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2. **Bada FO**, Blok N, Okpokoro E, et al. Cost comparison of nine-month treatment regimens with 20-month standardized care for the treatment of rifampicin-resistant / multi-drug resistant tuberculosis in Nigeria. *PLoS One*. 2020;15(9):1-14. doi:10.1371/journal.pone.0241065
3. Oga-Omenka C, **Bada F**, Agbaje A, Dakum P, Menzies D, Zarowsky C. Ease and equity of access to free DR-TB services in Nigeria- a qualitative analysis of policies, structures and processes. 2020:1-13. doi:10.21203/rs.3.rs-39365/v1
4. Olakunde BO, Adeyinka DA, Olakunde OA, Uthman OA, **Bada FO**, Nartey YA, et al. A systematic review and meta-analysis of the prevalence of hepatitis B virus infection among pregnant women in Nigeria. *PLoS One* [Internet].

2021;16(10):e0259218. Available from:
<http://dx.doi.org/10.1371/journal.pone.0259218>

5. Akolo C. **Bada F**, Okpokoro E, Nwanne O, Iziduh S, Usoroh E, Ali T, Ibeziako V, Oladimeji O, Odo M. Debunking the myths perpetuating low implementation of isoniazid preventive therapy amongst human immunodeficiency virus-infected persons. *World J Virol.* 2015;4(2):105. doi:10.5501/wjv.v4.i2.105

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ABSTRACT

Title of Dissertation: Effects of HIV Exposure and Maternal Antibodies to Hepatitis B Virus on the Immune Response of Nigerian Infants to Hepatitis B Vaccine

Florence O. Bada, PhD., 2022

Dissertation Directed by:

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Introduction: Chronic hepatitis B virus (HBV) infection leads to considerable morbidity and early mortality. Development of chronic HBV infection can be prevented by a three to four dose schedule of Hepatitis B vaccines in immunocompetent infants. However, HIV exposed uninfected infants (HEU) are thought to exhibit an attenuated immune response to some vaccines. In addition, a significant proportion of pregnant women in Nigeria have antibodies to HBV, specifically Hepatitis B surface antibodies (HBsAb). Maternal antibodies inhibit infant immune responses in some instances.

Objective: To estimate the effect of antenatal and perinatal exposure to maternal HIV, and the effects of maternal HBsAb on the immune response of Nigerian infants to hepatitis B vaccine.

Methods: Using a retrospective cohort design, we determined the relationship between infant HIV-exposure status, and infant exposure to detectable concentrations of maternal HBsAb, and infant immune response to Hepatitis B vaccine separately using general

linear models adjusted for potential confounders. Subsequently, we used Fisher's Exact tests to compare the proportion of infants with HBsAb concentrations above 10mIU/mL: for HEU as compared to HIV unexposed uninfected infants (HUU), and for infants exposed to detectable concentrations of maternal HBsAb as compared to infants unexposed to detectable concentrations of maternal HBsAb at 24 and 52 weeks of age separately.

Results: We found HIV exposure to be associated with infant immune response to hepatitis B vaccine at birth, Weeks 4 and 52 ($P < 0.0001$, $P = 0.05$ and $P < 0.0001$) and also to be associated with the proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$ at Week 52 ($P = 0.04$).

Exposure to detectable concentrations of maternal HBsAb was also associated with infant immune response at Weeks 24 and 52 respectively.

Conclusion: Though antenatal and perinatal exposure to HIV, and to detectable concentrations of maternal HBsAb were found to be associated with infant immune response to hepatitis B vaccine, these exposures did not appear to attenuate immune response. In addition, differences observed in the proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$ were small and do not appear to be clinically relevant.

Effects of HIV Exposure and Maternal Antibodies to Hepatitis B Virus on the Immune
Response of Nigerian Infants to Hepatitis B Vaccine

by
Florence Olufunmilayo Bada

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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DEDICATION

To my father, Dr. Eldred Ademola Wright, in memoriam.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ARV	Antiretroviral Medications
ART	Antiretroviral therapy
CD4	Cluster of Differentiation
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
HBcAg	Hepatitis B core Antigen
HBeAb	Hepatitis B e Antibody
HBeAg	Hepatitis B e antigen
HBIG	Hepatitis B Immunoglobulin
HBsAb	Hepatitis B surface Antibody
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HEU	HIV Exposed Uninfected Infants
HIB	Hemophilus Influenzae Type B
HIV	Human Immunodeficiency Virus
HUU	HIV Unexposed Uninfected Infants

IgG	Immunoglobulin G
IgM	Immunoglobulin M
INFANT	Innate, Adaptive and Mucosal Immune Responses in HIV-Exposed Uninfected Infants: A Human Model to Understand Correlates of Immune Protection
MatAbs	Maternal Antibodies
MTCT	Mother-to-Child Transmission
NRTI	Nucleoside Reverse Transcriptase Inhibitors
RNA	Ribonucleic Acid
RSA	Republic of South Africa
SSA	Sub-Saharan Africa
WHO	World Health Organization

CHAPTER I. INTRODUCTION

The objectives of this research were to determine the effects of antenatal and perinatal exposure to maternal HIV, and maternal antibodies to Hepatitis B virus (HBV) on the immune response of Nigerian infants to hepatitis B vaccine in a region hyperendemic with HBV. Though approximately 296 million persons live with chronic HBV infection¹ and are at risk of the substantial morbidity and early mortality that are associated with this infection,² insufficient attention is focused on preventing HBV infection which is amenable to prevention with a vaccine. The situation is particularly dire in sub-Saharan Africa which is the region of the world with the second highest prevalence of HBV infection.³

WHO recommends universal vaccination of medically stable infants with hepatitis B vaccine as close to birth as possible to minimize mother to child transmission (MTCT) of HBV and as part of a three-dose schedule that should proffer extended protection from HBV infection.^{4,5} This three-dose schedule with a birth dose has over 95% effectiveness in proffering extended protection⁵⁻⁸ and 69-98% effectiveness in preventing MTCT of HBV in immunocompetent infants.⁹⁻¹¹

Concurrent in SSA is the HIV epidemic which disproportionately affects women of reproductive age¹² and results in the birth of over one million children exposed to maternal HIV but without HIV themselves.¹³ These HIV-exposed uninfected infants (HEU) manifest disproportionate morbidity and mortality partially attributable to their exposure to maternal HIV and maternal antiretroviral medications. Maternal HIV and ARVs alter the function of these infant's immune systems.¹⁴⁻²³ In addition, maternal HIV

affects the levels of antibodies to vaccine-preventable diseases in pregnant women and the amount transferred to their infants.²⁴⁻²⁶ Maternal antibodies have been shown to interfere with infant immune response to vaccines in certain instances²⁷⁻³⁰ and are distributed differentially geographically and seasonally. Only 11 countries in SSA have introduced a birth dose of hepatitis B vaccine which is when maternal antibodies are at a peak.³¹

We utilized data and samples collected by the INFANT study which was a prospective cohort of mother-infant pairs at a tertiary hospital in North-Central Nigeria enrolled between 2013 and 2017.

The aims and hypotheses were as follows:

Aim 1. To determine the effect of antenatal and perinatal HIV exposure on humoral responses of Nigerian infants to three-dose hepatitis B vaccine.

Sub-aim 1.1. To determine the association between antenatal and perinatal HIV-exposure and the concentration of HBsAb at birth, 4, 24 and 52 weeks of age following hepatitis B vaccine given at birth, six and 14 weeks.

Hypothesis: Significantly more Nigerian HEU will exhibit an attenuated response to hepatitis B vaccine compared to Nigerian HIV-unexposed uninfected infants (HUU); Nigerian HEU will have lower mean concentrations of HBsAb at birth, 4, 24 and 52 weeks as compared to Nigerian HUU.

Sub-aim 1.2. To determine the association between antenatal and perinatal HIV-exposure and the proportion of infants with protective concentrations of HBsAb (HBsAb ≥ 10 mIU/mL) at 24 and 52 weeks of age respectively.

Hypothesis: A lower proportion of Nigerian HEU will have HBsAb concentrations ≥ 10 mIU/mL at 24 and 52 weeks respectively, as compared to Nigerian HUU.

Significance: This study has a larger sample size than previous studies (n=367 versus n=53-180) and can also account for factors that may confound the relationship between perinatal HIV-exposure and infants' humoral immune response to hepatitis B vaccine such as maternal clinical status, infant sex, maternal antibodies and infant nutritional status. This study will provide evidence on the immune response of infants to hepatitis B vaccine in Nigeria, and whether HIV-exposure should be taken into account in the timing of routine infant immunizations. This study will provide data to inform guidelines for optimal protection of HEU against HBV infection.

Aim 2: To determine the effect of maternal HBsAb on the immune response of Nigerian infants to three-dose hepatitis B vaccine.

Sub-aim 2.1. To determine the association between exposure to maternal HBsAb and the concentration of infant HBsAb at birth, 4, 24 and 52 weeks of age following hepatitis B vaccine given at birth, six and 14 weeks.

Hypothesis: Infants of mothers with detectable concentrations of HBsAb will have lower immune responses to hepatitis B vaccine. They will have higher HBsAb concentrations at birth but lower concentrations of HBsAb at 24 and 52 weeks.

Sub-aim 2.2. To determine the association between exposure to maternal HBsAb and the proportion of infants with protective concentrations of HBsAb (HBsAb $\geq 10\text{mIU/mL}$) at 24 and 52 weeks of age respectively.

Hypothesis: A lower proportion of infants exposed to detectable concentrations of maternal HBsAb will have HBsAb concentrations $\geq 10\text{mIU/mL}$ at 24 or 52 weeks respectively, as compared to infants not exposed to mothers with detectable concentrations of HBsAb.

Significance: This study will provide evidence on the proportion of pregnant women with antibodies to HBV, and if transferred maternal antibodies affect the infant's immune response to hepatitis B vaccine.

CHAPTER 2. BACKGROUND AND SIGNIFICANCE

2.1 Hepatitis B Virus - Epidemiology and Natural History

Hepatitis B virus (HBV) is a 42-nm, double-shelled deoxyribonucleic acid (DNA) virus of the Hepadnaviridae family that replicates in the liver and causes liver dysfunction.³² It is transmitted by percutaneous or mucous membrane contact with infected blood or other body fluids such as saliva, semen and vaginal fluids. Although it can be detected in other fluids such as urine, vomitus, sputum, and nasopharyngeal washings, these fluids are not efficient vehicles of transmission unless they contain blood. Also, HBV found in breastmilk is thought to be unlikely to lead to mother-to-child transmission (MTCT) of HBV.^{33,34} The primary routes of transmission are vertical (intrauterine transmission, transmission during labor) or through percutaneous exposure to blood or infectious body fluids as occurs through contact with blood or open sores on an infected person, sharing certain items like toothbrushes and razors which can break the skin or mucous membrane, unsafe injections, blood transfusions, organ transplants and sexual contact.

Hepatitis B can cause both acute and chronic infection; the acute phase is often asymptomatic (especially in children) but the chronic infection has three distinct phenotypes; an immune tolerant phenotype with minimal liver disease characterized by high HBV DNA and normal liver enzymes like alanine aminotransferase (ALT); an immune active phenotype with high HBV DNA and active liver inflammation evidenced by high ALT levels; and an inactive phenotype with HBV DNA < 2000 IU/mL and normal ALT levels with minimal inflammation and fibrosis.² There are several biomarkers that

indicate distinct stages of HBV infection and resolution; Hepatitis B surface antigen (HBsAg) indicates current HBV infection (either acute or chronic) while Hepatitis B e antigen (HBeAg) indicates viral replication and high infectivity. Hepatitis B surface antibody (HBsAb) indicates immunity to HBV infection, Immunoglobulin M Hepatitis B core antibody (IgM HBcAb) indicates acute HBV infection while IgG HBcAb indicates past infection, and Hepatitis B e antibody (HBeAb) indicates loss of replicating virus. (See Table 2:1 for interpretation of serologic results for HBV infection).

Table 2:1. Interpretation of serologic test results for hepatitis B virus infection

HBsAg	Total anti-HBc	IgM anti-HBc	HBsAb	HBV DNA	Interpretation
-	-	-	-	-	Never infected; susceptible
+	-	-	-	+ or -	Early acute infection
+	+	+	-	+	Acute infection
+ (+HBeAg)	+	+	-	+	Acute infection (with high viral replication and high infectivity)
-	+	+	+ or -	+ or -	Acute resolving infection
-	+	-	+	-	Recovered from past infection and immune
+	+	-	-	+	Chronic infection
+ (+HBeAg)	+	-	-	+	Chronic infection (with high viral replication and high infectivity)
-	+	-	-	+ or -	False positive; past infection; “low level chronic infection; or passive transfer of anti-HBc to infant born to HBsAg positive mother
-	-	-	+	-	Immune if anti-HBs is ≥ 10 mIU/mL after vaccine series completion; passive transfer after hepatitis B immune globulin and general IVIG administration

Abbreviations: -, negative; +, positive; anti-HBc, antibody to hepatitis B core antigen; HBsAb, antibody to hepatitis B surface antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus deoxyribonucleic acid; IgM, immunoglobulin class M; IVIG, intravenous immunoglobulin

Adapted from “Prevention of Hepatitis B virus infection in the United States: Recommendations of the Advisory Committee on Immunization Practices” Morbidity and Mortality Weekly Report, Jan 2018.

In 2021, the global prevalence of Hepatitis B was estimated at 3.6%, translating into 296 million persons living with chronic HBV infection.¹ This population was concentrated in the African and Western Pacific Regions where 6.2 and 6.1% of the adult population respectively, were infected.³ Globally, the epidemiology of HBV infection has traditionally been described as high, intermediate and low based on the proportion of the population that is seropositive.³⁵ Countries with high endemicity have an HBsAg seroprevalence that is greater or equal to 8%; countries with intermediate endemicity have a seroprevalence that is between 2-7% and countries with low endemicity have a HBsAg seroprevalence less than 2%.³⁵ Nigeria, which is a country in West Africa has a HBsAg seroprevalence of 8.1%.³⁶

Chronic HBV infection is associated with considerable morbidity and mortality – 15-40% of people with chronic hepatitis B go on to develop liver cirrhosis, liver failure, or hepatocellular carcinoma (HCC) and 15-25% die from HBV-related liver disease². Among 770,000 global cases of hepatocellular cancer in 2012, 56% were attributable to HBV;³⁷ and in 2015 alone, 887,000 deaths were attributable to HBV.³⁸ The risk of HBV infection becoming chronic is inversely related to age at infection; adults and children aged six years and above, have a 5% risk of becoming chronically infected with HBV; children aged five years and below have a 30% risk, and perinatally-infected infants have a 90% risk.^{39,40} (See Fig 2:1)

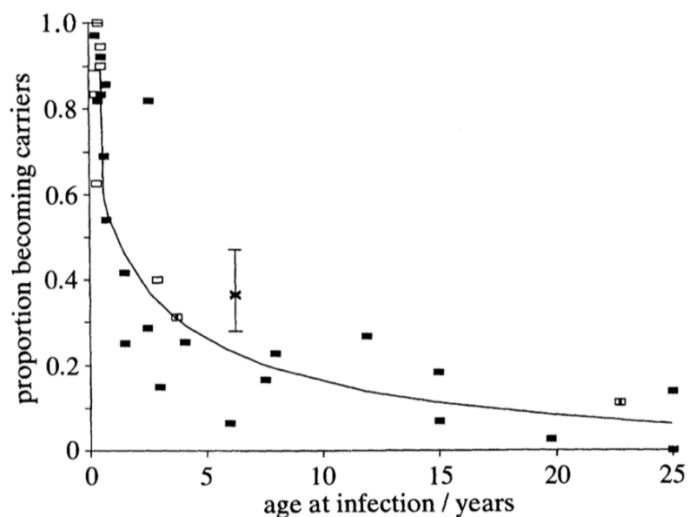


Figure 2.1.Relation between age at infection and the risk of becoming a carrier.

Source: The influence of age on the development of the hepatitis B carrier state. Edmunds et al. Proceedings: Biological sciences, 1993

MTCT of HBV is a significant mode of HBV transmission globally, particularly in resource-limited countries with high HBV endemicity and is responsible for 16% of new HBV infections in sub-Saharan Africa (SSA).⁴¹ The virus is most commonly transmitted from mother to child during delivery,³⁵ though horizontal transfer also plays a significant role, particularly in SSA.⁴² The global risk of MTCT of HBV among mothers with both HBsAg and HBeAg is estimated at 70-90%, while for mothers with only HBsAg, MTCT is estimated at 5-30% without intervention.^{35,43} However, in SSA, the risk of MTCT of HBV in mothers with HBsAg and HBeAg has been estimated to be 38.3% and for mothers with HBsAg alone, estimated at 4.8%;⁴⁴ this is thought to be due a reduced prevalence of the immune tolerant phenotype in SSA as compared to Asia.⁴¹ Though the risk of MTCT appears lower in SSA than that in regions with a similar prevalence of HBV, it is estimated that 1% of newborns in SSA (nearly 2 million infants from 2010 to 2015) are infected via

MTCT of HBV annually.⁴⁴ In West Africa, infants who are infected with HBV through MTCT have been found to have five times the risk of liver fibrosis as compared to people infected later in life via horizontal transmission.⁴¹ Finally, 54% of chronic HBV carriers with significant fibrosis, and 63% of carriers who need antiviral treatment in SSA have been determined to have been infected by MTCT.⁴¹

2.2 Perinatal HIV-exposure

The HIV epidemic is an epidemic concurrent with HBV in SSA, with approximately 25.3 million persons living with HIV in 2020, including approximately 900,000 persons newly infected that year.¹³ Though public health programs have reduced MTCT of HIV from 25% to less than 10% globally, over 1 million children exposed to maternal HIV antenatally and postnatally (though uninfected themselves) are born in SSA every year of which approximately 99,000 are born in Nigeria.¹³

There is evidence of higher morbidity and mortality among HIV-exposed uninfected infants (HEU) as compared to HIV-unexposed uninfected infants (HUU). HEU have over three times the mortality of HUU,^{45,46} and, as expected, lower mortality rates than HIV-infected infants. HEU also have a higher predisposition for infections as evidenced by four-fold higher rates of invasive *Streptococcus pneumoniae* and a 13-fold higher risk of Group B *Streptococcus* invasive infections, compared to the general infant population.^{47,48} Furthermore, HEU have more severe manifestations of infections, sometimes accompanied by clinical evidence of immune suppression,⁴⁹ and higher rates of sick clinic visits and hospital admissions for infections.^{46,50} HEU have also been found to

experience impaired linear and ponderal growth in their first 18 months of life,⁵¹ even after controlling for mode of infant feeding and maternal clinical status,⁵² and this stunting in growth is not corrected by nutritional supplementation.⁵³ The increased morbidity and mortality have been attributed to indirect effects of the clinical status of these infants whose mothers have HIV. Some of these predisposing factors are maternal illness and death,^{45,54} higher rates of colonization or higher numbers of virulent organisms,⁴⁸ lower household income,⁴⁵ reduced breast-feeding,⁵⁵ and poor maternal psychologic and physical health leading to poor child care.⁵⁶

However, these factors do not appear sufficient to account completely for the increased morbidity and mortality seen among HEU.⁴⁸ Another explanation encompasses two unique exposures of HEU, which have the potential to alter their developing immune systems and worsen infectious disease outcomes – which are exposure to maternal HIV infection, and to maternal antiretroviral medications (ARVs).

Effects of maternal HIV-infection

HIV infection appears to affect the level of antibodies to vaccine preventable diseases in pregnant women. HIV-infected mothers have been found to have higher mean levels of total IgG than non-infected mothers, and total serum levels of maternal IgG have been found to predict placental transfer of IgG antibodies independent of other factors.²⁴ There have however been varied reports on maternal levels of specific antibodies;^{24,25} HIV-infected mothers have been found less likely to have anti-*Hemophilus influenzae* type B (Hib) concentrations considered to be protective, but the proportions of women with protective antibody concentrations against pertussis, tetanus or hepatitis B were similar among HIV-infected and HIV-uninfected women.²⁵ Maternal HIV infection also appears

to affect the proportion of maternal-specific antibody transferred across the placenta to infants;²⁴⁻²⁶ HIV-infected women had significant reductions in placental transfer of 19% for measles, 23% for anti-Hib, 24% for *Streptococcus pneumoniae*, 31% for varicella zoster virus, 40% for pertussis and 27 - 52% for tetanus-specific antibodies compared with HIV-uninfected women, but no reduction in the transfer of herpes simplex virus.²⁴⁻²⁶ Both HIV-infected and HIV-uninfected mothers had statistically significant correlations between maternal and infant-specific antibodies for Hib, pertussis, pneumococcus and tetanus. However, no association was found between maternal CD4 count or viral load and placental transfer of maternal antibodies.²⁵

There are suggestions that an immune-deficient mother may have defective trans-placental transfer of hematopoietic cytokines, resulting in lower output of CD4 cells from the infant's thymus.^{57,58} A reduction in interleukin 12 cytokine production has been found in HEU as compared to HUU, and this is thought to delay immune cellular maturation and cause deficits in antigen-presenting cells.⁵⁹⁻⁶¹ HIV viral products are also thought to diffuse through the placenta and cause immune paresis.^{57,62}

Pregnant women who are co-infected with HIV and HBV are twice as likely as those only infected with HBV to test positive for HBeAg and more likely to have detectable HBV DNA, thereby greatly increasing their risk of MTCT of HBV.^{63,64}

Effects of antenatal exposure to maternal antiretroviral medications

ARVs such as nucleoside reverse transcriptase inhibitors (NRTIs) cross the placenta,^{20,23} and have been linked to many biological alterations that affect infant health.

