formation of a new HBV variant referred to as "escape mutant" has also been implicated in this[141,142,143]. Escape mutants arise from point mutations of HBsAg. By definition, escape mutants are replicating, infectious viruses that have mutated so that they are no longer susceptible to neutralizing immunity.

New HBV variants are of public health importance because they may lead to chronic carrier state. An HBV variant with one or two mutations in the pre-core region of the genome has been found to be responsible for chronic hepatitis in Greek and Italian patients [144, 145].

In the present study, subtyping or sequence analysis was not done. It is therefore not possible to give a definitive explanation for the co-occurrence of HBsAg and anti-HBs seen. It is possible that sequence analysis will be performed on these samples sometime in the future to help clarify the issue.
4.4.4; Co-occurrence of HBsAg and Anti-HBs in the Absence of Anti-HBc.

The fourth serologic profile revealed by this study was co-occurrence of HBsAg and anti-HBs in the absence of anti-HBc. The most likely explanation of this finding is that the HBsAg and anti-HBs are of different subtypes. A person gets infected with HBV. The primary infection leads to recovery with formation of a subtype anti-HBs. Because of the limited protection afforded by the subtype anti-HBs, the person remains susceptible to other subtypes of HBV. On exposure to any of these subtypes, reinfection occurs. At the time of blood sampling, the second infection was still at its presymptomatic stage, before anti-HBc is formed. The primary infection had occurred in the distant past and subsequently the subject lost anti-HBc reactivity.

Similarly, since subtyping or sequence analysis was not performed, it is not possible to confirm the explanations given. However, the most likely interpretation of this serologic profile is, early stages of HBV reinfections in persons who had HBV infections in the distant past.

4.4.5; Prevalence of HBsAg Chronic Carrier State.

Due to its sequelae, chronic carriage of HBsAg has
very serious public health implications [146]. The ramification of these implications varies from place to place, depending on the local prevalence of the chronic carrier state. This underscores the need to determine the prevalence of chronic carrier state.

In an attempt to distinguish between chronic carriers from non-chronic carriers and hence estimate the prevalence of chronic carrier state, determination of titers of anti-HBc was performed on sera that had undergone two-fold serial dilutions. Subjects were then classified according to their levels of anti-HBc.

All HBsAg positive subjects with titers of total anti-HBc equal or more than 1:160 were designated as chronic carriers. By this definition, 47 (56.6%) of 83 subjects who underwent this analysis were classified as chronic carriers. The results further showed that HIV positive individuals were significantly at more risk of having high titers of anti-HBc, a very common finding in chronic carrier state. Subsequently, 9 percent of HIV positive individuals were classified as chronic carriers compared to 3 percent of HIV negative individuals. The overall prevalence of chronic carriage was 4.9%, a figure that is lower than those reported from prospective studies [31].
The above finding suggests that HIV infection has the potential to promote chronic carriage of HBV. The implications of this is serious especially in populations with high prevalences of both HIV and HBV infections. First of all, promotion of chronic carrier state would increase the potential for further spread of HBV infection among the general population. Secondly, due to the subsequent increase in the incidence of HBV infection in the general population, morbidity and mortality due to complications or sequelae of chronic carrier state will also rise. These complications will be more serious in the HIV negative individuals who are expected to have longer life spans.

The definition of chronic carrier state used in this study may be less sensitive and specific than that commonly used in prospective studies, "persistence of HBsAg in serum for six months or longer". The cutoff value of titer of anti-HBc used in the classification, 1:150, may be too stringent, hence leading to some true chronic carriers being left out. Furthermore, total anti-HBc was used in the definition without consideration of IgM anti-HBc. This may have led to the definition being less specific. The prevalence rate reported could have been underestimated in some circumstances and overestimated in others. Caution
should therefore be taken when comparing the results of this study with those from prospective studies.

Unlike in previous studies [31], levels of CD4 counts had no significant association with chronic carrier state. Though the mean titer in the group with high titer of anti-HBc was lower, the difference was not statistically significant.

