KWAME NKRUMAH UNIVERISTY OF SCIENCE AND TECHNOLOGY, KUMASI

DEPARTMENT OF MOLECULAR MEDICNE

SEROPREVALENCE OF HEPATITIS B AMONG RECIPIENTS OF THE WHO-EPI VACCINE 10 YEARS AFTER THE INTEGRATION OF THE HEPTITIS B VACCINE IN TO THE EPI OF WA MUNICIPALITY, GHANA.

THESIS SUBMITTED TO THE DEPARTMENT OF MOLECULAR MEDICINE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE IN CHEMICAL PATHOLOGY

By

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DECLARATION

We hereby declare that the work presented, except for references to the other people's which have been duly acknowledged is entirely the product of our own effort carried out in the Department of Molecular Medicine under the supervision of Prof. Mrs. M. Frempong. This is an original research work which have neither in a whole nor part been submitted anywhere for any other degree.

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(Head of Department) Date: ……. . **DEPT. OF MOLECULAR MEDICINE**

DEDICATION

I dedicate this work to my dear mother, Mrs Salamatu Koray, whose love and support has brought me this far, to all my guardians and mentors and to all the children who participated in this study.

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To God Almighty who knows our end from the beginning, who is able to do just what He says He will do and has made all things beautiful in our lives be praise now and forever-Amen.

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ABBREVIATIONS

Acronyms & Abbreviations

CHPS: community health based planning and services

EPI: Expanded Program on Immunization

NHIS: National Health Insurance Scheme

HBV: Hepatitis B Virus

Hbsag: Hepatitis B surface antigen.

Hbsab /anti-hbs : Antibodies to hepatitis B surface antigen

Hbcab / anti-hbc: Antibodies to hepatitis B core protein

Anti-hbe: Antibody to hepatitis B e antigen;

Anti-hbs, : Antibody to hepatitis B surface antigen;

Anti-hbc: Antibody to hepatitis B core antigen;

EPI : Expanded Programme on Immunisation;

GMT: Geometric mean titre; HbeAg

: Hepatitis B e antigen;

HbsAg: Hepatitis B surface antigen;

HBV DNA : Hepatitis B virus deoxyribonucleic acid;

HBV: Hepatitis B virus;

HCC : Hepatocellular carcinoma

BMI:	Body mass index
GMT:	Geometric mean titers
CHPS:	Community base Health Planning Services
IU/L:	International Unit per liter.
%:	Percent
WHO:	World Health Organization
KNUST:	Kwame Nkrumah University of Science and Technology
UNICEF :	United Nations International Children's Emergency Fund
Ag (p):	pre-coated HBsAg
Ab(s):	Anti-HBs in standards or sample (Ag)ENZ:

HRP conjugated HBsAg.

ABSTRACT

BACKGROUND

Hepatitis B is a serious infectious disease of the liver and a major public health problem worldwide. It is however vaccine preventable as evidenced in the developed world. As per the WHO recommendations, Ghana and specifically, the Wa Municipal Health Administration included the hepatitis B vaccine (HB) in the national Expanded Programme of Immunization (EPI) in 2002. This study evaluated the sero prevalence of hepatitis B virus, anti- HBs levels and sero protection rates among children in the Wa Municipality 5 to 10 years after completing their primary HBV vaccination schedule as part of the EPI

METHODS

Two hundred and thirty three symptomatically healthy pupils who had received the hepatitis B vaccine as part of their primary infant immunization were followed up 5 to 10 years after their primary immunization schedule against the background of 234 pupils who were born before the integration of the HBV vaccine in to the EPI and so never received the HBV vaccine as part of the EPI. Blood samples were taken and tested for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb at the Upper West Regional Hospital laboratory. The samples from the children who had received the hepatitis B vaccine as part of their infant immunization were further tested quantitatively to determine the anti- HBs antibody levels by enzyme- linked immunosorbent assay (ELISA) at the Navrongo Institute of Tropical Research. Participants with anti HBs antibody titres greater than or equals to 10 mIU/mL considered seroprotected as per WHO and Kit manufacturer's

standards.

RESULTS

Prevalence of HBV measured by HBsAg positivity among the children decreased from 10.3 % among children who had not been vaccinated to 0.9% among the vaccinated children. The significant decrease in the prevalence of HBV among the two groups of participants could be attributed to the effectiveness of the pentavalent vaccine in preventing the transmission of HBV among the vaccinated children. The overall prevalence among the children in the Wa Municipality is 5.57 % based on HBsAg positivity. After vaccination one was less likely to be associated with HBV and more likely to test positive for anti HBs. Anti-HBs titres recorded ranged from 0.0mIU/mL to 462.7 mIU/mL. Only one participant recorded an anti- HBs level of 0mIU/mL indicating a 99.57% seroconversion rate. The protective immunity measured by anti – HBs levels greater than or equal to 10 mIU/mL is 27.04% among the vaccinated children. No significant difference was observed in the anti HBs antibody levels between the male and female vaccinated children. (p: 0.458). But it was evident that the anti HBs levels though extremely low, increased with increasing age for children between 6 years and 8years. This was supported by increasing GMT levels from 36.6mIU/mL in children between the ages of 5 to 7 years with a mean age 6.7 years to 48.4 mIU/mL in children between the ages of 8 to 10 years with a mean age of (8.4). ($P < 0.0001$).This was observed after an initial decrease in anti HBs levels with increasing age for children between the ages of 5 and 6 years and also in children between the ages of 8 to 10 years. This finding might be as a result of the fact that the vaccinated children with anti HBs levels below the protective range probably responded to the primary vaccination but the anti HBs levels have waned over the years to levels that are considered non protective. They might have developed an immune memory that recognizes and produces anti- HBs upon exposure to the hepatitis B virus. (Anamnestic Response). This might be the reason why older vaccinated 7 and 8 year old children who completed the primary

infant vaccination much earlier rather recorded higher anti HBs levels than children between the ages of 5 and 6 years who received their vaccination much later. Two (0.9%) vaccinated children demonstrated break through infections compared to 10.3% among the non vaccinated group suggesting the pentavalent vaccine within the frame work of the EPI is effective in protecting majority of the children in high endemic areas from HBV infection. It can be concluded that the penta valent vaccine given at 6, 10 and 14 weeks as part of the EPI for infants, is highly effective as it has shown a positive impact in the elimination of the HBV in children under 10 years of age.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND

Hepatitis B is a viral infection of the liver caused by the hepatitis B virus (HBV). It can be prevented through immunization but it remains a serious infectious disease. Hepatitis B virus infection is a key public health burden globally. It is projected that 2 billion individuals have been infected with Hepatitis B Virus, 350 million have become chronically infected, and that each year 4 million new acute infections will occur (Lavanchy, 2005). Hepatitis B Virus infection causes more than 50% of all cases of hepatocellular carcinoma and approximately 600,000 deaths per annum (Williams, 2006).

There is a varied disparity in the prevalence of chronic hepatitis B virus infection due to geographical area, and this is narrowly interlinked with the major ways of Hepatitis B Virus transmission (Maddrey, 2000). One of the principal factors that influence the incidence and occurrence of the chronic carriage status is the age at which Hepatitis B Virus infection occurs.

Approximately 90% of babies born to HBV surface antigen- (HBsAg) and hepatitis B e antigen (HBeAg)-positive mothers and almost 30% of children infected before 6 years of age turn out to be chronic carriers, matched with less than 10% of adults or grown-up children (Hyams, 1995; Ip et al., 1989). Children in early childhood can be horizontally infected or vertically infected from carrier mothers (perinatally). Three modes of Hepatitis B Virus transmission from HBsAg-positive mothers to babies have been proposed: (i) Trans placental intrauterine transmission; (ii) Contact with maternal infected fluids in the birth canal during delivery and (iii) Transmission through breast feeding and childcare during postnatal age (Ghendon, 1987; Shepard et al., 2006). Sub-Saharan Africa is

considered to be an area of high endemicity and more than 75% of adults have been exposed to Hepatitis B Virus infection, of which 5–25% are estimated as being chronic carriers.

Most children are being infected by the age of five because the predominant route of Hepatitis B Virus transmission is horizontal (Dumpis et al., 2001; Kew, 1996; Martinson et al., 1998). It has been observed that maternofetal transmission is the principal route of transmission in high prevalence endemic areas such as south-east Asia in contrast to what is observed in Africa where maternofetal transmission appears to play a minor role (Zhang et al., 1998).

Depending on the prevalence of hepatitis B surface antigen (HBsAg), three geographic classes of endemicity have been defined: areas with low (<2%), intermediate (2%–7%), and high (⩾8%), endemicity.(Shepard. et al, 2006, Custer. et al 2004, Lavanchy, 2005). The key roles in perpetuating the endemicity of HBV infection during childhood are vertical transmission and horizontal transmission. (Shepard. et al, 2006, Custer. et al 2004, Lavanchy, 2005). Promiscuous sexual activity and unsafe injection are other relevant means of transmission in countries with high or intermediate endemicity. (Shepard. et al, 2006, Custer. et al 2004, Lavanchy, 2005). In developing countries, it is projected that at least 30% of new Hepatitis B Virus infections are due to syringe reusage in the health care setting (Hauri, et al.2004). The most significant risk factors for infection in countries with low-level endemicity are multiple sexual partners and injection drug use (IDU). (Shepard et al, 2006, Custer. et al 2004, Lavanchy, 2005). Highly endemic populations may be existing in low endemic nations, and the effect of immigration is most likely overlooked in many countries that face portent large-scale migration (Shepard. et al, 2006, Williams, 2006, Giancchino et al, 2001). Through the use of volunteer donors and progressively sensitive HBsAg tests, the risk of Hepatitis B Virus transmission by blood transfusion has been steadily decreased (Alter and Houghton, 2000). In Ghana, Sarkodie

et.al, (2000) reported a hepatitis B virus prevalence of 15.3% among blood donors based on hepatitis B surface antigenemia.

Using HBsAg and of any HBV marker, the prevalence of HBsAg among Ghanaian rural children aged 1-16 years was 21% and 75% respectively. (Martison et al., 1998). The only most effective way of controlling hepatitis B disease is by immunization. The development of anti-HBs level greater than or equal to 10 mIU/mL is considered as protective immunity and any level less than 10 mIU/mL as non-protective is directly associated with protective immunity of HBV following vaccination. (Mahoney, 1995). After a complete vaccination, most individuals develop antibody titers greater than 100 IU/L within 6-8 weeks. Some healthy individuals seemingly do not demonstrate anti-HBs response or respond poorly to the vaccine and are considered as nonresponders or hypo responders with titer less than 10 IU/L and 10-100 IU/L respectively. (Zuckermann, 1996).

In agreement with references made by the World Health Organization (WHO, 1991) 110 countries have incorporated the Hepatitis B Virus vaccine into their Expanded Programme on Immunization (EPI) by April 2000 including only 8 were African countries, regardless of the high prevalence and carriage of Hepatitis B Virus infection in this continent. The pentavalent, diphtheria-pertussistetanus (DPT), hepatitis B (HBV), and *Haemophilus influenzae* type b (Hib) vaccine was incorporated into Ghana's immunization program in place of the conventional DPT vaccine by the Ministry of Health in Ghana while maintaining the immunization schedule at 6, 10, and 14 weeks after birth in 2002 as recommended by the WHO.

In several follow-up studies in Taiwan a steady yearly decline in antibody titers against the HBsAg among vaccinees has been noted, although the vaccination program has been very successful in reducing carriage rate of HBV in the world (Lu, et al, 2008), (Lee, et al, 1995). The antibody to HBsAg

(anti-HBs) seropositivity rate of the vaccinees declined from 99% at 1 year to 83% at 5 years, 71.1% at 7 years, 37.4% at 12 years, and 37% at 15-17 years. (Jan et al. 2010). The rate of seronegative three HBV viral markers which includes HBsAg, antibodies to HB core protein (antiHBc), and anti-HBs increased from 12.7% at 1 year to 62.6% at 15-17 years. (Jan et al, 2010).

1.2 STATEMENT OF PROBLEM

Worldwide infection persists, despite the availability of a vaccine, and in spite of recommendation by WHO to screen pregnant women for HBsAg in order to provide appropriate immunization for the babies of mothers who test positive. Until 2011, pregnant women within the Wa Municipality were not routinely screened for HBV as a step in preventing vertical transmission. Even now that it is done, the babies of HBV positive mothers are not vaccinated on the day of delivery to prevent them from getting infected. In 1995, a controlled study conducted to evaluate the immunogenicity of the HBV vaccine (Hepaccine-B, Cheil, Korea) reported that Hepaccine-B is extremely immunogenic, resulting in a seroprotection rate (anti-HBs≥10 mIU/ml) of 93.0% and geometric mean titre (GMT) of 257.6 mIU/ml (Aspinall and Kocks, 1998).

As the above studies were controlled in many ways, it remains unknown whether comparable results would be achieved when the HBV vaccine is monitored and administered by the public health personnel within the framework of the Ghanaian EPI. A study conducted in South Africa for UNICEF in 1995 revealed that the equipment used for storage and transportation of vaccines in certain provinces of South Africa, might cause freeze-sensitive vaccines to be frozen (Battersby,

1995). It is also imperious to establish surveillance programmes to monitor development of antibody resistant, or vaccine escape HBV strains, as recommended by the Viral Hepatitis Prevention Board (Viral Hepatitis Fact Sheet, 1999).

In other parts of the world, antibody resistant strains have been reported in some vaccinees and/or recipients of hepatitis B immunoglobulin (HBIG) therapy, (Fortuin et al, 1994), (Oon et al, 1995) and (Nainan et al, 1997).

1.3 JUSTIFICATION

There is no comprehensive and authoritative data regarding the prevalence of Hepatitis B infection and the sero-protective rate of the pentavalent vaccine 10 years after the integration of the HBV vaccine in to the EPI in Ghana and specifically, the Wa municipality. It is imperative establish surveillance programmes to observed emergence of antibody resistant, or vaccine escape HBV strains, as recommended by the Viral Hepatitis Prevention Board (Viral Hepatitis Fact Sheet, 1999).

Post vaccination testing for antibody titer within 1 -6 months after completion of vaccination schedule is recommended to detect non responders. Although antibody titers decline, it should be reasonably greater than 10 IU/L at any time in order to ensure immune protection among vaccinated people. (VHPB, 1996). No such surveillance has been conducted hence there is no evidence or data regarding the prevalence of HBV, the effectiveness of the vaccine, the seroprotection rate and duration of immunity among recipients of the vaccine in Ghana. This study seeks to determine prevalence of HBV among the vaccinated children and non vaccinated children born before the implementation of the HBV EPI in Ghana. It is also to evaluate the efficacy of the vaccine, the seroprotection rate and duration of immunity among recipients of the vaccine by determining the sero positivity or seronegativity of the HBV serological markers HBsAg, HBsAb, and HBcAb among recipients of the EPI vaccine 5 -10 years after completion of their primary vaccination schedule. It is also to find out whether the outcome of the study will be consistent or comparable with findings elsewhere in the

world. The outcome of this study will to a large extent inform and help in boosting our confidence in the HBV EPI as well as other vaccines.

1.4 AIM

The aim of this study was to determine the sero prevalence of HBV infection among recipients of the WHO- EPI vaccine 10 years after integration of the hepatitis B vaccine in to the EPI of the Wa Municipality.

