MAKERERE UNIVERSITY

Childhood Immune Responses to Hepatitis B Vaccine in Uganda

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2020/HD07/23175U

A Thesis Submitted to the Directorate of Research and Graduate Training in Partial Fulfillment of the Requirements for the Award of a Degree of Doctor of Philosophy of Makerere University

August, 2025

DECLRATAION

I Fahad Muwanda, do declare that I am the author of all the work in this thesis, any non-original work has been dully referenced by acknowledging the authors, and has not been submitted to this or any other academic institution for the award of any qualification.

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DEDICATION

I dedicate this thesis to my beloved parents, Mr. Noah Muwanda and Mrs. Aminah Nanyonga for their invaluable sacrifice and effort that have propelled me to this notable academic achievement. My family (wife; Sumayiya Nambejja Muwanda and children; Zariah Muwanda, Abdul-Hakim Muwanda, and Abdul-Razaq Muwanda) has additionally been a resounding and apparent source of inspiration; whenever it lacked. For the entire academic audience that this thesis attracts, may the Almighty Allah reward the aforementioned persons abundantly.

ACKNOWLEDGEMENT

I am greatly indebted to the remarkably commendable guidance from my supervisors; Dr. Bernard Ssentalo Bagaya, Dr. Hakim Sendagire, and Dr. Gerald Mboowa. Their mentorship has duly transformed me into an outstanding biomedical scientist. I am also grateful to my doctoral committee members (Dr. Freddie Bwanga, Dr. Bernard Ssentalo Bagaya, Dr. Hakim Sendagire, Assoc. Prof. David P. Kateete, Dr. Beatrice Achan, Assoc. Prof. Ezekiel Mupere, and Assoc. Prof. Paul Norman) for the unwavering support and advice they extended to me as I pursued and accomplished this important academic milestone.

I am profoundly honored to be a recipient of a study award from the Islamic Development Bank (IsDB) Scholarship Division and two grants from the Government of Uganda through the Makerere University Research and Innovation Fund (MakRIF). This funding adequately facilitated my doctoral study to completion.

I am grateful to the administration and staff of Child and Family Foundation-Uganda (CFU) medical center for the support they extended to me during the initial phase of data collection. Participant enrollment and sample collection was meticulously handled by the richly knowledgeable and experienced research support team at CFU.

I acknowledge the immense contribution of Assoc. Prof. Paul Norman of the University of Colorado Denver – Anschutz Medical Campus, USA. Assoc. Prof. Norman offered to sequence DNA extracted from our study samples for Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) genotyping. As a doctoral committee member, his technical and financial support was integral to the accomplishment of the PhD study.

I am greatly indebted to the entire staff in the Department of Medical Microbiology, at Islamic University in Uganda and in the Department of Immunology and Molecular Biology at Makerere University for their technical guidance. The relentless support provided by the staff of Kibuli Muslim Hospital and Kibuli Muslim Hospital Health Training Schools is very much appreciated.

TABLE OF CONTENTS

DECLRATAION i
COPYRIGHT STATEMENT ii
DOCTORAL COMMITTEE iii
DEDICATION iv
ACKNOWLEDGEMENT v
LIST OF TABLES xii
LIST OF FIGURES xiii
LIST OF ACRONYMS/ ABBREVIATIONS xiv
OPERATIONAL DEFINITIONS xvii
LIST OF PUBLICATIONS xviii
ABSTRACT xx
CHAPTER ONE 1
1.0 INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 3
1.3 Research Justification 5
1.4 Objectives of the Research 5
1.4.1 General objective 5
1.4.2 Specific objectives 5
1.5 Study Hypothesis 6
1.6 The Theoretical and Conceptual Framework 6
..... 7
1.7 Research Significance 8
CHAPTER TWO 9
2.0 LITERATURE REVIEW 9
2.1 Hepatitis B virus and hepatitis B disease pathogenesis 9
2.1.1 The structural composition of HBV 9
2.1.2 The pathogenesis of HBV infection 10
2.2 History of HBV vaccine development 11
2.3 HBV vaccine immunization schedules 12
2.4 Childhood hepatitis B vaccination coverage 14

2.5 Effectiveness of childhood hepatitis B vaccination 15
2.6 Associated risk factors for low childhood seroprotection from hepatitis B vaccination 17
2.6.1 Role of HLA and TCR genotype diversity in childhood response to hepatitis B vaccine 19
2.6.2 Role of T-Cell Immunophenotypes in childhood response to hepatitis B vaccine 22
2.7 Estimation of HBV vaccine immune responses 23
2.8 Duration of persistence of protective hepatitis B vaccine antibody titers 24
CHAPTER THREE 26
3.0 GENERAL METHODOLOGY/MATERIALS AND METHODS 26
3.1 Study Setting 26
3.1.1 The Child and Family Foundation – Uganda (CFU) Medical Center 26
3.1.2 Makerere University Biomedical Research Centre's (MakBRC's) Genomics, Molecular and Immunology Laboratories (GMI Labs) 26
3.1.3 The Integrated Biorespository of H3-Africa Uganda (IBRH3AU) 27
3.1.4 The Makerere University Infectious Diseases Institute (IDI) Core Laboratory 28
3.1.5 The Viral Load/EID Reference Laboratory and the National Biorepository 28
3.2 Study Population 29
3.3 Laboratory activities and experimentation 31
3.3.1 Serum/plasma processing 31
3.3.2 PBMC processing 31
3.3.3 Serum/plasma/PBMC storage 33
3.3.4 Determination of hepatitis B virus infection status 34
3.3.4.1 Interpretation of the hepatitis B serological test results 35
3.3.5 Determination anti-HBs using the electrochemiluminescence immunoassay (ECLIA) 36
3.4 Ethical considerations 37
3.4.1 Ethical approvals 37
3.4.2 Confidentiality and data security 38
3.4.3 Conflict of interest 38
3.4.4 Intended use of results 38
CHAPTER FOUR 39
Hepatitis B Vaccine Seroprotection Rates Among African Children of up to 17 Years of Age 39
4.1 Abstract 41
4.2 Background 42
4.3 Materials and methods 44

4.3.1 Systematic review protocol registration, information sources, and search strategies 44
4.3.2 Eligibility criteria and study selection 45
4.3.3 Quality assessment and data management 48
4.3.4 Risk of bias in individual studies 50
4.3.5 Publication bias and data synthesis 50
4.4 Results 51
4.4.1 Study selection 51
4.4.2 HBV vaccine sero-protection rate among children of up to 17 years of age 55
4.4.3 HBV vaccine sero-protection rates among children up to 17 years of age in the Northern, Western, and Southern Africa regions 56
4.4.4 HBV vaccine sero-protection rates among children up to 17 years of age from 1999 to 202160
CHAPTER FIVE 63
Serological Status of Vaccine and Hepatitis B Virus Exposure among Under 5-Year-Old Children and Adolescents in Kampala, Uganda 63
CHAPTER 5A 65
Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda 65
5A.1 Abstract 68
5A. 2 Background 69
5A.3 Materials and methods 71
5A.3.1 Study Design, Population, and Settings 71
5A.3.2 Inclusion and Exclusion Criteria 71
5A.3.3 Sample Size Estimation and Sampling Procedure 72
5A.3.4 Data Collection Procedures 72
5A.3.5 Sample Collection and Processing 72
5A.3.6 Laboratory Analysis 73
5A.3.7 Data Analysis 73
5A.4 Results 74
5A.4.1 Socio-demographic characteristics 74
5A.4.2 Concordance among NOVA TEST, Fastep, and Beright Diagnostic Rapid Tests 74
5A.4.3 Doses of Hepatitis B vaccine received 75
5A.4.4 Qualitative Anti-HBs Status and Prevalence of HBV Infection among Study Groups 76
CHAPTER 5B 79
5B.1 Abstract 82

5B.2 Background 83
5B.3 Materials and methods 86
5B.3.1 Study Design and Setting 86
5B.3.2 Study Population 87
5B.3.2.1 Inclusion and Exclusion Criteria 87
5B.3.2.2 Sample Size and sampling method 87
5B.3.3 Data collection procedures 88
5B.3.4 Serum/Plasma processing, storage 88
5B.3.5 Determination of titers/levels of anti-HBs antibodies in participant's serum/plasma 89
5B.3.6 Data analysis 90
5B.3.7 Interpretation of anti-HBs titer results 90
5B.4 Results 90
5B.4.1 Socio-demographic characteristics 90
5B.4.2 Anti-HBs antibodies among study population 91
5B.4.3 Anti-HBs antibody titers by number of vaccine doses and age of participants 92
5B.4.4 Comparative analysis of qualitative and quantitative assays for the estimation of anti-HBs 93
CHAPTER SIX 95
6.1 Abstract 97
6.2 Background 98
6.3 Materials and methods 99
6.3.1 Study design, setting and population 99
6.3.2 Sample size estimation 100
6.3.3 Sampling method 100
6.3.4 Meta-Data Collection 101
6.3.5 Specimen retrieval, transportation, storage and thawing 101
6.3.6 Determination of hepatitis B surface antibody titers 101
6.3.7 Determination of HBV infection status 102
6.3.8 Interpretation of hepatitis B virus infection status 102
6.3.9 Data analysis 103
6.4 Results 104
6.4.1 Demographic characteristics 104
6.4.2 Hepatitis B virus exposure according to the results of the Vaxpert HBV Combo tests 104
6.4.3 Hepatitis B surface antibody (anti-HBs) titer levels from the automated Cobas E411 105

6.4.4 Factors associated with protective effects on anti-HBs titers 105
CHAPTER SEVEN 107
7.0 DISCUSSION 107
7.1.2 Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda 114
7.2 General study Limitations 129
CHAPTER EIGHT 130
8.0 GENERAL CONCLUSION 130
CHAPTER NINE 131
9.0 RECOMMENDATIONS 131
REFERENCES 133
APPENDICES 149
Appendix 1.0: UNCST Approval 149
Appendix 1.1: Approval from School of Biomedical Sciences Higher Degrees Research and Ethics Committee 151
Appendix 1.2: Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee 153
Appendix 1.3: Second Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee 154
Appendix 1.4: Third Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee 155
Appendix 1.5: Approval of an amendment to the proposal 156
Appendix 2.0: Questionnaire 157
Appendix 3.0 Permission for authors 159
Appendix 4.0 Publications 162

LIST OF TABLES

Table 1 Interpretation of the hepatitis B serological test results 36

Table 2 The characteristics of eligible studies included in the meta-analysis.....46

Table 3 Detailed Newcastle-Ottawa Scale for each included study in the meta-analysis**.**.....49

Table 4 Meta-analyses results of pooled prevalence of HBV sero-protection rates by age, African geographical region and year of scientific paper publication**.** 54

Table 5 Concordance among the HBV Rapid Diagnostic Tests 75

Table 6 Characteristics of the study participants, Kawempe Division, May-August 2023.....75

Table 7 Clinical test characteristics of the study participants, Kawempe, May-August 2023 78

LIST OF FIGURES

Figure 1 Conceptual Framework.....7
Figure 2 The diagram showing the structure of hepatitis B virus 10
Figure 3 The PRISMA flow chart summarizes the databases searched, screening procedure and the eligible studies for inclusion in data synthesis.....52
Figure 4 Records eligible for inclusion in systematic review and meta-analysis; A: - by country, B: - by age. 53
Figure 5: HBV vaccine seroprotection rates and publication bias in studies of children under 15 (A and B) and 15-17 years of age (C and D). 56
Figure 6: HBV vaccine seroprotection rates and publication bias in studies of children of up to 17 years of age in the Southern (A and B), Northern (C and D), and Western Africa (E and F) regions. 58
Figure 7: HBV vaccine seroprotection rates and publication bias in studies of children 15-17 years of age in the Northern (A and B), and Western Africa (C and D) regions. 59
Figure 8: HBV vaccine seroprotection rates and publication bias in scientific articles investigating children under 15 years of age; published from 1999 to 2012 (A and B) and 2016 to 2021 (C and D). 61
Figure 9: HBV vaccine seroprotection rates and publication bias in scientific articles investigating children 15-17 years of age; published from 2002 to 2013 (A and B) and 2015 to 2016 (C and D).....62
Figure 10: HBV; (A) surface antibody (anti-HBs); (B) surface antigen (HBsAg); (C) Number of participants positive for HBsAg according to anti-HBs serostatus, among this study's participants in Kawempe Division, May–August 2023.....77
Figure 11: Anti-HBs; (A) by numbers of doses received; (B) Percent of those with seroprotective titers by number of doses received; (C) levels by age categorization, among the study participants in Kawempe Division, May-August 2023.....93
Figure 12: Proportions (%) of children with protective levels of anti-HBs (as determined on the Cobas E411 system) that were detected (anti-HBs positive) by the rapid diagnostic tests (RDTs) Assays. 94
Figure 13: Illustration of the methods used for sampling, assaying and data analysis 103

LIST OF ACRONYMS/ ABBREVIATIONS

Anti-HBc: Hepatitis B core antibody

Anti-HBe: Hepatitis B early antibody

Anti-HBs: Hepatitis B surface antibody

ARV: Anti-retro viral treatment

AST: Aspartate amino transferase

BD: Birth dose

cccDNA: Covalently closed circular DNA

CD: Cluster of differentiation

CD4: Cluster of differentiation-4

CHB: Chronic Hepatitis B

CHS: College of Health Sciences

CKD: Chronic kidney disease

CPHL: Central Public Health Laboratories (CPHL)

EDTA: Ethylene Diamine Tetra Acetic Acid

EPI: Extended programme on immunization

HAART: Highly active antiretroviral therapy

HBcAb: Hepatitis B core antibody

HBeAb: Hepatitis B pre-core antibody

HBeAg: Hepatitis B pre-core antigen

HBsAb: Hepatitis B surface antibody

HBsAg: Hepatitis B Surface Antigen

HBV: Hepatitis B virus

HCC: Hepatocellular carcinoma

HCV: Hepatitis C virus

HLA: Human Leucocyte Antigen

IDI: Infectious Diseases Institute

INF-γ: Interferon gamma

IU/L: International units per liter

IUIU: Islamic University in Uganda

KIR: Killer cell immunoglobulin-like receptors

LMICs: Low- and Medium-Income Countries

MoH: Ministry of Health

MakBRC: Makerere University Biomedical Research Centre

MTCT: Mother-to-Child Transmission

NHLDS: National Health Laboratory and Diagnostic Services

NPHC: National Population and Housing Census

PBMCs: Peripheral Blood Mononuclear Cells

PC: Pre-core

PCR: Polymerase chain reaction

RDT: Rapid Diagnostic Test

REC: Research and Ethics Committee

SBS: School of Biomedical Sciences

SDG: Sustainable Development Goal

SNPs: Single Nucleotide Polymorphisms

TCR: T cell receptor

TLR: Toll Like Receptor

TNF- α: Tumor Necrosis Factor alpha

UN: United Nations

UNCST: Uganda National Council for Science and Technology

UNEPI: Uganda National Extended Program on Immunizations

UNHLS: Uganda National Health Laboratory Services

UPHIA: Uganda Population Based HIV-Impact Assessment

WHO: World Health Organization

OPERATIONAL DEFINITIONS

Acute infection: Phase of active replication of the virus associated with increased hepatitis B virus DNA or viral load and characterized by concomitant HBsAg, HBeAg, and HBcAb IgM seropositivity.

Chronic infection: Persistence of the infection for 6 months after exposure characterized by both HBsAg and anti-HBc (IgG) seropositivity.

Genotype: An individual's, organism's or cellular genetic constitution/makeup which can pertain to all genes or to a specific gene of the individual, organism or cell.

Genotyping: Process of determining difference in the genotype of an individual by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence.

Hepatitis B vaccine non-responder: A person who fails to achieve a protective antibody titer (anti-HBs < 10 mIU/mL) after completing two full, documented series of the hepatitis B vaccine.

Occult hepatitis B infection: A hidden form of the hepatitis B virus where viral DNA stays in the liver or blood, but standard hepatitis B surface antigen tests show a negative result.

Resolved infection: Clearance of the virus following an acute infection, marked by HBsAg negative, anti-HBc (IgG) and anti-HBs (IgG) seropositivity.

Seroprotection rate: Percentage of vaccinated individuals who achieve a hepatitis B surface antibody level of anti-HBs ≥ 10 mIU/mL

LIST OF PUBLICATIONS

Main publications in support of this PhD thesis

I. **Muwanda Fahad;** Edward Kiyonga; Joan Nambafu; Agnes Turyamubona; Hussein Mukasa Kafeero; Edgar Kigozi; Harriet Mupere Babikako; Enock Wekiya; Gerald Mboowa; David Patrick Kateete; Hakim Sendagire; Paul J. Norman; and Bernard Ssentalo Bagaya (2024): *Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda.* **Livers** 2024, 4, 550–561. DOI: 10.3390/livers4040039

II. **Muwanda Fahad;** Hakim Sendagire; Gerald Mboowa; David Patrick Kateete; Beatrice Achan; Ezekiel Mupere; Hussein Mukasa Kafeero; and Bernard Ssentalo Bagaya (2023): *A systematic review reveals that African children of 15–17 years demonstrate low hepatitis B vaccine seroprotection rates.* **Scientific Reports** 2023, 13 (1), 22182. DOI: 10.1038/s41598-023-49674-1

III. **Muwanda Fahad;** Edward Kiyonga; Joan Nambafu; Agnes Turyamubona; Hussein Mukasa Kafeero; Edgar Kigozi; Harriet Mupere Babikako; Enock Wekiya; Gerald Mboowa; David Patrick Kateete; Hakim Sendagire; Paul J. Norman; and Bernard Ssentalo Bagaya (2025): *Hepatitis B Vaccine-Specific Antibody Titers Among Children of up to 17 Years in Kampala, Uganda.* **African Health Sciences** 2025. Manuscript ID: WKR0-2025-08-1661

IV. Turyamubona, Agnes; **Fahad Muwanda;** Aidah Nanvuma; Moses Ocan; Bosco Kafufu; Flaviano Mugalula; Isaac Ssewanyana; Barbara Castelnuovo; Rose Nabatanzi; and Bernard Ssentalo Bagaya (2025): *Hepatitis B virus exposure among adolescents living with HIV: a cross-sectional study of samples referred to the national viral load and early infant diagnosis reference laboratory in Uganda.* **BMC Infectious Diseases** 2025, 25 (1): 1-8. DOI: 10.1186/s12879-025-11244-3

V. Kafeero Hussein Mukasa; Hakim Sendagire; Dorothy Ndagire; Abdul Walusansa; Ali Kudamba; Fahad Muwanda; Mariam Namusoke; and Ponsiano Ocama (2026): *Correlates of hepatitis B virus awareness and disease specific knowledge in Uganda: a meta-analysis and meta-regression of studies published between 2018 and 2025.* **BMC Infectious Diseases** 2026 DOI: 10.1186/s12879-026-14019-6

VI. Kafeero Hussein Mukasa; Hakim Sendagire; Dorothy Ndagire; Abdul Walusansa; Ali Kudamba; Fahad Muwanda; Mariam Namusoke; and Ponsiano Ocama (2025): *The epidemiology of hepatitis b virus infection in Uganda after two decades of vaccination: a meta-analysis and meta regression.* **BMC Infectious Diseases** 2025, 25 (1): 1174. DOI: 10.1186/s12879-025-11582-2

VII. Kafeero Hussein Mukasa; Ponsiano Ocama; Dorothy Ndagire; Abdul Walusansa; Mariam Namusoke; Ali Kudamba; **Fahad Muwanda** and Hakim Sendagire (2025): *The status of occult HBV infection in a high endemic region: risk of community HBV transmission and reactivation.* **BMC Research Notes** 2025, 18 (1): 255. DOI: 10.1186/s13104-025-07337-6

VIII. Namaganda Maria Magdalene; Hussein Mukasa Kafeero; Joyce Nakatumba Nabende; David Patrick Kateete; Charles Batte; Misaki Wanyengera; Daudi Jjingo; Moses Joloba; Florence Kivunike; Isaac Ssewanyana; Yunus Miya; Darius Kato; Simple Ouma; Frederick Elishama Kakembo; Stephen Kanyerezi; Jupiter Marina Kabahiita; **Fahad Muwanda** and Gerald Mboowa (2025): *Prevalence and predictors of virological failure among the people living with HIV on antiretroviral treatment in East Africa: evidence from a systematic review with meta-analysis and meta-regression of published studies from 2016 to 2023.* **HIV Research & Clinical Practice** 2025 Dec 31; 26 (1): 2490774. DOI: 10.1080/25787489.2025.2490774

ABSTRACT

Introduction: Hepatitis B virus (HBV) infection is still a major global public health problem with approximately 296 million people infected. Sub-Saharan Africa is disproportionately over-burdened by HBV infection, with Uganda's national prevalence at 10%. The Ministry of Health integrated childhood hepatitis B vaccination into the Uganda National Extended Program on Immunizations (UNEPI) in 2002 to reduce high morbidity and mortality rates. Childhood HBV immunization remains fundamental to elimination of HBV but the levels of African/Ugandan paediatric non-response and the underlying reasons largely remain unknown. This doctoral study sought to determine the extent and persistence of immuno-protection accorded by the vaccine in Uganda.

Methods: A cross-sectional study design was utilized to conduct four sub studies. For the first sub study, a systematic review was conducted to synthesize data on African pediatric HBV vaccine responses, by including 19 eligible articles out of the 197 initially downloaded and published from 1999 to 2021 from African Journals Online (AJOL), EMBASE, Scopus, and, PubMed. The second and third sub studies qualitatively and quantitatively determined the serological status of vaccine and HBV exposure among childhood recipients of the hepatitis B vaccine who were under 5 and 15-17 years of age and enrolled on the UNEPI after the vaccine's inception in 2002. The fourth sub study analyzed archived plasma samples to determine HBV and vaccine serological status among adolescent children living with HIV. For sub study two, three, and four, we assessed participants' exposure to infection using a 5-panel qualitative hepatitis B Combo RDT methodology. Subsequently, immune responses to the hepatitis B vaccine were assessed in these participants' serum or plasma using the quantitative electrochemiluminescence immuno assay (ECLIA).

Results: Our systematic review revealed significantly higher proportion of HBV vaccine seroprotection (69.07%) among African children under 15 years of age than children 15 to 17 years

(32.368%), 95 % CI = [34.2454% to 39.0847%] ($p < 0.0001$) . Findings from sub studies 2 and 3 showed that qualitative seroprevalence of hepatitis B surface antibody (anti-HBs) was 33.1%, 18.4%, and 5.9% in the under-5, among the 15–17-year-old study groups, and in adolescents living with HIV, respectively. However, quantitative anti-HBs analysis revealed that post vaccination, 74.3% of children under 5 years retained protective anti-HBs levels compared to only 14.6% among adolescents and 16.3% in adolescents living with HIV. The overall prevalence of HBsAg was 1.52%, with higher rates in adolescents (2.78%) than in children under 5 years (0.8%) and 2.0% among adolescents living with HIV.

Conclusions: While infant HBV vaccination under UNEPI, like in most African extended programmes on Immunization (EPIs), provides commendable immune protection in children under 5 years, seroprotection wanes significantly by adolescence, increasing infection risks, especially in children 15-17 years of age and adolescents living with HIV. The apparent breakthrough HBV infections further suggest that the development of targeted adolescent strategies and guidelines addressing waning immunity will be essential to achieve the 2030 HBV elimination goals in Uganda and similar African settings.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Hepatitis B virus (HBV) infection is a major global public health problem with 296 million people infected, representing a global and WHO Africa regional prevalence of 3.9% and 3.0% respectively (Y.-C. Hsu, Huang, & Nguyen, 2023; Organization, 2017b; Razavi-Shearer et al., 2018). The infection is a major cause of chronic liver disease and the leading cause of hepatocellular carcinoma (HCC) worldwide (Organization, 2021). Sub-Saharan Africa is disproportionately over-burdened by HBV infection, with Uganda's national prevalence of 10% and variation in regional burden qualifying it to be highly endemic (Bwogi et al., 2009; Emmanuel Ochola et al., 2013b). Whereas a cross-sectional population-based study in Northern Uganda reported a prevalence of HBV infection in children as high as 21.9 %, national serosurveys have indicated prevalence of 4.3 % in the childhood and adult population.

The HBV is a blood-borne pathogen that can be transmitted vertically from the mother to the child (MTCT) or horizontally through exposure to contaminated blood or body fluids between toddlers and young children, household contacts, during sexual intercourse, or via injection during drug use, and nosocomial transmission (Sausen et al., 2022; Void-Holmes & Cartee, 2024). Adolescents living with HIV are at risk of HBV infection due to immunosuppression, poor adherence to antiretroviral therapy (ART), increased HIV viral loads (Bentouhami, Chakib, & El Fane, 2019; Hudelson & Cluver, 2015) and indulgence in risky practices. HBV/HIV coinfection complicates disease management (Ahmadi Gharaei et al., 2021), with additional risks for pregnant adolescents, including vertical transmission (P. K. Kitandwe et al., 2021) to the unborn child. The

infection outcome is dependent on viral, environmental, and host factors. Substantial variation in the course of hepatitis B disease clinical outcomes exist, depending on the transmission pattern, timing of infection, gender, immune status, and underlying diseases in infected individuals (Rehermann & Thimme, 2019).

To reduce on the unacceptably high morbidity and mortality rates, the Ministry of Health integrated childhood hepatitis B vaccination on the Uganda National Extended Program on Immunizations (UNEPI) in 2002, and has conducted mass vaccination campaigns especially in Northern and West Nile regions of the country known to have high endemicity (Bwogi et al., 2009; Emmanuel Ochola et al., 2013c). However, despite two decades of incorporation of HBV vaccine in UNEPI, Uganda still experiences sporadic outbreaks and epidemics of hepatitis B disease; including outbreaks in 2014 and 2018 in the Northern districts of Gulu, Adjumani, and Moyo (Ssekamatte et al., 2020). Moreover, the Ministry of Health through a Ministerial Statement to parliament on the progress of the control of Hepatitis B Virus in Uganda, developed statutory instruments that declared HBV infection a public health threat (Ssekamatte et al., 2020).

The presence of immunoglobulin G (IgG) to HBsAg (anti-HBs) >10 mIU/mL after vaccination is considered protective against infection (Jack, Hall, Maine, Mendy, & Whittle, 1999). However, persons previously exposed to HBV will also have detectable anti-HBc (antibody to core antigen) and may have antibody to HBeAg (anti-HBe). Approximately up to 20% of immunocompetent HBV vaccine recipients fail to respond to the full vaccine doses, leaving them susceptible with potential for the chronically infected to remain as reservoirs that hinder elimination of HBV (Nashibi et al., 2015a; Sahana, Sarala, & Prasad, 2017a; Tripathy, Sati, Puspa, Shankar, & Singh, 2011b; S. Yang et al., 2016a). The HBV seroprotection levels are probably lower in the African region when compared with the more developed countries (Fahad Muwanda et al., 2023),

and a paucity of studies particularly in Uganda have characterized immune responses to the HBV vaccine among childhood recipients (Kimera et al., 2025; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024; Fahad Muwanda et al., 2023).

After the initial HBV vaccination series, it would be imperative to monitor the long-term immune persistence of protective anti-HBs over time to assess the effectiveness of immunization programs and also to determine the timing for a booster dose, especially in high risk populations like teenagers, adolescents living with HIV, and suboptimal responders (Fahad Muwanda et al., 2023; Turyamubona et al., 2025). Childhood HBV immunization remains fundamental to elimination of HBV but the levels of African/Ugandan paediatric non-response and the underlying reasons largely remain unknown. A cumulative population of non-responsive childhood vaccine recipients constitutes a formidable high-risk population with the potential to propagate hepatitis B infection further, thus eroding regional and global vaccination eradication gains.

This study intended to determine the extent of non-responsiveness among hepatitis B vaccine childhood recipients, and persistence of immuno-protection accorded by the vaccine in Uganda. This study's outcomes were to inform public health strategies, including immunization programs targeting reduction of HBV incidence, improving population quality of life, better health and household incomes.

1.2 Problem statement

Whereas successful integration of the commendably efficacious HBV vaccine on the UNEPI in 2002 has been a major achievement in the National eradication of HBV infection,

stability and persistence of protective antibody titers is not coherent for all vaccine recipients (Kimera et al., 2025; Ocan et al., 2022; Okwakol, 2021; Turyamubona et al., 2025). Moreover, approximately up to 20% of HBV vaccine recipients in Uganda are known to be non-responders even after completion of three or four doses (Kimera et al., 2025; Ocan et al., 2022). The protective efficacy of the HBV vaccine is generally low in the East African region as indicated by a recent study in Ethiopia that reported close to 5% seroprevalence of breakthrough HBV infection among fully vaccinated children of 5–12 years of age (Adugna et al., 2023). Another recent study in Ethiopian children revealed only 14.4% and 2.6% of protective anti-HBs antibodies (>10 IU/mL) among under-5-year-olds and in ≥10-year-old children (Alemayehu, Daba, & Buonsenso, 2024).

The vivid suboptimal immune responses to the HBV vaccine and waning of anti-HBs antibodies below protective levels demonstrated among African children of up to 17 years of age predisposes them to breakthrough HBV infections (Fahad Muwanda et al., 2023; Quaye, Narkwa, Domfeh, Kattah, & Mutocheluh, 2021). Since vaccination failure can predispose vaccinees to HBV infection, promote periodic outbreaks, and stagnate HBV seroprevalence; as is the case in Uganda (Bwogi et al., 2009; P. K. Kitandwe et al., 2021; Emmanuel Ochola et al., 2013b), it is appropriate that post-vaccination surveillance is carried out to assess the effectiveness of HBV vaccination programs. However, to the best of our knowledge, there is limited data and information on HBV seroprotection among vaccinated children in Uganda (Kimera et al., 2025; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024; Emmanuel Ochola et al., 2013b; Turyamubona et al., 2025). Our study therefore intended to determine the long term immuno persistence in a high-risk cohort of childhood vaccine recipients, to assess the need and timing for administration of booster dose vaccination. The study sought to inform vaccination/immunization programs intended to reduce HBV incidence, chronicity, and

propagation; and suppress the accumulation of a potent high-risk population of non-responsive childhood vaccine recipients in Uganda.

1.3 Research Justification

The loss of life associated with an otherwise immunizable disease is unacceptable; especially that HBV vaccine is known to be very efficacious. However, despite two decades of incorporating HBV vaccine on the UNEPI, Uganda still experiences sporadic outbreaks and epidemics of hepatitis B disease. This highlights the suboptimal performance of the Ministry of Health's (MoH's) intervention towards this infectious disease, hence the urgent requirement to monitor and evaluate public health potency of vaccination on most infectious diseases, including hepatitis B. This study's findings can inform policy makers about the persistence and extent of immune protection accorded by the HBV vaccine in Uganda.

1.4 Objectives of the Research

1.4.1 General objective

To determine the extent of non-responsiveness among hepatitis B vaccine childhood recipients, and persistence of immuno-protection accorded by the vaccine in Uganda.

1.4.2 Specific objectives

1. To assess the hepatitis B vaccine seroprotection rates among African children of up to 17 years of age.

2. To determine the serological status of vaccine and hepatitis B virus exposure among under 5 year old children and adolescents in Kampala, Uganda.
3. To determine the hepatitis B virus exposure status and anti-Hepatitis B Virus surface antigen antibodies among adolescent children living with HIV in Uganda.

1.5 Study Hypothesis

Variations and or non-responsiveness in immunological responses to the hepatitis B vaccine among childhood UNEPI recipients can be suitably identified by accurate diagnosis of protective hepatitis B surface antibodies.

1.6 The Theoretical and Conceptual Framework

Our study is based on the ontological assumption that vaccination is the best strategy to infectious disease eradication, if adequately administered. The epistemological assumption is that variations in vaccine response are dependent on a number of host and non-host related factors. Our study drew its theory from the social ecological model (SEM); an evolution of Bronfenbrenner's model of ecological human development (Bronfenbrenner, 1977). The model addresses the interdependence between environmental, cultural, and biological determinants of health and states that health promotion determinants are multi-factorial, some of which people have direct control over and some of which they do not (Davison et al., 2015). HBV infection outcome and HBV vaccine response is dependent on viral, environmental, and host factors (Rehermann & Thimme, 2019). However, host immunogenetic factors contribute up to 77% to that effect (Newport et al., 2004); substantial variation in the course of hepatitis B disease clinical outcomes, and vaccine

response heterogeneity largely depends on host biological factors like genetics, gender, immune status, and underlying diseases in infected or vaccinated individuals (Rehermann & Thimme, 2019). This study utilized a Conceptual Framework (Figure 1) derived from constructs of the theoretical model to investigate the role of host immunogenetic factors in HBV vaccine responses. The theoretical model also guided the development of the data collection instruments, research paradigm, conduct of laboratory assays, and the interpretation of the findings.

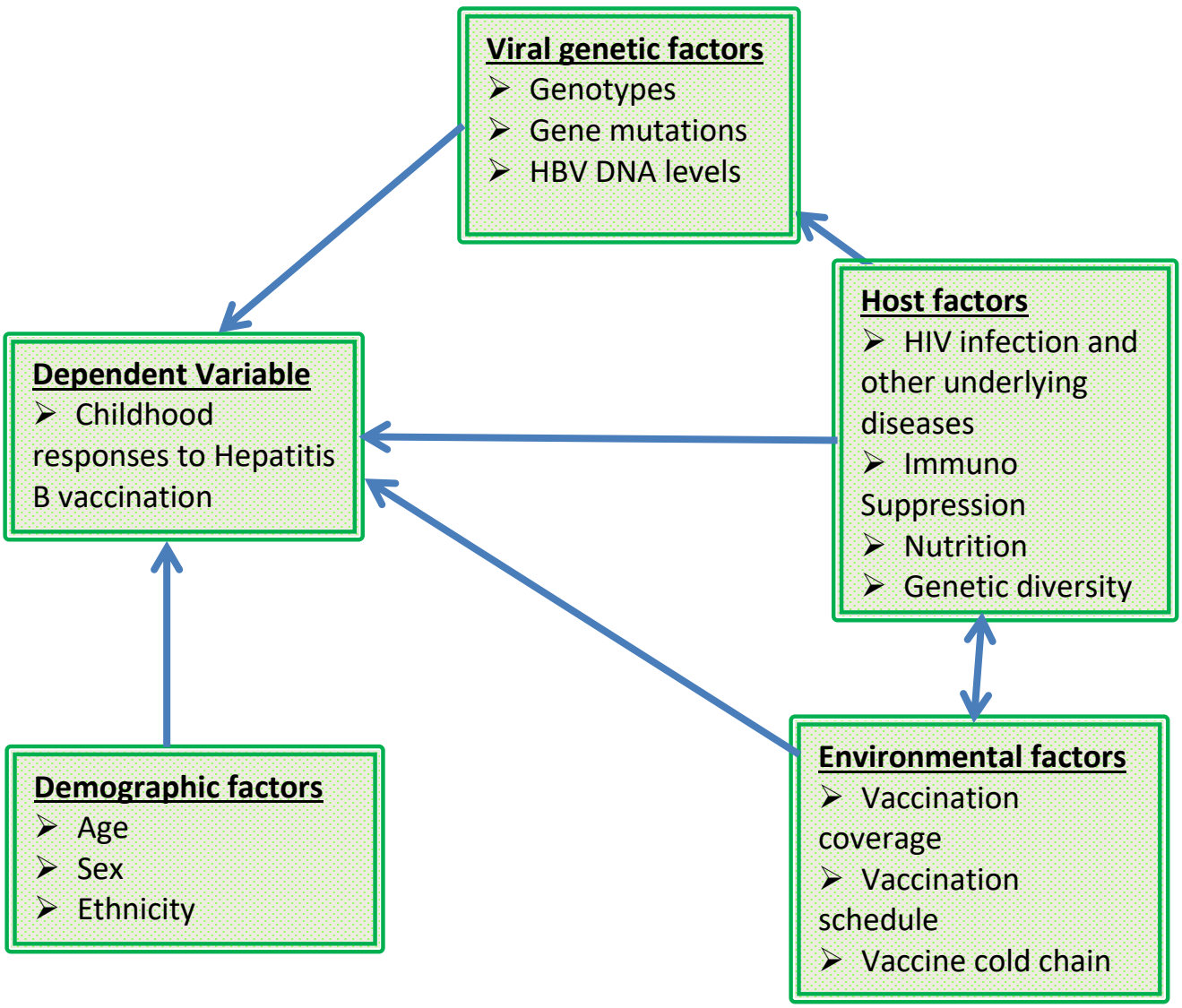


Figure 1 Conceptual Framework. *Childhood responses to HBV vaccine are dependent on viral, environmental, and largely host related factors. However demographic factors like age, sex, and ethnicity can variably confound or moderate responses to the vaccine, if they are not controlled.*

1.7 Research Significance

This study's quantification of immunological non-responsiveness to hepatitis B vaccine among childhood recipients of the vaccine less than 5 years of age and the persistence of sero-protection accorded by the vaccine until 15 to 17 years, will inform the UNEPI on evaluation of its efficacy and the need to administer booster dose vaccination to the high risk 15-to-17-year age group. This study avails data on HBV vaccine seroprotection to Uganda's Ministry of Health (MoH), UNEPI, and other stakeholders to inform policy formulation. Additionally, this study demonstrates the performance levels of qualitative Rapid Diagnostic Tests-based methods for the detection of anti-HBs antibodies. This study has also empowered me with skills transferable to my students in line with government's human capital development goal in Uganda's vision 2040 development agenda.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hepatitis B virus and hepatitis B disease pathogenesis

2.1.1 The structural composition of HBV

HBV is a partially double-stranded DNA virus that belongs to the *Hepadnaviridae* family. It consists of a complete virion (Dane particle) with a spherical double-shelled structure of approximately 42 nm in diameter. The Dane particle comprises two smaller spherical and filamentous particles consisting of the hepatitis B surface antigen (HBsAg) complexed with host lipids but lacking viral DNA (Mironova & Ghany, 2024). The viral genome and viral proteins are enclosed in the core particle, which is a nucleocapsid composed of core antigen (HBcAg). The core particle is surrounded by the Dane particle.

The HBV genome has four overlapping open reading frames that encode for seven viral proteins: core, polymerase, e antigen, large, middle, small surface antigen, and the x protein (C.-L. Lin & Kao, 2015). In hepatocytes, the primary replication site of HBV, the viral genome can integrate into the host genome by replication which occurs through an RNA intermediate. There are eight well-established genotypes, A-H, and two additional possible genotypes, I-J (Tong & Revill, 2016). These genotypes are based on sequence variability of the surface gene. There are four serotypes, *adw, ayw, ayr*, and *adr*, all of which share a common "a" determinant. Neutralizing anti-HBs target the common "a" determinant; explaining the broad protection provided by HBV vaccine (C.-L. Lin & Kao, 2015; Romanò et al., 2015; Tong & Revill, 2016).

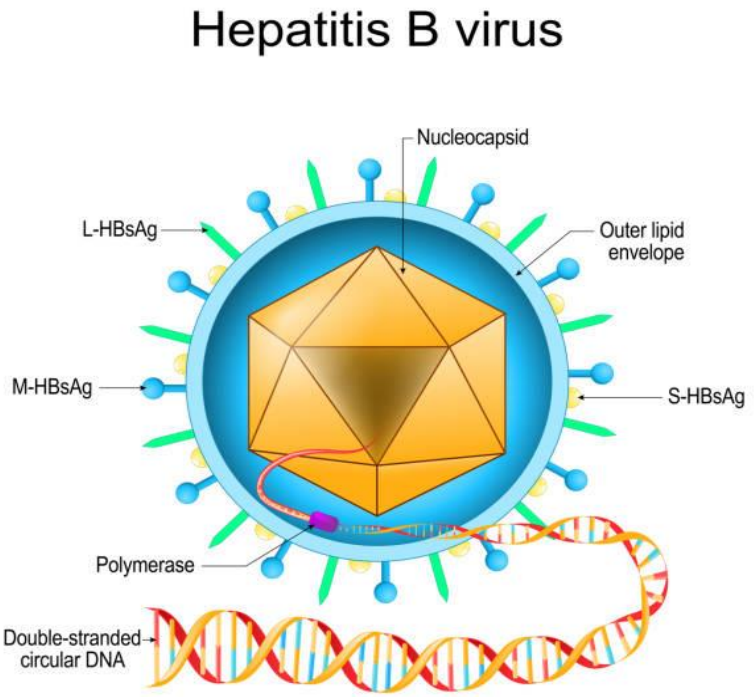


Figure 2 The diagram showing the structure of hepatitis B virus
(Source:https://www.istockphoto.com/vector/structure-of-hepatitis-b-virus-virion-anatomy-gm1907508727-554607663)

Abbreviations: - L-HBsAg: large hepatitis B virus surface antigen, M-HBsAg: middle hepatitis B virus surface antigen, S-HBsAg: small hepatitis B virus surface antigen, DNA: de-oxy ribose nucleic acid

2.1.2 The pathogenesis of HBV infection

HBV is a blood-borne virus that is transmitted vertically through mother-to-child transmission (MTCT) or horizontally, through exposure to contaminated blood or body fluids between toddlers and young children, household contacts, during sexual intercourse, via injection drug use, as well as nosocomial transmission (Sausen et al., 2022). Whereas the virus is primarily transmitted horizontally in low prevalence regions, variation in transmission occurs in high HBV prevalence regions. Notably, vertical transmission is predominant in Asia due to a high prevalence of hepatitis B e antigen (HBeAg) positivity among women of childbearing potential. In sub-Saharan

Africa the primary mode of transmission is horizontal between toddlers and children, with most being infected by school age (Sausen et al., 2022). The initial HBV infection is usually asymptomatic and anicteric, especially when the infection is acquired vertically. Approximately 30% of adults may present with jaundice and although acute liver failure is uncommon, it occurs in both adults and children (Chang, Lee, Chen, Hsu, & Lai, 1987; Wai et al., 2005). Development of chronic infection occurs in >90% of persons less than one year of age, 30% in those less than five years of age, and less than 10% in adults. It is estimated that 25% of persons with chronic HBV infection are at risk for developing cirrhosis (Noele P Nelson, Easterbrook, & McMahon, 2016). Much as approximately 20% of hepatocellular carcinoma (HCC) can develop in the absence of cirrhosis, the annual risk of HCC is 2-4% in the presence of cirrhosis.

2.2 History of HBV vaccine development

Blumberg and Millman initially proposed the concept for developing a hepatitis B vaccine using HBsAg derived from human plasma in 1969 (Maugh, 1975). Throughout the 1970s, efficacy of a purified, heat-inactivated plasma containing HBsAg obtained from the blood of HBsAg-positive persons, was tested for immunogenicity and efficacy in chimpanzees, and it was about 70% protective (Hilleman et al., 1975; Krugman, Giles, & Hammond, 1971; Maupas, Coursaget, Goudeau, Drucker, & Bagros, 1976; Purcell & Gerin, 1975). The first plasma-derived vaccine, Heptavax-B, was manufactured by Merck and approved by the Food and Drug Administration in 1981 (Krugman, 1982).

Challenges posed by the use of plasma-derived vaccine and the advent of recombinant DNA technology led to the development of the first human recombinant vaccine in 1986 (McAleer et al., 1984). The HBsAg was expressed in vector-transformed *Saccharomyces cerevisae* yeast cells and

the vaccine elicited similar anti-HBs seroconversion rates to the plasma-derived vaccine (Jilg et al., 1984; Valenzuela, Medina, Rutter, Ammerer, & Hall, 1982; Yamamoto, Kuroki, Kurai, & Iino, 1986). Recombinant vaccines like Recombivax-HB (Merck) and Engerix-B (GSK) have been used successfully for almost four decades.

To mitigate the challenges like inadequate seroprotection in certain populations, such as older and immunocompromised individuals and patients with chronic kidney disease (CKD of posed by these vaccines, and to reduce the number of doses, two third generation vaccines were developed. Heplisav-B, or Hep-CpG, containing the small S protein along with a novel cytosine phosphoguanine (CpG) immunostimulatory adjuvant (CpG 1018), obtained regulatory approval in the U.S. in 2017 (Sablan et al., 2012). PreHevbrio (initially licensed as Sci-B-Vac), containing the small S protein and enhanced by addition of large (preS1) and middle (pre-S2) proteins, was approved in 2021. A two-dose course of Heplisav-B was associated with a faster and more durable seroprotection compared to conventional recombinant vaccines, especially in populations with low response rates (Jackson et al., 2018; Janssen, Heyward, Martin, & Janssen, 2015; Sablan et al., 2012). Studies with a three-dose course of PreHevbrio demonstrated that seroprotection rates were non inferior to the conventional recombinant vaccines, and a study in patients with inflammatory bowel disease (IBD) reported higher seroprotection rates compared to that of mono-antigen vaccines (Etzion et al., 2016; Vesikari et al., 2021).

2.3 HBV vaccine immunization schedules

The HBV vaccine is available as a single-antigen formulation that may be administered at birth, infancy, childhood, and adulthood. It can be part of combination vaccines for infant and childhood immunizations like DTaP-HepB-IPV (Pediarix), consisting of diphtheria, tetanus toxoid,

and acellular pertussis adsorbed (DTaP); inactivated polio vaccine (IPV) and hepatitis B (recombinant); DTaP-IPV-Hib-HepB (Vaxelis), a hexavalent preparation containing diphtheria, tetanus toxoid, and acellular pertussis adsorbed; inactivated polio vaccine, haemophilus influenzae type b (Hib), and hepatitis B (recombinant); and in combination with hepatitis A vaccine for both children and adults (HepA-HepB, Twinrix) (Organization, 2019).

The HBV vaccine is not known to affect the immunogenicity of other vaccines and the standard adult dose of recombinant hepatitis B vaccine is 10–40 µg of HBsAg while the pediatric dose is 5–10 µg of HBsAg, and immunocompromised patients may be vaccinated with higher doses (Greenberg, Wong, Partridge, Howe, & Ward, 2002; Halperin, McDonald, Samson, & Danzig, 2002). The vaccine is administered by intramuscular injection into the anterolateral aspect of the thigh in infants or the deltoid muscle in older children and adults (Organization, 2019). The intradermal injection may be a potential alternative route of administration that has been shown to induce higher rates of seroconversion in populations with low response, such as patients with IBD and those on hemodialysis (HD) (Ko, Lam, Cheung, Hung, & Leung, 2022; Yousaf, Gandham, Galler, Spinowitz, & Charytan, 2015). For primary prophylaxis, the WHO recommends two vaccination schedules depending on individual national immunization programs. The standard schedule for infants is three doses at 0 (birth dose (BD) vaccine), 1, and 6 months. A four-dose vaccination schedule includes a BD vaccine followed by three doses of a combination vaccine as per the combination vaccine schedule.

In Africa, only 14 out of 47 countries have introduced BD vaccination into their immunization calendars (Mironova & Ghany, 2024), with a majority of countries starting immunization at 6 weeks. A pentavalent DTaP-Hib-HepB vaccine containing diphtheria, tetanus toxoid, and acellular pertussis adsorbed, haemophilus influenzae type b (Hib), and hepatitis B (recombinant) is administered by UNEPI; and Uganda, like most countries in sub-Saharan Africa

introduced the birth dose in 2023 to restructure the vaccination schedule to 0 (BD vaccine), 6, 10, and 14 weeks (de Villiers, Nayagam, & Hallett, 2021b; Memirie et al., 2020a; Mutyoba et al., 2021; Rakiep, 2023). A delayed schedule for children and adults is similar to the three-dose schedule for infants. If the second or third dose is delayed it should be administered as soon as possible, but the vaccination series should not be interrupted (Organization, 2019). An accelerated schedule can be considered in certain groups with a need for rapid immunity as demonstrated in immunocompromised persons, patients with cirrhosis (Wigg et al., 2019), patients on HD (Imam, 2017), and high-risk pregnant women (Sheffield et al., 2011).