This study is the only cross-sectional study done specifically to examine the association between HBV chronic carriage and HIV-1 infection. It is also the only study of its kind conducted in areas where HBV infection is highly endemic. The few studies that have examined the association between HBV chronic carriage and HIV-1 infection were done in the western world. Those studies also involved highly selected groups such as homosexual men. In addition, the studies were either prospective or retrospective in design. Thus, caution should be exercised when making a direct comparison of the results of the present study with those of studies just referred to. However, there is one common finding to all these studies. They all confirm that HIV infection has the potential to promote HBV chronic carriage, a finding confirmed by the present study. In Australia, Bodsworth et al reported a significantly higher
prevalence of HBV chronic carriage in HIV individuals [31]. The prevalences of HBV chronic carriage were 23% and 4% in HIV positive and HIV negative individuals respectively (p=0.026). In U.S.A, Hadler et al reported that HIV positive individuals were eight times more likely than HIV negative individuals to develop HBV chronic carriage [24].

4.4.6; The Relationship Between HBsAg Seropositivity and Levels of Anti-HBs.

When all the 765 subjects who had detectable levels of anti-HBs were grouped by their HIV sero-status, the results revealed that there was a trend for HIV positive individuals to be associated with a higher likelihood of having higher titers of anti-HBs, a finding which was not statistically significant. An opposite trend was however seen when only those with evidence of HBV infection that occurred in the distant past were considered. Of the 765 subjects with detectable levels of anti-HBs, 210 (27.5%) had evidence of HBV infection that occurred in the distant past (possession of anti-HBs in the absence of any other marker of HBV infection). Being HIV positive was significantly associated with less likelihood of being in this category. HIV positive individuals were 0.41 times less likely to have this form of HBV infection than their
HIV negative counterparts. It was further observed that within the group with evidence of HBV infection that occurred in the distant past, HIV infected people tended to be associated with less likelihood of having a protective level of anti-HBs, although the result was not statistically significant.

The above results seem to suggest that HIV negative individuals are more likely to have full recovery from HBV infection. Secondly, since presence of anti-HBs without HBsAg and anti-HBc is said to be most associated with a "self-limited primary antibody response without demostrable HBsAg", the finding further suggests that HBV infection in HIV negative individuals is more likely to be self-limited in nature [12]. That is, they are less likely to have chronic carrier state.

4.4.7; Hepatitis e Antigenemia.

This study shows that out of 152 HBsAg positive serum specimens tested for HBeAg, 23 (15%) were positive for the antigen. The study also shows that there was no significant association between HBe antigenemia and HIV sero-status, gender, age, CD4 count, titer of anti-HBc, fever, history of poor appetite and pain in the right hypochondrium. A multivariate analysis revealed that having yellow urine and
fatigue are independently predictive of HBeAg positivity.

The presence or absence of HBeAg provides useful information on the status of people (individuals) found to be HBsAg positive [147]. The antigen becomes detectable in serum after the rise of HBsAg, the time during which there is increased numbers of infectious virus (Dane particles). Thus, during the HBeAg positive stage, hepatitis B patients are at increased risk of transmitting the virus to their contacts [148]. Persistence of HBeAg in the HBV carriers is often associated with chronic active hepatitis. The findings of this study therefore suggest that the 15% of HBsAg positive individuals who were also positive for HBeAg are more capable of transmitting the virus than the other 85% of HBsAg positive individuals. Indeed, it has been suggested that HBeAg can be used as a marker or indicator of transmission of HBV [64]. In particular, maternal HBe antigenemia has been found to be a good predictor of vertical transmission of HBV [55, 66]. HBsAg carrier rates among babies born to HBeAg positive mothers have been found to be as high as 100% in some studies [60, 64]. In view of the fact that most of the subjects seen during the present study were women of child bearing age, an HBeAg positivity rate of 15% among HBsAg positive individuals seems to be an
important factor in vertical transmission of HBV in Uganda.

An HBeAg seroprevalence rate of 15% found during the present study is consistent with what is found in literature. In Africa, 10% or more of adult women may be HBV carriers, but fewer than 20% are HBeAg positive [51]. For instance, in Kenya, a neighboring country to Uganda, 10% of HBsAg positive sera were found to be HBeAg positive [132].
CHAPTER 5

5.0; CONCLUSIONS AND RECOMMENDATIONS.