1.4.1 SPECIFIC OBJECTIVES

1. To determine the seroprevalence of HBV markers (HBsAg, HBsAb and HBcAb) among recipients of the WHO- EPI vaccine.
2. To determine the seroprevalence of HBsAg among children born before the integration of the HBV vaccine in to the WHO-EPI policy in Ghana.
3. To estimate the seroprotection rates and HBsAb level among vaccinated children.
4. To estimate the impact of the WHO- EPI vaccine in reducing HBsAg carriage rates.

CHAPTER TWO

LITERATURE REVIEW

The earliest recognition of the public health importance of hepatitis B virus (HBV) infection is thought to have occurred when it appeared as an adverse event associated with a vaccination campaign. In 1883 in Bremen, Germany, 15 percent of 1,289 shipyard workers inoculated with a Smallpox vaccine made from human lymph fell ill with jaundice during the weeks following vaccination (Lurman, 1885). The etiology of ‘‘serum hepatitis,’’ as it was known for many years, was not identified until the 1960s (Blumberg et al, 1965), and only following the subsequent development of laboratory markers for infection was its significance as a major cause of morbidity and mortality worldwide fully appreciated (Beasley,1988). According to the most recent World Health Organization estimate, two billion people worldwide have serologic evidence of past or present HBV infection, and 360 million are chronically infected and at risk for HBV-related liver disease.

Approximately one third of all cases of cirrhosis and half of all cases of hepatocellular carcinoma can be attributed to chronic HBV infection. HBV is estimated to be responsible for 500,000–700,000 deaths each year (WHO, 2000, WHO, 2004, Perz et al 2004). Despite the vast population of infected persons, efforts to prevent and control HBV have met with increasing levels of success and hold promise for large reductions in disease burden in the future. A great deal of credit for achievements to date stems from the introduction of hepatitis B vaccines. First licensed in the United States in 1981, hepatitis B vaccine is now one of the most widely used vaccines in the world and is part of the routine vaccination schedule for many of the world’s infants and children. It is the world’s first cancer prevention vaccine and the first vaccine to prevent a sexually transmitted disease.

In countries where large-scale vaccination efforts were made in the first decade after introduction of the vaccine, the epidemiology of hepatitis B and HBV infection has been transformed, and there are early signs that the burden of HBV-related sequelae will be significantly reduced as vaccinated

populations age. In this comprehensive study of the prevalence of hepatitis B among children before and after the implementation of the HBV vaccine in to the Ghanaian EPI, we focus on studies that highlight current trends and policies and directions for the future regarding hepatitis B vaccination and the impact on HBV prevalence among children.

2.1 HEPATITIS B VIRUS

Hepatitis B Virus is a DNA virus categorized in the virus family Hepadnaviridae. The only known natural are host humans. Hepatitis B Virus enters the liver through the bloodstream, and replication take place only in liver tissue. The diameter of the whole infectious virus is 42–47 nm and concentrations as high as 10^8 virions per ml circulate in the bloodstream. The hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), partially double-stranded 3,200-nucleotide, and DNA molecule and DNA polymerase with reverse transcriptase activity are found in the inner core of the virus. Hepatitis B surface antigen (HBsAg) is found both on the surface of the virus and as self-assembling, non infectious spherical or tubular particles. (Shepard et al, 2006).

2.2 NATURAL HISTORY AND CLINICAL MANIFESTATIONS OF HBV INFECTION

Hepatitis B Virus infection can result in acute self-limited hepatitis, subclinical or asymptomatic infection, or fulminant hepatitis necessitating for transplantation of the liver. HBV infected individuals may also develop chronic HBV infection, which can results in cirrhosis or hepatocellular carcinoma. The possibility that newly HBV infected persons will develop chronic HBV infection is dependent on their age at the time of infection (McMahon et al, 1985).

About 25–50% of children infected between 1 and 5 years of age, more than 90% of infected infants and 6–10% of acutely infected older children and adults develop chronic infection.

Immunocompromised persons (for instance, hemodialysis patients and human immunodeficiency virus infected individual) are also at increased risk of developing chronic infection (Hadler et al, 1991, Hyams, 1995). Persons infected as children assume an extremely large burden of morbidity and mortality attributable to HBV due to the inverse relationship between risk of chronic infection and age. Up to Older children and 25% of infants who acquire HBV in due course develop HBV related Hepatocellular carcinoma or cirrhosis. Adults who acquire HBV infection in their childhood and have developed chronic HBV infection since childhood progress to primary hepatocellular carcinoma at a rate of 5% per decade, which is 100–300 times the rate among uninfected persons.
(Custer. et al 2004).

2.3 ACUTE HEPATITIS B

The average incubation period (time from exposure to onset of jaundice) is 90 days (range: 60–150 days) for newly infected individual who develop acute hepatitis, t (Krugman, et al. 1979, Hoofnagle and Dibisceglie 1991). The probability of developing signs of hepatitis as a result of a new Hepatitis B Virus infection is age-dependent. Over 90% of perinatal Hepatitis B Virus infections are asymptomatic, while the typical signs of acute hepatitis are prominent in 5–15% of newly infected young children (1–5 years) and in 33–50% of, adolescents, older children and adults (McMahon et al 1985). Individuals with acute hepatitis B are presented with signs and symptoms that comprise fever, nausea, abdominal pain, dark urine, jaundice, and vomiting, hepatomegaly or splenomegaly, and changes in stool color. HBsAg is the first serologic marker to become measurable in individuals with acute HBV infection and antibodies to hepatitis B core antigen. Immunoglobulin M antibodies to hepatitis B core antigen become undetectable in the 6–12 months after infection. Individuals who develop chronic infection as well as those who recover from infection produce total antibodies to

hepatitis B core antigen for life. Antibodies to hepatitis B surface antigen (anti-HBs) develops during convalescence and in persons who recover from Hepatitis B Virus infection, HBsAg is eliminated from the blood. Most people who recover from HBV infection will be positive for both anti-HBs and antibodies to hepatitis B core antigen, but anti-HBs becomes undetectable in due course. The existence of anti-HBs shows immunity to Hepatitis B Virus infection. Table 2.1 shows a summary of serologic markers present at different times after vaccination or during Hepatitis B Virus infection.

The presence of HBeAg shows active replication and higher chances of transmission.

Immuno-compromised individuals can develop reactivation of earlier recovered Hepatitis B Virus infection (Ortiz-Interian et al1990, Davis et al, 1995). Recovered acute infection is not a risk factor for succeeding hepatocellular carcinoma or cirrhosis (Seeff et al 1987).

Table 2.1 Interpretation of serologic test results for hepatitis B virus infection

HBsAg	Total antibodies to HBcAg	IgM antibodies to HBcAg	HBsAb	Interpretation
Negative	Negative	Negative	Negative	Susceptible; never infected

Positive	Negative	Negative	Negative	Early acute infection; transient (21 days) after vaccination
Positive	Positive	Positive	Negative	Acute infection
Negative	Positive	Positive	Negative	Acute resolving infection
Negative	Positive	Negative	Positive	Past infection; recovered and immune
Positive	Positive	Negative	Negative	Chronic infection
Negative	Positive	Negative	Negative	False-positive (i.e., susceptible); past infection; ‘‘low-level’’ chronic infection; passive transfer to an infant born to a mother who is positive for hepatitis B surface antigen
Negative	Negative	Negative	Positive	Immune if titer is >10 mIU/ml

From Shepard et al 2006, Epidemiology Review, Vol. 28,2006. Division of Viral Hepatitis, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.

2.4 CHRONIC HBV INFECTION

Chronic Hepatitis B Virus infection is defined as either the presence of HBsAg in individuals who test negative for immunoglobulin M antibodies to hepatitis B core antigen or the existence of HBsAg in the serum for at least 6 months. Persons with chronic HBV infection do not develop anti-HBs, and HBsAg typically persists for decades unlike individuals who recover from acute HBV infection. In the early stages of the illness, HBeAg, a marker of high viral replication activity which correlates with greater infectivity, is also usually present. HBeAg is undetectable at some point (usually 10 years or more) after an acute infection in many individuals with chronic infection; this change usually shows that viral replication is decreasing. About 0.5% of adults and a decreased proportion of children who

have had chronic Hepatitis B Virus infection will clear HBsAg and develop antiHBs annually (Alward et al, 1985, Liaw et al, 1991, Adachi et al 1992, McMahon et al, 1990.).

Chronic infection is accountable for most of the problems of diseases associated with Hepatitis B Virus infection although a significant proportion of chronically infected individuals will stay asymptomatic for decades and die of causes unrelated to HBV. Data reported from follow-up studies in individuals with HBV infection as young children or infants indicates that about 15–25% of individuals chronically infected die earlier from hepatocellular carcinoma or cirrhosis (Beasley et al 1981, McMahon et al 1990).

Individuals who have developed the HBV-related sequelae of hepatocellular carcinoma or cirrhosis may show no symptoms until diagnosis, or they may encounter intermittent flare-ups of signs and symptoms of acute hepatitis. Poly arthritis nodosa, membranous glomerulonephritis, and membrano proliferative glomerulonephritis are the extra hepatic complications which can also occur. Periodic medical evaluation should be done in individuals with chronic HBV infection and consistent screening for hepatocellular carcinoma using ultrasonography or *a*-fetoprotein as recommended by some authorities (Lok and MCMahon, 2001).

Treatments of chronic hepatitis B by approved therapeutic agents are now being used to achieve persistent suppression of Hepatitis B Virus replication and lessening of liver disease for some infected persons (Lok and MCMahon, 2001). However, the development of resistance to antiviral drugs and decreased rates of HBsAg clearance continue to be obstacles to treatment for many patients with chronic infection.

2.5 TRANSMISSION

Hepatitis B Virus is transmitted by mucosal contact or percutaneous to infected blood or other body fluids. Perinatal/mother-to-child; household (nonsexual); sexual; needle-sharing; and occupational/health-care-related are numerous forms of human contact through which HBV

transmitted. Blood and serum contain the highest concentrations of infectious HBV. Moreover, other serum-derived body fluids, for instance saliva and semen are also infective (Bond et al. 1977). The major reservoirs for transmission are individuals with chronic HBV infection, whiles any individuals testing positive for HBsAg is possibly infective to both sexual contacts and household. Transmission may occur indirectly via contaminated surfaces and other objects because Hepatitis B Virus can remain stable and very infective on environmental surfaces for at least 7 days.

One of the most common ways of HBV infection worldwide is transmission from a woman who is chronically infected to her infant during delivery. Most children may be infected perinatally from carrier mothers or horizontally in early childhood. The suggested three mechanisms of Hepatitis B Virus transmission from HBsAg-positive mothers to babies are: (i) transmission by contact with maternal infected fluids in the birth canal during delivery; (ii) trans placental intrauterine transmission and (iii) postnatal transmission during childcare or through breast feeding from mothers to infants (Ghendon, 1987; Shepard *et al.*, 2006). During the birth process, perinatal transmission of HBV most often occurs; it is rare but in-utero transmission can occur and accounts for less than 2% of perinatal transmissions (Wong et al, 1984, Xu et al.1985). If the mother is HBeAg-positive, the risk of perinatal infection is 70–90 % and 5–20% in babies born to HBsAg-positive mothers (Beasley et al. 1977).

In certain circumstances where there is regular and persistent close personal contact with an HBV infected individuals, HBV transmission can also occur (Davis et al. 1989). An estimation of about 16,000 children less than 10 years of age were infected yearly in the United States through contact with HBsAg-positive community contacts or household members prior to enactment of universal infant hepatitis B immunization, (Armstrong et al. 2001, Mast et al. 2004). Transmission is postulated to occur from body fluid exposures from parents or in apparent blood, siblings, or playmates that inoculate HBV into abrasions, cutaneous scratches, or other lesions or onto mucosal surfaces although the precise mode of transmission is unknown (Francis et al. 1981, Lauer et al. 1979).

Higher sero-prevalence of HBV infection has been reported in sexual contacts of chronically infected individuals than control populations, which include nonsexual contacts (households) of infected persons (Heathcote et al. 1974). Reports show that individuals with acute hepatitis B are more likely to have multiple heterosexual partners than controls, and the sero-prevalence of Hepatitis B Virus infection relates with increased numbers of current and lifetime hetero sexual partners (Alter et al. 1986, Alter et al. 1989). Gay men have long been known to exhibit high rates of the infection (Dietzman et al 1977), and they exhibit persistently increased HBV sero-prevalence rates than the general population (MacKeller et al. 2001).

Behaviors such as syringes, sharing of needles, and other drug paraphernalia among injection drug users put them at higher risk for HBV infection. In the United States and elsewhere most injection drug users have current HBV infection or past serologic evidence (Levine et al.1994). Moreover, the risk among injection drug users can differ depending on the occurrence of chronic Hepatitis B Virus infection in the community, as well as preparation practices and drug-sharing. During the mid-1990s in the United States, approximately 70% of injection drug users were infected after 5 years of injecting (Garfein et al.1996, Levine et al 1995). Besides injection drug use, such as tattooing and acupuncture; outbreaks linked to other percutaneous exposures, have been reported (Limentani et al. 1979, Kent et al. 1988).

Health-care-related transmission has long been acknowledged as a relevant source of new HBV infections worldwide. Patient-to-provider, provider-to-patient, and patient-to-patient transmission have all been reported, while the frequencies with which these kinds of transmission occur are widely contradictory.

Before the widespread hepatitis B vaccination of health care workers, patient-to-provider transmission was common; in the United States it is estimated that 12,000 health care workers per year were infected in the pre vaccine period (MMWR Morb Mortal Weekly Report, 1989). The level of blood and needle

exposure of a health care worker has shown to correlate to the risk of infection (Hadler et al. 1985). The risk of Hepatitis B Virus infection varies according to the volume and viral concentration of the infectious fluid following needle-stick exposure. The risk from inoculation is at least 30% after a needle stick with HBeAg-positive blood, but if blood is HBeAg-negative it's less than 6%. The major source of new HBV infections in the developing world is patient-to-patient HBV transmission. Patient-to patient HBV transmission can result from percutaneous exposure to contaminated equipment used for injections or from blood or mucosal exposure to contaminated medication or other procedures. Due to the lack of awareness of infection control practices, the purchase of new disposable equipment, and lack of resources for sterilization and economic incentives and cultural preferences favoring overuse of injections, exposures to contaminated equipment during scarification, circumcision or to contaminated therapeutic injection equipment are common in many settings in developing countries. Contaminated injections caused an estimated 21 million Hepatitis B Virus infections globally in 2000, accounting for 32% of all new infections

(Hauri, et al.2004). Outbreaks involving this kind of transmission remain a consistent problem as well, and they usually stem from lapses in infection control practice by health care workers in the advanced world. Associated mode of transmission include finger-stick devices, multi dose vials, acupuncture needles, and jet injection guns (Polish et al. 1992, Samadari et al. 2005,a, MMWR Morb Mortal Weekly Report, 2005, Williams et al. 2004).

Dialysis units particularly have been served as reservoir for HBV transmission in contaminated environmental surfaces in health care settings (MMWR Recommendation Report, 2001b). There has been largely elimination of transmission of HBV via transfusion of blood products in most parts of the world by screening blood donors and employing techniques that ensure viral inactivation of products made from blood, such as factor concentrates (Busch et al.2003). Provider-to-patient HBV

transmission has been barely reported. Majority of the events have been related with health care providers' performing invasive procedures, and most occurred before the general use of hepatitis B vaccine and the implementation of universal precautions in standard infection control practice (Gunson et al. 2003).