Zidovudine (ZDV) has been associated with mitochondrial toxicity^{14,15} and also impairs the HEU immune system by inhibiting the proliferation of human hematopoietic progenitor cells. As a result, ARV-exposed infants have lower lymphocyte counts than ARV unexposed infants.^{16,17} Combination ARVs have been associated with biologic alterations in HEU such as in cytokine expression,¹⁸ mitochondrial toxicity¹⁹ or mitochondrial depletion,²⁰ which are thought to lead to the production of abnormal lymphocytes.^{21,22} Use of maternal ART has also been found to be associated with preterm birth.^{65–67}

The effect of preterm birth on an infant's immune response to hepatitis B will be elaborated on in the section on factors influencing the immunogenicity of hepatitis B vaccine among infants.

2.3 Prevention of Perinatal Hepatitis B Infection

Several strategies and interventions have been recommended to prevent MTCT of HBV.⁶⁸ These include timely identification of HBV-infected mothers and exposed infants, with provision of a birth dose of hepatitis B vaccine^{68,69} and hepatitis B immune globulin (HBIG) to exposed infants both within 12 hours of birth where feasible.⁶⁸ Furthermore, pregnant women with HBV viral load $\geq 200,000$ IU/mL benefit from antiviral agents which decrease their viral load and reduce risk of MTCT to their infants.⁷⁰ However, the World Health Organization recommends universal vaccination of all medically stable infants regardless of their mother's HBV status.⁴

Safe and effective vaccines to prevent HBV infection have been available since 1982, and recombinant hepatitis B vaccines since 1986. Monovalent vaccines and

combination vaccines containing HBV antigens have been found to have over 95% efficacy in proffering extended protection from HBV infection⁵⁻⁸, although some studies from countries in SSA have shown lower vaccine efficacy.⁷¹ These vaccines, when given to newborns, also have 69 – 100% effectiveness in preventing mother-to-child transmission of HBV.⁹⁻¹¹ Three- or four-dose combinations are recommended, but one of the most important requirements to prevent perinatal transmission of HBV is a birth dose of hepatitis B vaccine, ideally within the first 12 hours of life.^{4,5} Countries that have implemented universal infant HBV vaccination have experienced drastic reductions in chronic HBV infection rates.^{72,73} However, in SSA, only 11 of 47 countries have introduced a birth dose of hepatitis B vaccine.³¹

Though administration of hepatitis B vaccine and HBIG within 12 hours of life reduces the rate of MTCT of HBV by 85-95% as compared to no intervention,⁷⁴ provision of HBIG to HBV-exposed infants is not feasible in many countries, due to complex production and storage needs and prohibitive costs.⁷⁵ However, the efficacy of a hepatitis B vaccine alone approaches that of hepatitis B vaccine plus HBIG, when a three dose series is initiated soon after birth.¹¹ The introduction of hepatitis B vaccines to existing immunization programs has also been found to be cost-effective.^{76,77}

Treatment of HBV-infected pregnant women with antivirals has not been adopted in low resource countries due to limited diagnostic testing capacity, inadequate access to antivirals and a lack of dedicated HBV public health programs.⁷⁸

Correlates of Protection

Evidence shows that most immunocompetent infants achieve extended protection once they are able to mount an anti-HBV antibody response of $\geq 10\text{mIU/mL}$ by one to three months after the third dose of hepatitis B vaccine.⁷⁹ This holds true even if the HBsAb drops below 10mIU/mL subsequently.⁸⁰

Nigeria's HBV Immunization Program

Hepatitis B vaccine was added to Nigeria's National Program of Immunization in 1995 but became available in 2004.⁸¹ Nigeria, which is hyperendemic for HBV,⁸² is one of eleven countries in SSA that have introduced a birth dose of hepatitis B vaccine³¹ with a coverage of 52% in 2019 for the birth dose and coverage of 57% for three doses of hepatitis B. At the time of the INFANT study, the approved hepatitis B vaccine schedule consisted of three doses of monovalent hepatitis B vaccine (Engerix B) to be given to infants at birth, six and fourteen weeks of life.⁸³

2.4 Factors that influence the immunogenicity of hepatitis B vaccine

Although a wide range of factors influence the immune response to vaccination (Fig 2:2), those specific to HBV vaccine for infants at birth are listed below. Some of these factors include perinatal host factors such as gestational age and birth weight; breastfeeding, maternal antibodies, and maternal infections during pregnancy; nutritional factors, intrinsic host factors like sex; and administration factors like vaccination schedule, site, and route.⁸⁴

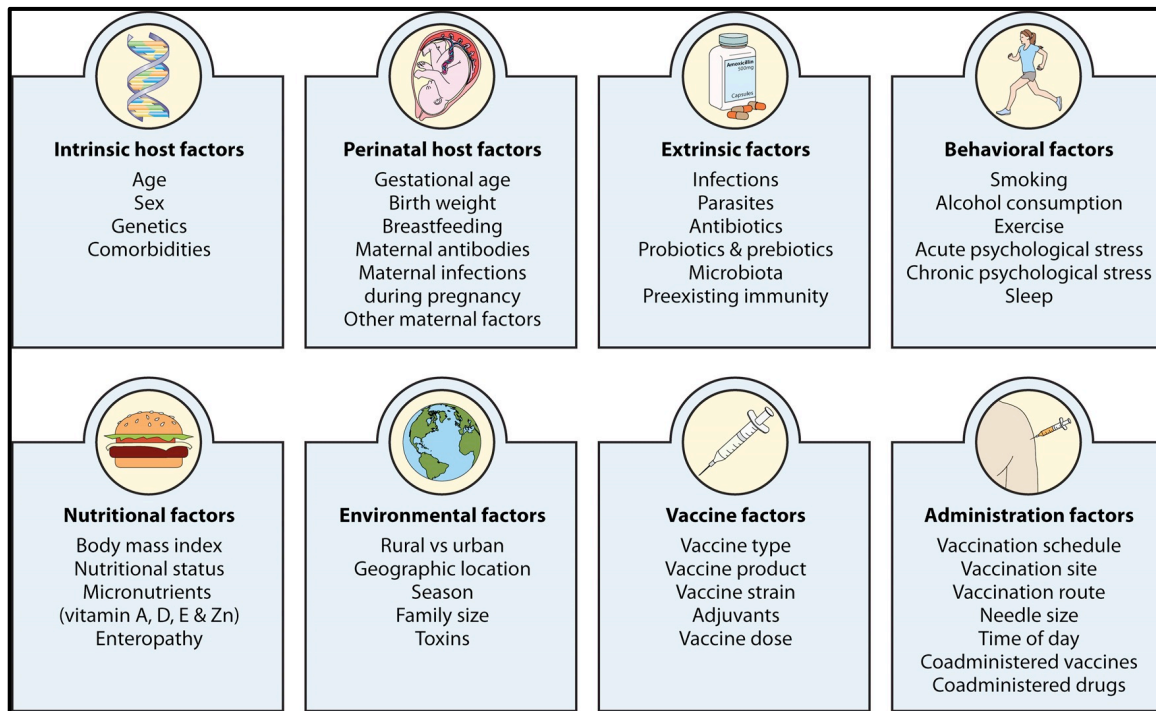


Figure 2:2. Factors that influence the immune response to vaccination.

Source: Clinical Microbiology Reviews; Zimmerman et al., 2019

Gestational age and birth weight - Reports on the immunogenicity of hepatitis B vaccine in preterm infants have been mixed.^{85,86} Pre-term infants are likely to have low birth weight which may also affect the immunogenicity of vaccines received, and this is not always taken into consideration in studies evaluating the immunogenicity of vaccines in pre-term infants.⁸⁷ A recent systematic review and meta-analysis by Fan et al. sought to examine associations between infant preterm birth and low birth weight with hepatitis B vaccine immunogenicity. They found that preterm birth appeared to be associated with an impaired immune response to hepatitis B vaccine, while low birth weight was not, when birth weight was dichotomized at 2500g.⁸⁷ In this study, pre-term infants (defined as infants born before

37 weeks of gestation) were found to be 1.36 times more likely to exhibit non-response to the hepatitis B vaccine (defined as anti-HBs titers <10mIU/mL) compared to their full-term counterparts.⁸⁷ Other studies that dichotomized low birth weight at 2000g or less showed that preterm infants had an impaired response to hepatitis B vaccine when it was administered before age 1 month⁸⁸⁻⁹⁰ but mount a response comparable to term infants if given the vaccine at age 1 month of age regardless of initial birth weight or gestational age.^{90,91} In a study by Patel et al., when preterm infants weighing <1500g were given a 3-dose series of hepatitis B vaccine with the first dose given at 3 days of age, only 68% mounted a response rate of >10mIU/mL, but when the first dose of the vaccine was given at 1 month of age, a 96% response rate was noted irrespective of birth weight and weight at the time of vaccination.⁹⁰

Nutrition – Malnourished children have been reported to have lower responses to vaccinations including Hepatitis B⁹² and mode of feeding (exclusive breast-feeding, mixed or formula feeding) has been associated with infant well-being with breast feeding found to mitigate the effects of antenatal HIV exposure among infants in their first year of life.⁹³

Maternal antibodies - Maternal antibodies (MatAbs) can interfere with an infant's response to vaccination.²⁷⁻³⁰ MatAbs are transmitted to infants transplacentally and via breastmilk. Infants who were IgA or IgG seropositive to rotavirus at base-line have been found to be less likely to seroconvert when vaccinated, compared to their negative counterparts.²⁷ The influence of MatAbs on the immune response of infants appears to be

dependent on the MatAb:vaccine ratio at the time of immunization.⁹⁴ This MatAb:vaccine ratio is the ratio of the concentration of MatAbs to the dose of the vaccine antigen. Infant immune responses are thought to be induced when MatAbs decline below the infant's response threshold.^{94,95} The amount of maternal antibody transferred to infants is affected by maternal antibody level, IgG subclass, gestational age and placental characteristics.^{24,26,96,97}

Maternal Infections during pregnancy - There are mixed reports on the effect of maternal infection with malaria, filariae, hookworm and Schistosoma parasites on infant antibody responses to Hepatitis B vaccine.^{98,99}

Infant sex – Female infants develop higher antibody responses to vaccines than males, and antibody responses can be twice as high in females compared to males of all ages.¹⁰⁰ Genetic factors are thought to play a role throughout the lifespan while sex hormones modulate immune response in a dose-dependent manner later in life.¹⁰¹

Administrative and vaccine-specific factors like vaccination schedule, dose, and specific vaccine administered all influence immune response.^{8,10,102,103}

2.5 Gaps in the Literature

The effect of antenatal fetal exposure to maternal HIV without being HIV-infected on an infant's immune response to hepatitis B vaccine is not well studied. Though several important studies have evaluated the immune responses of HEU and infants exposed to detectable concentrations of maternal HBsAb to hepatitis B vaccine, most of these previous studies had limitations including modest sample sizes (n=53 to n= 180). See Table 2.1 for a description of these studies and their limitations.

Nlend et al. and Abramczuk et al. enrolled infants after they had completed all three doses of hepatitis B vaccine^{104,105} and therefore had no maternal or infant pre-vaccination titers. Mancinelli et al. collected plasma samples from children aged 6, 12 and 24 months of age and did not have an internal cohort of HUU for comparison.¹⁰⁶ Hesselting et al. did not estimate maternal and infant pre-vaccination HBsAb but estimated infant HBsAb at 14, 24 and 52 weeks.¹⁰⁷

Another gap in the literature is the immune response of HEU when the first dose of hepatitis B vaccine is given at birth, which is required to prevent perinatal transmission of HBV from mother to child, but which is also when maternally transferred antibodies are highest. Jones et al., Mancinelli et al. and Hesselting et al. evaluated the immune response to vaccines given at 6, 10 and 14 weeks of age.^{25,106-108}

Table 2:2. Previous Studies on effects of exposure to maternal HIV or maternal antibodies to hepatitis B virus.

Study	Objective	Study Design	Timing of vaccinations	Timing of Outcome Assessment	Limitations
Reikie et al. ¹⁰⁸	To understand the responses of HEU to vaccinations	Prospective cohort study in RSA	6, 10 and 14 weeks	2 and 6 weeks, 3, 6, 12, 18, and 24 months	1. Modest sample size (n=53) 2. Different feeding modalities for HEU and HUU 3. Did not collect data on maternal clinical status
Hesseling et al. ¹⁰⁷	To investigate the effect of HIV exposure on vaccine antibody concentrations	A secondary question in a single-blinded exploratory randomized phase II trial	6, 10 and 14 weeks	14, 24 and 52 weeks	1. Modest sample size (n=180) 2. No data on pre-vaccination antibody concentrations
Abramczuk et. al. ¹⁰⁵	To evaluate humoral responses to hepatitis B vaccine in HEU and HUU	Prospective cohort study in Brazil	Birth, months 1 and 6	One month after third dose	1. Modest sample size (n=157) 2. Measured infant antibody response at only one point in time 3. Different feeding modalities for HEU and HUU
Jones et al. ²⁵	To study the association of maternal HIV with maternal and infant - specific antibody levels	Nested in a cohort study	6, 10 and 14 weeks	Birth and 16 weeks	1. Modest sample size (n=109) 2. Different feeding modalities for the exposure groups
Mancinelli et. al. ¹⁰⁶	To assess the immune response to hepatitis B vaccine in HEU and correlate it to HBV infection	An observational cohort study	6, 10 and 14 weeks	6, 12 and 24 months	1. Modest sample size (n=58 at 6 months; 144 at 12 months) 2. No HUU group for comparison
Nlend et al. ¹⁰⁴	To assess pediatric anti-hepatitis B vaccine response	A prospective observational study	6, 10 and 14 weeks	1 to 4 months after the third dose	1. Modest sample size (n=82) 2. Infants with HIV misclassified with HEU

Table 2:2. (cont)

Hu et al. ¹⁰⁹	To compare the immunogenicity of recombinant hepatitis B vaccine in infants exposed or not exposed to maternal HBsAb	A prospective cohort study	0, 1 and 6 months	Months 1, 3 and 7	1. Modest sample size (n=71)
Wang et al. ¹¹⁰	To compare the HBsAb response in children of mothers with and without HBsAb	Maternal HBsAb	0, 1 and 6 months	18 months, and five and a half years after three doses of hepatitis B vaccine	One measurement in each cohort thus not amenable to a longitudinal study
Chen et al. ¹¹¹	To determine if maternal anti-HBV affects infant immune response to hepatitis B vaccine.	A multicenter prospective cohort study	0, 1 and 6 months	Age 7-15 months and aged 16-24 months	One outcome measurement only (excluding measurements after booster doses.

Abbreviations: HBV, hepatitis B virus; HBsAb, hepatitis B surface antibody; HEU, HIV-exposed uninfected infant; HIV, human immunodeficiency virus; HUU, HIV-unexposed uninfected infant; RSA, Republic of South Africa.

Though several studies have evaluated the effect of maternal antibodies, they had mixed results with Wang et al. finding no effect of maternal antibodies,¹¹⁰ Hu et al. finding a response at month two but not at month seven¹⁰⁹ and Chen et al. not only finding an effect but an inverse relationship with infants born to mothers with HBsAb >500mIU/mL having the poorest responses while infants born to mothers with HBsAb <10mIU/mL having the best response post-vaccination.¹¹¹

In light of inconclusive evidence on the effects of antenatal and perinatal HIV exposure and of maternal anti-HBV antibodies on the infant immune responses to hepatitis B vaccine,^{25,26,96} additional studies are required. This proposed project will evaluate if antenatal or perinatal HIV exposure affects the immune response of Nigerian HEU to a

three-dose schedule of hepatitis B vaccine (that includes a birth dose) and elicit the effect of maternal antibodies to HBV on an infant's immune response to hepatitis B vaccine while controlling for identified potential confounders.

2.6 Innovation

This study proposes to take advantage of a large birth cohort in which biological samples were collected at numerous time points (See Fig 3:1) and with rigorous collection of data on potential confounders. This will enable an evaluation of several factors affecting infant immune response while controlling for potential confounders that were not available in previous studies.

Second, this proposed study will evaluate a hepatitis B vaccine schedule with a birth-dose of hepatitis B vaccine which is somewhat uncommon in SSA.³¹ SSA bears a disproportionate burden of HBV infection yet most countries in SSA utilize pentavalent vaccines which, although they include hepatitis B vaccine, are not recommended for use before six weeks of age. The birth dose is given at the time when maternal antibodies that have been transferred to their infants are at their peak.

CHAPTER 3. RESEARCH DESIGN AND METHODS

3.1 Study Design and Data Source

This study is a retrospective cohort study utilizing data collected from the “Innate, Adaptive and Mucosal Immune Responses in HIV-1 Exposed Uninfected Infants: A Human Model to Understand Correlates of Immune Protection” study (The INFANT Study) which is described below. Laboratory assays were conducted on stored samples from the parent study and the results included in this study. Approval for the use of de-identified data from the INFANT study was provided by the University of Maryland, Baltimore Institutional Review Board and the Plateau State Specialist Hospital in Nigeria.

3.2 Parent Study

The INFANT study was a prospective cohort study of mother-infant pairs at a tertiary hospital in Nigeria between 2013 and 2017.⁹³ Pregnant women living with and without HIV were offered enrollment as they attended antenatal clinic. All pregnant women were tested for HIV at the time of registration for antenatal care as part of standard of care.¹¹² For study participants who tested HIV-negative at the time of registration for antenatal care, they underwent repeated HIV testing between week 32 of gestation and delivery. All pregnant women testing HIV-positive received antiretroviral therapy (ART) according to Nigerian guidelines.¹¹² All HIV-exposed infants received post-exposure prophylaxis as per guidelines, and were prescribed cotrimoxazole from six weeks of age.¹¹² Mothers were encouraged to exclusively breastfeed infants during their first six months of life. Eight

study follow-up visits occurred at 1, 4, 7, 10, 15, 24, 36 and 52 weeks after enrollment at which serum was collected, processed, and stored. (See Fig 3:1).

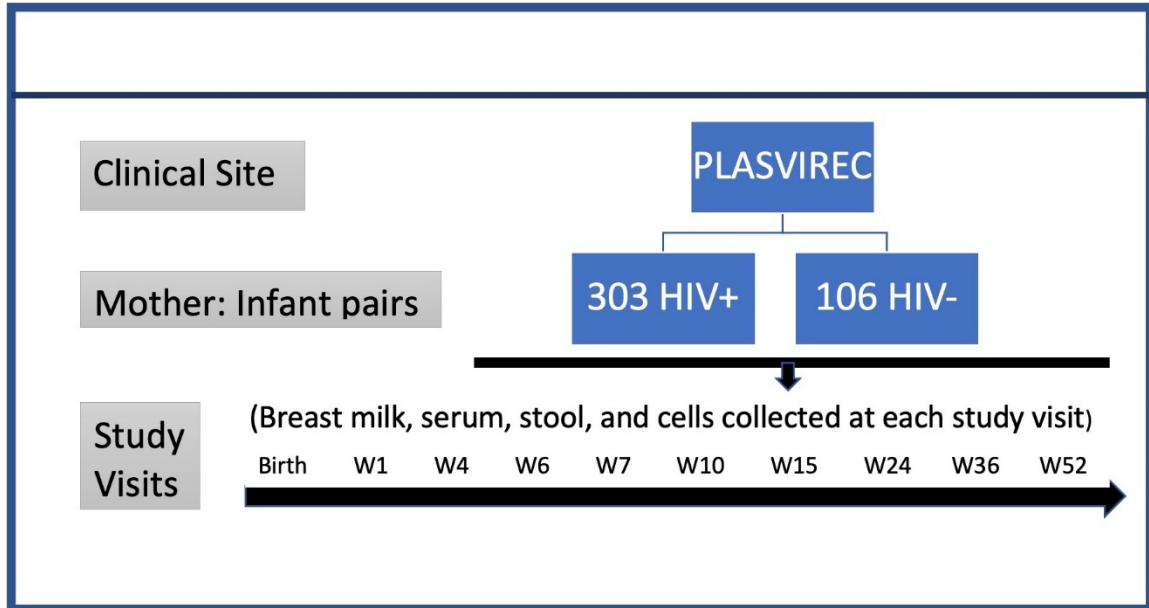


Figure 3:1. INFANT study prospective cohort.

Eligibility Criteria

Inclusion Criteria - Participating mothers were at least 18 years of age, voluntarily chose to breastfeed their infants and were able to meet the study assessment schedule.

