UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

The burden of Hepatitis B among people living with HIV in East Africa, evidence from 2016 -2019 Population-based HIV Impact Assessment surveys.

MITIKU TAMRE GENAMO

(GNMMIT001)

DISSERTATION IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
MASTER OF SCIENCE IN EPIDEMIOLOGY AND BIOSTATISTICS
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF CAPE TOWN

Supervisor:

Dr Hlengiwe Madlala

School of Public Health

Faculty of Health Sciences

University of Cape Town

Observatory, 7925
South Africa

Co-Supervisor

Professor Landon Myer

School of Public Health

Faculty of Health Sciences

University of Cape Town

Observatory, 7925
South Africa

DECLARATION

I, Mitiku Tamre Genamo, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: Signed by candidate MT Hermann

Date: 06/19/2025

ABSTRACT

Background:

Hepatitis B Virus (HBV) co-infection remains a critical public health challenge among people living with HIV (PLWH), particularly in sub-Saharan Africa (SSA). Africa, which is second to Asia in chronic HBV prevalence, globally it was reported approximately 60 million cases in 2017, with nearly 74% of global HBV/HIV co-infections occurring in the SSA region. In East Africa, hepatitis B surface antigen (HBsAg) prevalence rates are as follows: Ethiopia (6.03%), Uganda (9.19%), Kenya (5.16%), Rwanda (6.67%), and Tanzania (7.17%). In countries like Kenya, the infection rates surpass 5%, and Africa accounts for 70% of all new cases of HBV infections globally. Despite extensive research on the HIV epidemic, data on HBV co-infections among PLWH remain limited.

Objectives:

This study aimed to estimate the prevalence of HBV co-infection among PLWH in East Africa and identify related sociodemographic and clinical factors.

Methods:

Part A of this Minor dissertation details the research protocol, covering the rationale for the study in the introduction, objectives, methodology, statistical analysis plan, and ethical considerations. Part B provides a comprehensive literature review of studies conducted in sub-Saharan Africa (SSA), exploring the available research on the burden of Hepatitis B and its risk factors among people living with HIV (PLWH). Part C presents the study findings in a journal-formatted manuscript, including results, discussions, limitations, and recommendations. Part D covers the materials used in Parts A, B, and C as appendices, followed by the PLOS ONE journal's instructions to authors. Finally, Part E is the policy brief to inform decision-making. The entire thesis follows the Vancouver referencing style, in line with the requirements of the selected journal for manuscript formatting.

A cross-sectional analysis of secondary data from the PHIA surveys conducted in Ethiopia, Kenya, Rwanda, Tanzania, and Uganda from 2016 to 2019 by Colombia university. Data for secondary analysis was extracted from the PHIA dataset between April 2024 – August 2024. The study

employed stratified multistage probability sampling to select households and participants aged 15–64 years living with HIV. Data collection, conducted via mobile tablets, covered demographic, clinical, and HIV-related variables. HBV status was assessed through rapid diagnostic tests. Ethical approval was obtained for both primary data collection and secondary analysis per the Helsinki Declaration guidelines. Statistical analysis was performed in R, including descriptive measures, logistic regression, and significance testing ($P < 0.05$).

Results:

A total of 4,944 PLWH were included in this analysis, of the total 4,944 PLWH screened for HBV 248 (5.02%) were HBV-positive. The median age of PLWH participants was 36.5 years (IQR: 30–45), while HBV-negative participants had a median age of 38 years (IQR: 30–47). Males accounted for 42% of HBV-positive cases, compared to 29% among HBV-negative individuals. The overall HBV prevalence among PLWH was 5.02% (95% [CI]: 4.42% – 5.66%), with different country-specific prevalence: Rwanda 3.49% (95% CI: 2.40% – 4.89%), Tanzania 4.29% (95% CI: 0.89% – 12.02%), Kenya (4.99%), Ethiopia 5.54% (95% CI: 3.86% – 7.65%), and Uganda 5.67% (95% CI: 4.65% – 6.84%). Females had higher odds of HBV infection than males (aOR 1.78, P=0.003). Additionally, those who are HBV positive exhibited higher HIV viral loads (≥1,000 copies/mL; 39% vs. 31%) and lower median CD4 counts (455 vs. 488.5 cells/μL). Participants with viral suppression had 1.4 times higher odds of HBV infection than those without viral suppression (OR = 1.40; 95% CI: 1.08–1.82; P = 0.01). Active syphilis infections were more common among HBV-positive individuals (5%) than HBV-negative participants (3%).