2.6 GLOBAL PATTERNS OF TRANSMISSION

According to three classifications of endemicity, the worldwide epidemiology of Hepatitis B Virus infection has conventionally been described—high, intermediate, and low—subject to the percentage of the population that is seropositive for HBsAg. Countries with high endemicity are those where HBsAg seroprevalence is higher than or equal to 8%; countries with intermediate endemicity are those where sero-prevalence is 2–7%; and those with low endemicity are those where seroprevalence is less than 2%. The degree of HBV endemicity often associates with the major mode of transmission and HBsAg sero-prevalence has marked geographic disparities. Perinatal and horizontal routes are responsible for most disease transmission in highly endemic locations, and 70– 90% of the adult population has serologic evidence of prior infection (Lin et al.2003, Yao, 1996, Custer et al. 2004). Highly endemic countries have markedly higher rates of liver cancer than countries with lower endemicity because hepatocellular carcinoma is a potential sequela of chronic HBV infection, and hepatocellular carcinoma is a predominant cause of mortality in these areas.

More than 75% of adults have been exposed to HBV in areas of high endemicity such as SubSaharan Africa of which Ghana forms part, with an estimated 5–25% being chronic carriers. Horizontal transmision is the predominant route of HBV transmission with majority of children being infected by the age of five (Dumpis *et al.*, 2001; Kew, 1996; Martinson *et al.*, 1998). In Africa, maternofetal

transmission seems to play a minor role, in contrast to what is reported in other highprevalence endemic areas, such as south-east Asia, where maternofetal transmission is the principal vehicle of transmission (Zhang *et al.*, 1998). There is a mix of perinatal, horizontal, health-carerelated, sexual, and other forms of transmission in countries with intermediate endemicity. In low endemic countries, majority of new infections occur among young adults and are acquired through sexual contact or through injecting drug use. Depending upon sero-prevalence rates of immigrant groups and native/indigenous populations, highly endemic population subgroups may be present within low endemicity countries.

About 60% of the world's population lives in areas where Hepatitis B Virus infection is highly endemic, including Indonesia (222 million), China (total population, 1.3 billion), Nigeria (132 million), and much of the rest of Asia and Africa (Population Reference Bureau.2005). Taiwan, where 15–20% of the general population had chronic HBV infection and 30% of those chronically infected were HBeAg-positive is one of the notable examples of high pre-vaccine-era burdens of disease (Lin et al.1998, Sung et al.1982.), and Gambia, where the prevalence of chronic infection among children is 36% (Whittle et al 1983).

It has been reported that the prevalence of the surface antigen of hepatitis B virus (HBsAg) is 15.3% in among blood donors in Ghana (Sarkodie et al., 2001). Martinson et al reported that the prevalence of HBsAg among Ghanaian rural children aged 1-16 years was 21% using HBsAg and of any HBV marker was 75% (Martison FE et al., 1998). South Asia, Southern Europe, and the Middle East have an intermediate level of HBV endemicity. In India, HBsAg sero-prevalence is about 5 percent, and the main modes of HBV transmission are perinatal, child-related/ horizontal, and health-care-related, particularly unsafe injections (Kurien et al. 2005). The prevalence of chronic Hepatitis B Virus infection ranges from 3 percent to 10 percent in Italy, Russia, and Turkey, and unsafe injections have

been associated as a major route of HBV transmission (Iashina et al. 1992, Erden et al. 2003, Da Villa.,Sepe. 1999). The western Amazon basin, which includes Brazil and Peru, is a highly endemic area, with observed HBsAg sero-prevalence rates greater than 10 percent. However, most of Central and South America is considered a region of low HBV endemicity (Brasil et al 2003). Many advanced nations, including the United States, fall into the low endemicity category. Before the era of extensive hepatitis B vaccine use (1988–1994), 0.42% of the US population was HBsAgseropositive, and 4.9% had serologic markers of previous or current HBV infection (McQuillan et al.1999). Some native populations and immigrant groups in the United States had sero-prevalence rates similar to countries with high endemicity and members of these populations involved a disproportionate share of new HBV infections nationwide in the pre vaccine period (Armstrong et al. 2001). Research conducted among Southeast Asian families settled in the United States found that 5–10% of children aged 1–10 years and 15% of children aged 11-20 years were chronically infected in the 1980s (Frank et al. 1989, Mahoney et al.1995). In the 1970s studies conducted among Alaska Native children living in Alaska reported that 15% t had serologic evidence of infection and 6 % were infected chronically (McMahon et al.1985, Schreeder et al.1983). A recent pre-immunization

HBV survey established HBsAg carriage of 12.8% in 3-year-old South African children as South Africa is a region that was previously identified as endemic (7–10% HBsAg carrier rate) for HBV infection, (Mast et al. 2005.).

FIGURE 2.6. Geographic distribution of the prevalence of chronic hepatitis B virus infection, 2002. (Source: Mast et al. (28).)

2.7 PREVENTION

The most effective way to control hepatitis B disease is immunization. Numerous effective hepatitis B prevention measures had been employed to some degree, including screening of blood donors, preparation of plasma-derived products in a way that inactivates HBV virus, implementation of infection control measures, and administration of hepatitis B immune globulin following suspected exposure, especially for infants born to HBsAg-positive women before the introduction of hepatitis B vaccines. None have been as effective as active immunization with hepatitis B vaccine, which remains

the single best significant hepatitis B prevention measure, although all of these activities can lessen the risk of HBV transmission.

2.8. THE INVENTION OF THE HEPATITIS B VACCINE

Development of the vaccine began with the awareness that the Australia antigen was part of a virus that caused hepatitis (in 1968, by virologist Alfred Prince). In 1981, Maurice Hilleman at Merck used three treatments (pepsin, formaldehyde and urea) of blood serum together with rigorous filtration to yield a product that could be used as an innocuous vaccine and it was first licensed by the Food Drug Authority. Because clinicians knew that it was a product made from human blood serum it did not succeed in the marketplace. In 1986, it was withdrawn from the marketplace when Pablo DT Valenzuela, Research Director of Chiron Corporation succeeded in making the antigen in yeast and invented the first recombinant vaccine and it is the vaccine still in use today.

2.8.1 Hepatitis B Vaccine

The vaccine of Hepatitis B is developed for the prevention of hepatitis B virus infection. It comprises one of the viral envelope proteins, hepatitis B surface antigen (HBsAg) which is produced by yeast cells, into which the genetic code for HBsAg has been inserted (Hepatitis B vaccine from Merck, 2010). The leading vaccine became available in 1981. The Hepatitis B vaccine is given in a three-dose course(Beasley, 1988) with the second dose at least one month after the first dose and the third dose given six months after the first dose (Hepatitis B foundation 2009). Then an immune system antibody to HBsAg is established in the bloodstream. The antibody is known as *anti-HBsAg*. Immunity to hepatitis B infection is provided by this antibody and immune system memory (CDC viral Hepatitis

2009). Now varieties of vaccines are available in the market. Currently recombinant DNA vaccines are available, which means they are produced by introducing the gene for HBV into common baker's yeast where it is grown, harvested, and refined. These vaccines are given intramuscularly. Hepatitis B Virus infection cannot occur from receiving hepatitis B vaccine. Most recently available hepatitis B vaccines are produced by recombinant DNA technology where the first licensed hepatitis B vaccines were plasma derived and composed of purified HBsAg. In the US, vaccine formulations employing two- and four-dose schedules have also been licensed in some age groups but Hepatitis B vaccines are typically given in a three-dose series. Single antigen hepatitis B vaccine can only be given at birth and to newborns younger than 6 weeks of age. Other vaccines can be administered concurrently with Single-antigen vaccines at any age, and in the United States many combination vaccines containing hepatitis B antigens are approved and elsewhere. Hepatitis B vaccines are considered equivalent in their immunogenicity when used in the applicable age group and given at the manufacturer's endorsed dose, and effectiveness can be used interchangeably.

Reports show that there is a protective concentration of anti-HBs (10 mIU/ml) of adherence to licensed hepatitis B vaccination schedules in 90–100% of healthy children, infants, and adults.

2.8.2 Response To Vaccination

Blood test is usually taken after an interval of 1–4 months to establish if there has been an adequate response, which is defined as an anti-hepatitis B surface antigen (anti-Hbs) antibody level greater 100 mIU/ml following the primary course of 3 vaccinations. Such a full response occurs in about 8590% of individuals (Joint Committee on Vaccination and Immunization (2006; Immunization against Infectious Disease 2006). Antibody concentrations between 10 and 100 mIU/ml is considered a poor response. They do not need further retesting but these people should receive a single booster vaccination at this time.

Individuals who fail to respond (anti-Hbs antibody level below 10mIU/ml) should be tested to omit past or current Hepatitis B infection, and given a repeat course of three-dose series, followed by further retesting 1-4 months after the second course. Individuals who still do not respond to a second course of vaccination may respond to intradermal administration or to a double dose of a combined Hepatitis A and B vaccine or to a greater dose of vaccine (Levitz et al. 1995). People who still fail to respond will require hepatitis B immunoglobulin (HBIG) if later exposed to the hepatitis B virus. (Joint Committee on Vaccination and Immunization (2006), Immunization against Infectious Disease 2006). Obesity, smoking and being over the age of 40 years are mostly associated with poor responses (Roome et al., 1993) and also in alcoholics, especially if with progressive liver disease (Rosman et al., 1997). Patients who are immuno-compromised or on renal dialysis may respond less well and require larger or more frequent doses of vaccine. (Joint Committee on Vaccination and Immunization (2006), Immunization against Infectious Disease 2006). One study at least suggests that hepatitis B vaccination is less effective in patients with HIV. (Pasricha et al. 2006).

In a study to determine the efficacy of the vaccine, 90–100% of vaccinated persons who developed anti-HBs levels greater than or equal to 10 mIU/ml after a primary series were protected from HBV infection (Andre, 1995). Immuno-compromised individuals and adults over 40 years of age are less likely to develop protective concentrations (Averhoff et al., 1998.). Except under special circumstances post vaccination serologic testing in the United States is not indicated because hepatitis B vaccines are highly immunogenic,—for example, among infants born to HBsAg-positive women, persons with ongoing occupational exposure to blood, or persons with immunosuppressive conditions. Hepaccine-B is highly immunogenic, resulting in a sero-protection rate (anti-HBs≥10 mIU/ml) of 93.0% and geometric mean titre (GMT) of 257.6 mIU/ml as has been reported in a controlled study conducted in

1995 to investigate the immunogenicity of the HBV vaccine (Hepaccine-B, Cheil, Korea) indicated that (Aspinall and Kocks,1998).

A study conducted in South Africa (by Tsebe et al, 1999) found that the sero-protection rate of the HBV vaccine was 86.8%. No significant difference was observed in the effectiveness of the HBV vaccine between male and female vaccinated babies.

It was anticipated that a fair number of babies with anti-HBs titres<10 mIU/ml were not significantly non-responders to the HBV vaccine; they probably originally responded to the primary course of vaccination, but anti-HBs titres declined to lower levels in a cross-sectional study by Tsebe et al,1999). It was principally true for older babies, since analysis of anti-HBs in recently immunized young babies showed a seroprotection rate of 95.6%, an observation which clearly shows that the HBV vaccine is highly immunogenic within the framework of the South African EPI. If given as post exposure immune prophylaxis to prevent perinatal transmission, Hepatitis B vaccines are also highly effective. Hepatitis B vaccine and hepatitis B immune globulin administered within
12–24 hours after birth, followed by completion of a three-dose vaccine series, has been shown to be 89–98 percent effective in preventing acute and chronic HBV infection in infants born to women who are positive for both HBsAg and HBeAg (Greenberg,1993). The main determinant of the effectiveness of post exposure immune prophylaxis for infants of HBsAg-positive mothers is ontime administration of the initial doses of vaccine and hepatitis B immune globulin. Hepatitis B vaccine without hepatitis B immune globulin is as effective in preventing perinatal infection as vaccine alone in most studies and is used in areas where cost or other considerations make the use of hepatitis B immune globulin impractical (Greenberg,1993) (Marion et al.1994).

2.8.3 Vaccine Safety

Most published scientific studies do not support a causal association between hepatitis B vaccination and demyelinating diseases such as multiple sclerosis even though several studies have investigated for a significant relationship between recombinant hepatitis B vaccine (HBV) and multiple sclerosis (MS) in adults. Henan et al. 2004 reported a significant increase in risk within 3 years of vaccination. Some of these studies were evaluated for methodological problems. This disagreement generated public uncertainties about HB vaccination, and hepatitis B vaccination in children remained low in several countries. Vaccination does not seem to increase the risk of a first episode of MS in childhood in a study conducted in 2007 (Mikaeloff et al. 2007)

Research conducted in 2009 on the hepatitis B vaccine and associated risk of CNS inflammatory demyelination reported that the hepatitis B vaccine is to be generally safe; nonetheless the Engerix B vaccine seemed to triple the risk of CNS inflammatory demyelination in infant boys. (Neurology, 2009). Numerous reports have stated that the Hepatitis B vaccine is linked to Chronic Fatigue Syndrome - a syndrome marked by severe fatigue, brain fogs, and muscle pains among other symptoms. (Lanctot and Guylaine, 2011). Thiomersal is contained in the Engerix B vaccine, mercury containing vaccine preservative that is being phased out at the urging of the Public Health Service in the US. (**U.S**. Food and Drug Administration. 2010). The World Health Organization commends a pentavalent vaccine, combining vaccines against tetanus, diphtheria, pertussis and *Haemophilus influenzae* type B with the vaccine against hepatitis B. There has not been sufficient evidence yet on how effective this pentavalent vaccine is in association with the individual vaccines (Bar-On et al. 2009)

One hundred and ten (110) countries have introduced the HBV vaccine into their Expanded Programme on Immunisation (EPI) by April 2000 in accordance with recommendations made by the

World Health Organisation (WHO) (Joint Committee on Vaccination and Immunization (2006), Immunization against Infectious Disease 2006). Of these, only 8 were African countries in spite of the high prevalence and carriage of HBV infection in this continent. South Africa incorporated the HBV vaccine into the EPI in April 1995 (Battersby, 1995) and immunizations have been carried out using Hepaccine-B vaccine (Cheil) between 1995 and 1998, since the incorporation of the HBV vaccine into the EPI in April 1995. Engerix-B (Smithkline Beecham) substituted Hepaccine-B in 1999, as SmithKline Beecham won the government tender with a cheaper quote. Hence, most babies received Hepaccine-B to date. Around July 1999, majority of the clinics and hospitals were still using extra stock of Hepaccine-B. HBV vaccines are administered at 6, 10–14 weeks together along with diphtheria-tetanus-pertussis (DTP), the oral polio vaccine (OPV), and *Haemophilus influenzae type b* (Hib) vaccines.

In Ghana, the Ministry of Health in 2002 introduced the pentavalent, diphtheria-pertussis-tetanus (DPT), hepatitis B (HBV), and *Haemophilus influenzae* type b (Hib) vaccine into Ghana's immunization program in place of the conventional DPT vaccine while retaining the immunization schedule at 6, 10, and 14 weeks after birth. (Newton et al., 2007) as recommended by the WHO.