Exclusion Criteria - Prospective participants were excluded if they had complications during pregnancy or delivery (eclampsia, chorioamnionitis), if the subsequently delivered infant had neonatal asphyxia, seizures, or sepsis; if maternal gestational age was less than 36 weeks at enrollment, or if infant birth weight was less than 2.4 kg. All infants were tested for HIV via DNA polymerase chain reaction at birth (per study protocol) and

between 4-6 weeks of life (per standard of care). Infants testing positive for HIV were excluded from the study.⁹³

Study Procedures

Questionnaires detailing maternal socio-demographic, clinical and obstetric factors, and infant birth, feeding, clinical and vaccination data were completed by research personnel via maternal participant interviews and medical record abstraction. Physical examinations of infants were performed by pediatricians and pediatric nurses on the research team, and blood, stool, saliva and breast milk samples were collected by laboratory personnel on the research team at each study visit. The blood samples were processed and plasma aliquoted and stored in -80°C freezers within six hours of collection. Please see Table 3:1 for a description of the relevant variables collected during the INFANT study.

3.3 Study Participants

All mother-infant pairs enrolled into the INFANT study were eligible to be included in this study. This consisted of 303 mothers with HIV and their infants and 106 mothers without HIV and their infants recruited in the INFANT study and followed up until 12 months of age.

Exclusion criteria were:

- missing data on infant's immunization status

- a missing or insufficient sample at Week 24 on which to conduct the required laboratory assays and
- all second twins.

367 mother-infant pairs from the INFANT study were included. Please see Table 3.2 for a comparison of the mother-infant pairs included from the INFANT study and those excluded.

Table 3:1. Distribution and frequencies of relevant variables from the INFANT cohort.

Variable	N (%)
Maternal Age	
Mean	30.3 (5.1)
Median (IQR)	30 (27-34)
Marital Status	
Married	395 (96.5)
Living Together	2 (0.5)
Widowed	4 (1)
Divorced	0 (0)
Separated	2 (0.5)
Single/Never married	6 (1.5)
Maternal Education	
None	10 (2)
Elementary	126 (31)
Secondary	163 (40)
Tertiary	110 (27)
Maternal Occupation	
Housewife	106 (26)
Salaried Government	33 (8)
Salaried Private	36 (9)
Domestic Housekeeper	10 (2)
Farmer	6 (1)
Self-employed	189 (46)
Unemployed	10 (2)
Student	11 (3)
Other	8 (2)

Table 3:1 (cont)

<i>Maternal Obstetric</i>	
Parity	
0	56 (14)
1	80 (20)
2	94 (23)
3	83 (20)
4	41 (10)
5	25 (6)
6	14 (3)
7	8 (2)
8	4 (1)
9	3 (1)
10	1 (0)
Primiparous	
Yes	56 (14)
No	353 (86)
Mode of Delivery	
Vaginal	371 (91)
Cesarean	38 (9)
Maternal HIV Status	
Positive	303 (74)
Negative	106 (26)
Maternal CD4	
Mean (SD)	599 (270)
Median (IQR)	570 (408-742)
Missing=50	
Maternal Viral Load	
Undetectable	174 (57)
20-100	57 (19)
101-2000	24 (8)
2001-10,000	17 (6)
>10,000	22 (7)
Missing= 9	9 (3)

Table 3:1. (cont)

<i>Infant Factors</i>	
Infant Sex	
Male	204 (50)
Female	205 (50)
Duration of breastfeeding	
No breastfeeding	6 (1)
4-7 days	4 (1)
4 weeks	8 (2)
7 weeks	4 (1)
10 weeks	7 (2)
15 weeks	4 (1)
24 weeks	23 (6)
36 weeks	87 (21)
52 weeks	245 (60)
18 months	15 (4)
Missing = 6	6 (1)
Age at HBVac1 vaccine	
Mean (SD)	4.5 (9.4)
Median (IQR)	2 (1-4)
Age at HBVac2 vaccine	
Mean (SD)	49.3 (22.3)
Median (IQR)	44 (43-47)
Age at HBVac3 vaccine	
Mean (SD)	80 (18.1)
Median (IQR)	73 (71-84)
Number of hepatitis B vaccinations received	
0	5 (1)
1	14 (3)
2	6 (1)
3	384 (94)
Birthweight	
Mean (SD)	3039 (411)
Median (IQR)	3000 (2700-3300)

Table 3:1. (cont)

Weight at Week 10	
Mean (SD)	5327
Median (IQR)	5300 (4800-5900)
Head circumference at birth	
Mean (SD)	34.8 (2.6)
Median (IQR)	35 (34-36)
Length at birth	
Mean (SD)	47.5 (3.2)
Median (IQR)	48 (46-50)

Abbreviations: CD4, cluster of differentiation; HBVac1, First dose of hepatitis B vaccine; HBVac2, second dose of hepatitis B vaccine; HBVac3, third dose of hepatitis B vaccine; IQR, interquartile range; n, number; SD, standard deviation.

Table 3:2. Comparison of baseline variables among Included versus not Included from the INFANT study.

	All N = 405	Included n = 367	Excluded (n=38)	P-value
Characteristics	n (column %)	n (column %)	n (column %)	
<i>Maternal</i>				
Maternal Age (years)				0.62
≤ 30	209 (52)	188 (51)	21 (55)	
>30	196 (48)	179 (49)	17 (45)	
Marital Status				
Married, n (%)	391 (97)	356 (97)	35 (92)	0.10†
Education				
Elementary or less	134 (33)	126 (34)	8 (21)	0.10
At least secondary	271 (67)	241 (66)	30 (79)	
Employment Status				0.83
Employed, n (%)	273 (67)	248 (68)	25 (66)	
Mode of Delivery				0.41
Vaginal, n (%)	367 (91)	334 (91)	33 (87)	
Previous Pregnancies, n (%)				0.14
0	57 (14)	48 (13)	9 (24)	
1-3	252 (62)	229 (62)	23 (60)	
≥ 4	96 (24)	90 (25)	6 (16)	
Maternal HIV				0.11
Positive	300 (74)	276 (75)	24 (63)	
Negative	105 (26)	91 (25)	14 (37)	
CD4 (cells/μL)				0.08*
Mean (SD)	599 (270)	605 (273)	452 (103)	
Median (IQR)	570 (408-742)	580 (411-749)	465 (380 – 495)	
Missing = 50				
HIV Viral Load (copies/mL)				0.003†
Undetectable	174 (57)	166 (60)	8 (31)	
20-100	57 (19)	50 (18)	7 (27)	
101-2000	24 (8)	22 (8)	2 (8)	
2001-10,000	17 (6)	15 (5)	2 (8)	
>10,000	22 (7)	19 (7)	3 (12)	
Missing = 9	9 (3)	5 (2)	4 (15)	

Table 3.2. (cont)

<i>Infant</i>				
Sex				0.48
Female	203 (50)	186 (51)	17 (45)	
Birth Weight (grams)				0.15
≤ 3000	216 (53)	200 (54)	16 (42)	
>3000	189 (47)	167 (46)	22 (58)	
Duration of breastfeeding				<0.0001†
< 6 months	32 (8)	10 (3)	22 (58)	
≥ 6 months	371 (92)	356 (97)	15 (39)	
Missing = 2	2 (0)	1 (0)	1 (3)	
Birth dose received				0.10
Yes	134 (33)	117 (32)	17 (45)	
No	271 (67)	250 (68)	21 (55)	
Number of hepatitis B vaccinations received				<0.0001†
1	22 (5)	2 (1)	20 (53)	
2	6 (1)	0 (0)	6 (16)	
3	377 (93)	365 (99)	12 (32)	

†Fisher's Exact test, *Wilcoxon rank sum test

Abbreviations: CD4, cluster of differentiation; HIV, human immunodeficiency virus; IQR, interquartile range; n, number; SD, standard deviation.

3.4 Study Variables

Outcome Measures – Aim 1

The primary outcome measures were the concentration of infant HBsAb at birth, 4, 24 and 52 weeks of age. HBsAb was quantified using enzyme-linked immunosorbent assay MBS3800892 (MyBiosource Inc, San Diego, USA). This assay uses an antigen “sandwich” method in which polystyrene microwell strips are pre-coated with recombinant HBsAg. After performing the assay according to the manufacturer’s specifications,¹¹³ a spectrophotometer was used to read the absorbance at 450nm. This optical density was then converted to ng/mL using a standard curve of optical density versus HBsAb concentration produced using calibration standards provided with each ELISA kit (Fig 2:3.).¹¹³ Results in ng/mL were then converted to mIU/mL by multiplying the result in ng/mL by six; conversion criteria were provided by MyBiosource Incorporated.

The secondary outcomes were the proportion of infants who developed protective concentrations of HBsAb, defined as HBsAb concentrations ≥ 10 mIU/mL at Weeks 24 and Weeks 52 respectively.

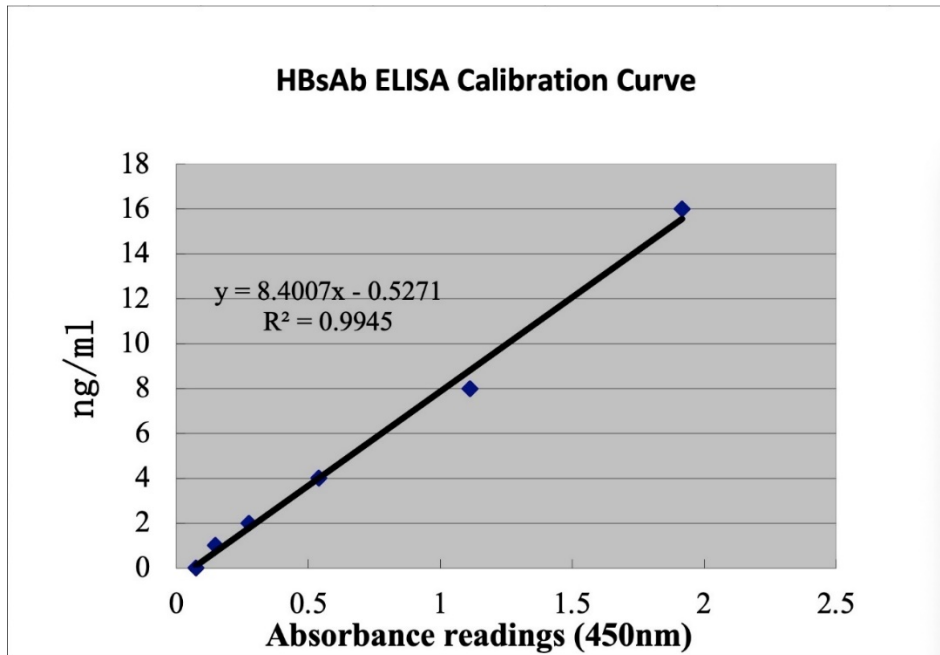


Figure 3:2. Standard curve for calculating concentration of HBsAb in a sample.

Exposure Variables – Aim 1

The primary exposure for Aim 1 was each infant’s antenatal or perinatal exposure to maternal HIV infection. It was categorized as exposed or unexposed. HIV exposure (vs infection) status was determined by identifying pregnant women with HIV and then testing their infants to ensure that the latter remained HIV-negative. Rapid HIV tests were performed for all pregnant women based on the national sequential testing algorithm ; a positive Determine (Abbott Laboratories, Japan) screening test combined with a positive second test-Unigold (Trinity Biotech, USA) was considered a positive result.¹¹² For discordant HIV results, a tiebreaker test was done with StatPak (ChemBio Diagnostic Systems, USA).¹¹² For the study, infants were tested by DNA polymerase chain reaction

performed at birth, 3-4 weeks, six weeks and at one year of age, or six weeks post cessation of breast feeding if breast feeding continued for longer than one year.¹¹²

The primary exposure for Aim 2 was each infant's exposure to maternal HBsAb. It was categorized as detectable or undetectable. Exposure to maternal HBsAb was determined from a maternal blood sample taken at delivery. Detectable concentrations of HBsAb were determined using the ARIA Hepatitis B Combination Test. The ARIA test is a lateral flow chromatographic immunoassay consisting of five test panel strips assembled in one cassette. (See Fig. 3:4) It can detect the following biomarkers: HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. Each strip panel is composed of a sample pad, colloidal gold conjugate pad, a nitrocellulose membrane strip coated with a control line (C-line) and a test line (T-line), as well as an absorbent pad. The HBsAb strip is an antigen-based sandwich immunoassay with HBsAg conjugated with colloidal gold and the nitrocellulose membrane precoated with un-conjugated HBsAg. If HBsAb is present, the specimen will bind to the HBsAg-gold conjugates leading to the formation of a burgundy line. Detection limits for the five biomarkers using the Aria test are listed in Table 3:3

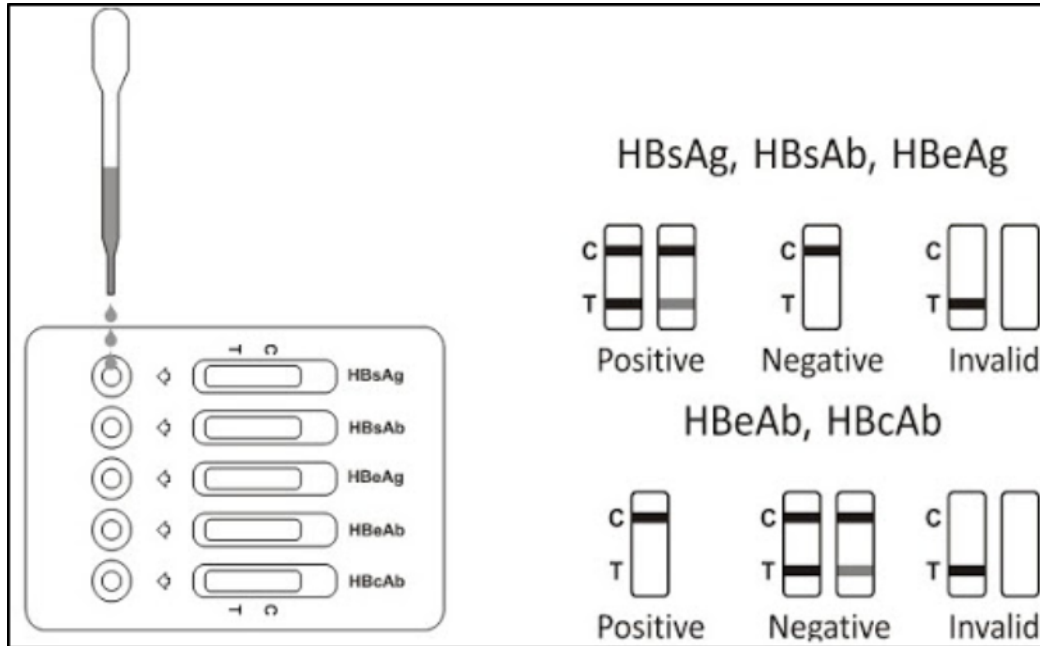


Figure 3:3. HBV combination test results.

Source: Adapted from CTK Biotech Inc., Aria HBV-5 Rapid Test Instructions for Use

Table 3:3. Analytical Sensitivity for Aria HBV Combination Test.

Test	Detection Limit
HBsAg	1ng/mL
HBsAb	30mIU/mL
HBeAg	2NCU/mL
HBeAb	2NCU/mL
HBcAb	2NCU/mL

Source: Adapted from CTK Biotech Inc., Aria HBV-5 Rapid Test Instructions for Use

Potential Confounding Variables as measured in the study

Aim 1

Maternal

Maternal demographic variables identified in the literature as being indirectly associated with infant immune response and available from the INFANT dataset were evaluated.

- Maternal age in years recorded at enrollment. Maternal age ranged from 18 to 45 years. Maternal age in years did not have a linear relationship with HBsAb. It was therefore categorized into young (≤ 30 years) and older (> 30 years).
- Maternal occupation was recorded at enrollment and categorized into employed and unemployed.
- Maternal educational status collected at enrollment and categorized as low (Primary education and below) and high (secondary education and above)
- Maternal parity was included and described as number of previous pregnancies and categorized as primiparous (0 previous pregnancies), 1-3 previous pregnancies and ≥ 4 previous pregnancies
- Maternal HBsAb antibodies in mIU/mL. Identified as part of the current study using Aria hepatitis B combination test and categorized as detectable or undetectable with a lower detection limit of 30mIU/mL.

Infant

- Infant sex – collected at delivery and categorized as male or female
- Infant birth weight was collected at delivery and recorded in grams. Due to exclusion criteria of the parent study, infant weights ranged from 2400 to 4700 grams. Infant weight in grams was not found to have a linear relationship with infant HBsAb as such it was categorized. Due to the exclusion criteria, it was not practical to use the clinically relevant cut-off point for underweight children (2500g) as this would have resulted in very few infants in the category <2500g. As such, 3000g was used as the cut-off point.
- Infant nutritional Status -measured using duration of breast feeding and weight for age z-scores at week 10. Information on breastfeeding was collected at every study visit during which the mother would be asked if she was still breastfeeding her infant. Infant weights were also taken at every visit.

Aim 2

Maternal

Maternal demographic variables identified in the literature as being indirectly associated with infant immune response and available from the INFANT dataset were evaluated.

- Maternal age in years recorded at enrollment. Maternal age ranged from 18 to 45 years. Maternal age in years did not have a linear relationship with HBsAb. It was therefore categorized into young (≤ 30 years) and older (> 30 years).

- Maternal occupation was recorded at enrollment and categorized into employed and unemployed.
- Maternal educational status collected at enrollment and categorized as low (Primary education and below) and high (secondary education and above)
- Maternal parity was included and described as number of previous pregnancies and categorized as primiparous (0 previous pregnancies), 1-3 previous pregnancies and ≥ 4 previous pregnancies
- Maternal HIV status – recorded at enrollment and modified if necessary, between week 32 of gestation and delivery for mothers who were negative initially and then tested HIV positive on re-testing

Infant

- Infant sex – collected at delivery and categorized as male or female
- Infant birth weight was collected at delivery and recorded in grams. Due to exclusion criteria of the parent study, infant weights ranged from 2400 to 4700 grams. Infant weight in grams was not found to have a linear relationship with infant HBsAb as such it was categorized. Due to the exclusion criteria, it was not practical to use the clinically relevant cut-off point for underweight children (2500g) as this would have resulted in very few infants in the category $<2500g$. As such, 3000g was used as the cut-off point.
- Infant nutritional Status -measured using duration of breast feeding and weight for age z-scores at week 10. Information on breastfeeding was collected at every study

visit during which the mother would be asked if she was still breastfeeding her infant. Infant weights were also taken at every visit.

3.5 Data Management and Statistical Analysis

A de-identified data set was obtained from the data repository of the INFANT study in accordance with the ethical requirements for such transfers.

Analysis -Sub-aim 1.1

Univariate analyses were conducted on all variables. We assessed frequencies and proportions for categorical variables and distributions and measures of central tendency for continuous variables. All continuous variables were examined for normality and linearity. Maternal age and infant birth weight were found to lack a linear relationship with infant HBsAb at Week 24 and as such were categorized. Influential outliers were identified using studentized residuals and Cook's distance.

Potential confounding was explored by examining the association between exposure variables and covariates of interest and between the primary endpoint infant HBsAb and covariates of interest. For bivariate analyses of the association between infant HIV exposure status and covariates of interest, Pearson's chi-square was used and for variables with expected cell counts less than five, Fisher's Exact test was used. In the same vein, general linear models were used to explore the relationship between infant HBsAb and covariates of interest. The proc mixed procedure was used with restricted maximum likelihood approach. A repeated statement was used to indicate repeated measurements on

individuals and maternal ID was used to identify the clusters. An unstructured correlation structure was utilized.

Potential confounders explored included baseline variables and other covariates identified a priori from the literature as being associated with the exposure and the outcomes of interest. These include maternal factors namely age, occupation, educational status, number of previous pregnancies, mode of delivery and presence of detectable maternal antibodies to HBV and infant factors namely sex, birthweight, duration of breastfeeding and receipt of a birth dose of hepatitis B vaccine. We used the following criteria to identify potential confounders: Covariates that were associated with both the exposure and the outcome with a p-value of 0.10 were deemed significant. On including any identified potential confounder in model building, any covariates that yielded at least a 10% change in the crude estimate of infant HBsAb were considered confounders and retained in the final model.

General Linear models accounting for clustering within individuals were used to assess the relationship between infant HIV-exposure status and their immune response to hepatitis B vaccine and adjusted for identified confounding variables. We utilized a saturated model with no intercept which yielded mean estimates for all timepoints of interest by exposure group.

The model was as follows:

$$E(Y_{ij}) = \beta_1(G=1, T=1) + \beta_2(G=1, T=2) + \beta_3(G=1, T=3) + \beta_4(G=1, T=4) + \beta_5(G=2, T=1) + \beta_6(G=2, T=2) + \beta_7(G=2, T=3) + \beta_8(G=2, T=4)$$

G1 = HIV -exposed

G2 = HIV- unexposed

T1 = Week 0 (birth)

T2 = Week 4

T3 = Week 24 and

T4 = Week 52

Contrast statements were used to perform statistical tests to evaluate differences in mean responses comparing exposure groups at the four time points.

Model fit criteria

Akaike information criterion, Bayesian information criterion and -2 Restricted Log Likelihood are used to evaluate model fit when using general linear models. The smaller the value, the better the fit. We used these criteria to see which model fits the data better.