This study’s focal point has been determination of the prevalence of HBV and HDV markers in the study population as an attempt to examine the relationship between HIV infection and HBV chronic carrier state. Based on the results obtained, some conclusions are drawn. Subsequently, recommendations are made. Finally, a brief comment is presented in lieu of limitations of the study.

5.1; Conclusions.

The conclusions that follow have important policy implications. They will be used as stepping stones for defining future directions.

5.1.1; Uganda has High Endemicity of HBV Infection.

The study revealed that the prevalence of HBsAg is 15.7% while that of total HBV infection is 66.9%. This confirms that HBV infection is highly endemic in Uganda.

5.1.2; High Prevalence of Protective Levels of Anti-HBs.

A protective level of anti-HBs is defined as a level of anti-HBs equal to or more than 10mIU/mL [94,122]. By this definition, almost two thirds of those respondents with detectable levels of anti-HBs had a protective level of this antibody. Thus, this group of subjects seems to
have an adequate natural antibody response following HBV infection.

5.1.3; Concurrent Circulation of HBsAg and Anti-HBs.

Over 10% of HBsAg positive subjects had concurrently circulating anti-HBs. In view of the fact that almost all these subjects had anti-HBs in excess of 10mIU/mL, it can be concluded that presence of a protective level of anti-HBs may not necessarily ensure protection against all reinfections. Secondly, though the co-circulation of HBsAg and anti-HBs may be due the HBsAg and anti-HBs being of different subtypes, presence of unusual HBV variants known as "escape mutants" is a possibility. There may be some unusual variant(s) of HBV in this community that is(are) capable of evading neutralization by anti-a.

5.1.4; Insignificant Infection with Delta Agent.

The prevalences of HDV infection among HBsAg positive individuals and the study population were 2.3% and 0.3% respectively. From this, it can be concluded that HDV infection seems not to be a problem in this group of subjects, those they represent and probably in Southern Uganda.

5.1.5; HIV Infection Seems to have the Potential to

Promote HBsAg Chronic Carrier State.
The results presented show that HIV infection is associated with an increased likelihood of HBsAg chronic carrier state. First, the prevalence of HBsAg chronic carriage is significantly higher in subjects who are HIV positive. Secondly, HIV positive individuals are more likely to have high titers of anti-HBc, a common finding in HBsAg chronic carriers. Third, the more advanced stage of HIV infection tends to be associated with high titers of anti-HBc. Fourth, HIV negative individuals were more likely to have full recovery from past HBV infection, thus providing an indirect evidence to support the hypothesis that HIV infection has the potential to promote HBV chronic carrier state.

5.1.6; Independent Predictors of HBsAg Sero-status.

Of all the variables considered, only two turned out to be good predictors of HBsAg sero-status. These are, male gender and a newly created variable designated "risk" (risk=yes if a subject has history of both fever and unusual coloration of urine, and risk=no if otherwise). Among the two, "risk" was the strongest predictor of HBsAg positivity. Since none of the individual signs and symptoms of HBV infection came out as a good predictor, it may be reasonable to conclude that use of a composite variable
that takes into account a number of these variables may be more beneficial in identification of independent predictors of HBsAg sero-status.

5.2; Recommendations.

This study has made revelations that are of public health importance. Arising from this are a number of recommendations.

5.2.1; Institution of Hepatitis B Vaccination Programme.

In the light of the high prevalence of HBV infection revealed by this study, institution of an HB vaccination programme is recommended to stop further spread of hepatitis B. In order to minimize costs and promote acceptability, this programme must be part and partial of the already existing Expanded Programme on Immunization. Furthermore, Uganda has very limited resources to be used in the implementation of this recommendation. Any strategies to be drawn must take this factor into account.

Since vertical transmission and horizontal transmission during childhood seem to be the most important routes of transmission in Uganda, the following strategies are recommended: a; Universal infant immunization. Hepatitis B vaccination should be given to all infants regardless of the HBsAg status of the mother. Since there
is no interference with antibody response of the other vaccines following administration of hepatitis B vaccine [149], the vaccine should be incorporated into the vaccination schedule for children. This approach has been successfully used elsewhere [150]. The first dose can be given at birth together with BCG and OPV-0, the second dose at six weeks with DPT-1 and OPV-1 and the last dose at 9 months with measles vaccine. This schedule will not require setting up additional immunization clinics. The number of visits parents make to immunization clinics for the already ongoing childhood immunization will also remain the same.