Conclusion:

HBV co-infection remains a significant concern among PLWH in East Africa, with notable country-level variations. It remains a burden that poses an additional challenge to the national health system, which is already battling various infectious and non-infectious diseases. The findings underscore the importance of enhanced HBV screening, vaccination, and integrated treatment approaches to reduce the dual burden of HIV and HBV.

ACKNOWLEDGEMENT

First and foremost, I would like to express my profound appreciation to my primary supervisor, Dr Hlengiwe Madlala, for her constant guidance, support, and insightful advice during this study. My academic development has been greatly influenced by your leadership, and I appreciate all of your support, and I am thankful for the patience and encouragement along this journey.

I would also like to express my heartfelt appreciation to my co-supervisor, Professor Landon Myer, for your support and advice during the initial phase of this dissertation, constructive feedback and for giving me some directions. Your expertise and encouragement have significantly contributed to the completion of this work, and I am truly grateful for your mentorship.

A particular thank you to SOS-Kinderdörfer weltweit Hermann-Gmeiner-Fonds Deutschland e.V for providing me with financial assistance throughout my studies and giving me the great opportunity to further my educations at the best and world class universities. This experience has provided me with the knowledge and skills that I can apply in the real world to make a significant impact in society. I am forever grateful to you for believing in me and investing in my future.

To my friends who have grown to be like brothers and sisters, I am grateful for your support and friendship, as well as for constantly pushing me to become a better person. This adventure has been even more remarkable because of what you have done. I look forward to witnessing your success and the meaningful contributions you will make to a world that so desperately needs healing.

Finally, I am grateful to everyone who contributed to my academic career, whether directly or indirectly.

LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
HBV	Hepatitis B Virus
HCV	Hepatitis C virus
FDA	Food and Drug Administration
HCC	Hepatocellular carcinoma
STDs	Sexually transmitted diseases
STIs	sexually transmitted infections
VLS	Viral load suppression
INH	Isoniazid
TB	Tuberculosis
ARVs	Antiretrovirals
ART	Antiretroviral therapy
LLOQ	Lower limit of quantification
CD4	Cluster of Differentiation 4 (a marker for immune cells)
HREC	Human Research Ethics Committee
SSA	Sub-Saharan Africa
PLHIV	People living with HIV
UCT	University of Cape Town
EAs	Enumeration areas
PSUs	Primary sampling units
ODK	Open Data Kit
IRBs	Institutional Review Boards
ICAP	International Center for AIDS Care and Treatment Programs
SEC	Scientific and Ethics Committee
CDC	Centers for Disease Control and Prevention
KEMRI	Kenya Medical Research Institute
EPHI	Ethiopia Public Health Institute
MOH	Ministry of Health
UVRI	Uganda Virus Research Institute

UBOS	Uganda Bureau of Standards
TACAIDS	Tanzania Commission for AIDS
ZAC	Zanzibar AIDS Commission
NASCOP	National AIDS and STI Control Programme
RBC	Rwanda Biomedical Center
PECO	Population, Exposure, Comparison, and Outcome
PWID	People who inject drugs
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase chain reaction
HBsAg	Hepatitis B surface antigen
ANCs	Antenatal clinics
MSM	Men who have sex with Men
IDU	Injection drug use
PHIA	Population-Based HIV Impact Assessment
UPHIA	Uganda Population-based HIV Impact Assessment
RPHIA	Rwanda Population-based HIV Impact Assessment
AASLD	American Association for the Study of Liver Diseases
MTCT	Mother-to-child transmission
WHO	World health organization
UNAIDS	United Nations Programme on HIV and AIDS
USAID	The United States Agency for International Development
OR	Odds ratio
aOR	Adjusted odds ratio
BMJ	British medical journal
CI	Confidence interval

TABLE OF CONTENTS

LIST OF TABLES

RESEARCH PROTOCOL

LITRETURE REVIEW

MANUSCRIPT

Manuscript Supplemental Tables (subgroup analysis tables)