Sub-aim 1.2

Fisher's exact tests were used to compare the proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$ by HIV-exposure group.

Sub-Analyses

Mothers with HIV were categorized based on HIV viral load and evaluated to see if mean infant HBsAb concentrations were different comparing infants born to mothers with detectable HIV viral load (VL) to infants born to mothers with undetectable HIV VL. A crude analysis using a general linear model was performed first and then a saturated model based on the presence or absence of detectable HIV VL was created.

Contrast tests were performed to test if the presence of detectable maternal HIV VL had an effect on infant's immune response.

Analysis -Sub-aim 2.1

Univariate analyses were conducted on all variables. We assessed frequencies and proportions for categorical variables and distributions and measures of central tendency for continuous variables. All continuous variables were examined for normality and linearity. Maternal age and infant birth weight were found to lack a linear relationship with infant HBsAb at Week 24 and as such were categorized. Influential outliers were identified using studentized residuals and Cook's distance.

Potential confounding was explored by examining the association between exposure variables and covariates of interest and between the primary endpoint infant HBsAb and covariates of interest. For bivariate analyses of the association between infant exposure status to detectable concentrations of maternal HBsAb and covariates of interest, Pearson's chi-square was used and for variables with expected cell counts less than five, Fisher's Exact test was used. In the same vein, general linear models were used to explore the relationship between infant HBsAb and covariates of interest. The proc mixed procedure was used with restricted maximum likelihood approach. A repeated statement was used to indicate repeated measurements on individuals and maternal ID was used to identify the clusters. An unstructured correlation structure was utilized.

Potential confounders explored included baseline variables and other covariates identified a priori from the literature as being associated with the exposure and the outcomes of interest. These include maternal factors namely age, occupation, educational

status, number of previous pregnancies, mode of delivery and maternal HIV status, and infant factors namely sex, birthweight, duration of breastfeeding and receipt of a birth dose of hepatitis B vaccine. We used the following criteria to identify potential confounders: Covariates that were associated with both the exposure and the outcome with a p-value of 0.10 were deemed significant. On including any identified potential confounder in the course of model building, any covariates that yielded at least a 10% change in the crude estimate were considered confounders and retained in the final model.

General Linear models accounting for clustering within individuals were used to assess the relationship between infant exposure to detectable concentrations of maternal HBsAb and their immune response to Hepatitis B vaccine adjusted for identified confounding variables. We utilized a saturated model with no intercept which yielded mean estimates for all timepoints of interest by exposure group.

The model was as follows:

$$E(Y_{ij}) = \beta_1(G=1, T=1) + \beta_2(G=1, T=2) + \beta_3(G=1, T=3) + \beta_4(G=1, T=4) + \beta_5(G=2, T=1) + \beta_6(G=2, T=2) + \beta_7(G=2, T=3) + \beta_8(G=2, T=4)$$

G1 = Exposed to detectable concentrations of maternal HBsAb

G2 = Unexposed to detectable concentrations of maternal HBsAb

T1 = Week 0 (birth)

T2 = Week 4

T3 = Week 24 and

T4 = Week 52

Contrast statements were used to perform statistical tests to evaluate differences in mean responses comparing exposure groups at the four time points.

Model fit criteria

Akaike information criterion, Bayesian information criterion and -2 Restricted Log Likelihood are used to evaluate model fit when using general linear models. The smaller the value, the better the fit. We used these criteria to see which model fits the data better.

Sub-aim 2.2

Fisher's exact tests were used to compare the proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$ by exposure status to detectable concentrations of maternal HBsAb.

Sample Size and Power

409 mothers and 419 infants were included in the parent study. After excluding 1 from each of ten sets of twins and infants without outcome results at Week 24, 367 mother-infant pairs were included in this study.

$$Z_{\beta} = Z_{\alpha} - \left\{ \left[\frac{(\mu_e - \mu_u)}{\sigma} \right] \sqrt{[(1+n-1) \rho] \left(\frac{1}{N_e n} + \frac{1}{N_u n} \right)} \right\}$$

$Z_{\alpha} = 1.96$ (2-tailed .05 hypothesis test)

μ_e = Mean HBsAb in HUU

μ_u = Mean HBsAb in HEU

ES = Effect size = $-\left[\frac{(\mu_e - \mu_u)}{\sigma} \right]$

ρ = correlation of repeated outcomes

N_e = Number of HUU

N_u = Number of HEU

n = number of time points = 4

Calculations were repeated with an assumption of two different correlations ($\rho=0.1$ and $\rho=0.2$) and assumption of two or three visits ($n=2$ or $n=3$)

Results are presented in Table 3:4.

Table 3:4. Power analyses for Sub-Aim 1.1 – Repeated measures with a continuous outcome.

Number of Exposed	Number of Unexposed	Number of visits	ICC	Power	
				ES:0.2	ES:0.5
276	91	4	0.1	0.82	1
276	91	4	0.2	0.74	1
276	91	3	0.1	0.74	1
276	91	3	0.2	0.67	1
276	91	2	0.1	0.60	1
276	91	2	0.2	0.57	1

Abbreviations: ES, Effect size; ICC, intraclass correlation

Sample Size and Power for Sub-aim 2.1

409 mothers and 419 infants were included in the parent study. After excluding 1 from each of ten sets of twins and infants without data on the exposure or without outcome results at Week 24, 367 mother-infant pairs were included in this study.

$$Z_{\beta} = Z_{\alpha} - \{[(\mu_e - \mu_u)/\sigma] \sqrt{[(1+n-1) \rho] (1/N_e n + 1/N_u n)}\}$$

$$Z_{\alpha} = 1.96 \text{ (2-tailed .05 hypothesis test)}$$

μ_e = Mean HBsAb in infants with mothers with detectable concentrations of HB sAb

μ_u = Mean HBsAb in infants with mothers with undetectable concentrations of HB sAb

$$\text{ES} = \text{Effect size} = - [(\mu_e - \mu_u)/\sigma]$$

ρ = correlation of repeated outcomes

N_e = Number of infants with mothers with detectable concentrations of HB sAb

N_u = Number of infants with mothers with undetectable concentrations of HB sAb

n = number of time points = 4

Calculations were repeated with an assumption of two different correlations ($\rho=0.1$ and $\rho=0.2$) and assumption of two or three visits ($n=2$ or $n=3$)

Results are presented in Table 3:5.

Table 3:5. Power analyses for Sub-Aim 2.1 – Repeated measures with a continuous outcome.

Number of Exposed	Number of Unexposed	Number of visits	ICC	Power	
				ES:0.2	ES:0.5
52	315	4	0.1	0.65	1
52	315	4	0.2	0.56	1
52	315	3	0.1	0.56	1
52	315	3	0.2	0.50	1
52	315	2	0.1	0.44	0.99
52	315	2	0.2	0.40	0.99

Abbreviations: ES, Effect size; ICC, intraclass correlation

CHAPTER 4. EFFECTS OF HIV EXPOSURE ON THE IMMUNE RESPONSE OF NIGERIAN INFANTS TO HEPATITIS B VACCINE

ABSTRACT

Background: Chronic hepatitis B virus (HBV) infection is associated with considerable morbidity and mortality. Risk of chronicity is highest if HBV infection is acquired in the perinatal period. Mothers in sub-Saharan Africa (SSA) with two biomarkers of HBV infection, namely Hepatitis B surface antigen (HBsAg) and Hepatitis B e antigen (HBeAg) have a nearly 40% risk of transmitting HBV to their infants when no intervention is undertaken.

In SSA, concurrent with HBV is the HIV epidemic, which also disproportionately affects women of reproductive age. Over 1 million children exposed to maternal HIV antenatally though not infected themselves are born in SSA every year. HIV-exposed but uninfected infants (HEU) manifest disproportionate morbidity and mortality when compared to HIV-unexposed uninfected infants (HUU) thought to be partially attributable to exposure to maternal HIV infection and antiretroviral (ARV) medications. Maternal HIV is thought to reduce transplacental transfer of cytokines needed for infant hematopoiesis and also lead to immune paresis of infants through the transplacental transfer of HIV viral products. Maternal ARVs also cross the placenta and alter the function of the immune system of HEU. Though there are effective vaccines to prevent mother-to-child transmission (MTCT) of HBV, vaccine effectiveness is reduced among persons with compromised immune systems.

We evaluated the effects of antenatal and perinatal HIV exposure on the immune response of 367 Nigerian infants to a three-dose schedule of hepatitis B vaccine, while controlling for identified confounders.

Methods: We conducted a retrospective cohort study utilizing data from the INFANT study. INFANT was a prospective cohort study of mother-infant pairs at a tertiary hospital in North-Central Nigeria between 2013 and 2017. General linear models were used to estimate the relationship between infant HIV-exposure status and their immune response to Hepatitis B vaccine. Pairwise comparisons of mean HBsAb concentrations by week and HIV exposure group among infants were conducted. Fisher's Exact tests were used to compare the proportion of infants with HBsAb concentrations above 10mIU/mL in HEU as compared to HUU at 24 and 52 weeks of age separately. Infants born to mothers with HIV were evaluated further to see if exposure to detectable concentrations of HIV viral load had an effect on infant immune response to hepatitis B vaccine.

Results: HIV-exposure was associated with infant immune response to hepatitis B vaccine at birth, Week 4 and Week 52, with HEU having higher adjusted mean concentrations of 31 vs 23mIU/mL at birth ($p=0.0002$), 42 vs 38 at Week 4 ($p=0.03$), and 40 vs. 31 mIU/mL at Week 52 ($p<0.0001$) as compared to HUU. The proportion of infants with HBsAb concentrations ≥ 10 mIU/mL was similar for HEU and HUU at Week 24, with 99 vs 100% having HBsAb concentrations greater than 10mIU/mL ($p=1.00$); this differed slightly at Week 52 with 96% of HEU vs 100% of HUU having HBsAb concentrations greater than 10mIU/mL ($p=0.04$)

Conclusion: Though exposure to maternal HIV can affect an infant's immune system and have other health related effects, in this study of Nigerian infants, nearly all HEU and

HUU had responses to a 3-dose hepatitis B vaccine that would predict protection, even when adjusted for potential confounders.

Introduction

Chronic hepatitis B virus (HBV) infection is associated with considerable morbidity and mortality,² and there are approximately 296 million persons living with chronic HBV infection globally¹. The risk of HBV infection becoming chronic is inversely related to age at infection, with perinatally-infected infants having a 90% risk of developing chronic hepatitis B, while adults and children aged six years and above have a 5% risk.^{39,40} Mothers in sub-Saharan Africa (SSA) with two biomarkers of HBV infection, namely Hepatitis B surface antigen (HBsAg) and Hepatitis B e antigen (HBeAg) have a nearly 40% risk of transmitting HBV to their infants when no intervention is undertaken.⁴⁴

In SSA, concurrent with HBV is the HIV epidemic, which also disproportionately affects women of reproductive age.¹² Though public health programs have reduced mother-to-child transmission (MTCT) of HIV to less than 10% globally, over 1 million children exposed to maternal HIV antenatal though not infected themselves are born in SSA every year; 99,000 in Nigeria.¹³ HIV-exposed but uninfected infants (HEU) manifest disproportionate morbidity and mortality when compared to HIV-unexposed uninfected infants (HUU).⁴⁵⁻⁴⁹ The higher likelihood of these poor outcomes appears to be due to indirect effects of maternal HIV, namely maternal death or illness^{45,54}, higher rates of colonization or higher numbers of virulent organisms,⁴⁸ lower household income,⁴⁵ reduced breast-feeding,⁵⁵ and poor maternal psychological and physical health, leading to poor child care⁵⁶. In addition, a proportion of this excess morbidity and mortality is thought to be due to exposure to maternal HIV infection and antiretroviral (ARV) medications. Among pregnant women, HIV affects the concentrations of antibodies to vaccine-preventable diseases and also the amount transferred to their infants.²⁴⁻²⁶ Maternal ARVs

cross the placenta and have been found to alter the function of the immune system of HEU¹⁴⁻²³ through mitochondrial toxicity and the inhibition of human hematopoietic progenitor cells.^{16,17}

Hepatitis B vaccines are safe and effective (69 – 100% effectiveness) in preventing mother-to-child transmission of HBV infection,⁹⁻¹¹ however, vaccine effectiveness is reduced among persons with compromised immune systems.¹¹⁴

The response of infants living with HIV to hepatitis B vaccine has been studied extensively,^{115,116} but an area that needs further elucidation is HEU response to a birth dose-inclusive three – dose schedule of hepatitis B vaccine. Previous studies had modest sample sizes of 53-180^{25,104,105,107,108} and did not control for some of the factors that could independently influence the immune response of HEU to hepatitis B vaccines,^{25,104-108} resulting in mixed findings.

We evaluated the effects of antenatal and perinatal HIV exposure on the immune response of 367 Nigerian infants to a three-dose schedule of hepatitis B vaccine, while controlling for identified confounders.

Methods

We conducted a retrospective cohort study utilizing data from the INFANT (Innate, Adaptive and Mucosal Immune Responses in HIV-1 Exposed Uninfected Infants: A Human Model to Understand Correlates of Immune Protection) study. INFANT was a prospective cohort study of mother-infant pairs at a tertiary hospital in North-Central Nigeria between 2013 and 2017.⁹³ Approval for the use of de-identified

data from the INFANT study was provided by the University of Maryland, Baltimore Institutional Review Board and the Plateau State Specialist Hospital in Nigeria.

Study Setting

Nigeria, is a country with a population of over 211 million persons¹¹⁷ with an HBV seroprevalence of 6% among pregnant women.^{36,118} Nigeria expanded its National Program of immunization to include Hepatitis B vaccine in 1995, but the vaccine was not widely available for administration until 2004.⁸⁰ At the time of the 2013-2017 INFANT study, the approved hepatitis B vaccine schedule consisted of three doses of monovalent hepatitis B vaccine given to infants at birth, and at 6 and 14 weeks of life.⁸³

Study Participants

All mother-infant pairs enrolled into the INFANT study were eligible to be included in this study. From 303 mothers with HIV and their infants and 106 mothers without HIV and their infants recruited in the INFANT study and followed up until 12 months of age, we excluded infants missing data on their immunization status, all second twins and any infants with a missing or insufficient sample at Week 24. 367 mother-infant pairs from the INFANT study were included.

Parent Study

INFANT study participants have been fully described previously.⁹³ Briefly, pregnant women living with and without HIV were offered enrollment into the INFANT study as they attended antenatal clinic. All pregnant women were tested for HIV at the time of registration for antenatal care as part of standard of care.¹¹² For study participants who tested HIV-negative at the time of registration for antenatal care, they underwent repeated HIV testing between week 32 of gestation and delivery. All pregnant women testing HIV-positive received antiretroviral therapy (ART) according to Nigerian guidelines.¹¹² All HIV-exposed infants received post-exposure prophylaxis as per guidelines, and were prescribed cotrimoxazole from six weeks of age.¹¹² Mothers were encouraged to exclusively breastfeed infants during their first six months of life. Eight study follow-up visits occurred at 1, 4, 7, 10, 15, 24, 36 and 52 weeks after enrollment at which serum was collected, processed, and stored.

Inclusion Criteria - Participating pregnant women were at least 18 years of age, voluntarily chose to breastfeed their infants, and were able to meet the study assessment schedule.

Exclusion Criteria - Prospective participants were excluded if they had complications during pregnancy or delivery (e.g., eclampsia, chorioamnionitis), if the subsequently delivered infant had neonatal asphyxia, seizures, or sepsis; if infant gestational age was less than 36 weeks at birth, or if infant birth weight was less than 2.4 kg. All infants were tested for HIV via DNA polymerase chain reaction at birth, per study protocol, and between 4-6 weeks of life per standard of care. Infants testing positive for HIV were excluded from the study.⁹³

Study Procedures

Questionnaires detailing maternal socio-demographic, clinical and obstetric factors, and infant birth, feeding, clinical and vaccination data were completed by research personnel via maternal participant interviews and medical record abstraction. Physical examinations of infants were performed by pediatricians and pediatric nurses on the research team, and blood, stool, saliva and breast milk samples were collected by laboratory personnel on the research team at each study visit. The blood samples were processed and plasma aliquoted and stored in -80°C freezers within six hours of collection.

Statistical Analysis

The primary outcome measures were the concentration of infant HBsAb at birth, 4, 24 and 52 weeks of age. The secondary outcomes were the proportion of infants who developed protective concentrations of HBsAb, defined as HBsAb concentrations ≥ 10 mIU/mL at Week 24 or Week 52 respectively. HBsAb was quantified using enzyme-linked immunosorbent assay MBS3800892 (MyBiosource Inc, San Diego, USA). This assay uses an antigen “sandwich” method in which polystyrene microwell strips are pre-coated with recombinant HBsAg. After performing the assay according to the manufacturer’s specification,¹¹³ a spectrophotometer is used to read the absorbance at 450nm. This optical density is then converted to ng/mL using a standard curve of optical density versus HBsAb concentration produced using calibration standards provided with each ELISA kit.¹¹³ Ng/mL were then converted to mIU/mL by multiplying the result in ng/mL by six based on the specifications of Mybiosource Incorporated.

The primary exposure was each infant's antenatal and perinatal exposure to maternal HIV infection. HIV exposure (vs infection) status was determined by identifying pregnant women with HIV and then testing their infants to ensure that the latter remained HIV-negative. Rapid HIV tests were performed for all pregnant women based on the national sequential testing algorithm ; a positive Determine (Abbott Laboratories, Japan) screening test combined with a positive second test-Unigold (Trinity Biotech, USA) was considered a positive result.¹¹² For discordant HIV results, a tiebreaker test was done with StatPak (Chembio Diagnostic Systems, USA).¹¹² For the study, infants were tested by DNA polymerase chain reaction performed at birth, 3-4 weeks, six weeks and at one year of age, or six weeks post cessation of breast feeding if breast feeding continued for longer than one year.¹¹²

Baseline variables and other covariates identified a priori as being associated with infant immune response were analyzed for frequency, distribution and missing data. Influential outliers (identified using studentized residuals and Cook's distance) were omitted. The covariates of interest were maternal demographic factors namely age, occupation and educational status; maternal obstetric factors namely number of previous pregnancies, mode of delivery, and presence of detectable surface antibodies to Hepatitis B; and infant factors namely infant sex, birthweight, duration of breastfeeding, and if a birth dose of hepatitis B vaccine was received. Bivariate analyses of these covariates by exposure groups were conducted to identify potential confounders. The criteria for identifying confounders were association with both the exposure and outcome with a p-value ≤ 0.10 .

The relationship between infant HIV-exposure status and their immune response to Hepatitis B vaccine was estimated using general linear models, which account for repeated measurements on the same individual and permit adjusting for potential confounders. We used the proc mixed procedure with the restricted maximum likelihood approach. A repeated statement was used to indicate repeated measurements on individuals and maternal ID was used to identify the clusters. An unstructured correlation structure was utilized.

Four time points were of interest: baseline (at birth) and Weeks 4, 24, and 52 of life. The Week 4 sample is four weeks after the first hepatitis B vaccine dose, while the Week 24 sample is 10 weeks after the third hepatitis B vaccine dose. Baseline covariates found to be potential confounders were investigated further by introducing them into a model assessing the crude association between infant HIV exposure status and infant antibody response to hepatitis B vaccine, namely hepatitis B surface antibody (HBsAb). A 10% change in the crude response was required for the variable to be retained. Maternal educational status which fulfilled these criteria was investigated as a potential confounder. Pairwise comparisons of mean HBsAb concentrations by week and HIV exposure group among infants were conducted.

Secondary Outcome Analysis

Fisher's Exact tests were used to compare the proportion of infants with HBsAb concentrations above 10mIU/mL in HEU as compared to HUU at 24 and 52 weeks of age separately.

Sub-Analysis

Mothers with HIV were categorized based on HIV viral load and evaluated to see if mean infant HBsAb concentrations following hepatitis B vaccinations were different comparing infants born to mothers with detectable HIV viral load to infants born to mothers with undetectable HIV viral load. A saturated model based on the presence or absence of detectable HIV VL at the four time points of interest was created. Contrast tests were performed to test if the presence of detectable maternal HIV VL had an effect on infant's immune response.

Results

A total of 409 pregnant women and 419 infants were recruited into the INFANT study. For our analysis, we included 367 mother-infant pairs, after we excluded one twin each from 10 pairs of twins, 22 mother-infant pairs lost to follow-up before Week 24, six infants for lack of a sample at Week 24, one mother-infant pair with an underaged mother (age<18 years), and 13 infants with HBsAb concentrations considered influential outliers. (Please see Table 3:2 for differences between infants included in the study and those excluded.)

On comparing baseline maternal and infant factors across HIV-exposure groups, the groups only differed in terms of maternal educational status, number of previous pregnancies and infant birth weight (Table 4:1). Maternal educational status was also found to be associated with infant HBsAb and as such was evaluated as a potential confounder. Maternal educational status did change the crude relationship between HBsAb and maternal HIV status by approximately 10% and thus was retained in the final model. (Table 4:2).