2.8.4 Duration Of Immunity

Anti-HBs is easily measurable and it correlate with vaccine induced protection; it also declines in the years following vaccination, making confirmation of vaccine-induced immunity in persons vaccinated years ago unrealistic, if not impossible. However, reports from numerous long-term follow-up studies show that immunized individuals are still protected against HBV infection despite declines in anti-HBs to levels that are less than protective. In 10- to 22-year follow-up studies among immune competent vaccinated populations no clinical cases of hepatitis B was observed but rare chronic infections have only been documented (Banatvala and van Danme, 2003, Dentiger and Walden. 2005

and Milne et al. 1992). Majority of the vaccines in long-term (decade or more years after vaccination) follow-up studies will develop a fast rise in antibody (anamnestic response) if given an extra (booster) dose of hepatitis B vaccine, regardless of pre booster anti-HBs concentrations below 10 mIU/ml. This reaction simulates a response that would occur after exposure to HBV and provides indirect confirmation of protective immune memory (86–92).

However, it was earlier believed and advocated that the vaccination would only provide effective cover of between five and seven years but it is currently believed that the HBV vaccine provides indeterminate protection (Krugman and Davidson, 1987, Petersen et al. 2004). Subsequent testing and administration of booster doses is not required in successfully vaccinated immuno competent individuals since it has been appreciated that long-term immunity derives from immunological memory can outlasts the loss of antibody levels (Gabbuti et al. 2007 and Lancet 2000). With the passage of time and longer experience, protection has been shown for at least 25 years in individuals who showed a sufficient initial response to the primary course of vaccinations, (Vandamme and Van, 2007) and UK guidelines now suggest that only a single booster is advocated at 5 years for initial responders who need ongoing protection, such as for healthcare workers, (Joint Committee on Vaccination and Immunization (2006), Immunization against Infectious Disease 2006.

2.9 HEPATITIS B VIRUS VARIANTS

The failure of the various vaccines is due to HBV variants with mutations in the small surface protein (S) gene (S mutants) that occurs in perinatally exposed newborns who received hepatitis B vaccine or hepatitis B immune globulin appropriately and have concentrations of anti-HBs that are usually protective (Hsu et al. 1999). Concerns have been raised about these HBV variants, which are sometimes impervious to the neutralizing effect of anti-HBs, could threaten the effectiveness of

hepatitis B immunization programs and that immunization may hasten the creation of HBV variants (Carman 1997). There are numerous reasons to rely on that hepatitis B vaccination will continue to lessen disease burden despite these concerns.

S mutants implicated in perinatally exposed newborn vaccine failures were usually of maternal origin and not induced by vaccination; this was reported in a study using sensitive detection methods (Ngui et al. 1997). In addition, mothers of infants who responded to vaccination were as likely to have these surface antigen variants as mothers of infants who did not respond, which suggest that infections among vaccinated children with S mutants represented immunoprophylaxis failures and infection with maternal viral variants rather than breakthrough infections among successfully vaccinated infants (Nainan et al. 2002). Moreover, vaccinated chimpanzees are protected from challenge with the most common surface antigen variant (Ogata et al. 1999).

The ongoing surveillance for S mutants is made possible because most commercially available assays employ polyclonal anti-HBs that can detect S mutants (Hussain et al. 2003).

2.9.1 Hepatitis B Vaccine Recommendations And Program Implementation

The World Health Organization in 1992 endorsed the incorporation of hepatitis B vaccine into the national immunization programs in countries with high endemicity by 1995 and all other countries by 1997 (WHO, 2005). More than 150 (78%) of 192 World Health Organization member states had implemented universal childhood hepatitis B vaccination policies as of 2004 (WHO, 2004). Particularly absent are numerous highly endemic countries and most of them are located in subSaharan Africa, including the heavily populated and hastily growing nation of Nigeria. South Africa incorporated the HBV vaccine into the EPI in April 1995(Aspinall 1995) and Ghana in 2002. Most advanced countries with low endemicity, which include the United Kingdom, Japan, and the Scandinavian countries, do not consistently vaccinate children but have instead created policies aiming

at immigrant groups from highly endemic parts of the world, adolescents, and adults with risk factors for HBV infection (kane, 1998). Countries who adopted and implemented universal infant hepatitis B immunization included Taiwan (1984), Israel (1989), the Gambia (1990), Malaysia (1990), Italy, Spain, and the United States (all 1991).

Vaccination in each of these countries covered more than 80% within a few years of implementation and has been persistent at that level (WHO, 2005). By the end of the 1990s, most of the world's population still lived in countries without universal infant vaccination, largely because of the expense of hepatitis B vaccine despite several countries' success in implementing broad vaccination policies. Several resource-poor countries have been able to begin implementing universal infant hepatitis B vaccination programs in the last 5 years. A new organization such as the Global Alliance for Vaccines and Immunization, formed in 2000 has been providing key assistance (Global Alliance for Vaccines and Immunization, 2005). Seven had hepatitis B immunization programs before 2000; as of December 2004 of 70 countries entitled for grants from this initiative based on a per-capita gross national income of less than US$1,000 per year, 50 countries had been permitted for funding for hepatitis B vaccine programs. The World Health Organization approximates that global threedose infant vaccination coverage in 2004 was 48% (WHO, 2005).

Initial recommendations for use of hepatitis B vaccine in the United States were centered on adults at high risk of getting HBV infection, such as health care workers, injection drug users, hemodialysis patients, and persons with multiple sex partners, risk-factor-based HBsAg screening as well as newborns to HBV-infected women identified through prenatal screening. Nevertheless, this policy did not have a relevant effect on hepatitis B incidence. In the early part of the 1980s, a complete immunization policy for the United States was established which assimilated each of the following: 1) avoidance of mother-to-child HBV infection through repetitive HBsAg screening of all pregnant

women, then suitable post exposure immunoprophylaxis of newborns of HBsAg-positive females (1988); 2) infants will be immunized routinely (1992); 3) adolescents not previously immunized will also be routinely vaccinated (1995); and 4) all formerly unvaccinated children under age 19 years will be vaccinated (1999). Moreover, the US hepatitis B immunization strategy has been consistently focusing on populations with a greater occurrence of chronic Hepatitis B Virus infection, which includes Pacific Islanders, Alaska Natives, and progenies of immigrant or refugee families from countries with high HBV endemicity (MMWR Morb Mortal Wkly Rep 1988, MMWR Recomm Rep 1991, MMWR Morb Mortal Wkly Rep 1995 and MMWR Morb Mortal Wkly Rep 1999). Infant vaccination has been the most efficacious implementation of the several aspects of the US approach for eradicating Hepatitis B Virus infection.

From 1991 to 2004 Three-dose vaccination series for children aged 19–35 months increased from 16% to 92 % (MMWR Morb Mortal Wkly Rep 2005, MMWR Morb Mortal Wkly Rep 2002, Yusuf, et al. 2001). There has also been headway towards comprehensive enactment of routine immunization of adolescents who have not been vaccinated earlier: From 1993 to 2004, exposure of vaccination among adolescents aged 13–15 years augmented from almost zero to 74 percent (Centers for Disease Control and Prevention, unpublished data). Laws needing vaccination preceding to school entry has been introduced in the United States and it has been observed to correspond with an increase in the coverage of vaccination (MMWR Morb Mortal Wkly Rep 2001), and school-entry laws are currently in effect in most US states (Immunization Action Coalition, 2004).

Prenatal HBsAg screening is done in almost all pregnant women in the United States to detect those at risk of mother-to-child transmission to their infants. In 1998, a population-based research conducted several US sites among pregnant women reported that 97% received HBsAg testing preceding to delivery (Schrag et al. 2003). There is significant discrepancy, however, in the application of perinatal post exposure prophylaxis. In Louisiana, only 78–83% of exposed newborns received correct

immunoprophylaxis (Kohn et al. 1996) but in a large California health maintenance organization, 99.8% of perinatally exposed newborns were vaccinated against HBV infection and hepatitis B immune globulin within 12 hours of delivery (MMWR Morb Mortal Wkly Rep 1997). Hepatitis B vaccination coverage among all US adults (with and without indications for immunizations) is projected to be 35% on the foundation of data from the 2004 National Health

Interview Survey (MMWR Morb Mortal Wkly Rep 2006). Results of the study show that coverage are greatest among individuals between the ages 18–20 years and decreases with age, demonstrating the cohort influence of the childhood vaccination program. The utmost immunization coverage rates among adults with a sign for vaccination have been perceived among individuals with likely occupational contact to HBV. In 2002–2003, among health-care workers with consistent or possible exposure to blood from a representative sample of US hospitals, 75 percent had been vaccinated according to data from their medical records (Simard et al. 2005). Among staff members at dialysis centers in the United States, vaccination coverage was 90% in 2002, well higher than the 56% coverage reported among dialysis patients (Finelli et al. 2005). Outcomes of other studies are also consistent with low vaccination coverage among adults at risk for disease. Report from the National Health Interview Survey indicated that immunization analysis among individuals defined as ‘‘high risk’’ for sexually transmitted and blood borne diseases was only 30% in 2000 and 45% in 2004 (MMWR Morb Mortal Wkly Rep 2006).

In 1998, among 1,755 MSM attending a San Diego, California, sexually transmitted disease clinic, 16% were immunized; of 1,106 individuals reporting injection drug use at the same clinic, only 6% were immunized (MMWR Morb Mortal Wkly Rep 2002). Between 1994 and 1998, only 9% had been vaccinated against hepatitis B among 3,432 young MSM in a survey in seven US metropolitan areas (MacKeller et al. 2001). A study conducted in San Diego in 1997 to evaluate adherence to vaccination

recommendations among adult household and sexual contacts of persons with chronic hepatitis B reported that only 13% had been vaccinated against hepatitis B (Weinberg et al. 2001)

2.9.2 Impact Of Hepatitis B Vaccination

The main aim of hepatitis B vaccination is avoidance of chronic infection, which prevents sequelae such as hepatocellular carcinoma and cirrhosis. Adults who were infected with HBV as children develop HBV-related cirrhosis and hepatocellular carcinoma; decades must pass before the most important benefits of HBV vaccination are comprehended which creates hindrances in monitoring the effect of hepatitis B vaccination programs.

Demonstration of a reduction in the HBV-related disease burden in a short period relies on direct processes such as surveillance for acute hepatitis B, which epitomizes a small but constant proportion of new infections, and serial cross-sectional seroprevalence studies in people targeted for immunization. In the long period countries with entrenched cancer surveillance organizations and registries declines in mortality and incidence rates from HBV-related hepatocellular carcinoma.

2.9.3 Countries With High Endemicity

Taiwan was possibly the finest instance of an area with high endemicity with a significant and assessable decrease in disease problem resulting from an established policy of general childhood hepatitis B vaccination. In 1984, HBsAg sero-prevalence among Taiwanese children declined from 9.8%, the year when universal infant vaccination started, to 0.7% in 1999 (Chan et al. 2004). The mean yearly incidence of hepatocellular carcinoma among children between the ages 6 to14 years in

1981–1986 was 0.7 per 100,000 before the vaccine era, while in 1990–1994 it was 0.36 per 100,000 ($p < 0.01$) (Chang et al. 1997).

Hepatocellular carcinoma mortality decreased from 70% to 60% among children between the term prior to routine immunization (1974–1983) and the post vaccination era (1984–1999) (Lee et al. 2003). Moreover, mortality from fulminant hepatitis among infants declined significantly (Kao et al. 2001). Childhood HBsAg sero-prevalence has declined from 10% to 0.6% ever since the introduction of routine infant and childhood vaccination in Gambia in 1986 (Viviani et al. 1999, Chotard et al 1992). In South Africa where the endemicity of HBV infection was high, a proof of the effect of routine vaccination has been shown through innovative means: a statistically significant decline in the incidence of HBV-related membranous nephropathy among children at a single hospital in 2000–2001 as compared with incidence during the before the vaccination era (Bhimma et al. 2003).

Countries with intermediate endemicity, HBsAg sero-prevalence among school children (between 7–12 years) declined from 1.6% in 1997 to 0.3% in 2003 in Malaysia which introduced universal infant vaccination in 1990 (Ng et al. 2005). Population-based surveillance data indicated a decline in the incidence of acute hepatitis B from 11 per 100,000 populations in 1987 to three per 100,000 populations in 2000 in Italy (Bonanni et al 2003). Moreover, the general prevalence of chronic HBV infection reduced from 13.4% in 1978 to 3.7% in 1997 (Namgyal, 2003).

In Bristol Bay, Alaska, prior to routine immunization, 7.6% of children had serologic evidence of recovered infection by 9 years of age and 3.2% of children were infected chronically. No child under 10 years of age was chronically infected after ten years since the begining of routine immunization, and only 1.5% had evidence of recovered infection (Harpaz et al. 2000). In other US societies with traditionally high rates of disease, similar sero-prevalence declines have been observed. Seroprevalence of markers of Hepatitis B Virus infection declined from more than 20% before 1992 to

1.9% in 2001 among children of Asian immigrants residing in Georgia, (Fiore et al. 2003). Nationwide surveillance for acute hepatitis B in the United States is also in line with the general decrease in new HBV infections. The incidence of acute hepatitis B was 2.1 per 100,000 populations (6,212 cases) in 2004, the lowest ever reported in the United States, demonstrating a 75% decline since 1990. The utmost intense declines have happened among children to whom endorsements for routine infant and adolescent catch-up immunization have applied (MMWR Morb Mortal Wkly Rep 2004). The incidence of HBV has declined by 94% (from 3.0 per 100,000 to 0.19 per 100,000) in children under 20 years of age from 1990 to 2004.

Cases of acute hepatitis B have become progressively concentrated in young adult males in recent years. The male: female rate ratio, although traditionally higher than 1.0 and stable at 1.5 through the 1990s, has augmented to and persisted at 1.7 since 2000. Decreases in the incidence of acute hepatitis B among US adults have been marginally less intense than the decline among US children: acute hepatitis B incidence dropped to 74% and 30%, respectively among persons aged 20–39 years and persons aged 40 years.

A cumulative percentage of acute hepatitis B cases in the United States occur in individuals who report injection drug use and sexual behaviors related with increased risk of HBV transmission. Between 1990 and 2004, the percentage of people reporting multiple sex partners (two or more partners in the 6 months proceeding to diagnosis) rose from 14% to 29%. The percentages of cases classifying themselves as MSM rose from 7% to 14% over the same time period, with about 50% of all MSM also reporting multiple sex partners. These outcomes are supported by sero-prevalence data collected in a cohort of young MSM which proposed that many MSM become infected with HBV during late adolescence and early adulthood. Serologic profiles demonstrating past or present HBV infection were prominent among 2% of 15-year-olds and 17% of 22-year olds in this cohort.

2.9.4 Challenges To Hepatitis B Prevention

Due to the introduction of hepatitis B immunization, there has been a significant decline in the number of new HBV infections each year in the United States. Moreover, implementation of the complete strategy to eradicate HBV transmission has been rutted, but transmission of HBV still occurs among individuals for whom vaccine indications have been in place for more than 10 years.

The main obstacles in the control and prevention of hepatitis B in the United States is complete implementation of existing vaccinations recommendations, predominantly those associated to the prevention of perinatally acquired infections and infections acquired in adulthood through sexual and needle-sharing exposures.