LIST OF FIGURES

RESEARCH PROTOCOL

LITRETURE REVIEW

MANUSCRIPT

PART A: RESEARCH PROTOCOL

1. Introduction

Hepatitis B virus (HBV) is a potent, viral human pathogen that causes both acute and chronic liver infection with long-lasting adverse health impact (1). The HBV infection can lead to progressive liver damage and eventually death from complications resulting from cirrhosis and hepatocellular carcinoma (HCC) (2). Although effective antiviral medications and FDA-approved vaccines against the hepatitis B virus (HBV) are available at large for the public, persistent infection with the virus remains an enormous public health challenge (3). Globally, around 1.5 million new infections and 887,000 deaths were attributed to the virus as of 2019 (4). An investigation of publicly accessible data from 161 countries recorded between 1965 and 2013 projected the global average prevalence of hepatitis B to be 3.61%. According to a 2016 global report, a revised estimation revealed that HBV infection prevalence climbed to 3.9% (95% confidence interval, 3.4 to 4.6%) (5). In 2019, the global estimated prevalence of chronic HBV infection rose to 4.1% which corresponds to 316 million individuals globally (5).

The highest prevalence levels were seen in Africa (8.83%) and the Western Pacific areas (5.26%) (5). This suggests that HBV infection rates were not declining. In the WHO Africa Region, HBV infection is most prevalent in Sub-Saharan Africa(SSA), with around 80 million people chronically infected with the virus (6). Roughly 87,890 deaths were recorded in 2015 as a result of either cirrhosis, hepatocellular cancer, or acute hepatitis B (7). Over 8% of adults in SSA are thought to be chronically infected, making the region the most endemic for HBV (8). According to a meta-analysis and systematic review done in Ethiopia (2016), the prevalence of the HBV virus (HBV) with pooled HBV seropositivity was 7.4% (9). Nonetheless, this varies from 5% to 11% depending on the subgroup; for case in point, 5.2% of people living with HIV(PLHIV) infection and 8.0% of people in community-based research (9). In Kenya, the meta-analysis comprised of fifty studies found the overall national pooled prevalence projection for HBV was 7.8%, and it varies across different groups such as the pooled prevalence was 4.1% for blood donors, 41.7% for jaundice patients, and 6.5% for pregnant women (10). The national study, Uganda Population-based HIV Impact Assessment (UPHIA) 2016–2017, found that the prevalence of HBV infection among adult Ugandans was 4.3%, with the highest prevalence (4.6%) found in the country's north and the lowest prevalence (0.8%) in the southwest (8). Approximately 52% of adult Ugandans in 2015 showed signs of prior or current HBV infection (8). In 2018, the overall population in Rwanda had a chronic HBV prevalence of 3.9%, according to data collected countrywide from 24

of the 30 administrative regions (11). The most current Rwanda Population-based HIV Impact Assessment (RPHIA) survey, which included every district in the country, found a 2% prevalence of HBV (12).

Despite improved health outcomes, people living with HIV (PLWH) remain susceptible to multiple comorbidities. Roughly 7.4% of PLWH worldwide are thought to have a chronic HBV infection simultaneously. Over 70% of these people with co-infections between HBV and HIV live in sub-Saharan Africa (4). The clinical burden of hepatitis B is significantly increased by co-infection with HIV, an estimated 36 million people living with HIV reside in SSA (13). SSA countries account for around 2.6 million HIV-HBV co-infected individuals. The co-infection of HIV and HBV is linked to a more aggressive course of chronic hepatitis B and raises the possibility of HBV transmission during pregnancy (13). Among PLHIV, there are wide variations in the prevalence of HBV infection. According to one meta-analysis of SSA countries, Tanzania has coinfection rates that vary from 5 to 17% among various population groups (14). The percentage of HBV/HIV coinfections among PLWH who participated in the latest surveys conducted in 2016 in Morogoro and Mwanza has been estimated to be 6.6% and 7.3%, respectively (14). Although PLWH accounts for a significant portion of HBV infections since both viruses share common transmission pathways, The study done in Kenya did not include PLHIV. This might have resulted in underreporting of the burden of HBV in the country, since, Kenya has seen one of the most severe HIV outbreaks (10). A cross-sectional study conducted in Mwanza, Tanzania in 2024 found the overall prevalence of HBV to be 5.4%, additional studies in this region found a prevalence of 7.9% among healthcare workers and 6.9% among PLWH (15). Other institutional studies conducted within certain Rawndan subpopulations report a prevalence of 2.9% among Health care workers from one referral hospital, 4.3% among HIV-positive people (16), 4.1% among blood donors (16), and 3.7% among pregnant women (17).