2.4 Childhood hepatitis B vaccination coverage

The WHO aims to eliminate viral hepatitis by the year 2030 (Organization, 2016a). Elimination of HBV transmission requires prevention of infections acquired perinatally, during childhood, and those acquired by adolescents and adults. Much as prevention by immunization remains the most effective way to control the spread of HBV infection; particularly in developing countries, it is estimated that annually, 27 million (3%) children worldwide do not receive the basic package of immunizations and yet 25% mortality of children under five years is due to infectious diseases preventable by vaccine (Alssamei et al., 2017a; Bhonsla et al., 2019). WHO–UNICEF estimated that in 2015, only 77% of infants in Africa received full vaccine coverage (Spearman et al., 2017; WHO, 2017). The WHO/UNICEF estimates of National Immunization Coverage (WUENI) notably improved to 89% for the 3rd dose in 2020 (Cherian & Mantel, 2020; Danovaro-Holliday, Gacic-Dobo, Diallo, Murphy, & Brown, 2021; Organization, 2021).

In Uganda, only 55% of children aged 12–23 months were found to be fully vaccinated, with coverage being relatively higher in urban areas (61%) than rural areas (50%) (Malande et al.,

2019). As a recommendation by WHO; to universally vaccinate all neonates and adolescents as well as adults who have not been previously vaccinated, hepatitis B vaccines were included on the Uganda National Expanded Programme on Immunization in 2002; and is administered to children at 0, 6, 10, and 14 weeks (Kimera et al., 2025; Malande et al., 2019; Mutyoba et al., 2021). Much as national acceptable coverage for every vaccine is 90%, the Uganda Demographic Health Survey (UDHS-2022) indicated notable declining trends in immunization coverage. This was envisaged by high infant mortality rates attributable to vaccine-preventable diseases like hepatitis B; whose vaccine coverage was 70% for all doses in the vaccination series. Whereas 68.1% of vaccinated children in Uganda, aged 12-33 months receive immunization services from a health unit and 10.6% from outreaches, 45% of children do not complete the immunization schedule as many parents drop out or fail to go for routine vaccination, potentially due to inadequacy in awareness and mobilization (Malande et al., 2019). Notably also, much as almost 7 in 10 children aged 12-33 months access vaccination at health facilities, there is evidence of parental apathy and misconceptions about the vaccination (Nsubuga et al., 2019).

2.5 Effectiveness of childhood hepatitis B vaccination

The WHO adopted a global hepatitis strategy of increasing vaccination coverage and effectiveness with the goal of eliminating viral hepatitis as a public health threat by 2030; targeting to reduce 90% and 65% of new cases of chronic hepatitis B and C, and mortality due to HBV and HCV infection respectively (Organization, 2016a). Globally, vaccination programmes have been effective in countries where both the vaccine coverage and immunological responsive rates are commendable. In rural China, neonatal vaccination reduced the mortality of infant fulminant hepatitis and severe end-stage liver disease with efficacies of 69% (95% CI 34–85) and 70% (15–

89), respectively in a HBV intervention study. Vaccination also reduced hepatocellular carcinoma incidence with an 84% efficacy (95% CI 23–97) (Qu et al., 2014). In Taiwan, universal vaccination, together with a catch-up vaccination programme and improved maternal screening, resulted in a decrease in the prevalence of HBsAg positivity in children younger than 15 years from 9·8% in 1984 to 0·3% in 2009 (Chen et al., 1996; H.-M. Hsu et al., 1988). A study conducted by Fuad et al. (2017) on children under five years in rural areas of Taiz, Yemen revealed a moderate protective rate against HBV infection. With a coverage rate of 87.3%, 72.2% of children responded to the vaccine with anti-HBs level ≥ 10 IU/L while 27.8% had non-protective anti-HBs levels of < 10 IU/L (Alssamei et al., 2017a).

Generally, there is a paucity of data/information on evaluation of EPIs in Africa and HBV vaccine seroprotection rates are profoundly reduced (Fahad Muwanda et al., 2023). In South Africa, following the introduction of universal HBV vaccination into the extended programme on immunization (EPI) in April, 1995, overall HBsAg seroprevalence declined from 12·8% in 1995 to 3% in 2009 in children younger than 5 years (Burnett, Kramvis, Dochez, & Meheus, 2012). In Uganda, childhood hepatitis B vaccine coverage is at 70% for all the doses in the vaccination series (UDHS-2022), and data/information on monitoring the effectiveness of vaccination programs is not conclusive. Whereas a recent study by Kimera et al. (2025) reported HBV seroprotection rates of 77.4 % among children under 5 years (Kimera et al., 2025), another recent study by Muwanda et al. (2024) revealed qualitative sero prevalence of anti-HBs at 33.1% and 18.4% in children under 5 and 15-17 years of age, respectively (Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024).

2.6 Associated risk factors for low childhood seroprotection from hepatitis B vaccination

The degree and duration of sero protection accorded by hepatitis B vaccine depends on a number of several endogenous and exogenous factors apparent at the time of administration of the initial vaccination series. These factors include: age, body mass index, sex, concomitant diseases (like HIV infection, diabetes, chronic liver/kidney disease), psychological stress, nutrition, smoking, alcohol consumption, race, type and quantity of vaccine administered, and vaccination schedule. (Bauer & Jilg, 2006; Gara et al., 2015; Walayat et al., 2015). Cohort studies have suggested that being male, a family history of hepatocellular carcinoma, cirrhosis, high HBsAg concentration, high HBV DNA concentration, HBV genotypes A and C, basal core-promoter mutations, and aflatoxin exposure are all important risk factors for low seroprotection from hepatitis B vaccination in sub-Saharan Africa (Kew, 2015; Spearman et al., 2017). A meta-analysis by Yang et al. (2016) revealed that male gender, both in Asians and non-Asians may be associated with non-response to the vaccine, owing to the opposing effects of the sex hormone androgen and estrogen in genetic regulation (S. Yang et al., 2016a). However, the hormones' joint effects on the epigenetic adjustment of genetic expression, and gene structure on the X chromosome differing between XX females and XY males, only partly and non-significantly account for the heterogeneity in vaccine response due to gender (Klein, Marriott, & Fish, 2015).

In consideration of timing and vaccination compliance, the conventional 0-1-6 month vaccination schedule could still be more recommendable in comparison with the 0-1-12 schedule. Conversely, some studies (De Schryver et al., 2011; Yao, Qiu, et al., 2015) observed no difference in seroconversion rates between these two schedules; nearly 100% in all cases, while another study (Yao, Li, et al., 2015) showed a higher seroconversion rate in individuals with the 0-1-12 schedule. The standard schedule as recommended by WHO for infants of three doses at 0 (BD vaccine), 1,

and 6 months, or a four-dose vaccination schedule including a BD vaccine followed by three doses of a combination vaccine as per the combination vaccine schedule demonstrates significantly improved seroconversion rates (Mironova & Ghany, 2024).

In a twins study assessing the genetic determinants of heterogeneity in response to the vaccines against hepatitis B, 60% of the phenotypic variance was interpreted for the anti-HBs immune response by additive genetic while 40% by non-shared environmental effects (Höhler et al., 2002). A meta-analysis by Yang et al. (2016) revealed that the percentage of non-responders after hepatitis B vaccine remarkably varied in ethnic groups, which may result from the difference of environmental surroundings, the mutation rate and genetic variability, especially at the human leucocyte antigen (HLA) genetic region (S. Yang et al., 2016a). However, because it is hard to accurately locate the variation affecting the HBV response in the HLA locus as a result of the long-range linkage disequilibrium in this area, it requires further studies to explore (Mentzer, O'Connor, Pollard, & Hill, 2015).

Efficient hemodialysis (HD), age, nutritional status, and systemic inflammation are determinants of an adequate response to HBV vaccination (Dede Sit, Atay, & Kayabaşı, 2015). However, Hashemi et al. (2011) stated that duration of dialysis, hemoglobin, parathyroid hormone level, and accompanying HCV infection do not affect immune response to HBV vaccination (B. Hashemi et al., 2011).

In patients with HIV, the seroconversion rate on vaccine administration varies from 18%-72% depending on the immune status of the patient. In patients not receiving highly active antiretroviral therapy (HAART), the rate of immune response can vary from 30%-50% while in those receiving HAART the response increases to 60%-70% and is directly proportional to the CD-4 count and inversely proportional to the viral load (Rubin et al., 2014). A recent study that we conducted in Uganda indicates that vaccination-induced immunity was found in only 4.9% (20/405)

of adolescents living with HIV referred for viral load testing at the national reference laboratory (Turyamubona et al., 2025).

Intradermal administration of the vaccine, which involves presentation of antigen into the dermis enhances the potential for activation of the immune cascade and development of protective antibodies, although less likely to achieve seroprotection on comparison with intramuscular administration (Sangaré, Manhart, Zehrung, & Wang, 2009). Rahman et al. (2000) compared the efficacy of intradermal vaccine and showed that it resulted in a significantly higher immune response as compared to the intramuscular (IM) form (Rahman et al., 2000). Both T and B cell responses are higher with intradermal form as compared to IM form, suggesting that the intradermal presentation of the antigen to dendritic cells, macrophages or Langerhans cells in the dermis might result in trapping of the antigen in the skin, resulting in a more robust and more sustained humoral and cell mediated response (Filippelli et al., 2014).

2.6.1 Role of HLA and TCR genotype diversity in childhood response to hepatitis B vaccine

Polymorphisms in the genes encoding the HLA and T-cell receptor (TCR) have been implicated as cardinal genetic factors in hepatitis B vaccine non-response (Godkin, Davenport, & Hill, 2005). The human leukocyte antigen (HLA) plays an important role in the presentation of the viral peptides to CD-4 T-helper cells, influencing the quality of both cell mediated and humoral immune responses. HBV vaccine non-responders may harbor unique mutations or defects in their HLA molecules, altering the context of antigen presentation and subsequent stimulation of T-helper cells. T-cell activation and proliferation modulates nearly all steps in immune responses to HBV vaccination (Saffar et al., 2014). Polymorphisms in the TCR influence the expression levels of IL-4, IL-6, and IL-10 following vaccination, and malfunctioning helper T-cells due to genetic

polymorphism in the T-cell receptor has been related to non-response to the vaccine (Xie et al., 2016b).

Certain HLA types are associated with increased or reduced antibody response to hepatitis B vaccines in different individuals (L. Wang, Zou, & Wang, 2016). HLA-A∗33, B62, DRB1∗04, and DRB1∗07 alleles showed positive associations with non-responsiveness (< 10 mIU/mL) or low antibody titers (< 100 mIU/mL) while alleles A∗02 and DRB1∗08 showed negative associations; in Korean infants who received HBV vaccination (Yoon et al., 2014). Notably, after stratification by other associated alleles at different loci, B62 and DRB1∗07 in this study were still independently associated with non-responsiveness.

A meta-analysis by Z.-K. Li, J.-J. Nie, J. Li, and H. Zhuang (2013) assessed the effect of HLA on immunological response to hepatitis B vaccines in healthy individuals, and showed that, for DRB1 alleles, the three HLA variants DRB1∗01, DRB1∗1301, and DRB1∗15 are associated with significantly increased antibody response to hepatitis B vaccines. However, DRB1∗03 (DRB1∗0301), DRB1∗04, DRB1∗07, and DRB1∗1302 showed opposite results. For DQB1 alleles, DQB1∗05 (DQB1∗0501), DQB1∗06, and DQB1∗0602 were shown to be associated with markedly increased antibody response to hepatitis B vaccine; DQB1∗02 (pooled OR = 0.27) showed opposite results (Li, Nie, Li, & Zhuang, 2013). In Turkey, a study by Gurkan et al. (2014) found positive correlations between four HLA-DR (HLA-DRB1∗04X, DRB1∗0401X, DRB1∗11/13, and DRB1∗0401X0201) haplotypes and non-responders but a negative correlation with the allele HLA-B13 (Mert, Sengul, Gul, Karakas, & Eyigun, 2014).

Significantly diminished secretion of both Th1 (IFN-γ) and Th2 (IL-4, IL-10) cytokines was observed in HBsAg-stimulated PBMCs from healthy Iranian neonate vaccinees expressing the HLA-DR7 compared to DR7 negative vaccinees (Jafarzadeh & Shokri, 2012). A genome-wide

association study of HBV vaccine induced response in an Indonesian population suggested that the BTNL2 gene might be associated with the immune response to hepatitis B vaccination. Tag single nucleotide polymorphisms (SNPs) rs9277535 and rs3135363, encompassing the HLA-DP, HLA-DR, and the BTNL2 gene, were the first to emerge from the conditional analysis and exhibited strongest association with the immune response to hepatitis B vaccination (Png et al., 2011).

An Iranian study investigated the possible association of *KIR (Killer cell immunoglobulin-like receptors)* genes, their known *HLA* ligands, and compound *KIR–HLA* genotypes with hepatitis B virus (HBV) infection. The study indicated that frequencies of the *KIR2DL5A*, *KIR2DS1*, and *KIR3DS1* genes were significantly elevated in recovered individuals when compared with both control and patient groups (Shah-Hosseini et al., 2017). Similarly, a study conducted in Cameroon showed that frequencies of *KIR2DL2* and *KIR3DS1* were significantly higher in uninfected healthy controls, compared with those from HBV infected group (Torimiro et al., 2020).

Upon evaluating the response to HBV vaccination, it is imperative that different HLA types of distinct ethnic groups are taken into consideration (Zeng et al., 2016). A study by Pan et al. (2012) evaluated two independent case–control study populations, and found that the SNP rs12133337 in the CD3Z gene was associated with low immune response to hepatitis B vaccination in both populations and that the abnormal expression or false assembly of the TCR-CD3 complex can result in T lymphocyte dysfunction (Pan et al., 2012). Conversely, findings from a study by Soroosh et al. (2003) are not consistent with the notion that defective TCR repertoire may be an explanation for unresponsiveness to recombinant HBsAg vaccine. This is because T-cell response to HBsAg is generally oligoclonal and involves multiple BV (beta chain variable gene segment) families. Additionally, overexpressed individual TCR BV genes and CDR3 (complementarity-determining region 3) length distributions in response to HBsAg are subject-dependent (Soroosh, Shokri, Azizi, & Jeddi-Tehrani, 2003).

2.6.2 Role of T-Cell Immunophenotypes in childhood response to hepatitis B vaccine

The activation and proliferation of T cells are important steps in the immune response to HBV vaccination (Lambert & Pinschewer, 2015; Saffar et al., 2014). The immune response is largely controlled by T cells, which can recognize the HBsAg and regulate other immune cells to maintain the production of hepatitis B surface antibodies (HBsAb), as one of the indicators of successful HBV vaccination (H.-C. Wang, Perry, & Sun, 2009). HBV vaccination stimulates specific antibody production by the activation of B cells, which is mediated by CD8+ cytotoxic T cells and CD4+ helper T cells (Rahman et al., 2000). Long-term protection by hepatitis B vaccination is dependent on the persistence of strong immunologic memory, which is a key characteristic of specific immune response and resides in memory B and T lymphocytes; sensitized through an initial exposure to a specific antigen (Saffar et al., 2014).

The immune response manifests itself as cell-mediated and humoral immunity, and the T cell immune response is vital for sufficient control and clearance of HBV. While the CD4+ T cells have a preferential Th-1 profile, which is specific to multiple epitopes of HBV antigens, CD8+ T cells eliminate HBV-infected cells via their cytotoxicity and antiviral activity by secreting antiviral cytokines such as interferons (IFNs) and tumor necrosis factor- (TNF-) α without cytolytic activity (Chawansuntati et al., 2018). To validate HBsAg-specific T- and B-cell reactivity and memory in vaccinees, a study by Simons et al. (2016) indicated that all vaccinees tested positive for TNF-α, IL-10, or IL-6 production by HBV surface antigen-specific T-Cells, regardless of anti-HBs level. The study additionally demonstrated that the frequency of natural killer T-Cells correlated with the level of anti-HBs level (Simons et al., 2016). The study further revealed that Vaccine recipients with anti-HB titers < 10 mIU/mL exhibited more frequencies of exhausted and regulatory T-Cells and less natural killer cells and B cells; compared to vaccinees with protective

anti-HB titers. Another study suggested that the standard HBV vaccination schedule might induce better production of TNF-α and IL-2 from CD4+ T cells in healthy individuals (Chawansuntati et al., 2018).

T-cell response of good responders to HBsAg vaccine is multispecific and polyclonal; numerous epitopes and restricting elements whereas the T-cell response of poor and non-responders is paucispecific and oligoclonal; with few epitopes and restricting elements (Desombere, Gijbels, Verwulgen, & Leroux-Roels, 2000). Diminished proliferation capacity and impaired IL-2, IL-10 and IFN-γ production of non-responder T cells to HBsAg may be caused by defective T-cell recognition of HBsAg and not inadequate antigen presentation by APC (Kardar, Jeddi-Tehrani, & Shokri, 2002). Failure of T cells to respond to HBsAg may also be explained by an excess of antigen-specific suppression mediated by regulatory T cells. This hypothesis is not well explained, as increased numbers of regulatory T cells (CD4+ CD25+ FoxP3+) were demonstrated in the blood of non-responders after normal hepatitis B vaccination, compared to high-responders (Z. Yang et al., 2008), and in hepatitis B vaccine non-responsive HIV-infected subjects (del Pozo Balado et al., 2010).

2.7 Estimation of HBV vaccine immune responses

Vaccine responses are measured by utilizing whole blood, plasma or serum samples to detect HBsAg and/or to quantify HBV DNA or neutralizing anti-HBs antibody titers in laboratory assays including; 1) enzyme immunoassays (EIA) like enzyme linked immunosorbent assay (ELISA), Lateral flow point of care (LPOC) assays e.g. the automated Immune Analytic Architect System, and the Electrochemiluminescence Immunoassay (ECLIA), 2) HBsAg rapid test strips like hepatitis B combo test, and 3) HBV genotyping. The enzyme immunoassays like the Architect

System, ELISA, and ECLIA are as highly sensitive and specific as the HBV genotyping assays although expensive. On the contrary the rapid test strips are less sensitive, specific, cheaper, and less time consuming and laborious to perform. Rapid test strips are antigen based, qualitative and screening assays while enzyme immunoassays are antibody based, quantitative and confirmatory assays for HBV. Additionally, genotyping assays are quantitative and confirmatory assays for HBV that are antigen based. (Malik, Zakaria, Majeed, & Ismail, 2022) (Zong, Gao, Chen, Luo, & Jiang, 2022) (Kafeero et al., 2022).

2.8 Duration of persistence of protective hepatitis B vaccine antibody titers

Much as universal vaccination with hepatitis B vaccine has been very effective in preventing infection with HBV and at reducing the development of chronic infection in young children from perinatal or early childhood exposures to HBV, the duration of protection accorded by the vaccine to infants, older children, and adults is not well understood (Bruce et al., 2016). A study by Bruce et al. (2016) demonstrated that of the 56% Alaska native adults and children that responded to the original primary vaccine series, but received no subsequent doses during a 30 year period, 51% had an anti-HBs level ≥ 10 mIU/mL. This study correlated initial anti-HBs level after the primary vaccine series with higher anti-HBs levels at 30 years; and in conclusion estimated that ≥ 90% of the participants had evidence of protection 30 years later, and that booster doses were not needed (Bruce et al., 2016).

Conversely, a study by Pileggi et al. (2017) revealed that over 25% of HBV vaccine recipients had an anti-HBs titer < 10 mIU/mL after 18 years or more from the primary vaccination. Findings from this study further suggested that in case a booster dose would be needed, the vaccination strategy should prefer administration of a vaccine adult dose during early adolescence,

as it could offer longer protection through adulthood (Pileggi, Papadopoli, Bianco, & Pavia, 2017). Zhao et al. (2019) established that primary vaccination provided sufficient protection among 17-20 year old Chinese childhood vaccine recipients and that there was no urgent need for booster immunization (Zhao et al., 2019a).

Currently, a booster dose, or subsequent vaccination after a primary vaccination series, is not recommended by the WHO or CDC for fully vaccinated immunocompetent individuals (Mironova & Ghany, 2024). Despite waning anti-HBs levels, the rapid and robust response to a booster dose among the majority (~90%) of healthy adults with anti-HBs < 10–12 mIU/mL suggests persistence of an amnestic response up to 30 years following primary vaccination. How long immune memory lasts is unknown and requires additional long-term follow-up studies. Booster doses, however, are recommended for persons receiving HD or who are otherwise immunocompromised if anti-HBs levels decline (Mironova & Ghany, 2024).

CHAPTER THREE

3.0 GENERAL METHODOLOGY/MATERIALS AND METHODS

The thesis research was done in four sub studies that are presented as stand-alone chapters (4, 5A, 5B, and 6). This chapter presents general methods that cut across two or more sub studies. Sub study specific methods and designs are presented in their respective chapters.

3.1 Study Setting

3.1.1 The Child and Family Foundation – Uganda (CFU) Medical Center

Participants for sub-studies described in chapter 5 (sub section 5A.3.1) were recruited from Kawempe Division of Kampala Capital City Authority, at the CFU Medical Center (www.cfufoundation.org). The CFU medical center is located at Mpererwe-Ttula, is a Christian-based private not-for-profit (PNFP) Non-Government Organization (NGO) that serves a catchment population of approximately 63,467 children (NPHC, 2024). The medical center opens 24 hours daily and receives an average of 226 children per month in its different clinics; including child health, nutrition, and outpatient clinics. In a bid to identify and increase access to vulnerable groups, the center runs integrated community and static outreach clinics, involving families, youth and children of Kawempe Division and countrywide to improve their health, nutrition, and livelihood.

3.1.2 Makerere University Biomedical Research Centre's (MakBRC's) Genomics, Molecular and Immunology Laboratories (GMI Labs)

The GMI Labs are located at Makerere University College of Health Sciences encompassing both the Genomics & Molecular Unit and the Immunology Unit. These laboratories provide

specialized services to healthcare providers, researchers, students, and policymakers, under the Department of Immunology and Molecular Biology; ensuring high-quality analysis for infectious disease studies. The Immunology Unit is located on the first floor of the School of Medicine building and offers a range of immunological assays and diagnostics like ELISA tests for markers such as CRP, HIV, Toxoplasmosis, Rubella, and HBsAg and also serology tests including COVID-19 rapid testing, HIV rapid test, RPR, TPHA, serum HCG and urine lam detection. The Genomics & Molecular Unit is on the third floor of the Pathology-Microbiology Building, and is equipped with state-of-the-art equipment including an Illumina MiSeq sequencer and a QIAxcel robotic PCR setup, supporting diagnostic, research, and training activities.

3.1.3 The Integrated Biorespository of H3-Africa Uganda (IBRH3AU)

The IBRH3AU is housed within the Makerere University Biomedical Research Centre (MakBRC). It is a state-of-the-art facility designed to serve as a world-class biorepository, providing well-annotated stored biospecimens to the H3Africa Consortium, African, and international researchers for genomic discovery projects. This facility enables the development and testing of new diagnostic tests, personalized treatment options, and innovative treatment methods through genomic analysis of its diverse collection, which includes DNA, RNA, PBMCs, serum, plasma, and associated phenotypic data accessible via the H3Africa Data and Biospecimen Access Committee (DBAC). The IBRH3AU has built capacity for biorepository services, including collection, processing, storage, and shipping of biospecimens to international standards, while also offering training resources in biorepository science and management to support its aims of maintaining infrastructure, enhancing capacity, and performing standardized operations for the H3Africa Consortium.

3.1.4 The Makerere University Infectious Diseases Institute (IDI) Core Laboratory

The Makerere University IDI Core Laboratory is a state-of-the-art laboratory located within the Infectious Diseases Institute on Mulago Hill in Kampala and was established from a collaboration between Makerere University and Johns Hopkins University. It is situated between the Mulago National Referral Hospital and the Makerere University College of Health Sciences. As a lead partner in strengthening laboratory systems, the laboratory is a College of American Pathologists (CAP) and Ministry of Health (MoH)–accredited facility and is renowned for its excellence in medical diagnostics and research. It supports both clinical and research work, conducting over 180,000 routine and complex tests annually, including HIV viral load testing, hepatitis B serology and antibody titers, SARS-CoV2- PCR tests, etc. It plays a vital role in supporting more than 100 ongoing research studies, thanks to its skilled staff, advanced equipment, and robust maintenance systems.

3.1.5 The Viral Load/EID Reference Laboratory and the National Biorepository

The Viral Load/EID Reference Laboratory serves as the national hub for HIV viral load testing and early infant diagnosis in Uganda, centrally managed by the Central Public Health Laboratories (CPHL) under the National Health Laboratory and Diagnostic Services (NHLDS)—formerly the Uganda National Health Laboratory Services (UNHLS). Blood samples collected from health facilities across the country are referred, processed, and archived in the Uganda National Biorepository at –80 °C. These archived plasma specimens are then retrieved by the Makerere University IDI Core Laboratory, where they undergo further testing such as hepatitis B serology and antibody titers.

3.2 Study Population

The PRISMA flow chart (Figure 3) summarizes the method for selection of articles included in first sub-study (systematic review presented as chapter 4). Initially, 197 records were retrieved through the primary database searches: PubMed (52), Scopus (20), EMBASE (20), AJOL (80), and Willey library (25). Of the 197 records, 50 records were selected based on the relevance of their titles and abstracts to HBV seroprotection in children. From these, 15 articles were excluded for dwelling on prevalence of HBV infection rather than vaccine seroprotection. Ultimately, 35 articles met the inclusion criteria from which the following studies were removed with reasons; 2 were not published in English, 9 were conducted beyond Africa, and 5 had inaccessible or insufficient data. In total, 19 studies with a total sample size of 10,730 were included in the meta-analysis (Table 2). Of these, 6 studies (31.6%) were from Egypt, 2 (10.5%) from Tunisia, 1 (5.26%) from Nigeria, 1 (5.26%) from Ethiopia, 3 (15.8%) from South Africa, 1 (5.26%) from Ghana, 1 (5.26%) from Senegal, 1 (5.26%) from Cameroon and 3 (15.8%) from Gambia (Figure 4). Consequently 9 (47.4%), 6 (31.6%), and 3(15.8%) records from North, West and Southern Africa regions were respectively included in the data synthesis.

Only a paucity of pediatric seroprotection studies has been published in the East African region. Therefore, one record from the East African region was not included in sub-group analysis. Additionally, there were no identifiable studies from the Southern Africa region for the children 15-17 years of age. Overall, 15 (65%) records covered children under 15 years of age and 6 (26%) for the children between 15-17 years of age with publication years ranging from 1999 to 2021, and 2002 to 2016 respectively. However, 2 (9%) records contained data for both the under 15 and 15-17-year-old children (Figure 3 and 4).

A cross-sectional study design was used to enroll 789 children for sub-studies two and three (presented as chapters 5A and 5B, respectively) between May and August 2023 from the Child and Family Foundation (CFU) medical center catchment area in Kawempe Division. The CFU medical center catchment area is located in Kawempe division, one of the five divisions of Kampala city. Kawempe division has 22 administrative parishes. CFU Medical Center conducts both statistics and mobile immunization services under the UNEPI. The statistical immunization services are scheduled for Monday and Friday, while the outreaches are conducted twice a month in the different parishes. All immunization services provided are recorded in the Ministry of Health's (MOH) health management information system (HMIS). This study population included children under 5 who had received the hepatitis B vaccine as indicated by the child health card and adolescents (15 to 17 years) who received the hepatitis B vaccine during childhood. All 789 children were qualitatively tested for HBV sero-markers in sub-study two, while 710 children were successfully tested for anti-HBs antibodies in sub-study three.

Sub-study four (presented as chapter 6) adopted a cross-sectional study design and utilized leftover archived clinical samples from the National Reference Laboratory for Viral Load/Early Infant Diagnosis (VL/ EID) of the Central Public Health Laboratories, Department of National Health Laboratories and Diagnostic Services (CPHL/NHLDS), Ministry of Health, Uganda. A total of 6,872 samples from adolescent (10–19 years old) patients were referred from hospitals across the country between January and September 2023 for HIV viral load testing at the CPHL/NHLDS. Among these, 532 adolescents and their guardians/parents provided verbal assent and written consent, respectively, for future use of their leftover samples for research. The samples were stored at −80°C in the Uganda National Biorepository located within the CPHL/NHLDS. Qualitative

hepatitis B serology and HBsAb titer determination were performed at the Makerere University Infectious Diseases Institute Core Laboratory (IDI Core Laboratory).

3.3 Laboratory activities and experimentation

3.3.1 Serum/plasma processing

Blood was collected from all participants by a designated study clinician or phlebotomist following pre-developed standard operating procedures (SOPs). For the under 5-year-old participants, 6 mL of venous blood was aseptically collected by venipuncture in the following order: One 4 mL Heparinized blood (Becton and Dickson Co. Ltd., Nairobi, Kenya) tube, and a 2 mL plain serum separation tube (Becton and Dickson Co. Ltd., Nairobi, Kenya). For the 15–17-year-olds, 4 mL of venous blood in a plain serum separation tube was collected. Serum separation was then conducted by centrifugation of the blood tubes at 1500× *g* for 15 min at room temperature CFU medical center laboratory where it was temporarily stored and shipped in batches to the Integrated Biorepository of H3 Africa-Uganda (IBRH3AU) for long term storage and testing later. HIV testing was also done at the CFU medical center laboratory. Plasma processing and storage was done at the MakBRC's Immunology Laboratory. To separate PBMCs, whole blood samples were layered; without centrifugation directly into a Falcon tube on top of Ficoll or Histopaque in a 1:2 ratio, with the tube slanted to avoid mixing of the blood and Ficoll layers. The plasma on top of the PBMC layer was then harvested using a Pasteur pipette and collected in well labelled cryotubes.

3.3.2 PBMC processing

Upon collection at the CFU medical center laboratory, whole blood samples were immediately transported to the MakBRC's Immunology Laboratory for peripheral blood

mononuclear cell (PBMC) isolation and storage. Isolation of PBMCs using the Ficoll over-layering method was carried out by ensuring that all reagents, except the freezing media, were at room temperature (18–25°C) before starting. Cryotubes for plasma storage were labeled with the sample ID, date, and patient's initials, with the number of tubes depending on client specifications or sample volume. Polypropylene tubes were also labeled with the sample ID, one for each sample. Blood samples were layered immediately without prior centrifugation to remove plasma. The layering was performed in Falcon tubes, with the blood sample placed on top of Ficoll or Histopaque in a 1:2 ratio. The Falcon tubes were slanted, and the blood sample, either diluted in PBS or whole blood, was carefully overlaid on the Ficoll without mixing the layers. For example, 30 ml of blood was layered over 15 ml of Ficoll Hypaque. The Falcon tubes were then transferred to a centrifuge and spun at 1500–1800 rpm for 30 minutes without brakes. After centrifugation, two layers were observed: plasma at the top and PBMCs (white buffy coat) just above the Ficoll layer. The plasma layer was harvested using a Pasteur pipette and stored in cryovials at –16 to –25°C. The white buffy coat was then gently harvested using a Pasteur pipette or 1 ml pipette tip and transferred into a labeled 15 ml Falcon tube containing 10 ml of complete RPMI to wash the PBMCs. The Falcon tubes were centrifuged for 10 minutes at 1500 rpm.

Afterward, in a biosafety cabinet, the media was poured off, and the cell pellet was gently resuspended by tapping the tube bottom. Five milliliters of complete media were added, and 10 µl of PBMCs were aliquoted into a cap for staining. Meanwhile, the tubes underwent a final centrifugation for 5 minutes at 1500 rpm to further wash the cells. Cell staining was performed by mixing the resuspended cells with trypan blue in a 1:1 ratio (e.g., 10 µl of resuspended cells with 10 µl of trypan blue). After mixing, 10 µl of the solution was transferred to a Corning cell counter's Cytosmart slide for automated counting. Live cells appeared colorless, whereas dead cells absorbed

the stain and appeared blue. PBMCs consisted of lymphocytes (7–10 µm), NK cells (6–7 µm), and dendritic cells (10–15 µm). Cells were considered for cryopreservation only if they exhibited at least 90% viability.

3.3.3 Serum/plasma/PBMC storage

All serum, plasma, and PBMC recoverable was stored at IBRH3AU until later testing. Following PBMC isolation, the plasma layer above the PBMC interface was harvested using a Pasteur pipette and transferred into labeled cryovials. The cryovials were stored in a freezer maintained at –16 to –25 °C. Cryopreservation was performed using cold freezing media containing DMSO as a cryoprotectant, as DMSO is toxic to cells at high temperatures. The prepared freezing media was stored at 2–8 °C for up to seven days before use. After the final wash step, the supernatant was poured off and the PBMC pellet was resuspended. Freezing media was then added according to the number of cryovials required. Each cryovial contained 1 ml of the PBMC and freezing media mixture; for example, 5 ml of freezing media was added to the resuspended cells in a Falcon tube when preparing five cryovials, and 1 ml of the mixture was transferred to each vial. Cells were not kept at room temperature in freezing media for more than 10 minutes. The cryovials were placed into a Mr. Frosty (cool cell) at room temperature and transferred into a –80 °C freezer, allowing the temperature to decrease steadily at a rate of 1 °C per minute. After approximately 24 hours, the cryovials were removed from the Mr. Frosty and transferred into liquid nitrogen (–197 °C) for long-term storage. The total turnaround time for PBMC processing and cryopreservation was approximately five hours. In cases where red blood cells were present in the PBMC preparation, RBC lysis was performed by adding 5 ml of RBC lysis buffer to the cells after pouring off the wash buffer, followed by incubation at room temperature for 10 minutes. The mixture was then centrifuged at 1800 rpm for 10 minutes, the supernatant was discarded, and the cells underwent a final wash with RPMI before proceeding with cryopreservation.

3.3.4 Determination of hepatitis B virus infection status

HBV infection rapid diagnostic tests (RDTs) were done in the MakBRC's Genomics & Molecular Laboratory. The HBV Combo Rapid Test is a qualitative, membrane-based immunoassay that we used to detect for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb or anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb or anti-HBe), and for hepatitis B core antibody (HBcAb or anti-HBc) in plasma or serum. Serum/plasma samples were tested using three and one different Hepatitis B Combo RDT test kits for sub studies described in Chapters 5A and 6 respectively: NOVA TEST-One Step Diagnostic Rapid Test (Global Science, Malaysia; Jaya, Selangor D.E., Malaysia, Cat#: 20221811HBV), Fastep Rapid Diagnostic Test (Healgen Scientific Limited Liability Company, Houston, TX, USA, Cat#: 21076412), Beright Diagnostic Rapid Test (Medhealth Biotechnology Limited, Shanghai, China, Cat#: 12216453), and Vaxpert Hepatitis B Combo tests (Catalog no. HBV23120001) with manufacturer reported sensitivity and specificity of (91.43% and 98.28%), (68% and 77%), (98.92% and 99.46%), (99.9% and 99.4%) respectively. Positive and negative controls (prepared and distributed to health facilities by the Uganda Virus Research Institute (UVRI)) were included in all kits and testing runs. Children who tested positive for HBsAg were referred to the CFU Medical Center for appropriate clinical management. During testing, the test cassettes were removed from the pouch and labeled with a sample identifier, and three drops of the serum/plasma were dropped on each sample port via the provided dropper. The results were read after fifteen minutes and no later than 20 minutes after sample addition. The thawed plasma/serum specimens reacted with recombinant antigens or specific antibodies on the test kit. The mixture then migrated laterally on each of the membranes chromatographically by capillary action and reacted with

recombinant antigen or antibody complexes on the membrane in the testline region. When the specimen contained the relevant antigen tested for, one coloured line appeared in the test line region. This indicated a positive result for each test. When the specimen did not contain the relevant antigen or antibody tested for, no coloured line appeared in the test line region, hence indicating a negative result. To serve as a procedural control, a coloured line always appeared in the control line regions indicating that proper volume of specimen had been added and membrane wicking had occurred.

3.3.4.1 Interpretation of the hepatitis B serological test results

Interpretation of serological results in relation to HBV exposure or vaccine status was performed according to the Australasian Society for HIV, Viral Hepatitis, and Sexual Health Medicine (ASHM)'s 2024 Clinical Tool for Decision Making in Hepatitis B (www.testingportal.ashm.org.au/hbv, accessed on 24 May 2024). Study participants with detectable HBsAg were considered HBV infected, with additional positive anti-HBc or HBeAg interpreted as having chronic or acute HBV infection, respectively. Participants who were negative for HBsAg, anti-HBc, with or without detectable anti-HBs were considered to have never been exposed to HBV infection. Those participants who were positive for anti-HBc and were also either positive or negative for both HBsAg and anti-HBs were considered to have been exposed to HBV infection. However, participants who were negative for HBsAg and positive for anti-HBc and positive or negative for anti-HBs were considered to have ever been exposed to and resolved HBV infection (Table 1).

Table 1 Interpretation of the hepatitis B serological test results

SN	HBV seromarkers				Serological interpretation	Immune status
	HBsAg	Anti-HBc	Anti-HBs	HBe		
1.	Positive	Positive	Negative/Positive	Negative/Positive	Chronic HBV infection	Non- immune
2.	Positive	Negative/Positive	Negative/Positive	Positive	Acute HBV infection	Non-immune
3.	Negative	Negative	Negative/Positive	Negative	Never been exposed	Susceptible or non-immune
4.	Negative/Positive	Positive	Negative/Positive	Negative/Positive	Ever been exposed	Immune/non-immune
5.	Negative	Positive	Negative/Positive	Negative	Exposed and resolved	Immune

www.testingportal.ashm.org.au/hbv

3.3.5 Determination anti-HBs using the electrochemiluminescence immunoassay (ECLIA)

Tittering for the anti-HBs was done at Makerere University IDI Core Laboratory. We used the electrochemiluminescence immunoassay (ECLIA) for the in vitro quantitative determination of anti-HBs in our participants' serum/plasma samples. The ECLIA is based on a sandwich principle where anti-HBs in the sample, biotinylated HBsAg, and HBsAg labeled with a ruthenium complex react to form a sandwich complex in the first incubation phase. After addition of streptavidin-coated microparticles in the second incubation phase, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are then

determined via a calibration curve which is specifically generated by the immunoassay analyzer as a 2-point calibration and master curve provided via the reagent barcode or e-barcode.

We automatically measured anti-HBs using the Elecsys Anti-HBs II (ECLIA) test reagent kits (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany, Cat#: 11876317122) on a cobas e 411 immunoassay analyzer (serial number: 6111-04). The kits had manufacturer-reported sensitivity and specificity of 100% and 99.62%, respectively. All test procedures followed the manufacturer's analyzer-specific assay instructions [https://www.roche.com/]. All kits and testing runs included positive and negative controls run at least once every 24 hours and following calibration of each reagent lot. The analyzer run 40 µL of each thawed sample and reported the anti-HBs concentration in international units per Liter (IU/L). Participants' samples with anti-HBs titers <10 mIU/ml were interpreted as Non-reactive/negative for anti-HBs and hence non-responsive to the HBV vaccine. Participants' samples with anti-HBs titers ≥ 10 mIU/ml were interpreted as reactive/positive for anti-HBs and therefore responsive to the vaccine.

3.4 Ethical considerations

3.4.1 Ethical approvals

Ethical approval to conduct this study was sought and obtained from the Research and Ethics Committee of the School of Biomedical Sciences, Makerere University College of Health Sciences (SBS-REC) and from the Uganda National Council for Science and Technology (UNCST) under registration numbers SBS-2022-184 and HS2604ES respectively. This study only enrolled participants whose parents/guardians were willing to give informed consent for their children. For our teenage study population, children provided assent in addition to the parental/guardian's consent.

3.4.2 Confidentiality and data security

For anonymity, participants' names were excluded from the samples. The samples were instead identified by unique laboratory identifiers.

3.4.3 Conflict of interest

The student had no apparent conflict of interest. The purpose of this study was to have an in-depth understanding of childhood responses to hepatitis B vaccine in Uganda and for academic award of a PhD in Biomedical Sciences.

3.4.4 Intended use of results

Notable publications have been derived from this thesis and a manuscript has been submitted for review in the *African Health Sciences* journal. The findings of this study will be used by the Uganda's Ministry of Health (MoH), UNEPI, and other integral stakeholders involved in developing policies and interventions aimed at reducing the public health threat and incidence of HBV infection. Additionally, findings will inform future hepatitis B virus related research to address notable knowledge gaps.

CHAPTER FOUR

Hepatitis B Vaccine Seroprotection Rates Among African Children of up to 17 Years of Age

Preamble

Hepatitis B virus (HBV) infection remains a significant public health concern in Africa, where perinatal and early childhood transmission contributes substantially to the disease burden. Despite the inclusion of the HBV vaccine in routine immunization schedules across many African countries, variations in vaccine uptake, immunization schedules, and health system capacities have resulted in differences in population-level immunity. Evaluating the extent and persistence of seroprotection in African children is critical for guiding immunization policy, determining the need for booster doses, and identifying gaps in vaccine coverage or immune response. Such evaluation is particularly important in high-endemic settings, where vaccine non-responders and those with declining immunity may remain susceptible to breakthrough infections, thereby sustaining HBV transmission (Adugna et al., 2023; Quaye et al., 2021). This chapter presents a systematic review aimed at synthesizing available evidence on HBV vaccine-induced seroprotection rates among children aged 0–17 years in Africa, providing insights into the effectiveness of current vaccination programs and informing future public health interventions. By collating and analyzing data from diverse settings, the review provides robust estimates of immunity levels across age groups, highlights trends in antibody persistence, and identifies research and programmatic gaps.

This work was published by the *Scientific Reports* journal (Springer Nature): See Appendix 4

DOI: 10.1038/s41598-023-49674-1

A Systematic Review Reveals that African Children of 15 to 17 Years Demonstrate Low Hepatitis B Vaccine Seroprotection Rates

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Disclosures

None

Acknowledgements

We acknowledge the ICT system management at Islamic University in Uganda- Kampala Campus for authorizing access to the database. We are also grateful to Dr. Ntanda K Moses, Makerere University College of Computing and Information Sciences for the ICT technical support.

Competing Interests

The authors declare no competing interests.

4.1 Abstract

Childhood HBV immunization remains globally fundamental to the elimination of hepatitis B virus (HBV). However, monitoring proportions of HBV vaccine seroprotection and their determinants among African Pediatric recipients is crucial. This study sought to verify extent of immune protection accorded by the HBV vaccine in African children of up to 17 years of age by pooling the prevalence of seroprotection reported by primary studies conducted in the Northern, Western, and Southern African regions. We included 19 eligible articles out of the 197 initially downloaded, published from 1999 to 2021 from African Journals Online (AJOL), EMBASE, Scopus, and PubMed. The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO), University of York Centre for Reviews and Dissemination, under the registration number CRD42022361277. Significantly higher ($p < 0.0001$) proportion of HBV vaccine seroprotection (69.07%) was found among children under 15 years of age than children 15-17 years (32.368%), 95 % CI = [34.2454% to 39.0847%]. Whereas successful integration of the HBV vaccine on the extended programs on immunizations (EPI) has been a major achievement in the reduction of HBV infection in Africa, markedly reduced HBV vaccine seroprotection is persistently demonstrated among adolescent children 15-17 years of age. Future studies are required to clarify the need for booster dose vaccination in most at risk populations and age groups.

Key words: Anti-HBs antibody, hepatitis B vaccine, seroprotection, Africa

4.2 Background

Hepatitis B virus (HBV) infection is still a major global public health problem, with 292 million people infected, translating into a global prevalence of 3.9% (Razavi-Shearer et al., 2018). Although universal vaccination had decreased the prevalence of HBV in children younger than 5 years from 4.7% in the pre-vaccination era (1980s to early 2000s) to 1.3% by 2015, prevalence in the WHO Africa region remains at 3%. Sub-Saharan Africa is disproportionately over-burdened (WHO, 2017). In highly endemic countries like Uganda, HBV national prevalence can be as high as 10% (Emmanuel Ochola et al., 2013b). In most of Sub-Saharan Africa, the HBV vaccine is administered in early infancy at 6, 10, and 14 weeks, but pediatric seroprotection proportions and rates are not determined or documented as part of national EPIs monitoring. Additionally, up to 20% of immunocompetent HBV vaccine recipients may fail to respond even to the full three doses (Nashibi et al., 2015a; Sahana et al., 2017a; Tripathy et al., 2011b; S. Yang et al., 2016a). For instance, Rey-Cuille et al., (2012) revealed as low as 43% and 58% HBV vaccine seroprotection rates among the under 4-year-olds in Dakar, Senegal in 2007 and 2010 respectively. However, in general the African pediatric HBV vaccine seroprotection remain variably characterized and inconclusive.

Monitoring of the long-term persistence of HBV vaccine responses informs design, review, and implementation of effective vaccination programs for adequate control, and or elimination of HBV infection. Adoption of, and for timing of booster doses, especially in suboptimal responders may in part be informed by such studies. Even in countries located within the same geographical settings, studies continue to find wildly differing HBV vaccine immune responses and rates of waning. In Tunisia, Ghana and Egypt, studies documented HBV seroprotection of 68.8%, 56% and 8.9% respectively among 17-year-olds (Chaouch et al., 2016; Eladawy, Gamal, Fouad, & El-

Faramawy, 2015; Quaye et al., 2021). The alarmingly low seroprotection proportion in Egypt was accompanied by a recommendation for a booster dose. A recent (2023) review of the Mauritanian Infant Hepatitis B vaccination Program revealed a seroprotective proportion of 93% among those under 2 years, but protection was only 58% and 29% among the 4-5 year, and 10-12 year old children respectively (El Hachimi et al., 2023). Therefore, as much as contemporary literature reports a 20 year or possibly lifelong seroprotection conferred by HBV vaccination (Romanò et al., 2017; Saffar et al., 2014; Zhao et al., 2019b), the periodic outbreaks of HBV and the stagnated HBV seroprevalence in Africa justify a need for investigation of proportions of HBV seroprotection in the era of infant vaccination.

Many African countries integrated HBV vaccination into their national extended programs for Immunization (EPI) by 2002, a 15–17-year age group gave us an opportunity to probe the persistence of HBV seroprotection in this age group as a surrogate for effectiveness of the immunization programs. Fledgling emancipation, combined with peer pressures exposes this age group to behaviors associated with increased HBV infection such as sexual activity, tattooing, and sharing of injectable or sharps. It is worth mentioning that populations at heightened risk of infection benefit most from having the optimal sero-protective levels of the HBV vaccine responses. Therefore, if boosting is needed, it should preferably be administered in the adolescent population.

Much as childhood HBV immunization remains fundamental to elimination of HBV, characterization of immune responses, their persistence to late childhood and their determinant factors are crucial to deployment of strategies to elimination of HBV infections. A cumulative population of non-responsive childhood vaccine recipients constitutes a formidable pool of high-risk population with the potential to sustain propagation of HBV infections further, thus eroding gains in regional and global prevention efforts. A meta-analysis to verify the extent of immune

protection accorded by the HBV vaccines to African children, and persistence up to 17 years is important and informs vaccinologists and programs to deploy optimized interventions, prior to later years associated with increased HBV transmission exposures and decreased protective immunity. This meta-analysis provides comprehensive findings on HBV vaccine seroprotection proportions among African children of up to 17 years of age. This will in turn inform public health strategies, including immunization programs targeting reduction of HBV incidence, improving population quality of life, better health, and household incomes.

4.3 Materials and methods

4.3.1 Systematic review protocol registration, information sources, and search strategies

This study sought to determine the proportions of seroprotection against hepatitis B vaccine among HBV vaccine childhood recipients of under 15, and 15-17 years in Africa. The study protocol was submitted for registration with the International Prospective Register of Systematic Reviews (PROSPERO), University of York Centre for Reviews and Dissemination (https://www.crd.york.ac.uk/PROSPERO). The study required no ethical review to be conducted. The findings of the review were reported based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 statement checklist (Page et al., 2021). The search scope included electronic databases; African Journals Online (AJOL), EMBASE, Willey library, Scopus, and PubMed, for studies published from 1999 to 2021 that investigated the seroprotection accorded by hepatitis B vaccine among childhood recipients under 15, and 15 to 17 years in Africa.