2.9.5 Perinatal Transmission

In the United States, despite increased rates of prenatal HBsAg screening, detecting pregnant women who are HBV carriers chronically has proven problematic. Based on data from the national seroprevalence, an estimation of 23,000 HBsAg-positive women deliver each year, but only 9,000 HBsAg positive women are essentially identified and reported yearly through prenatal screening (Euler et al. 2003). This difference proposes that the small proportion of pregnant women devoid of prenatal HBsAg screening have high prevalence of chronic HBV infection. In addition, HBsAg status often remains unidentified, even when unscreened women are hospitalized during labor and delivery (Thomas et al. 2004). Moreover, the provision of a birth dose of hepatitis B vaccine, which could serve as a protection net for infants whose mothers' HBsAg testing was not done or was incorrectly recorded, is provided to only 33% of US infants, according to data collected in 2000 (Luman et al 2004). Local health departments using improved case management schemes to advance detection and prevention of perinatal hepatitis B have shown promising results in addressing these burdens, now identifying a much bigger proportion (85%) of the expected number of perinatally exposed infants in their regions

and far more house hold and sexual contacts for vaccination per perinatally exposed infant (Euler et al. 2003, Roome et al.2000). The Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices in 2005 endorsed the institution of specific policies and procedures in delivery hospitals to guarantee routine administration of a birth dose to all medically stable infants in an attempt to improve the proportion of infants receiving their first dose of hepatitis B vaccine at birth, ,unless there is a doctor's order to defer administration and a copy of the laboratory report indicating that in the mother's medical record (MMWR Recomm Rep 2005).

CHAPTER THREE

METHODOLOGY

3.1 STUDY AREA

The study was conducted within the Wa municipality of the Upper West Region, Ghana. Wa Municipality is the Regional capital of the Upper West Region and according to the 2010 annual Health review has a population of 118205. The majority of the peop1e are cash crop and subsistence farmers. An ethnographic characteristic of the peop1e in the Wa municipality and the region at large is universa1 triba1 scarification, ear piercing and male circumcision. These traditional practices where different sizes of scars are made on the face or other parts of the body of the individuals using unsterile sharp knives and implements is a common practice across all cultures in the region. Within the Upper West Region, Wa municipality has well established primary health care facilities which are well patronized. The Municipality is endowed with 1 regional hospital, 2 mission/private hospitals, and 22 minor health facilities consisting of health centers, clinics and community health based programs and services. (CHPS compounds). The Municipal Health Authority caries out public health services in all health facilities in the district. These include maternal and child health services, family planning and adolescent reproductive health services. Paramount among the services provided by the primary health facilities is the immunization exercise carried out both within the facilities and on outreach basis in the communities.

Eleven primary schools were randomly selected to participate in this study, namely, Tendaamba primary , Bamahu primary, Limanyiri primary, TI Ahmadiyya primary A and B, SDA primary, Fongo LA primary, St. Andrews Catholic primary, Nuzrat Jahan Demonstration, Falaahiyat primary

B, C and D and Huuriya primary school. The Wa Municipality was chosen because of its well established primary health care facilities hence given it a relatively high coverage as far as EPI is concerned.

3.2 STUDY DESIGN

The study was a cross sectional case control study involving elementary school children in the Wa municipality. The study used serum from 467 healthy or asymptomatically healthy pupils. This included 233 children who completed the HBV Vaccine WHO EPI 7-10 years ago for the vaccinated group and 234 children between the age of 10 and 16 for the group born before the integration of the HBV Vaccine WHO EPI policy in 2002. Written informed consent was obtained from the legal guardians of the participants before samples were collected. The infant immunization records of the vaccinated group were inspected to ascertain whether all 3 doses of the HBV vaccine had been duly completed. Only children who met this criterion were included in the vaccinated group. The following demographic information were recorded: sex, date of birth, HBV vaccination dates, residential address. In the case of the group born before the implementation of the policy the infant immunization records were inspected to confirm they were not vaccinated.

3.3 SAMPLE SIZE

A study conducted on the prevalence of HBsAg among Ghanaian rural children between the ages of 1-16 years was 21% and any of HBV markers was 75%. If the prevalence of HBV in the study group is 21 %, with a power of 80% and a $p <= 0.05$ and 95% confidence interval yielded a sample size of 255. We chose to enroll 300 children for each category making a total of 600 participants. However

for the vaccinated group only 233 children voluntarily agreed to participate and for the nonvaccinated group only 245 out of the 300 anticipated earlier volunteered to participate.

3.4. ETHICAL APPROVAL

The study protocol was approved by the Committee on Human Research Publications and Ethics, School of medical sciences, College of Health Science, Kwame Nkrumah University of Science and Technology, Kumasi. (Ref: CHRPE/AP/116/13), the Municipal Director of Health Services, the Municipal Director of Education services and the Heads of the participating Schools. The purpose and the protocol of the study were clearly explained. Informed consent and infant immunization records (Road to Health Cards) were obtained from the parents or guardians before a blood specimen was collected from each participant. Only children of consenting parents/guardians were included in the study.

3.5 STUDY POPULATION

3.5.1. Vaccinated children

A total of 233 children who took their infant immunization as part of the EPI between 2002 -2007 using the Penta valent vaccine were enrolled. The children were drawn from randomly sampled primary schools within the Wa municipality. The children had received three doses of penta valent vaccine (1.0 µg per 0.5 ml dose) between 2002 and 2007, as part of childhood routine immunizations. The mean age was 7.9 years (range: 5-10 years) with a male to female ratio of approximately 1:1 (107:126). For each child in this group, an infant immunization record (Road-toHealth card) was examined to ascertain that the three doses of the HBV vaccine were administered, and only children who met this criterion were included.

The following parameters were recorded by the research: name, date of birth, age, sex of the child and HBV vaccination dates and batch numbers of vaccines. Five milliliters of venous blood was aseptically drawn from each participant using 5mL disposable needle and syringe after disinfecting the selected venepuncture site with 70% ethanol. The blood samples were put in vacutainer tubes and labeled appropriately with participant's identification number and date of sample collection. Each child was given either a chocolate drink on a sunny day or a boiled egg when the weather is cold and a cold drink was not appropriate. This was just to show appreciation to the kids and help nourish and replenish the blood taken. The samples were kept at 25°C for 10 minutes to allow clotting and centrifuged for 10 minutes at 3000rpm with Sure-Sep II, silicon based serum–plasma separator (Organon Teknika Corporation, North Carolina). Serum fractions were separated in to two different vacutainers labeled A and B, labeled and stored at −20°C until needed. The sera in vacutainers labeled A were tested for HBV serological markers: HBsAg, anti-HBs and anti-HBc, HBeAg and anti-HBe. The sera in vacutainers labeled B were tested for quantitative levels of antiHBs.

3.5.2. Non vaccinated Children

A total of 234 children born before the integration of the HBV vaccine in to the EPI were enrolled. The children were drawn from the randomly sampled primary schools within the Wa municipality. The children were born before the integration of the HBV vaccine in to the EPI and so did not receive any HBV vaccine at infancy as indicated by the type of immunization record (road to Health card) they held. The mean age of participants in this group was 12.3 years (range: 10-16 years) with a male to female ratio of approximately 1:1 (115:119). For each child in this group, the infant immunization records (Road-to-Health card) were examined to ascertain that they had not taken HBV vaccine, held the immunization record that were used before the integration of the HBV vaccine in to the EPI and belongs to the non-vaccinated group and children who were clearly older than 10 years. Only children

who met with this criterion were included in this group. The following parameters were recorded by the research: date of birth, age, sex of the child. Five milliliters of venous blood was aseptically drawn from each participant using 5mL disposable needle and syringe after disinfecting the selected venepuncture site with 70% ethanol. The blood samples were put in vacutainer tubes and labeled appropriately with participant's identification number and date of sample collection. Each child was given either a chocolate drink on a sunny day or a boiled egg when the weather is cold and a cold drink was not appropriate. This was just to show appreciation to the kids and help nourish and replenish the blood taken. The samples were kept at 25°C for 10 minutes to allow clotting and centrifuged for 10 minutes at 3000rpm with Sure-Sep II, silicon based serum–plasma separator (Organon Teknika Corporation, North Carolina). Serum fractions were removed, labeled appropriately and stored at −20°C until needed. Samples were tested for HBsAg, anti-HBs and anti-HBc, HBeAg, anti-HBe.

3.6 ASSAYS FOR SEROLOGICAL TESTING

Sera were tested for HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, using commercial Wondfo one step HBV serum/plasma test kits (Guangzhou Wondfo Biotech Co. Ltd, Wondfo Scientech park, South China University of Technology, China). Quantitative levels of anti-HBs were determined using anti-HBs quantitative ELISA kit (Fotress diagnostics Limited, United Kingdom). Manufacturers claimed sensitivity and specificity of 99.0 and 99.8% respectively. Assay was performed as per the manufacturer's instruction. For the quantitative analysis of anti-HBs each specimen was ran in duplicates and results were averaged to get anti-HBs levels for each sample. Specimens that gave negative were recorded as 0.

Anti-HBs titres were only considered protective if values were≥10 mIU/ml. Chi-square calculations were performed using Stata version 11.

3.6.1 Qualitative Analysis of HBV serological markers: HBsAg, HBsAb, HBeAg, HBeAb and HBcAb.

Principle

The presence of the HBV serological markers were determined using the Wondfo one step HBV serum/plasma test kit. This kit is a rapid immunochromatographic assay designed for qualitative determination of the 5 HBV markers in human serum or plasma. The kit is designed for in vitro use only. Wondfo one step HBV serum tests a rapid immunochromatographic test for visual detection of HBV antigen and antibodies in serum in the diagnosis of hepatitis B infection. The HBV markers, HBsAg, HBsAb and HBeAg adopt the sandwich method while HBeAb and HBcAb adopt competitive assay. When serum is added to the sample wells in these test devices, the serum is absorbed on to the device by capillary action, mixes with the antigen/antibody-dye conjugate and flows across the pre-coated membrane. In testing for HBsAg, HBsAb and HBeAg, if the antigen levels are zero or below the detection limit, there is usually no visible coloured band in the test region of the device. This indicates a negative result. In contrast, if there is a coloured band in the Test band, it indicated a positive result.

PROCEDURE

- The sera labeled A were allowed to attain room temperature (25°C).
- The test devices were removed from the foil pouch, labeled appropriately and placed on a level surface of the serology bench.
- 100uL of serum was added to each well and allowed to stand for 15minutes at room temperature.
- For HBsAg, HBeAb and HBcAb, a rose pink band is visible in both the test and control bands.

- For the HBeAb and HBcAb, a rose pink band is visible in the control region. No colour band appears in the test region.
- For a negative result in the case of HBsAg, HBeAb and HBcAb, a rose pink band is visible in the control region. No colour band appears in the test region.
- In the case of HBeAb and HBcAb, Rose pink bands are visible in both control and test regions.
- A test was considered invalid if no visible band appeared at the control region or the band only appeared at the test region.

3.6.2. Quantitative Determination Of Anti-Hbs (ELISA).

Fortress anti- HBs (Quantitative) ELISA KIT, manufactured by Fortress Diagnostics Limited, United Kingdom was used. This assay is for the quantitative determination of antibodies to hepatitis B virus. The kit uses microplates coated with a preparation of highly purified HBsAg. Following the addition of samples and subsequent washing, a different HBsAg conjugated to peroxide was added that binds to a 2nd site on the antibody. The enzyme specifically binds to wells by acting on the substrate/chromogen mixture, generating an optical signal proportional to the amount of Hep B antibodies in the sample and detectable by an ELISA reader. The concentration of antibodies is then estimated by means of a standard curve calibrated against the WHO reference preparation using a master Plex ELISA reader.

PRINCIPLE

The anti-HBs ELISA kit uses a polysterene microwell strips pre-coated with recombinant HBsAg. Serum is added to the microwell together with a second recombinant HBsAg conjugated with Horseradish peroxidase (HRP). In case of the presence of anti-HBs in the sample, the two antigens bind to the two variable domains of the antibody and during incubation the specific immune complex formed is captured on a solid phase. After washing to remove the sample and unbound conjugates,

chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In the presence of the antigen-anti-body-antigen sandwich complex, the colourless chromogens are hydrolysed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping reaction with sulfuric acid. The amount of colour can then be measured and is directly proportional to the amount of antibody in the sample. Wells containing samples negative for anti-HBs remain colourless.

Double antigen sandwich ELISA

Ag (p) + Ab (s) + (Ag) ENZ → [Ag (p) – Ab(s) – (Ag) ENZ] → Blue→ Yellow Colour Positive

Ag (p) + (Ag) ENZ → [Ag (p)] → No Colour Negative

Incubation Immobilized Complex Coloring

60 min. 15min.

Ag(p)–pre-coated HBsAg; Ab(s)– anti-HBs in sample; (Ag)ENZ–HRP conjugated HBsAg

PROCEDURE

- The serum labeled B and the Fotress ELISA reagents were allowed to reach room temperature (25°C).
- The stock wash buffer was diluted 20 times with distilled water.
- The strips to be used were set in the strip holder labeled appropriately. Each microplate well was labeled to include the 6 calibrators (standards) B1-G1:B2-G2) and one blank (A1).
- Each plate had 2 designated blank wells, 12 designated standard wells for the 6 standards run in duplicates and 82 designated sample wells for the 41samples run in duplicates.
- In all 6 ELISA micro plates were used and 50 uL of calibrator standards and 50 uL of specimen were pippeted in to their respective wells using separate disposable pipette tips for each specimen or standard. Both samples and standards were run in duplicates. Neither sample nor HRP-conjugate was added to the Blank well

- 50 uL of HRP-conjugate reagent was then added to each of the wells in step 3 above and gently mixed except for the blank wells where nothing was added.
- The plates were then covered with a plate sealer and incubated for 60 minutes at 37 ^{o}C.
- After 60 minutes of incubation, the plate sealer was removed and the wells washed 5 times with the diluted buffer. At each wash, the wells were allowed to soak for 60 seconds. After the final wash, the strip plate was blotted on an absorbent tissue to remove any excess buffer.
- 50 uL of chromogen A and 50 uL of chromogen B were pippeted in to each well including the blank well. The plates were then covered with micro plate sealer and mixed gently by tapping the plate.
- The plates were then incubated for 15 minutes at 37° C. The enzymatic reaction between the chromogen A/B solutions and the HRP-conjugate produced a blue colour in the wells containing the standards and anti-HBs positive wells.
- After 15 minutes of incubation, 50 uL of 2.0M sulpheric acid added to each well to stop the reaction and turn blue coloured wells yellow.
- The ELISA plate reader was then calibrated with the blank well at 450nm and the absorbance of standards and samples then read within 10 minutes after stopping the reaction.
- Records of the absorbance were obtained from the print report of the ELISA micro plate reader and the corresponding concentrations obtained using a Master Plex ELISA OD converter.

STATISTICAL ANALYSIS

Statistical analysis was performed using software Graphpad Prism, version 5.0 (Graph Pad Software Inc., Los Angeles) for windows. The Mann Whitney U test was used to compare minimum HbsAb

levels between the two groups. Two by two categorical variable were compared using Fischer exact test. Data were reported as mean ± standard deviation (SD) for demographic continuous data, as median (interquartile range) for marker levels and as a frequency (percentage) for categorical data. $p<0.05$ was considered as statistically significant different.