Hepatitis B can be prevented by modifiable risk factors. While a small number of prevalence surveys are carried out in developing countries, the great majority of research on risk factors for hepatitis B virus infection occurs in developed countries (18). An investigation into a retrospective cohort study conducted in Uganda revealed high-risk sexual behaviours as a risk factor that raises the possibility of contracting STDs, HIV, and HBV (8). Similarly, South Africa's case-control study found having more than two or multiple sexual partners increases the risk

of acquiring HBV in both genders (19). Another case-control study conducted in Ethiopia indicated a clear difference in HBV infection risk variables in cases and controls (20). A history of blood transfusions (7.3% vs.3.7%), STDs (7.7% vs.3%), body tattooing (20.7% vs.11.3%), multiple sexual partners (29.7% vs.13%), sharing sharp objects (31% vs.10.7%), a family history of HBV (12% vs.4%), and a history of jaundice (6% vs.1.3%) were associated with cases more frequently than controls (20). Additionally, the following risk factors were found in a meta-analysis of the factors that predict HBV infection in East Africa: age, gravidity, marital status, STD, education level, blood transfusion, scarification, alcohol consumption, HIV serostatus, body piercing, surgery, having sex with multiple partners, gender, and blood transfusion (21). The prevalence of HBV was substantially correlated with factors such as being a man (8.561%), being single (8.644%), scarification (8.102%), blood transfusion (11.057%), HIV seropositivity (12.131%), having sex with numerous partners (9.53%), scarification (8.102%), and being multigravid (8.104%). However, in this meta-analysis, there was no discernible correlation between the risk of HBV infection and age, occupation, education level, alcohol consumption, body piercings, or STDs (21).

East Africa lacks data on the disease burden associated with HBV and other types of hepatitis, however large hospital admissions and mortality rates were attributed to acute viral hepatitis, chronic hepatitis, and cirrhosis of the liver (9). Individuals with HIV are vulnerable to acquiring additional illnesses, such as TB, hepatitis B, syphilis, and other sexually transmitted infections (22) . HIV and HBV share similar transmission routes, and co-infection with both viruses often results in a hastened progression of HBV disease to cirrhosis and increased liver disease-related mortality (23). The PHIA study provided population-based estimates of HBV prevalence among people with HIV, which can aid in developing practical policy recommendations for screening and treatment, and may help evaluate the effectiveness of national HBV vaccination initiatives. HBV infection rates in East Africa are underestimated due to insufficient screening and surveillance for the disease, and HBV continues to be neglected. Ethiopia is among the countries that do not have a nationwide viral hepatitis surveillance strategy so do other East African countries in this study. A preponderance amount of HBV reports came from research conducted in institutions and among high-risk individuals. Population-based studies are still scarce, despite the fact that a small number of community-based research on the seroepidemiology of HBV prevalence in East Africa have been conducted in the past and have shown that the disease is endemic with regional variation in

the continent. Due to inadequate record keeping and underreporting, it has thus been challenging to accurately estimate the burden of HBV in the Horn of Africa. In addition, a dearth of financing and inadequate infrastructure have resulted in a relatively small number of research that attempt to explain the prevalence of HBV infections (10).

2. Aim

This study aimed to investigate the burden and risk factors associated with Hepatitis B among individuals living with HIV in East Africa using evidence from the 2016–2019 population-based HIV impact assessment surveys.

2.1. Objectives

1. To assess the prevalence of Hepatitis B among PLWH in the overall sample.
2. To examine whether the burden of Hepatitis B among PLWH differs among East African countries, and
3. To investigate the demographic and clinical risk factors associated with hepatitis among PLWH.

3. Methodology

3.1. Study setting and population

There are thirteen countries in East Africa: Burundi, Comoros, Djibouti, Ethiopia, Eritrea, Kenya, Rwanda, Seychelles, Somalia, South Sudan, Sudan, Tanzania, and Uganda. The PHIA data is available for only five of these countries, so this study will focus on the available data. These five countries include Ethiopia, Kenya, Rwanda, Tanzania and Uganda. In 2023, it is estimated that Ethiopia had a population of 123 million. In 2024, the latest population figures, based on Worldometer's elaboration of the most recent United Nations data, are as follows: Kenya's population stands at 56,153,297; Rwanda has a population of 14,400,058; the United Republic of Tanzania's population has reached 69,321,072; and Uganda's population is 49,858,955 (24). Data collection was conducted in urban and rural areas for Kenya, Uganda, Rwanda, and Tanzania. In Ethiopia, data collection was conducted only in urban areas, including large and small urban areas.