Weekly reviewers' evaluation meetings were conducted for detailed review of accessible full journal articles, titles and abstracts. Resolution of disagreements among the reviewers; Fahad

Muwanda, Hussein Mukasa Kafeero, and Bernard Ssentalo Bagaya (FM, HMK, and BSB) was done by consensus. Only qualified full text articles were retained for data extraction after explicit interrogation by FM, HMK, and BSB to identify duplicate records and other anomalies.

The population, comparison, and outcome (PCO) model were utilized as a search strategy. All studies assessing seroprotection to HBV that used study participants from Northern, Western, and Southern Africa regions were searched for data extraction. For comparison, the data on the prevalence of seroprotection to HBV infection were searched and then compared among children below 15 years and those between 15 to 17 years. The key outcome was sero-protective level of immune responses to HBV vaccine in the population, while the supplementary outcomes were the relative sero-protective proportions based on the diagnostic methods used for anti-HBs antibody detection (ELISA, EIA, ECLIA, and MEIA) and factors associated with the sero-protective levels.

Search terms were used either in isolation or in combination using the Boolean operators 'OR' and 'AND'. The titles and abstracts were searched for the keywords related to HBV vaccine seroprotection proportion or rate while the full text of each article was searched for the keywords related to protective anti-HBs titers and associated risk factors. The authors (FM, HMK, and BSB) separately extracted the following data: first author, year of publication, country, sampling technique, study design, sample size, method of detection and quantification of anti-HBs antibodies, and seroprotection rate (Table 2).

4.3.2 Eligibility criteria and study selection

Records were included in the systematic review and meta-analysis if they were full text, cross sectional or longitudinal study designs determining sero-protective anti-HBs titers (≥ 10 mIU/ml), conducted in Northern, Western, or Southern Africa, and published in English in peer reviewed journals between January of 1999 and December, 2021. We excluded case reports;

reviews; conference abstracts; studies with insufficient or inaccessible data; pre-prints; studies with a sample size < 100; studies that never described their sampling technique; studies that investigated seroprotection against other types of viral hepatitis (A, C, D, or E); studies in languages other than English; and studies published before January, 1999 or after December, 2021 (Table 2)

Table 2: The characteristics of eligible studies included in the meta-analysis.

First author	Year	Country	Sampling technique	Study design	Method of Analysis	Quality Score	Sample size	Sero-protection rate; %	
								<15	15-17
Makhlouf, N. A(Makhlouf et al., 2016)	2016	Egypt	Purposive	Cross sectional	MEIA and flow cytometry	9	440	63.9	15.9
Eladawy, M(Eladawy et al., 2015)	2015	Egypt	Purposive	Cross sectional	ELISA	8	225		8.9
Shaaban, F. M(Shaaban, Hassanin, Samy, Salama, & Said, 2007)	2007	Egypt	Consecutive	Cross sectional	ELISA	8	242	39.4	
El Sawy, I. M(El Sawy & Mohamed, 1999)	1999	Egypt	Consecutive	Cross sectional	MEIA	6	180	81.3	
Abushady, E. A(Abushady et al., 2011)	2011	Egypt	Purposive	Cross sectional	ELISA	8	700	42.8	
Tfifha, M(Tfifha, Kacem, Naija, et al., 2019)	2019	Tunisia	Purposive	Cross sectional	ECLIA	6	180	77.2	
Odusanya, O. O(Odusanya, Alufohai, Meurice, & Ahonkhai, 2011)	2011	Nigeria	Purposive	Cross sectional, case-controlled, descriptive	Computer processed lab results analyzed by Epi-Info software	8	822	61	

Chaouch, H(Chaouch et al., 2016)	2016	Tunisia	Stratified, two-stage random cluster	Cross sectional	MEIA, and ECLIA	9	1422	75.1	60.8
Teshome, S(Teshome et al., 2019)	2019	Ethiopia	Multistage probability	Prospective cross sectional	ELISA	8	450	54.3	
Prabdial-Sing, N(Prabdial-Sing et al., 2019)	2019	South Africa	Convenience	Cross sectional	ECLIA	7	450	67.3	
Tsebe, K. V(Tsebe et al., 2001)	2001	South Africa	Purposive	Cross sectional	Imx or Axsym kits (Abbort labs)	8	598	86.8	
Madhi, S. A(Madhi et al., 2019)	2019	South Africa	Purposive	Longitudinal	ECLIA	8	567	96.1	
Quaye, T(Quaye et al., 2021)	2021	Ghana	Purposive	Cross sectional	ELISA	8	350	56	
Van der Sande, M. A(Van der Sande et al., 2007)	2007	Gambia	Purposive/Random	RCT/Cross sectional	Commercial EIA	8	492		31.2
Rey-Cuille, M. A(Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012)	2012	Senegal	Purposive/consecutive	Cross-sectional	Commercial EIA	8	242	92	
Rey-Cuille, M. A(Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012)	2012	Cameroon	Purposive/consecutive	Cross sectional	Commercial EIA	8	242	58	
Whittle, H(Whittle et al., 2002)	2002	Gambia	Simple random/purposive	Cross sectional	ELISA	8	852		64
Mendy, M(Mendy et al., 2013)	2013	Gambia	Purposive	Cross sectional	ELISA	8	1276		22.4
Reda, A(Reda et al., 2003a)	2003	Egypt	Purposive (Systematic/stratified random	Cross sectional	ELISA	8	1000	67	

4.3.3 Quality assessment and data management

The Newcastle-Ottawa Scale (NOS) was used to assess quality (Stang, 2010). The three dimensions of selection, comparability and exposure were considered, as described in the scale. Articles were assigned one point for each attribute they adequately addressed within the above-mentioned dimensions. For this meta-analysis, the selection assessed the representativeness of the sample, the sampling technique, the sample size, the inclusion criteria, and the scope of the study. Comparability assessed the inclusion of seroprotection prevalence in the primary studies and compared them between vaccine recipients below 15 years and those 15-17 years. Sero -protection proportions across the Northern, Western, and Southern regions of Africa were also compared. Assessment of inclusion of the exposure in the primary studies was done by looking at the outcome of the study, and a statistical tool used for the analysis (Table 3). According to the scale, studies with scores of 9–8 were considered very high quality; 7–6, high quality; and 5–4, moderate quality. Those with scores ≤3 were considered unsatisfactory and were rejected. The three reviewers (FM, HMK, and BSB) independently assessed the articles for their overall methodological quality. The authors individually participated in daily entry of data derived from research papers, into the spreadsheet. The authors additionally removed duplicate papers after comparing their archives. During the review process (screening, eligibility, and inclusion in meta-analysis), the primary studies were independently assessed by three hepatitis B virus specialists (FM, HMK, and BSB). From each study, the following data were extracted: first author, year of publication, country, sampling technique, sample size, quality score (QS), method of detection and quantification of anti-HBs antibodies, and seroprotection rate.

Table 3 Detailed Newcastle-Ottawa Scale for each included study in the meta-analysis.

Study	Selection					Comparability		Exposure		QS
	Representativeness of the sample	Sampling technique	Sample size	Inclusion criteria stated	Scope of the study defined	Assessed for sero-protection	Compared HBV sero-protection among study groups	Assessment of outcome	Statistical test	
(Makhlouf et al., 2016)	1	1	1	1	1	1	1	1	1	9
(Eladawy et al., 2015)	1	1	1	1	1	1	0	1	1	8
(Shaaban et al., 2007)	1	1	1	1	1	1	0	1	1	8
(El Sawy & Mohamed, 1999)	0	1	0	1	1	1	0	1	1	6
(Abushady et al., 2011)	1	1	1	1	1	1	0	1	1	8
(Tfifha, Kacem, Naija, et al., 2019)	0	1	0	1	1	1	0	1	1	6
(Odusanya et al., 2011)	1	1	1	1	1	1	0	1	1	8
(Chaouch et al., 2016)	1	1	1	1	1	1	1	1	1	9
(Teshome et al., 2019)	1	1	1	1	1	1	0	1	1	8
(Prabdial-Sing et al., 2019)	1	0	1	1	1	1	0	1	1	7
(Tsebe et al., 2001)	1	1	1	1	1	1	0	1	1	8
(Madhi et al., 2019)	1	1	1	1	1	1	0	1	1	8
(Quaye et al., 2021)	1	1	1	1	1	1	0	1	1	8
(Van der Sande et al., 2007)	1	1	1	1	1	1	0	1	1	8
(Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012)	1	1	1	1	1	1	0	1	1	8
(Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012)	1	1	1	1	1	1	0	1	1	8

(Whittle et al., 2002)	1	1	1	1	1	1	0	1	1	8
(Mendy et al., 2013)	1	1	1	1	1	1	0	1	1	8
(Reda et al., 2003a)	1	1	1	1	1	1	0	1	1	8

4.3.4 Risk of bias in individual studies

To subdue the effects of selection bias, we reviewed the data collection procedures in the primary studies (retrospective or prospective), study design (cohort, cross-sectional, or case control), and recruitment strategy (from the community or from the hospital). For the information bias, we extracted information on methods used to detect and quantify anti-HBs antibodies (ELISA, ELISA, EIA, ECLIA, MEIA and other immune assays). When more than one method was used to detect and quantify anti-HBs antibodies, data were extracted using the more accurate method, as indicated by the evaluator. All studies with a sample size less than 100 were excluded to avoid biasing the results.

4.3.5 Publication bias and data synthesis

The Begg's test, which uses Kendall's rank correlation coefficient between the meta-analysis effect size and the study weight, was used to assess publication bias (Becker, 1997). Funnel plots further verified the existence of overall publication bias. The Begg's test determined funnel plot asymmetry between the effect size and the meta-analysis study weight. There was insufficient evidence to detect publication bias even when P was greater (>) than 0.05. The test I2 statistic was used to assess heterogeneity among studies (Table 4). Sources of heterogeneity were assessed by sub-group analysis, sensitivity analysis, and meta-regression. Pooled proportions, test of

proportions, relative risk (RR), and the corresponding 95% CI were used to assess seroprotection rates various geographical regions. The random-effects model (REM) was used to pool the seroprotection rates. All the calculations were done using the Medcalc software version 19.1.3. Seroprotection rates and its associated factors, and heterogeneity analyses among studies were done at 95% CI, and a $p < 0.05$ was considered significant. Forest plots were used to graphically present representative results (Figures 5: A, C; 6: A, C, E; 7: A, C; 8: A, C; 9: A, C). The seroprotection rate in each study in the forest plot is indicated by a blue square. The size of the square represents the weight contributed by each study in the meta-analysis. The pooled seroprotection rate for the REM is shown by the blue diamond.

4.4 Results

4.4.1 Study selection

The PRISMA flow chart (Figure 3) summarizes the method for selection of articles included in this meta-analysis. We initially retrieved 197 records through the primary database searches: PubMed (52), Scopus (20), EMBASE (20), AJOL (80), and Willey library (25). Of the 197 records, 50 records were selected based on the relevance of their titles and abstracts to HBV seroprotection in children. From these, 15 articles were excluded for dwelling on prevalence of HBV infection rather than vaccine seroprotection. Ultimately, 35 articles met the inclusion criteria from which the following studies were removed with reasons; 2 were not published in English, 9 were conducted beyond Africa, and 5 had inaccessible or insufficient data.

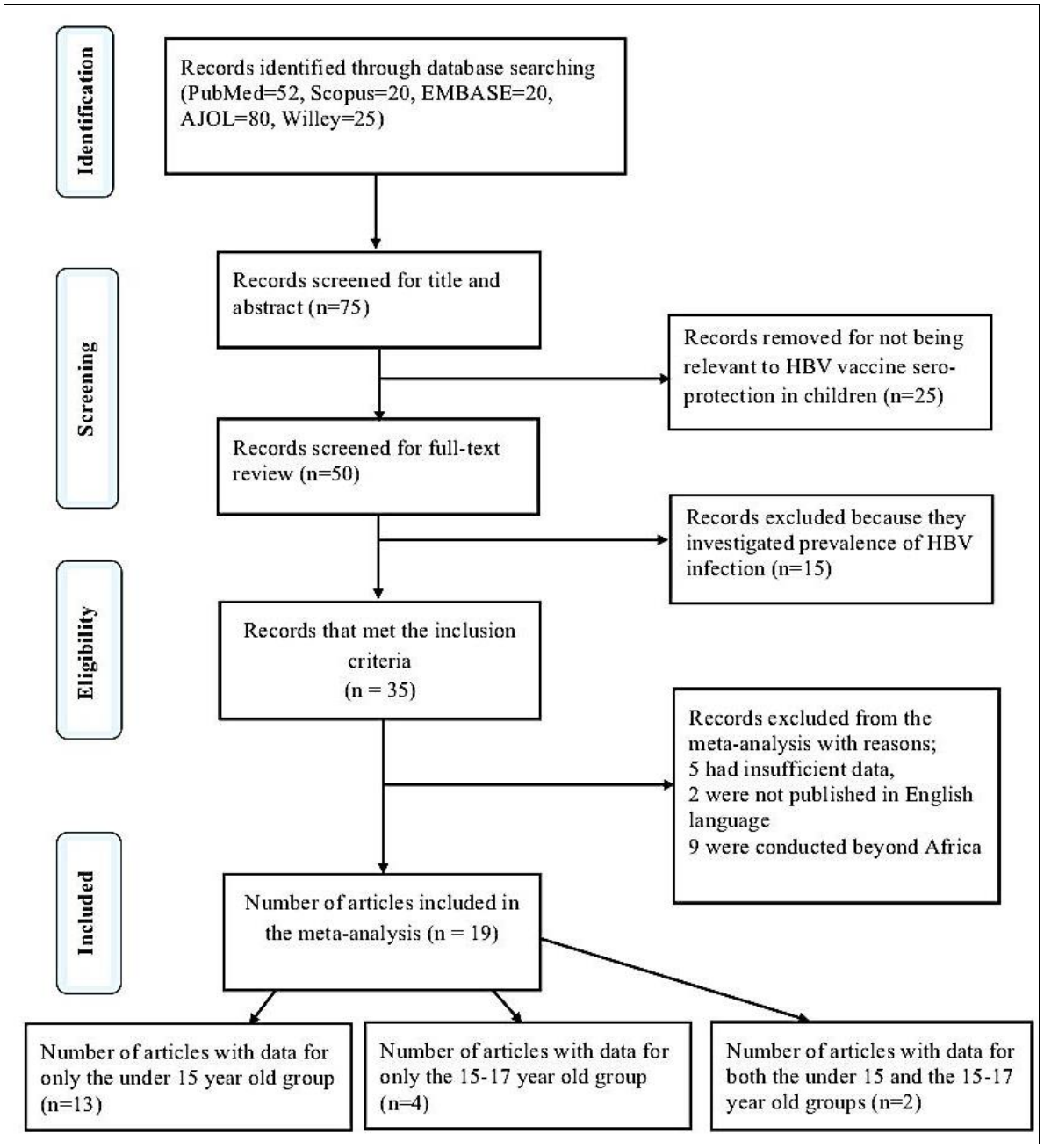


Figure 3 The PRISMA flow chart summarizes the databases searched, screening procedure and the eligible studies for inclusion in data synthesis.

In total, 19 studies with a total sample size of 10,730 were included in our meta-analysis (Table 2). Of these, 6 studies (31.6%) were from Egypt, 2 (10.5%) from Tunisia, 1 (5.26%) from Nigeria, 1 (5.26%) from Ethiopia, 3 (15.8%) from South Africa, 1 (5.26%) from Ghana, 1 (5.26%)

from Senegal, 1 (5.26%) from Cameroon and 3 (15.8%) from Gambia (Figure 4). Consequently 9 (47.4%), 6 (31.6%), and 3(15.8%) records from North, West and Southern Africa regions were respectively included in the data synthesis. Only a paucity of pediatric seroprotection studies has been published in the East African region. Therefore, one record from the East African region was not included in sub-group analysis. Additionally, there were no identifiable studies from the Southern Africa region for the children 15-17 years of age.

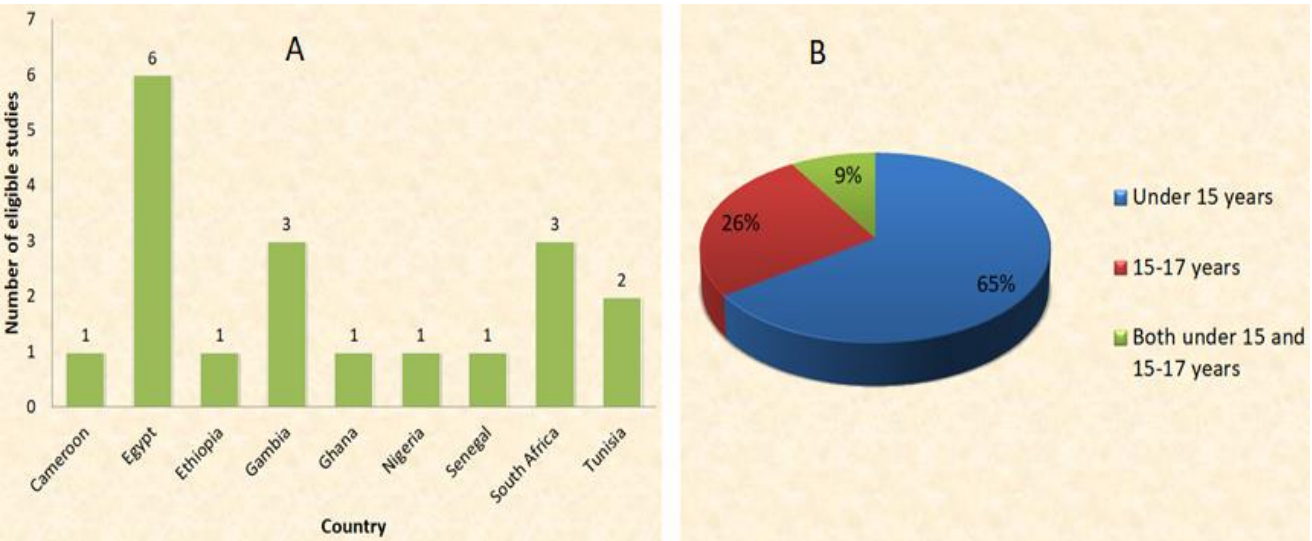


Figure 4 Records eligible for inclusion in systematic review and meta-analysis; A: - by country, B: - by age.

Overall, 15 (65%) records covered children under 15 years of age and 6 (26%) for the children between 15-17 years of age with publication years ranging from 1999 to 2021, and 2002 to 2016 respectively. However, 2 (9%) records contained data for both the under 15 and 15-17-year-old children (Figure 3 and 4). The number of participants in the records included in the meta-analysis was 5,370 and 1,940 participants for the under 15 years and those 15-17 years of age were respectively (Table 2).

Our results showed that children under 15 years of age had a higher proportion of seroprotection to HBV as compared to those 15-17 years of age in Africa. Additionally, children in the Western Africa region had a higher proportion of HBV vaccine seroprotection than their counterparts in the Northern region. The HBV vaccine seroprotection among children in the Southern Africa region is markedly higher than that demonstrated in both the Western and Northern Africa regions (Table 4 and Figures 6: C, D, E, F; 7: A, B, C, and D).

Table 4 Meta-analyses results of pooled prevalence of HBV sero-protection rates by age, African geographical region and year of scientific paper publication.

sub groups					
Variable	**Category**	**No**	**Prevalence% (95%CI)**	**p value(95%CI)**	**I2% (95%CI)**
	Age				
Age	below 15	15	69.07 (60.068 to 77.395)		98.58 (98.25 to 98.86)
	15-17	6	32.368 (15.560 to 51.952)	< 0.0001 (34.2454 to 39.0847)	99.45 (99.29 to 99.58)
	Region (under 15)				
Region	South	3	85.221 (66.250 to 97.162)		98.82 (98.02 to 99.30)
	North	7	64.242 (52.857 to 74.866)	< 0.0001 (18.312 to 23.5317)	98.09 (97.27 to 98.66)
	West	3	70.566 (43.867 to 91.267)	< 0.0001 (10.5225 to 18.9504)	98.44 (97.24 to 99.12)
	Region (15-17))				

	North	3	26.253(1.951 to 64.813)		99.60 (99.42 to 99.72)
	West	3	38.704 (14.724 to 66.079)	< 0.0001 (9.7793 to 15.0838)	99.49 (99.24 to 99.66)
	Under 15 years				
Year	1999-2012	8	67.421(54.100 to 79.445)		98.66 (98.20 to 99.00)
	2016-2021	7	71.475 (58.482 to 82.872)	< 0.0013 (1.5894 to 6.5158)	98.56 (98.00 to 98.96)
	15-17 years				
	2002-2013	2	4.306 (0.228 to 13.097)		98.19 (95.88 to 99.21)
	2015-2016	3	26.253 (1.951 to 64.813)	< 0.0001(19.8746 to 24.03)	99.60 (99.42 to 99.72)

4.4.2 HBV vaccine sero-protection rate among children of up to 17 years of age

We included 15 records for children below 15 years of age and 6 records for the children 15 to 17 years of age in our meta-analysis (Table 4 and Figures 5: A, B, C, and D). Heterogeneity was persistently high ($I^2 \geq 98\%$, $p < 0.0001$) for all the analyses and hence the random effect model was used to determine prevalence of seroprotection. Both the Egger's and Begg's test found no evidence for sources of publication bias ($p > 0.1$). Our results showed a significantly higher ($p < 0.0001$) HBV vaccine seroprotection among children under 15 years of age (69.07%) than children 15-17 years (32.368%) (Table 4 and Figures 5: A, B, C, and D).

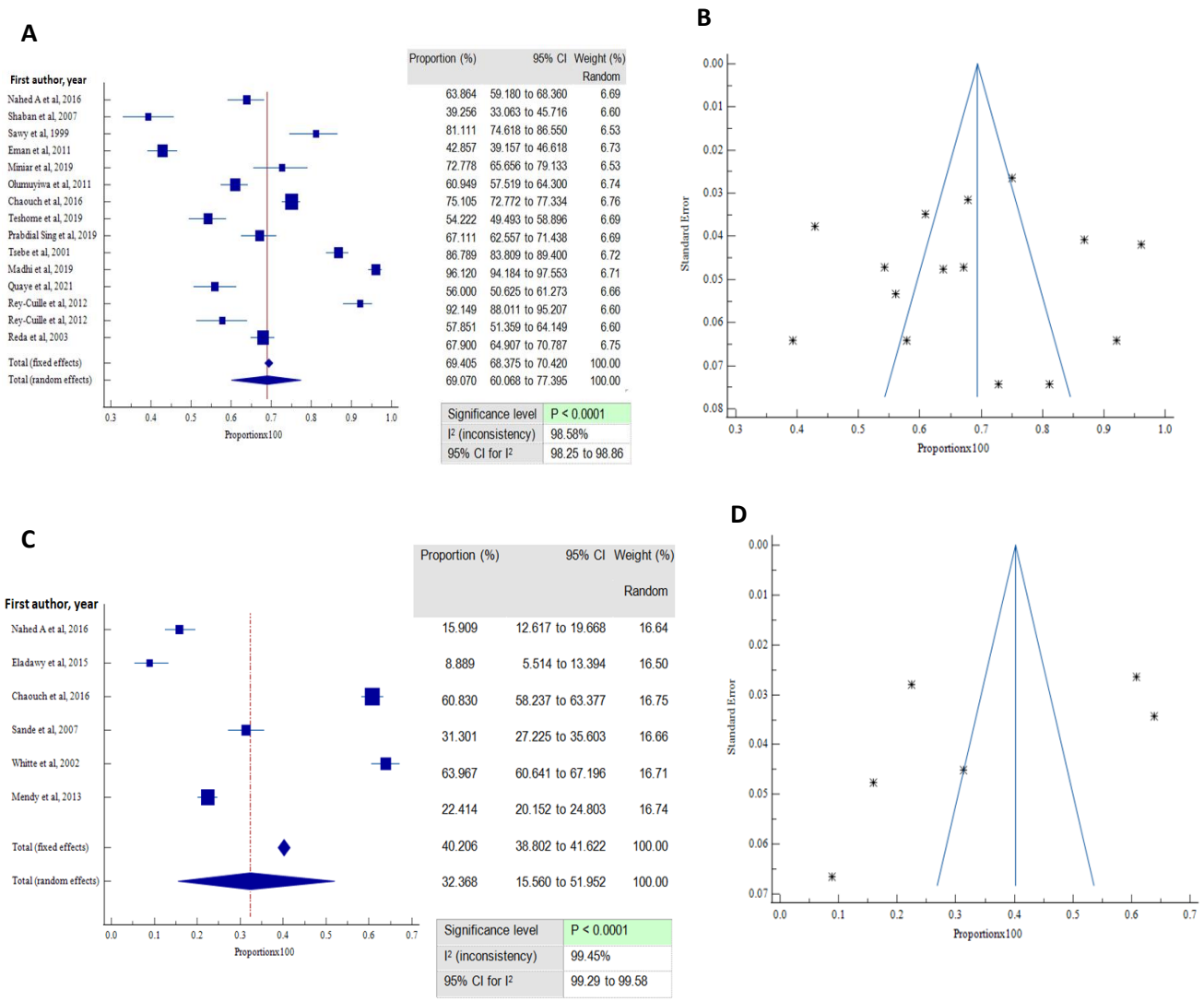


Figure 5: HBV vaccine seroprotection rates and publication bias in studies of children under 15 (A and B) and 15-17 years of age (C and D).

4.4.3 HBV vaccine sero-protection rates among children up to 17 years of age in the Northern, Western, and Southern Africa regions

Our analysis included 3, 7 and 3 records for children under 15 years from the Southern, Northern and Western Africa regions respectively (Table 4 and Figures 6: A, B, C, D, E, and F). For children 15-17 years of age, 3 records from the Northern and 3 from the Western Africa regions

were included in our analysis. Similarly, the heterogeneity was high for all the studies ($I2 \geq 98\%$, $p< 0.0001$). Consequently, a random effect model was utilized to determine the prevalence. Egger's and Begg's test were used to assess the bias and there was no evidence of publication bias ($p > 0.1$). Additionally, the funnel plots in which the standard error of prevalence of each study was plotted against its prevalence displayed a symmetrical spread of the plots, suggesting little or no evidence of publication bias (Figures 6: B, D, and F). Our results showed that children under 15 years from the Southern Africa region demonstrated significantly higher proportion of HBV seroprotection (85.221%, $p < 0.0001$) than their counterparts in Northern and Western Africa. The Northern region demonstrated the least proportion of seroprotection (64.242%, $p < 0.0001$) among this age group (Table 5 and Figures 6: C and D). Children 15-17 years of age from the Western Africa region demonstrated a low HBV vaccine seroprotection (38.704%, $p < 0.0001$) but was higher than proportions for the Northern Africa region (26.253%, $p < 0.0001$) (Table 4 and Figures 6: A, B, C, and D).

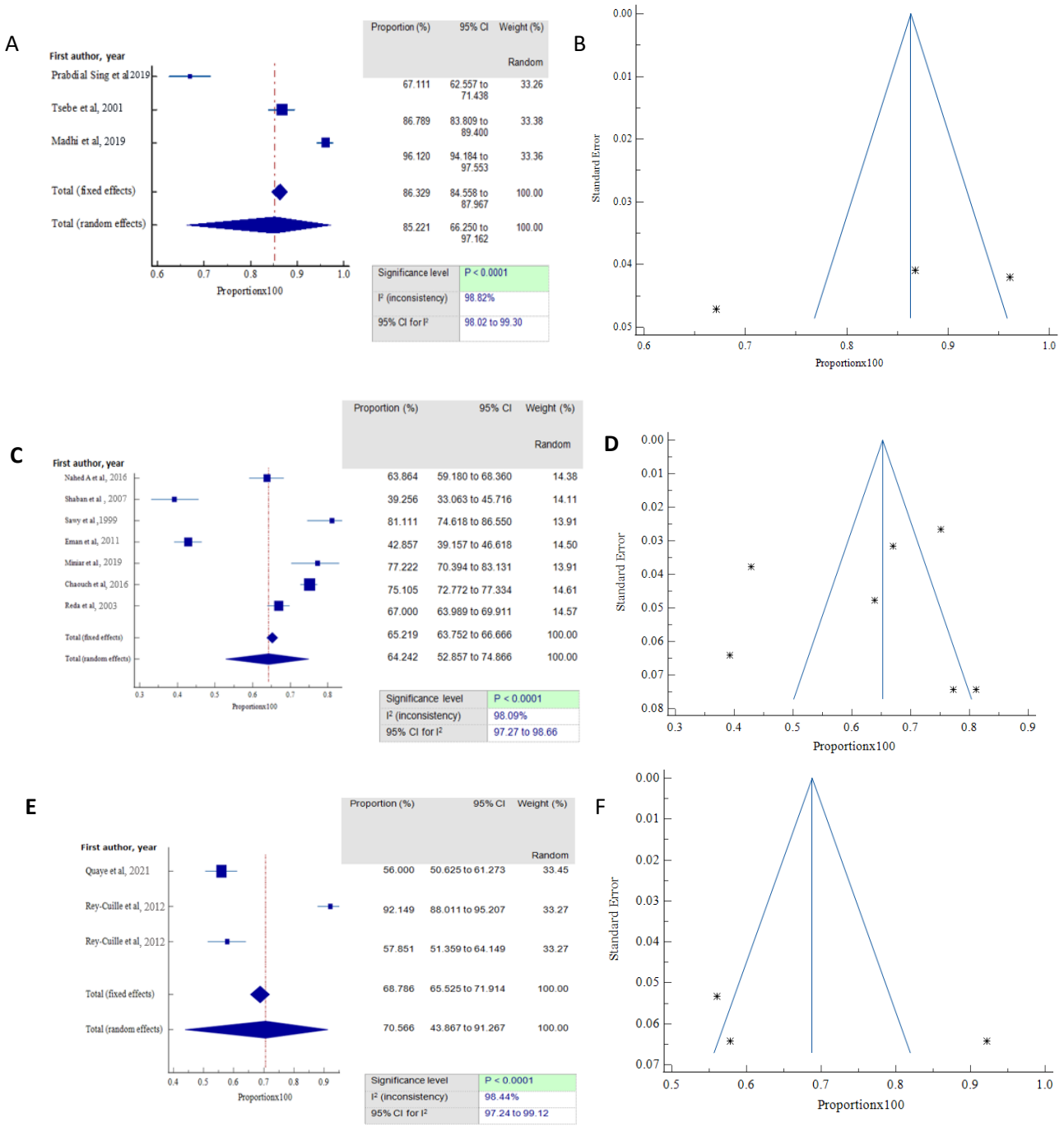


Figure 6: HBV vaccine seroprotection rates and publication bias in studies of children of up to 17 years of age in the Southern (A and B), Northern (C and D), and Western Africa (E and F) regions.

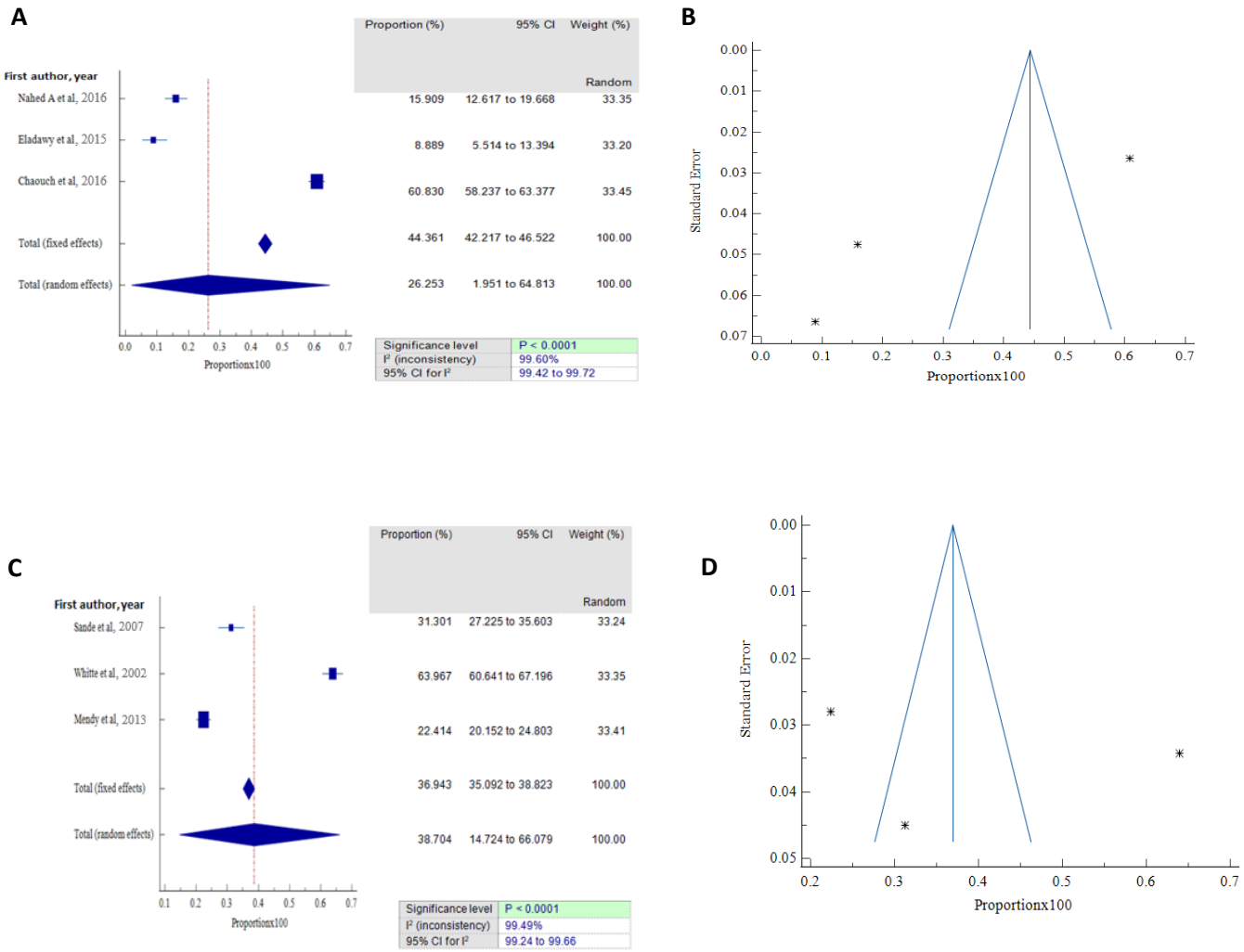


Figure 7: HBV vaccine seroprotection rates and publication bias in studies of children 15-17 years of age in the Northern (A and B), and Western Africa (C and D) regions.

4.4.4 HBV vaccine sero-protection rates among children up to 17 years of age from 1999 to 2021

We included 15 and 5 studies in our meta-analysis for children under 15 years and children 15 to 17 years of age respectively. For children under 15 years, 8 records published between 1999 and 2012 were analyzed, while 7 records published between 2016 and 2021 were also analyzed. However, for children 15 to 17 years, 2 records published between 2002 and 2013 were analyzed, and additionally 3 records published between 2015 and 2016 were also analyzed. All studies used to determine the prevalence illustrated a high heterogeneity ($I2 > 98\%$, $p < 0.0001$) and hence the random effect model was used (Table 4). Our analysis indicated that proportion of HBV vaccine seroprotection was high (67.421% and 71.475 respectively, $p < 0.0013$) (Table 4 and Figures 8: A, B, C, and D) in children under 15 years when comparing studies published between 1999 and 2012 and between 2016 and 2021. However, studies for children 15-17 years published between 2015 and 2016 showed low but significantly higher HBV vaccine seroprotection (26.253%, $p < 0.0001$) than studies published between 2002 and 2013 (4.306%) (Table 2 and Figures 9: A, B, C, and D). Publication bias was evidenced in studies for children 15-17 years published between 2002 and 2013 as demonstrated by the Egger's ($p = 0.0001$) but not the Begg's test ($p = 0.3173$). However, there was symmetrical spread of studies from the funnel plot of the standard error of prevalence against its prevalence (Figure 8: B).

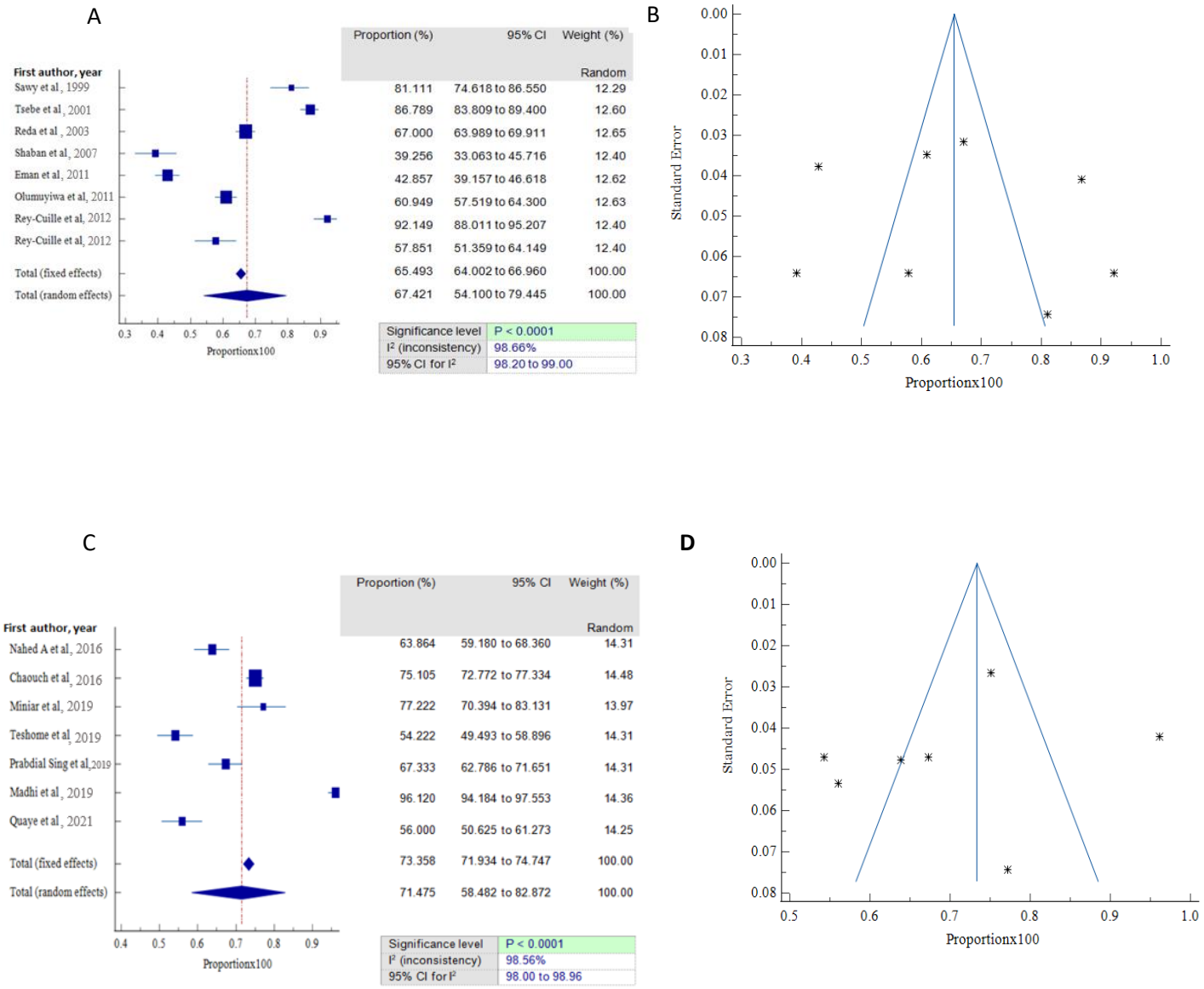


Figure 8: HBV vaccine seroprotection rates and publication bias in scientific articles investigating children under 15 years of age; published from 1999 to 2012 (A and B) and 2016 to 2021 (C and D).

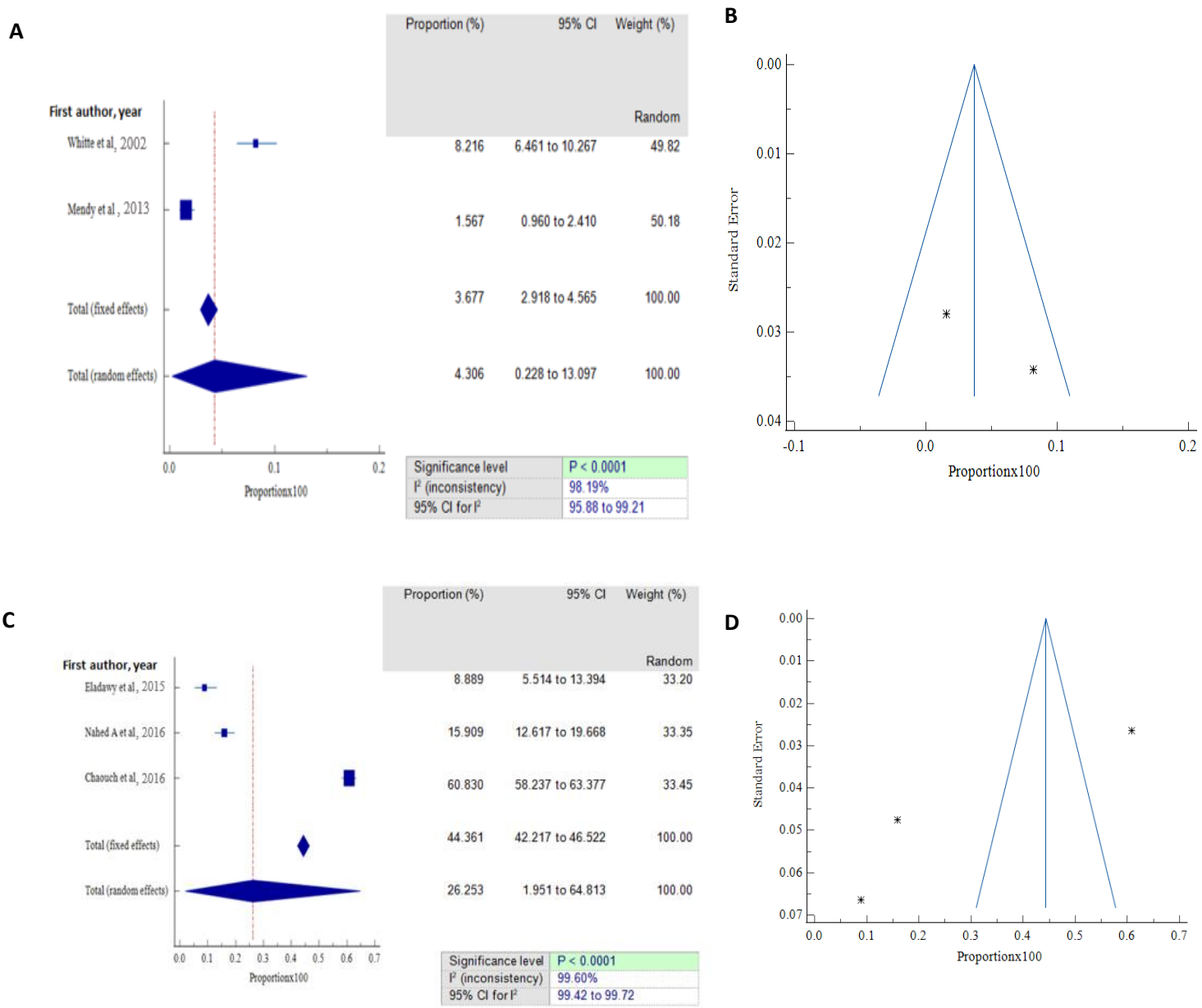


Figure 9: HBV vaccine seroprotection rates and publication bias in scientific articles investigating children 15-17 years of age; published from 2002 to 2013 (A and B) and 2015 to 2016 (C and D).

CHAPTER FIVE

Serological Status of Vaccine and Hepatitis B Virus Exposure among Under 5-Year-Old Children and Adolescents in Kampala, Uganda

Preamble

There is a paucity of monitoring and evaluation reports and publications on the hepatitis B vaccine seroprotection, from national EPIs in the East African region (Fahad Muwanda et al., 2023). Since the implementation of Hepatitis B vaccination in Uganda's EPI in 2002, we found no data on the systematic review of the performance of this program by the MoH or other independent researchers. The efficacy of the hepatitis B Vaccine is high, yet the downside of it is that up to 20% of immunocompetent individuals may not respond to it. This, when coupled to other programmatic and socio-behavioral challenges, can affect individual and community level (herd) immunity. Uganda is among the highest hepatitis B burdened countries; sub-optimal response to the vaccine would mean that a significant proportion of vaccinated children in infancy or even in adolescent years remains susceptible to infection. This chapter presents findings from a community level study conducted among childhood recipients of the hepatitis B vaccine enrolled on the Uganda National Extended Program on Immunizations (UNEPI), who were under 5 and 15-17 years of age. We assessed participants' exposure to infection using a 5-panel qualitative hepatitis B Combo RDT methodology. Immune responses to the hepatitis B vaccine were assessed in participants' serum or plasma using quantitative anti-HBs methods. Findings of this community study are presented in two sub-chapters;

Chapter 5a: Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda

This work was published in *Livers*: See Appendix 4

DOI: 10.3390/livers4040039

Chapter 5b: Hepatitis B Vaccine-Specific Antibody Titers Among Children of up to 17 Years in Kampala, Uganda

This manuscript is under review in the *African Health Sciences Journal*: Submission number: **WKR0-2025-08-1661**: See Appendix 4

CHAPTER 5A

Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda

Preamble

Although the hepatitis B vaccine, integrated into the Uganda National Extended Program on Immunizations (UNEPI) in 2002, up to 20% of immunocompetent recipients fail to achieve protective antibody levels (Nashibi et al., 2015b; Sahana, Sarala, & Prasad, 2017b). Moreover, multiple studies in Africa have reported substantial waning of hepatitis B surface antibody (anti-HBs) titers and occurrence of breakthrough infections years after full vaccination (Adugna et al., 2023; Alemayehu et al., 2024). This is particularly concerning among adolescents, whose increased risk behaviors—such as unprotected sexual activity, tattooing, and sharing sharp instruments—heighten HBV exposure risk (Chaouch et al., 2016; Eladawy et al., 2015). Despite these concerns, national HBV surveillance in Uganda does not routinely evaluate long-term vaccine-induced immunity or the prevalence of non-response among childhood recipients. Additionally, while qualitative rapid diagnostic tests (RDTs) for anti-HBs detection are widely used due to their low cost and accessibility, their diagnostic utility for assessing vaccine protection has not been validated at the national level. Without such data, susceptibility in vaccinated populations may be underestimated, enabling ongoing HBV transmission and chronic disease progression. This study addresses these critical gaps by determining the qualitative HBV seroprotection status of vaccinated

children under 5 years and adolescents aged 15–17 years in Kampala, Uganda, and by assessing the concordance of multiple HBV RDT brands.

Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda

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Disclosures

None

Acknowledgements

We thank the management of the School of Medical Laboratory Technology, Kibuli Muslim Hospital Health Training Schools—for providing us with the necessary stationery and ICT technical support. We appreciate the clinicians at Child and Family Foundation (CFU) Medical Center and the village health teams (VHTs) of the respective study areas of Kawempe Division of Kampala Capital City Authority who supported the consenting and mobilization of study participants.

Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of this study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

5A.1 Abstract

Background: Pediatric hepatitis B virus (HBV) serostatus remains variably characterized, hardly determined at times, or documented as part of national monitoring of the Extended Programs for Immunization (EPI).

Methods: We cross-sectionally characterized the seroprevalence of HBV vaccine and/or infection status among 501 and 288 children <5 and 15–17 years old, respectively, in Kawempe Division, Kampala, Uganda, between May and August 2023. These children received HBV vaccination under the Uganda National Extended Program on Immunizations (UNEPI). Samples were qualitatively screened for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb or anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb or anti-HBe), and for hepatitis B core antibody (HBcAb or anti-HBc) using three different commercial HBV Combo test rapid immunochromatographic diagnostic tests: Nova, Fastep, and Beright.