Table 4:1. Characteristics of women with and without HIV and their uninfected infants.

Characteristics	All N = 367 n (column %)	HIV Exposed Uninfected N = 276 n (column %)	HIV Unexposed Uninfected N = 91 n (column %)	P-value
Maternal				
Maternal Age (years)				0.29
≤ 30	188 (51)	137 (50)	51 (56)	
>30	179 (49)	139 (50)	40 (44)	
Marital Status				0.31†
Married, n (%)	356 (97)	266 (96)	90 (99)	
Education				0.004
Elementary or less	126 (34)	106 (38)	20 (22)	
At least secondary	241 (66)	170 (62)	71 (78)	
Employment Status				0.37
Employed, n (%)	248 (68)	183 (66)	65 (71)	
Mode of Delivery				0.23
Vaginal, n (%)	334 (91)	254 (92)	80 (88)	
Previous Pregnancies, n (%)				0.03
0	48 (13)	30 (11)	18 (20)	
1-3	229 (62)	171 (62)	58 (64)	
≥ 4	90 (25)	75 (27)	15 (16)	
Maternal HBV Biomarkers				0.001
Previous Infection/ vaccination	72 (20)	43 (16)	29 (32)	
Current infection	43 (12)	30 (11)	13 (14)	
No biomarkers	252 (69)	203 (74)	49 (54)	
Maternal HBsAb				<0.0001
Detectable	51 (14)	25 (9)	26(29)	
Undetectable	316 (86)	251 (91)	65 (71)	
CD4 (cells/μL)				
Median (IQR)	580 (411-749)	580 (411-749)	N/A	
Missing = 34				

Table 4:1. (cont)

Viral Load (copies/mL)				
Undetectable	166 (61)	166 (61)		
20-100	50 (18)	50 (18)		
101-2000	22 (8)	22 (8)	N/A	
2001-10,000	15 (6)	15 (6)		
>10,000	19 (7)	19 (7)		
Missing = 5				
<i>Infant</i>				
Sex				
Female	186 (51)	141 (51)	45 (49)	0.79
Birth Weight (grams)				
≤ 3000	200 (55)	163 (59)	37 (41)	0.002
>3000	167 (45)	113 (41)	54 (59)	
Weight-for-age z-score at week 10				
≤-2	8 (2)	7 (3)	1 (1)	0.76†
>-2	358 (98)	268 (97)	90 (99)	
Missing=1	1 (0)	1(0)		
Duration of breastfeeding				
< 6 months	10 (3)	9 (3)	1 (1)	0.59†
≥ 6 months	356 (97)	266 (97)	90 (99)	
Missing = 1	1 (0)	1 (0)		
Birth hepatitis B dose received				
Yes	117 (32)	91 (33)	26 (29)	0.43
No	250 (68)	185 (67)	65 (71)	
Number of hepatitis B vaccines received				
<3	2 (1)	1 (0)	1 (1)	0.43†
3	365 (99)	275 (100)	90 (99)	

†Fisher's Exact Test

Abbreviations: CD4, cluster of differentiation; HIV, human immunodeficiency virus; IQR, interquartile range; n, number; SD, standard deviation.

Median infant HBsAb concentrations were 31, 41, 34 and 40 mIU/mL at birth, 4, 24 and 52 weeks of life. These differed significantly by HIV-exposure group at baseline, Week 4 and Week 52, with HEU having higher adjusted mean concentrations of 31 vs 23mIU/mL at birth ($p<0.0001$), 42 vs 38mIU/mL at week 4 ($p=0.05$), and 40 vs. 31 mIU/mL at week 52 ($p<0.0001$) as compared to HUU (Please see Table 4:3 and Fig 4:1).

Table 4:2. Estimate of mean infant HBsAb concentrations by week and HIV Exposure.

Parameter	Unadjusted		Adjusted*	
	Estimate	S.E	Estimate	S.E
HEU Week 0	33.2	0.92	30.6	1.06
HEU Week 4	45.1	1.20	42.4	1.30
HEU Week 24	34.8	0.77	32.2	0.93
HEU Week 52	42.1	0.93	39.5	1.05
HUU Week 0	26.4	1.59	23.0	1.72
HUU Week 4	41.1	2.06	37.8	2.15
HUU Week 24	37.2	1.35	33.9	1.49
HUU Week 52	34.5	1.62	31.2	1.72
Maternal Educational Status				
Elementary or less	Ref		Ref	
At least secondary	3.7	0.85	4.3	0.85

*Adjusted for maternal educational status

(HEU, HIV-exposed uninfected infant; HUU, HIV unexposed uninfected infant; S.E, standard error)

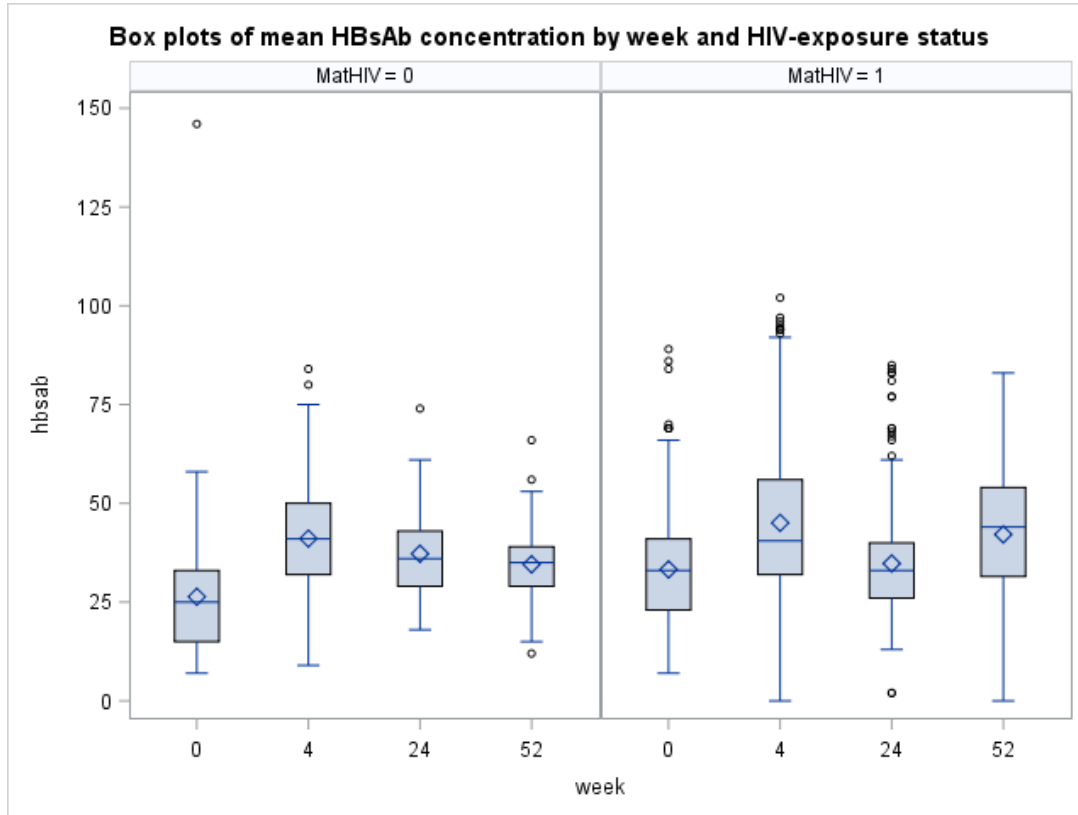


Figure 4:1. Box plots showing mean hepatitis B surface antibodies by week and HIV exposure status

Table 4:3. Pairwise comparisons of mean HBsAb by week and HIV exposure group.

Contrast	P-value (F Test)
Mean HBsAb for Week 0 in HEU as compared to HUU	<0.0001
Mean HBsAb for Week 4 in HEU as compared to HUU	0.05
Mean HBsAb for Week 24 in HEU as compared to HUU	0.26
Mean HBsAb for Week 52 in HEU as compared to HUU	<0.0001

Abbreviations: HEU= HIV-exposed uninfected infant; HUU= HIV unexposed uninfected infant.

Comparison of the proportions of HEU versus HUU with HBsAb concentrations $\geq 10\text{mIU/mL}$ at weeks 24 and 52

The proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$ was similar for HEU and HUU at Week 24, with 99 vs 100% having HBsAb titers greater than 10mIU/mL at Week 24 ($p=1.00$); this differed slightly at Week 52 with 96% of HEU vs 100% of HUU having HBsAb titers greater than 10mIU/mL ($p=0.04$). Please see Table 4:4. For the results for the comparison for exposure groups for the proportion of infants with HBsAb concentrations $\geq 10\text{mIU/mL}$.

Table 4:4. Proportions of infants with protective HBsAb concentrations at Weeks 24 and 52.

Parameter	HEU n (%)	HUU n (%)	P-value†
HBsAb at Week 24			1.00
$\geq 10\text{mIU/mL}$	275 (99)	91 (100)	
$< 10\text{mIU/mL}$	2 (1)	0 (0)	
HBsAb at Week 52			0.04
$\geq 10\text{mIU/mL}$	264 (96)	91 (100)	
$< 10\text{mIU/mL}$	12 (4)	0 (0)	

†Fisher's Exact Test

Abbreviations: HEU, HIV-exposed uninfected infant; HUU, HIV unexposed uninfected infant; n, number

Comparison of mean HBsAb concentrations in infants born to mothers with detectable HIV viral load as compared to infants born to mothers without

Mean infant HBsAb concentrations ranged from 28-42mIU/mL (Please see Table 4:4.). On comparing infants born to mothers with detectable HIV viral load as compared to infants born to mothers without, the former had significantly lower HBsAb concentrations at baseline 27.7mIU/mL as compared to 31.7mIU/mL ($P=0.03$). (Please see Table 4:5.)

Table 4:5. Estimate of mean infant HBsAb concentrations by week and Exposure to detectable HIV viral load.

Parameter	Unadjusted		Adjusted*	
	Estimate	S.E	Estimate	S.E
Detectable Week 0	30.9	1.4	27.7	1.6
Detectable Week 4	45.3	2.1	42.1	2.2
Detectable Week 24	35.6	1.4	32.4	1.5
Detectable Week 52	40.6	1.8	37.4	1.8
Undetectable Week 0	35.0	1.1	31.7	1.3
Undetectable Week 4	44.9	1.7	41.7	1.8
Undetectable Week 24	34.4	1.1	31.1	1.2
Undetectable Week 52	42.9	1.4	39.7	1.5
Maternal Educational Status				
Elementary or less	Ref	Ref	Ref	Ref
At least secondary	3.7	0.9	5.2	1.1

*Adjusted for maternal educational status

Abbreviations: S.E.= Standard Error; Ref=reference

Table 4:6. Pairwise comparisons of mean HBsAb by week and exposure to detectable HIV viral load.

Contrast	P-value (F Test)
Mean HBsAb for Week 0 in exposed versus unexposed	0.03
Mean HBsAb for Week 4 in exposed versus unexposed	0.86
Mean HBsAb for Week 24 in exposed versus unexposed	0.45
Mean HBsAb for Week 52 in exposed versus unexposed	0.31

Abbreviations: HBsAb, hepatitis B surface antibodies;

Definitions: exposed= born to a mother with detectable HIV viral load; unexposed= born to a mother with detectable HIV

Discussion

The timely provision of hepatitis B vaccine has 69-98% effectiveness in preventing HBV among immunocompetent vaccinated infants, including those born to mothers with HBeAg in regions hyperendemic for HBV^{10,11} A standard hepatitis B vaccine schedule of three doses is usually sufficient to stimulate an adequate immune response, however immunocompromised individuals may not mount an adequate response^{115,116} and may need additional or higher doses of hepatitis B vaccine to stimulate a sufficient response.¹¹⁵ We sought to determine if antenatal or perinatal exposure to maternal HIV could blunt infants' immune responses to hepatitis B vaccine.

We found that HEU had significantly higher HBsAb concentrations at birth as compared to HUU (31 vs 23 mIU/mL $p < 0.0001$). This was in contrast to the findings by Jones et al. and Hesselning et al. from community-based cohort studies in South Africa, in which HEU had lower specific antibodies than HUU at birth.^{25,107} We explored possible factors that could explain these differences. Though Jones et al. and Hesselning et al. evaluated vaccines given at weeks six, 10 and 14, this does not seem sufficient to explain the differences in our results. One important difference is the clinical status of our cohorts; only 50% of mothers with HIV in the 2009 to 2010 and 58% in the 2006 to 2008 South African studies were on ART, in contrast to our 2013 to 2017 study in which 99% of the mothers with HIV were on ART, with 60% virally suppressed for HIV and a median CD4 count of 580 cells/ μ L. Another difference is the mode of feeding with 97% of all infants in our cohort breastfed for over six months while only HUU were breastfed in the study by Jones et al. in South Africa²⁵ with infant nutrition known to play a

substantial role in infant immune response.^{92,93} The absence of a statistically significant difference in HBsAb concentration in HEU as compared to HUU at Week 24 is similar to findings by Hesselting et. al, who reported no significant difference at Week 24 although their results still differed from ours because they reported HEU having lower concentrations than HUU at 14 and 52 weeks respectively.¹⁰⁷

Overall HBsAb concentrations in our study were lower than those described by Mancinelli et al among a cohort of HEU in Malawi, which had a median GMC of 384 and 108 at 6 months and 12 months of age, respectively.¹⁰⁶ Part of this difference may be due to the use of a different assay, in their case the Enzygnost anti-HBs assay (Siemens Healthcare, Erlangen Germany). Other factors that may contribute to these differences are the timing of the vaccinations and collection of samples. In Malawi, infant hepatitis B vaccine was provided at 6, 10 and 14 weeks of age.¹⁰⁶ In our study, infants were to have been vaccinated at birth, six and 14 weeks but instead of receiving the third hepatitis B vaccine at Week 14, the median age at receipt of the third hepatitis B vaccine was at 10 weeks, which meant that by assessing HBsAb at Week 24, we may have missed the peak immune response after the third vaccine dose.

The majority of infants in our study mounted immune responses substantially above 10mIU/mL, which is considered the threshold for extended protection against HBV infection. Though a lower proportion of HEU had protective concentrations of HBsAb (≥ 10 mIU/mL) as compared to HUU, the difference was small and not likely to be relevant from the public health perspective, with 99 vs 100% and 96 vs 100% of infants respectively ($p > 0.99$ and $p = 0.04$) with protective concentrations at weeks 24 and 52 respectively. The proportion of infants with HBsAb concentrations above 10mIU/mL was

higher than concentrations reported by Nlend et al. and Rey-cuille in Cameroon and Senegal^{71,104} but similar to that of other studies in Malawi, Brazil and South Africa^{105–107} Nlend et al reported 52% of HEU mounted protective responses.¹⁰⁴ This, however included some infants up to four years after completing their hepatitis B vaccine schedule. Rey-cuille et al. reported 58% of infants in Senegal mounted protective responses but they had a cohort in which 66% of the infants had moderate to severe malnutrition defined as a weight for age z score ≤ -2 ⁷¹ while only 2% of the infants in our cohort had moderate to severe wasting. Mancinelli et al. in Malawi reported 93.2, 87.5, and 80.1% of HEU with HBsAb concentrations above 10mIU/mL at six months, 12 months and 24 months respectively,¹⁰⁶ Abramczuk et al reported 93.3% of HEU and 96.4% of HUU achieving HBsAb concentrations above 10mIU/mL. in Brazil,¹⁰⁵ and Hesselting et al. reported all HEU in a study in South Africa having protective concentrations of HBsAb at weeks 14 and 52.¹⁰⁷

On comparing infants born to mothers with detectable HIV viral loads (VL) to those without, mean infant HBsAb was significantly different only at birth with infants born to mothers with detectable HIV VL having lower mean HBsAb concentrations. This is in contrast to findings from Jones et al. who did not find any correlation between viral load and any specific antibody level.²⁵ However, Jones et al only correlated viral load with maternal antibodies and not to their infants' antibodies.²⁵

This study is limited by the use of data from a single center. Also, as a retrospective study utilizing data collected for the INFANT study, only data collected for the primary study was available, and no data was collected on maternal hepatitis B vaccination history in the initial study. We however assumed that maternal hepatitis B

vaccination history would be similar in our two (HEU and HUU) infant HIV exposure groups, who were similar in maternal age though they differed in maternal educational status. That said, the parent INFANT study collected data to address a similar research question; *“The relationship between infant microbial structure and infant humoral response to rotavirus, oral polio and BCG vaccines.”* and as such collected most of the data needed to conduct rigorous research into infant immune responses. Second, with the serial testing algorithms to determine maternal HIV status and verify absence of HIV among our infant cohort, it is unlikely that there was misclassification based on the exposure of interest, ie, HIV. Finally, we utilized general linear models, which accounted for repeated measurements on the same individual and permitted adjusting for potential confounders.

Conclusion

Though exposure to maternal HIV can affect an infant’s immune system and have other health related effects, in this study of Nigerian infants, nearly all HEU and HUU had responses to a 3-dose hepatitis B vaccine that would predict protection, even when adjusted for potential confounders. However, longer, prospective studies that explore the rate of attenuation of infant vaccine responses beyond the first year of life and evaluate the duration of protection provided by infant hepatitis B vaccinations could provide evidence of the need for booster doses among HEU later in childhood or in adulthood. Of further value would be studies with large maternal cohorts containing a substantial number of mothers with HBV infection, to evaluate the functionality of the antibodies produced and the protective effect of hepatitis B vaccine in preventing HBV infection among HEU as compared to HUU.

CHAPTER 5. THE EFFECTS OF MATERNAL ANTIBODIES TO HEPATITIS B VIRUS ON THE IMMUNE RESPONSE OF NIGERIAN INFANTS TO HEPATITIS B VACCINE.

ABSTRACT

Background: Universal infant vaccination with hepatitis B vaccine has drastically reduced the prevalence of chronic hepatitis B virus (HBV) infection. Though efforts to improve the coverage of hepatitis B vaccination among infants and at-risk persons must be expanded, another consideration is the effect of maternal antibodies which are transferred to infants transplacentally and via breastmilk and are known to interfere with an infant's response to vaccination. The Nigerian immunization schedule provided monovalent Hepatitis B at birth, six and fourteen weeks resulting in infants being vaccinated at a very young age when maternal antibodies are at a peak in a region with high endemicity for Hepatitis B. We sought to evaluate the effects of maternal antibodies to Hepatitis B surface antigen on the immune response of infants given hepatitis B vaccine at birth, six and 14 weeks of age in a region with high endemicity of Hepatitis B.

Methods: We conducted a retrospective cohort study utilizing data from the INFANT study. INFANT was a prospective cohort study of mother-infant pairs at a tertiary hospital in North-Central Nigeria between 2013 and 2017. General linear models were used to estimate the relationship between infant exposure to maternal HBsAb and their immune response to Hepatitis B vaccine. Pairwise comparisons of mean HBsAb concentrations by week and maternal HBsAb exposure groups among infants were conducted. Fisher's Exact tests were used to compare the proportion of infants with

HBsAb concentrations above 10mIU/mL in infants exposed as compared to infants unexposed to maternal HBsAb at 24 and 52 weeks of age separately.

Results: Mean infant HBsAb ranged from 31 mIU/mL at birth among infants whose mothers had undetectable HBsAb to 45mIU/mL at week 4 among infants with mothers with detectable HBsAb. Mean HBsAb concentrations were significantly different in exposed versus unexposed infants at Weeks 24 and 52 respectively with exposed infants having higher concentrations at week 24 and unexposed infants having higher concentrations at week 52 (P=0.007 and 0.04). However, the proportion of infants with protective concentrations of HBsAb did not differ significantly in infants exposed to detectable concentrations of maternal HBsAb as compared to unexposed infants; 96-100% of infants had HBsAb concentrations \geq 10mIU/mL at Week 24 and Week 52 respectively.

Conclusion: Though exposure to detectable concentrations of maternal antibodies to HBsAb was associated with the immune response of Nigerian infants to hepatitis B vaccine, the differences observed were small, not likely to be clinically relevant and thus do not provide evidence to support a change in the hepatitis B vaccination schedule for infants exposed to detectable concentrations of maternal HBsAb. Future studies should explore the duration of protection against HBV infection in infants exposed to detectable concentrations of maternal HBsAb.

Background

Although universal infant vaccination with Hepatitis B vaccine has drastically reduced the prevalence of chronic hepatitis B virus (HBV) infection,^{72 73} approximately 296 million persons are currently living with chronic HBV infection globally.¹ Efforts to improve the coverage of hepatitis B vaccination among infants and at-risk persons must be expanded, but another consideration is the effect of maternal antibodies on infants immune systems. Maternal antibodies are known to interfere with an infant's response to vaccination.^{25,27-30,119} They vary geographically and seasonally,¹²⁰ and are transferred to infants trans-placentally and via breastmilk.