The national survey population included women and men 0–64 years of age, and this analysis will only focus on adult individuals living with HIV aged 15–64 years.

3.2.Sampling

The sampling strategy used for the Population-based HIV Impact Assessment (PHIA) in East Africa employed a stratified multistage probability sample design. This entailed defining strata as the urban areas in 11 regions of Ethiopia, subdividing the 47 counties by urban-rural status for Kenya, and categorising the five provinces of Rwanda, the 31 regions of Tanzania, and the 10 regions of Uganda. The sample design was structured into three stages of sampling units: enumeration areas (EAs) within the strata as the first-stage units, households within EAs as the second-stage units, and eligible persons within households as the final units.

Within each sampling stratum, the first-stage sampling units, also referred to as "primary sampling units" or PSUs were selected with probabilities proportionate to the number of households in the PSU. These probabilities were based on data from the Population and Housing Census of the respective countries. The allocation of the sample PSUs to the EA sampling strata aimed to achieve specified precision levels for regional estimates of viral load suppression (VLS) rates among people living with HIV (PLWH) adults aged 15 and older.

Furthermore, the second-stage sampling units were selected from lists of dwelling units/households compiled by trained staff for each of the sampled PSUs. Following the listing process, random systematic samples of specified numbers of dwelling units/households were chosen from each PSU. Finally, eligible individuals within households were sampled in the PHIA dataset. For this study, all eligible adults living with HIV aged 15 to 64 within the sampled households will be included in the analysis.

3.3.Study design

This is a household-based cross-sectional study which analyses secondary data collected from the PHIA dataset between April 2024 – August 2024.

3.4.Inclusion criteria

The eligible participants for this secondary analysis will be filtered out from the main PHIA biomarker survey dataset based on their HIV status.

- Women and men aged 15 – 64 years who are living with HIV(PLWH). Individuals with available HBV test results.

3.5.Exclusion criteria

- HIV-negative individuals
- person younger than the age of 15 and older than the age of 64
- If someone did not wish to know the results of their HIV test, they were considered to have refused the survey. Thus they will also be excluded from the analysis.

3.6.Procedures and data collection tools

Data was collected between October 2017 and April 2018 for Ethiopia, in 2018 for Kenya, between 2018 and 2019 for Rwanda, and from 2016 to 2017 for Tanzania and Uganda. The PHIA team conducted HIV testing using the national rapid diagnostic testing protocol and confirmed the results in the laboratory. In-person interviews were conducted using the adult questionnaire to eligible participants aged 15 and older. The adult questionnaire was composed of three sections, including demographic/behavioural, and clinical information (Appendix 1). Data were collected electronically using mobile tablets. The data collection application used was Open Data Kit (ODK) which is programmed for mobile tablet devices.

3.6.1. Assessment of exposure status of interest

Exposures of interests are HIV status and Hepatitis B. Whole blood collected into vacutainers by trained survey nurses was sent for HIV testing to the national diagnostic laboratory that uses validated instruments and standardized protocols. Blood samples were labelled with a unique barcoded participant identification number and stored in temperature-controlled cooler boxes. If study participants tested nonreactive on the screening test, they were considered HIV-negative. If their screening test showed a reactive result, they had to undergo confirmatory testing. Individuals with reactive results on both the screening and confirmatory tests were classified as HIV positive. Testing for the Hepatitis B virus (HBV) was carried out using the Determine HBsAg rapid HBV test from Abbott Molecular Inc. for all HIV-positive adults to check for the HBV surface antigen. A positive result on the Determine HBV test meant that the participant was classified as HBV positive, while a non-reactive result meant that the participant was classified as HBV negative.

3.6.2. Assessment of risk factors of interest

Variables included in this analysis are selected from the PHIA dataset, including adult questionnaires codebook each with specific categories for measurement. The selection of variables is based on the literature and other scientific evidence to examine their relationship with HBV(8,16,19,21).The dependent (outcome) variable for this study is HBV status among HIV-positive respondents 15–64 years of age. The risk factors of interest for HBV included sociodemographic and behavioural factors as well as HIV clinical biomarkers.