Results: The seroprevalence of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc was 1.52%, 27.75%, 0.88%, 0.63%, and 0.76%, respectively, for the combined study age groups. The HBsAg seroprevalence of 2.78% was almost 3.5-fold higher among adolescents when compared to the 0.8% observed in the under-5-year-olds. The qualitative seroprevalence of anti-HBs was 33.1% and 18.4% in the under-5 and among the 15–17-year-old study groups, respectively.

Conclusion: The proportion of qualitatively detectable anti-HBs in both groups of vaccinated children is low and probably indicates reduced seroprotection. Consequently, a large proportion of

children who received the hepatitis B vaccine under UNEPI may be at risk of HBV infection, especially adolescents. A booster dose of the Hepatitis B Vaccine may be required for adolescents.

Keywords: hepatitis B virus; serological status; combo test; hepatitis B vaccine; Kampala-Uganda

5A. 2 Background

Chronic hepatitis B virus (HBV) infection continues to be a global public health threat. It is estimated that 296 million people are currently living with the virus (Y.-C. Hsu et al., 2023). The global prevalence of HBV infection is 3.9%, and the WHO Africa region remains substantially burdened at a prevalence of 3.0% (Organization, 2017b; Razavi-Shearer et al., 2018). In highly endemic countries such as Uganda, the national prevalence of HBV infection is 10% (Emmanuel Ochola et al., 2013b).

Many African countries integrated HBV vaccination into their national extended programs for immunization (EPI) by 2002. In Uganda, like most of Sub-Saharan Africa, the HBV vaccination schedule is at birth, 6, 10, and 14 weeks (Akbar et al., 2021; de Villiers et al., 2021b; Memirie et al., 2020a; Safadi et al., 2021). Immunization is conducted at all levels of public healthcare, at all private hospitals, and in accredited health facilities of private practitioners. However, following vaccination, pediatric HBV serostatus remains variably characterized or documented as part of the national EPI monitoring. Approximately 20% of immunocompetent HBV vaccine recipients fail to respond to the complete vaccination (Nashibi et al., 2015b; Sahana et al., 2017b; Tripathy, Sati, Puspa, Shankar, & Singh, 2011a; S. Yang et al., 2016b). In addition, effective control measures for HBV infection and disease are underutilized. For example, HBV infection is generally underdiagnosed, and coverage of fully vaccinated children remains low, especially in low-income and high-burdened countries (Y.-C. Hsu et al., 2023).

As is with other vaccines, the protective efficacy of the Hepatitis B vaccine is probably lower in the East African region when compared with the more developed countries. A recent study in Ethiopia has reported close to 5% seroprevalence of breakthrough HBV infection among fully vaccinated children of 5–12 years of age (Adugna et al., 2023). Another recent study in Ethiopian children revealed only 14.4% and 2.6% of protective anti-HBs antibodies (>10 IU/mL) among under-5-year-olds and in ≥10-year-old children (Alemayehu et al., 2024). There is a rising belief presence of breakthrough infections among children previously vaccinated may suggest a genuine lack of response rather than a mere drop in antibody titers below the protective levels (Adugna et al., 2023; Alemayehu et al., 2024; M.-L. Huang, Liao, & Ho, 2007; Kedir et al., 2019; Leuridan & Van Damme, 2011; Shih et al., 1999; Yonghao et al., 2015).

Since Uganda integrated the hepatitis B vaccine into UNEPI in 2002, we assumed that persistent detectable anti-HBs antibodies among adolescents (15–17 years) would be a good surrogate for the effectiveness of the immunization program (Fahad Muwanda et al., 2023), at least in our study jurisdiction. Fledgling emancipation, combined with peer pressures, exposes this age group to behaviors (such as sexual activity, body tattooing, and sharing of injectables) associated with an increased risk of HBV infection (Chaouch et al., 2016; Eladawy et al., 2015; Makhlouf et al., 2016; Fahad Muwanda et al., 2023). HBV seroprevalence in such populations with a heightened risk of infection must be keenly monitored for effective evaluation of vaccination programs to inform adequate control and/or elimination of HBV infection (Y.-C. Hsu et al., 2023; Organization, 2017b). Although acceptable national coverage for every vaccine is 90% (Malande et al., 2019; Organization, 2020b), the Uganda Demographic Health Survey (UDHS-2016) indicated a low (79%) immunization coverage of the childhood hepatitis B vaccine (Malande et al., 2019). However, the periodic outbreaks of HBV and the stagnated HBV seroprevalence in Uganda (Bwogi

et al., 2009; P. K. Kitandwe et al., 2021; Emmanuel Ochola et al., 2013b; Organization, 2020b) justify the need to investigate the seroprotective efficacy of the HBV vaccine among children under 5 who have been vaccinated. Further, there is a paucity of data on both qualitative and quantitative determination of HBV serostatus among vaccinated children in Uganda. To the best of our knowledge, immune responses among the children previously vaccinated are variable and inconclusively studied in Uganda (Bwogi et al., 2009; P. K. Kitandwe et al., 2021; Ocan et al., 2022; Emmanuel Ochola et al., 2013b; Ssekamatte et al., 2020). This study therefore sought to determine the seroprevalence of HBV vaccine and/or serological status of HBV exposure among children under 5 years and 15–17 years of age. We used qualitative HBV Rapid Diagnostic tests as a baseline for the quantitative estimation to infer the HBV vaccination protection in Kawempe Division, Kampala, Uganda.

5A.3 Materials and methods

5A.3.1 Study Design, Population, and Settings

In a cross-sectional study, we enrolled 789 children between May and August 2023 from the Child and Family Foundation (CFU) medical center catchment area in Kawempe Division. Our study population included children under 5 and 15 to 17 years of age.

5A.3.2 Inclusion and Exclusion Criteria

We included children under five years of age who had received at least one of the three doses of the HBV vaccine, as evidenced by their UNEPI immunization card. For the 15–17-year-old participants, we included participants who had received at least one hepatitis B vaccine dose verifiable with a UNEPI immunization card or, if not available, based on parents/guardians'

reporting. For both study groups, we excluded children who were anemic as assessed by a study clinician. We also excluded children living with or testing positive for HIV as well as those reporting to have or deemed to have any form of allergy by a study clinician.

5A.3.3 Sample Size Estimation and Sampling Procedure

For both group sample sizes, we estimated using Kish Leslie's formula (Kalton & Heeringa, 2003) for cross-sectional studies. We arrived at a sample size of 501 and 288 participants for the under-5 years and 15–17-year-olds, respectively. For the under-5-year-old participant group, we enrolled consecutively eligible children seeking services at the CFU medical center until the sample size was accrued. For the 15–17-year-old study group, eligible study participants were identified during CFU's scheduled health home visits and community medical outreaches and were referred to the medical center for consenting and conduct of other study procedures until the sample size accrued consecutively.

5A.3.4 Data Collection Procedures

A pretested structured questionnaire was administered to parents/legal guardians of participants in the under-5-year-old study group and directly to the participants in the 15-17-year-old group with a provision for consulting their parents/legal guardians if they wished. The questionnaire collected data on the children's socio-demographic characteristics, such as age, gender, medical history, and hepatitis B vaccination status.

5A.3.5 Sample Collection and Processing

Blood was collected from all participants by a designated study clinician or phlebotomist following pre-developed standard operating procedures (SOPs). For the under 5-year-old participants, 8 mL of venous blood was aseptically collected by venipuncture in the following order: 1 EDTA anticoagulant tube of 4 mL, 2 mL Heparinized blood (Becton and Dickson Co. Ltd., Nairobi,

Kenya), and a 2 mL plain serum separation tube (Becton and Dickson Co. Ltd., Nairobi, Kenya). For the 15–17-year-olds, 4 mL of venous blood in a plain serum separation tube was collected. Serum and Plasma separation was then conducted by centrifugation of the blood tubes at 1500× g for 15 min at room temperature at the Immunology Laboratory of the School of Biomedical Sciences, Makerere University, Kampala, Uganda. All sera, plasma, and PBMCs recoverable were stored in labeled cryovial tubes in volumes of 0.5 mL at −20 ◦C or lower until analysis at the Integrated Biorepository of H3Africa-Uganda (IBRH3AU), Makerere University, Kampala, Uganda.

5A.3.6 Laboratory Analysis

Serum samples were tested for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb using three different Hepatitis B Combo RDT test kits: NOVA TEST One Step Diagnostic Rapid Test (Global Science, Malaysia; Jaya, Selangor D.E., Malaysia, Cat#: 20221811HBV), Fastep Rapid Diagnostic Test (Healgen Scientific Limited Liabil ity Company, Houston, TX, USA, Cat#: 21076412), and Beright Diagnostic Rapid Test (Medhealth Biotechnology Limited, Shanghai, China, Cat#: 12216453), with manufacturer reported sensitivity and specificity of (91.43% and 98.28%), (68% and 77%), and (98.92% and 99.46%), respectively. Positive and negative controls (prepared and distributed to health facilities by the Uganda Virus Research Institute (UVRI)) were included in all kits and testing runs. Children who tested positive for HBsAg were referred to the CFU Medical Center for appropriate clinical management.

5A.3.7 Data Analysis

The interpretation of the HBV serological results was performed according to the Australasian Society for HIV, Viral Hepatitis, and Sexual Health Medicine (ASHM)'s 2024 Clinical Tool for Decision Making in Hepatitis B (www.testingportal.ashm.org.au/hbv) (Table 1).

All the participants' data for hepatitis B Combo RDT results were consistent on all three test kits and were therefore included in the analysis. Data was entered for cleaning into EpiData software version 3.1 and exported to SPSS version 25 for descriptive analysis. The results were presented as frequency tables and graphs. The main outcome of interest was HBV serostatus, which was expressed as a percentage. Concordance among the 3 serological tests was analyzed according to Langton et al. (2002) as the percentage of chance that they would yield the same results (Langton, Chevennement, Nagelkerke, & Lombard, 2002) (Table 5).

5A.4 Results

5A.4.1 Socio-demographic characteristics

A total of 789 children participated in this study: 288 and 501 in the 15–17-year-old and under 5-year-old groups, respectively. The mean age of this study's participants was 16.18 years and 2.93 years for the 15–17-year-old and 0–5-year-old groups, respectively. While more female children (51.7%) were enrolled in the under-5-year-old group, more males (51.74%) were enrolled in the 15-17-year-old group (Table 6).

5A.4.2 Concordance among NOVA TEST, Fastep, and Beright Diagnostic Rapid Tests

Testing all this study's participants' blood sera (n = 789) with each of the three hepatitis B Combo RDT tests yielded 100% concordance and a similar number of positive and negative results for the five HBV seromarkers: HBsAg (12), anti-HBs (219), HBeAg (7), anti-HBe (5), and HBcAb (6) (Table 5).

Table 5 Concordance among the HBV Rapid Diagnostic Tests

Serial Number	Rapid Diagnostic Test	HBV markers (n=789): number positive (%)				
		HBsAg	Anti-HBs	HBeAg	Anti-HBe	Anti-HBc
1.	**NOVA TEST**	12 (1.52)	219 (27.75)	7 (0.88)	5 (0.63)	6 (0.76)
2.	**Fastep**	12 (1.52)	219 (27.75)	7 (0.88)	5 (0.63)	6 (0.76)
3.	**Beright**	12 (1.52)	219 (27.75)	7 (0.88)	5 (0.63)	6 (0.76)

5A.4.3 Doses of Hepatitis B vaccine received

Of the 789 study participants, 760 (96.32%) received a verified and documented number of the HBV vaccine doses; 100% (501 of 501) and 89.93% (259 of 288) among the 0-5 and 15-17-year-old groups respectively. Of the verified vaccine recipients (n=760), 629 (82.76%), 79 (10.39%), and 52 (6.84%) received 3, 2, and 1 doses of the HBV vaccine respectively.

Table 6 Characteristics of the study participants, Kawempe Division, May-August 2023

Variables	**15–17 Years, *n* = 288**	**0–5 Years, *n* = 501**	**Combined, *n* = 789**
Age Mean (SD)	16.18 (0.8836)	2.93 (1.3)	
Gender *n* (%)			
Male	149 (51.74)	242 (48.3)	391 (49.55)
Female	139 (48.26)	259 (51.7)	398 (50.44)
Doses	**15–17 years, *n* = 259**	**0–5 years, *n* = 501**	**Combined, *n* = 760**
One	26 (10.04)	26 (5.19)	52 (6.84)
Two	45 (17.37)	34 (6.79)	79 (10.39)
Three	188 (72.59)	441 (88.02)	629 (82.76)

Notably, relatively more children in the 0-5-year-old group (88.02% [441 of 501]) than those in the 15-17-year-old group (72.59% [188 of 259]) received the 3 HBV vaccine doses, unlike the 1 and 2-dose vaccine recipients who were predominant in the 15-17-year-old group (Table 6).

5A.4.4 Qualitative Anti-HBs Status and Prevalence of HBV Infection among Study Groups

Among the entire study participants, 27.75% (219 of 789) had detectable anti-HBs; 33.10% of the children in the 0–5-year-old age cohort (166 of 501) and 18.40% (53 of 288) in the 15–17-year-old age cohort, respectively (Figure 10A). The overall prevalence of HBsAg (active HBV infection) among all study participants was 1.52% (12 of 789); 2.77% (8 of 288) and 0.80% (4 of 501) in the 15–17-year-old and 0–5-year-old groups, respectively (Figure 10B). Acute HBV infection was demonstrated in 25% (2 of 8); the rest (6 of 8) of the 15–17-year-old participants were chronically HBV infected. All 100% (4 of 4) of children 0–5- years -old were acutely HBV infected; that is, none had chronic infection at the time of study (Table 7). There was no significant difference in proportion of HBV infection between the 15–17 and 0–5 year-old age groups ($p = 0.087$).

Two of the 8 (25%) children infected with HBV (qualitatively detectable HBsAg) among the 15–17-year-old group also had qualitatively detectable anti-HBs on the HBV Combo cassette. Also, in the 0–5-year-old group, one (25%) out of the 4 children with HBV infection had qualitatively detectable anti-HBs on the HBV Combo RDT cassette (Figure 10C). In the entire study population, 72.24% (570 of 789) did not possess detectable anti-HBs; 68.86% (335 of 501) in the 0–5-year-old and 81.59% (235 of 288) in the 15–17-year-old groups, respectively (Table 7).

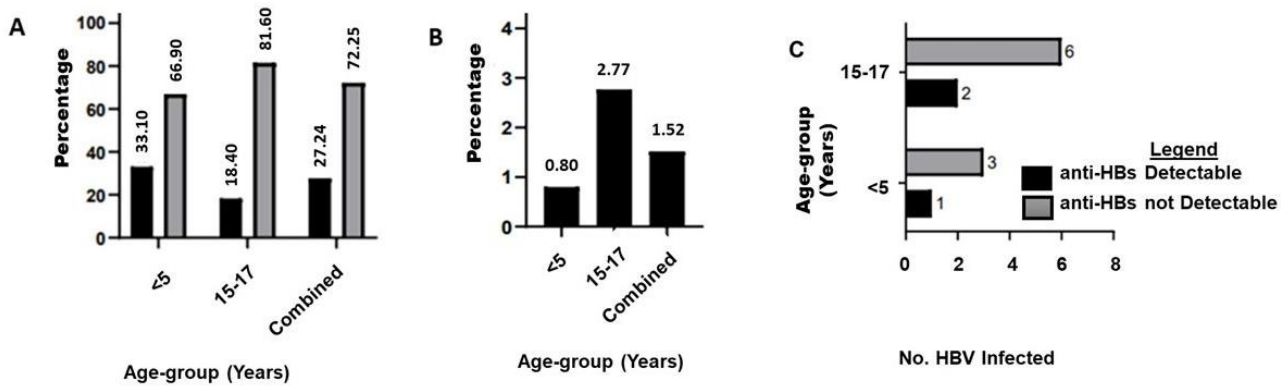


Figure 10: HBV; (A) surface antibody (anti-HBs); (B) surface antigen (HBsAg); (C) Number of participants positive for HBsAg according to anti-HBs serostatus, among this study's participants in Kawempe Division, May–August 2023.

Of the 11 children in the 15–17-year-old group who had evidence of ever being exposed to HBV, 27.3% (3 of 11) had serological evidence of having resolved the infection. However, none (0 of 4) of the children in the 0–5-year-old group who had ever been infected by HBV had serological evidence of having resolved the infection. Overall, 98.09% (774 of 789) of children in the entire study population had not been exposed to HBV infection before; 99.2% (497 of 501) and 96.18% (277 of 288) in the 0–5 and 15–17-year-old groups, respectively (Table 7).

Table 7 Clinical test characteristics of the study participants, Kawempe, May-August 2023

Variables	15–17 Years, *n* = 288	0–5 Years, *n* = 501	Combined, *n* = 789
HBV markers combo *n* (%)			
HBsAg	8(2.78)	4 (0.8)	12 (1.52)
HBsAb	53(18.4)	166 (33.1)	219 (27.75)
HBeAg	3(1.04)	4 (0.8)	7 (0.88)
HBeAb	2(0.69)	3 (0.6)	5 (0.63)
HBcAb	6(2.08)	0 (0)	6 (0.76)
Infection status *n* (%)			
Never been exposed	277 (96.18)	497 (99.2)	774 (98.09)
Ever been exposed	11 (3.8)	4 (0.80)	15 (1.9)
Exposed and resolved	3 (1.04)	0 (0)	3 (0.38)

CHAPTER 5B

Levels of Hepatitis B Vaccine Specific Antibodies among Children of Up To 17 Years of Age in Kawempe Division, Kampala

Preamble

Whereas childhood HBV vaccination has contributed significant gains in the global eradication of HBV infection, the WHO African region remains largely burdened. Moreover, HBV vaccine seroprotection among children is variably or hardly documented but generally low (Adugna et al., 2023; Makhlouf et al., 2016; Fahad Muwanda et al., 2023). Age brackets of under 5 and 15 to 17 years of age demonstrate increased risk of HBV infection and constitute a population that requires critical monitoring by EPIs post vaccination if the WHO's 2030 elimination targets are to be realized. Uganda, like most low and middle income countries (LMICs) has limited capacity to verify seroprotection by using tittering methods that are costly and complex to conduct. HBV rapid diagnostic tests (RDTs) are good alternatives to the titer methods if validated. This study sought to determine the levels of anti-HBs antibodies among children of up to 17 years in Kampala and to assess the utility of RDTs by analyzing data on their potential use as surrogates for tittering methods in the determination of HBV vaccine seroprotection.

Hepatitis B Vaccine-Specific Antibody Titers Among Children of up to 17 Years in Kampala, Uganda

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Disclosures

None

Acknowledgements

We thank the management of the School of Medical Laboratory Technology, Kibuli Muslim Hospital Health Training Schools—for providing us with the necessary stationery and ICT technical support. We appreciate the clinicians at Child and Family Foundation (CFU) Medical Center and the village health teams (VHTs) of the respective study areas of Kawempe Division of Kampala Capital City Authority who supported the consenting and mobilization of study participants.

Conflicts of Interest

The authors declare no conflicts of interest.

5B.1 Abstract

Background: Childhood HBV vaccination is pivotal to elimination of HBV infection but the extent of the vaccine seroprotection is unclear and variably characterized by most Extended Programs for Immunization (EPI). This study sought to characterize the seroprevalence of HBV vaccine specific antibodies among children of up to 17 years in Kampala, Uganda.

Methods: In a cross-sectional study, we characterized the seroprevalence of HBV vaccine specific antibodies among 443 and 267 children <5 and 15-17 years old respectively, in Kawempe Division, Kampala, Uganda, between May and August 2023. We quantitatively analyzed serum/plasma samples for hepatitis B surface antibody (anti-HBs) using an electro chemiluminescence immunoassay (ECLIA) on the Roche Cobas e 411 immunoassay analyzer system.

Results: The seroprevalence of protective levels of anti-HBs antibodies was 51.83% (368 of 710); 74.29% of the children in the 0-5-year-old age cohort (329 of 443) and 14.61% (39 of 267) in the 15-17-year olds respectively. The qualitative Hepatitis B Combo RDT test assays demonstrated low sensitivity; they detected only 42.12% (155/368) of all protective anti-HBs titers that were determined by the quantitative Cobas E411 system (368/710).

Conclusions: The markedly reduced seroprotection in the 15-17 year olds identifies a clear adolescent immunity gap after infant HBV vaccination in Kampala.

Keywords: Hepatitis B Virus; Seroprotection; Combo Test; Hepatitis B Vaccine; Kampala-Uganda

5B.2 Background

More than 250 million people globally are chronically infected with the hepatitis B virus (HBV); the highest contributor to mortality-related liver cirrhosis and cancer cases annually (Y.-C. Hsu et al., 2023). Sub-Saharan Africa is disproportionately affected (Razavi-Shearer et al., 2018; WHO, 2017; J. D. Yang et al., 2015), with high endemicity occurring in countries like Uganda where the national prevalence of HBV infection is 10% (Emmanuel Ochola et al., 2013b). The WHO through a series of globally published health sector strategies (Organization, 2016a, 2022) and guidelines for hepatitis B prevention, testing, care, and treatment (Hellard, Chou, & Easterbrook, 2017; Hou et al., 2017; Organization, 2024b); aims at eliminating HBV infection by 2030. If this goal is to be realized, it is, among others a strong recommendation by the WHO that reporting and monitoring systems for national extended programs for Immunization (EPI) be strengthened (Organization, 2024b). Adequate EPI monitoring can ably verify the approximately 20% of immunocompetent HBV vaccine recipients who fail to respond to the complete vaccination (Nashibi et al., 2015a; Sahana et al., 2017a; Tripathy et al., 2011b; S. Yang et al., 2016a). In Uganda, the HBV vaccination was integrated into the national EPI in 2002 and the current schedule is given at birth, 6, 10, and 14 weeks (Akbar et al., 2021; de Villiers, Nayagam, & Hallett, 2021a; Memirie et al., 2020b; Safadi et al., 2021). However, like in most low-income and high-burdened countries, hepatitis B vaccination coverage, and completion of all recommended doses among vaccinated children remains low (Y.-C. Hsu et al., 2023). Following vaccination, unlike in adult high risk vaccination programs such as among healthcare workers, pediatric HBV serostatus is not or remains variably characterized as part of the national EPI monitoring. It is apparent that the proportion of childhood vaccinees with protective levels of the hepatitis B surface antibody (anti-HBs) is hardly

documented in Uganda and East Africa as a whole (Kimera et al., 2025; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024).

National sero-surveillance studies conducted to monitor the efficacy of EPIs in Africa show reduced HBV vaccine seroprotection rates, including among children of 15-17 years (Fahad Muwanda et al., 2023). Whereas various studies in northern (Chaouch et al., 2016; El Sawy & Mohamed, 1999; Odusanya et al., 2011; Tfifha, Kacem, Ben Rejeb, et al., 2019), western (Ephesians N Anutebeh et al., 2021a; Quaye et al., 2021; Rey-Cuille, Seck, Njouom, Chartier, Sow, Mamadou, et al., 2012), and southern Africa (Madhi et al., 2019; Prabdial-Sing et al., 2019; Tsebe et al., 2001) reported increased seroprotective rates following hepatitis B vaccination of infants, a few studies conducted in the East African region (Adugna et al., 2023; Alemayehu et al., 2024; Kimera et al., 2025; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024) demonstrated varied anti-HBs seroprotection levels. In Uganda, Kimera et al., (2025) reported good protective immune response of 77.4 % among children under five years attending Mulago hospital (Kimera et al., 2025). On the contrary, a recent study in Ethiopian children revealed as low as 14.4 % and 2.6 % of protective anti-HBs antibodies (>10 IU/mL) among under 5 year olds and in ≥10 years children (Alemayehu et al., 2024), respectively. It is probable that a lack of response to the HBV vaccine or waning of antibody titers below the protective levels significantly predisposes previously vaccinated children to breakthrough infections (Adugna et al., 2023; Alemayehu et al., 2024; Huang MeiLiang, Liao WenLing, & Ho MeiShang, 2007; Kedir et al., 2019; Leuridan & Van Damme, 2011; Shih et al., 1999; Yonghao et al., 2015). The markedly prevalent risk factors associated with transmission of HBV infection among children underscores the need for effective HBV vaccination among infants and continued EPI monitoring and evaluation (Chaouch et al., 2016; Eladawy et al., 2015; Makhlouf et al., 2016;

Fahad Muwanda et al., 2023; Organization, 2024b). Moreover, studies have documented many factors that result in suboptimal immune responses to childhood vaccination (Adugna et al., 2023; Alemayehu et al., 2024; Hammitt et al., 2007; Kimera et al., 2025; A. W.-c. Lin & Wong, 2016; Fahad Muwanda et al., 2023; Ni et al., 2007; Turyamubona et al., 2025). Determining and keenly monitoring the proportion of HBV vaccine seroprotection among populations with a heightened risk of infection can inform adequate control and/or elimination of HBV infection (Y.-C. Hsu et al., 2023; Organization, 2017a).

The Uganda Demographic Health Survey (UDHS-2022) indicated a low (70%) 3-dose immunization coverage of childhood hepatitis B vaccine (Malande et al., 2019), compared with the recommended 90% coverage for every vaccine (Malande et al., 2019; Organization, 2016b). Additionally, Uganda continues to experience periodic outbreaks of HBV infection and a stagnated HBV infection seroprevalence (Bwogi et al., 2009; Paul Kato Kitandwe et al., 2021; Emmanuel Ochola et al., 2013a; Organization, 2016b). Elimination of hepatitis B virus infection will require a strong and successful national childhood immunization program, justifying the need to monitor the levels of anti-HBs among children vaccinated in infancy for effective evaluation of the seroprotective efficacy of the HBV vaccine. There is generally limited data on estimation of HBV vaccine seroprotection among vaccinees in Uganda. A study conducted in selected health facilities in northern Uganda revealed that protective immune response persists for over ten years following hepatitis B vaccination among healthcare workers (Ocan et al., 2022). Much as Muwanda et al., (2024) reported a low prevalence of children with qualitatively detectable anti-HBs antibodies, they were not able to determine the extent of utility of these qualitative Rapid Diagnostic Test-based methods (RDTs) nor quantify the Anti-HBs titers (Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024). Although a recent study by

Kimera et al., (2025) among children under 5 years of age attending Mulago hospital revealed seroprotection rate of 77.4%, immune response among the children previously vaccinated is variable and remains inconclusively studied in Uganda (Bwogi et al., 2009; Paul Kato Kitandwe et al., 2021; Ocan et al., 2022; Emmanuel Ochola et al., 2013a; Ssekamatte et al., 2020). This study therefore sought to evaluate the efficacy of HBV RDTs and to quantitatively determine the seroprevalence of anti-HBs antibodies among high risk vaccinated children under 5 years and 15-17 years of age in Kawempe Division, Kampala, Uganda.

5B.3 Materials and methods

5B.3.1 Study Design and Setting

We enrolled 789 children in a cross-sectional study between May and August 2023 from the Child and Family Foundation (CFU) medical center's catchment areas in Kawempe Division, Kampala Capital City Authority. Kawempe division has 22 administrative parishes. The CFU medical center conducts both static and mobile immunization services under the UNEPI. The static immunization services are scheduled for Monday and Friday while the outreaches are done twice a month, rotational in the different parishes. All immunization services provided are recorded in the Ministry of Health (MOH) health management information system (HMIS). Our study population included children under 5 who had received the hepatitis B vaccine as indicated by the child health card, and adolescents (15 to 17 years) who received the hepatitis B vaccine during childhood. All 789 children were qualitatively tested for HBV sero-markers, while 710 children were successfully tested for anti-HBs antibodies.

5B.3.2 Study Population

We enrolled 789 children in a cross-sectional study between May and August 2023 from the Child and Family Foundation (CFU) medical center's catchment areas in Kawempe Division, Kampala Capital City Authority. Kawempe division has 22 administrative parishes. The CFU medical center conducts both static and mobile immunization services under the UNEPI. The static immunization services are scheduled for Monday and Friday while the outreaches are done twice a month, rotational in the different parishes. All immunization services provided are recorded in the Ministry of Health (MOH) health management information system (HMIS). Our study population included children under 5 who had received the hepatitis B vaccine as indicated by the child health card, and adolescents (15 to 17 years) who received the hepatitis B vaccine during childhood. All 789 children were qualitatively tested for HBV sero-markers, while 710 children were successfully tested for anti-HBs antibodies.

5B.3.2.1 Inclusion and Exclusion Criteria

We included children under five years of age who had received at least one dose of the HBV vaccine, evidenced on their UNEPI immunization card, and were not anaemic or immunocompromised as assessed by a study clinician. For the 15-17-year-old participants, we included non-anaemic or immunocompromised participants who had received at least one Hepatitis B vaccine dose verifiable with a UNEPI immunization card or if not available, based on parents/guardians' reporting.

5B.3.2.2 Sample Size and sampling method

Using Kish Leslie's formula (Kalton & Heeringa, 2003), we arrived at a sample size of 501 and 288 participants for the under 5 years and 15-17-year olds participant groups respectively. For the under-5-year-olds, we enrolled consecutively eligible children among those seeking services at

the CFU medical center until the sample size was accrued. For the 15-17-year-old study group, eligible study participants were identified during CFU's scheduled health home visits and community medical outreaches and were referred to the medical center for consenting and conduct of other study procedures until the sample size accrued consecutively.

5B.3.3 Data collection procedures

A pretested structured questionnaire was administered to parents/legal guardians of participants in the under 5-year-old study group, and directly to the participants in the 15-17-year-old group with a provision for consulting their parents/legal guardians if they wished. The questionnaire collected data on the children's socio-demographic characteristics like age, gender, medical history, and hepatitis B vaccination status.

5B.3.4 Serum/Plasma processing, storage

Blood was collected from all participants by a designated study clinician or phlebotomist following pre-developed standard operating procedures (SOPs). For the under 5-year-old participants, 6 mL of venous blood was aseptically collected by venipuncture in the following order; 1 Heparin anticoagulant tube of 4 mLs (Becton & Dickson Co. Ltd., Nairobi, Kenya) and a 2 mL plain serum separation tube (Becton & Dickson Co. Ltd., Nairobi, Kenya). For the 15-17-year-olds, 4 mL venous blood in a plain serum separation tube was collected. Serum and Plasma separation was then done by centrifugation of the blood tubes at 1500g for 15 minutes at room temperature, at the Immunology Laboratory of the School of Biomedical Sciences, Makerere University, Kampala, Uganda. All sera, plasma, and PBMCs recoverable were stored in labeled cryovial tubes in volumes of 0.5 mL at -20 ˚C or lower until analysis; at the at the Integrated Biorepository of H3Africa-Uganda (IBRH3AU), Makerere University, Kampala, Uganda. Qualitative assessment of all

participants' serum/plasma samples for HBV seromarkers was conducted, and sufficient aliquots re-archived for quantitative anti-HBs estimation.

5B.3.5 Determination of titers/levels of anti-HBs antibodies in participant's serum/plasma

This followed qualitative detection of anti-HBs. Serum samples were tested for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb using three different Hepatitis B Combo RDT test kits; NOVA TEST-One Step Diagnostic Rapid Test (Global Science, Malaysia; Jaya, Selangor D.E, Malaysia, Cat#: 20221811HBV), Fastep Rapid Diagnostic Test (Healgen Scientific Limited Li-ability Company, Houston, TX77047-USA, Cat#: 21076412) and Beright Diagnostic Rapid Test (Medhealth Biotechnology Limited, China, Cat#: 12216453) with manufacturer re-ported sensitivity and specificity of (91.43% and 98.28%), (68% and 77%), and (98.92% and 99.46%) respectively. Children who tested positive for HBsAg were referred to the CFU Medical Center for appropriate clinical management. All sufficient plasma samples volumes, including those that were qualitatively positive for HBsAb were further quantitatively confirmed by measurement of anti-HBs using the Elecsys Anti-HBs II electrochemiluminescence immunoassay (ECLIA) test reagent kits (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany, Cat#: 11876317122) on a cobas e 411 immunoassay analyzer (serial number: 6111-04). The kits have manufacturer-reported sensitivity and specificity of 100% and 99.62%, respectively. For anti-HBs, we quantitatively analyzed 443 and 267 serum samples for the under 5 years and 15-17-year-olds respectively. All test procedures followed the manufacturer's instructions [https://www.roche.com/]. All kits and testing runs included positive and negative controls (prepared and distributed to health facilities by the Uganda Virus Research Institute (UVRI)).

5B.3.6 Data analysis

The determined anti-HBs quantification was reported in international units (IU). Data were entered for cleaning into EpiData software version 3.1 and exported to SPSS version 25 for descriptive analysis. Categorical variables and continuous variables were presented as frequency tables and graphs. Anti-HBs titers were presented with the median and as a percentage with the corresponding 95% confidence interval. Mann-whitney U test was used to compare groups. A p-value of less than 0.05 was considered statistically significant.

5B.3.7 Interpretation of anti-HBs titer results

HBV vaccine seroprotection/response is categorized as the number/proportion of children with anti-HBs titers ≥ 10 mIU/ml (Chathuranga, Noordeen, & Abeykoon, 2013). Therefore, the lack of HBV vaccine protection/non-response was characterized by anti-HBs titers < 10 mIU/ml. The proportion of seroprotection determined by an anti-HBs estimation assay defined its positivity rate. Anti-HBs levels between 10 and 100 mIU/ml were considered as hyporesponse and more than 100 mIU/ml was considered as a high level of immunity following vaccination (Chathuranga et al., 2013).

5B.4 Results

5B.4.1 Socio-demographic characteristics

Out of the 789 enrolled, a total of 710 children participated in this study; 267 and 443 in the 15-17-year-old and under 5-year-old groups, respectively. Following qualitative HBV seroprevalence estimation, 79 samples did not retain sufficient amounts to quantify the detectable anti-HBs. The mean age of the study participants was 16.1 years and 3 years for the 15-17-year-old and 0-5-year-old groups, respectively (Table 8). Whereas more female children (53.05%) were

enrolled in the under-5-year-old group, more males (51.69%) were enrolled in the 15-17-year-old group (Table 8).

Table 8. Characteristics of the study participants, Kawempe Division, May-August 2023

Variable	0 - 5 years, *n*=443	15 - 17 years, *n*=267	Combined, *n*=710
Age Mean (SD)	3.01 (1.32)	16.17 (0.82)	
Gender n (%)			
Male	208 (46.95)	138 (51.69)	346 (48.73)
Female	234 (53.05)	129 (48.31)	363 (51.12)

5B.4.2 Anti-HBs antibodies among study population

Among participants successfully tittered, prevalence of protective levels of anti-HBs antibodies was 51.83% (368 of 710); 74.29% of the children in the 0-5-year-old age group (329 of 443) and 14.61% (39 of 267) in the 15-17-year olds respectively. The median anti-HBs titers were 74.12 mIU/mL (IQR: 8-330.8 mIU/mL) and 2.48 mIU/mL (IQR: 2-2.48 mIU/mL) (Table 9). These results translate into a 48.17% proportion of participants with no detectable or less than protective levels of antibodies against hepatitis B virus; 25.73% (114 of 443) in the 0-5-year-old and 85.39% (228 of 267) in the 15-17-year-old groups respectively. We also found that none (0) and 2 children under 5 years and 15–17-year-old children respectively had protective anti-HBs levels resulting from hepatitis B virus exposure or infection.

Table 9. Anti-HBs among the study participants in Kawempe Division, May-August 2023

Variable	Overall, n=710	0 - 5 years, n=443	15 - 17 years, n=267
Anti-HBs titer Median (IQR)		74.12 (8-330.8)	2.48 (2-2.48)
Protective Anti-HBs n (%)	368 (51.83)	329 (74.29)	39 (14.61)

5B.4.3 Anti-HBs antibody titers by number of vaccine doses and age of participants

Of the 412 HBV vaccine responders; 363, 28, and 21 received 3, 2, and 1 doses, respectively. The proportion of those with protective levels as well as titers of HBV vaccine-specific anti-HBs antibodies increased with number of doses received (See Figure 11 A & B). The anti-HBs antibody titers were significantly higher among those who completed 3 doses when compared to single $p = 0.009$ or two $p = 0.003$ dose recipients.

Within the first 5 years, we saw a gradual decrease in the median anti-HBs levels but titers were significantly higher among the under 5 year group when compared with titers of the 15-17 year old children $p < 0.05$ (Figure 11 C).

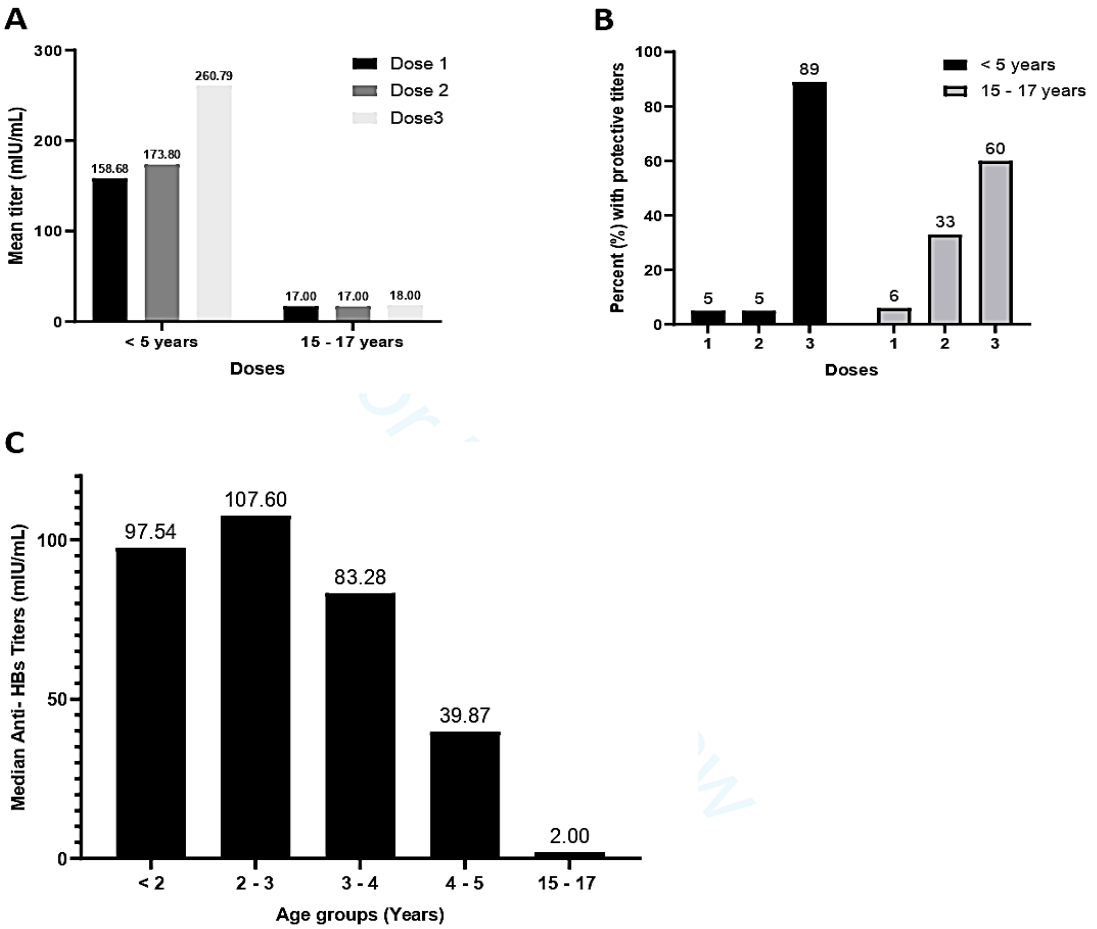


Figure 11: Anti-HBs; (A) by numbers of doses received; (B) Percent of those with seroprotective titers by number of doses received; (C) levels by age categorization, among the study participants in Kawempe Division, May-August 2023.

5B.4.4 Comparative analysis of qualitative and quantitative assays for the estimation of anti-HBs

The qualitative Hepatitis B Combo RDT test assays detected only 42.12% (155/368) of all protective anti-HBs titers that were determined by the quantitative Cobas E411 system (368/710) (Figure 12). The lowest titer as determined by the Cobas E411 system and also detectable on the RDT was 10.28 mIU/mL.

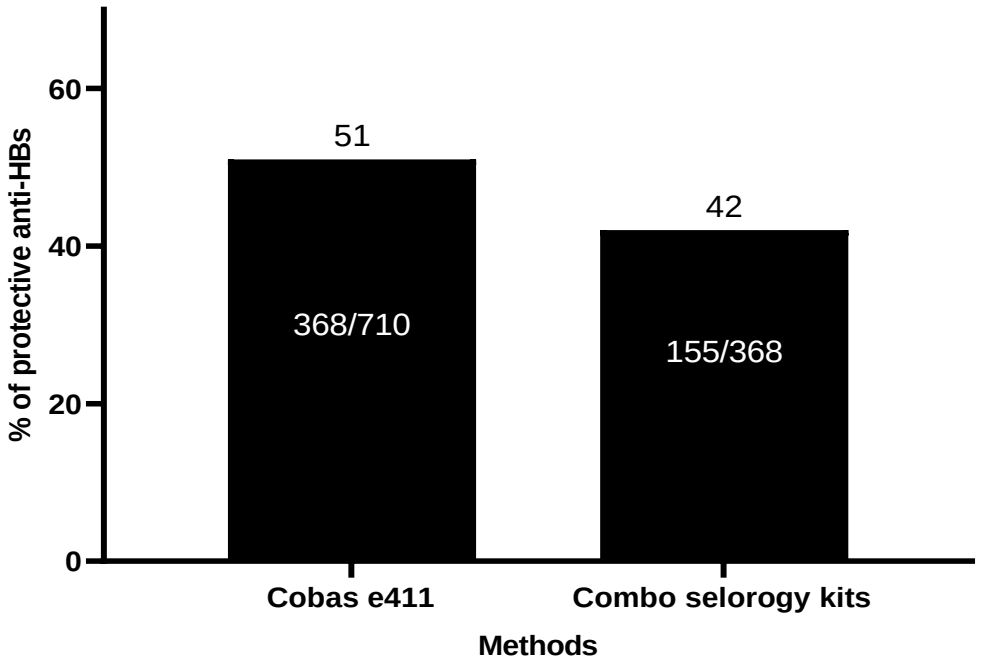


Figure 12: Proportions (%) of children with protective levels of anti-HBs (as determined on the Cobas E411 system) that were detected (anti-HBs positive) by the rapid diagnostic tests (RDTs) Assays.

CHAPTER SIX

Hepatitis B Virus Exposure Status and Anti-Hepatitis B Virus Surface Antigen Antibodies Among Adolescent Children Living with HIV in Uganda

Preamble

In Uganda, HBV/HIV coinfection rates range between 4–17% (Seremba et al., 2021). Adolescents living with HIV are particularly vulnerable due to factors such as immunosuppression, poor adherence to antiretroviral therapy (ART), high HIV viral loads, and engagement in high-risk behaviors. Current data/information in Uganda on HBV immunity among HIV-positive adolescents is scarce, leaving gaps in understanding their actual immune protection and risk of HBV acquisition. Furthermore, although qualitative rapid diagnostic tests (RDTs) are widely used for HBV screening in low-resource settings, their sensitivity for detecting protective anti-HBs levels is potentially limited, potentially leading to underestimation of immunity. This raises questions about the reliability of current screening approaches for guiding vaccination policy in HIV care programs. Given the high burden of both HIV and HBV in Uganda, the unclear extent of long-term vaccine-induced protection among HIV-positive adolescents, and the limitations of available screening tools, it is crucial to generate robust data on HBV exposure status and protective anti-HBs prevalence in this population. Such evidence is essential to inform national guidelines for HBV risk assessment, targeted vaccination strategies, and the development of improved diagnostic approaches, ultimately contributing to the WHO goal of eliminating viral hepatitis as a public health threat by 2030.

This work was published by *BMC Infectious Diseases*: See Appendix 4

DOI: 10.1186/s12879-025-11244-3

Hepatitis B Virus Exposure among Adolescents Living with HIV: A Cross-sectional Study of Samples Referred to the National Viral Load and Early Infant Diagnosis Reference Laboratory in Uganda

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Disclosures

None

Acknowledgements

We thank the management and staff of the School of Biomedical Sciences, Uganda National Health Laboratories, and Diagnostic Services; UNHLDs; the Infectious Disease Institute (IDI); and the study participants.

Competing interests

The authors declare that they have no competing interests.

6.1 Abstract

Background: Adolescents living with HIV are vulnerable to hepatitis B coinfection. However, the prevalence of hepatitis B virus (HBV) exposure and the persistence of childhood vaccine-induced HBV antibodies in this population remain unclear. This study assessed HBV exposure and the prevalence of protective Hepatitis B Surface Antibody (anti-HBs) levels among adolescents living with HIV referred for viral load testing at the national reference laboratory in Uganda.

Methods: A cross-sectional analysis was conducted on 405 archived plasma samples collected between January and September 2023 at the CPHL-VL/EID laboratory of UNHLDS. HBV exposure was determined via a 5-panel Vaxpert HBV combo cassette, and protective anti-HBs levels were quantified via the Roche Cobas e411 system.

Results: The mean age of the participants was 15±2.7 years, with 52.5% (212/405) being female. Among them, 8.7% (35/405) had an unsuppressed HIV viral load. The following HBV markers were detected: HBsAg (2.0%), anti-HBs (24/405, 5.9%), HBeAg (2.2%), anti-HBc (1.2%), and anti-HBe (3.45%). Overall, 7% (28/405) had evidence of HBV exposure, including 11 (2.7%) with acute infection, 13 (3.2%) with chronic infection, and 4 (1%) with resolved infection. Vaccination-induced immunity (anti-HBs-positive only) was found in 4.9% (20/405) of the participants. Of the 301 samples tested for anti-HBs titers using the Cobas E411 system, only 49 (16.3%) had protective levels (≥10 IU/L), with a median titer of 77.7 IU/L (IQR: 18.43–589.2). All 24 samples that were qualitatively anti-HBs-positive on the Vaxpert rapid test had protective titers, 21 of which were >100 IU/L. Among 277 samples that were anti-HBs-negative by the rapid test, 25 (9%) showed protective titers upon quantitative testing, highlighting potential false-negative results and suggesting limited sensitivity of the rapid assay. All the demographic and clinical factors available and assessed in this study population were not significantly associated with anti-HBs levels**.**

Conclusions: The low prevalence of protective anti-HBs levels and evidence of HBV exposure highlight the ongoing risk of HBV infection in this population. Our findings suggest a need for Uganda to establish specific guidelines for HBV risk assessment, screening, and management among people living with HIV.

Keywords: Adolescents, hepatitis B, antibodies, HIV, coinfection, acute & chronic, vaccination & exposure

6.2 Background

Hepatitis B virus (HBV) remains a significant global public health challenge, with an estimated 254 million chronic infections and 1.1 million deaths in 2022. The WHO African region bears a substantial burden, accounting for 63% of the global estimate of 1.2 million new infections in 2022(Organization, 2024a). Uganda is highly endemic, with an estimated national prevalence of 10% (E. Ochola et al., 2013). Hepatitis B virus infection is common among populations, especially in Africa, and often leads to unwanted outcomes, including liver cirrhosis, hepatocellular carcinoma (HCC), and death (N. P. Nelson, Jamieson, & Murphy, 2014).

Among people living with HIV (PLWH), HBV coinfection is a major contributor to morbidity and mortality (Ganesan, Poluektova, Kharbanda, & Osna, 2019; Ruta, Grecu, Iacob, Cernescu, & Sultana, 2023). Globally, the prevalence of HBV/HIV coinfection is estimated to be 7.6% (Ahmadi Gharaei et al., 2021) and ranges from 8–20% in sub-Saharan Africa (Day et al., 2013) and from 4–17% in Uganda (Seremba et al., 2021). Adolescents living with HIV are at risk of HBV infection due to immunosuppression, poor adherence to antiretroviral therapy (ART), increased HIV viral loads (Bentouhami et al., 2019; Hudelson & Cluver, 2015) and indulgence in risky practices. Risk factors such as shared transmission routes, tattooing, piercings, and injection drug use further exacerbate this risk (Bentouhami et al., 2019; Seremba et al., 2021) in this population. HBV/HIV coinfection complicates disease management (Ahmadi Gharaei et al., 2021), with additional risks for pregnant adolescents, including vertical transmission (P. K. Kitandwe et al., 2021) to the unborn child.