Nigeria, a country with a population of over 211 million persons,¹¹⁷ has an HBV seroprevalence of approximately 8% and a seroprevalence of 6% among pregnant women.^{36,118} As part of Nigeria's National Programme on Immunization, monovalent hepatitis B vaccine was given to infants at birth, and at 6 and 14 weeks of life.⁸³ Provision of a birth dose resulted in infants being vaccinated at a very young age when maternal antibodies are at a peak. We sought to evaluate the effects of detectable concentrations of maternal hepatitis B surface antigen on the immune response of 367 Nigerian infants given hepatitis B vaccine at birth, six and 14 weeks of age in a region with a high endemicity of HBV infection.

Materials and methods

We conducted a retrospective cohort study utilizing data from the INFANT (Innate, Adaptive and Mucosal Immune Responses in HIV-1 Exposed Uninfected Infants: A Human Model to Understand Correlates of Immune Protection) study. INFANT was a prospective cohort study of mother-infant pairs at a tertiary hospital in North-Central Nigeria enrolled between 2013 and 2017.⁹³ Approval for the use of de-identified data from the INFANT study was provided by the University of Maryland Baltimore, Institutional Review Board and the Plateau State Specialist Hospital in Nigeria.

Study setting

Nigeria added hepatitis B vaccine to its National Programme of immunization in 1995, but the vaccine was not widely available until 2004.⁸⁰ At the time of the INFANT study, the approved hepatitis B vaccine schedule consisted of three doses of monovalent hepatitis B vaccine to be given to infants at birth, and at six and 14 weeks of life.⁸³

Study participants

All mother-infant pairs enrolled into the INFANT study were eligible to be included in this study. From 303 mothers with HIV and their infants and 106 mothers without HIV and their infants recruited in the INFANT study and followed up until 12 months of age,

we excluded infants missing data on their immunization status, all second twins and any infants with a missing or insufficient sample at Week 24. 367 mother-infant pairs from the INFANT study were included.

Parent Study

INFANT study participants have been fully described previously.⁹³ Briefly, pregnant women living with and without HIV were offered enrollment into the INFANT study as they attended antenatal clinic. At the time of registration for antenatal care, all pregnant women were tested for HIV as part of standard of care.¹¹² For women recruited into the study who tested HIV-negative at the time of registration for antenatal care, the INFANT study procedures included repeat HIV testing between week 32 of gestation and delivery. Eight study follow-up visits occurred at 1, 4, 7, 10, 15, 24, 36 and 52 weeks after enrollment.

Inclusion Criteria - Participating pregnant women were to be at least 18 years of age, to have voluntarily chosen to breastfeed their infants and able to meet the study assessment schedule.

Exclusion Criteria - Prospective participants were excluded if they had complications during pregnancy or delivery (e.g., eclampsia, chorioamnionitis), if the subsequently delivered infant had neonatal asphyxia, seizures, or sepsis; if infant gestational age was less than 36 weeks at birth, or if infant birth weight was less than 2.4 kg. All infants were tested for HIV via DNA polymerase chain reaction at birth per study protocol, and between

4-6 weeks of life per standard of care, and any infants found to be HIV-infected were also excluded from the study⁹³.

Study Procedures

Questionnaires detailing maternal socio-demographic, clinical and obstetric factors, and infant birth, feeding, clinical and vaccination data were completed by research personnel via maternal participant interviews and medical record abstraction. Physical examinations of infants were performed by pediatricians and pediatric nurses on the research team, and blood, stool, saliva and breast milk collected by laboratory personnel on the research team at each study visit. The blood samples were processed and plasma aliquoted and stored in -80°C freezers within six hours of blood collection.

Statistical analysis

The primary outcome measures were the concentration of infant HBsAb at birth, 4, 24 and 52 weeks of age. The secondary outcomes were the proportion of infants who developed protective concentrations of HBsAb, defined as HBsAb concentrations $\geq 10\text{mIU/mL}$ at either 24 or 52 weeks of life. HBsAb was quantified using enzyme-linked immunosorbent assay MBS3800892 (MyBiosource Inc, San Diego, USA). This assay uses an antigen “sandwich” method in which polystyrene microwell strips are pre-coated with recombinant HBsAg. After performing the assay according to the manufacturer’s specifications,¹¹³ a spectrophotometer was used to read the absorbance at 450nm. This optical density was then converted to ng/mL using a standard curve of optical density versus HBsAb concentration produced using calibration standards provided with each

ELISA kit.¹¹³ Results in ng/mL were then converted to mIU/mL by multiplying the result in ng/mL by six based on the specifications of MyBiosource Incorporated.

The primary exposure was each infant's exposure to detectable concentrations of maternal HBsAb determined from a maternal sample taken at delivery. Detectable concentrations of HBsAb were determined using ARIA which detects HBsAb concentrations ≥ 30 mIU/mL. Exposure was categorized as detectable and undetectable.

Baseline variables and other covariates identified a priori as being associated with infant immune response were analyzed for frequency, distribution and missing data. Influential outliers (identified using studentized residuals and cook's distance) were omitted. Please see Table 3:2 for the differences between variables included and those excluded from the INFANT study. The covariates of interest were maternal demographic factors namely age, occupation and educational status; maternal obstetric factors namely number of previous pregnancies, mode of delivery; and infant factors namely infant sex, birthweight, duration of breastfeeding, and if a birth dose of hepatitis B vaccine was received. Bivariate analyses of these covariates by exposure groups were conducted to identify potential confounders. The criteria for identifying potential confounders were covariates associated with both the exposure and the outcome with a p-value ≤ 0.10 .

The relationship between infant exposure to detectable concentrations of maternal HBsAb and infant immune response to hepatitis B vaccine was determined using general linear models which account for repeated measurements on the same individual and permit adjusting for potential confounders. We used the proc mixed procedure with restricted maximum likelihood approach. A repeated statement was used to indicate repeated

measurements on individuals and maternal ID was used to identify the clusters. An unstructured correlation structure was utilized.

Four time points were of interest: baseline (at birth) and Weeks 4, 24, and 52 of life. The Week 4 sample was four weeks after the first hepatitis B vaccine dose, while the Week 24 sample was to be 10 weeks after the third hepatitis B vaccine dose. Baseline covariates found to be potential confounders were investigated further by introducing them into a model assessing the crude association between infant exposure to detectable concentrations of maternal HBsAb and infant antibody response to hepatitis B vaccine, namely hepatitis B surface antibody (HBsAb). A 10% change in the crude response was required for the variable to be retained. Maternal HIV status was a factor identified through bivariate analysis that was explored as a potential confounder. Adjusting for maternal HIV status did not result in a 10% change in infant mean HBsAb concentrations (See supplementary Table 1), as such, it was not retained in the final model. Pairwise comparisons of mean HBsAb concentrations by week and exposure status (to detectable concentrations of maternal HBsAb) among infants were conducted.

Secondary Outcome Analysis

Fisher's Exact tests were used to compare the proportion of infants with HBsAb concentrations above 10mIU/mL in exposed versus unexposed infants at 24 and 52 weeks of age separately.

Results

A total of 409 pregnant women and 419 infants were recruited into the INFANT study. For our analysis, we included 367 mother-infant pairs, after we excluded one twin each from 10 pairs of twins, 22 mother-infant pairs lost to follow-up before Week 24, six infants for lack of a sample at Week 24, one mother-infant pair with an underaged mother (age<18 years), and 13 infants with HBsAb concentrations considered influential outliers.

On comparing baseline maternal and infant factors across exposure groups, the groups differed in terms of maternal HIV status and the proportion of infants that received a timely birth dose of hepatitis B vaccine; a lower proportion of mothers with HIV had detectable concentrations of HBsAb and a lower proportion of infants exposed to maternal HBsAb received a timely birthdose of hepatitis B vaccine. (Please see Table 5:1 for the results of bivariate analyses comparing covariates of interest by exposure groups). Maternal educational status, maternal HIV status, and infant sex were found to be associated with infant HBsAb (See Results of bivariate analysis: covariates and infant hbsab in Appendix I). Infants of mothers with at least secondary education had mean HBsAb concentrations almost 3.7mIU/mL higher than infants of mothers with primary education or less ($P<0.0001$). Infants whose mothers did not have HIV had mean HBsAb concentrations 3.1mIU/mL higher than mean HBsAb concentrations in infants whose mothers had HIV and males had mean HBsAb concentrations 1.8mIU/mL higher than females ($P=0.03$). We therefore evaluated maternal HIV status as a potential confounder but found that it did not change mean HBsAb by up to 10%. Therefore, it was not retained in the final model. (Please see Appendix II for Adjusted mean estimates of infant HBsAb by week and exposure status to detectable concentrations of maternal HBsAb.

Table 5:1. Characteristics of mothers with and without detectable antibodies to HBV and their infants.

	Total	Exposed to detectable maternal HBsAb	Unexposed to detectable maternal HBsAb	P-value
<i>Maternal</i>				
Maternal Age (years)				0.57
≤ 30	188 (51)	28 (55)	160 (51)	
>30	179 (49)	23 (45)	156 (49)	
Marital Status				0.37†
Married	356 (97)	51 (100)	305 (97)	
Education				0.15
Elementary or less	126 (34)	13 (25)	113 (36)	
At least secondary	241 (66)	38 (75)	203 (64)	
Employment Status				0.26
Employed	248 (68)	31 (61)	217 (69)	
Mode of Delivery				1.00†
Vaginal	334 (91)	47 (92)	287 (91)	
Previous Pregnancies				0.28
0	48 (13)	7 (14)	41 (13)	
1-3	229 (62)	36 (71)	193 (61)	
≥ 4	90 (25)	8 (16)	82 (26)	
Maternal HIV				<0.0001
Positive	276 (75)	25 (49)	251 (79)	
Negative	91 (25)	26 (51)	65 (21)	
<i>Infant</i>				
Sex				0.25
Female	186 (51)	22 (43)	164 (52)	
Birthweight (grams)				0.95
≤ 3000	200 (55)	28 (55)	172 (54)	
>3000	167 (45)	23 (44)	144 (46)	
Range: 2400-4700				
Duration of breastfeeding				0.46†
< 6 months	10 (3)	0 (0)	10 (3)	
≥ 6 months	356 (97)	51 (100)	305 (97)	
Missing=1	1 (0)		1 (0)	

Table 5:1. (cont)

Birth hepatitis B dose received				0.04
Yes	117 (32)	10 (20)	107 (34)	
No	250 (68)	41 (80)	209 (66)	
Number of hepatitis B vaccines received				1.00†
<3	2 (1)	0 (0)	2 (1)	
3	365 (99)	51 (100)	314 (99)	

Abbreviations: HBsAb= hepatitis B surface antibodies

Mean infant HBsAb ranged from 31.3mIU/mL at birth among infants whose mothers had undetectable HBsAb, to 45.3mIU/mL at Week 4 among infants with mothers with detectable HBsAb (See Table 5:2 and Fig 5:1.). Exposure to detectable concentrations of maternal HBsAb was found to be associated with mean infant HBsAb concentrations at Weeks 24 and 52 respectively with exposed infants having higher concentrations at week 24 and unexposed infants having higher concentrations at week 52 (P=0.007 and 0.04). (Please see Table 5:3. for the results of the pairwise comparisons). However, infant exposure to detectable concentrations of maternal HBsAb was not found to be associated with the proportion of infants with protective concentrations of HBsAb at either Week 24 or Week 52 (Table 5.4).

Table 5:2. Unadjusted mean estimate of Infant HBsAb by week and HIV exposure status (n=367).

Parameter	Estimate	SE
Infants with detectable MatAbs in Week 0	32.5	2.2
Infants with detectable MatAbs in Week 4	45.3	2.8
Infants with detectable MatAbs in Week 24	39.7	1.8
Infants with detectable MatAbs in Week 52	36.0	2.2
Infants with undetectable MatAbs in Week 0	31.3	0.9
Infants with undetectable MatAbs in Week 4	43.9	1.1
Infants with undetectable MatAbs in Week 24	34.7	0.7
Infants with undetectable MatAbs in Week 52	40.9	0.9

Abbreviations: MatAbs= Maternal antibodies to HBsAb

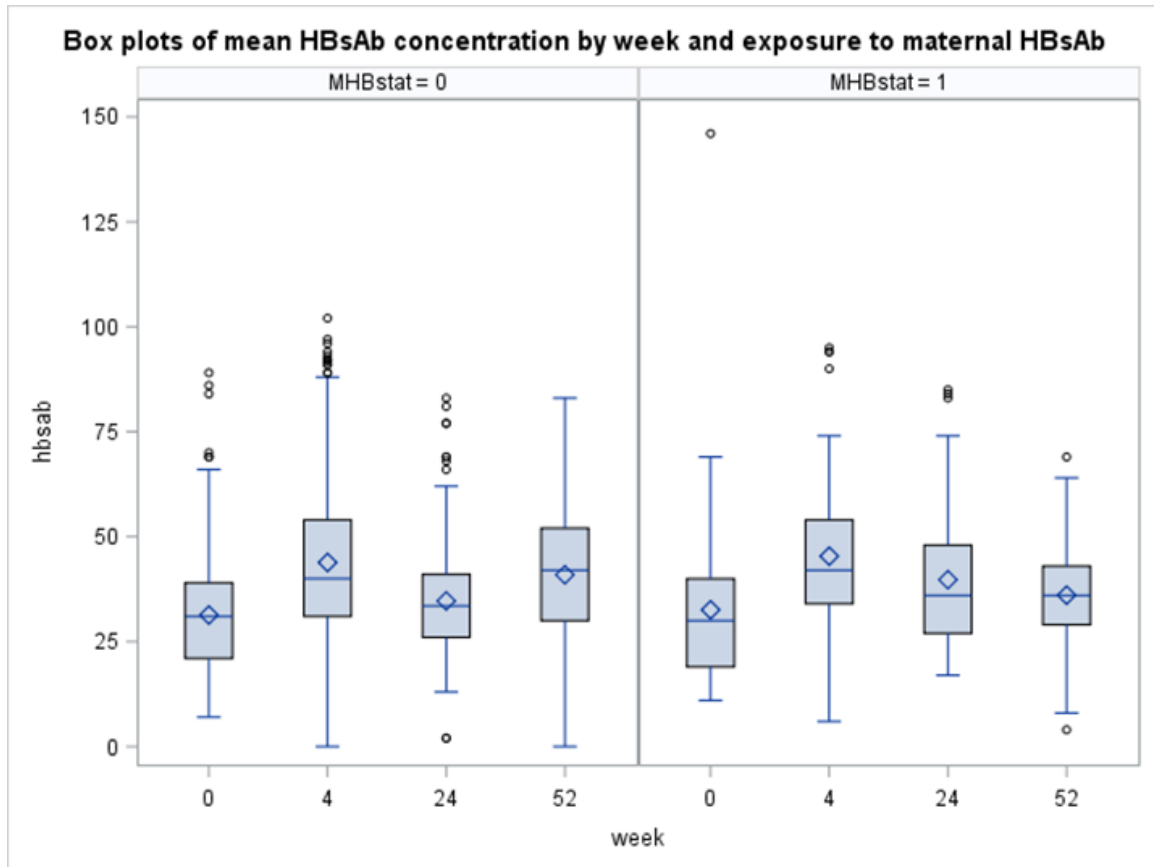


Figure 5:1. Box plots showing mean HBsAb concentration by week and exposure to maternal HBsAb.

Table 5:3. Pairwise comparisons of mean HBsAb by week and presence or absence of detectable maternal HBsAb.

Contrast	P-value
Mean HBsAb for Week 0 in infants of mothers exposed versus unexposed to detectable HbsAb	0.61
Mean HBsAb for Week 4 in infants of mothers exposed versus unexposed to detectable HbsAb	0.62
Mean HBsAb for Week 24 in infants of mothers exposed versus unexposed to detectable HbsAb	0.009
Mean HBsAb for Week 52 in infants of mothers exposed versus unexposed to detectable HbsAb	0.04

Abbreviations: HBsAb= hepatitis B surface antibodies

Table 5:4. Proportions of infants with protective concentrations of HBsAb at Weeks 24 and Week 52 respectively.

	Total	Exposed to detectable concentrations of HBsAb	Unexposed to detectable concentrations of HBsAb	P-value (Fisher's Exact)
Week 24				
<10mIU/mL	2 (1)	0 (0)	2 (1)	1.0
≥ 10mIU/mL	365 (99)	51 (100)	314 (99)	
Week 52				
<10mIU/mL	12 (3)	2 (4)	10 (3)	0.68
≥ 10mIU/mL	355 (97)	49 (96)	306 (97)	

Abbreviations: HBsAb= hepatitis B surface antibodies

Discussion

We found that infant immune response was associated with exposure to detectable concentrations of maternal HBsAb at Weeks 24 and 52. We found higher mean HBsAb concentrations in infants exposed to maternal HBsAb at Week 24 and lower at Week 52. This was in contrast to findings by Hu et al. who found higher HBsAb concentrations in infants unexposed to detectable maternal HBsAb at two months after the second hepatitis B vaccination (approximately Week 12)¹⁰⁹ and Wang et al. who found comparable HBsAb geometric mean concentrations in infants with detectable maternal HBsAb as compared to those without.¹¹⁰ Some of these differences could be attributed to the detection limits of the tests used to identify detectable concentrations of HBsAb. The test we used (Aria HBV combination test, CTK Biotech Incorporated, USA), had a detection limit of 30mIU/mL and thus could have misclassified mothers with detectable concentrations of HBsAb thus leading to bias of the association between exposure to maternal HBsAb and infant immune response to hepatitis B towards the null. The test used by Wang et al. had a detection limit of 0.01mIU/mL.

On evaluating the proportion of infants with protective concentrations of HBsAb, the differences we found were similar to previous studies^{105–107,109,110} and not clinically relevant. Majority of the infants (96-100%) of both exposed and unexposed infants had HBsAb concentrations above protective concentrations at Week 24 or Week 52 respectively. These concentrations were higher than those reported for other studies though these differences were likely due to the timing of the outcome assessment which ranged from two months after the second hepatitis B vaccination to five and a half years after

receipt of the third hepatitis B vaccine.^{109,110} Though we found no difference in the proportion of infants with protective concentrations when comparing exposed to unexposed, Chen et al. found an inverse relationship with infants exposed to the highest concentration of maternal antibodies having the poorest response and vice versa.¹¹¹ Our failure to find a difference could be linked to the detection limit of the Aria test which at 30mIU/mL could have resulted in misclassification of mothers with HBsAb thus causing bias of our results towards the null.

Although no data was collected on maternal hepatitis B vaccination history in the initial study and differences in vaccination status could influence maternal HBsAb concentrations, exposure status was determined downstream by HBsAb concentration status and as such could not be confounded by maternal vaccination status. The use of a quantitative ELISA assay to determine maternal HBsAb concentrations would have made it possible to classify mothers appropriately and minimize bias. We would also have been able to assess the correlation between maternal antibody concentrations and infant concentrations at birth. However, because the parent study was a prospective cohort study evaluating a similar research question on immune response to rotavirus, BCG and tetanus vaccines, we had access to robust data on potential confounders with minimal missing data and had samples available for testing at our four time points of interest. Our use of general linear models which account for repeated measures and can be adjusted for potential confounders increased the precision of our results.

Conclusion

Though exposure to detectable concentrations of maternal antibodies to HBsAb was associated with the immune response of Nigerian infants to hepatitis B vaccine, the differences observed were small and not likely to be not clinically relevant. They do not provide evidence to support a change in the hepatitis B vaccination schedule for infants exposed to detectable concentrations of maternal HBsAb. However, longer, prospective studies that explore the rate of attenuation of infant vaccine responses beyond the first year of life and evaluate the duration of protection provided by infant hepatitis B vaccinations could provide evidence of the need for booster doses in late childhood or adulthood among infants exposed to detectable concentrations of maternal HBsAb perinatally. Another area that may warrant further research is a comparison of the effects of maternal HBsAb from natural infection to that obtained through vaccination as more persons vaccinated as infants grow to child-bearing age.

CHAPTER 6. DISCUSSION OF DISSERTATION RESULTS

We sought to evaluate two factors that could potentially inhibit infant immune response to hepatitis B vaccine even with the availability of highly effective hepatitis B vaccines.¹¹¹⁰ We evaluated the effect of antenatal or perinatal exposure to maternal HIV and made the following observations:

Exposure to HIV antenatally or in the perinatal period was found to be associated with infant immune response. HEU had significantly higher HBsAb concentrations at birth as compared to HUU (30 vs 23 mIU/mL $p=0.0002$). This was in contrast to findings by Jones et al. from a community-based cohort study in RSA, in which HEU had lower specific antibodies to Hib, pertussis, pneumococcus, and tetanus than HUU at birth.²⁵ This may be due to differences in our cohorts and timing of the studies; only 50% of mothers with HIV in the 2009 to 2010 South African study were on ART, in contrast to our 2013 to 2017 study in which 99% of the mothers with HIV were on ART, with 60% virally suppressed for HIV and a median CD4 count of 580 cells/ μ L. Another possibly consequential difference is the mode of feeding of the infants in our respective studies; 97% of the infants in our cohort were breastfed for over six months while HEU in the study by Jones et al. were formula fed.²⁵ Breastfeeding is vital for infant well-being in lower resource countries and has been found to mitigate the effects of HIV exposure in HEU.⁹³

We found no difference in mean HBsAb at Week 24 but higher HBsAb concentrations in HEU at Weeks 4 and 52. Though this finding of no difference at Week 24 is similar to findings by Hesselning et. al from a study in RSA, who reported no significant difference at Week 24, the study by Hesselning et al. still differed from ours because they reported HEU having lower mean concentrations than HUU at 14 and 52

weeks respectively.¹⁰⁷ The lower proportion of mothers on ART with 15% having CD4 counts <200 cells/ μ L in their study may play a role in this.¹⁰⁷

Overall, HBsAb concentrations in our study were lower than those described by Mancinelli et al. among a cohort of HEU in Malawi, which had a median GMC of 384 and 108 at 6 months and 12 months of age, respectively.¹⁰⁶ Part of this difference may be due to the use of a different assay, in their case the Enzygnost anti-HBs assay (Siemens Healthcare, Erlangen Germany). Other factors that may contribute to these differences are the timing of the vaccinations and collection of samples. In Malawi, infant hepatitis B vaccine was provided at 6, 10 and 14 weeks of age.¹⁰⁶ In our study, infants were to have been vaccinated at birth, six and 14 weeks but instead of receiving the third hepatitis B vaccine at Week 14, the median age at receipt of the third hepatitis B vaccine was 10 weeks, which meant that by assessing HBsAb at Week 24, we may have missed the peak immune response after the third vaccine dose. This, however, does not fully explain the difference in magnitude in our results.