CHAPTER FOUR

RESULTS

To investigate the prevalence of Hepatitis B infection among children in the Wa municipality, a total of four hundred and sixty seven (467) children between the ages of 5 and sixteen years (mean age of 9.99 years) were studied. Out of which 49.89% (233) constituted the vaccinated group, comprising of children between the ages of 5 and 10 years (with a mean age of 7.89 years) who received 3 doses of the hepatitis B vaccine as part of their routine infant immunization at 6 weeks, 10 weeks and 14 weeks after birth. The remaining 50.1% (234) of the children constituted the non vaccinated group. This group comprised of children born before the integration of the Hepatitis B vaccine in to the EPI and hence never received the Hepatitis B vaccination at infancy. **(Table 4.0)** shows the demographic characteristics of the study participants. Non-vaccinated children were significantly older (mean age 12.07 years) than their counterparts who were vaccinated (mean age, 7.89 year) ($p<0.0001$). Out of the 467 participants (54.4%) were females while 45.6% of them were males. There was no significant difference between the number of participants in the vaccinated and non-vaccinated groups as well as across gender. ($p=0.4586$).

Table 4.0: Demographic Characteristics of study participants

Variables	Total (n=467)	Non-Vaccinated (n=234)	Vaccinated (233)	p-value
Age range (Mean)	(5-16)yrs 9.99	(10-16)yrs 12.07	(5-10)yrs 7.89	< 0.0001
Gender n (%) Male	213(45.61)	115(49.1)	107(45.9)	0.4586
female	254(54.39)	119(50.9)	126 (54.1)	

n (%): Frequency (proportion);

4.0 Prevalence of HBV infection among children in the Wa municipality.

4.1 HBV infection markers among non-vaccinated children in the Wa Municipality.

To investigate the distribution of the HBV infection markers among non vaccinated children in the Wa municipality, a total of 234 children who never received the HBV vaccine as part of their EPI were studied. Age range was between 10 and 16 years with a mean of 12.7 years. Out of the 234 children, 50.1% were girls. The distribution of HBV serological markers in the 234 participants are as displayed in table 4.1.. Out of the 234 participants: 24(10.3%) were positive for HBsAg, 6 (2.6%) were positive for HBsAb, 4(1.7%) were positive for HBsAg, HBeAg and HBcAb, 4(1.7%) were positive for HBsAg, HBeAb and HBcAb and 6(2.6%) were positive for HBsAb and HBcAb. The total exposure measured by the presence of any of the HBV markers was 31 (13.25%) mean age 12.64 years, while a total of 203(86.75%) children with a mean age 12.63 years were negative for all markers. Across the gender 14 boys representing 12.61% of the non-vaccinated male and 5.98 % of the entire non vaccinated group tested positive for HBsAg whilst 10 girls representing 8.13 % nonvaccinated female group and 4.11% of the entire non vaccinated participants tested positive for HBsAg.

For the HBsAb, 4 boys representing 1.7% of the entire population and 3.2% of the entire non vaccinated male group were positive whilst 5 girls representing 2.04% of the entire population and 4.2% of the female non vaccinated group were positive as displayed in Table 4.1..

Table 4.1. Distribution of HBV infection markers among non vaccinated children.

HBV markers	Total (n=234)	males (n=111)	females (n=123)	p.value
HBsAg n (%)	24(10.26)	14 (5.98)	10 (4.27)	(0.8923)
HbsAb n (%)	6(2.56)	3 (2.7)	3 (2.44)	(0.8823)
HBsAg + HBeAg n (%) +HBcAb	4(1.7) 31(13.25)			
Total exposure HBsAg (%) +HBsAb +HBcAb				

4.1.2. Distribution of HBV infection markers among vaccinated children.

To investigate the distribution of the HBV infection markers, seroprotection rate, anti- HBs levels as well as investigates the effectiveness of the HBV infant immunization programme among children who received the HBV vaccine under the EPI immunization programme, 233 children who received 3 doses of the vaccine at infancy were studied. The distribution of HBV serological markers in the 233 children were as follows: 2(0.86%) were positive for HBsAg, 66(28.3%) were positive for HBsAb, 1(0.43%) was positive for HBeAg, 1 (0.43%) was positive for HBeAb, 3(1.29%) were positive for HBcAb, 1(0.43%) was positive for HBsAg, HBeAg and HBcAB, 1(0.43%) was positive for HBsag, HBeAb and HBcAb and 1(0.43%) was positive for HBsAb and HBcAb. 1(0.43%) was positive for HBsAg, HBsAb and HBcAb. 1(0.43%) was positive for both HBsAb and HBcAb, 2 (0.86%) were positive for both HBsAg and HBcAb. The total exposure, as measured by the presence of HBsAg, HBsAb, or HBcAb was 19.7% (46/233), while a total of 80.3% (187/233) of children were negative for all markers (Table 4.1). Of interest is that there was a significant difference in HBsAg carriage between the vaccinated group and the non vaccinated group. The non vaccinated participants who tested positive to HBsAg reported with a higher prevalence (10.3%) of infection compared to the vaccinated participants who recorded a significantly lower prevalence (0.9%) 10 or less years after vaccination ($p<0.0001$). In contrast, total exposure to HBV measured by the presence of the 3 markers increased significantly from (13.25%) in the non vaccinated group to 19.7 % in the vaccinated group. ($p.< 0.0001$).

After vaccination, participants were less likely to be associated with HBV (CoR 0.076 95% CI (0.017 to 0.324). Immuno protection in the non vaccinated group, measured by HbsAb positivity is (2.6%) whilst their vaccinated counterparts reported with a significantly higher immunoprotection rate (28.3%) ($p<0.0001$). After vaccination participant were more likely to be tested positive for HBsAb

(CoR 15.02 95% CI (6.358 to 35.470). There were no statistically significant differences in prevalence among non-vaccinated verses vaccinated participants who tested positive to HBeAg (1.7% vs 1.3%; p=0.9979), HBeAb (2.1% vs 0.4%; p=0.2157), and HBcAb (4.7% vs 1.3%; p=0.0537). Participants after vaccination were less likely to test positive to HBeAg (CoR=0.750 95%CI (0.166 to 3.390), HBeAb (CoR=0.197 95%CI (0.023 to 1.704), and HBcAb (CoR=0.26495% CI (0.073 to 0.961).

Table 4.2. Distribution of HBV infection markers among children vaccinated at infancy.

HBV markers	Total (n=233)	Males (n=107)	females (n=126)	(p.value)
HBsAg n (%)	2(0.86)	1 (0.43)	1(0.43)	(0.8923)
HbsAb n (%)	66(28.46)	24 (10.3)	42(18.03)	(0.8923)
	1(0.43)	1(0.43)	0	
HBsAg + HBeAg n (%) +HBcAb				
Total exposure HBsAg (%) +HBsAb +HBcAb	26(11.2)			
None of the markers	188 (80.69)	89(32.2)	99 (42.49)	

Combining the distribution of HBV markers from the two groups, the total number of children is 467 with 26 of them representing 5.44% testing positive for HBsAg whilst 52 representing 10.88% tested positive for HBsAb.

Table 4.3. Seroprevalence of HBV markers among children in the Wa Municipality

HBV markers	Total (n=467)	Non-Vaccinated (n=234)	Vaccinated (n=233)	Crudes Odd ratio (95% CI)	p-value
HBsAg n (%)					
Negative	441 (94.4)	210(89.7)	231(99.1)		< 0.0001
Positive	26 (5.6)	24(10.3)	2 (0.9)	0.076 (0.017 to 0.324)	
HbsAb n (%)					
Negative	395(84.6)	228(97.4)	167(71.7)		< 0.0001
Positive	72 (15.4)	6(2.6)	66 (28.3)	15.02 (6.358 to 35.470)	
HBeAg n (%)					
Negative	460 (98.5)	230 (98.3)	230 (98.7)		0.9979
Positive	7(1.5)	4 (1.7)	3 (1.3)	0.750 (0.166 to 3.390)	
HBeAb n (%)					
Negative	461(98.7)	229 (97.9)	232 (99.6)		0.2157
Positive	6 (1.3)	5 (2.1)	1 (0.4)	0.197 (0.023 to 1.704)	
HBcAbn (%)					
Negative	453(97.0)	223 (95.3)	230 (98.7)		0.0537
Positive	14 (3.0)	11 (4.7%)	3 (1.3)	0.264 (0.073 to 0.961)	

Values are presented as n(%): frequency (prevalence).

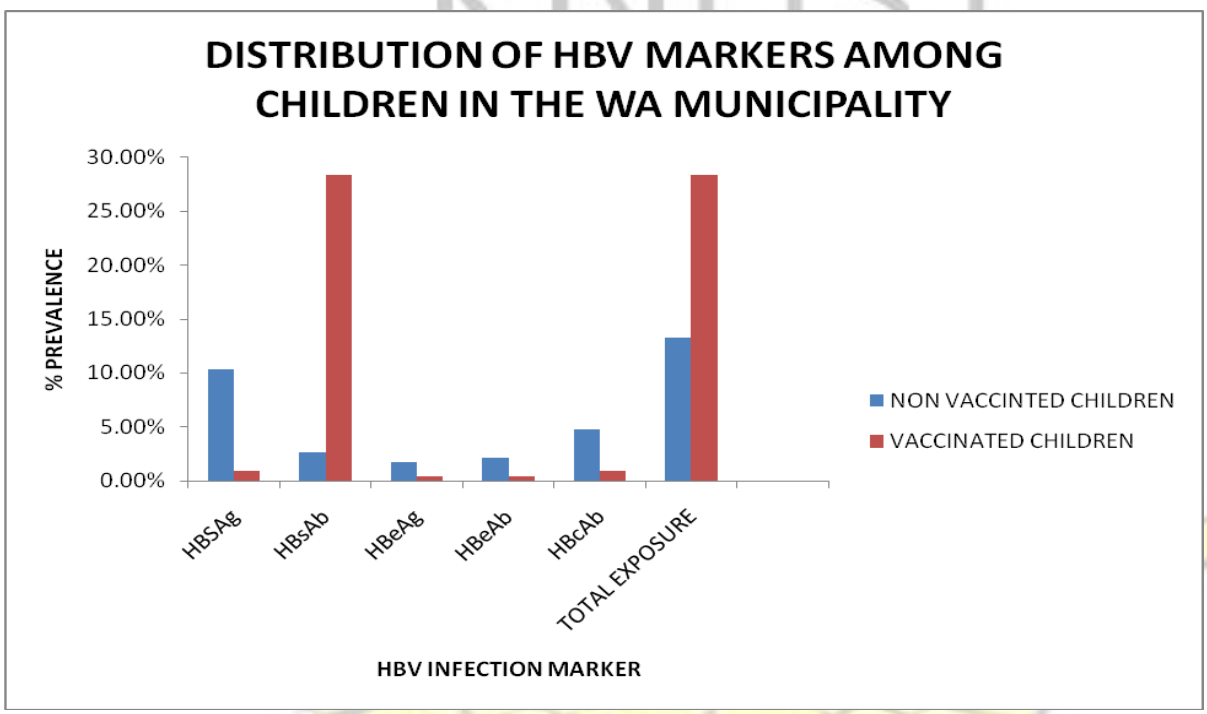

FIGURE 4.1. A GRAPH ILLUSTRATING THE DISTRIBUTION OF HEPATITIS B VIRAL MARKERS AMONG CHILDREN IN THE WA MUNICIPALITY.

4.2 DISTRIBUTION OF HBV INFECTION MARKER ACROSS AGE GROUPS AMONGST VACCINATED CHILDREN

Stratifying the data for different age groups, it became evident that the seroprotection rate measured by HBsAg positivity increased significantly (p<0.05) with increasing age, from 30.2% for children 5 and 7 years (mean age 6.7 years) to a 69.8 % in children between 8-10 year (mean age 8.4) (Table 4.4). This was supported by increasing GMT values, from 36.93 mIU/ml in children between 5-7 years; mean age 6.7years) to 48.4 mIU/ml for children aged between 8-10 years (mean: 8.4 years).

Table 4.4. Distribution of HBV infection marker across age groups amongst vaccinated children

Age group	Number positive for HBsAg	Number positive for HBsAb	Number positive for HBeAg	Number positive for HBeAb	Number positive for HBcAb	Males	females
5	0	1	0	0	0	0	1
6	0	3	0	0	0	7	12
7	1	9	1	0	2	22	31
8	1	22	0	1	1	44	50
9	0	8	0	0	0	31	29
10	0	0	0	0	0	3	2
total	2	43	1	1	3	107	126

4.3.0 Distribution of anti – HBs among vaccinated children.

Anti – HBs levels in vaccinated children.

The anti-HBs titres recorded ranged from 0.0IU/L to 462.7 mIU/mL. Only one participant recorded an anti- HBs level of 0mIU/mL. This data indicated a 99.57% seroconversion rate following primary immunization. Among the participants, 0.43 % were non responders and 71.67 % appeared to be hypo responders with anti- HBs been < 10 mIU/mL. The overall anti-HBs seroprotection rate was 27.89% (65/233), based on a titre of ≥10 mIU/ml. Of these, 20.63% had anti-HBs titres≥100 mIU/ml and 79.37% had anti-HBs titres between 10–99.9 mIU/ml. 72.96% (170/233) had anti-HBs levels below the seroprotection level (i.e. <10 mIU/ml). The minimum titre was 0.01; maximum titre is

462.7 with a geometric mean titre of 42.64 mIU/mL

Anti HBs levels across age and gender

Table 4.3 shows the minimum HbsAb concentration stratified by gender and age among vaccinated participants. Male and Female participant who tested positive to HbsAb had a significantly higher median minimum concentration compared to those who tested negative (p<0.0001). Similarly, there were higher concentration among all age groups who tested positive to HbsAb compared to those who tested negative (p<0.0001) (Table 4.3). Although females reported with higher median minimum concentration than the male counterparts, the difference was not statistically significant. (p=0.8823)

Table 4.5 Minimum HbsAb concentration (mIU/mL) in relation to gender and age

	HBsAb		
	Negative (n=170)	Positive (n=63)	
Variables	Median (IQR)	Median (IQR)	P-value
Gender			
Male	2.48 (0.90 - 3.77)	29.45 (18.70 - 80.19)	<0.0001
Female	2.02 (0.62 - 3.11)	39.22 (16.37 - 87.53)	<0.0001
Age (years)			
≤6	2.00 (0.61 - 4.21)	31.84 (11.65 - 180.9)	<0.0001
7	2.25 (0.45 - 4.35)	22.74 (13.72 - 81.08)	<0.0001
8	1.77 (0.63 - 3.40)	53.25 (27.61 - 110.3)	<0.0001
9	2.48 (1.70 - 3.29)	24.67 (14.87 - 64.61)	<0.0001
10	2.66 (0.61 - 4.82)	28.00 (18.61 - 98.91)	<0.0001

Values are presented as Median (25th quartile- 75th quartile range). IQR: interquartile range.