3.6.3. Demographic and behavioural

The demographic and behavioural risk factors are relevant to HBV among people living with HIV (PLWH), these variables will include gender (male, female), age (an integer), ever attended school (yes, no, don't know), highest level of education attended (none, primary/preprimary, secondary, tertiary), nationality (Ethiopia, Kenya, Tanzania, Rwanda, Uganda), current marital status (married/cohabiting/living together, divorced, separated, widowed, never-married), the number of sexual partners in the last 12 months, male circumcision status (yes, no, don't know), condom use frequency (always, most of the time, sometimes, rarely, never), engagement in sexual relationships for material benefits (yes, no, don't know), HIV testing as a couple (yes, no, don't know), individual HIV testing history (yes, no, don't know), awareness of individual HIV test results (positive, negative, uncertain/indeterminate, did not receive the result, don't know). pregnancy status (currently pregnant, not currently pregnant), use of isoniazid (INH) medication for TB prevention (yes, no, don't know), clinic visits and treatment for TB (yes, no, don't know), urban area indicator (for the other countries: urban area indicators are urban and, rural but for Ethiopia, urban area indicator is divided based on population size into small and large), and wealth quartile (lowest, second, middle, fourth, highest) (Table 1). The association of these variables with HBV will provide valuable insights for the identification of individuals who are at high risk of the disease.

3.6.4. HIV Clinical Biomarkers

These variables included indicators of whether an individual has an active syphilis infection which is categorized as (Yes, NO), Results of test for Efavirenz in blood are categorized as positive and negative. Additional variables are antiretroviral (ARVs) detected, Viral Load (< LLOQ: 400, <

LLOQ: 40, < LLOQ: 20, Target not detected Values(TND)), An adult is on ART (On ART, Not on ART), Duration of time on ART (On ART 24 months or more, On ART 12-23 months, On ART < 12 months, Not on ART), an individual has their viral load suppressed (Viral load suppressed (mL), Not viral load suppressed), CD4 count (CD4 count: instrument reading preferred over ODK transcription) and CD4 categories(CD4 less than 100, CD4 greater than or equal to 100 and less than 200, CD4 greater than or equal to 200 and less than 350, CD4 greater than or equal to 350 and less than 500, CD4 greater than or equal to 500) (Table 1).

Demographic characteristics	**Scale of measurement**	**Categories**
Gender	Categorical, nominal	Male, Female
Age	Numerical, continuous	15, 16, 17, ..., 80
Ever attended school	Categorical, binary	Yes, No
Highest level of education attended	Categorical, ordinal	None, Primary/Preprimary, Secondary, Tertiary
Nationality	Categorical, nominal	Kenyan, Ugandan, Tanzanian, Ethiopian, Rwanda
Currently married	Categorical, nominal	Married, Living Together, Widowed, Divorced, Separated
Number of partners	Numerical, continuous	Integer
Male circumcision	Categorical, binary	Yes, No
Number of people they had sex within the last 12 months	Numerical, continuous	Integer
Condom use (past 12 months)	Categorical, nominal	Always, Most of The Time, Sometimes
Beneficiary Sexual relationships	Categorical, binary	Yes, No
HIV tests as a couple	Categorical, binary	Yes, No
Ever tested for HIV	Categorical, binary	Yes, No
Awareness of HIV (if they know their status)	Categorical, nominal	Positive, Negative, Uncertain/Indeterminate, Did not receive the result
Pregnancy	Categorical, nominal	Yes, No, Don't Know/Unsure
Taking Isoniazid (INH)	Categorical, binary	Yes, No,
TB clinic visit	Categorical, binary	Yes, No
Ever was treated for TB	Categorical, binary	Yes, No
Urban area indicator	Categorical, nominal	Small (50,000)/Rural, Large (>50,000)/Urban
Wealth quintile	Categorical, ordinal	Lowest, Second, Middle, Fourth, Highest
Biomarkers		
Active syphilis infection	Categorical, binary	Yes, No
HIV status	Categorical, binary	HIV positive, HIV negative
ARVs detected	Categorical, binary	Yes, No
Viral Load	Categorical, ordinal	Tnd - target not detected values <= 10000000 < lloq: 20 - less than lower limit of detection of 20 < lloq: 40 - less than lower limit of detection of 40 < Lloq: 400 - less than lower limit of detection of 400
Efavirenz in blood	Categorical, binary	Positive, Negative
Hepatitis B Status	Categorical, binary	Positive, Negative
On ART	Categorical, binary	Yes, NO
Duration of time on ART	Categorical, ordinal	< 12 months, 12-23 months, 24 months or more, not on art
Viral load suppressed	Categorical, binary	Yes, No
CD4count	Numerical, continuous	Integer
CD4 categorical	Categorical, ordinal	< 100, >=100, <200, >=200, < 350, >= 350, <500, and >= 500

Table 1: Variables selected from the PHIA dataset codebook for the purpose of this analysis.