In response to HBV infection, Uganda incorporated the HBV vaccine into its National Expanded Program on Immunization (UNEPI) in 2002, which targeted infants with a three-dose regimen at 0, 1, and 6 months (Mast et al., 2006; E. Ochola et al., 2013). As of 2019, the national

vaccine coverage for three doses reached 85% (Pattyn, Hendrickx, Vorsters, & Van Damme, 2021). A hepatitis B surface antibody (anti-HBs) titer ≥10 IU/L is considered protective (Giacomet et al., 2018); however, in our setting, 5–10% of vaccinated children fail to reach this threshold (E. N. Anutebeh et al., 2021), and response rates are further reduced among PLWH (Seremba et al., 2021). Among HIV-positive individuals, protection rates range from 34% to 88.6%, whereas they are ≥90% in the general population (Seremba et al., 2021) (Chonwattana et al., 2016). The waning of protective antibody levels is a concern, with rates ranging from 13% to 60% over the years after the initial protective anti-HBs titers among healthy individuals (Giacomet et al., 2018). Recently, reports have shown that adolescents 15–17 years of age in Africa have a very low prevalence of protective anti-HBs (Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, Kateete, et al., 2024), potentially due to the waning of antibody responses (Giacomet et al., 2018; Vivian Efua, Armah, & Delali Adwoa, 2023).

Among HIV-positive individuals, the extent of waning of the protective HB antibody response is unclear. Up to 66.7% of infant recipients of the HBV vaccine have been reported to lose protective titers of antibodies after 15 years (Klinger, Chodick, & Levy, 2018). As such, HBV remains a major HIV coinfection and cause of mortality among PLWH (Ruta et al., 2023).
Therefore, the aim of this study was to determine hepatitis B virus exposure and the prevalence of protective anti-HBs in samples collected from adolescents living with HIV in Uganda.

6.3 Materials and methods

6.3.1 Study design, setting and population

We conducted a cross-sectional study utilizing leftover archived clinical samples from a total of 6,872 samples from adolescent (10–19 years old) patients that were referred to the National

Biorepository from hospitals across the country between January and September 2023, for HIV viral load testing at the CPHL-VL/EID laboratory of UNHLDS (refer to Chapter 3, sub section 3.1.5). Qualitative hepatitis B serology and anti-HBs titer determination were performed at the Makerere University IDI Core Laboratory (refer to Chapter 3, sub section 3.1.4).

6.3.2 Sample size estimation

For an estimated 170,000 adolescents living with HIV in Uganda (Akankunda et al., 2022) and at an estimated margin of error (e) of 5%, we calculated the sample size of 400 plasma samples via the Taro Yamane formula (Adam, 2020) although 405 samples were analyzed.

6.3.3 Sampling method

We utilized Microsoft Excel-aided simple random sampling within an archive of 532 samples that met the inclusion criteria (adolescent, living with HIV and consented for future research use of samples) to get the 400 samples. A total of 27 of the 400 samples selected were excluded and replaced with another randomly sampled plasma due to inadequate sample volume, ineligible label on containers or being leaky. To assess the prevalence of protective hepatitis B surface antibody (anti-HBs) levels (≥10 IU/L), we analyzed a total of 301 archived plasma samples. These included all 24 samples that tested positive for anti-HBs using the qualitative HBV Vaxpert Combo test cassette (purposively included to maximize detection of protective titers), and a randomly selected subset of 277 samples from the remaining 381 anti-HBs-negative samples. This sampling strategy was employed to ensure a representative and epidemiologically balanced cohort while optimizing available laboratory resources. Given budgetary and logistical constraints, it was not feasible to test all 405 samples quantitatively using the Cobas e411 system. Therefore, random sampling of the anti-HBs-negative group minimized selection bias while maintaining the statistical

power necessary for subgroup analyses, including assessment of correlates such as age, sex, and HIV viral load. An illustration of the sampling, laboratory testing and data analysis is summarized in Figure 13.

6.3.4 Meta-Data Collection

All samples arriving at the CPHL/NHLDS are accompanied by an individual standardized viral load/EID testing request form and a sample chain of custody forms that summarizes a list of samples in the transportation package. Data on both the request forms and the sample chain of custody forms are entered into the CPHL/NHLDS Viral Load/EID Laboratory Information Management System/Dashboard. We developed and utilized a data collection form to extract participants' age, sex, pregnancy status and breastfeeding status, HIV viral load and ART adherence from the dashboard and/or test request form. The completed data collection forms were transported from CPHL/NHLDS to Makerere University College of Health Sciences, where they were double entered into an Epidata-based data entry tool.

6.3.5 Specimen retrieval, transportation, storage and thawing

Frozen samples were retrieved from the -80°C freezers and transported in an insulated container with dry ice to the Makerere University IDI Core Laboratory for batch testing. The samples were normalized to room temperature for at least one hour before assaying.

6.3.6 Determination of hepatitis B surface antibody titers

The Elecsys HBsAb II reagent (catalog no. 69612201) run on the Roche Cobas e411 system was used to measure the levels of HBsAb in the samples following the manufacturer's instructions. Daily Internal Quality control was performed by running calibrators provided within the reagents procured, Anti-HBs G2 ELECSYS COBAS E 100 V2 (Batch number # 69612201 & 71433301)

and also running commercial ELECSYS PRECICONTROL ANTI-HBS (Batch #: 72302401) on the analyzer each day of sample analysis. The Roche Cobas e411 analyzer used in the study participates in the HBsAb proficiency testing (PT) scheme of the College of American Pathologists (CAP). The analyzer software automatically calculated the analyte concentration of each sample, reported in IU/L.

6.3.7 Determination of HBV infection status

Five-panel HBV Combo test cassettes (Vaxpert, Cata log no. HBV23120001) were used to test for the presence or absence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb) and hepatitis B core antibody (HBcAb) according to the manufacturer's instructions. The Vaxpert Hepatitis B Combo test cassettes have a manufacturer's declared sensitivity of 99.9%, specificity of 99.4% and accuracy of 99.7% for the detection of markers of hepatitis B virus exposure. Briefly, the test cassettes were removed from the pouch and labeled with a sample identifier, and three drops of the plasma were dropped on each sample port via the provided dropper. The results were read after fifteen minutes and no later than 20 min after sample addition.

6.3.8 Interpretation of hepatitis B virus infection status

The interpretation of the HBV serological results was performed according to the Australasian Society for HIV, Viral Hepatitis, and Sexual Health Medicine (ASHM)'s 2024 Clinical Tool for Decision Making in Hepatitis B (www.testingportal.ashm.org.au/hbv, accessed on 24 May 2024) (Table 1).

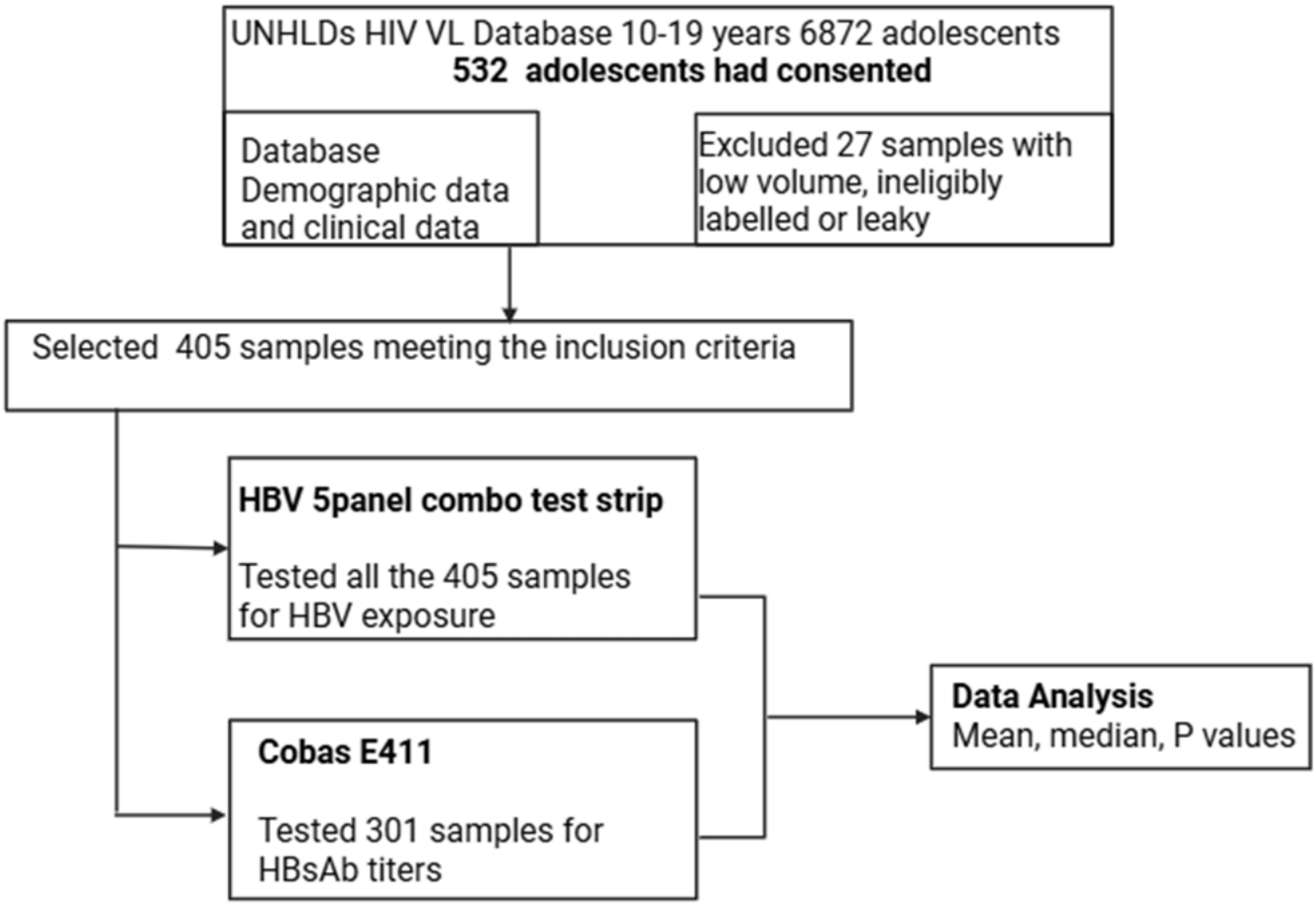


Figure 13: Illustration of the methods used for sampling, assaying and data analysis

6.3.9 Data analysis

Data analysis was performed via GraphPad Prism software 10.1.2. Descriptive statistics were used for continuous variables to obtain medians and means, whereas the Wilcoxon rank sum test was used to compare parameters between the groups with protective and nonprotective anti-HBs levels, deriving p values for statistical significance and confidence intervals. Categorical variables were analyzed using the fisher's exact test. The analysis was performed at a 95% level of significance. The prevalence of each hepatitis B virus infection status and protective antibody level was calculated as the proportion of adolescents with the parameter of interest, divided by the total

number tested, and expressed as a percentage. Titers were measured in international units, and adolescents with anti-HBs titers ≥10 IU/L were considered immune to hepatitis B virus.

6.4 Results

6.4.1 Demographic characteristics

A total of 405 samples from adolescent participants were included in the study. The mean age of the sample donors was 15±2.7 years. Over a half, 52.5% (212/405) of the sample donors were female, one of whom 1/405 (0.6%) was pregnant and 3/405 (1.7%) were breastfeeding. A total of 35/405 (8.7%) had no suppressed HIV viral load.

6.4.2 Hepatitis B virus exposure according to the results of the Vaxpert HBV Combo tests

Of the 405 adolescents included in the study, 8 (2.0%) were positive for HBsAg, 24 (5.9%) for anti-HBs, 9 (2.2%) for HBeAg, 5 (1.2%) for anti-HBc, and 14 (3.5%) for anti-HBe. Based on the results above, 48/405 (11.9%) adolescent participants were reactive to at least one or more serological marker. We found that 20/405 (4.9%) participants were positive for only anti-HBs and no other hepatitis B virus marker, indicating antibody responses due to vaccination. The number of adolescents with evidence of current or previous exposure (acute, chronic or resolved) to the hepatitis B virus was 28/405 (7.0%), with 4/28 (14.3%) having recovered/resolved from the infection (positive for anti-HBs and anti-HBc, with or without anti-HBe), 13/28 (46.4%) having chronic infection (positive for HBsAg and anti-HBc, with or without HBeAg and anti-HBe) and 11/28 (39.3%) having acute infection (positive for HBsAg, anti-HBc and HBeAg).

6.4.3 Hepatitis B surface antibody (anti-HBs) titer levels from the automated Cobas E411

Among the 301 samples tested for anti-HBs titers using the automated Cobas E411 analyzer, only 49 (16.3%) had protective antibody levels (≥10 IU/L). The overall median anti-HBs titer was 77.70 IU/L (IQR: 18.43–589.20 IU/L). All 24 samples that were qualitatively anti-HBs-positive on the Vaxpert hepatitis B virus Combo cassette had protective titers, with 21 of them showing values above 100 IU/L. Among the 277 samples that were qualitatively anti-HBs-negative, only 25 (9%) had protective anti-HBs levels, with a median titer of 2.00 IU/L (IQR: 2.00–3.17 IU/L).

6.4.4 Factors associated with protective effects on anti-HBs titers

Among all the demographic factors assessed in this study, none showed a statistically significant association with protective hepatitis B surface antibody (anti-HBs) levels in the adolescent population. Specifically, age, sex, pregnancy, and breastfeeding were not significantly associated with protective anti-HBs titers, as presented in Table 10.

Table 10 Association of demographic characteristics to anti-HBs titers

Total No = 301		Protective n=49	Nonprotective n=252	P value
Age (years), Median		15 (10 -19)	15 (10 -19)	0.4313
Sex	Male	25(51.02%)	121 (48.02%)	0.7558
	Female	24 (48.98%)	131 (51.98%)	
Pregnancy	Yes	0	1	>0.999
	No	18	114	
Breast feeding	Yes	01	02	0.3751
	No	18	111	

Similarly, clinical factors evaluated in this study, including HIV viral load suppression and ART adherence did not demonstrate any significant association with protective anti-HBs levels. These findings are summarized in Table 11 below.

Table 11; Clinical factors associated with protective anti-HBs titers among the study population

Total No = 301		Protective n=49	Non-protective n=252	P value
HIV Viral Load	Suppression	47	228	0.2759
	Non-suppression	02	24	
Art adherence	Good ≥ 95%	31	191	>0.9999
	Fair (84-94%)	0	02	

CHAPTER SEVEN

7.0 DISCUSSION

7.1.1 Hepatitis B Vaccine Seroprotection Rates Among African Children of up to 17 Years of Age

The robustness and duration of seroprotection accorded by hepatitis B vaccine depends on a number of baseline endogenous and exogenous factors such as age, body mass index, sex, concomitant diseases (like HIV infection, diabetes, and chronic liver/kidney disease), psychological stress, nutrition, smoking, alcohol consumption, race, vaccine type, dose, and vaccination schedule (Bauer & Jilg, 2006; Gara et al., 2015; Walayat et al., 2015). Studies have been conducted globally to evaluate the immune protection accorded by HBV vaccine and its persistence among the pediatric population (Abushady et al., 2011; Chaouch et al., 2016; El-Sayed, El-Guindi, El-Shaarawy, Salama, & Sobhy, 2011; El Sawy & Mohamed, 1999; Eladawy et al., 2015; Madhi et al., 2019; Makhlouf et al., 2016; Mendy et al., 2013; Odusanya et al., 2011; Pileggi et al., 2017; Prabdial-Sing et al., 2019; Quaye et al., 2021; Reda et al., 2003a; Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012; Romanò et al., 2017; Saffar et al., 2014; Shaaban et al., 2007; Teshome et al., 2019; Tfifha, Kacem, Naija, et al., 2019; Tsebe et al., 2001; Van der Sande et al., 2007; Whittle et al., 2002; Zhao et al., 2019b). However, there is paucity of such studies in Africa. Where such studies have been done, their findings are inconclusive and significantly vary due to the different sample sizes, geographical regions, methods and other study parameters. In this meta-analysis we provide a holistic evaluation of HBV vaccine seroprotection proportions in Africa among childhood recipients, of up to 17 years of age. We have used a pooled sample size of 10,730, significantly improving statistical power that affects other similar studies (El-Sayed et al., 2011; Eladawy et al., 2015; Tfifha, Kacem, Ben Rejeb, et al., 2019).

Here, we report that the proportion of children under 15 years of age with HBV vaccine-specific seroprotection is significantly higher ($p < 0.0001$) (69.07%) than their counterparts 15 to 17 years of age (32.368%). This finding is in agreement with Egyptian (Makhlouf et al., 2016) and Tunisian(Chaouch et al., 2016) studies. Immune protection accorded by HBV vaccine wanes over time, perhaps contributing to the significantly reduced proportion of seroprotection demonstrated in the 15-17 year olds age group (Eladawy et al., 2015; Makhlouf et al., 2016; Mendy et al., 2013). More so, the 15-17-year olds received the HBV vaccination in the early to mid-2000s; a time when many African countries were just initiating and or rolling out HBV vaccination into their national EPIs. It is possible that challenges in the early phases of HBV vaccine integration and roll-out in the national EPIs affected coverage and completion of doses, that in part could explain the lower proportion seen among the 15-17 years old children (Eladawy et al., 2015). What is apparent though is that there is a big pool of children 15-17 years of age who are at increased risk of contracting HBV on exposure yet this age bracket correlates with onset of socio-behavioral activities associated with increased risk to HBV infection. We report that studies among the under 15-year-olds done between 1999-2012 and then from 2016 – 2021, proportions of HBV vaccination seroprotection were very similar (67.421% and 71.475% respectively, $p < 0.0013$), indicating that challenges of implementing a nascent HBV vaccination program, if any, did not significantly impact on proportions of children with induced seroprotection. A number of studies (Bruce et al., 2016; Romanò et al., 2017; Saffar et al., 2014; Zhao et al., 2019b) have also reported relatively high proportions of seroprotection for children under 5 years. The United Nations Sustainable Development Goals aim to eliminate viral hepatitis by the year 2030 (Organization, 2016a), and the WHO-UNICEF, continues to advocate for improved vaccination coverage and proportions of seroprotection that meet the herd immunity threshold. The results above indicate a concerted effort

for African national EPIs to attain the desired coverage right from the start of HBV integration in childhood vaccination programs.

Regardless of the age groups, we generally saw low proportions of HBV vaccine seroprotection in much of Africa. We have found studies reporting even lower proportions of seroprotection (39.4% and 42.8%) among Egyptian children under 15 years (Abushady et al., 2011; Shaaban et al., 2007). These reduced proportions of seroprotection in HBV vaccination are contrary to those reported in Western developed countries. Studies conducted in higher income countries other than Africa report significantly higher proportions of seroprotection among adolescent children. Probably the profoundly reduced HBV infection rates in such countries are attributable to higher seroprotection rates compared to Africa. A study that assessed the immune persistence conferred by a Chinese hamster ovary (CHO)-derived hepatitis B vaccine (HepB) 17 to 20 years after primary immunization during early life, demonstrated seroprotection rates of 74.5 % among the participants (Zhao et al., 2019b). Similarly, a study conducted in Germany confirmed that paediatric doses of hepatitis B vaccine confer long-term protection against hepatitis B in adolescents up to 15–16 years; with pre-booster dose seroprotection rates of 65.4%, and produce robust immune memory (Olivier Van Der Meeren, Ulrich Behre, & Priya Crasta, 2016). Reduced sero protective levels in Africa have been attributed to several factors. Waning of immunity over time, often measured as reduction or loss of detectable levels of anti-HBs antibody titers has been cited by many (Makhlouf et al., 2016; Prabdial-Sing et al., 2019; Shaaban et al., 2007; Teshome et al., 2019). Evidence for waning responses is supported by our results showing that articles published between 1999 and 2012, and those published between 2016 and 2021 show relatively high and comparable proportions of seroprotection (67.421% and 71.475% respectively, $p < 0.0013$) among children who received the HBV vaccine less than 15 years ago, while the reverse is true in the 15–17-year-olds.

The correlation of antibodies with seroprotection is driven by the belief that neutralizing antibodies are the major correlate of protection against HBV infection, and several infectious agents (Coppola et al., 2015; Frosch & John, 2012; Roshell Muir et al., 2023; van Dorst et al., 2023). However, another side of the argument is that levels of anti-HBs neutralizing antibodies may not accurately correlate with seroprotection because recall or anamnestic responses may ensure effective protection against HBV upon exposure (Bruce et al., 2016; Papadopoli, De Sarro, Torti, Pileggi, & Pavia, 2020; Schwarz et al., 2019; Van Damme et al., 2019; Zhao et al., 2019b). In this context, the waning of seroprotection may not be of clinical or epidemiological consequence. Also, the type of vaccination schedule adopted has been associated with variations in proportions and magnitude of seroprotection induced. Some national EPIs in Africa were subscribing to the 2, 4 and 6 months schedule (Chaouch et al., 2016; Eladawy et al., 2015; Makhlouf et al., 2016; Reda et al., 2003b; Shaaban et al., 2007) while others were implementing the 6, 10 and 14 weeks schedule (Odusanya et al., 2011; Prabdial-Sing et al., 2019; Quaye et al., 2021; Rey-Cuille, Seck, Njouom, Chartier, Sow, Mamadou, et al., 2012; Whittle et al., 2002). However, many recent national EPIs have moved to a modified version of the latter schedule that starts with a HBV vaccine shot given at birth that is now thought to be more effective (Akbar et al., 2021; de Villiers et al., 2021b; Memirie et al., 2020b; Safadi et al., 2021). Another source of reduced proportions of seroprotection in Africa has been the plethora of current, past, and future exposures to several infectious agents. Particularly, parasitic infections such as *Schistosoma mansoni* and *Plasmodium falciparum* have been associated with blunted responses to vaccines (Frosch & John, 2012; Julg & Alter, 2018; Qadri, Bhuiyan, Sack, & Svennerholm, 2013; van Dorst et al., 2023), including to HBV vaccination (Roshell Muir et al., 2023).

Even within Africa, we report seeing variations in proportions of HBV vaccine seroprotection. For instance, children under 15 years from the Southern Africa region demonstrate significantly higher HBV vaccine seroprotection proportions (85.221%, $p < 0.0001$) than their counterparts in the Northern and Western Africa. Equally, children 15 -17 years of age from the Western Africa region demonstrate higher HBV vaccine seroprotection proportion (38.704%, $p < 0.0001$) than those from the Northern Africa region (26.253%, $p < 0.0001$).Northern Africa demonstrates the least proportions in seroprotection (64.242%, $p < 0.0001$) among this age group (Madhi et al., 2019; Prabdial-Sing et al., 2019; Tsebe et al., 2001) (Mendy et al., 2013; Quaye et al., 2021; Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012; Van der Sande et al., 2007; Whittle et al., 2002) (Abushady et al., 2011; Chaouch et al., 2016; El Sawy & Mohamed, 1999; Eladawy et al., 2015; Makhlouf et al., 2016; Odusanya et al., 2011; Reda et al., 2003a; Shaaban et al., 2007; Tfifha, Kacem, Naija, et al., 2019). A closer look at the HBV vaccine schedule implemented by EPIs in Southern and Western Africa regions shows that it has been the 6, 10, and 14 weeks schedule (Quaye et al., 2021; Rey-Cuille, Seck, Njouom, Chartier, Sow, Ka, et al., 2012; Tsebe et al., 2001) that is associated with better seroprotective responses. Northern Africa EPIs, on the other hand have been implementing the less effective 2, 4, and 6 months schedule (Chaouch et al., 2016; Mansour, Aylward, & Cummings, 1997). Sub-optimal or outright non-response to HBV vaccination also has a genetic dimension. Contemporary literature continues to indicate that individuals with a genetic predisposition for non-response to HBV vaccine may have a defect in their Human Leukocyte Antigen (HLA) and T-cell receptor (TCR), and hence dysfunctional antigen presentation and stimulation of T Helper cells, respectively (Walayat et al., 2015). Certain HLA types are associated with increased or reduced antibody response to hepatitis B vaccines in different individuals (L. Wang et al., 2016). To the best of our knowledge, such studies have not been conducted to conclusion in Africa, yet the African people and generally people of the black race

are known to be the most genetically diverse (Agyemang, Bhopal, & Bruijnzeels, 2005; Just, King, Thomson, & Klitz, 1997; Maróstica et al., 2022; Tshabalala, Mellet, & Pepper, 2015). Inherent heterogeneity therein can significantly determine the variations in proportions of HBV sero protection among children under 15 years in Southern, Western and Northern Africa.

We also indicate here that studies for children 15 to 17 years which were published between 2015 and 2016 showed low but significantly higher proportions of HBV vaccine seroprotection (26.253%, $p < 0.0001$) than studies published between 2002 and 2013 (4.306%). Here we report publication bias in studies for children 15 to 17 years published between 2002 and 2013 as demonstrated by the Egger's ($p = 0.0001$) but not the Begg's test ($p = 0.3173$). These two studies were conducted in the same country; Gambia, and done in 2002 and 2013, respectively, hence probably vindicating the noted bias. However, there was symmetrical spread of studies from the funnel plot of the standard error of prevalence against its prevalence (Supplementary Fig. S20). As earlier noted, the 15–17-year age group is a high-risk cohort for HBV and other sexually transmitted diseases yet it is demonstrating lower HBV vaccine seroprotection. However, studies published between 2015 and 2016 were probably conducted on children 15-17 years of age who were immunized in the HBV vaccination era (WHO, 2017), and after effective integration of HBV vaccines on EPIs of all countries (1997) (Lavanchy, 2004). Studies published between 2002 and 2013 were probably conducted on 15–17-year-old children who were ineffectively and inadequately vaccinated in the pre-vaccination era, and perhaps markedly limiting the HBV seroprotection rates.

We acknowledge some limitations in our study. Limited number of EPI monitoring and evaluation reports and publications on HBV vaccine seroprotection in Africa may have limited geographical coverage or representations of the meta-data used, particularly the East Africa regions.

We could not confirm if all the study participants enrolled in the primary studies had completed all the WHO recommended three HBV vaccine doses. This could certainly have led to an underestimation of the seroprotective proportions reported in the primary, and hence in this study. To mitigate both of the limitations above, we recommend meta-analyses that consider only the better designed national EPI evaluation data as they become more available. We considered no genetic diversity across the African continent in explaining the regional variability in sero-proportions observed. Therefore, the differences in seroprotection observed across the African regions may be due to genetics rather than vaccination strategy. All these study limitations notwithstanding, we pooled the prevalence of seroprotection to commendably enhance the representativeness and power of our meta-analysis.

This meta-analysis establishes proportions of HBV seroprotection among children up to 17 years of age in Africa. Children under 15 years of age demonstrate a significantly higher proportion of HBV vaccine seroprotection than those 15-17 years of age. The proportion of HBV vaccine seroprotection in children under 15 years are relatively high with the Southern Africa region demonstrating significantly higher proportion than its counterparts in the Northern and Western Africa. Notably, African children 15-17 years of age generally present with reduced proportion of HBV vaccine seroprotection. We further demonstrate generally higher proportions of HBV vaccine seroprotection in children under 15 years reported by studies conducted between 1999 and 2012, and those published between 2016 and 2021. However, studies among children 15-17 years published between 2015 and 2016 show low proportions of HBV vaccine seroprotection, although higher than proportions reported by studies published between 2002 and 2013. Whereas successful integration of the efficacious HBV vaccine on the EPIs has been a major achievement in the reduction of HBV infection in Africa, this meta-analysis indicates profoundly reduced HBV

vaccine seroprotection rates among current adolescents of 15-17 years of age. Systematically designed EPI evaluation studies may be required to supplement and augment our findings and perhaps establish and clarify the need for booster dose vaccination, particularly in the most at risk pediatric African populations.

7.1.2 Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda

This study aimed to determine the proportion of vaccinated children with qualitatively detectable anti-HBs antibodies in Kampala, Uganda. In 2016, the World Health Assembly (WHA) adopted a resolution for the elimination of viral hepatitis by 2030, setting elimination targets of a 90% reduction in incidence and a 65% reduction in mortality for hepatitis B by 2030 (Easterbrook et al., 2021). Expanding both vaccine coverage and third dose completion to more than 90% of infants, diagnosing more than 90% of estimated chronic hepatitis virus infections, and enrolling them in care are key national-level strategies for the elimination of HBV infections.

Uganda integrated the HBV vaccine into UNEPI in 2002 (Bwogi et al., 2009; Lewis et al., 2008), continuously increasing coverage over time. Our teenage and under 5-year-old study participants (average age 16.2 and 2.9 years, respectively) received the hepatitis B vaccine in 2006–2008 and 2018–2020, respectively, approximately 10–12 years apart. In this timeframe, our results suggest a 16% increase in hepatitis B vaccine third dose coverage (from 72.6% to 88%), with an approximate halving of the numbers of children stopping at the first and second dose(s). Our data compares with the WHO/UNICEF Estimates of National Immunization Coverage (WUENI) of 2008 and 2020 (71% and 89% for the 3rd dose in 2008 and 2020, respectively) (Casey et al., 2017; Mironova & Ghany, 2024), affirming Uganda's commitment to improving hepatitis B vaccine coverage to meet the global targets. Although the 2020 third dose coverage fell below the 90%

global target, official data shows that Uganda surpassed and consistently maintained third dose coverage at 93% and above (Organization, 2020a) by 2016. The 2020 drop in coverage coincides with the COVID-19 pandemic, which disrupted healthcare systems globally, including vaccination services (Kabore et al., 2022).

The Hepatitis B vaccine is known to be very efficacious, and response rates are high, ranging between 85% and 100%, eliciting antibody titers above the 10 mIU/mL protective limit (Jack et al., 1999). However, asymptomatic breakthrough infections (detected by the presence of anti-HBc, anti-HBs, or HBV DNA in serum) have been reported in vaccinated persons with a documented initial protective antibody response (Lu et al., 2004; McMahon et al., 2005). Age at infection greatly determines whether HBV infection progresses into a chronic state: about 90% of infants will develop chronic infection when infected within the first year of life, compared with 30% of children aged 1–4 years and less than 5% of adults (Organization, 2017c). Therefore, it is of paramount importance that vaccine responses in infancy can prevent HBV infection. To meet the global targets for the elimination of HBV infections, ideally, countries would need to perform anti-HBs titer determination to confirm elicitation of protective levels among vaccine recipients, as is the case in vaccinated workers at risk (Canton, 2021).

The anti-HBs titer test availability as well as cost are prohibitive in low- and middle-income countries (LMICs) and would translate into huge programmatic costs, a situation that may divert funds meant for expanding and maintaining optimal vaccination coverage. On the contrary, qualitative strip or cassette-based chromatographic tests that detect anti-HBs antibodies (singly or in combination with other markers of HBV infection) are readily and cheaply available in LMICs. While qualitative anti-HBs cassette tests may not give levels of anti-HBs titers, in resource-limited settings they may be a surrogate for the tittering methods. Using this assumption and qualitative

testing, we were surprised to find that only one-third (33.1%) of vaccinated children under 5 years and 18.4% of the 15–17-year-old participants had qualitatively detectable anti-HBs antibodies. Both proportions seemed to be very low for such populations at high risk for HBV infection (Organization, 2017b), but are in accordance with findings elsewhere. Long-term follow-up studies of persons vaccinated as infants have reported the absence of detectable anti-HBs antibodies in 50–70% of persons 15–30 years later (Hammitt et al., 2007; A. W.-c. Lin & Wong, 2016; Lu et al., 2004; Ni et al., 2007). In the African setting, a recent study by Alemayehu, T et al., 2024, found that only 14.4% of children had protective anti-HBs antibodies (Alemayehu et al., 2024). Equally, our systematic review of 2023 reported low seroprotection rates across Africa (Fahad Muwanda et al., 2023).

Taken together, these results confirm low proportions of detectable anti-HBs antibodies in our study groups, which should concern Uganda's hepatitis B vaccination program as studies have reported a lack of anamnestic responses in close to half of those vaccinated in infancy (Hammitt et al., 2007), and infections continue to be reported in children in as short a period as 5 years post completion of vaccination (Norizuki et al., 2019). Contrary to our findings, some studies have reported moderate to high seroprotection rates for children under five years of age (Alssamei et al., 2017b; Ephesians N Anutebeh et al., 2021b; Madhi et al., 2019; Prabdial-Sing et al., 2019; Quaye et al., 2021; Rey-Cuille, Seck, Njouom, Chartier, Sow, Mamadou, et al., 2012; Tfifha, Kacem, Ben Rejeb, et al., 2019) and among teenagers (Chaouch et al., 2016; Eladawy et al., 2015; Makhlouf et al., 2016; Mendy et al., 2013; Romanò et al., 2017; Whittle et al., 2002; Zhao et al., 2019a) respectively. Several factors could have contributed to this notably improved vaccine efficacy, including administration of birth doses and enhanced vaccine coverage in this study setting.

Our study also showed HBV infection prevalence of 0.8% among the under-5-year-olds, which more than tripled to 2.77% in the 15-17-year-old participants. While all four infected under-5-year-olds were still acutely infected, six of the eight HBV-infected 15–17-year-olds were chronically infected, demonstrating the high tendency of pediatric HBV infections to progress into a chronic state (Organization, 2017c). A higher prevalence in adolescents versus those under 5 years is in line with the chronicity of pediatric infections and the evidence that adolescents are more likely to have low seroprotection (Alemayehu et al., 2024), hence more prone to breakthrough infections. Adolescence-associated socio-behaviors are also known to increase the risk of HBV exposure and infection (Mangowi et al., 2024; Melhem, Rahhal, Charide, Kreidieh, & El-Khatib, 2015; Organization, 2012). Our study's 2.77% prevalence in adolescents is comparable to those reported in studies among similar age groups that mostly range between 2.3–2.6% (Adugna et al., 2023; Alemayehu et al., 2024; Barro et al., 2019; Hagan et al., 2018). However, the seroprevalence of HBV infection reported by our study is relatively higher than that reported in low (Ethiopia; 0.4% (Teshome et al., 2019), Egypt; 0.6% (Shatat, Kotkat, Farghaly, Omar, & Zayton, 2005), Egypt; 0.4% (Hamid & Said, 2014)) and high-income countries (Italy; 0.6% (Mele et al., 2001), Iran; 0.6% (S. Hashemi, Moghadami, BAGHERI, Alborzi, & Mahboudi, 2010), Uzbekistan-Japan; 0.8% (Avazova et al., 2008)). The varying levels of breakthrough infections may be attributed to EPI vaccination coverage rates and evaluation, host immunogenetic factors, vaccination schedule adopted, and the method used to detect HBsAg.

A combined total of fifteen breakthrough infections is testament to suboptimal levels of protection induced by the hepatitis B vaccine in some members of both groups or of waning levels of protection (Hammitt et al., 2007; Lu et al., 2004). In Uganda, 5–17% of vaccine recipients are known to be non-responders regardless of completion of all 3 doses (Ocan et al., 2022; Okwakol,

2021). Our data showed that the majority of those with active HBV infection (6 of 8, and 3 of 4 among 15–17 year and under 5-year groups, respectively) were among those with no qualitatively detectable anti-HBs antibodies, pointing to the fact that suboptimal or non-response to, or significant waning of vaccine responses leaves children susceptible to HBV infection. This is supported by Reda AA et al.'s (2003) study in Egyptian 6-year-old children (Reda et al., 2003a) that reported a three-fold higher prevalence of HBsAg positivity (2.2% versus 0.8%) among vaccine non-responders in comparison to those with detectable anti-HBs.

Results of this study strengthen our idea that in the absence of access to tittering methods or financial constraints, qualitative Rapid Diagnostic Tests-based methods for the detection of anti-HBs antibodies may be valuable in identifying those susceptible and at high risk of HBV infection. The extent of utility of such qualitative anti-HBs detection methods may be better among children who are recent recipients of the vaccine and therefore expected to still have high levels of vaccine-induced anti-HBs antibodies detectable even on the less sensitive cassette-based RDT methods. Determination of the true utility of these qualitative RDT cassette-based methods would require studies to determine lower limits of detection (Lubell-Doughtie, Bhatt, Wong, & Shankar, 2022; Park et al., 2021), based on anti-HBs antibody titers. Clinical trials that estimate the proportion of vaccinated children with titers below detection levels who would otherwise be missed by relying on the qualitative method alone would also provide the public health risk of such an approach. Such studies would provide the extent of measurement uncertainty or confidence with which to credit qualitative test results concerning screening vaccine immune responses or susceptibility to HBV infection.

Rapid Diagnostic Test-based technologies (RDTs) have dominated Africa’s clinical laboratories and expanded access to cheap, timely, and reliable diagnostic services in the region,

including at point-of-care settings. The challenge of RDT-based tests is the lack of national-level policy or validation data to guide the choice of a better test brand for use, market stockouts of validated test kit brands, and the rapid emergence of new brands altogether. Laboratories implementing quality management systems do carry out some level of performance verification of emerging RDT brands at a local level, but the high turnover of brands on the market makes the exercise daunting. We provide reassuring data that the three brands used in this study were concordant and therefore may be used interchangeably to address stockouts in the Ugandan market.

Whereas our study boasts a good sample size, this study was conducted in only one of the five administrative divisions of Kampala and may not represent the entire city or be extrapolable to the rest of Uganda. Additionally, we were unable to perform quantitative measurements of anti-HBs titers. We also did not conduct HBV viral load testing; instead, participants found to be infected were referred to a public health facility for specialist care, usually provided at no cost to the patient. Also, not all of this study's participants enrolled in our study completed all three WHO-recommended HBV vaccine doses. Finally, we did not determine and exclude congenital exposure to HIV, a factor that is known to affect quality and magnitude of immune responses in a subsection of exposed infants.

We report a low prevalence of children with qualitatively detectable anti-HBs antibodies (33.3% among under-5-year-olds and 18.4% among 15-17-year-olds). The proportions above and being much lower in the 15–17-year-old participants are evidence of a rapid waning of anti-HBs antibodies over time. Most HBV infections are happening in those with no detectable anti-HBs antibodies, which affirms increased susceptibility to infection as well as a tendency to progress into the chronic state in children. The three Hepatitis B Combo test kit brands (NOVA TEST, Fastep, and Beright Diagnostic Rapid Tests) were perfectly concordant for all markers of HBV infection.

Anti-HBs titer determination and the determination of the extent of the utility of qualitative anti-HBs methods were outside the scope of this study but are recommended in the future or for other researchers to undertake.

7.1.3 Levels of Hepatitis B Vaccine Specific Antibodies among Children of Up To 17 Years of Age in Kawempe Division, Kampala

This study aimed to assess levels of HBV vaccine-specific seroprotective antibodies among children under 5 years and in those of 15-17 years in Kampala, Uganda. We have found that the prevalence of anti-HBs antibodies of 74.29% among children under-5 years of age is comparable with that (77.4%) of another study conducted in Uganda (Kimera et al., 2025), but falls short of WHO's recommended minimum of 90 % (Organization, 2024a). For children of the same age bracket, studies in similar populations elsewhere have reported much higher prevalence of protection of up to 97.5 % in some instances (Ephesians N Anutebeh et al., 2021b; Madhi et al., 2019; Organization, 2024a). Many reasons have been put forward to explain sub-optimal responses to vaccination, not only to HBV but other vaccines too. Under performance of the UNEPI due to multiple challenges like dose completion and coverage, vaccine cold chain, skills of nurses administering the vaccines, malnutrition, and lower economic standards, among others are notable reasons (Malande et al., 2019). For instance, immune studies among children of the fisherfolk of Lake Victoria, Uganda, have found that immune responses to measles and tetanus vaccines are equally sub-optimal (Anderson et al., 2012; Dennison Kizito et al., 2013; Natukunda et al., 2024; Prentice et al., 2015; Zirimenya et al., 2020), pointing to underlying global effects to vaccination in this part of the world.

Our findings revealed 14.6% prevalence of anti-HBs antibodies among children 15-17-years of age. This prevalence compares with what is reported in several African countries (Alemayehu et

al., 2024; Makhlouf et al., 2016; Fahad Muwanda et al., 2023) but is lower than global averages of 70–85% in European follow-up studies (Olivier Van Der Meeren et al., 2016; Zhao et al., 2019a). Socio-behavioral activities associated with increased risk to HBV and other sexually transmitted infections and reduced vaccination coverage/full dose completion rates are integral environmental factors that predispose to sub-optimal or non-response, and ultimately sustained HBV infection transmission in this age group (Adugna et al., 2023; Kafeero et al., 2025; Mangowi et al., 2024; Turyamubona et al., 2025). Low adolescent protection risks perpetuation of HBV cycles, hindering the WHO's 2030 targets of 90% incidence reduction.

Our study revealed an increment in anti-HBs titers and proportion of vaccinated individuals with seroprotection as the number of administered doses were also increased. This was particularly observed with in the first five years of vaccination prior to substantial waning of anti-HBs in the later years of life. Increased dosing enhances B cell priming, germinal center stimulation, plasma cell and neutralizing antibody production, while promoting memory B cell formation (Safadi et al., 2021; Tfifha, Kacem, Ben Rejeb, et al., 2019). This study and several others augment the notion that anti-HBs titers increase with HBV vaccine dose administration (Makhlouf et al., 2016; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024; Quaye et al., 2021). The observed increase in third-dose coverage from 72.6% (2006-2008 cohorts) to 88% (2018-2020 cohorts) reflects Uganda's progress toward 90% global targets, corroborated by WHO/UNICEF Estimates of National Immunization Coverage (WUENI) (71% in 2008 to 89% in 2020) (Kabore et al., 2022; Organization, 2024a). Additionally, Uganda’s 90% HBV vaccine coverage in some districts surpasses African averages (34%) (Kabore et al., 2022). However, the 2020 dip, linked to COVID-19 disruptions, underscores vulnerabilities in immunization systems, as seen in regional declines (Kabore et al., 2022).

We have also shown evidence of waning of anti-HBs, visible even among the under 5 year olds, while children 15-17 years demonstrate titers as low as 2 mIU/mL. Our findings are consistent with studies showing 40-90% antibody loss post-vaccination in adolescents (Alemayehu et al., 2024; Makhlouf et al., 2016; Fahad Muwanda et al., 2023). Indeed waning of anti-HBs antibodies over time, has been reported severally (Makhlouf et al., 2016; Prabdial-Sing et al., 2019; Teshome et al., 2019). However, it has also been argued that waning of neutralizing antibodies is not a major correlate of vaccine seroprotection because anamnestic responses can ensure effective seroprotection (Bruce et al., 2016; Papadopoli et al., 2020; Van Damme et al., 2019; Zhao et al., 2019a). We therefore advocate for enhanced HBV vaccine coverage and full dose completion rates, especially within the first five years of vaccination, when a robust immune response and recall memory can be potentiated. We also advocate for booster dose administration in adolescents as recommended in the 2024 WHO guidelines for high-risk groups (Kimera et al., 2025; Organization, 2024a, 2024c; Turyamubona et al., 2025). Much as the HBV vaccine demonstrates initial efficacy in infancy, rapid anti-HBs antibody waning, particularly in adolescents, calls for strengthened EPI monitoring to achieve WHO's 2030 elimination targets (Kimera et al., 2025; Organization, 2024a).

Low and middle-income countries (LMICs) demonstrate poor access to tittering as methods to verify attainment of protection against HBV infection. Factors like high costs, complexity, and access to remote areas limit the utility of titer methods. However, HBV Rapid Diagnostic Tests (RDTs) revolutionized access to diagnostics and can be a surrogate for tittering if validated. RDTs are designed for qualitative detection, and manufacturers are not obligated to make them sensitive. Our research group however, nurtured ambitions of collecting data on the use of RDTs as alternatives to tittering methods. The hope is that RDT manufacturers or other Biotechnology companies will gain interest in improving RDTs to achieve commendable utility in detecting HBV

seroprotection. Our findings showed that of the 368 children with protective anti-HBs as determined by the standard electro chemiluminescence immunoassay (ECLIA) run on the Cobas E411 system, only 155 (42.12%) were anti-HBs positive by the HBV RDT assays. This questions the RDTs' surrogate role for anti-HBs titer estimation in low and middle-income countries (LMICs) (Lubell-Doughtie et al., 2022; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, & Kateete, 2024), but perhaps stimulates RDT manufacturers to further improve RDT performance levels and their overall utility in the estimation of HBV and vaccine seroprotection.

Our reliance on self-reported vaccination for adolescents who lacked immunization cards predisposed our findings to confounders like potential recall bias. Our cross-sectional design precluded causality and therefore, future studies should incorporate longitudinal follow-up to elucidate sub-optimal response. Our large sample size and consecutive enrollment from Kawempe Division enhance representativeness, though urban bias limits generalizability to rural Uganda. Finally, not all of the study participants enrolled in our study completed all three WHO-recommended HBV vaccine doses.

The study identifies a clear adolescent immunity gap after infant HBV vaccination in Kampala, demonstrating reduced prevalence of HBV vaccine seroprotection in children under 5 years (74.29%), and particularly among the 15-17-year olds (14.6%). We also indicate that qualitative anti-HBs assays underestimate seroprotection relative to quantitative methods. To align with the 2030 HBV elimination targets, we advocate for enhanced HBV vaccination coverage and targeted adolescent strategies like evaluating the adoption of booster vaccine dose administration in Uganda.

7.1.4 Hepatitis B Virus Exposure Status and Anti-Hepatitis B Virus Surface Antigen Antibodies Among Adolescent Children Living with HIV in Uganda

In this study, we aimed to determine the prevalence of exposure to hepatitis B virus as well as the protective effects on anti-HBs titers among adolescents living with HIV in Uganda. Despite being an average age of 15 years, a few cases of pregnancy and breastfeeding in the adolescent population exemplify an already sexually active group, especially in a country where the age of adulthood starts at 18 years (Muwanguzi et al., 2021). This highlights the need for integrated comprehensive sexual health education and services for adolescents living with HIV in Uganda. In the context of the HIV program indicators, a suppression prevalence of 91.3% shows that even among this young population, Uganda is on the way to achieving the Joint United Nations Program on HIV/AIDS (UNAIDS) 95–95–95–95 targets for elimination of HIV (Woldesenbet et al., 2022).

The prevalence of HBV exposure (active or resolved) of 7.0% in this population corresponds with data from Sub-Saharan Africa, where Uganda is a hyperendemic (≥8% prevalence) region (Zampino et al., 2015). Our active HBV infection prevalence of 5.9% (2.7% for acute and 3.2% for chronic infection) agrees with similar adolescent and/or pediatric HIV/HBV coinfection studies in similar populations (Y. C. Huang et al., 2022; Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, Kateete, et al., 2024). In a systematic review of HBV among a 15–17-year-old population in sub-Saharan Africa, the prevalence of HBV infection was approximately half (2.77%) that of the general population (Muwanda, Kiyonga, Nambafu, Turyamubona, Kafeero, Kigozi, Babikako, Wekiya, Mboowa, Kateete, et al., 2024). Given that both HIV and HBV can be transmitted perinatally as well as during breast feeding, the incidence of HBV infection among children and adolescents living with HIV plausibly exceeds that of the general population (Organisation, March 2024; Zampino et al., 2015). Importantly, the

adolescent children in this study were born to mothers predating the introduction of the HBV vaccine into the Uganda National Expanded Program on Immunization (UNEPI) in 2002 (Bwogi et al., 2009; E. Ochola et al., 2013); hence, a higher prevalence of HBV infection is likely, and the virus could have been transmitted to these children. Almost half of the hepatitis B virus (HBV) actively infected adolescents were in a chronic state, supporting existing evidence that most pediatric infections progress to chronic infections rather than be resolved (Rouet et al., 2008). In light of these findings, the 2024 WHO guidelines recommend routine screening for HBsAg, anti-HBs, and anti-HBc among individuals newly diagnosed with HIV, with vaccination advised for those found to be non-immune. This strategy is critical for reducing HBV acquisition and transmission in this vulnerable population (Organisation, March 2024).