However, the majority of infants in our study mounted immune responses above 10mIU/mL, which is considered the threshold for extended protection against HBV infection. Though a lower proportion of HEU had protective concentrations of HBsAb (HBsAb \geq 10mIU/mL) as compared to HUU, the difference was small and likely to be irrelevant from a public health perspective, with 99 vs 100% and 96 vs 100% of infants respectively with protective concentrations ($p=1.00$ and $p=0.04$). The proportion of infants with HBsAb concentrations above 10mIU/mL was higher than concentrations reported by Nlend et al. and Rey-cuille in Cameroon and Senegal¹⁰⁴⁷¹ but similar to that of other studies in Malawi, Brazil and RSA.¹⁰⁶¹⁰⁵¹⁰⁷ Nlend et al reported 52% of HEU

mounted protective responses.¹⁰⁴ This, however included some infants up to four years after completing their hepatitis B vaccine schedule. Rey-cuille et al. reported 58% of infants in Senegal mounted protective responses but they had a cohort in which 66% of the infants had moderate to severe malnutrition defined as a weight for age z score ≤ -2 .⁷¹ Mancinelli et al. in Malawi reported 93.2, 87.5, and 80.1% of HEU with HBsAb concentrations above 10mIU/mL at six months, 12 months and 24 months respectively.¹⁰⁶ Abramczuk et al. reported 93.3% of HEU and 96.4% of HUU achieving HBsAb concentrations above 10mIU/mL. in Brazil,¹⁰⁵ and Hesselning et al. reported all HEU in a study in RSA having protective concentrations of HBsAb at weeks 14 and 52.¹⁰⁷

We examined the effect of detectable concentrations of maternal HBsAb on the immune response of Nigerian infants to hepatitis B vaccine. We found that infant exposure to detectable concentrations was associated with infant immune response to hepatitis B vaccine at Weeks 24 and 52 in our case. In Week 24, infants exposed to detectable maternal HBsAb had higher mean HBsAb while at Week 52, infants exposed to detectable maternal HBsAb had lower mean HBsAb. This was in contrast to findings by Hu et al. who found higher HBsAb in infants born to mothers with undetectable HBsAb at two months after the second hepatitis B vaccination (approximately Week 12)¹⁰⁹ and Wang et al. who found comparable HBsAb geometric mean concentrations in infants with detectable maternal HBsAb as compared to those without at eighteen months and five and a half years after three-dose hepatitis B vaccine.¹¹⁰ Some of these differences could be attributed to the detection limit of the tests used to identify detectable concentrations of HBsAb. The test we used had a detection limit of 30mIU/mL and thus could have misclassified mothers with detectable concentrations of HBsAb thus leading to bias of the association between

exposure to maternal HBsAb and infant immune response to hepatitis B towards the null. The test used by Wang et al. had a detection limit of 0.01mIU/mL. Another reason for this difference could be the timing of the tests; Wang et al. compared their exposure groups 18 months and five and a half years after they had received three doses of hepatitis B vaccine.¹¹⁰

On evaluating the proportion of infants with protective concentrations of HBsAb, the differences we found were similar to previous studies^{105–107,109,110} and not likely to be clinically relevant. Majority of the infants (96-100%) of both exposed and unexposed infants had HBsAb concentrations above protective levels at Week 24 or Week 52 respectively (P=1.00 and P=0.68).

Limitations and strengths

Pregnant women who agreed to participate in the parent study which was a prospective cohort study implemented over 18 months at a tertiary hospital and which involved the recurrent collection of biologic samples from themselves and their infants, are likely to differ from the general population. However, we answered a biological question and evaluated potential confounding variables like maternal age, maternal occupation, maternal educational status and duration of breastfeeding which could differ when comparing our study cohort to the general population. Only maternal educational status was found to confound the relationship between infant HIV exposure status and immune response and to counteract this, it was controlled for. Also, as a retrospective study relying on data collected for a different study, we could only access data collected for the primary study. Though the data set lacked pertinent information like maternal hepatitis B

vaccination history, it contained most of the data we would have collected for the purpose of a study like ours. For example, the dates all pediatric immunizations in the National Programme of Immunization were given, the number of doses given and potential confounders namely mode of feeding, duration of breastfeeding and anthropometric measurements like weight, height, and head circumference at every clinic visit in a prospective manner. This was because one aim of the parent study was to assess the relationship between infant microbial structure and infant humoral response to rotavirus, oral polio and BCG vaccines. Also, because the parent study was a prospective study with frequent follow-up visits, there was minimal missing data.

Second, HIV exposure status was determined at two time points. Mothers were tested for HIV at the time of registration for ante-natal care and again between 32 weeks gestation and delivery if they were negative during the first test making it unlikely that there was misclassification based on the exposure for Aim 1. Infants too were tested at birth, 3-4 weeks, six weeks and at one year of age, or six weeks post cessation of breast feeding if breast feeding continued for longer than one year, so they are not likely to have been HIV-infected themselves.

The use of stored samples determined the quantity and quality of plasma available for use to elicit the concentration of HBsAb in Nigerian infants. Some of the samples experienced several freeze-thaw cycles and some samples were no longer available for testing. However, this only reduced the available sample at our primary end point by about 2%. Though the sample size is larger than for previous studies, it is still insufficient to correlate antibody concentrations with clinical outcome measures adjusted for potential confounders such as the proportion of infants infected with HBV within their first year of

life. This was because only 42 mothers (11%) had evidence of HBV infection (Please see Table 4:1.). Also, due to the small numbers of mothers with HBsAb, it was not possible to evaluate HIV exposure as an effect measure modifier in our study.

Overall, the ELISA results followed an unusual pattern. At birth, all infants had detectable HBsAb concentrations (detection limit=0.6mIU/ml) using ELISA MBS3800892 (MyBiosource Inc., San Diego, USA). However, after hepatitis B vaccines were provided, the concentrations were lower than those measured in infants vaccinated with hepatitis B vaccine in other studies.^{106,111} Another unusual finding was that the highest concentrations were measured at Week 4 in our cohort. We also failed to see fold increments following subsequent doses of hepatitis B vaccine. There may be several reasons for these findings:

1. The vaccines: Vaccines licensed by several pharmaceutical companies and manufactured by many more biological companies are used to vaccinate infants. Some are imported, others made locally with different degrees of immunogenicity. This could lead to different magnitudes of responses among vaccinated infants. There is also the need to maintain the potency of the vaccines and where there is sub-optimal storage or transportation of the vaccines, they may lose their potency and in such cases, vaccinated infants may not mount the expected response.
2. Vaccination – Vaccinating the infants as and when due; ensuring the appropriate quantity of vaccine, in the designated site and verifying that records of vaccinations on immunization cards reflect vaccinations received. In our study,

study visits were designated to coincide with routine childhood vaccinations and the data on vaccinations received collected at each visit. It is not likely that vaccinations were recorded and not given to the infants.

3. The ELISA tests and ELISA testing – ELISA tests are made by different laboratories and need to be utilized following strict protocols, with highly specialized equipment. Very precise measurements are required and exact timing of steps. When any of these requirements is flouted, variability can be introduced. In our study, ELISA kits were bought in several batches using different vendors who delivered them to the testing laboratory using different modes of transportation. These could have introduced some variability in the results though this is not likely as a faulty kit is likely to be flagged by the internal calibrators provided by the manufacturers to be used in creating standard curves. Laboratory staff are also a key component of testing. Three different laboratory scientists performed the testing for our study. Though the three testers were competent in the protocol and were supervised by a single laboratory manager, having three testers too could have introduced interobserver bias. However, the laboratory in which the testing was carried out is accredited and has procedures in place to ensure the quality of the testing and the results.

Another factor to consider is the method we used for determining the threshold for a positive result. We relied on tests performed at the laboratory providing the ELISA test kits in San Diego, USA to determine results for persons confirmed to be negative. Perhaps if these tests were done at the laboratory

carrying out the testing for our study the cut-off point would have resulted in a higher threshold for a positive result.

4. The samples – have been in storage in -80 degree freezers for many years and some of the batches of samples have undergone several freeze-thaw cycles. These factors too may play a role in the results.

Public health Importance and Future Directions

Our study demonstrated significantly different immune responses to hepatitis B vaccine in HEU as compared to HUU at three timepoints and significant differences in the proportion of infants with protective concentrations of HBsAb at one year of life, but these differences were small and not likely to be clinically relevant. They do not provide evidence to support a change in the hepatitis B vaccination schedule for HEU. However, longer, prospective studies that explore the rate of attenuation of infant vaccine responses beyond the first year of life and evaluate the duration of protection provided by infant hepatitis B vaccinations could provide evidence of the need for booster doses among HEU in late childhood or adulthood. Of further value would be studies with large maternal cohorts containing a substantial number of mothers with HBV infection, to evaluate the functionality of the antibodies produced and the protective effect of hepatitis B vaccine in preventing HBV infection among HEU as compared to HUU.

CHAPTER 7. Appendix I

Results of Bivariate Analysis: Covariates versus infant HBsAb

Parameter	Estimate (mIU/mL)	Standard Error	Type 3 test P-value
Maternal HBsAb			
Detectable	37.4	0.45	0.30
Undetectable	36.2	1.20	ref
Maternal HIV			
Positive	34.1	0.82	0.001
Negative	37.2	0.95	ref
Maternal Age			
≤ 30 years	36.2	0.58	Ref
>30 years	36.6	0.83	0.61
Marital Status			
Unmarried	33.5	2.39	Ref
Married	36.5	2.42	0.22
Maternal Education			
Elementary or less	34.1	0.69	Ref
At least secondary	37.8	0.85	<0.0001
Maternal Occupation			
Unemployed	36.1	0.73	Ref
Employed	36.5	0.89	0.65
Previous Pregnancies			
0	35.6	1.14	Ref
1-3	37.0	1.26	0.27
≥ 4	35.4	1.41	0.89
Mode of Delivery			
Cesarean	35.4	1.39	Ref
Vaginal	36.5	1.45	0.47
Infant Sex			
Male	37.3	0.58	Ref
Female	35.5	0.83	0.03
Duration of breastfeeding			
< 6 months	36.0	2.50	Ref
≥ 6 months	36.3	2.53	0.90
Birth weight			
≤ 3000g	36.5	0.56	Ref
> 3000g	36.3	0.83	0.75
Birth dose received			
Yes	36.6	0.50	0.82
No	36.4	0.89	ref

CHAPTER 8. Appendix II

Table showing unadjusted and adjusted mean estimates of infant HBsAb by week and exposure to detectable concentrations of maternal HBsAb

Parameter	Unadjusted		Adjusted	
	Estimate	Standard Error	Estimate	Standard Error
Exposed Week 0 (birth)	32.5	2.2	30.8	2.2
Exposed Week 4	45.3	2.8	43.6	2.8
Exposed Week 24	39.7	1.8	38.0	1.9
Exposed Week 52	36.0	2.2	34.3	2.2
Unexposed Week 0 (birth)	31.3	0.9	28.6	1.1
Unexposed Week 4	43.9	1.1	41.1	1.4
Unexposed Week 24	34.7	0.7	31.9	1.1
Unexposed Week 52	40.9	0.9	38.2	1.2
Maternal HIV				
Positive	3.1	0.9	3.5	1.0
negative	ref		ref	

CHAPTER 9. REFERENCES

1. Organization WH. Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. *Who*. 2021;53(9):1689-1699. <https://www.who.int/publications/i/item/9789240027077>
2. McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology*. 2009;49(SUPPL. 5):45-55. doi:10.1002/hep.22898
3. World Health Organization. *Global Hepatitis Report, 2017.*; 2017.
4. World Health Organization W. Hepatitis B Vaccines: WHO position paper. *Wkly Epidemiol Rec*. 2017;(27):369-392. <http://www.who.int/wer>
5. Greenberg DP. Pediatric experience with recombinant hepatitis B vaccines and relevant safety and immunogenicity studies. *Pediatr Infect Dis J*. 1993;12(5):438-445.
6. Greenberg D, Vadhein C, Marcy M, et al. Safety and Immunogenicity of a recombinant hepatitis B vaccine administered to infants at 2, 4 and 6 onths of age. *Vaccine*. 1996;14(8):811-816. [papers2://publication/uuid/9D0225A8-C051-45C8-AD40-9323D6E8C47A](https://pubmed.ncbi.nlm.nih.gov/9D0225A8-C051-45C8-AD40-9323D6E8C47A)
7. Greenberg DP, Wong VK, Partridge S, Howe BJ. Safety and immunogenicity of a combination pertussis-hepatitis B vaccine administered at two , four and six months of age compared with monovalent hepatitis B vaccine administered at birth , one month and six months of age. *Pediatr Infect Dis J*. 2002;21(8):769-776. doi:10.1097/01.inf.0000023959.66684.61
8. Coates T, Wilson FR, Patrick G, et al. Hepatitis B Vaccines : Assessment of the Seroprotective Efficacy of Two Recombinant DNA Vaccines. (3):392-403.
9. Mele A, Tancredi F, Romano L, et al. Effectiveness of Hepatitis B Vaccination in Babies Born to Hepatitis B Surface Antigen – Positive Mothers in Italy. Published online 1991:905-908.
10. Schillie SF, Murphy T V. Seroprotection after recombinant hepatitis B vaccination among newborn infants : A review &. *Vaccine*. 2013;31(21):2506-2516. doi:10.1016/j.vaccine.2012.12.012
11. Lolekha S, Warachit B, Hirunyachote A, Bowonkiratikachorn P, West DJ, Poerschke G. Protective efficacy of hepatitis B vaccine without HBIG in infants of HBeAg-positive carrier mothers in Thailand. *Vaccine*. 2002;20(31-32):3739-3743. doi:10.1016/S0264-410X(02)00358-4
12. United Nations Programme on HIV/AIDS. UNAIDS. *UNAIDS Data 2021.*; 2021. https://www.unaids.org/sites/default/files/media_asset/JC3032_AIDS_Data_book_2021_En.pdf
13. UNAIDS. *UNAIDS Data.*; 2020. https://www.unaids.org/sites/default/files/media_asset/2020_aids-data-book_en.pdf

14. Brinkman K, Ter Hofstede HJM, Burger DM, Smeitink JAM, Koopmans PP. Adverse effects of reverse transcriptase inhibitors: Mitochondrial toxicity as common pathway. *Aids*. 1998;12(14):1735-1744. doi:10.1097/00002030-199814000-00004
15. Yamaguchi T, Katoh I, Kurata SI. Azidothymidine causes functional and structural destruction of mitochondria, glutathione deficiency and HIV-1 promoter sensitization. *Eur J Biochem*. 2002;269(11):2782-2788. doi:10.1046/j.1432-1033.2002.02954.x
16. Gribaldo L. Inhibition of CFU-E/BFU-E by 3'-Azido-3'-deoxythymidine, Chlorpropamide, and Protoporphirin IX Zinc (II): A Comparison between Direct Exposure of Progenitor Cells and Long-Term Exposure of Bone Marrow Cultures. *Toxicol Sci*. 2000;58(1):96-101. doi:10.1093/toxsci/58.1.96
17. Fowler DA, Xie MY, Sommadossi JP. Protection and rescue from 2',3'-dideoxypyrimidine nucleoside analog toxicity by hemin in human bone marrow progenitor cells. *Antimicrob Agents Chemother*. 1996;40(1):191-195. doi:10.1128/aac.40.1.191
18. Faye A, Pornprasert S, Mary JY, et al. Characterization of the main placental cytokine profiles from HIV-1-infected pregnant women treated with anti-retroviral drugs in France. *Clin Exp Immunol*. 2007;149(3):430-439. doi:10.1111/j.1365-2249.2007.03411.x
19. Barret B, Tardieu M, Rustin P, et al. Persistent mitochondrial dysfunction in HIV-1-exposed but uninfected infants: Clinical screening in a large prospective cohort. *Aids*. 2003;17(12):1769-1785. doi:10.1097/00002030-200308150-00006
20. Poirier, Miriam Divi, Rao Al-Harthi L. Long-Term Mitochondrial Toxicity in HIV-Uninfected Infants Born to HIV-Infected Mothers. *J Acquir Immune Defic Syndr*. 2003;33:175-183.
21. Bunders M, Thorne C, Newell ML, et al. Maternal and infant factors and lymphocyte, CD4 and CD8 cell counts in uninfected children of HIV-1-infected mothers. *Aids*. 2005;19(10):1071-1079. doi:10.1097/01.aids.0000174454.63250.22
22. Borges-Almeida E, Milanez HMBPM, Vilela MMS, et al. The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. *BMC Infect Dis*. 2011;11:8-14. doi:10.1186/1471-2334-11-38
23. Aldrovandi GM, Chu C, Shearer WT, et al. Antiretroviral exposure and lymphocyte mtDNA content among uninfected infants of HIV-1-infected women. *Pediatrics*. 2009;124(6). doi:10.1542/peds.2008-2771
24. Moraes-pinto AMI De, Almeida ACM, Kenj G, et al. Placental Transfer and Maternally Acquired Neonatal IgG Immunity in Human Immunodeficiency Virus Infection. *Oxford*. 1996;173(5):1077-1084. <http://www.jstor.org/stable/30123006>
25. Jones CE, Beer C De. Maternal HIV Infection and Antibody Responses Against Vaccine-Preventable Diseases in Uninfected Infants. *JAMA*. 2011;305(6).

26. Cumberland P, Shulman CE, Maple PAC, et al. Maternal HIV Infection and Placental Malaria Reduce Transplacental Antibody Transfer and Tetanus Antibody Levels in Newborns in Kenya. *J Infect Dis*. 2007;196(4):550-557. doi:10.1086/519845
27. Chilengi R, Simuyandi M, Beach L, et al. Association of maternal immunity with rotavirus vaccine immunogenicity in Zambian Infants. *PLoS One*. 2016;11(3):1-12. doi:10.1371/journal.pone.0150100
28. Appaiahgari MB, Glass R, Singh S, et al. Transplacental rotavirus IgG interferes with immune response to live oral rotavirus vaccine ORV-116E in Indian infants. *Vaccine*. 2014;32(6):651-656. doi:10.1016/j.vaccine.2013.12.017
29. Mwila K, Chilengi R, Simuyandi M, Permar S, Becker-Dreps S. Contribution of Maternal Immunity to Decreased Rotavirus Vaccine performance in Low and Middle income countries. *Clin Vaccine Immunol*. 2017;24(1):1-8.
30. Bjorkholm B, Gransrom M, Taranger J, Wahl M, Hagberg L. Influence of high titers of maternal antibody on the serological response of infants to diphtheria vaccination at three, five and twelve months of age. *Pediatr Infect Dis J*. 1995;14(10):846-850.
31. Breakwell L, Tevi-benissan C, Childs L, et al. The status of hepatitis B control in the African region. 2017;27(Supp 3):1-11. doi:10.11604/pamj.supp.2017.27.3.11981
32. Seeger C, Mason WS. Molecular biology of hepatitis B virus infection. *Virology*. 2015;479-480:672-686. doi:10.1016/j.virol.2015.02.031
33. World Health Organization. *Hepatitis B and Breastfeeding*.; 1996. doi:10.1089/bfm.2009.0093
34. Shi Z, Yang Y, Wang H, et al. Breastfeeding of newborns by mothers carrying hepatitis B virus: A meta-analysis and systematic review. *Arch Pediatr Adolesc Med*. 2011;165(9):837-846. doi:10.1001/archpediatrics.2011.72
35. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: Epidemiology and vaccination. *Epidemiol Rev*. 2006;28(1):112-125. doi:10.1093/epirev/mxj009
36. Federal Ministry of Health N. *Nigeria HIV/AIDS Indicator and Impact Survey 2018: Technical Report*.; 2019. www.ciheb.org/PHIA
37. Maucourt-Boulch D, de Martel C, Franceschi S, Plummer M. Fraction and incidence of liver cancer attributable to hepatitis B and C viruses worldwide. *Int J Cancer*. 2018;142(12):2471-2477. doi:10.1002/ijc.31280
38. World Health Organization. *Hepatitis B Fact Sheet*.; 2020. <http://www.who.int/mediacentre/factsheets/fs204/en/>. Accessed on November 24, 2020
39. Edmunds WJ, Medley GF, Nokes DJ, Hall AJ, Whittle HC. The influence of age on the development of the hepatitis B carrier state. *Proc R Soc B Biol Sci*.