In addition, because of the 4 to 6 months spacing between the second and third doses, the recommended schedule will maximize seroconversion following immunization. b; Immunization of women of child bearing age. A great number of respondents were women of child bearing age. Furthermore, 15% of HBsAg positive subjects were HBeAg positive, with a tendency of women being more likely to test positive for the antigen. These results help re-emphasize the need to immunize women of child bearing age.

5.2.2; Prevention of HBV Infection through Blood Screening.

In Uganda, donated blood is obtained from volunteers.
These volunteers are usually young people apparently in good health. Patients can also receive blood from family members or friends. If any of these donors is HBsAg positive, the recipients of blood would be at increased risk of HBV infection.

The population studied is composed of potential blood donors. In view of the very high prevalence of HBsAg, continuation of the already ongoing screening of blood for HBsAg and HIV will help reduce the incidence of HBV infection, and thus chronic carrier state.

5.2.3; Proper Sterilization of Injection and Surgical Equipments.

Translation of the HBsAg seroprevalence rate shown by this study into an absolute number indicates that approximately 1 in every 6 patients seeking for health care at a government's hospital will be HBsAg positive. Most of these patients come with various medical conditions. More often than not, they end up receiving parenteral therapy. Those pregnant end up in labor wards when due for delivery. During delivery, surgical equipments may be used during episiotomy or when cutting the umbilical cord. Thus, injection and surgical equipments seem to be additional potential sources of HBV infection in this community.
Proper sterilization of injection and surgical equipments is recommended.

5.2.4; Prevention of HIV Infection Through Counselling.

The study demonstrated that HIV infected individuals are more likely to have chronic carrier state than individuals uninfected with HIV. The chronic carriers also form reservoir of infection for the uninfected persons. A continued programme of prevention of HIV infection through counselling is necessary to reduce the risk of HBV infection and subsequently that of HBV chronic carrier state and its sequelae.

5.2.5; Further Research Needed.

Ensuing from the findings of this study, some grey areas have been unravelled, hence need for further research. a; More research is required to elucidate the subject of co-occurrence of HBsAg and anti-HBs. This can be accomplished through simple subtyping of HBsAg and anti-HBs or through sequence analysis. The latter approach is also capable of identification of unusual HBV variants, if any. Unusual HBV variants have been reported in other settings, including Africa [142,150,151]. b; More research is required in the area of maternal transmission of HBV to find out how maternal transmission of HBV is impacted by
HIV infection.

5.3; Limitations of the Study.

This section highlights the limitations of the study in a comprehensive manner. The limitations may have already been discussed individually in previous chapters, where applicable.

5.3.1; Generalisability of the Study Results.

The study population may not be representative of the entire Ugandan population. It should, however be representative of people who usually get medical care in any government's hospital located in an urban center. In addition, since Entebbe General hospital has a wide encatchment area, and the study population is composed of all ethnic groups in the country, the data presented should have a much wider generalisability.

5.3.2; Association Between HIV Infection and Hepatitis Delta Virus Infection.

Only 4 patients were with evidence of HDV infection. Due to this small number, it was not possible to study the association between HIV and HDV infections. This question remains unanswered.

5.3.3; Definition of HBV Chronic carrier State.

HBsAg chronic carrier state was defined as presence of
HBsAg associated with high titers of anti-HBc. In comparison to defining HBV chronic carriage as "persistence of HBsAg for six months or more", the definition used may be less sensitive and possibly less specific.

5.3.4; Samples not Examined.

Of the 1506 samples collected, 77 (5.1%) never came from Uganda. Though there are no differences in the demographic characteristics of the 77 respondents and those whose blood samples were analyzed, a very remote possibility of bias cannot be ruled out in case the 77 respondents have much lower or much higher prevalences of HBV markers. Secondly, a slight reduction in the power of the study may have resulted from this.