Unsurprisingly, anti-HBs attributable to HBV vaccination was only qualitatively evident in 16.3% of our study population, regardless of HIV viral suppression. The 16.3% seroprotective level in our population is in line with another study that reported protective anti-HBs levels at a prevalence of 18.2% at 2.8 years after neonatal HBV vaccination (Y. C. Huang et al., 2022). Another study reported a significant decline in anti-HBs titers over time among infants vaccinated against hepatitis B virus (Mancinelli et al., 2018). These low levels of protection in our settings contrast with those observed elsewhere in developed countries (O. Van Der Meeren, U. Behre, & P. Crasta, 2016; Zhao et al., 2019c).

The exact reasons for the low proportions of adolescents with protective anti-HBs levels are several and likely multifactorial. Among the starter-receiving HBV vaccine recipients, approximately 5%-10% of vaccinated individuals are immunocompetent and do not respond to the vaccine, and a decrease in the number of vaccine-specific antibodies to the HBV surface protein (the vaccine antigen) occurs in vaccinated individuals (Pierre Van Damme, 2021). By adolescence,

the decrease in vaccine-specific anti-HBs to undetectable levels can reach 70% (Bekele, Berzofsky, & Chiodi, 2021). The challenges of both poor primary immunological response to the HBV vaccine and the rapid waning of elicited immune responses are much greater among HIV-infected recipients (Bekele et al., 2017). However, it is also possible that vaccination coverage at the time these adolescents should have received their pediatric HBV vaccines was still suboptimal, since coverage between 2004 and 2013, when our 10-year–19-year-old adolescents may have received their vaccines under UNEPI coverage in Uganda, was reported to be between 62% and 84% (Whorld Health Organisation). We have recently shown that the majority of Sub-Saharan African 15–17-year-olds, born around the period of integration of HBV vaccines in many national EPIs in the region and therefore most likely receiving childhood vaccination, lacked protective anti-HBs levels (F. Muwanda et al., 2023). Research in Uganda has also shown generally poor childhood responses to infant vaccines, including measles, tetanus and hepatitis B (Elliott et al., 2010; D. Kizito et al., 2013; R. Muir et al., 2023). Underlying preexisting infections, especially malaria and helminths (Malhotra et al., 2015), poor childhood nutritional status (Rytter, Kolte, Briend, Friis, & Christensen, 2014) and programmatical challenges in the implementation of EPIs (Eladawy et al., 2015) have also been cited. These studies also suggested that antibody levels decline post vaccination, which further explains the results of our study. The presence of quantitatively undetectable anti-HBs may indicate that the majority of adolescents living with HIV (252 of 301, 83.7%) are vulnerable to HBV infection (Seremba et al., 2021). These findings aligned with those of Le et al., who reported a decrease in hepatitis B immunity despite increased vaccination rates during infancy (Le et al., 2020). However, a ray of hope from recent studies seems to suggest that a significant proportion of HBV vaccine recipients with waned undetectable anti-HBs immune responses seroconvert upon receiving a booster HBV vaccine dose, clearly showing that an

anamnestic response to the HBV vaccine is present in immunocompetent individuals despite a seronegative status (https://doi.org/10.1016/j.vaccine.2018.03.028).

Our study revealed no associations between ART adherence or suppressed viral loads and protective levels of anti-HBs, supporting other findings that these factors may not prevent antibodies from waning (Seremba et al., 2021). Similarly, sex played no role in the protection of HBs antibodies, suggesting that both male and female adolescents living with HIV were equally prone to either waning protective anti-HBs or susceptibility to HBV infection. While the number of pregnant and breastfeeding adolescents was too small for association analysis with anti-HBs levels or protection, they themselves are evidence of a sexually active population, and sexual contact is a major route of HBV transmission (Kalapila & Dhanireddy, 2020; P. K. Kitandwe et al., 2021).

Finally, although not the focus of this study, we examined the results of the qualitative serology of the 5-panel HBV combo cassette and observed that 88.1% (357 of 405) had no anti-HBs, a result that correlated well with the 83.7% proportion of those without protective anti-HBs, as determined via the quantitative tittering method. The qualitative HBV Combo RDT cassette was able to detect anti-HBs in only 24 of the 49 samples quantitatively at protective levels, indicating the reduced sensitivity of the immunochromatographic lateral flow strip assays (Cruz et al., 2017; Poiteau et al., 2017; Wu et al., 2016). A closer examination of the raw data revealed that samples with anti-HBs titers less than 80 IU/L were consistently negative for anti-HBs on the qualitative Combo test cassette. This finding aligns with a study by Poiteau et al., which reported that the sensitivity of RDTs for detecting anti-HBs was low, especially for titers below 100 IU/L (Poiteau et al., 2017). The limited sensitivity of the qualitative Combo test cassette in detecting anti-HBs reduces its reliability for accurately assessing immunity. Therefore, it should not be used as a

standalone tool in vaccine policy decision-making, as it may underestimate true levels of seroprotection. In many low-resource settings in Sub-Saharan Africa, access to quantitative methods for determining titer levels of anti-HBs in vaccine recipients is limited in geographical and cost terms. Alternative approaches to screen for those immune or susceptible to HBV infection remain needed, and immune-chromatographic-based methods are the best choice, as they solve access to diagnostics in Africa. The 80 IU/L threshold level may have been used for the Vaxpert hepatitis Combo test but should be considered in future carefully designed studies focused on addressing this challenge.

Our study had several limitations that could have affected the generalizability and conclusions of the findings; the samples were drawn from patients who provided consent for the use of their leftover clinical samples for research, a very small fraction of the samples from adolescents whose samples were received for HIV viral load testing during the study period. Moreover, the metadata accessible for this study is limited to those provided only on the test request forms and not the entire medical records. Another limitation of this study was the lack of hepatitis B vaccination history, which constrained our ability to distinguish between vaccine-induced and naturally acquired anti-HBs immunity. However, interpretation of serological patterns from the qualitative assay provided some indication, presence of anti-HBs alone reflected prior vaccination, while combinations with other HBV markers suggested past exposure or infection. The Vaxpert hepatitis B Combo test used in the screening was not verified for sensitivity and specificity and could have resulted in an unknown number of false negatives and or false positives. Low amounts of resources meant that we were only able to perform anti-HBs titers for 301 out of the 405 samples available for the study.

Our study revealed a low prevalence of protective anti-HBs (16.3%) among adolescents living with HIV. Serological evidence of exposure (7.0%) to hepatitis B virus is evidence of a continuous risk of HBV infection in a significant proportion of adolescents living with HIV who may be potentially vulnerable to HBV infection. Therefore, our findings suggest a need for Uganda to develop a guideline for assessing the risk, screening and management of hepatitis B virus infection, which is specific for persons living with HIV if it is to achieve the WHO targets of elimination of viral hepatitis by 2030. The qualitative HBV Combo RDT cassette results indicate our aspiration to attract the attention of researchers, innovators and manufacturers in developing immunochromatographic lateral flow-based assays as surrogates for screening susceptibility and/or protective immune responses in low-resource HBV endemic countries.

7.2 General study Limitations

Much as this study boasted of strengths like its large sample size, and use of both qualitative and quantitative anti-HBs detection methods, it was not short of general non-sub study specific limitations. First, its cross-sectional design precluded causal inference on the determinants of antibody persistence. Secondly, we relied on caregiver recall for some vaccination histories in adolescents lacking immunization cards and not all of our participants completed all the three WHO-recommended HBV vaccine doses. Finally, we did not evaluate host genetic determinants like polymorphisms in the T-cell receptor and particularly in HLA class II alleles that account for much of the heterogeneity in anti-HBs responses (Newport et al., 2004; Xie et al., 2016a). Despite these constraints that could have affected the study's generalizability and conclusions, the findings offer critical evidence for optimizing Uganda's HBV immunization strategy.

CHAPTER EIGHT

8.0 GENERAL CONCLUSION

This doctoral work shows that while infant HBV vaccination under UNEPI provides robust short-term anti-HBs protection (74.3%) in children under 5 years, seroprotection wanes significantly by adolescence, increasing infection risks. Approximately only 15% of 15–17-year-olds tested had protective anti-HBs by ECLIA despite earlier vaccination, and HBsAg prevalence was approximately 3.5-fold higher in adolescents than in under-5s from the same communities. Protective immunity was especially low (16.3%) among adolescents living with HIV, who demonstrated profound prior HBV exposure. These findings, together with our Africa-wide systematic review, showed systematically lower HBV vaccine seroprotection in 15–17-year-olds. Much as national efforts have advanced HBV diagnosis and vaccination coverage, targeted adolescent strategies involving sustained progress in addressing waning immunity will be essential to reach the 2030 HBV elimination goals in Uganda and similar African settings.

CHAPTER NINE

9.0 RECOMMENDATIONS

Our study finding that adolescents across several African datasets often fall below HBV vaccine seroprotective thresholds has direct public health implications. It strategically points to the need to fully implement and monitor timely hepatitis B birth dose; within 24 hours and on-time 6-10-14-week doses; audit timeliness and coverage sub-nationally and address bottlenecks like cold chain, stockouts, and birth outside health facilities.

While qualitative assays are logistically attractive, their limited sensitivity argues for incorporating periodic quantitative testing (ECLIA tests) into EPI monitoring frameworks, at least in sentinel surveillance sites. Programs should rely on validated quantitative immunoassays for impact evaluations, while reserving validated RDTs for limited screening scenarios only when sensitivity thresholds are clearly understood.

Much as routine boosters are not recommended for fully immunocompetent persons (Organization, 2024c), the identification of breakthrough infections suggests that HBV elimination strategies in Uganda could evaluate the requirement to implement routine adolescent booster doses in UNEPI to sustain seroprotection, integrate vaccination with strengthened post screening for anti-HBs, early diagnosis, and antiviral treatment programs, especially in high-risk groups like 15-17 year-olds and HIV-positive children.

Systematically designed EPI evaluation studies may be required to supplement and augment our findings. Future research studies should incorporate longitudinal follow-up and genetic analyses

to further elucidate non-response. Nationwide genomic studies on HLA/TCR variants could identify non-responders and guide tailored vaccine production. Additional operational studies could compare screen-and-revaccinate and age-based booster vaccination strategies in adolescents with sub-optimal response to the HBV vaccine. Evaluation studies directly comparing anti-HBs RDTs and quantitative assays to determine the extent of utility of these methods should also guide future research.

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APPENDICES

Appendix 1.0: UNCST Approval

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS2604ES **23 December 2022**

Fahad Muwanda
Makerere University College of Health Sciences, School of Biomedical Sciences, Department of Immunology and Molecular Biology
Kampala

Re: Research Approval: **Childhood responses to Hepatitis B vaccine in Uganda: the role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity**

I am pleased to inform you that on **23/12/2022**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **23/12/2022** to **23/12/2025**.

Your research registration number with the UNCST is **HS2604ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

Please note that this approval includes all study related tools submitted as part of the application as shown below:

No.	Document Title	Language	Version Number	Version Date
1	Data Collection Tools	English	PDF	21 December 2022
2	Project Proposal	English	PDF	
3	Approval Letter	English		
4	Administrative Clearance	English		
4	English Assent Form	English	Pdf	21 December 2022
5	English Parental Informed Consent Form	English	Pdf	21 December 2022
6	Luganda Assent Form	Luganda	Pdf	21 December 2022
7	Luganda ICF	Luganda	2	21 December 2022

Yours sincerely,



Hellen Opolot
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P.O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
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Appendix 1.1: Approval from School of Biomedical Sciences Higher Degrees Research and Ethics Committee

MAKERERE UNIVERSITY
P.O. Box 7072 Kampala, Uganda
E-mail:biomedicalresearch62@gmail.com
Tel: + 256 779340363/+256701940363
COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
RESEARCH ETHICS COMMITTEE

05/12/2022

To: Fahad Muwanda

Makerere University
+256 703 515339 / +256 782 233418

Type: Initial Review

Re: SBS-2022-184: Childhood responses to Hepatitis B vaccine in Uganda; the role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity , ,

I am pleased to inform you that at the **120** convened meeting on **15/09/2022**, the MAK School of Biomedical Sciences REC (SBSREC), committee meeting, etc voted to approve the above referenced application. Approval of the research is for the period of **05/12/2022** to **05/12/2023.**

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **05/12/2023** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the list of all documents approved in this application by MAK School of Biomedical Sciences REC (SBSREC):

No.	Document Title	Language	Version Number	Version Date
1	Clean; revised PhD proposal	English	PDF	2022-11-10
2	Informed Consent forms	Luganda	PhD (ACADEMIC), PDF	2022-05-26
3	Informed Consent forms	Luganda	PhD (ACADEMIC), PDF	2022-05-26
4	Informed Consent forms	English	PhD (ACADEMIC), PDF	2022-05-26
5	Informed Consent forms	English	PhD (ACADEMIC), PDF	2022-05-26
6	Data collection tools	English	PhD (ACADEMIC), PDF	2022-05-26
7	Data collection tools	English	PhD (ACADEMIC), PDF	2022-05-26

Yours Sincerely



Assoc Prof. Erisa Mwaka
For: MAK School of Biomedical Sciences REC (SBSREC)

Appendix 1.2: Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee

MAKERERE UNIVERSITY

P.O. Box 7072 Kampala, Uganda
E-mail: biomedicalresearch62@gmail.com

Phone:0751828420
Fax: 256 414 532204

COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
RESEARCH ETHICS COMMITTEE

8th Feb 2024

File: SBS-2022-184

To Mr. Muwanda Fahad
Principal Investigator
Department of Immunology and Molecular Biology

Category of review
[ ] Initial review
[X] Continuing review
[ ] Amendment
[ ] Termination of study
[ ] SAEs

In the matter concerning continuing review of a research proposal entitled **"Childhood responses to Hepatitis B vaccine in Uganda: the role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity"**

The School of Biomedical Sciences Research Ethics Committee (**SBS-REC**) reviewed the progress report of the above study and found the report satisfactory and granted continuing **APPROVAL** valid until 4th **Dec 2024.**

Please note that the annual report and the request for renewal where applicable should be submitted 6 weeks before expiry date of approval.

Any problems of a serious nature related to the execution of the research protocol should be promptly reported to the **SBS-REC**, and any changes to the research protocol should not be implemented without approval from **SBS-REC** except when necessary to eliminate apparent immediate hazards to the research participant(s).

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
APPROVED
VALID UNTIL
04 DEC 2024
RESEARCH & ETHICS COMMITTEE
P. O. BOX 7072, KAMPALA

Signed.....

Dr. Ocan Moses
Chairperson, School of Biomedical Sciences Research Ethics Committee

Appendix 1.3: Second Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee

MAKERERE UNIVERSITY

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COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
RESEARCH AND ETHICS COMMITTEE

January 29, 2025

File: **SBS-2022-184**

To: Mr. Muwanda Fahad
Principal Investigator

Category of review
[ ] Initial review
[X] Continuing review
[ ] Amendment
[ ] Termination of study
[ ] SAEs

In the matter concerning, continuing review of a research proposal entitled **"Childhood Responses to Hepatitis B Vaccine in Uganda: The role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity".**

The School of Biomedical Sciences Research and Ethics Committee (**SBS-REC**) reviewed the progress report of the above study and found the report satisfactory and granted continuing **APPROVAL** valid until **04th December 2025.**

Please note that the annual report and the request for renewal where applicable should be submitted two months before expiry date of approval.

Any problems of a serious nature related to the execution of the research protocol should be promptly reported to the **SBS-REC**, and any changes to the research protocol should not be implemented without approval from **SBS-REC**, except when necessary to eliminate apparent immediate hazards to the research participant (s).

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
APPROVED
VALID UNTIL
★ 04 DEC 2025 ★
RESEARCH & ETHICS COMMETTEE
P. O. BOX 7072, KAMPALA

Signed .....

Dr. Moses Ocan
Chairperson, School of Biomedical Sciences Research and Ethics Committee

Appendix 1.4: Third Renewal of Approval from school of Biomedical Sciences Higher Degrees Research and Ethics Committee

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COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
RESEARCH AND ETHICS COMMITTEE

February 20, 2026

File: **SBS-2022-184**

To: Mr. Muwanda Fahad
Principal Investigator

Category of review
[ ] Initial review
[X] Continuing review
[ ] Amendment
[ ] Termination of study
[ ] SAEs

In the matter concerning, continuing review of a research proposal entitled **"Childhood Responses to Hepatitis B Vaccine in Uganda: The role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity".**

The School of Biomedical Sciences Research and Ethics Committee (**SBS-REC**) reviewed the progress report of the above study and found the report satisfactory and granted continuing **APPROVAL** valid until **04th December 2026.**

Please note that the annual report and the request for renewal where applicable should be submitted two months before expiry date of approval.

Any problems of a serious nature related to the execution of the research protocol should be promptly reported to the **SBS-REC,** and any changes to the research protocol should not be implemented without approval from **SBS-REC,** except when necessary to eliminate apparent immediate hazards to the research participant (s).

Signed .....

Dr. Moses Ocan
Chairperson, School of Biomedical Sciences Research and Ethics Committee

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
APPROVED
VALID UNTIL
★ 04 DEC 2026 ★
RESEARCH & ETHICS COMMETTEE
P. O. BOX 7072, KAMPALA

Appendix 1.5: Approval of an amendment to the proposal

MAKERERE UNIVERSITY

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COLLEGE OF HEALTH SCIENCES
SCHOOL OF BIOMEDICAL SCIENCES
RESEARCH ETHICS COMMITTEE

February 25, 2026

File: **SBS-2022-184**

To: **Mr. Muwanda Fahad**
Principal Investigator

Category of review
[ ] Initial review
[ ] Continuing review
[X] Amendment
[ ] Termination of study
[ ] SAEs

Re: Approval of an amendment to the proposal entitled **"Childhood Responses to Hepatitis B Vaccine in Uganda: The role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity".**

Your proposal entitled **"Childhood Responses to Hepatitis B Vaccine in Uganda: The role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity"** was initially reviewed and approved by School of Biomedical Sciences Research Ethics Committee in a letter dated **05th December 2022.**

On 30th January 2026, you requested permission to make the following modification.

List of changes.

- Change of the research topic from **"Childhood Responses to Hepatitis B Vaccine in Uganda: The role of Human Leukocyte Antigen (HLA) and T-Cell Receptor (TCR) diversity"** to **"Childhood Immune Responses to Hepatitis B Vaccine in Uganda".**

The committee considered this amendment as it was endorsed by the department and doesn't affect participant safety.

On behalf of the committee, I am glad to inform you that this amendment has been approved. You may now proceed with the study but forward regular reports to the committee.

Please ensure to notify the Uganda National Council for Science and Technology.

Signed.....

MAKERERE UNIVERSITY
SCHOOL OF BIOMEDICAL SCIENCES
25 FEB 2026
RESEARCH ETHICS COMMITTEE
P.O.BOX 7072, KAMPALA

Dr. Moses Ocan
Chairperson, School of Biomedical Sciences Research Ethics Committee

Appendix 2.0: Questionnaire

Childhood immune responses to hepatitis B vaccine in Uganda

Dear parent/guardian,

My name is Fahad Muwanda; I am a PhD student at the Department of Immunology and Molecular Biology, Makerere University College of Health Sciences. We are kindly requesting you (for the child under 5 years) and your child (15-17 years) to answer these questions as part of the "Childhood immune responses to hepatitis B vaccine in Uganda" study.

Volunteer identification number_____ Date._____

Kindly provide precise, correct answers, exact figures and tick in the right box appropriately

SECTION A: Socio-demographics

1. Child's age_____

2. Child's Gender

Male ☐ Female ☐

3. Place of residence

District…….. Sub-county……

Parish…… Village/Ward…….

SECTION B: Medical history

1. Does the child have any allergy that you know of?

No ☐ Yes ☐

If yes, kindly mention it/them……...

2. Has the child ever received Hepatitis B vaccine?

Yes ☐ ☐ No

If yes, how many doses? _____

Kindly mention dates when the doses were received……

3. Do you have the Child Immunization card?

Yes ☐ No ☐

If yes, could you share it with our staff so that we verify the status of child's Hepatitis B immunization?

_____

SECTION C: Check list

Serial number	**Study ID**	**Volunteer ID**	**Response to HBV vaccine**	**TCR genotype**	**HLA genotype**

KEY (Response to HBV vaccine)

R- Responder (participant with anti-HBs titers ≥ 10 IU/ml)

NR- Non-responder (participant with anti-HBs titers <10 IU/ml)

Thank you for participating in this study

Appendix 3.0 Permission for authors

Open Access This article (*A systematic review reveals that African children of 15–17 years demonstrate low hepatitis B vaccine seroprotection rates.* Scientific Reports 2023, 13 (1), 22182. DOI: 10.1038/s41598-023-49674-1) is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. © The Author(s) 2023.

Appendix 3.0 Permission for authors-continued

© **The Author(s) 2025.** Open Access This article (*Hepatitis B virus exposure among adolescents living with HIV: a cross-sectional study of samples referred to the national viral load and early infant diagnosis reference laboratory in Uganda*. **BMC Infectious Diseases** 2025, 25 (1): 1-8. DOI: 10.1186/s12879-025-11244-3) is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Appendix 3.0 Permission for authors-continued

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article (*Serological Status of Vaccine and Hepatitis B Virus Exposure among Children Under 5 and Aged 15–17 Years in Kampala, Uganda*. **Livers** 2024, 4, 550–561. DOI: 10.3390/livers4040039) is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license http://creativecommons.org/licenses/by/4.0/.

Appendix 4.0 Publications

livers

MDPI

Article

Serological Status of Vaccine and Hepatitis B Virus Exposure Among Children Under 5 and Aged 15–17 Years in Kampala, Uganda

Fahad Muwanda [1,2,3,*], Edward Kiyonga [1], Joan Nambafu [1], Agnes Turyamubona [1], Hussein Mukasa Kafeero [2], Edgar Kigozi [1], Harriet Mupere Babikako [4,5], Enock Wekiya [1], Gerald Mboowa [6], David Patrick Kateete [1,7], Hakim Sendagire [8], Paul J. Norman [9] and Bernard Ssentalo Bagaya [1,7,10,11,*]

[1] Department of Immunology and Molecular Biology, School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala P.O. Box 7072, Uganda; edwardkiyonga@gmail.com (E.K.); joannambafu6@gmail.com (J.N.); turyamubonaagnes@gmail.com (A.T.); kigoziedgar@gmail.com (E.K.); wekiaeric@gmail.com (E.W.); davidkateete@gmail.com (D.P.K.)
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[6] The African Center of Excellence in Bioinformatics and Data-Intensive Sciences, Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala P.O. Box 22418, Uganda; gmboowa@gmail.com
[7] Integrated Biorepository of H3Africa-Uganda (IBRH3AU), Department of Immunology and Molecular Biology, School of Biomedical Sciences, Makerere University College of Health Sciences, Kampala P.O. Box 7072, Uganda
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[10] Department for Research, BMK Medical Laboratory Services, Mityana P.O. Box 36639, Uganda
[11] Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE), Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, K-RITH Tower Building, Level 3, Durban 4001, South Africa
* Correspondence: muwandafahad@gmail.com (F.M.); bernard.bagaya@mak.ac.ug (B.S.B.)

Abstract: Background: Pediatric hepatitis B virus (HBV) serostatus remains variably characterized, hardly determined at times, or documented as part of national monitoring of the Extended Programs for Immunization (EPI). **Methods**: We cross-sectionally characterized the seroprevalence of HBV vaccine and/or infection status among 501 and 288 children <5 and 15–17 years old, respectively, in Kawempe Division, Kampala, Uganda, between May and August 2023. These children received HBV vaccination under the Uganda National Extended Program on Immunizations (UNEPI). Samples were qualitatively screened for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb or anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb or anti-HBe), and for hepatitis B core antibody (HBcAb or anti-HBc) using three different HBV Combo test rapid immunochromatographic diagnostic tests: Nova, Fastep, and Beright. **Results**: The seroprevalence of HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc was 1.52%, 27.75%, 0.88%, 0.63%, and 0.76%, respectively, for the combined study age groups. The HBsAg seroprevalence of 2.78% was almost 3.5-fold higher among adolescents when compared to the 0.8% observed in the under-5-year-olds. The qualitative seroprevalence of anti-HBs was 33.1% and 18.4% in the under-5 and among the 15–17-year-old study groups, respectively. **Conclusions**: The proportion of qualitatively detectable anti-HBs in both groups of vaccinated children is low and probably indicates reduced seroprotection. Consequently, a large proportion of children who received the hepatitis B vaccine under UNEPI may be at risk of HBV infection, especially adolescents. A booster dose of the Hepatitis B Vaccine may be required for adolescents.

check for updates

Citation: Muwanda, F.; Kiyonga, E.; Nambafu, J.; Turyamubona, A.; Kafeero, H.M.; Kigozi, E.; Babikako, H.M.; Wekiya, E.; Mboowa, G.; Kateete, D.P.; et al. Serological Status of Vaccine and Hepatitis B Virus Exposure Among Children Under 5 and Aged 15–17 Years in Kampala, Uganda. *Livers* **2024**, *4*, 550–561. https://doi.org/10.3390/livers4040039

Academic Editor: Melanie Deutsch

Received: 30 July 2024
Revised: 29 September 2024
Accepted: 9 October 2024
Published: 30 October 2024

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Keywords: hepatitis B virus; serological status; combo test; hepatitis B vaccine; Kampala-Uganda

1. Introduction

Chronic hepatitis B virus (HBV) infection continues to be a global public health threat. It is estimated that 296 million people are currently living with the virus [1]. The global prevalence of HBV infection is 3.9%, and the WHO Africa region remains substantially burdened at a prevalence of 3.0% [2,3]. In highly endemic countries such as Uganda, the national prevalence of HBV infection is 10% [4].

Many African countries integrated HBV vaccination into their national extended programs for Immunization (EPI) by 2002. In Uganda, such as most of Sub-Saharan Africa, the HBV vaccination schedule is at birth, 6, 10, and 14 weeks [5–8]. Immunization is conducted at all levels of public healthcare, at all private hospitals, and in accredited health facilities of private practitioners. However, following vaccination, pediatric HBV serostatus remains variably characterized or documented as part of the national EPI monitoring. Approximately 20% of immunocompetent HBV vaccine recipients fail to respond to the complete vaccination [9–12]. In addition, effective control measures for HBV infection and disease are underutilized. For example, HBV infection is generally underdiagnosed, and coverage of fully vaccinated children remains low, especially in low-income and high-burdened countries [1].

As is with other vaccines, the protective efficacy of the Hepatitis B vaccine is probably lower in the East African region when compared with the more developed countries. A recent study in Ethiopia has reported close to 5% seroprevalence of breakthrough HBV infection among fully vaccinated children of 5–12 years of age [13]. Another recent study in Ethiopian children revealed only 14.4% and 2.6% of protective anti-HBs antibodies (>10 IU/mL) among under-5-year-olds and in ≥10-year-old children [14]. There is a rising belief that the presence of breakthrough infections among children previously vaccinated may suggest a genuine lack of response rather than a mere drop in antibody titers below the protective levels [13–19].

Since Uganda integrated the hepatitis B vaccine into UNEPI in 2002, we assumed that persistent detectable anti-HBs antibodies among adolescents (15–17 years) would be a good surrogate for the effectiveness of the immunization program [20], at least in our study jurisdiction. Fledgling emancipation, combined with peer pressures, exposes this age group to behaviors (such as sexual activity, body tattooing, and sharing of injectables) associated with an increased risk of HBV infection [20–23]. HBV seroprevalence in such populations with a heightened risk of infection must be keenly monitored for effective evaluation of vaccination programs to inform adequate control and/or elimination of HBV infection [1,2]. Although acceptable national coverage for every vaccine is 90% [24,25] the Uganda Demographic Health Survey (UDHS-2016) indicated a low (79%) immunization coverage of the childhood hepatitis B vaccine [24]. However, the periodic outbreaks of HBV and the stagnated HBV seroprevalence in Uganda [4,25–27] justify the need to investigate the seroprotective efficacy of the HBV vaccine among children under 5 who have been vaccinated. Further, there is a paucity of data on both qualitative and quantitative determination of HBV serostatus among vaccinated children in Uganda. To the best of our knowledge, immune responses among the children previously vaccinated are variable and inconclusively studied in Uganda [4,26–29]. This study therefore sought to characterize the seroprevalence of HBV vaccine and/or serological status of HBV exposure among children under 5 years and 15–17 years of age. We used qualitative HBV Rapid Diagnostic tests as a baseline for the quantitative estimation to infer the HBV vaccination protection in Kawempe Division, Kampala, Uganda.

2. Materials and Methods

2.1. Study Design, Population, and Settings

In a cross-sectional study, we enrolled 789 children between May and August 2023 from the Child and Family Foundation (CFU) medical center catchment area in Kawempe Division. The CFU medical center catchment area is located in Kawempe division, one of the five divisions of Kampala city. Kawempe division has 22 administrative parishes. CFU Medical Center conducts both statistics and mobile immunization services under the UNEPI. The statistical immunization services are scheduled for Monday and Friday, while the outreaches are conducted twice a month in the different parishes. All immunization services provided are recorded in the Ministry of Health's (MOH) health management information system (HMIS). Our study population included children under 5 who had received the hepatitis B vaccine as indicated by the child health card and adolescents (15 to 17 years) who received the hepatitis B vaccine during childhood.

2.2. Inclusion and Exclusion Criteria

We included children under five years of age who had received at least one of the three doses of the HBV vaccine, as evidenced by their UNEPI immunization card. For the 15–17-year-old participants, we included participants who had received at least one Hepatitis B vaccine dose verifiable with a UNEPI immunization card or, if not available, based on parents/guardians' reporting. For both study groups, we excluded children who were anemic as assessed by a study clinician. We also excluded children living with or testing positive for HIV as well as those reporting to have or deemed to have any form of allergy by a study clinician.

2.3. Sample Size Estimation and Sampling Procedure

For both group sample sizes, we estimated using Kish Leslie's formula [30] for cross-sectional studies. We arrived at a sample size of 501 and 288 participants for the under-5 years and 15–17-year-olds, respectively. For the under-5-year-old participant group, we enrolled consecutively eligible children seeking services at the CFU medical center until the sample size was accrued. For the 15–17-year-old study group, eligible study participants were identified during CFU's scheduled health home visits and community medical outreaches and were referred to the medical center for consenting and conduct of other study procedures until the sample size accrued consecutively.

2.4. Data Collection Procedures

A pretested structured questionnaire was administered to parents/legal guardians of participants in the under-5-year-old study group and directly to the participants in the 15–17-year-old group with a provision for consulting their parents/legal guardians if they wished. The questionnaire collected data on the children's socio-demographic characteristics, such as age, gender, medical history, and hepatitis B vaccination status.

2.5. Sample Collection and Processing

Blood was collected from all participants by a designated study clinician or phlebotomist following pre-developed standard operating procedures (SOPs). For the under 5-year-old participants, 8 mL of venous blood was aseptically collected by venipuncture in the following order: 1 EDTA anticoagulant tube of 4 mL, 2 mL Heparinized blood (Becton and Dickson Co. Ltd., Nairobi, Kenya), and a 2 mL plain serum separation tube (Becton and Dickson Co. Ltd., Nairobi, Kenya). For the 15–17-year-olds, 4 mL of venous blood in a plain serum separation tube was collected. Serum and Plasma separation was then conducted by centrifugation of the blood tubes at $1500 \times g$ for 15 min at room temperature at the Immunology Laboratory of the School of Biomedical Sciences, Makerere University, Kampala, Uganda. All sera, plasma, and PBMCs recoverable were stored in labeled cryovial tubes in volumes of 0.5 mL at -20 °C or lower until analysis at the Integrated Biorepository of H3Africa-Uganda (IBRH3AU), Makerere University, Kampala, Uganda.

2.6. Laboratory Analysis

Serum samples were tested for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb using three different Hepatitis B Combo RDT test kits: NOVA TEST-One Step Diagnostic Rapid Test (Global Science, Malaysia; Jaya, Selangor D.E., Malaysia, Cat#: 20221811HBV), Fastep Rapid Diagnostic Test (Healgen Scientific Limited Liability Company, Houston, TX, USA, Cat#: 21076412), and Beright Diagnostic Rapid Test (Medhealth Biotechnology Limited, Shanghai, China, Cat#: 12216453), with manufacturer reported sensitivity and specificity of (91.43% and 98.28%), (68% and 77%), and (98.92% and 99.46%), respectively. Positive and negative controls (prepared and distributed to health facilities by the Uganda Virus Research Institute (UVRI)) were included in all kits and testing runs. Children who tested positive for HBsAg were referred to the CFU Medical Center for appropriate clinical management.

2.7. Data Analysis

Interpretation of HBV infection status was indicated in Supplementary Table S1 and as described in Section 2.8 below. All the participants' data for hepatitis B Combo RDT results were consistent on all three test kits and were therefore included in the analysis. Data were entered for cleaning into EpiData software version 3.1 and exported to SPSS version 25 for descriptive analysis. The results were presented as frequency tables and graphs. The main outcome of interest was HBV serostatus, which was expressed as a percentage. Concordance among the 3 serological tests was analyzed according to Langton et al. (2002) as the percentage of chance that they would yield the same results [31] (Supplementary Table S2).

2.8. Interpretation of the Hepatitis B Serological Test Results

Interpretation of serological results in relation to HBV exposure or vaccine status was performed according to the Australasian Society for HIV, Viral Hepatitis, and Sexual Health Medicine (ASHM)'s 2024 Clinical Tool for Decision Making in Hepatitis B (www.testingportal.ashm.org.au/hbv, accessed on 24 May 2024). Study participants with detectable HBsAg were considered HBV infected, with additional positive anti-HBc or HBeAg detected interpreted as having chronic or acute HBV infection, respectively. Participants who were negative for HBsAg, anti-HBc, with or without detectable anti-HBs were considered to have never been exposed to HBV infection. Those participants who were positive for anti-HBc and were also either positive or negative for both HBsAg and anti-HBs were considered to have ever been exposed to HBV infection. However, participants who were negative for HBsAg and positive for anti-HBc and positive or negative for anti-HBs were considered to have ever been exposed to and resolved HBV infection (Supplementary Table S1).

3. Results

3.1. Socio-Demographic Characteristics

A total of 789 children participated in this study: 288 and 501 in the 15–17-year-old and under 5-year-old groups, respectively. The mean age of this study participants was 16.18 years and 2.93 years for the 15–17-year-old and 0–5-year-old groups, respectively. While more female children (51.7%) were enrolled in the under-5-year-old group, more males (51.74%) were enrolled in the 15–17-year-old group.

3.2. Concordance Among NOVA TEST, Fastep, and Beright Diagnostic Rapid Tests

Testing all this study participants blood sera (n = 789) with each of the three hepatitis B Combo RDT tests yielded 100% concordance and a similar number of positive and negative results for the five HBV seromarkers: HBsAg (12), anti-HBs (219), HBeAg (7), anti-HBe (5), and HBcAb (6) (Supplementary Table S2).

3.3. Doses of Hepatitis B Vaccine Received

Of the 789 study participants, 760 (96.32%) received a verified and documented number of the HBV vaccine doses; 100% (501 of 501) and 89.93% (259 of 288) among the 0–5 and 15–17-year-old groups, respectively. Of the verified vaccine recipients (n = 760), 629 (82.76%), 79 (10.39%), and 52 (6.84%) received 3, 2, and 1 dose of the HBV vaccine, respectively.

Notably, relatively more children in the 0–5-year-old group (88.02% [441 of 501]) than those in the 15–17-year-old group (72.59% [188 of 259]) received the 3 HBV vaccine doses, unlike the 1 and 2-dose vaccine recipients who were predominant in the 15–17-year-old group (Table 1).

Table 1. Characteristics of this study participants, Kawempe Division, May–August 2023.

Variables	15–17 Years, n = 288	0–5 Years, n = 501	Combined, n = 789
Age Mean (SD)	16.18 (0.8836)	2.93 (1.3)	
Gender n (%)			
Male	149 (51.74)	242 (48.3)	391 (49.55)
Female	139 (48.26)	259 (51.7)	398 (50.44)
Doses	**15–17 years, n = 259**	**0–5 years, n = 501**	**Combined, n = 760**
One	26 (10.04)	26 (5.19)	52 (6.84)
Two	45 (17.37)	34 (6.79)	79 (10.39)
Three	188 (72.59)	441 (88.02)	629 (82.76)

3.4. Qualitative Anti-HBs Status and Prevalence of HBV Infection Among Study Groups

Among the entire study participants, 27.75% (219 of 789) had detectable anti-HBs; 33.10% of the children in the 0–5-year-old age cohort (166 of 501) and 18.40% (53 of 288) in the 15–17-year-old age cohort, respectively (Figure 1A). The overall prevalence of HBsAg (active HBV infection) among all study participants was 1.52% (12 of 789); 2.77% (8 of 288) and 0.80% (4 of 501) in the 15–17-year-old and 0–5-year-old groups, respectively (Figure 1B). Acute HBV infection was demonstrated in 25% (2 of 8); the rest (6 of 8) of the 15–17-year-old participants were chronically HBV infected. All 100% (4 of 4) of children 0–5- years -old were acutely HBV infected; that is, none had chronic infection at the time of study (Table 2). There was a significant difference in proportion of HBV infection between the 15–17 and 0–5 year-old age groups (p = 0.087).

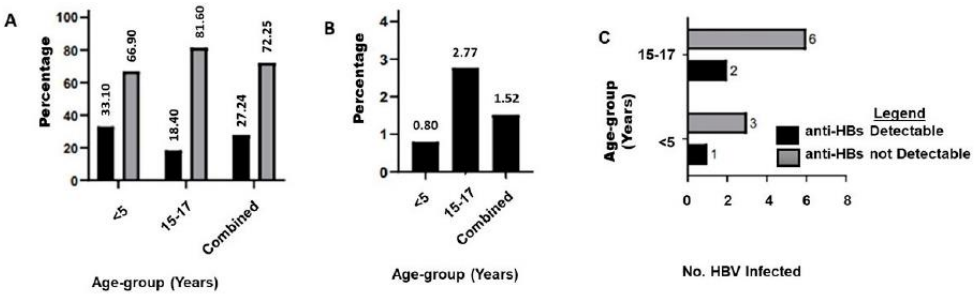


Figure 1. HBV; (**A**) surface antibody (anti-HBs); (**B**) surface antigen (HBsAg); (**C**) Number of participants positive for HBsAg according to anti-HBs serostatus, among this study participants in Kawempe Division, May–August 2023.

Two of the 8 (25%) children infected with HBV (qualitatively detectable HBsAg) among the 15–17-year-old group also had qualitatively detectable anti-HBs on the HBV Combo cassette. Also, in the 0–5-year-old group, one (25%) out of the 4 children with HBV infection had qualitatively detectable anti-HBs on the HBV Combo RDT cassette (Figure 1C). In the entire study population, 72.24% (570 of 789) did not possess detectable anti-HBs; 68.86%

(335 of 501) in the 0–5-year-old and 81.59% (235 of 288) in the 15–17-year-old groups, respectively (Table 2).

Table 2. Clinical test characteristics of this study participants, Kawempe, May–August 2023.

Variables	15–17 Years, *n* = 288	0–5 Years, *n* = 501	Combined, *n* = 789
HBV markers combo *n* (%)			
HBsAg	8 (2.78)	4 (0.8)	12 (1.52)
HBsAb	53 (18.4)	166 (33.1)	219 (27.75)
HBeAg	3 (1.04)	4 (0.8)	7 (0.88)
HBeAb	2 (0.69)	3 (0.6)	5 (0.63)
HBcAb	6 (2.08)	0 (0)	6 (0.76)
Infection status *n* (%)			
Never been exposed	277 (96.18)	497 (99.2)	774 (98.09)
Ever been exposed	11 (3.8)	4 (0.80)	15 (1.9)
Exposed and resolved	3 (1.04)	0 (0)	3 (0.38)

Of the 11 children in the 15–17-year-old group who had evidence of ever being exposed to HBV, 27.3% (3 of 11) had serological evidence of having resolved the infection. However, none (0 of 4) of the children in the 0–5-year-old group who had ever been infected by HBV had serological evidence of having resolved the infection. Overall, 98.09% (774 of 789) of children in the entire study population had not been exposed to HBV infection before; 99.2% (497 of 501) and 96.18% (277 of 288) in the 0–5 and 15–17-year-old groups, respectively (Table 2).

4. Discussion

This study aimed to determine the proportion of vaccinated children with qualitatively detectable anti-HBs antibodies in Kampala, Uganda. In 2016, the World Health Assembly (WHA) adopted a resolution for the elimination of viral hepatitis by 2030, setting elimination targets of a 90% reduction in incidence and a 65% reduction in mortality for hepatitis B by 2030 [32]. Expanding both vaccine coverage and third dose completion to more than 90% of infants, diagnosing more than 90% of estimated chronic hepatitis virus infections, and enrolling them in care are key national-level strategies for the elimination of HBV infections.

Uganda integrated the HBV vaccine into UNEPI in 2002 [26,33], continuously increasing coverage over time. Our teenage and under 5-year-old study participants (average age 16.2 and 2.9 years, respectively) received the hepatitis B vaccine in 2006–2008 and 2018–2020, respectively, approximately 10–12 years apart. In this timeframe, our results suggest a 16% increase in hepatitis B vaccine third dose coverage (from 72.6% to 88%), with an approximate halving of the numbers of children stopping at the first and second dose(s). Our data compares with the WHO/UNICEF Estimates of National Immunization Coverage (WUENI) of 2008 and 2020 (71% and 89% for the 3rd dose in 2008 and 2020, respectively) [34,35], affirming Uganda's commitment to improving hepatitis B vaccine coverage to meet the global targets. Although the 2020 third dose coverage fell below the 90% global target, official data shows that Uganda surpassed and consistently maintained third dose coverage at 93% and above [36] by 2016. The 2020 drop in coverage coincides with the COVID-19 pandemic, which disrupted healthcare systems globally, including vaccination services [37].

The Hepatitis B vaccine is known to be very efficacious, and response rates are high, ranging between 85% and 100%, eliciting antibody titers far and above the 10 mIU/mL protective limit [38]. However, asymptomatic breakthrough infections (detected by the presence of anti-HBc, anti-HBs, or HBV DNA in serum) have been reported in vaccinated persons with a documented initial antibody response [39,40]. Age at infection greatly determines whether HBV infection progresses into a chronic state: about 90% of infants will develop chronic infection when infected within the first year of life, compared with 30%

of children aged 1–4 years and less than 5% of adults [41]. Therefore, it is of paramount importance that vaccine responses in infancy can prevent HBV infection. To meet the global targets for the elimination of HBV infections, ideally, countries would need to perform anti-HBs titer determination to confirm elicitation of protective levels among vaccine recipients, as is the case in vaccinated workers at risk [42]. The anti-HBs titer test availability as well as cost are prohibitive in low- and middle-income countries (LMICs) and would translate into huge programmatic costs, a situation that may divert funds meant for expanding and maintaining optimal vaccination coverage. On the contrary, qualitative strip or cassette-based chromatographic tests that detect anti-HBs antibodies (singly or in combination with other markers of HBV infection) are readily and cheaply available in LMICs. While qualitative anti-HBs cassette tests may not give levels of anti-HBs titers, in resource-limited settings they may be a surrogate for the tittering methods. Using this assumption and qualitative testing, we were surprised to find that only one-third (33.1%) of vaccinated children under 5 years and 18.4% of the 15–17-year-old participants had qualitatively detectable anti-HBs antibodies. Both proportions seemed to be very low for such populations at high risk for HBV infection [2], but are in accordance with findings elsewhere. Long-term follow-up studies of persons vaccinated as infants have reported the absence of detectable anti-HBs antibodies in 50–70% of persons 15–30 years later [39,43–45]. In the African setting, a recent study by Alemayehu, T et al., 2024, found that only 14.4% of children had protective anti-HBs antibodies [14]. Equally, our systematic review of 2023 reported low seroprotection rates across Africa [20]. Taken together, these results confirm low proportions of detectable anti-HBs antibodies in our study groups, which should concern Uganda's hepatitis B vaccination program as studies have reported a lack of anamnestic responses in close to half of those vaccinated in infancy [43], and infections continue to be reported in children in as short a period as 5 years post completion of vaccination [46]. Contrary to our findings, some studies have reported moderate to high seroprotection rates for children under five years of age [47–53] and among teenagers [21–23,54–57] respectively. Several factors could have contributed to this notably improved vaccine efficacy, including administration of birth doses and enhanced vaccine coverage in this study setting.

Our study also showed HBV infection prevalence of 0.8% among the under-5-year-olds, which more than tripled to 2.77% in the 15–17-year-old participants. While all four infected under-5-year-olds were still acutely infected, six of the eight HBV-infected 15–17-year-olds were chronically infected, demonstrating the high tendency of pediatric HBV infections to progress into a chronic state [41]. A higher prevalence in adolescents versus those under 5 years is in line with the chronicity of pediatric infections and the evidence that adolescents are more likely to have low seroprotection [14], hence more prone to breakthrough infections. Adolescence-associated socio-behaviors are also known to increase the risk of HBV exposure and infection [58–60]. Our study's 2.77% prevalence in adolescents is comparable to those reported in studies among similar age groups that mostly range between 2.3–2.6% [13,14,61,62]. However, the seroprevalence of HBV infection reported by our study is relatively higher than that reported in low (Ethiopia; 0.4% [63], Egypt; 0.6% [64], Egypt; 0.4% [65]) and high-income countries (Italy; 0.6% [66], Iran; 0.6% [67], Uzbekistan-Japan; 0.8% [68]). The varying levels of breakthrough infections may be attributed to EPI vaccination coverage rates and evaluation, host immunogenetic factors, vaccination schedule adopted, and the method used to detect HBsAg.

A combined total of fifteen breakthrough infections is testament to suboptimal levels of protection induced by the hepatitis B vaccine in some members of both groups or of waning levels of protection [39,43]. In Uganda, 5–17% of vaccine recipients are known to be non-responders regardless of completion of all 3 doses [28,69]. Our data showed that the majority of those with active HBV infection (6 of 8, and 3 of 4 among 15–17 year and under 5-year groups, respectively) were among those with no qualitatively detectable anti-HBs antibodies, pointing to the fact that suboptimal or non-response to, or significant waning of vaccine responses leaves children susceptible to HBV infection. This is supported by

Reda AA et al.'s (2003) study in Egyptian 6-year-old children [70] that reported a three-fold higher prevalence of HBsAg positivity (2.2% versus 0.8%) among vaccine non-responders in comparison to those with detectable anti-HBs.

Results of this study strengthen our idea that in the absence of access to tittering methods or financial constraints, qualitative Rapid Diagnostic Tests-based methods for the detection of anti-HBs antibodies may be valuable in identifying those susceptible and at high risk of HBV infection. The extent of utility of such qualitative anti-HBs detection methods may be better among children who are recent recipients of the vaccine and therefore expected to still have high levels of vaccine-induced anti-HBs antibodies detectable even on the less sensitive cassette-based RDT methods. Determination of the true utility of these qualitative RDT cassette-based methods would require studies to determine lower limits of detection [71,72], based on anti-HBs antibody titers. Clinical trials that estimate the proportion of vaccinated children with titers below detection levels who would otherwise be missed by relying on the qualitative method alone would also provide the public health risk of such an approach. Such studies would provide the extent of measurement uncertainty or confidence with which to credit qualitative test results concerning screening vaccine immune responses or susceptibility to HBV infection.

Rapid Diagnostic Test-based technologies (RDTs) have dominated Africa's clinical laboratories and expanded access to cheap, timely, and reliable diagnostic services in the region, including at point-of-care settings. The challenge of RDT-based tests is the lack of national-level policy or validation data to guide the choice of a better test brand for use, market stockouts of validated test kit brands, and the rapid emergence of new brands altogether. Laboratories implementing quality management systems do carry out some level of performance verification of emerging RDT brands at a local level, but the high turnover of brands on the market makes the exercise daunting. We provide reassuring data that the three brands used in this study were concordant, and therefore may be used interchangeably to address stockouts in the Ugandan market.