- 1993;253(1337):197-201. doi:10.1098/rspb.1993.0102
40. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: A review. *Clin Infect Dis*. 1995;20(4):992-1000. doi:10.1093/clinids/20.4.992
 41. Shimakawa Y, Lemoine M, Njai HF, et al. Natural history of chronic HBV infection in West Africa: A longitudinal population-based study from The Gambia. *Gut*. 2016;65(12):2007-2016. doi:10.1136/gutjnl-2015-309892
 42. Kiire CF. The epidemiology and prophylaxis of hepatitis B in sub-Saharan Africa : a view from tropical and subtropical Africa. *Gut*. 1996;38(suppl 2).
 43. Visvanathan K, Dusheiko G, Giles M, et al. Managing HBV in pregnancy . Prevention , prophylaxis , treatment and follow-up : position paper produced by Australian , UK and New Zealand key opinion leaders. *Gut*. Published online 2016:340-350. doi:10.1136/gutjnl-2015-310317
 44. Keane E, Funk AL, Shimakawa Y. Systematic review with meta-analysis: the risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. *Aliment Pharmacol Ther*. 2016;44(10):1005-1017. doi:10.1111/apt.13795
 45. Marinda E, Humphrey JH, Iliff PJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatr Infect Dis J*. 2007;26(6):519-526. doi:10.1097/01.inf.0000264527.69954.4c
 46. Koyanagi A, Humphrey JH, Ntozini R, et al. Morbidity among human immunodeficiency virus-exposed but uninfected, human immunodeficiency virus-infected, and human immunodeficiency virus-unexposed infants in zimbabwe before availability of highly active antiretroviral therapy. *Pediatr Infect Dis J*. 2011;30(1):45-51. doi:10.1097/INF.0b013e3181ecbf7e
 47. Adler C, Haelterman E, Barlow P, Marchant A, Levy J, Goetghebuer T. Severe Infections in HIV-Exposed Uninfected Infants Born in a European Country. *PLoS One*. 2015;10(8):1-14. doi:10.1371/journal.pone.0135375
 48. Epalza C, Goetghebuer T, Hainaut M, et al. High incidence of invasive group B streptococcal infections in HIV-exposed uninfected infants. *Pediatrics*. 2010;126(3). doi:10.1542/peds.2010-0183
 49. Slogrove AL, Cotton MF, Esser MM. Severe infections in HIV-exposed uninfected infants: Clinical evidence of immunodeficiency. *J Trop Pediatr*. 2009;56(2):75-81. doi:10.1093/tropej/fmp057
 50. Slogrove A, Reikie B, Naidoo S, et al. HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. *J Trop Pediatr*. 2012;58(6):505-508. doi:10.1093/tropej/fms019
 51. Hankin C, Thorne C, Newell ML. Does exposure to antiretroviral therapy affect growth in the first 18 months of life in uninfected children born to HIV-infected women? *J Acquir Immune Defic Syndr*. 2005;40(3):364-370. doi:10.1097/01.qai.0000162417.62748.cd
 52. Jumare J, Datong P, Osawe S, et al. Compromised Growth among HIV-exposed

- Uninfected Compared with Unexposed Children in Nigeria. *Pediatr Infect Dis J*. 2019;38(3):280-286. doi:10.1097/INF.0000000000002238
53. Filteau S, Baisley K, Chisenga M, Kasonka L, Gibson RS. Provision of micronutrient-fortified food from 6 months of age does not permit HIV-exposed uninfected Zambian children to catch up in growth to HIV-unexposed children: A randomized controlled trial. *J Acquir Immune Defic Syndr*. 2011;56(2):166-175. doi:10.1097/QAI.0b013e318201f6c9
 54. Kuhn L, Kasonde P, Sinkala M, et al. Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? *Clin Infect Dis*. 2005;41(11):1654-1661. doi:10.1086/498029
 55. Filteau S. The HIV-exposed, uninfected African child. *Trop Med Int Heal*. 2009;14(3):276-287. doi:10.1111/j.1365-3156.2009.02220.x
 56. Mast TC, Kigozi G, Wabwire-Mangen F, et al. Immunisation coverage among children born to HIV-infected women in Rakai district, Uganda: Effect of voluntary testing and counselling (VCT). *AIDS Care - Psychol Socio-Medical Asp AIDS/HIV*. 2006;18(7):755-763. doi:10.1080/09540120500521053
 57. Clerici M, Saresella M, Colombo F, et al. T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. *Blood*. 2000;96(12):3866-3871. doi:10.1182/blood.v96.12.3866.h8003866_3866_3871
 58. Weinhold KJ, Lyerly HKIM, Stanley SD, Austin AA, Matthews TJ, Bolognesi DANP. HIV-1 GP120-mediated immune suppression and lymphocyte destruction in the absence of viral infection . Matthews and D P Bolognesi Information about subscribing to The Journal of Immunology is online at : HIV-1 GP120-MEDIATED IMMUNE SUPPRESSION AND LYMPHOC. *Immunology*. Published online 1989.
 59. Rich KC, Siegel JN, Jennings C, Rydman RJ, Landay AL. Function and phenotype of immature CD4+ lymphocytes in healthy infants and early lymphocyte activation in uninfected infants of human immunodeficiency virus-infected mothers. *Clin Diagn Lab Immunol*. 1997;4(3):358-361. doi:10.1128/cdli.4.3.358-361.1997
 60. Kuhn L, Coutsooudis A, Moodley D, et al. T-helper cell responses to HIV envelope peptides in cord blood: Protection against intrapartum and breast-feeding transmission. *Aids*. 2001;15(1):1-9. doi:10.1097/00002030-200101050-00003
 61. Kuhn L, Meddows-taylor S, Gray G, Tiemessen C. Human Immunodeficiency Virus (HIV)– Specific Cellular Immune Responses in Newborns Exposed to HIV In Utero. 2002;10032(December 2001):267-276.
 62. Legrand, F Nixon, D Loo C. Strong HIV-1-Specific T Cell Responses in HIV-1-Exposed Uninfected Infants and Neonates Revealed after Regulatory T Cell Removal. *PLoS One*. 2006;(1). doi:10.1371/journal.pone.0000102
 63. Kourtis, AP Bulterys M Hu, DJ Jamieson D. HIV-HBV co-infection - a global challenge. *N Engl J Med*. Published online 2012:1749-1752.
 64. Hoffman CJ, Thio CL. Clinical implications of HIV and hepatitis B co-infection in

- Asia and Africa. *Lancet Infect Dis*. 2007;7:402-409.
65. Spira R, Lepage P, Msellati P, et al. Natural history of human immunodeficiency virus type 1 infection in children: a five-year prospective study in Rwanda. Mother-to-Child HIV-1 Transmission Study Group. *Pediatrics*. 1999;104(5). doi:10.1542/peds.104.5.e56
 66. Grosch-Woerner I, Puch K, Maier RF, et al. Increased rate of prematurity associated with antenatal antiretroviral therapy in a German/Austrian cohort of HIV-1-infected women. *HIV Med*. 2008;9(1):6-13. doi:10.1111/j.1468-1293.2008.00520.x
 67. European Collaborative Study. Combination antiretroviral therapy and duration of pregnancy. The European Collaborative Study and the Swiss Mother + Child HIV Cohort Study. *Aids*. 2000;14(June):2913-2920.
 68. Dionne-odum J, Tita A, Siverman N. SMFM Consult Series: Hepatitis B in pregnancy screening, treatment, and prevention of vertical transmission. *Am J Obstet Gynaecol*. 2016;(January):6-14. doi:10.1016/j.ajog.2015.09.100
 69. Nelson NP, Weng MK, Hofmeister MG, et al. Prevention of hepatitis A virus infection in the United States: Recommendations of the advisory committee on immunization practices, 2020. *MMWR Recomm Reports*. 2020;69(1):1-38. doi:10.15585/MMWR.RR6905A1
 70. Brown RS, McMahon BJ, Lok ASF, et al. Antiviral therapy in chronic hepatitis B viral infection during pregnancy: A systematic review and meta-analysis. *Hepatology*. 2016;63(1):319-333. doi:10.1002/hep.28302
 71. Sow HD, Vray M, Unal G, et al. Low Immune Response to Hepatitis B Vaccine among Children in Dakar , Senegal. *PLoS One*. 2012;7(5):5-8. doi:10.1371/journal.pone.0038153
 72. Viviani S, Jack A, Hall AJ, et al. Hepatitis B vaccination in infancy in The Gambia: Protection against carriage at 9 years of age. *Vaccine*. 1999;17(23-24):2946-2950. doi:10.1016/S0264-410X(99)00178-4
 73. Wiesen E, Diorditsa S, Li X. Progress towards hepatitis B prevention through vaccination in the Western Pacific, 1990-2014. *Vaccine*. 2016;34(25):2855-2862. doi:10.1016/j.vaccine.2016.03.060
 74. André FE, Zuckerman AJ. Protective efficacy of hepatitis B vaccines in neonates. *J Med Virol*. 1994;44(2):144-151. doi:10.1002/jmv.1890440206
 75. Onakewhor JUE, Charurat M, Matthew O, Osagie E, Asemota MO, Omoigberale A. Immunologic pattern of hepatitis B infection among exposed and non-exposed babies in A PMTCT program in low resource setting: Does every exposed newborn require 200IU of hepatitis B immunoglobulin? *J Vaccines Vaccin*. 2013;4(7). doi:10.4172/2157-7560.1000207
 76. Hall AJ, Roberston RL, Crivelli PE, et al. Cost-effectiveness of hepatitis B vaccine in The Gambia. Published online 1993.

77. Griffiths UK, Hutton G, Das Dores Pascoal E. The cost-effectiveness of introducing hepatitis B vaccine into infant immunization services in Mozambique. *Health Policy Plan.* 2005;20(1):50-59. doi:10.1093/heapol/czi006
78. Wilson P, Parr JB, Jhaveri R, Meshnick SR. Call to Action: Prevention of Mother-to-Child Transmission of Hepatitis B in Africa. *J Infect Dis.* 2018;217(8):1180-1183. doi:10.1093/infdis/jiy028
79. Jack AD, Hall AJ, Maine N, Mendy M, Whittle HC. What Level of Hepatitis B Antibody Is Protective? *J Infect Dis.* 1999;179(2):489-492. doi:10.1086/314578
80. Banatvala JE, Van Damme P. Hepatitis B vaccine - Do we need boosters? *J Viral Hepat.* 2003;10(1):1-6. doi:10.1046/j.1365-2893.2003.00400.x
81. Moturi E, Tevi-Benissan C, Hagan J, et al. Implementing a Birth Dose of Hepatitis B Vaccine in Africa: Findings from Assessments in 5 Countries. *J Immunol Sci.* 2018;2(SI1):31-40. doi:10.29245/2578-3009/2018/si.1104
82. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. *Lancet.* 2015;386(10003):1546-1555. doi:10.1016/S0140-6736(15)61412-X
83. Sadoh AE, Eregie CO. Timeliness and completion rate of immunization among Nigerian children attending a clinic-based immunization service. *J Heal Popul Nutr.* 2009;27(3):391-395. doi:10.3329/jhpn.v27i3.3381
84. Zimmermann P. Factors that affect the immune response to vaccination. *Clin Microbiol Rev.* 2019;32(2):1-50.
85. Freitas Da Motta MS, Mussi-pinhata MM, Jorge SM, et al. Immunogenicity of Hepatitis B vaccine in preterm and full term infants vaccinated within the first week of life. *Vaccine.* 2002;20(11-12):1557-1562. doi:10.1016/S0264-410X(01)00493-5
86. Blondheim O, Bader D, Abend M, et al. Immunogenicity of plasma-derived hepatitis B vaccine in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 1998;79(3):F206-F208. doi:10.1007/BF02802630
87. Fan W, Zhang M, Zhu YM, Zheng YJ. Immunogenicity of Hepatitis B Vaccine in Preterm or Low Birth Weight Infants: A Meta-Analysis. *Am J Prev Med.* 2020;59(2):278-287. doi:10.1016/j.amepre.2020.03.009
88. Lau YL, Tam AYC, Ng KW, et al. Response of preterm infants to hepatitis B vaccine. *J Pediatr.* 1992;121(6):962-965. doi:10.1016/S0022-3476(05)80352-X
89. Losonsky GA, Wasserman SS, Stephens I, et al. Hepatitis B vaccination of premature infants: a reassessment of current recommendations for delayed immunization. *Pediatrics.* 1999;103(2). doi:10.1542/peds.103.2.e14
90. Patel DM, Butler J, Feldman S, Graves GR, Rhodes PG. Immunogenicity of hepatitis B vaccine in healthy very low birth weight infants. *J Pediatr.* 1997;131(4):641-643. doi:10.1016/S0022-3476(97)70078-7

91. Huang F-Y, Lee P-I, Lee C-Y, Huang L-M, Chang L-Y, Liu S-C. Hepatitis B vaccination in preterm infants. *Arch Dis Child*. 1997;77:F135-F138. doi:10.1007/s004310051075
92. El-Gamal Y, Aly RH, Hossny E, Afify E, El-Taliawy D. Response of Egyptian infants with protein calorie malnutrition to hepatitis B vaccination. *J Trop Pediatr*. 1996;42(3):144-145. doi:10.1093/tropej/42.3.144
93. Tchakoute CT, Sainani KL, Osawe S, et al. Breastfeeding mitigates the effects of maternal HIV on infant infectious morbidity in the Option B R era. *AIDS*. 2018;32:2383-2391. doi:10.1097/QAD.0000000000001974
94. Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: Review of hypotheses and definition of main determinants. *Vaccine*. 2003;21(24):3406-3412. doi:10.1016/S0264-410X(03)00342-6
95. Cutts FT, Nyandu B, Markowitz LE, et al. Immunogenicity of high-titre AIK-C or Edmonston-Zagreb vaccines in 3.5-month-old infants, and of medium-or high-titre Edmonston-Zagreb vaccine in 6-month-old infants, in Kinshasa, Zaire. *Vaccine*. 1994;12(14):1311-1316. doi:10.1016/S0264-410X(94)80057-7
96. De Moraes-Pinto MI, Verhoeff F, Chimsuku L, et al. Placental antibody transfer: Influence of maternal HIV infection and placental malaria. *Arch Dis Child Fetal Neonatal Ed*. 1998;79(3):202-205. doi:10.1136/fn.79.3.F202
97. De Voer RM, Van Der Klis FRM, Nooitgedagt JE, et al. Seroprevalence and placental transportation of maternal antibodies specific for Neisseria meningitidis Serogroup C, Haemophilus influenzae Type B, diphtheria, tetanus, and pertussis. *Clin Infect Dis*. 2009;49(1):58-64. doi:10.1086/599347
98. Nash S, Mentzer AJ, Lule SA, et al. The impact of prenatal exposure to parasitic infections and to anthelmintic treatment on antibody responses to routine immunisations given in infancy: Secondary analysis of a randomised controlled trial. *PLoS Negl Trop Dis*. 2017;11(2):1-13. doi:10.1371/journal.pntd.0005213
99. Malhotra I, Mckibben M, Mungai P, Mckibben E, Wang X, Labeaud D. Effect of Antenatal Parasitic Infections on Anti-vaccine IgG Levels in Children : A Prospective Birth Cohort Study in Kenya. Published online 2015:1-18. doi:10.1371/journal.pntd.0003466
100. Klein SL, Jedlicka A, Pekosz A. The Xs and Y of immune responses to viral vaccines. *Lancet Infect Dis*. 2010;10(5):338-349. doi:10.1016/S1473-3099(10)70049-9
101. Kleina SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. *Trans R Soc Trop Med Hyg*. 2014;109(1):9-15. doi:10.1093/trstmh/tru167
102. Goldfarb, Johanna Baley, Jill Vanderbrug Medendorp, Sharon Seto, Dexter Garcia, Haydee Toy, Pearl Watson, Barbara Gooch, Manford Krause D. Comparative study of two dosing schedules for Engerix B.pdf. *Pediatr Infect Dis J*. 1994;13(1):18-22.

103. Ekra D, Herbinger KH, Konate S, et al. A non-randomized vaccine effectiveness trial of accelerated infant hepatitis B immunization schedules with a first dose at birth or age 6 weeks in Côte d'Ivoire. *Vaccine*. 2008;26(22):2753-2761. doi:10.1016/j.vaccine.2008.03.018
104. Nlend AE, Nguwoh PS, Ngounouh CT, et al. HIV-Infected or -Exposed Children Exhibit Lower Immunogenicity to Hepatitis B Vaccine in Yaoundé , Cameroon : An Appeal for Revised Policies in Tropical Settings ? *PLoS One*. Published online 2016:1-13. doi:10.1371/journal.pone.0161714
105. Abramczuk BM, Mazzola TN, Maria Y, et al. Impaired Humoral Response to Vaccines among HIV-Exposed Uninfected Infants □. 2011;18(9):1406-1409. doi:10.1128/CVI.05065-11
106. Mancinelli S, Pirillo MF, Liotta G, et al. Antibody response to hepatitis B vaccine in HIV-exposed infants in Malawi and correlation with HBV infection acquisition. 2018;(December 2017):1172-1176. doi:10.1002/jmv.25049
107. Hesseling AC, Blakney AK, Jones CE, et al. Delayed BCG immunization does not alter antibody responses to EPI vaccines in HIV-exposed and -unexposed South African infants. *Vaccine*. 2016;34(32):3702-3709. doi:10.1016/j.vaccine.2016.03.081
108. Reikie BA, Naidoo S, Ruck CE, et al. Antibody responses to vaccination among South African HIV-exposed and unexposed uninfected infants during the first 2 years of life. *Clin Vaccine Immunol*. 2013;20(1):33-38. doi:10.1128/CVI.00557-12
109. Hu Y, Wu Q, Xu B, Zhou Z, Wang Z, Zhou Y. Influence of maternal antibody against hepatitis B surface antigen on active immune response to hepatitis B vaccine in infants. 2008;26:6064-6067. doi:10.1016/j.vaccine.2008.09.014
110. Wang Z, Zhang S, Luo C, et al. Transplacentally Acquired Maternal Antibody against Hepatitis B Surface Antigen in Infants and its Influence on the Response to Hepatitis B Vaccine. 2011;6(9). doi:10.1371/journal.pone.0025130
111. Chen X, Gui XCX, Huang LZ, et al. Maternal anti- - HBVs suppress the immune response of infants to hepatitis B vaccine. *Viral Hepat*. 2016;23(February):955-960. doi:10.1111/jvh.12572
112. Federal Ministry of health NACP. *Federal Republic of Nigeria National Guidelines for HIV Prevention, Treatment and Care.*; 2014. <https://www.childrenandaids.org/sites/default/files/2017-05/Nigeria-Integrated-National-Guidelines-For-HIV-Prevention-treatment-and-care-2014.pdf>
113. Incorporated MB. Human anti-hepatitis B virus surface antibody (HBsAb) ELISA Kit. Published online 2022. <https://www.mybiosource.com/human-elisa-kits/anti-hepatitis-b-virus-surface-hbsab/3800892>
114. Schillie S, Vellozi C, Reingold A, et al. *Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices*. Vol 67.; 2018.
115. Zuin G, Principi N, Tornaghi R, et al. Impaired response to hepatitis B vaccine in

- HIV infected children. *Vaccine*. 1992;10(12):857-860. doi:10.1016/0264-410X(92)90050-T
116. Lao-araya M, Puthanakit T, Aurpibul L, Taecharoenkul S, Sirisanthana T, Sirisanthana V. Prevalence of protective level of hepatitis B antibody 3 years after revaccination in HIV-infected children on antiretroviral therapy. *Vaccine*. 2011;29(23):3977-3981. doi:10.1016/j.vaccine.2011.03.077
 117. Bureau PR. World Population Data Sheet. Published 2021. Accessed December 22, 2021. <https://www.prb.org/wp-content/uploads/2021/08/print-at-home-2021-world-population-data-sheet.pdf>
 118. Olakunde BO, Adeyinka DA, Olakunde OA, et al. A systematic review and meta-analysis of the prevalence of hepatitis B virus infection among pregnant women in Nigeria. *PLoS One*. 2021;16(10):e0259218. doi:10.1371/journal.pone.0259218
 119. Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. The relationship between concentration of specific antibody at birth and subsequent response to primary immunization. *Vaccine*. 2014;32(8):996-1002. doi:10.1016/j.vaccine.2013.11.104
 120. Novak D, Svennerholm AM. A comparison of seasonal variations in rotavirus antibodies in the breast milk of Swedish and Bangladeshi mothers. *Acta Paediatr Int J Paediatr*. 2015;104(3):247-251. doi:10.1111/apa.12841