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APPENDIX 1
QUESTIONNAIRE FOR A STUDY OF THE INFLUENCE OF HIV INFECTION
ON HEPATITIS B CHRONIC CARRIAGE AND DELTA VIRUS INFECTION

Date of interview_____/_____/_____  Serial number {   }
   day month year

1. Sex of respondent (interviewer to note)  1. Male  [   ]
   2. Female

2. How old were you during your last birthday?______________
   years
   7. Don't know  [   ]
   8. Refused

3. Where do you reside?____________________________

4. Have you ever been to school?  1. Yes  [   ]
   2. No [SKIP TO 7]

5. How many years did you spend in school?
   ________________________________ years
   9. Not applicable [   ]

6. What level of qualification did you achieve?
   1. None
   2. Certificate
   3. Diploma  [   ]
   4. First degree
   5. Postgraduate
   8. Refused
   9. Not applicable

7. Are you employed?  1. Yes  [   ]
   2. No [SKIP TO 9]

8. What is your occupation?____________________________
   9. Not applicable [   ]

9. What is your tribal origin?____________________________
   8. Refused [   ]
10. What is your religion?
   1. Islam
   2. Anglican church
   3. Catholic
   4. Orthodox church
   5. Others
   specify
   6. Pagan
   8. Refused

11. To the best of your knowledge, have you ever received blood transfusion?
   1. Yes
   2. No [SKIP TO 14]

12. How many bottles of blood did you receive?
   1. One
   2. Two
   3. Three
   4. Four
   5. Five
   6. Over five
   7. Don't know
   8. Refused
   9. Not applicable

13. When was your last blood transfusion?
   1. Within the last six months
   2. More than six months ago
   7. Don't know (can't remember)
   9. Not applicable

14. Have you ever received injection in a place other than a hospital, a health center or a dispensary?
   1. Yes
   2. No [SKIP TO 16]

15. How many times?
   1. One
   2. Two
   3. Three
   4. Four
   5. Five
   6. Over five
   7. Don't know
   8. Refused
   9. Not applicable
16. Have you ever had treatment through administration of medicine direct on a cut on your skin deliberately made for that purpose?

1. Yes
2. No [SKIP TO 19] [ ]
8. Refused [SKIP TO 19]

17. How many times?
1. One
2. Two
3. Three
4. Four [ ]
5. Five
6. Over five
7. Don’t know
8. Refused
9. Not applicable

18. To the best of your knowledge, do you feel that the cutting instrument was sterilized?

1. Yes
2. No [ ]
7. Don’t know
8. Refused
9. Not applicable

19. Have you ever had a cut on your skin to make traditional marks?

1. Yes
2. No [SKIP TO 21] [ ]
7. Don’t know [SKIP TO 21]
8. Refused [SKIP TO 21]

20. How many times?
1. One
2. Two
3. Three
4. Four [ ]
5. Five
6. Over five
7. Don’t know
8. Refused
9. Not applicable
21. Have you ever suffered from hepatitis (yellow coloration of the eye associated with pain in the right upper abdomen)?
   1. Yes
   2. No [SKIP TO 23]
   7. Don't know

22. When was it? ____________________________________________

   9. Not applicable

23. What about your family members, do you know any who has had hepatitis?
   1. Yes
   2. No

24. Right now, are you experiencing or suffering from fever?
   1. Yes
   2. No
   7. Don't know
   8. Refused

25. What about your appetite for food, do you feel that it is lower than usual?
   1. Yes
   2. No
   7. Don't know
   8. Refused

26. Do you have any discomfort on the right upper part of your abdomen?
   1. Yes
   2. No

27. Do you have any feeling of general malaise?
   1. Yes
   2. No
28. What about your urine, does it appear to be darker than usual?
   1. Yes
   2. No
   7. Don’t know
   8. Refused

29. Have you ever suffered from sexually transmitted diseases such as gonorrhoea, syphilis or genital herpes (sores)?
   1. Yes
   2. No [SKIP TO 31]
   8. Refused [SKIP TO 31]

30. When was that?
   1. Within the last six months
   2. More than six months ago
   7. Don’t know
   8. Refused
   9. Not applicable

31. What about sex for money, have you ever had sex for money?
   1. Yes
   2. No [SKIP TO 33]
   3. Refused [SKIP TO 33]