5. Conclusions

We report a low prevalence of children with qualitatively detectable anti-HBs antibodies (33.3% among under-5-year-olds and 18.4% among 15–17-year-olds). The proportions above and being much lower in the 15–17-year-old participants are evidence of a rapid waning of anti-HBs antibodies over time. The majority of HBV infections are happening in those with no detectable anti-HBs antibodies, which affirms increased susceptibility to infection as well as a tendency to progress into the chronic state in children. The three Hepatitis B Combo test kit brands (NOVA TEST, Fastep, and Beright Diagnostic Rapid Tests) were perfectly concordant for all markers of HBV infection. Anti-HBs titer determination and the determination of the extent of the utility of qualitative anti-HBs methods were outside the scope of this study but are recommended in the future or for other researchers to undertake.

6. Limitations

Whereas our study boasts a good sample size, this study was conducted in only one of the five administrative divisions of Kampala and may not represent the entire city or be extrapolable to the rest of Uganda. Additionally, we were unable to perform quantitative measurements of anti-HBs titers. We also did not conduct HBV viral load testing; instead, participants found to be infected were referred to a public health facility for specialist care, usually provided at no cost to the patient. Also, not all of this study participants enrolled in our study completed all three WHO-recommended HBV vaccine doses. Finally, we did not determine and exclude congenital exposure to HIV, a factor that is known to affect quality and magnitude of immune responses in a subsection of exposed infants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/livers4040039/s1. Table S1: Interpretation of the hepatitis B serological test results; Table S2: Concordance among the HBV Rapid Diagnostic Tests.

Author Contributions: Conceptualization, F.M., H.M.K., G.M., H.S., P.J.N. and B.S.B.; Data curation, E.K. (Edward Kiyonga) and B.S.B.; Formal analysis, F.M., H.M.K., E.W., H.S. and B.S.B. Funding acquisition, P.J.N. and B.S.B.; Investigation, F.M., E.K. (Edward Kiyonga), J.N., A.T., H.M.B., G.M., P.J.N. and B.S.B.; Methodology, F.M., E.K. (Edward Kiyonga), A.T., H.M.K., H.M.B., G.M., H.S., P.J.N. and B.S.B.; Project administration, H.M.B., D.P.K. and B.S.B.; Resources, F.M., E.K. (Edgar Kigozi), D.P.K. and B.S.B.; Supervision, E.K. (Edgar Kigozi), G.M., H.S. and B.S.B.; Visualization, B.S.B.; Writing—original draft, F.M. and B.S.B.; Writing—review & editing, F.M., J.N., H.M.K., H.M.B., E.W., G.M., D.P.K., H.S. and B.S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was conducted with funds availed to the Principal Investigator, F.M., by Makerere University Research and Innovation Fund (Mak-RIF) and the Islamic Development Bank Scholarship Division (Award number: 600040776). B.S.B.: receives support from the Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE), which is funded by the Science for Africa Foundation to the Developing Excellence in Leadership, Training, and Science in Africa (DELTAS Africa) program [Del-22-007] with support from Wellcome Trust and the UK Foreign, Commonwealth & Development Office and is part of the EDCPT2 program supported by the European Union; the Bill & Melinda Gates Foundation [INV-033558]; and Gilead Sciences Inc. (19275). All content contained within is that of the authors and does not necessarily reflect the positions or policies of any SANTHE funder. For the purpose of open access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.

Institutional Review Board Statement: Ethical approval to conduct this study was sought and obtained from the Research and Ethics Committee of the School of Biomedical Sciences, Makerere University College of Health Sciences (SBS-REC) under a registration number SBS-2022-184; dated 5 December 2022 to 5 December 2023, and from the Uganda National Council for Science and Technology (UNCST) under a registration number HS2604ES; dated 23 December 2022 to 23 December 2025.

Informed Consent Statement: This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: All this study-generated data are included in this research article and the Supplementary Materials.

Acknowledgments: We thank the management of the School of Medical Laboratory Technology, Kibuli Muslim Hospital Health Training Schools—for providing us with the necessary stationery and ICT technical support. We appreciate the clinicians at Child and Family Foundation (CFU) Medical Center and the village health teams (VHTs) of the respective study areas of Kawempe Division of Kampala Capital City Authority who supported the consenting and mobilization of study participants.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of this study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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A systematic review reveals that African children of 15–17 years demonstrate low hepatitis B vaccine seroprotection rates

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Childhood HBV immunization remains globally fundamental to the elimination of hepatitis B virus (HBV). However, monitoring proportions of HBV vaccine seroprotection and their determinants among African Pediatric recipients is crucial. This study sought to verify extent of immune protection accorded by the HBV vaccine in African children of up to 17 years of age by pooling the prevalence of seroprotection reported by primary studies conducted in the Northern, Western, and Southern African regions. We included 19 eligible articles out of the 197 initially downloaded, published from 1999 to 2021 from African Journals Online (AJOL), EMBASE, Scopus, and PubMed. The study protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO), University of York Centre for Reviews and Dissemination, under the registration number CRD42022361277. Significantly higher ($p < 0.0001$) proportion of HBV vaccine seroprotection (69.07%) was found among children under 15 years of age than children 15–17 years (32.368%), 95% CI [34.2454–39.0847%]. Whereas successful integration of the HBV vaccine on the extended programs on immunizations (EPI) has been a major achievement in the reduction of HBV infection in Africa, markedly reduced HBV vaccine seroprotection is persistently demonstrated among adolescent children 15–17 years of age. Future studies are required to clarify the need for booster dose vaccination in most at risk populations and age groups.

Abbreviations

AJOL	African Journal Online
Anti-HBs	Antibody against the hepatitis B surface antigen
EIA	Enzyme immuno assay
ECLIA	Electrochemiluminiscence immunoassay
ELISA	Enzyme linked immunosorbent assay
EPI	Extended program on immunization
FEM	Fixed effect model
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
MEIA	Micro-particle enzyme immuno assay
NOS	Newcastle–Ottawa scale
PRISMA	Preferred reporting items for systematic review and meta-analysis
QS	Quality assessment

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REM	Random effect model
WHO	World Health Organization

Hepatitis B virus (HBV) infection is still a major global public health problem, with 292 million people infected, translating into a global prevalence of 3.9%[1]. Although universal vaccination had decreased prevalence of HBV in children younger than 5 years from 4.7% in the pre-vaccination era (1980s to early 2000s) to 1.3% by 2015, prevalence in the WHO Africa region remains at 3%. Sub-Saharan Africa is disproportionately over-burdened[2]. In highly endemic countries like Uganda, HBV national prevalence can be as high as 10%[3]. In most of Sub-Saharan Africa the HBV vaccine is administered in early infancy at 6, 10 and 14 weeks, but pediatric seroprotection proportions and rates are not determined or documented as part of national EPIs monitoring. Additionally, up to 20% of immunocompetent HBV vaccine recipients may fail to respond even to the full three doses[4–7]. For instance, Rey-Cuille et al.[26] revealed as low as 43% and 58% immune response to HBV vaccine among the under 4-year-olds in Dakar, Senegal in 2007 and 2010 respectively. However, in general the African pediatric HBV vaccine seroprotection remain variably characterized and inconclusive.

Monitoring of the long-term persistence of HBV vaccine responses informs design, review and implementation of effective vaccination programs for adequate control and or elimination of HBV infection. Adoption of, and for timing of booster doses, especially in suboptimal responders may in part be informed by such studies. Even in countries located within the same geographical settings, studies continue to find wildly differing HBV vaccine immune responses and rates of waning. In Tunisia, Ghana and Egypt, studies documented HBV seroprotection of 68.8%, 56% and 8.9% respectively among 17-year-olds[8–10]. The alarmingly low seroprotection proportion in Egypt was accompanied by a recommendation for a booster dose. A recent (2023) review of the Mauritanian Infant Hepatitis B vaccination Program revealed a seroprotective proportion of 93% among those under 2 years, but protection was only 58% and 29% among the 4–5 year, and 10–12 year old children respectively[11]. Therefore, much as contemporary literature reports a 20 year or possibly lifelong seroprotection conferred by HBV vaccination[12–14], the periodic outbreaks of HBV and the stagnated HBV seroprevalence in Africa justify a need for investigation of proportions of HBV seroprotection in the era of infant vaccination.

Many African countries integrated HBV vaccination onto their national extended programs for Immunization (EPI) by 2002, a 15–17-year age group gives us an opportunity to probe the persistence of HBV seroprotection in this age group as a surrogate for effectiveness of the immunization programs. Fledgling emancipation, combined with peer pressures exposes this age group to behaviors associated with increased HBV infection such as sexual activity, tattooing and sharing of injectable or sharps. It is worth mentioning that populations at heightened risk of infection benefit most from having the sero-protective levels of the HBV vaccine responses. Therefore, if boosting is needed, it should be preferably administered in the adolescent population.

Much as childhood HBV immunization remains fundamental to elimination of HBV, characterization of immune responses, their persistence to late childhood and their determinant factors are crucial to deployment of strategies to elimination of HBV infections. A cumulative population of non-responsive childhood vaccine recipients constitutes a formidable pool of high-risk population with the potential to sustain propagation of HBV infections further, thus eroding gains in regional and global prevention efforts. A meta-analysis to verify the extent of immune protection accorded by the HBV vaccines to African children, and persistence to 17 years is important and informs vaccinologists and programs to deploy optimized interventions prior to later years associated of increased HBV transmission exposures and decreased protective immunity. This meta-analysis provides comprehensive findings on HBV vaccine seroprotection proportions among African children of up to 17 years of age. This will in turn inform public health strategies, including immunization programs targeting reduction of HBV incidence, improving population quality of life, better health and household incomes.

Results

Study selection

The PRISMA flow chart (Fig. 1) summarizes the method for selection of articles included in this meta-analysis. We initially retrieved 197 records through the primary database searches; PubMed (52), Scopus (20), EMBASE (20), AJOL (80), and Willey library (25). Of the 197 records, 50 records were selected based on relevancy of their titles and abstracts to HBV seroprotection in children. From these, 15 articles were excluded for dwelling on prevalence of HBV infection rather than vaccine seroprotection. Ultimately, 35 articles met the inclusion criteria from which the following studies were removed with reasons; 2 were not published in English, 9 were conducted beyond Africa, and 5 had inaccessible or insufficient data.

In total, 19 studies with a total sample size of 10,730 were included in our meta-analysis (Table 1). Of these, 6 studies (31.6%) were from Egypt, 2 (10.5%) from Tunisia, 1 (5.26%) from Nigeria, 1 (5.26%) from Ethiopia, 3 (15.8%) from South Africa, 1 (5.26%) from Ghana, 1 (5.26%) from Senegal, 1 (5.26%) from Cameroon and 3 (15.8%) from Gambia (Fig. 2). Consequently 9 (47.4%), 6 (31.6%), and 3(15.8%) records from North, West and Southern Africa regions were respectively included in the data synthesis. Only a paucity of pediatric seroprotection studies has been published in the East African region. Therefore, one record from the East African region was not included in sub-group analysis. Additionally, there were no identifiable studies from the Southern Africa region for the children 15–17 years of age.

Overall, 15 (65%) records covered children under 15 years of age and 6 (26%) for the children between 15 and 17 years of age with publication years ranging from 1999 to 2021, and 2002 to 2016 respectively. However, 2 (9%) records contained data for both the under 15 and 15–17-year-old children (Figs. 1 and 2). The number of participants in the records included in the meta-analysis was 5,370 and 1,940 participants for the under 15 years and those 15–17 years of age were respectively (Table 1).

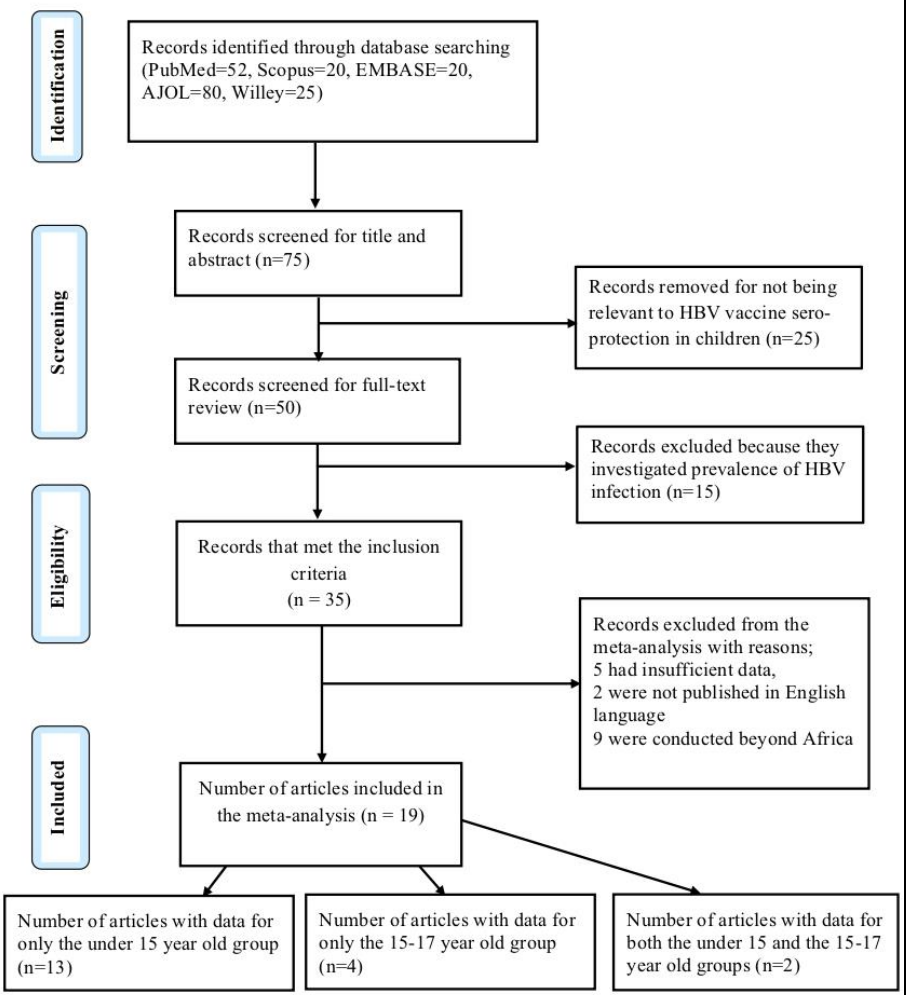


Figure 1. The PRISMA flow chart summarizing the data base searched, screening procedure and the eligible studies for inclusion in data synthesis.

Our results showed that children under 15 years of age had a higher proportion of seroprotection to HBV as compared to those 15–17 years of age in Africa. Additionally, children in the Western Africa region had a higher proportion of HBV vaccine seroprotection than their counterparts in the Northern region. The HBV vaccine seroprotection among children in the Southern Africa region is markedly higher than that demonstrated in both the Western and Northern Africa regions (Table 2 and Supplementary Figs. S7, S8, S9, S10, S11, S12, S13 and S14).

Proportion of HBV vaccine seroprotection among children up to 17 years of age

We included 15 records for the children below 15 years of age and 6 records for the children 15–17 years of age in our meta-analysis (Table 2, Supplementary Figs. S1, S2, S3 and S4). Heterogeneity was persistently high ($I^2 \geq 98\%$, $p < 0.0001$) for all the analyses and hence the random effect model was used to determine prevalence of seroprotection. Both the Egger's and Begg's test found no evidence for sources of publication bias ($p > 0.1$). Our results showed a significantly higher ($p < 0.0001$) HBV vaccine seroprotection among children under 15 years of age (69.07%) than children 15–17 years (32.368%) (Table 2, Supplementary Figs. S1, S2, S3 and S4).

Proportion of HBV vaccine seroprotection among children up to 17 years of age in the Northern, Western, and Southern Africa regions

Our analysis included 3, 7 and 3 records for children under 15 years from the Southern, Northern and Western Africa regions respectively (Table 2, Supplementary Figs. S5, S6, S7, S8, S9 and S10). For children 15–17 years

First author	Year	Country	Sampling technique	Study design	Method of Analysis	Quality Score	Sample size	Seroprotection rate; %	
								<15	15–17
Makhlouf[15]	2016	Egypt	Purposive	Cross sectional	MEIA and flow cytometry	9	440	63.9	15.9
Eladawy[10]	2015	Egypt	Purposive	Cross sectional	ELISA	8	225		8.9
Shaaban[16]	2007	Egypt	Consecutive	Cross sectional	ELISA	8	242	39.4	
El Sawy[17]	1999	Egypt	Consecutive	Cross sectional	MEIA	6	180	81.3	
Abushady[18]	2011	Egypt	Purposive	Cross sectional	ELISA	8	700	42.8	
Tfifha[19]	2019	Tunisia	Purposive	Cross sectional	ECLIA	6	180	77.2	
Odusanya[20]	2011	Nigeria	Purposive	Cross sectional, case-controlled, descriptive	Computer processed lab results analyzed by Epi-Info software	8	822	61	
Chaouch[8]	2016	Tunisia	Stratified, two-stage random cluster	Cross sectional	MEIA, and ECLIA	9	1422	75.1	60.8
Teshome[21]	2019	Ethiopia	Multistage probability	Prospective cross sectional	ELISA	8	450	54.3	
Prabdial-Sing[22]	2019	South Africa	Convenience	Cross sectional	ECLIA	7	450	67.3	
Tsebe[23]	2001	South Africa	Purposive	Cross sectional	Imx or Axsym kits (Abbort labs)	8	598	86.8	
Madhi[24]	2019	South Africa	Purposive	Longitudinal	ECLIA	8	567	96.1	
Quaye[9]	2021	Ghana	Purposive	Cross sectional	ELISA	8	350	56	
Van der Sande[25]	2007	Gambia	Purposive/Random	RCT/Cross sectional	Commercial EIA	8	492		31.2
Rey-Cuille[26]	2012	Senegal	Purposive/consecutive	Cross-sectional	Commercial EIA	8	242	92	
Rey-Cuille[26]	2012	Cameroon	Purposive/consecutive	Cross sectional	Commercial EIA	8	242	58	
Whittle[27]	2002	Gambia	Simple random/purposive	Cross sectional	ELISA	8	852		64
Mendy[28]	2013	Gambia	Purposive	Cross sectional	ELISA	8	1276		22.4
Reda[29]	2003	Egypt	Purposive (Systematic/stratified random	Cross sectional	ELISA	8	1000	67	

Table 1. The characteristics of eligible studies included in the meta-analysis.

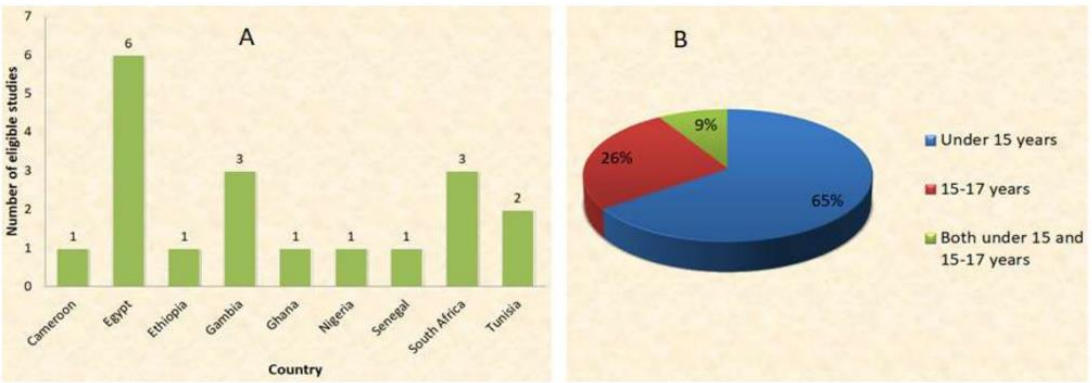


Figure 2. Records eligible for inclusion in the systematic review and meta-analysis; (**A**):—by country, (**B**):—by age.

of age, 3 records from the Northern and 3 from the Western Africa regions were included in our analysis. Similarly, the heterogeneity was high for all the studies ($I2 \geq 98\%$, $p < 0.0001$). Consequently, a random effect model was utilized to determine the prevalence. Egger's and Begg's test were used to assess the bias and, there was no evidence of publication bias ($p > 0.1$). Additionally, the funnel plots in which the standard error of prevalence of each study was plotted against its prevalence, displayed a symmetrical spread of the plots, suggesting little or no evidence of publication bias (Supplementary Figs. S6, S8 and S10). Our results showed that children under 15 years from the Southern Africa region demonstrated significantly higher proportion of HBV seroprotection (85.221%, $p < 0.0001$) than their counterparts in the Northern and Western Africa. The Northern region demonstrated the least proportion of seroprotection (64.242%, $p < 0.0001$) among this age group (Table 2, Supplementary Figs. S7 and S8). Children 15–17 years of age from the Western Africa region demonstrated a low HBV vaccine seroprotection (38.704%, $p < 0.0001$) but was higher than proportions for the Northern Africa region (26.253%, $p < 0.0001$) (Table 2, Supplementary Figs. S11, 12, 13 and 14).

Sub groups					
variable	category	No	Prevalence% (95%CI)	*p* value (95%CI)	I 2% (95%CI)
	Age				
Age	Below 15	15	69.07 (60.068–77.395)		98.58 (98.25–98.86)
	15–17	6	32.368 (15.560–51.952)	< 0.0001 (34.2454–39.0847)	99.45 (99.29–99.58)
	Region (under 15)				
Region	South	3	85.221 (66.250–97.162) Ref		98.82 (98.02–99.30)
	North	7	64.242 (52.857–74.866)	< 0.0001 (18.312–23.5317)	98.09 (97.27–98.66)
	West	3	70.566 (43.867–91.267)	< 0.0001 (10.5225–18.9504)	98.44 (97.24–99.12)
	Region (15–17)				
	North	3	26.253 (1.951–64.813)		99.60 (99.42–99.72)
	West	3	38.704 (14.724–66.079)	< 0.0001 (9.7793–15.0838)	99.49 (99.24–99.66)
	Under 15 years				
Year	1999–2012	8	67.421 (54.100–79.445)		98.66 (98.20–99.00)
	2016–2021	7	71.475 (58.482–82.872)	< 0.0013 (1.5894–6.5158)	98.56 (98.00–98.96)
	15–17 years				
	2002–2013	2	4.306 (0.228–13.097)		98.19 (95.88–99.21)
	2015–2016	3	26.253 (1.951–64.813)	< 0.0001 (19.8746–24.03)	99.60 (99.42–99.72)

Table 2. Meta-analyses results of pooled prevalence of HBV seroprotection rates by age, African geographical region and year of scientific paper publication.

Proportion of HBV vaccine seroprotection among children up to 17 years of age from 1999 to 2021

We included 15 and 5 studies in our meta-analysis for children under 15 years and children 15–17 years of age respectively. For children under 15 years, 8 records published between 1999 and 2012 were analyzed, while 7 records published between 2016 and 2021 were also analyzed. However, for children 15–17 years, 2 records published between 2002 and 2013 were analyzed, and additionally 3 records published between 2015 and 2016 were also analyzed. All studies used to determine the prevalence illustrated a high heterogeneity ($I2 > 98\%$, $p < 0.0001$) and hence the random effect model was used (Table 2). Our analysis indicated that proportion of HBV vaccine seroprotection was high (67.421% and 71.475 respectively, $p < 0.0013$) (Table 2, Supplementary Figs. S15, S16, S17 and S18) in children under 15 years when comparing studies published between 1999 and 2012 and between 2016 and 2021. However, studies for children 15–17 years published between 2015 and 2016 showed low but significantly higher HBV vaccine seroprotection (26.253%, $p < 0.0001$) than studies published between 2002 and 2013 (4.306%) (Table 2, Supplementary Figs. S19, S20, S21 and S22). Publication bias was evidenced in studies for children 15–17 years published between 2002 and 2013 as demonstrated by the Egger's ($p = 0.0001$) but not the Begg's test ($p = 0.3173$). However, there was symmetrical spread of studies from the funnel plot of the standard error of prevalence against its prevalence (Supplementary Fig. S20).

Discussion

The robustness and duration of seroprotection accorded by hepatitis B vaccine depends on a number of baseline endogenous and exogenous factors such as age, body mass index, sex, concomitant diseases (like HIV infection, diabetes, and chronic liver/kidney disease), psychological stress, nutrition, smoking, alcohol consumption, race, vaccine type, dose and vaccination schedule[30–32].

Studies have been conducted globally to evaluate the immune protection accorded by HBV vaccine and its persistence among the pediatric population[8–10,12–29,33,34]. However, there is paucity of such studies in Africa. Where such studies have been done, their findings are inconclusive and significantly vary due to the different sample sizes, geographical regions, methods and other study parameters. In this meta-analysis we provide a holistic evaluation of HBV vaccine seroprotection proportions in Africa among childhood recipients, of up to 17 years of age. We have used a pooled sample size of 10,730, significantly improving statistical power that affects other similar studies[10,19,34].

Here, we report that the proportion of children under 15 years of age with HBV vaccine-specific seroprotection is significantly higher ($p < 0.0001$) (69.07%) than their counterparts 15–17 years of age (32.368%). This finding is in agreement with Egyptian[15] and Tunisian[8] studies. Immune protection accorded by HBV vaccine wanes over time, perhaps contributing to the significantly reduced proportion of seroprotection demonstrated in the 15–17 year olds age group[10,15,28]. More so, the 15–17 year olds received the HBV vaccination in the early to mid-2000s; a time when many African countries were just initiating and or rolling out HBV vaccination into their national EPIs. It is possible that challenges in the early phases of HBV vaccine integration and roll-out in the national EPIs affected coverage and completion of doses that in part could explain the lower proportion seen among the 15–17 years old children[10]. What is apparent though is that there is a big pool of children 15–17 years of age who are at increased risk of contracting HBV on exposure yet this age bracket correlates with onset of socio-behavioral activities associated with increased risk to HBV infection. We report that studies among the under 15-year-olds done between 1999–2012 and then from 2016 to 2021, proportions of HBV vaccination

seroprotection were very similar (67.421% and 71.475 respectively, $p < 0.0013$), indicating that challenges of implementing a nascent HBV vaccination program, if any, did not significantly impact on proportions of children with induced seroprotection. A number of studies[12–14,35] have also reported relatively high proportions of seroprotection for children under 5 years. The United Nations Sustainable Development Goals aim to eliminate viral hepatitis by the year 2030[36], and the WHO-UNICEF, continues to advocate for improved vaccination coverage and proportions of seroprotection that meet the herd immunity threshold. The results above indicate a concerted effort for African national EPIs to attain the desired coverage right from the start of HBV integration in childhood vaccination programs.

Regardless of the age groups, we generally are seeing low proportions of HBV vaccine seroprotection in much of Africa. We have found studies reporting even lower proportions of seroprotection (39.4% and 42.8%) among Egyptian children under 15 years[16,18]. These reduced proportions of seroprotection in HBV vaccination are contrary to those reported in Western developed countries. Studies conducted in higher income countries other than Africa that report significantly higher proportions of seroprotection among adolescent children. Probably the profoundly reduced HBV infection rates in such countries are attributable to higher seroprotection rates compared to Africa. A study that assessed the immune persistence conferred by a Chinese hamster ovary (CHO)-derived hepatitis B vaccine (HepB) 17–20 years after primary immunization during early life, demonstrated seroprotection rates of 74.5% among the participants[13]. Similarly a study conducted in Germany confirmed that paediatric doses of hepatitis B vaccine confer long-term protection against hepatitis B in adolescents up to 15–16 years; with pre-booster dose seroprotection rates of 65.4%, and produce robust immune memory[37]. Reduced sero protective levels in Africa have been attributed to several factors. Waning of immunity over time, often measured as reduction or loss of detectable levels of anti-HBs antibody titers has been cited by many[15,16,21,22]. Evidence for waning responses is supported by our results showing that articles published between 1999 and 2012, and those published between 2016 and 2021 show relatively high and comparable proportions of seroprotection (67.421% and 71.475 respectively, $p < 0.0013$) among children who received the HBV vaccine less than 15 years ago while the reverse is true in those 15–17 year olds. The correlation of antibodies with seroprotection is driven by the belief that neutralizing antibodies are the major correlate of protection against HBV infection, and several infectious agents[38–41]. However, another side of the argument is that levels of anti-HBs neutralizing antibodies may not accurately correlate with seroprotection because recall or anamnestic responses may ensure effective protection against HBV upon exposure[13,35,42–45]. In this context, the waning of seroprotection may not be of clinical or epidemiological consequence. Also, the type of vaccination schedule adopted has been associated with variations in proportions and magnitude of seroprotection induced. Some national EPIs in Africa were subscribing to the 2, 4 and 6 months schedule[8,10,15,16,29] while others were implementing the 6, 10 and 14 weeks schedule[9,20,22,27,46]. However, recently many national EPIs have moved to a modified version of the later schedule that starts with a HBV vaccine shot given at birth that is now thought to be more effective[45,47–49]. Another source of reduced proportions of seroprotection in Africa has been the plethora of current, past and future exposures to several infectious agents. Particularly, parasitic infections such as *Schistosoma mansoni* and *Plasmodium falciparum* have been associated with blunted responses to vaccines[38,40,50,51], including to HBV vaccination[39].

Even within Africa we report seeing variations in proportions of HBV vaccine seroprotection. For instance, children under 15 years from the Southern Africa region demonstrate significantly higher HBV vaccine seroprotection proportions (85.221%, $p < 0.0001$) than their counterparts in the Northern and Western Africa. Equally, children 15–17 years of age from the Western Africa region demonstrate higher HBV vaccine seroprotection proportion (38.704%, $p < 0.0001$) than those from the Northern Africa region (26.253%, $p < 0.0001$).Northern Africa demonstrates the least proportions in seroprotection (64.242%, $p < 0.0001$) among this age group[8–10,15–20,22–29]. A closer look at the HBV vaccine schedule implemented by EPIs in Southern and Western Africa regions shows that it has been the 6, 10, and 14 weeks schedule[9,23,26] that is associated with better seroprotective responses. Northern Africa EPIs on the other hand have been implementing the less effective 2, 4, and 6 months schedule[8,52]. Suboptimal or outright non-response to HBV vaccination also has a genetic dimension. Contemporary literature continues to indicate that individuals with a genetic predisposition for non-response to HBV vaccine may have a defect in their Human Leukocyte Antigen (HLA) and T-cell receptor (TCR) and hence dysfunctional antigen presentation and stimulation of T Helper cells respectively[32]. Certain HLA types are associated with increased or reduced antibody response to hepatitis B vaccines in different individuals[53]. To the best of our knowledge, such studies have not been conducted to conclusion in Africa, yet the African people and generally people of the black race are known to be the most genetically diverse[54–57]. Inherent heterogeneity therein can significantly determine the variations in proportions of HBV sero protection among children under 15 years in the Southern, Western and Northern Africa.

We also indicate here that studies for children 15–17 years which were published between 2015 and 2016 showed low but significantly higher proportions of HBV vaccine seroprotection (26.253%,%, $p < 0.0001$) than studies published between 2002 and 2013 (4.306%). Here we report publication bias in studies for children 15 to 17 years published between 2002 and 2013 as demonstrated by the Egger's ($p = 0.0001$) but not the Begg's test ($p = 0.3173$). These two studies were conducted in the same country; Gambia, and done in 2002 and 2013 respectively, hence probably vindicating the noted bias. However, there was symmetrical spread of studies from the funnel plot of the standard error of prevalence against its prevalence (Supplementary Fig. S20). As earlier noted, the 15–17-year age group is a high risk cohort for HBV and other sexually transmitted diseases yet it is demonstrating lower HBV vaccine seroprotection. However, studies published between 2015 and 2016 were probably conducted on children 15–17 years of age who were immunized in the HBV vaccination era[2], and after effective integration of HBV vaccines on EPIs of all countries (1997)[58]. Studies published between 2002 and 2013 were probably conducted on 15–17-year-old children who were ineffectively and inadequately vaccinated in the pre-vaccination era, and perhaps markedly limiting the HBV seroprotection rates.

This meta-analysis establishes proportions of HBV seroprotection among children up to 17 years of age in Africa. Children under 15 years of age demonstrate a significantly higher proportion of HBV vaccine seroprotection than those 15–17 years of age. The proportion of HBV vaccine seroprotection in children under 15 years are relatively high with the Southern Africa region demonstrating significantly higher proportion than its counterparts in the Northern and Western Africa. Notably, African children 15–17 years of age generally present with reduced proportion of HBV vaccine seroprotection. We further demonstrate generally higher proportions of HBV vaccine seroprotection in children under 5 years reported by studies conducted between 1999 and 2012, and those published between 2016 and 2021. However, studies among children 15–17 years published between 2015 and 2016 show low proportions of HBV vaccine seroprotection, although higher than proportions reported by studies published between 2002 and 2013. Whereas successful integration of the efficacious HBV vaccine on the EPIs has been a major achievement in the reduction of HBV infection in Africa, this meta-analysis indicates profoundly reduced HBV vaccine seroprotection rates among current adolescents of 15–17 years of age. Systematically designed EPI evaluation studies may be required to supplement and augment our findings, and perhaps establish and clarify the need for booster dose vaccination, particularly in the most at risk pediatric African populations.

We acknowledge some limitations in our study. Limited number of EPI monitoring and evaluation reports and publications on HBV vaccine seroprotection in Africa may have limited geographical coverage or representations of the meta-data used, particularly the East Africa regions. We could not confirm if all the study participants enrolled in the primary studies had completed all the WHO recommended three HBV vaccine doses. This could certainly have led to an underestimation of the seroprotective proportions reported in the primary, and hence in this study. To mitigate both of the limitations above, we recommend meta-analyses that consider only the better designed national EPI evaluation data as they become more available. We considered no genetic diversity across the African continent in explaining the regional variability in sero-proportions observed. Therefore, the differences in seroprotection observed across the African regions may be due to genetics rather than vaccination strategy. All these study limitations notwithstanding, we pooled the prevalence of seroprotection to commendably enhance the representativeness and power of our meta-analysis.

Methods

Systematic review protocol registration, information sources, and search strategies

This study sought to determine the proportions of immune protection against hepatitis B vaccine among HBV vaccine childhood recipients of under 15, and 15–17 years in Africa. The study protocol was submitted for registration with the International Prospective Register of Systematic Reviews (PROSPERO), University of York Centre for Reviews and Dissemination (https://www.crd.york.ac.uk/PROSPERO). The study required no ethical review to be conducted. The findings of the review were reported based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 statement checklist[59]. Our search scope included electronic databases; African Journals Online (AJOL), EMBASE, Willey library, Scopus, and PubMed, for studies published from 1999 to 2021 that investigated the seroprotection accorded by hepatitis B vaccine among childhood recipients under 15, and 15–17 years in Africa.

Weekly reviewers' evaluation meetings were conducted for detailed review of accessible full journal articles, titles and abstracts. Resolution of disagreements among the reviewers (FM, HMK, and BSB) was done by consensus. Only qualified full text articles were retained for data extraction after explicit interrogation by FM, HMK, and BSB to identify duplicate records and other anomalies.

We utilized the population, comparison and outcome (PCO) model as a search strategy. All studies assessing seroprotection to HBV studies that used study participants from Northern, Western, and Southern Africa regions were searched for data extraction. For comparison, the data on the prevalence of seroprotection to HBV infection were searched and then compared among children below 15 years and those between 15 and 17 years. The key outcome was sero-protective level of immune responses to HBV vaccine in the population, while the supplementary outcomes were the relative sero-protective proportions basing on the diagnostic methods used for anti-HBs antibody detection (ELISA, EIA, ECLIA, and MEIA) and factors associated with the sero-protective levels.

The search terms used are presented in Supplementary Information File, Table S1. These were used either in isolation or in combination using the Boolean operators 'OR' and 'AND'. The titles and abstracts were searched for the keywords related to HBV vaccine seroprotection proportion or rate while the full text of each article was searched for the keywords related to protective anti-HBs titers and associated risk factors. The three authors (FM, HMK, and BSB) separately extracted the following data: first author, year of publication, country, sampling technique, study design, sample size, method of detection and quantification of anti-HBs antibodies, and seroprotection rate (Table 1).

Eligibility criteria and study selection

Records were included in the systematic review and meta-analysis if they were full text, cross sectional or longitudinal study designs determining sero-protective anti-HBs titers (≥ 10 mIU/ml), conducted in Northern, Western, or Southern Africa, and published in English in peer reviewed journals between January of 1999 and December, 2021. We excluded case reports; reviews; conference abstracts; studies with insufficient or inaccessible data; pre-prints; studies with a sample size < 100; studies that never described their sampling technique; studies that investigated seroprotection against other types of viral hepatitis (A, C, D, or E); studies in languages other than English; and studies published before January, 1999 or after December, 2021 (Table 1).

Quality assessment and data management

The Newcastle–Ottawa Scale (NOS) was used to assess quality[60]. The three dimensions of selection, comparability and exposure were considered, as described in the scale. Articles were assigned one point for each attribute they adequately addressed within the above-mentioned dimensions. For this meta-analysis, the selection assessed the representativeness of the sample, the sampling technique, the sample size, the inclusion criteria, and the scope of the study. Comparability assessed the inclusion of seroprotection prevalence in the primary studies and compared them between vaccine recipients below 15 years and those 15–17 years. Sero -protection proportions across the Northern, Western, and Southern regions of Africa were also compared. Assessment of inclusion of the exposure in the primary studies was done by looking at the outcome of the study, and a statistical tool used for the analysis (Supplementary Information File, Table S2). According to the scale, studies with scores of 9–8 were considered very high quality; 7–6, high quality; and 5–4, moderate quality. Those with scores ≤ 3 were considered unsatisfactory and were rejected. The three reviewers (FM, HMK, and BSB) independently assessed the articles for their overall methodological quality. The authors individually participated in daily entry of data derived from research papers, into the spreadsheet. The authors additionally removed duplicate papers after comparing their archives. During the review process (screening, eligibility, and inclusion in meta-analysis), the primary studies were independently assessed by three hepatitis B virus specialists (FM, HMK, and BSB). From each study, the following data were extracted: first author, year of publication, country, sampling technique, sample size, quality score (QS), method of detection and quantification of anti-HBs antibodies, and seroprotection rate.

Risk of bias in individual studies

To subdue the effects of selection bias, we reviewed the data collection procedures in the primary studies (retrospective or prospective), study design (cohort, cross-sectional, or case control), and recruitment strategy (from the community or from the hospital). For the information bias, we extracted information on methods used to detect and quantify anti-HBs antibodies (ELISA, ELISA, EIA, ECLIA, MEIA and other immune assays). When more than one method was used to detect and quantify anti-HBs antibodies, data were extracted using the more accurate method, as indicated by the evaluator. All studies with a sample size less than 100 were excluded to avoid biasing the results.

Publication bias and data synthesis

The Begg's test, which uses Kendall's rank correlation coefficient between the meta-analysis effect size and the study weight, was used to assess publication bias[61]. Funnel plots further verified the existence of overall publication bias. The Begg's test determined funnel plot asymmetry between the effect size and the meta-analysis study weight. In this test, $p > 0.05$ signified a lack of publication bias (not significant). The test I2 statistic was used to assess heterogeneity among studies (Table 2). Sources of heterogeneity were assessed by sub-group analysis, sensitivity analysis, and meta-regression. Pooled proportions, test of proportions, relative risk (RR), and the corresponding 95% CI were used to assess seroprotection rates various geographical regions. As a result of high heterogeneities ($I2 > 73\%$ and p het < 0.05), the random-effects model (REM) was used to pool the seroprotection rates. All the calculations were done using the Medcalc software version 19.1.3. Seroprotection rates and its associated factors, and heterogeneity analyses among studies were done at 95% CI, and a $p < 0.05$ was considered significant. Forest plots were used to graphically present representative results (Supplementary Information File, Figs. S1, S3, S5, S7, S9, S11, S13, S15, S17, S19 and S21). The seroprotection rate in each study in the forest plot is indicated by a blue square. The size of the square represents the weight contributed by each study in the meta-analysis. The pooled seroprotection rate for the REM is shown by the blue diamond.

Data availability

All data generated or analyzed during this study are included in this published article.

Received: 15 December 2022; Accepted: 11 December 2023
Published online: 13 December 2023

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Acknowledgements
We acknowledge the ICT system management at Islamic University in Uganda- Kampala Campus for authorizing access to the database. We are also grateful to Dr. Ntanda K Moses, Makerere University College of Computing and Information Sciences for the ICT technical support.

Author contributions
F.M., B.S.B., H.S., G.M., and H.M.K. conceived the idea. F.M., B.S.B., H.S., G.M., and H.M.K. participated in the search for the articles from the databases. F.M., B.S.B., H.S., G.M., and H.M.K. participated in the data presentation, analysis, and discussion. F.M. wrote the final manuscript draft. All authors reviewed the manuscript draft and approved the publication of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-49674-1.

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Turyamubona *et al. BMC Infectious Diseases* (2025) 25:903
https://doi.org/10.1186/s12879-025-11244-3

BMC Infectious Diseases

RESEARCH Open Access

Hepatitis B virus exposure among adolescents living with HIV: a cross-sectional study of samples referred to the national viral load and early infant diagnosis reference laboratory in Uganda

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Abstract

Background Adolescents living with HIV are vulnerable to hepatitis B coinfection. However, the prevalence of hepatitis B virus (HBV) exposure and the persistence of childhood vaccine-induced HBV antibodies in this population remain unclear. This study assessed HBV exposure and the prevalence of protective Hepatitis B Surface Antibody (HBsAb) levels among adolescents living with HIV referred for viral load testing at the national reference laboratory in Uganda.

Methods A cross-sectional analysis was conducted on 405 archived plasma samples collected between January and September 2023 at Uganda National Health Laboratories and Diagnostic Services (UNHLDs). HBV exposure was determined via a 5-panel Vaxpert HBV combo cassette, and protective HBsAb levels were quantified via the Roche Cobas e411 system.

Results The mean age of the participants was 15 ± 2.7 years, with 52.5% (212/405) being female. Among them, 8.7% (35/405) had an unsuppressed HIV viral load. The following HBV markers were detected: HBsAg (2.0%), HBsAb (24/405, 5.9%), HBeAg (2.2%), HBcAb (1.2%), and HBeAb (3.45%). Overall, 7% (28/405) had evidence of HBV exposure, including 11 (2.7%) with acute infection, 13 (3.2%) with chronic infection, and 4 (1%) with resolved infection. Vaccination-induced immunity (HBsAb-positive only) was found in 4.9% (20/405) of the participants.
Of the 301 samples tested for HBsAb titers using the Cobas E411 system, only 49 (16.3%) had protective levels (≥ 10 IU/L), with a median titer of 77.7 IU/L (IQR: 18.43–589.2). All 24 samples that were qualitatively HBsAb-positive on the Vaxpert rapid test had protective titers, 21 of which were > 100 IU/L. Among 277 samples that were HBsAb-negative by the rapid test, 25 (9%) showed protective titers upon quantitative testing, highlighting potential

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false-negative results and suggesting limited sensitivity of the rapid assay. All the demographic and clinical factors available and assessed in this study population were not significantly associated with HBsAb levels.

Conclusions The low prevalence of protective HBsAb levels and evidence of HBV exposure highlight the ongoing risk of HBV infection in this population. Our findings suggest a need for Uganda to establish specific guidelines for HBV risk assessment, screening, and management among people living with HIV.

Keywords Adolescents, Hepatitis B, Antibodies, HIV, Coinfection, Acute & chronic, Vaccination & exposure

Background

Hepatitis B virus (HBV) remains a significant global public health challenge, with an estimated 254 million chronic infections and 1.1 million deaths in 2022. The WHO African region bears a substantial burden, accounting for 63% of the global estimate of 1.2 million new infections in 2022 [1]. Uganda is highly endemic, with an estimated national prevalence of 10% [2]. Hepatitis B virus infection is common among populations, especially in Africa, and often leads to unwanted outcomes, including liver cirrhosis, hepatocellular carcinoma (HCC), and death [3].

Among people living with HIV (PLWH), HBV coinfection is a major contributor to morbidity and mortality [4, 5]. Globally, the prevalence of HBV/HIV coinfection is estimated to be 7.6% [6] and ranges from 8 to 20% in sub-Saharan Africa [7] and from 4 to 17% in Uganda [8]. Adolescents living with HIV are at risk of HBV infection due to immunosuppression, poor adherence to antiretroviral therapy (ART), increased HIV viral loads [9, 10] and indulgence in risky practices. Risk factors such as shared transmission routes, tattooing, piercings, and injection drug use further exacerbate this risk [8, 9] in this population. HBV/HIV coinfection complicates disease management [6], with additional risks for pregnant adolescents, including vertical transmission [11] to the unborn child.

In response to HBV infection, Uganda incorporated the HBV vaccine into its National Expanded Program on Immunization (UNEPI) in 2002, which targeted infants with a three-dose regimen at 0, 1, and 6 months [2, 12]. As of 2019, the national vaccine coverage for three doses reached 85% [13]. A hepatitis B surface antibody (HBsAb) titer ≥ 10 IU/L is considered protective [14]; however, in our setting, 5–10% of vaccinated children fail to reach this threshold [15], and response rates are further reduced among PLWH [8]. Among HIV-positive individuals, protection rates range from 34 to 88.6%, whereas they are ≥ 90% in the general population [8, 16]. The waning of protective antibody levels is a concern, with rates ranging from 13 to 60% over the years after the initial protective HBsAb titers among healthy individuals [14]. Recently, reports have shown that adolescents 15–17 years of age in Africa have a very low prevalence of protective HBsAbs [17], potentially due to the waning of antibody responses [14, 18].

Among HIV-positive individuals, the extent of waning of the protective HB antibody response is unclear. Up to 66.7% of infant recipients of the HBV vaccine have been reported to lose protective titers of antibodies after 15 years [19]. As such, HBV remains a major HIV coinfection and cause of mortality among PLWH [4].

Therefore, the aim of this study was to determine hepatitis B virus exposure and the prevalence of protective HBsAb in samples collected from adolescents living with HIV in Uganda.

Methods

Study design, setting and population

We conducted a cross-sectional study utilizing leftover archived clinical samples from the National Reference Laboratory for Viral Load/Early Infant Diagnosis (VL/EID) of the Central Public Health Laboratories, Department of National Health Laboratories and Diagnostic Services (CPHL/NHLDS), Ministry of Health, Uganda. A total of 6,872 samples from adolescent (10–19 years old) patients were referred from hospitals across the country between January and September 2023 for HIV viral load testing at the CPHL/NHLDS. Among these, 532 adolescents and their guardians/parents provided verbal assent and written consent, respectively, for future use of their leftover samples for research. The samples were stored at −80°C in the Uganda National Biorepository located within the CPHL/NHLDS. Qualitative hepatitis B serology and HBsAb titer determination were performed at the Makerere University Infectious Diseases Institute Core Laboratory (IDI Core Laboratory).

Sample size estimation

For an estimated 170,000 adolescents living with HIV in Uganda [20] and at an estimated margin of error (e) of 5%, we calculated the sample size of 400 plasma samples via the Taro Yamane formula [21] although 405 samples were analyzed.

Sampling method

We utilized Microsoft Excel-aided simple random sampling within an archive of 532 samples that met the inclusion criteria (adolescent, living with HIV and consented for future research use of samples) to get the 400 samples. A total of 27 of the 400 samples selected were

excluded and replaced with another randomly sampled plasma due to inadequate sample volume, ineligible label on containers or being leaky.