4.3.1 Sero protection rates across gender

Table 4.6 Distribution of seroprotection rates across gender

Gender	No. Tested	Anti-HBs < 10 mIU/ml	Anti-HBs >10 mIU/ml
Males	**107**	**84**	**23**
Females	**126**	**86**	**40**
Sub Total	**233**	**170**	**63**

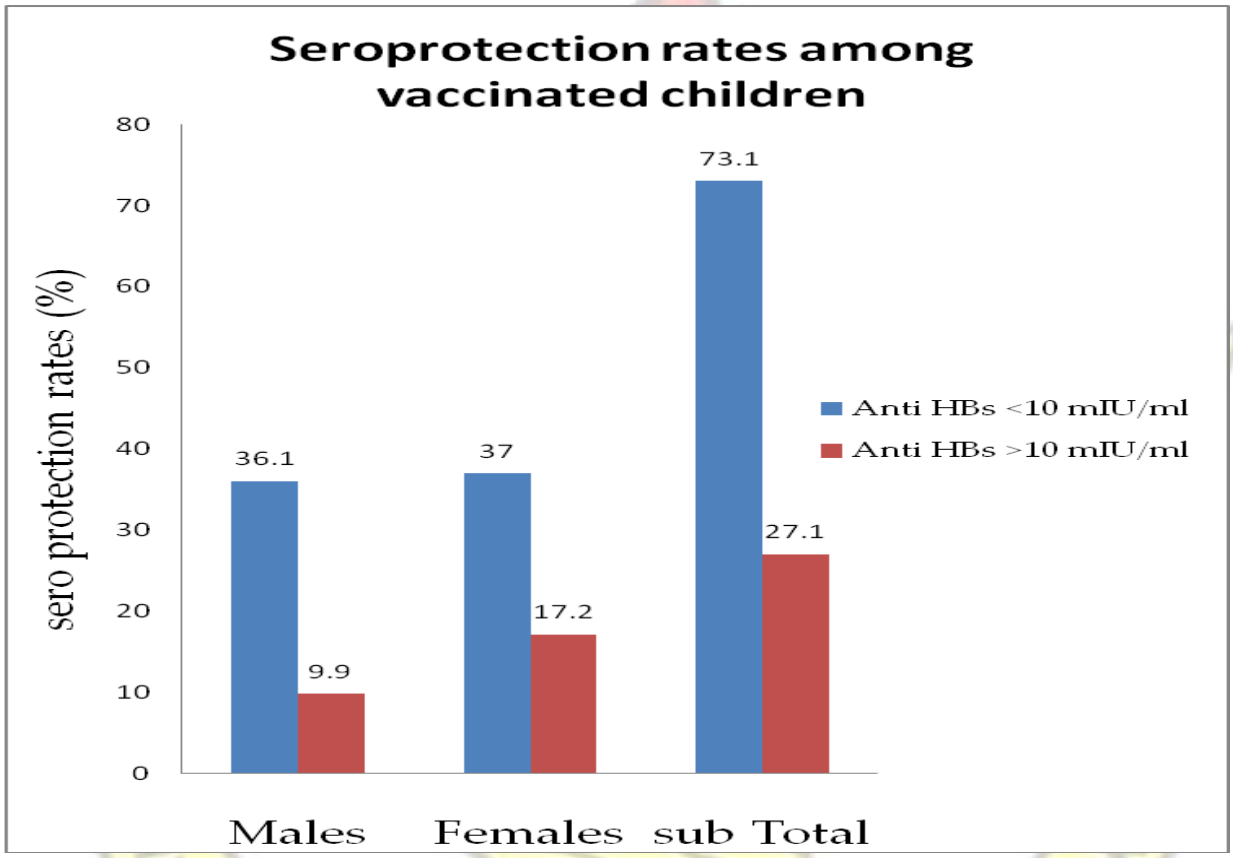

Figure 4.3.

4.3.2. Seroprotection rates across age groups

Stratifying the data for different age groups, it became evident that the seroprotection rate based on anti-HBs levels decreased with increasing age from 5 to 6 years but however increased from 33.3%

(21/63) for children between 5-7 years, to 68.2% in children 8 years or older. This was supported by increasing GMT values, from 36.93 mIU/ml in children between5-7 years; mean age 6.7years) to 48.4 mIU/ml for children aged between 8-10 years (mean: 8.4 years).

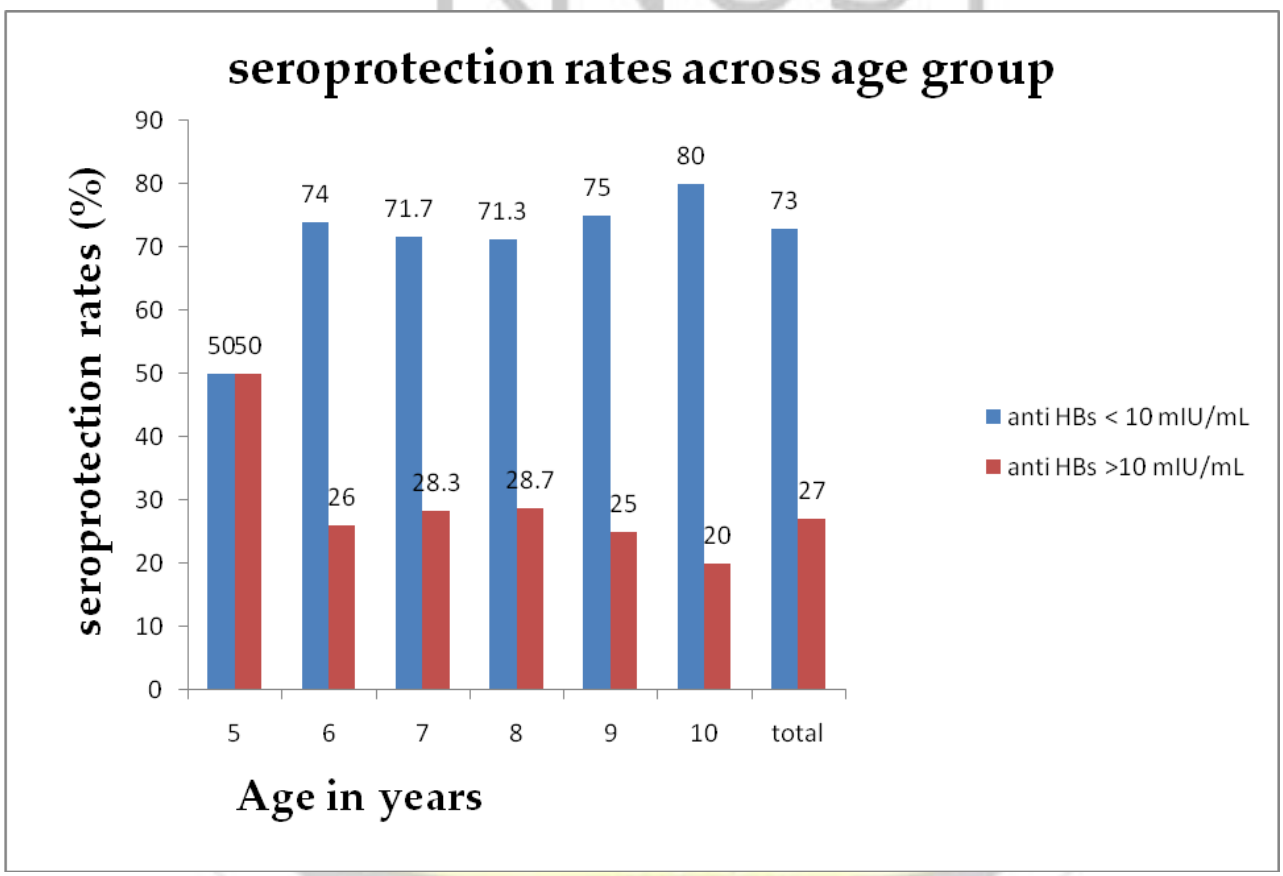

FIGURE 4.4. Distribution of anti- HBs in different age groups.

4.3.3 Compliance with the vaccine dosages

Of the 233 vaccinated children, 166 (71.24%) received the HBV vaccine as scheduled in the EPI (i.e. at 6, 10 and 14 weeks, along with OPV, DTP and Hib). The remaining 67 (28.76%) received the vaccine

in an 'unscheduled' manner as follows: 65 received all 3 doses within 1year and 2 received the vaccine within 1.5 years. It was interesting to observe that 58 (88.3%) of the 67 babies received the first dose at 6 weeks, but the second and third doses were administered at random. Nevertheless, there was no significant difference in the seroprotection rate and GMT values between babies who received the 'scheduled' and 'unscheduled' course of the HBV vaccine.

Table 4.7. Compliance with the vaccine dosages.

Vaccination Schedule	No. Tested	Anti-HBs < 10 mIU/ml	Anti-HBs >10 mIU/ml
Standard	166	107	59
Unscheduled	67	63	4
Subtotal	233	170	63

Standard: Received hepatitis B vaccine at 6, 10 and 14 weeks, along with OPV, DTP and Hib.

Unscheduled: vaccines were administered at unscheduled dates according to the Road-to-Health cards (immunization cards)

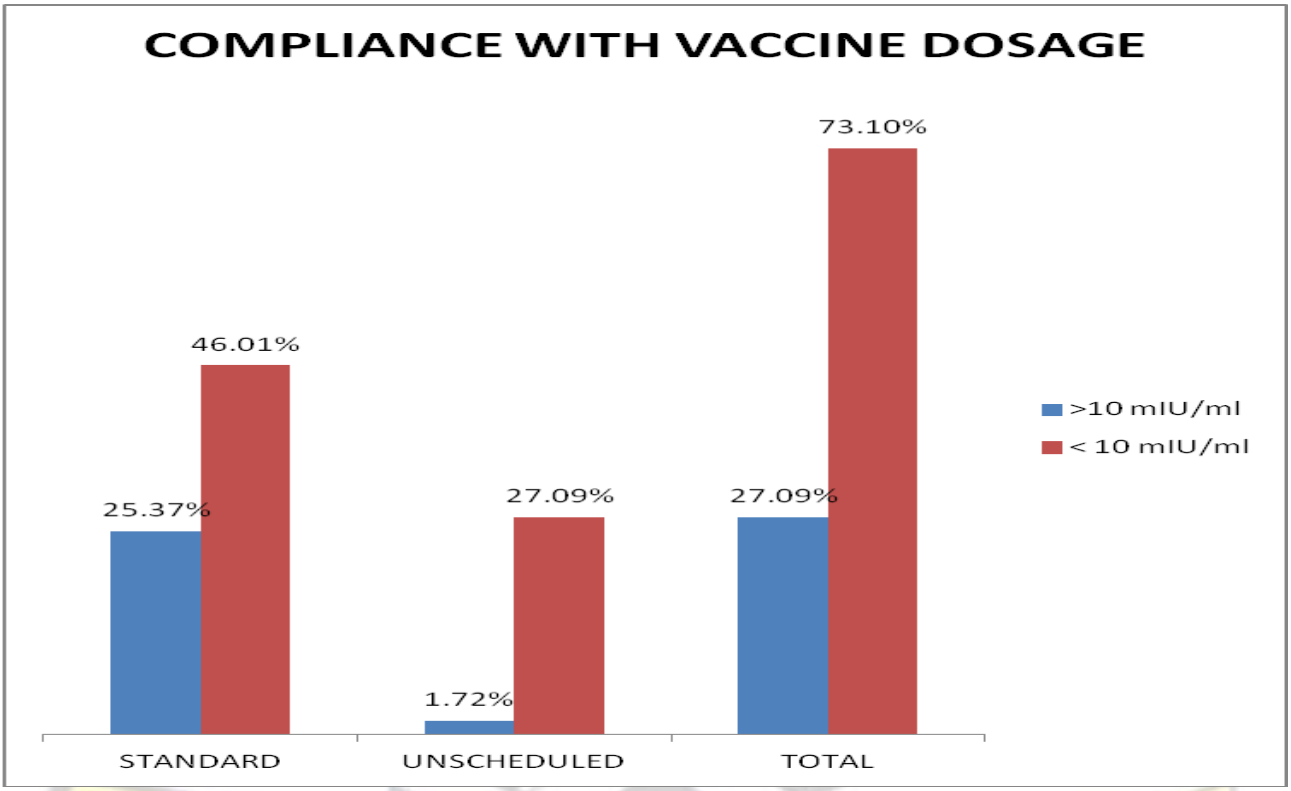

Figure 4.5. A graph illustrating the compliance of participants with vaccination schedules.

4.4 Protective efficacy of the HBV vaccine among vaccinated children in the Wa Municipality.

Two (2) of the vaccinated babies experienced breakthrough infections resulting in HBs antigenemia. The seroprotection rate was based on anti-HBs levels greater than 10 mIU/Ml 27.89%. among children who received the WHO EPI penta valent vaccine 5-10 years ago.

4.4.1 Impact of the pentavalent vaccine in reducing the prevalence of hepatitis B virus infection

The impact of the hepatitis B vaccine in the penta valent vaccine as measured by comparing the total number of children who were Positive for HBsAg and HBsAb among the vaccinated and non vaccinated children revealed the vaccine has successfully reduced the prevalence of HBsAg from

10.3 % in the non vaccinated group to 0.9% in the vaccinated group and evidently increased the protective immunity rate, measured by HBsAb positivity from 3.67% in the non vaccinated group to 27.89 % in the vaccinated group. Though protective immunity wanes with time, the HBV vaccine given under the EPI at 6 weeks, 10 weeks and 14 weeks has demonstrated evidence in preventing the transmission of HBV in the Wa municipality.

CHAPTER FIVE DISCUSSION

Prevalence of HBV among children in the Wa municipality

This study investigated the prevalence of hepatitis B infection amongst children in the Wa municipality years before and 10 years after the integration of the HBV vaccine in to the Ghanaian EPI. It also estimated the anti- HBs levels, sero protection rates as well the impact of the universal childhood Hepatitis B Virus immunization programme in reducing HBsAg carriage rate. The prevalence of HBV measured by HBs antigenemia (HBsAg) among the children decreased from

10.3% in the non-vaccinated children to 0.9 % in the vaccinated group. This significant decrease in HBsAg prevalence among the vaccinated and non-vaccinated groups might be as a result of the effectiveness of the HBV infant immunization in preventing the horizontal transmission of the virus to the vaccinated children. As has been stated earlier that the predominant route of transmission in sub-Saharan Africa is through the horizontal route with most children infected by age of 6 years. These findings are consistent with findings by (Yen- Hsuan et al, 2001) who in a study to examine the Hepatitis B infection in children and adolescents in an area with high endemicity 15 years after mass vaccination reported the prevalence of HBsAg among children younger than 15 years of age decreased from 9.8% to 0.7% in the 15 years since enactment of the immunization program universally. The discoveries are also consistent with outcomes by Tsebe et al, (2001) who in an evaluation of the first 5 years of universal infant Hepatitis B immunization reported that the vaccine is highly effective in eliminating the HBsAg carriage rates of children in South Africa. The overall prevalence among the children in the Wa municipality, both vaccinated and non-vaccinated is 5.44% based on HbsAg positivity. All persons with anti-HBc seropositivity have had HBV infection, but only some are carriers while others experience complete recovery. There was one instance in the vaccinated group where HBsAg was negative whilst HBcAb was positive explaining the discrepancy between the

prevalence of anti-HBc and HBsAg. This could be explained by the fact that the 1 person that was infected with HBV did not become chronic carriers but rather recovered but has anti HBc being positive as an indication of exposure. Total HBV exposure among the vaccinated children 11.2% (26/233) is not significantly different from 13.2% (31/234) recorded for the nonvaccinated group.