32. How many times?
   1. One
   2. Two
   3. Three
   4. Four
   5. Five
   6. Over five
   7. Don’t remember
   8. Refused
   9. Not applicable

33. Thank you for answering my questions. Please, could you now prepare so that some little blood can be drawn from your arm.
   1. Blood taken
   8. Refused
APPENDIX II

EXPLANATION FORM FOR POTENTIAL PARTICIPANTS IN A STUDY TO DETERMINE THE IMPACT OF HIV INFECTION ON HEPATITIS B CHRONIC CARRIAGE

Good morning/afternoon to you. I of am currently conducting a study on men and women aged 18 years or more who are attending the outpatient clinics of this hospital. The study aims at determining how many people have chronic hepatitis B virus infection. The information to be obtained will help to plan how to prevent hepatitis. Hepatitis is a disease that causes liver problems, usually with yellow coloration of the eye associated with pain in the right upper part of the abdomen and a feeling of general malaise.

The study involves interviewing participants in regard to some important risk factors for acquiring hepatitis B virus infection. In addition to the interview, about 10 c.c of venous blood will be drawn for both hepatitis and HIV tests. The test results will only be revealed to you upon your personal request and any necessary counselling rendered. In order to ensure
strict confidence in lieu of information gathered from you, no records of names or personal identification will be kept. Instead, each participant will be assigned a code number that is unique and only known to himself/herself. I would also like to inform you that the usual complications of blood drawing are limited to discomfort due to needle prick and possibly some slight bleeding. Precautions will however be taken to ensure that any possible discomfort due to needle prick is minimized.

Your participation in this study is completely voluntary and be reassured that whether or not you agree to participate will not affect the health care you and your family receive. Feel free to discontinue any time you so wish.

The whole process (interview and blood drawing) will take about 30 to 45 minutes to complete. I and other interviewers are and will be available to answer any questions that you may have or come up with.

**Thank you**

(This explanation will be translated into the appropriate local language, using terms understood by lay people)
SAMPLE SIZE ESTIMATION:

An important objective of the study is to test for any significant differences between different proportions ($d^*$). In view of this, the appropriate formula for sample size estimation is:

$$n = \frac{(Z_{a/2} + Z_B)^2 \bar{p}(1-\bar{p})(r+1)}{(d^*)^2 r}$$

where $Z_{a/2}$ is the level of significance, $Z_B$ is the study power, $p_1$ is the proportion of exposed (HIV-1 infected) individuals who have HBV infection, $p_0$ is the proportion of unexposed (non-HIV-1 infected) individuals who have HBV infection, $r$ is the ratio of the number of unexposed individuals studied to the number of exposed individuals and $\bar{p}$ is the weighted average of $p_1$ and $p_0$ and is given by the formula:

$$\bar{p} = \frac{p_1 + rp_0}{1 + r}$$

The specific figures used for $r$ is based on HIV-1 sero-positivity rate in a Uganda based hospital outpatients' clinic. $p_1$, $p_0$ and $d^*$ are derived from published data (Hudson et al, 1989). Hudson et al conducted a study in western Uganda, the results of which showed that twelve out of 15 (80%) anti-HIV seropositive outpatients and AIDS patients were anti-HBc
positive compared with 81 of 138 (59%) anti-HIV seronegatives.

Thus, given an alpha-level = 5%, power = 90%, \( p_1 = 0.80 \), \( p_0 = 0.59 \), \( d^* = 0.10 \) instead of \( (0.80 - 0.59) = 0.21 \) and \( r = 3 \), then

\[
\bar{p} = \frac{0.80 + (0.59)3}{1 + 3} = 0.6425
\]

and

\[
n = \frac{(1.96 + 1.28)^2(0.3575)(0.6425)(3+1)}{(0.10)^2 \times 3}
\]

= 321.5 or 322 = the number of HIV infected people required. A sample of 1288 (322 HIV infected and 966 non-HIV infected) is therefore required. In consideration of a possibility of loss or inadequacy of some blood specimens and the HIV sero-positivity in the study subjects being lower than the rate used for sample size estimation, the figure 1288 is rounded up to 1500.

If a study power of 80% is adopted instead of 90%, a total of 964 subjects will be required (241 HIV infected individuals and 723 non-HIV infected individuals).