To assess the prevalence of protective hepatitis B surface antibody (HBsAb) levels (≥ 10 IU/L), we analyzed a total of 301 archived plasma samples. These included all 24 samples that tested positive for HBsAb using the qualitative HBV Vaxpert Combo test cassette (purposively included to maximize detection of protective titers), and a randomly selected subset of 277 samples from the remaining 381 HBsAb-negative samples. This sampling strategy was employed to ensure a representative and epidemiologically balanced cohort while optimizing available laboratory resources. Given budgetary and logistical constraints, it was not feasible to test all 405 samples quantitatively using the Cobas e411 system. Therefore, random sampling of the HBsAb-negative group minimized selection bias while maintaining the statistical power necessary for subgroup analyses, including assessment of correlates such as age, sex, and HIV viral load. An illustration of the sampling, laboratory testing and data analysis is summarized in Fig. 1.

Meta-data collection

All samples arriving at the CPHL/NHLDS are accompanied by an individual standardized viral load/EID testing request form and a sample chain of custody forms that summarizes a list of samples in the transportation package. Data on both the request forms and the sample chain of custody forms are entered into the CPHL/NHLDS Viral Load/EID Laboratory Information Management System/Dashboard. We developed and utilized a data collection form to extract participants' age, sex, pregnancy status and breastfeeding status, HIV viral load and ART adherence from the dashboard and/or test request form.

The completed data collection forms were transported from CPHL/NHLDS to Makerere University College of Health Sciences, where they were double entered into an Epidata-based data entry tool.

Specimen retrieval, transportation, storage and thawing

Frozen samples were retrieved from the – 80°C freezers and transported in an insulated container with dry ice to the Makerere University IDI Core Laboratory for batch testing. The samples were normalized to room temperature for at least one hour before assaying.

Determination of hepatitis B surface antibody titers

The Elecsys HBsAb II reagent (catalog no. 69612201) run on the Roche Cobas e411 system was used to measure the levels of HBsAb in the samples following the manufacturer's instructions. Daily Internal Quality control was performed by running calibrators provided within the reagents procured, Anti-HBs G2 ELECSYS COBAS E 100 V2 (Batch number # 69612201 & 71433301) and also running commercial ELECSYS PRECICONTROL ANTI-HBS (Batch #: 72302401) on the analyzer each day of sample analysis. The Roche Cobas e411 analyzer used in the study participates in the HBsAb proficeiency testing (PT) scheme of the College of American Pathologists (CAP). The analyzer software automatically calculated

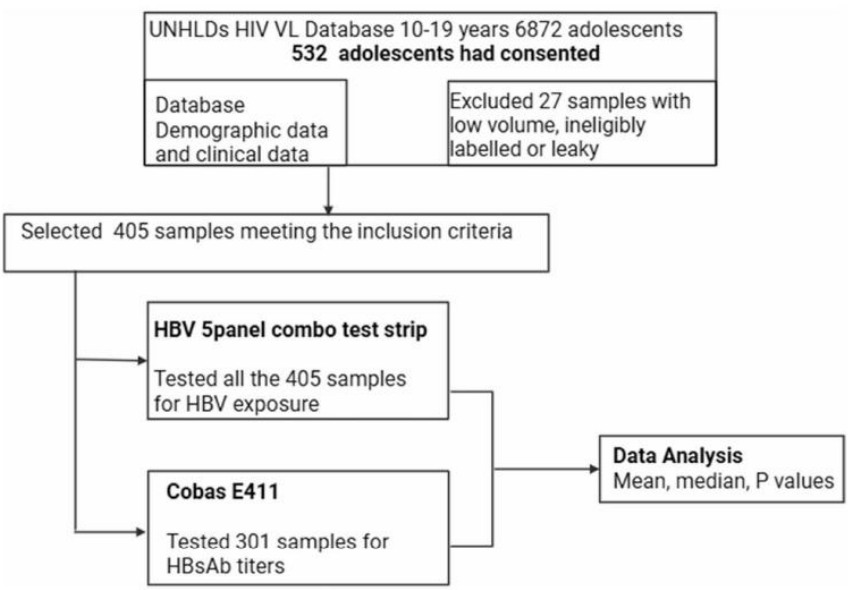


Fig. 1 Illustration of the methods used for sampling, assaying and data analysis

the analyte concentration of each sample, reported in IU/L.

Determination of HBV infection status

Five-panel HBV Combo test cassettes (Vaxpert, Catalog no. HBV23120001) were used to test for the presence or absence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb) and hepatitis B core antibody (HBcAb) according to the manufacturer's instructions. The Vaxpert Hepatitis B Combo test cassettes have a manufacturer's declared sensitivity of 99.9%, specificity of 99.4% and accuracy of 99.7% for the detection of markers of hepatitis B virus exposure. Briefly, the test cassettes were removed from the pouch and labeled with a sample identifier, and three drops of the plasma were dropped on each sample port via the provided dropper. The results were read after fifteen minutes and no later than 20 min after sample addition.

Interpretation of hepatitis B virus infection status

The interpretation of the HBV serological results was performed according to the Australasian Society for HIV, Viral Hepatitis, and Sexual Health Medicine (ASHM)'s 2024 Clinical Tool for Decision Making in Hepatitis B (www.testingportal.ashm.org.au/hbv, accessed on 24 May 2024). A summary of the interpretation is provided in Supplementary Table 1.

Data analysis

Data analysis was performed via GraphPad Prism software 10.1.2. Descriptive statistics were used for continuous variables to obtain medians and means, whereas the Wilcoxon rank sum test was used to compare parameters between the groups with protective and nonprotective HBsAb levels, deriving p values for statistical significance and confidence intervals. Categorical variables were analyzed using the fisher's exact test. The analysis was performed at a 95% level of significance. The prevalence of each hepatitis B virus infection status and protective antibody level was calculated as the proportion of adolescents with the parameter of interest, divided by the total number tested, and expressed as a percentage.Titers were measured in international units, and adolescents with HBsAb titers ≥ 10 IU/L were considered immune to hepatitis B virus.

Results

Demographic characteristics

A total of 405 samples from adolescent participants were included in the study. The mean age of the sample donors was 15±2.7 years. Over a half, 52.5% (212/405) of the sample donors were female, one of whom 1/405 (0.6%) was pregnant and 3/405 (1.7%) were breastfeeding. A total of 35/405 (8.7%) had no suppressed HIV viral load.

Hepatitis B virus exposure according to the results of the Vaxpert HBV combo tests

Of the 405 adolescents included in the study, 8 (2.0%) were positive for HBsAg, 24 (5.9%) for HBsAb, 9 (2.2%) for HBeAg, 5 (1.2%) for HBcAb, and 14 (3.5%) for HBeAb. On the basis of the results above, 48/405 (11.9%) adolescent participants were reactive to at least one or more serological marker. We found that 20/405 (4.9%) participants were positive for only HBsAb and no other hepatitis B virus marker, indicating antibody responses due to vaccination. The number of adolescents with evidence of current or previous exposure (acute, chronic or resolved) to the hepatitis B virus was 28/405 (7.0%), with 4/28 (14.3%) having recovered/resolved from the infection (positive for HBsAb and HBcAb, with or without HBeAb), 13/28 (46.4%) having chronic infection (positive for HBsAg and HBcAb, with or without HBeAg and HBeAb) and 11/28 (39.3%) having acute infection (positive for HBsAg, HBcAb and HBeAg).

Hepatitis B surface antibody (HBsAb) titer levels from the automated Cobas E411

Among the 301 samples tested for HBsAb titers using the automated Cobas E411 analyzer, only 49 (16.3%) had protective antibody levels (≥ 10 IU/L). The overall median HBsAb titer was 77.70 IU/L (IQR: 18.43–589.20 IU/L). All 24 samples that were qualitatively HBsAb-positive on the Vaxpert hepatitis B virus Combo cassette had protective titers, with 21 of them showing values above 100 IU/L. Among the 277 samples that were qualitatively HBsAb-negative, only 25 (9%) had protective HBsAb levels, with a median titer of 2.00 IU/L (IQR: 2.00–3.17 IU/L).

Factors associated with protective effects on HBsAb titers

Among all the demographic factors assessed in this study, none showed a statistically significant association with protective hepatitis B surface antibody (HBsAb) levels in the adolescent population. Specifically, age, sex, pregnancy, and breastfeeding were not significantly associated with protective HBsAb titers, as presented in Table 1.

Similarly, clinical factors evaluated in this study, including HIV viral load suppression and ART adherence, did not demonstrate any significant association with protective HBsAb levels. These findings are summarized in Table 2 below.

Table 1 Association of demographic characteristics to HBsAb titers

Total No = 301		Protective *n* = 49	Nonprotective *n* = 252	*P* value
Age (years), Median		15 (10–19)	15 (10–19)	0.4313
Sex	Male	25(51.02%)	121 (48.02%)	0.7558
	Female	24 (48.98%)	131 (51.98%)	
Pregnancy	Yes	0	1	> 0.999
	No	18	114	
Breast feeding	Yes	01	02	0.3751
	No	18	111	

Table 2 Clinical factors associated with protective HBsAb titers among the study population

Total No = 301		Protective *n* = 49	Non-protective *n* = 252	*P* value
HIV Viral Load	Suppression	47	228	0.2759
	Non-suppression	02	24	
Art adherence	Good ≥ 95%	31	191	> 0.9999
	Fair (84–94%)	0	02	

Discussion

In this study, we aimed to determine the prevalence of exposure to hepatitis B virus as well as the protective effects on HBsAb titers among adolescents living with HIV in Uganda. Despite being an average age of 15 years, a few cases of pregnancy and breastfeeding in the adolescent population exemplify an already sexually active group, especially in a country where the age of adulthood starts at 18 years [22]. This highlights the need for integrated comprehensive sexual health education and services for adolescents living with HIV in Uganda. In the context of the HIV program indicators, a suppression prevalence of 91.3% shows that even among this young population, Uganda is on the way to achieving the Joint United Nations Program on HIV/AIDS (UNAIDS) 95–95–95–95 targets for elimination of HIV [23].

The prevalence of HBV exposure (active or resolved) of 7.0% in this population corresponds with data from Sub-Saharan Africa, where Uganda is a hyperendemic (≥ 8% prevalence) region [24]. Our active HBV infection prevalence of 5.9% (2.7% for acute and 3.2% for chronic infection) agrees with similar adolescent and/or pediatric HIV/HBV coinfection studies in similar populations [17, 25]. In a systematic review of HBV among a 15–17-year-old population in sub-Saharan Africa, the prevalence of HBV infection was approximately half (2.77%) that of the general population [17]. Given that both HIV and HBV can be transmitted perinatally as well as during breast feeding, the incidence of HBV infection among children and adolescents living with HIV plausibly exceeds that of the general population [24, 26]. Importantly, the adolescent children in this study were born to mothers predating the introduction of the HBV vaccine into the Uganda National Expanded Program on Immunization (UNEPI) in 2002 [2, 27]; hence, a higher prevalence of HBV infection is likely, and the virus could have been transmitted to these children. Almost half of the hepatitis B virus (HBV)- actively infected adolescents were in a chronic state, supporting existing evidence that most pediatric infections progress to chronic infections rather than be resolved [28]. In light of these findings, the 2024 WHO guidelines recommend routine screening for HBsAg, anti-HBs, and anti-HBc among individuals newly diagnosed with HIV, with vaccination advised for those found to be non-immune. This strategy is critical for reducing HBV acquisition and transmission in this vulnerable population [26]. Unsurprisingly, HBsAb attributable to HBV vaccination was only qualitatively evident in 16.3% of our study population, regardless of HIV viral suppression. The 16.3% seroprotective level in our population is in line with another study that reported protective HBsAb levels at a prevalence of 18.2% at 2.8 years after neonatal HBV vaccination [25]. Another study reported a significant decline in HBsAb titers over time among infants vaccinated against hepatitis B virus [29]. These low levels of protection in our settings contrast with those observed elsewhere in developed countries [30, 31]. The exact reasons for the low proportions of adolescents with protective HBsAb levels are several and likely multifactorial. Among the starter-receiving HBV vaccine recipients, approximately 5–10% of vaccinated individuals are immunocompetent and do not respond to the vaccine, and a decrease in the number of vaccine-specific antibodies to the HBV surface protein (the vaccine antigen) occurs in vaccinated individuals [32]. By adolescence, the decrease in vaccine-specific HBsAb to undetectable levels can reach 70%. The challenges of both poor primary immunological response to the HBV vaccine and the rapid waning of elicited immune responses are much greater among HIV-infected recipients [33]. However, it is also possible that vaccination coverage at the time these adolescents should have received their pediatric HBV vaccines was still suboptimal, since coverage between 2004 and 2013, when our

10-year–19-year-old adolescents may have received their vaccines under UNEPI coverage in Uganda, was reported to be between 62% and 84% [34]. We have recently shown that the majority of Sub-Saharan African 15–17-year-olds, born around the period of integration of HBV vaccines in many national EPIs in the region and therefore most likely receiving childhood vaccination, lacked protective HBsAb levels [35]. Research in Uganda has also shown generally poor childhood responses to infant vaccines, including measles, tetanus and hepatitis B [36–38]. Underlying preexisting infections, especially malaria and helminths [39], poor childhood nutritional status [40] and programmatical challenges in the implementation of EPIs [41] have also been cited. These studies also suggested that antibody levels decline post vaccination, which further explains the results of our study. The presence of quantitatively undetectable HBsAb may indicate that the majority of adolescents living with HIV (252 of 301, 83.7%) are vulnerable to HBV infection [8]. These findings aligned with those of Le et al., who reported a decrease in hepatitis B immunity despite increased vaccination rates during infancy [42]. However, a ray of hope from recent studies seems to suggest that a significant proportion of HBV vaccine recipients with waned undetectable HBsAb immune responses seroconvert upon receiving a booster HBV vaccine dose, clearly showing that an anamnestic response to the HBV vaccine is present in immunocompetent individuals despite a seronegative status (https://doi.org/10.1016/j.vaccine.2018.03.028).

Our study revealed no associations between ART adherence or suppressed viral loads and protective levels of HBsAb antibodies, supporting other findings that these factors may not prevent antibodies from waning [8]. Similarly, sex played no role in the protection of HBs antibodies, suggesting that both male and female adolescents living with HIV were equally prone to either waning protective HBsAb antibodies or susceptibility to HBV infection. While the number of pregnant and breastfeeding adolescents was too small for association analysis with HBsAb levels or protection, they themselves are evidence of a sexually active population, and sexual contact is a major route of HBV transmission [11, 43].

Finally, although not the main focus of this study, we examined the results of the qualitative serology of the 5-panel HBV combo cassette and observed that 88.1% (357 of 405) had no HBsAb, a result that correlated well with the 83.7% proportion of those without protective HBsAb, as determined via the quantitative titering method. The qualitative HBV Combo RDT cassette was able to detect HBsAb antibodies in only 24 of the 49 samples quantitatively at protective levels, indicating the reduced sensitivity of the immunochromatographic lateral flow strip assays [44–46]. A closer examination of the raw data revealed that samples with HBsAb titers less than 80 IU/L were consistently negative for HBsAb on the qualitative Combo test cassette. This finding aligns with a study by Poiteau et al., which reported that the sensitivity of RDTs for detecting HBsAb was low, especially for titers below 100 IU/L [44]. The limited sensitivity of the qualitative Combo test cassette in detecting HBsAb reduces its reliability for accurately assessing immunity. Therefore, it should not be used as a standalone tool in vaccine policy decision-making, as it may underestimate true levels of seroprotection. In many low-resource settings in Sub-Saharan Africa, access to quantitative methods for determining titer levels of HBsAb in vaccine recipients is limited in geographical and cost terms. Alternative approaches to screen for those immune or susceptible to HBV infection remain needed, and immune-chromatographic-based methods are the best choice, as they solve access to diagnostics in Africa. The 80 IU/L threshold level may have been used for the Vaxpert hepatitis Combo test but should be considered in future carefully designed studies focused on addressing this challenge.

Our study had several limitations that could have affected the generalizability and conclusions of the findings; the samples were drawn from patients who provided consent for the use of their leftover clinical samples for research, a very small fraction of the samples from adolescents whose samples were received for HIV viral load testing during the study period. Moreover, the metadata accessible for this study are limited to those provided only on the test request forms and not the entire medical records. Another limitation of this study was the lack of hepatitis B vaccination history, which constrained our ability to distinguish between vaccine-induced and naturally acquired anti-HBs immunity. However, interpretation of serological patterns from the qualitative assay provided some indication, presence of HBsAb alone reflected prior vaccination, while combinations with other HBV markers suggested past exposure or infection. The Vaxpert hepatitis B Combo test used in the screening was not verified for sensitivity and specificity and could have resulted in an unknown number of false negatives and or false positives. Low amounts of resources meant that we were only able to perform HBsAb titers for 301 out of the 405 samples available for the study.

Conclusion

Our study revealed a low prevalence of protective HBsAb antibodies (16.3%) among adolescents living with HIV. Serological evidence of exposure to 7.0% hepatitis B virus is evidence of a continuous risk of HBV infection in a significant proportion of adolescents living with HIV who may be potentially vulnerable to HBV infection. Therefore, our findings suggest a need for Uganda

to develop a guideline for assessing the risk, screening and management of hepatitis B virus infection, which is specific for persons living with HIV if it is to achieve the WHO targets of elimination of viral hepatitis by 2030. The qualitative HBV Combo RDT cassette results indicate our aspiration to attract the attention of researchers, innovators and manufacturers in developing immunochromatographic lateral flow-based assays as surrogates for screening susceptibility and/or protective immune responses in low-resource HBV endemic countries.

Abbreviations

CPHL	Central public health laboratories
Ccc DNA	Covalently closed circular DNA
EQA/IQC	External quality assurance/internal quality control
CHS	College of health sciences
UNAIDS	Joint united nations programme on HIV/AIDS
Et al.	And others
HBcAb	Hepatitis B core antibody
HBeAb	Hepatitis B precore antibody
HBeAg	Hepatitis B precore antigen
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular cancer
HIV	Human immunodeficiency virus
PLWHIV	People living with HIV
RDTs	Rapid diagnostic tests
UNEPI	Uganda national expanded program on immunization
IU/L	International units per liter
IDI	Infectious disease institute
Ab	Antibody
Ag	Antigen
NHLDs	National health laboratory and diagnostic services
MOH	Ministry of health

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11244-3.

Supplementary Material 1.

Acknowledgements

We thank the management and staff of the School of Biomedical Sciences, Uganda National Health Laboratories, and Diagnostic Services; UNHLDs; the Infectious Disease Institute (IDI); and the study participants.

Authors' contributions

Conceptualization was by A.T., R.N., and B.S.B.; Data curation, A.T., F. Mugalula and B.S.B.; Formal analysis, A.T., R.N., F.M., F. Mugalula., and B.S.B. Funding acquisition, A.T., A.N., B.C., R.N. and B.S.B.; Investigation, A.T., F.M., A.N., M.O., B.K., F.M., I.S., B.C., R.N. and B.S.B.; Methodology, A.T., F.M., M.O. and B.S.B.; Project administration, A.T., and B.S.B.; Resources, A.T., A.N., B.K., B.C. and B.S.B.; Supervision, B.C., I.S., M.O., R.N. and B.S.B.; Visualization, A.T. and B.S.B.; Writing original draft, A.T. and B.S.B.; Review & editing, A.T., F.M., M.O., B.C., and B.S.B. All the authors have read and agreed to the published version of the manuscript.

Funding

Support for research was provided by the Fogarty International Centre, National Institutes of Health (grant # D43TW009771 "HIV coinfections in Uganda: TB, Cryptococcus and Viral Hepatitis").

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Makerere University College of Health Sciences, School of Biomedical Sciences Research and Ethics Committee (SBS-REC) under approval number SBS-2023-447. A waiver of consent was granted by SBS-REC for the use of archived samples, given that verbal assent and parental/guardian consent for future use of samples had already been obtained through the Uganda National Biorepository's broad consent process.

The study was also registered with the Uganda National Council for Science and Technology (UNCST), registration number HS3679ES. Administrative approvals were obtained from the Uganda National Health Laboratory and Diagnostic Services (UNHLDS) and the Infectious Diseases Institute Core Laboratory (IDI Core Lab). This study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki for medical research involving human subjects. Participant confidentiality and data protection were maintained throughout the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 27 March 2025 / Accepted: 12 June 2025
Published online: 09 July 2025

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Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

African Health Sciences
Makerere Medical School

Hepatitis B Vaccine-Specific Antibody Titers Among Children of up to 17 Years in Kampala, Uganda

Journal:	*African Health Sciences*
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	MUWANDA, FAHAD; Makerere University College of Health Sciences, Immunology and Molecular Biology; Islamic University in Uganda, Medical Microbiology; Kibuli Muslim Hospital, Health Training Schools-School of Medical Laboratory Technology Kiyonga, Edward; Makerere University College of Health Sciences, Immunology and Molecular Biology Nambafu, Joan; Makerere University College of Health Sciences, Immunology and Molecular Biology Turyamubona, Agnes; Makerere University College of Health Sciences, Immunology and Molecular Biology Kafeero, Hussein; Islamic University in Uganda, Medical Microbiology; Kyambogo University, Biomedical and Mechatronics Engineering Kigozi, Edgar; Makerere University College of Health Sciences, Immunology and Molecular Biology Babikako, Harriet; Makerere University College of Health Sciences, Child Health and Development Center Wekiya, Enock; Republic of Uganda Ministry of Health, Tuberculosis and Leprosy Control; Makerere University College of Health Sciences, Immunology and Molecular Biology Gerald, Mboowa; Makerere University College of Health Sciences, Immunology and Molecular Biology Kateete, David; Makerere University College of Health Sciences, Immunology and Molecular Biology Sendagire, Hakim; Makerere University College of Health Sciences, Microbiology Norman, Paul J. ; University of Colorado Anschutz Medical Campus School of Medicine, Biomedical Informatics Bagaya, Benard; Makerere University College of Health Sciences, Immunology and Molecular Biology, School of Biomedical Sciences
Keywords:	Hepatitis B Virus, Seroprotection, Hepatitis B Vaccine

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Article

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Abstract: Background: Childhood HBV vaccination is pivotal to elimination of HBV infection but the extent of the vaccine seroprotection is unclear and variably characterized by most Extended Programs for Immunization (EPI). This study sought to characterize the seroprevalence of HBV vaccine specific antibodies among children of up to 17 years in Kampala, Uganda. **Methods:** In a cross-sectional study, we characterized the seroprevalence of HBV vaccine specific antibodies among 443 and 267 children <5 and 15-17 years old respectively, in Kawempe Division, Kampala, Uganda, between May and August 2023. We quantitatively analyzed Serum/plasma samples for hepatitis B surface antibody (anti-HBs) by running the electrochemiluminescence immunoassay (ECLIA) on the Roche Cobas e 411 immunoassay analyzer system. **Results:** The seroprevalence of protective levels of anti-HBs antibodies was 51.83% (368 of 710); 74.29% of the children in the 0-5-year-old age cohort (329 of 443) and 14.61% (39 of 267) in the 15-17-year olds respectively. The qualitative Hepatitis B Combo RDT test assays detected only 42.12% (155/368) of all protective anti-HBs titers that were determined by the quantitative Cobas E411 system (368/710). **Conclusions:** The markedly reduced seroprotection in the 15-17 year olds identifies a clear adolescent immunity gap after infant HBV vaccination in Kampala.

Keywords: Hepatitis B Virus; Seroprotection; Combo Test; Hepatitis B Vaccine; Kampala-Uganda

For Peer Review

1. Introduction

More than 250 million people globally are chronically infected with the hepatitis B virus (HBV); the highest contributor to mortality-related liver cirrhosis and cancer cases annually [1]. Sub-Saharan Africa is disproportionately affected [2-4], with high endemicity occurring in countries like Uganda where the national prevalence of HBV infection is 10% [5]. The WHO through a series of globally published health sector strategies [6, 7] and guidelines for hepatitis B prevention, testing, care, and treatment [8-10]; aims at eliminating HBV infection by 2030. If this goal is to be realized, it is, among others a strong recommendation by the WHO that reporting and monitoring systems for national extended programs for Immunization (EPI) be strengthened [10]. Adequate EPI monitoring can ably verify the approximately 20% of immunocompetent HBV vaccine recipients who fail to respond to the complete vaccination [11-14]. In Uganda, the HBV vaccination was integrated into the national EPI in 2002 and the current schedule is given at birth, 6, 10, and 14 weeks [15-18]. However, like in most low-income and high-burdened countries, hepatitis B vaccination coverage, and completion of all recommended doses among vaccinated children remains low [1]. Following vaccination, unlike in adult high risk vaccination programs such as among healthcare workers, pediatric HBV serostatus is not or remains variably characterized as part of the national EPI monitoring. It is apparent that the proportion of childhood vaccinees with protective levels of the hepatitis B surface antibody (anti-HBs) is hardly documented in Uganda and East Africa as a whole [19, 20].

National sero-surveillance studies conducted to monitor the efficacy of EPIs in Africa show reduced HBV vaccine seroprotection rates, including among children of 15-17 years [21]. Whereas various studies in northern [22-25], western [26-28], and southern Africa [29-31] reported increased seroprotective rates following hepatitis B vaccination of infants, a few studies conducted in the East African region [19, 20, 32, 33] demonstrated varied anti-HBs seroprotection levels. In Uganda, Kimera *et al.,* (2025) reported good protective immune response of 77.4 % among children under five years attending Mulago hospital [19]. On the contrary, a recent study in Ethiopian children revealed as low as 14.4 % and

2.6 % of protective anti-HBs antibodies (>10 IU/mL) among under 5 year olds and in ≥10 years children [33] respectively. It is probable that a lack of response to the HBV vaccine or waning of antibody titers below the protective levels significantly predisposes previously vaccinated children to breakthrough infections [32-38]. The markedly prevalent risk factors associated with transmission of HBV infection among children underscores the need for effective HBV vaccination among infants and continued EPI monitoring and evaluation [10, 21, 25, 39, 40]. Moreover, studies have documented many factors that result in suboptimal immune responses to childhood vaccination [19, 21, 32, 33, 41-44]). Determining and keenly monitoring the proportion of HBV vaccine seroprotection among populations with a heightened risk of infection can inform adequate control and/or elimination of HBV infection [1, 45].

The Uganda Demographic Health Survey (UDHS-2022) indicated a low (70%) 3-dose immunization coverage of childhood hepatitis B vaccine [46], compared with the recommended 90% coverage for every vaccine [46, 47]. Additionally, Uganda continues to experience periodic outbreaks of HBV infection and a stagnated HBV infection seroprevalence [47-50]. Elimination of hepatitis B virus infection will require a strong and successful national childhood immunization program, justifying the need to monitor the levels of anti-HBs among children vaccinated in infancy for effective evaluation of the seroprotective efficacy of the HBV vaccine. There is generally limited data on estimation of HBV vaccine seroprotection among vaccinees in Uganda. A study conducted in selected health facilities in northern Uganda revealed that protective immune response persists for over ten years following hepatitis B vaccination among healthcare workers [51]. Much as Muwanda et al., (2024) reported a low prevalence of children with qualitatively detectable anti-HBs antibodies, they were not able to determine the extent of utility of these qualitative Rapid Diagnostic Test-based methods (RDTs) nor quantify the Anti-HBs titers [20]. Although a recent study by Kimera et al., (2025) among children under 5 years of age attending Mulago hospital revealed seroprotection rate of 77.4%, immune response among the children previously vaccinated is variable and remains inconclusively studied in Uganda [48-52]. This study therefore sought to evaluate the efficacy of HBV RDTs and to quantitatively determine the seroprevalence of anti-HBs antibodies among high risk vaccinated children under 5 years and 15-17 years of age in Kawempe Division, Kampala, Uganda.

2. Materials and Methods

2.1. Study design, population, and settings

We enrolled 789 children in a cross-sectional study between May and August 2023 from the Child and Family Foundation (CFU) medical center's catchment areas in Kawempe Division, Kampala Capital City Authority. Kawempe division has 22 administrative parishes. The CFU medical center conducts both static and mobile immunization services under the UNEPI. The static immunization services are scheduled for Monday and Friday while the outreaches are done twice a month, rotational in the different parishes. All immunization services provided are recorded in the Ministry of Health (MOH) health management information system (HMIS). Our study population included children under 5 who had received the hepatitis B vaccine as indicated by the child health card, and adolescents (15 to 17 years) who received the hepatitis B vaccine during childhood. All 789 children were qualitatively tested for HBV sero-markers, while 710 children were successfully tested for anti-HBs antibodies.

2.2. Inclusion and exclusion criteria

We included children under five years of age who had received at least one dose of the HBV vaccine, evidenced on their UNEPI immunization card, and were not anaemic or immunocompromised as assessed by a study clinician. For the 15-17-year-old participants, we included non-anaemic or immunocompromised participants who had

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received at least one Hepatitis B vaccine dose verifiable with a UNEPI immunization card or if not available, based on parents/guardians' reporting.

2.3. Sample size estimation and sampling procedure

Using Kish Leslie's formula[53], we arrived at a sample size of 501 and 288 participants for the under 5 years and 15-17-year olds participant groups respectively. For the under-5-year-olds, we enrolled consecutively eligible children among those seeking services at the CFU medical center until the sample size was accrued. For the 15-17-year-old study group, eligible study participants were identified during CFU's scheduled health home visits and community medical outreaches and were referred to the medical center for consenting and conduct of other study procedures until the sample size accrued consecutively.

2.4. Data collection procedures

A pretested structured questionnaire was administered to parents/legal guardians of participants in the under 5-year-old study group, and directly to the participants in the 15-17-year-old group with a provision for consulting their parents/legal guardians if they wished. The questionnaire collected data on the children's socio-demographic characteristics like age, gender, medical history, and hepatitis B vaccination status.

2.5. Sample collection and processing

Blood was collected from all participants by a designated study clinician or phlebotomist following pre-developed standard operating procedures (SOPs). For the under 5-year-old participants, 6 mL of venous blood was aseptically collected by venipuncture in the following order; 1 Heparin anticoagulant tube of 4 mLs (Becton & Dickson Co. Ltd., Nairobi, Kenya) and a 2 mL plain serum separation tube (Becton & Dickson Co. Ltd., Nairobi, Kenya). For the 15-17-year-olds, 4 mL venous blood in a plain serum separation tube was collected. Serum and Plasma separation was then done by centrifugation of the blood tubes at 1500g for 15 minutes at room temperature, at the Immunology Laboratory of the School of Biomedical Sciences, Makerere University, Kampala, Uganda. All sera, plasma, and PBMCs recoverable were stored in labeled cryovial tubes in volumes of 0.5 mL at -20 ˚C or lower until analysis; at the at the Integrated Biorepository of H3Africa-Uganda (IBRH3AU), Makerere University, Kampala, Uganda. Qualitative assessment of all participants' serum/plasma samples for HBV seromarkers was conducted, and sufficient aliquots re-archived for quantitative anti-HBs estimation.

2.6. Laboratory analysis

Serum samples were tested for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb using three different Hepatitis B Combo RDT test kits; NOVA TEST-One Step Diagnostic Rapid Test (Global Science, Malaysia; Jaya, Selangor D.E, Malaysia, Cat#: 20221811HBV), Fastep Rapid Diagnostic Test (Healgen Scientific Limited Liability Company, Houston, TX77047-USA, Cat#: 21076412) and Beright Diagnostic Rapid Test (Medhealth Biotechnology Limited, China, Cat#: 12216453) with manufacturer reported sensitivity and specificity of (91.43% and 98.28%), (68% and 77%), and (98.92% and 99.46%) respectively. Children who tested positive for HBsAg were referred to the CFU Medical Center for appropriate clinical management. All sufficient plasma samples volumes, including those that were qualitatively positive for HBsAb were further quantitatively confirmed by measurement of anti-HBs using the Elecsys Anti-HBs II electrochemiluminescence immunoassay (ECLIA) test reagent kits (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany, Cat#: 11876317122) on a cobas e 411 immunoassay analyzer (serial number: 6111-04). The kits have manufacturer-reported sensitivity and specificity of 100% and 99.62%, respectively. For anti-HBs, we quantitatively analyzed 443 and 267 serum samples for the under 5 years and 15-17-year-olds respectively. All test procedures followed the manufacturer's instructions

[https://www.roche.com/]. All kits and testing runs included positive and negative controls (prepared and distributed to health facilities by the Uganda Virus Research Institute (UVRI)).

2.7. Data analysis

All the participants' data for hepatitis B Combo RDT results were concordant on all three test kits and were therefore included in the analysis. Concordance among the 3 serological tests was analyzed according to Langton et al., (2002) as the percentage of chance that they would yield the same results [54] (Supplementary Table 2). The determined anti-HBs quantification was reported in international units (IU).

Data were entered for cleaning into EpiData software version 3.1 and exported to SPSS version 25 for descriptive analysis. Categorical variables and continuous variables were presented as frequency tables and graphs. Anti-HBs titers were presented with the median and as a percentage with the corresponding 95% confidence interval. Mann-whitney U test was used to compare groups. A p-value of less than 0.05 was considered statistically significant.

2.8. Interpretation of anti-HBs titer results

Whereas HBV vaccine seroprotection/response was categorized as the number/proportion of children with anti-HBs titers ≥ 10 mIU/ml, lack of HBV vaccine protection/non-response was characterized by anti-HBs titers < 10 mIU/ml. The proportion of seroprotection determined by an anti-HBs estimation assay defined its positivity rate. Anti-HBs levels between 10 and 100 mIU/ml were considered as hyporesponse and more than 100 mIU/ml was considered as a high level of immunity following vaccination [55].

3. Results

3.1. Socio-demographic characteristics

Out of the 789 enrolled, a total of 710 children participated in this study; 267 and 443 in the 15-17-year-old and under 5-year-old groups respectively. Following qualitative HBV seroprevalence estimation, 79 samples did not retain sufficient amounts to quantify the detectable anti-HBs. The mean age of the study participants was 16.1 years and 3 years for the 15-17-year-old and 0-5-year-old groups respectively (Table 1). Whereas more female children (53.05%) were enrolled in the under-5-year-old group, more males (51.69%) were enrolled in the 15-17-year-old group (Table 1).

Table 1. Characteristics of the study participants, Kawempe Division, May-August 2023

Variable	0 - 5 years, *n*=443	15 - 17 years, *n*=267	Combined, *n*=710
Age Mean (SD)	3.01 (1.32)	16.17 (0.82)	
Gender n (%)			
Male	208 (46.95)	138 (51.69)	346 (48.73)
Female	234 (53.05)	129 (48.31)	363 (51.12)

3.2. Anti-HBs antibodies among study population

The prevalence of hepatitis B virus exposure status among the 789 study participants, determined by the hepatitis B Combo test RDT cassettes, has previously been reported elsewhere [32, 33, 56-58]. Among participants successfully tittered, prevalence of protective levels of anti-HBs antibodies was 51.83% (368 of 710); 74.29% of the children in the 0-5-year-old age group (329 of 443) and 14.61% (39 of 267) in the 15-17-year olds respectively. The median anti-HBs titers were 74.12 mIU/mL (IQR: 8-330.8 mIU/mL) and

For Peer Review

2.48 mIU/mL (IQR: 2-2.48 mIU/mL) (Table 2). These results translate into a 48.17% proportion of participants with no detectable or less than protective levels of antibodies against hepatitis B virus; 25.73% (114 of 443) in the 0-5-year-old and 85.39% (228 of 267) in the 15-17-year-old groups respectively. We also found that none (0) and 2 children under 5 years and 15–17-year-old children respectively had protective anti-HBs levels resulting from hepatitis B virus exposure or infection.

Table 2. Anti-HBs among the study participants in Kawempe Division, May-August 2023

Variable	Overall, n=710	0 - 5 years, n=443	15 - 17 years, n=267
Anti-HBs titer Median (IQR)		74.12 (8-330.8)	2.48 (2-2.48)
Protective Anti-HBs n (%)	368 (51.83)	329 (74.29)	39 (14.61)

3.3. *Anti-HBs antibody titers by number of vaccine doses and age of participants*

Of the 412 HBV vaccine responders; 363, 28, and 21 received 3, 2, and 1 doses, respectively. The proportion of those with protective levels as well as titers of HBV vaccine-specific anti-HBs antibodies increased with number of doses received (See Figure 1 A & B). The antiHBs antibody titers were significantly higher among those who completed 3 doses when compared to single $p = 0.009$ or two $p = 0.003$ dose recipients.

Within the first 5 years, we saw a gradual decrease in the median anti-HBs levels but titers were significantly higher among the under 5 year group when compared with titers of the 15-17 year old children $p < 0.05$ (Figure 1 C).

For Peer Review

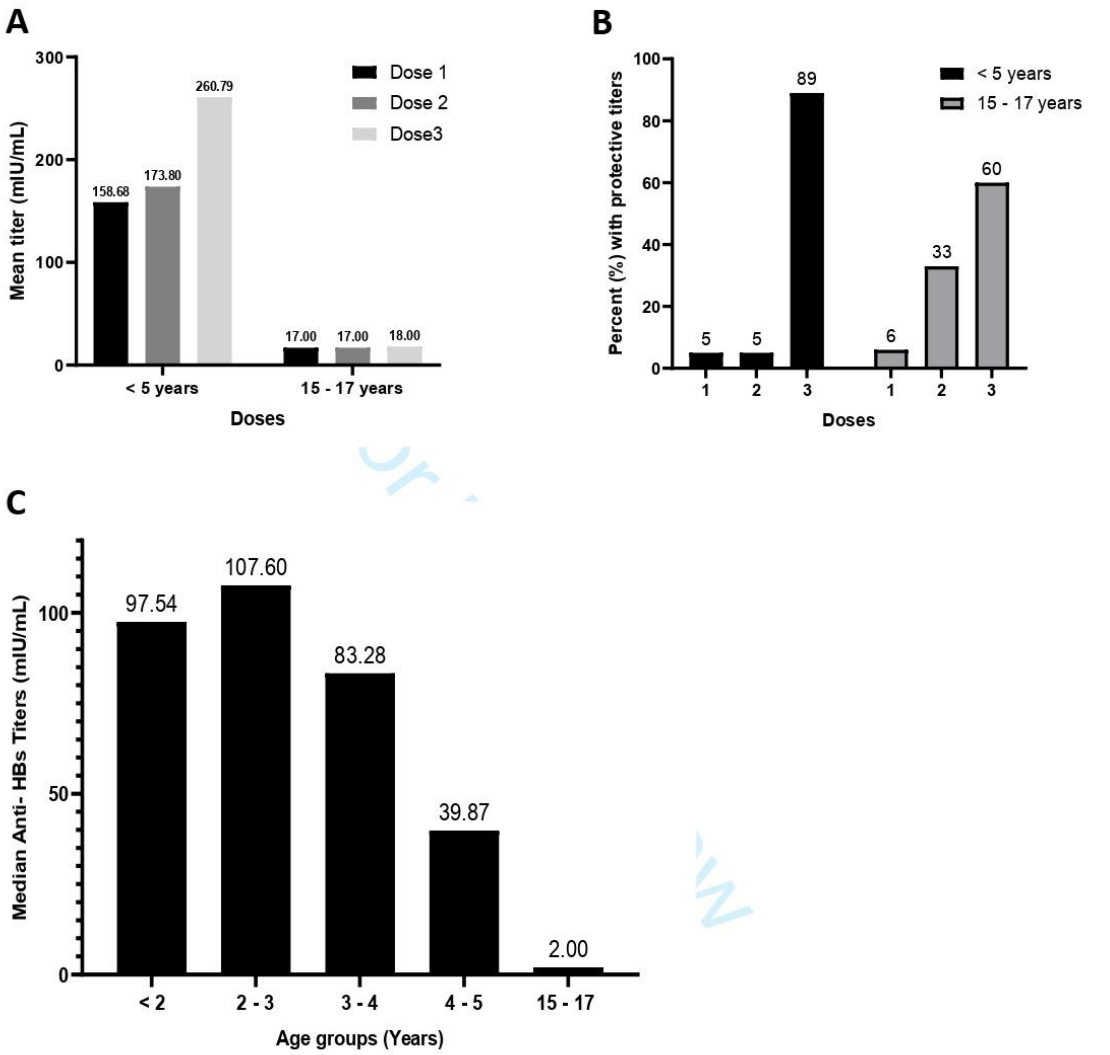


Figure 1. Anti-HBs; **(A)** by numbers of doses received; **(B)** Percent of those with seroprotective titers by number of doses received; **(C)** levels by age categorization, among the study participants in Kawempe Division, May-August 2023.

3.4. Comparative analysis of qualitative and quantitative assays for the estimation of anti-HBs

The qualitative Hepatitis B Combo RDT test assays detected only 42.12% (155/368) of all protective anti-HBs titers that were determined by the quantitative Cobas E411 system (368/710) (Figure 2). The lowest titer as determined by the Cobas E411 system and also detectable on the RDT was 10.28 mIU/mL.

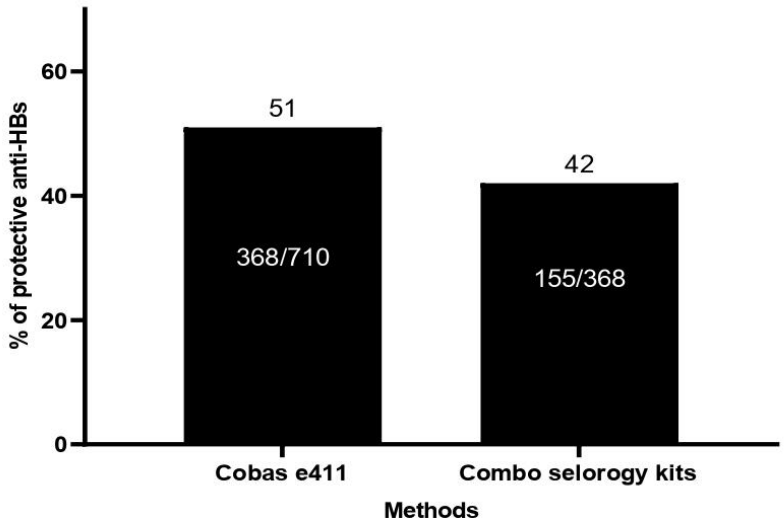


Figure 2. Proportions (%) of children with protective levels of anti-HBs (as determined on the Cobas E411 system) that were detected (anti-HBs positive) by the rapid diagnostic tests (RDTs) Assays.

4. Discussion

This study aimed to assess levels of HBV vaccine-specific seroprotective antibodies among children under 5 years and in those of 15-17 years in Kampala, Uganda. We have found that the prevalence of anti-HBs antibodies of 74.29% among children under-5 years of age is comparable with that (77.4%) of another study conducted in Uganda [19], but falls short of WHO's recommended minimum of 90 % [59]. For children of the same age bracket, studies in similar populations elsewhere have reported much higher prevalence of protection of up to 97.5 % in some instances [29, 59, 60]. Many reasons have been put forward to explain sub-optimal responses to vaccination, not only to HBV but other vaccines too. Under performance of the UNEPI due to multiple challenges like dose completion and coverage, vaccine cold chain, skills of nurses administering the vaccines, malnutrition, and lower economic standards, among others are notable reasons. For instance, immune studies among children of the fisherfolk of Lake Victoria, Uganda, have found that immune responses to measles and tetanus vaccines are equally sub-optimal [61-65], pointing to underlying global effects to vaccination in this part of the world.

Our findings revealed 14.6% prevalence of anti-HBs antibodies among children 15-17-years of age. This prevalence compares with what is reported in several African countries [21, 33, 40] but is lower than global averages of 70–85% in European follow-up studies [66, 67]. Socio-behavioral activities associated with increased risk to HBV and other sexually transmitted infections and reduced vaccination coverage/full dose completion rates are integral environmental factors that predispose to sub-optimal or non-response, and ultimately sustained HBV infection transmission in this age group [32, 41, 68, 69]. Low adolescent protection risks perpetuation of HBV cycles, hindering the WHO's 2030 targets of 90% incidence reduction.

For Review Only

Our study revealed an increment in anti-HBs titers and proportion of vaccinated individuals with seroprotection as the number of administered doses were also increased. This was particularly observed with in the first five years of vaccination prior to substantial waning of anti-HBs in the later years of life. Increased dosing enhances B cell priming, germinal center stimulation, plasma cell and neutralizing antibody production, while promoting memory B cell formation. This study and several others augment the notion that anti-HBs titers increase with HBV vaccine dose administration [20, 27, 40]. The observed increase in third-dose coverage from 72.6% (2006-2008 cohorts) to 88% (2018-2020 cohorts) reflects Uganda's progress toward 90% global targets, corroborated by WHO/UNICEF Estimates of National Immunization Coverage (WUENI) (71% in 2008 to 89% in 2020) [59, 70]. Additionally, Uganda's 90% HBV vaccine coverage in some districts surpasses African averages (34%) [70]. However, the 2020 dip, linked to COVID-19 disruptions, underscores vulnerabilities in immunization systems, as seen in regional declines [70].

We have also shown evidence of waning of anti-HBs, visible even among the under 5 year olds, while children 15-17 years demonstrate titers as low as 2 mIU/mL. Our findings are consistent with studies showing 40-90% antibody loss post-vaccination in adolescents [21, 33, 40]. Indeed waning of anti-HBs antibodies over time, has been reported severally [31, 40, 71]. However, it has also been argued that waning of neutralizing antibodies is not a major correlate of vaccine seroprotection because anamnestic responses can ensure effective seroprotection [66, 72-74]. We therefore advocate for enhanced HBV vaccine coverage and full dose completion rates, especially within the first five years of vaccination, when a robust immune response and recall memory can be potentiated. We also advocate for booster dose administration in adolescents as recommended in the 2024 WHO guidelines for high-risk groups [19, 41, 59, 75]. Much as the HBV vaccine demonstrates initial efficacy in infancy, rapid anti-HBs antibody waning, particularly in adolescents, calls for strengthened EPI monitoring to achieve WHO's 2030 elimination targets [19, 59].

Low and middle-income countries (LMICs) demonstrate poor access to tittering as methods to verify attainment of protection against HBV infection. Factors like high costs, complexity, and access to remote areas limits the utility of titer methods. However, HBV Rapid Diagnostic Tests (RDTs) revolutionized access to diagnostics and can be a surrogate for tittering if validated. RDTs are designed for qualitative detection, and manufacturers are not obligated make them sensitive. Our research group however, nurtured ambitions of collecting data on the use of RDTs as alternatives to tittering methods. The hope is that RDT manufacturers or other Biotechnology companies will gain interest in improving RDTs to achieve commendable utility in detecting HBV seroprotection. Our findings showed that of the 368 children with protective anti-HBs as determined by the standard electro chemiluminescence immunoassay (ECLIA) run on the Cobas E411 system, only 155 (42.12%) were anti-HBs positive by the HBV RDT assays. This questions the RDTs' surrogate role for anti-HBs titer estimation in low and middle-income countries (LMICs) [20, 76], but perhaps stimulates RDT manufacturers to further improve RDT performance levels and their overall utility in the estimation of HBV and vaccine seroprotection.

5. Conclusions

The study identifies a clear adolescent immunity gap after infant HBV vaccination in Kampala, demonstrating reduced prevalence of HBV vaccine seroprotection in children under 5 years (74.29%), and particularly among the 15-17 year olds (14.6%). We also indicate that qualitative anti-HBs assays underestimate seroprotection relative to quantitative methods. To align with the 2030 HBV elimination targets, we advocate for enhanced HBV vaccination coverage and targeted adolescent strategies like the adoption of booster vaccine dose administration in Uganda.

For Peer Review

6. Limitations

Our reliance on self-reported vaccination for adolescents who lacked immunization cards predisposed our findings to confounders like potential recall bias. Our cross-sectional design precluded causality and therefore, future studies should incorporate longitudinal follow-up to elucidate sub-optimal response. Our large sample size and consecutive enrollment from Kawempe Division enhance representativeness, though urban bias limits generalizability to rural Uganda. Finally, not all of the study participants enrolled in our study completed all three WHO-recommended HBV vaccine doses.

Institutional Review Board Statement: Ethical approval to conduct this study was sought and obtained from the Research and Ethics Committee of the School of Biomedical Sciences, Makerere University College of Health Sciences (SBS-REC) under a registration number SBS-2022-184 and from the Uganda National Council for Science and Technology (UNCST) under a registration number HS2604ES.

Informed Consent Statement: The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All the study-generated data are included in this research article.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

For Peer Review

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