Anti- HBs level and sero protection rates among vaccinated children

The anti-HBs titres recorded ranged from 0.0IU/L to 462.7 IU/L. Only one participant recorded an anti- HBs level of 0IU/L. This data indicated a 99.57% seroconversion rate following primary immunization. Among the participants, 0.43 % were non responders and 71.67 % appeared to be hypo responders with anti- HBs been < 10 mIU/mL. The protective immunity measured by anti-HBs levels ≥10 mIU/ml is 27.04% among the vaccinated children. No significant difference was observed in the protective immunity between male and female vaccinated children (p: 0.458). But it was evident that across age groups the anti HBs levels though extremely low, declined from age 5 years to age 6 years after which it increased with increasing age peaking among 8 year olds and then declining with increasing age through 9 year olds to 10 year old among the vaccinated children; this was supported by the decreasing GMT levels 171.81 mIU/L at among 5 year olds to 13.1 mIU/L among 6 year olds and from 8 year olds through 9year olds to 10 year olds This decline in seroprotection rates and anti HBs levels with increasing age from 5 to 6 years and from 8 through 9 years to 10 years is in line with findings by Yen- Hsuan et al, (2001) who stated that the rate of antiHBs seropositivity gradually diminished from 1 to 12 years of age regardless of a sturdy vaccination coverage rate of higher than 85%. This is perhaps due to the waning of anti-HBs titers over time. The findings are also consistent with findings by (Tsebe, et al 2001) who found that the seroprotection rates among young vaccinated

south African children declined with increasing age; the younger the age and the more recent the vaccination, the higher the protective anti-HBs and GMT values. This claim by Tsebe et al 2001, was supported by the fact that Seroprotection in recently vaccinated babies (mean age of 10.3 months) was 95.6% (with GMT of 285.4 mIU/ml) which is comparable to that reported previously (93.0%; GMT was 257.6 mIU/ml) in a controlled study by Aspinall & Kocks . Hence the decrease in seroprotection rates and anti HBs levels from ages 5 to 6 years could be due to waning imunity of anti HBs levels with time. The overall generally low anti HBs levels among the vaccinated group can also be attributed to waning immunity with increasing age, (declining anti-HBs titre values with age). A finding consistent with findings by (Lu, CY et al, 2008), (Lee, P I et al, 1995) and (Lin et al, 2003) that although the vaccination program has been very successful in reducing carriage rate of HBV in the world, a gradual yearly decline in antibody titers against the HBsAg among vaccinees has been noted in several follow-up studies. It is therefore possible that the 231 vaccinated children probably responded to the vaccine but the anti HBs-levels decreased with increasing age as a result of waning immunity.

Contrary to the findings by Tsebe et al 2001, Aspinall and Kock and Yen- Hsuan et al, (2001) s, seroprotections rates in the present study were found to increase with increasing age from 6 year olds through 7years to a peak at among 8 year old children. This remarkable increase in seroprotection rates with increasing age was supported by increasing GMT values, from 36.93 mIU/ml in younger children with mean age 6.7 years to 48.4 mIU/ml in older babies with a mean age of 8.4 years. This finding could be the result of an anamnestic response of the older vaccinated children following recent exposure to the virus. This might especially account for the reason why younger vaccinated 5 years old children rather recorded low anti- HBs levels and older children (8 years) rather recorded high anti-HBs levels. Therefore one might conclude that even though most of the children in the vaccinated group have very low anti- HBs levels, they have intact immune memory that recognizes and stimulates

the production of anti-HBs upon exposure to the virus. Hence the 188 children with anti-HBs titres <10 mIU/ml might not necessarily be non-responders to the pentavalent vaccine; they probably originally responded to the primary course of vaccination, but anti-HBs titers have declined over the years to lower levels and this is probably what accounted for the significantly low anti HBs levels and the sero-protection rate of 27.04%.

In a cross-sectional study by Tsebe et al, it was assumed that a fair number of babies with anti-HBs titres<10 mIU/ml were not necessarily non-responders to the HBV vaccine; they probably originally responded to the primary course of vaccination, but anti-HBs titres declined to lower levels. This was particularly true for older babies, since analysis of anti-HBs in recently immunized young babies demonstrated a sero-protection rate of 95.6%, an observation that clearly indicates that the HBV vaccine is highly immunogenic within the framework of the South African EPI.

In 1995, UNICEF conducted a study which reported that the equipment used for storage and transportation of vaccines in certain provinces of South Africa might cause freeze-sensitive vaccines to be frozen (Battersby. 1995). This data therefore show that the immunogenicity of the pentavalent vaccine could have been affected as it is distributed by the government transport system, stored in government refrigerators (2–8°C), and monitored and administered by health care personnel in various clinics and hospitals especially given the fact that not all the health facilities have fridges and so may have challenges maintaining the cold chain of vaccines. It is therefore important that a closer look is given to the storage of the vaccine, the response and efficacy of the pentavalent vaccine as administered within the frame work of the Ghanaian EPI.

The sero-protection rate amongst vaccinated children in the Wa municipality measured by HBsAb seropositivity decreased from 50% at 5 years to 27.8% at 6 year, 31.4% at 7 year olds, 29.03% at 8

year olds, 25.4% at 9 years and 16.7% among children who took the vaccine. The sharp decrease in anti HBs positivity observed from 50% at 5 years to 27 % at 6 years, and 8 to 9 years could attributed to waning immunity as well as difference in number of participants in each group. These findings are consistent with findings by Jan et al. 2010 that the (anti-HBs) sero positivity rate of the vaccinees decreased from 99% at 1 year to 83% at 5 years, 71.1% at 7 years, 37.4% at 12 years, and 37% at 15-17 years, The increase observed between ages 6 years and 7 years could probably be due to recent exposure. A study by Tshatsinde et al. 1999, revealed a decrease in sero-protection rates among vaccinated babies with time. In the said study (Tshatsinde et al. 1999), vaccinated babies were followed-up from 1995–1998. The sero-protection decreased from 93% (1995), to 74.2% (1997) and 76.8% (1998). Similarly, GMT values decreased with time.

Compliance with vaccination schedule

It was interesting to observe that 71.24% (166/233) of children received the standard course of HBV vaccine at 6, 10 and 14 weeks, in parallel with OPV, DTP and Hib. Only 28.76% (67/233) received 'unscheduled' dosages of the vaccine after the first dose at 6 weeks (Table 4.5.). However, there was no significant difference in the sero protection rates between these two groups (p;0.4658)., perhaps indicating that the first dose is sufficient to prime the immune system, while the second and third doses act as boosters, irrespective of the wide spacing of dosages. Two (0.86%) of the vaccinated children demonstrated breakthrough infection compared to 10.3% among the non-vaccinated group suggesting the pentavalent vaccine within the frame work of the EPI is effective in protecting the majority of children in hyper endemic areas from HBV infection and from becoming chronic carriers within the Wa Municipality. The 2 vaccine failures probably were non responders or the result of a vertical

transmission from HBV positive mothers or horizontal infection from close associates or through scarification at infancy or even through blood transfusion.

Impact of the WHO-EPI vaccine in reducing the HBsAg carriage rates

The impact of the hepatitis B vaccine in the penta valent vaccine as measured by comparing the total number of children Positive for HBsAg and HBsAb among the vaccinated and non vaccinated children revealed the vaccine has successfully reduced the prevalence of HBsAg from 10.3 % in the non vaccinated group to 0.86% in the vaccinated group and evidently increased the protective imunity rate, measured by HBsAb positivity from 3.67% in the non vaccinated group to 27.89 % in the vaccinated group. Two (2) of the vaccinated babies experienced breakthrough infections resulting in HBs antigenemia compared to 24 (10.3%) in the non vaccinated group. Hence the HBV vaccine is 99.1% effective in preventing the transmission of HBV in the Wa municipality. These finding are consistent with findings by Viviani et al. 1999, Chotard et al 1992 in Gambia where Childhood HBsAg sero-prevalence has declined from 10% to 0.6% ever since the introduction of routine infant and childhood vaccination in Gambia in 1986 (Viviani et al. 1999, Chotard et al 1992). In South Africa where the endemicity of HBV infection, proof of the effect of routine vaccination has been shown through innovative means: a statistically significant decline in the incidence of HBV-related membranous nephropathy among children at a single hospital in 2000–2001 as compared with incidence during the before the vaccination era (Bhimma et al. 2003). Countries with intermediate endemicity HBsAg sero-prevalence among school children (between 7–12 years) declined from 1.6% in 1997 to 0.3% in 2003 in Malaysia which introduced universal infant vaccination in 1990 (Ng et al. 2005). Population-based surveillance data indicated a decline in the incidence of acute hepatitis B from 11 per 100,000 populations in 1987 to three per 100,000 populations in 2000 in Italy (Bonanni et al 2003). Moreover, the general prevalence of chronic HBV infection reduced from 13.4% in 1978 to 3.7% in 1997

(Namgyal, 2003). In Bristol Bay, Alaska, prior to routine immunization, 7.6% of children had serologic evidence of recovered infection by 9 years of age and 3.2% of children were infected chronically. No child under 10 years of age was chronically infected after ten years since the beginning of routine immunization, and only 1.5% had evidence of recovered infection (Harpaz et al. 2000). In other US societies with traditionally high rates of disease, similar sero-prevalence declines have been observed. Sero-prevalence of markers of Hepatitis B Virus infection declined from more than 20% before 1992 to 1.9% in 2001 among children of Asian immigrants residing in Georgia, (Fiore et al. 2003).

The overall data unequivocally demonstrated the protective efficacy of the HBV vaccine and has demonstrated its impact in reducing hepatitis B carriage among children in the Wa municipality of the Upper West region, a region that was previously identified as endemic (15.3% HBsAg carrier rate among blood donors and 21% among pregnant women) for HBV infection (Sarkodie et al., 1997). It was shown previously that urbanization plays an important role in the natural control of HBV in Soweto, a huge township in Johannesburg (Kew.1996,) It is therefore possible that improvement in socio-economic status of most people in the Wa municipality may have had a positive impact on the control of HBV infection being the regional capital and having the highest number of primary health care facilities within the region. It appears that the success of HBV vaccination in the Wa municipality is largely due to the unique, predominant childhood horizontal transmission (versus vertical and perinatal transmission in Asia) of HBV in sub-Saharan Africa; the majority of children become infected between 6 months and 5 years (Kew.1996,), Kiire.1996, and Vardas et al, 1999). A pre-immunization HBV survey demonstrated HBsAg carriage of 12.8% in 3year-old South African children (Vardas et al, 1999). Therefore, giving the vaccine within a few weeks after delivery is an advantage to newborns; the babies are already protected by the time they become exposed to HBV infection. The HBV vaccine is already showing a positive impact on the control and prevention of

HBV infection in children within the Wa municipality; our study demonstrate a marked reduction of the HBsAg carrier rate in children less than 10 years compared to those above 10 years who did not get the opportunity to take the pentavalent vaccine. Two (0.86%) of the vaccinated children demonstrated breakthrough infection. There are some reported cases of HBV vaccine failures in the Far East, Europe and the USA, regardless of whether the vaccine is given alone immediately after delivery, or in combination with HBIG therapy. For example, Carman W.F et al 1990. reported vaccine failures in 44 (2.8%) of 1590 vaccinated babies. These babies were born to HBsAg (some being HBeAg positive) carrier mothers, and received HBIG passive immunization immediately after birth and at 1 month, as well as HBV vaccine at 0, 1 and 6 months, or 3, 4 and 9 months. The babies developed protective antibodies induced by immunoprophylaxis; nevertheless, they became positive for HBsAg, and other serological markers of HBV infection.

It appears that vertical and/or perinatal transmissions contribute to the emergence of vaccine escape mutants. Babies become exposed to HBV infections before receiving either active (vaccine) or passive (HBIG) prophylaxis (or a combination of both). This puts the virus under immune pressure and the virus will mutate in an attempt to escape the pressure. Nevertheless, the benefits of universal HBV vaccination greatly outweigh the potential threat posed by HBV vaccine escape mutants at present. For example, in 1984, HBsAg sero-prevalence among Taiwanese children declined from

9.8%, the year when universal infant vaccination started, to 0.7% in 1999 (Chan et al. 2004). The mean yearly incidence of hepatocellular carcinoma among children between the ages 6 to14 years in 1981–1986 was 0.7 per 100,000 before the vaccine era, whiles in 1990–1994 it was 0.36 per 100,000 ($p < 0.01$) (Chang et al. 1997). These impressive data clearly showing that even in countries where vertical and perinatal transmissions of HBV do occur, vaccination still remains an effective tool of curbing the hepatitis B disease.

5.2 CONCLUSSION

It was found out that the prevalence of Hepatitis measured by HBsAg positivity among children between the ages of 5 and 10 years who had received the HBV vaccine as part of the EPI was 0.86 % in the Wa Municipality against the background of 10.3% among children between the ages of 10 and 16 who were born before the integration and so never received any such vaccine. Hence one can conclude that the incorporation of the HBV vaccine in to the EPI in the Wa municipality has succeeded in reducing the prevalence of the virus and therefore has the potential of converting the prevalence of the HBV infection in the Wa municipality from high endemicity to low endemicity.

Out of 233 children, 27.04% (63/233) had anti-HBs titres > 10 IU/L with a GMT of 42.64 IU/L, hence can be said to be seroprotected while 72.96% (170/233) had anti-HBs titres <10 IU/L can be said to be non-protected. There was no significant difference in the seroprotection rates between males and females (p value= 0.458). Despite the fact that the sero protection rates based on anti HBsAb levels were low due to waning immunity generally, the in higher titres recorded by children 7 years and 8 year old who received the vaccine much earlier than the younger ones could be attributed to anamnestic response of the older children to recent exposure to the Hepatitis B virus. It can be concluded that HBV vaccine given at 6, 10 and 14 weeks as part of routine immunization for HBV infection, is highly effective within the framework of the EPI in the Wa municipality since it have successfully reduced the prevalence of HBsAg from 10.3 % among non vaccinated children to 0.86% among vaccinated children and 5.4% among the entire study population. The results clearly indicate that universal childhood HBV vaccination can drastically reduce the rate of new infections, and thereby reduce the burden of HBV related liver disease, especially cirrhosis and hepatocellular carcinoma in Ghanaian children.

5.3 LIMITATIONS

The data was a one point source data and so could not evaluate the trends observed per vaccinee over time.

5.4 RECOMMENDATIONS

Post vaccination surveillance is recommended to identify non responders and vaccine escape antigens.

A more comprehensive study is recommended to evaluate the trends that were observed per vaccinee over time.

More efforts are needed to educate mothers to send their children for immunization on the scheduled dates whether they deliver at home or health facility.

More efforts are needed to improve on the identification and administration of birth dose to children born to Hepatitis B positive carrier mothers.

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APPENDIX

APPENDIX 1

3.6 MATERIALS AND INSTRUMENTS.

- 5 mL needle and syringes
- Vacutainer tubes
- 70% alcohol
- Sterile cotton wool
- Disposable gloves
- Torniquet
- Centrifuge
- Timer
- Multi Channel micro pipettes and disposable tips
- Absorbent tissue
- Water bath
- Waste container
- Distilled Water

- Micro shaker for dissolving and mixing conjugate with samples.
- Microwell reader
- Microwell aspiration /wash system
- Fortress anti – HBs for the quantitative determination of Ant-HBs. (4)
- Wondfo one step HBV serum/plasma test kits

Reagents provided in the Fotress ELISA kit

- microwell plates: (12 × 8)wells per plate
- Caliberation standards with concentrations :0mIU/mL, 10mIU/mL, 20mIU/mL, 40mIU/mL,80mIU/mL,160mIU/mL
- HRP-Conjugate reagents
- Wash buffer
- Chromogen solution A
- Chromogen solution B
- Stop solution (2.0M Sulpheric Acid)
- Card board plate sealer: to cover plates during incubation.