3.7.Statistical analysis

The data will be analyzed using the Windows version of R statistical software. We will summarize the study population's characteristics using descriptive statistics. This will include reporting measures of central tendency (such as mean and median) and dispersion (such as standard deviation and interquartile range) for continuous variables. For categorical variables, we will provide the frequencies and percentages to address objectives 1 and 2.

To address objective 3 we will perform a bivariate analysis to examine the relationships between specific risk factors and HBV status. This will involve the use of either independent t-tests or Mann-Whitney U tests for continuous variables, depending on their distribution, and Chi-square tests or Fisher's exact tests for categorical variables. We will apply the Shapiro-Wilk test to check the distribution of continuous variables, which will then guide the selection of the right parametric or non-parametric statistical tests. We plan to utilize a binary logistic regression model to pinpoint the critical factors contributing to HBV prevalence, taking into account potential confounding variables. We will regard findings as statistically significant if they are within a 95% confidence interval ($P < 0.05$). To ensure the reliability of our results, we will perform sensitivity analyses. This will involve running the logistic regression analysis with various subsets of data and examining the effects of potential outliers and influential observations.

4. Ethical consideration

All PHIA surveys received ethical clearance from Institutional Review Boards (IRBs) in both the implementing country and at the institutions conducting the surveys, which is ICAP (International Center for AIDS Care and Treatment Programs) at Columbia University. Furthermore, national ethics committees and health authorities within the respective countries also approved the PHIA survey to ensure compliance with local regulations and ethical standards.

These entities include the Rwanda Scientific and Ethics Committee (SEC), The Centers for Disease Control and Prevention (CDC; Atlanta, USA), the Institutional Review Boards at Kenya Medical Research Institute (KEMRI), Westat (a statistical survey research organization), the Ethiopia Public Health Institute (EPHI). UPHIA was coordinated by Uganda's Ministry of Health (MOH), with technical assistance from the US CDC, Uganda Virus Research Institute (UVRI), the Uganda Bureau of Standards (UBOS), Uganda National Council for Science and Technology, Tanzania

National Institute for Medical Research, and Zanzibar Medical Research and Ethics Committee before the initiation of data collection (Tanzania Commission for AIDS (TACAIDS) & Zanzibar AIDS Commission (ZAC), 2018; Ethiopian Public Health Institute (EPHI), 2020; Ministry of Health, Uganda, 2019; National AIDS and STI Control Programme (NASCOP), 2022; Rwanda Biomedical Center (RBC), 2020).

All survey staff, including laboratory technologists, nurse interviewers, and supervisors, underwent training on good clinical and laboratory practices, and ethical protection of survey respondents, and signed a data confidentiality agreement. Additionally, staff members from the Institutional Review Boards or Ethics Committees oversee the conduct of these surveys to ensure the protection of the rights of participants. The PHIA study adheres to the ethical principles outlined in the Helsinki Declaration, emphasising respect for individuals, informed consent, and the protection of participants in medical research (Tanzania Commission for AIDS (TACAIDS) & Zanzibar AIDS Commission (ZAC), 2018; Ethiopian Public Health Institute (EPHI), 2020; Ministry of Health, Uganda, 2019; National AIDS and STI Control Programme (NASCOP), 2022; Rwanda Biomedical Center (RBC), 2020).

This study involves secondary data analysis using publicly available PHIA datasets. To access the datasets permission is granted by the PHIA team (Appendix 2). Additionally, Ethical approval was obtained from the University of Cape Town Human Research Ethics Committee(HREC REF 788/2024). For the main study, ethical clearance for the study protocol, screening forms, primary data collection materials including adult questionnaires and biomarker tests, consent forms, and digital documentation of consent was granted by the Centers for Disease Control and Prevention (CDC), Columbia University, and all respective Public Health Institutes in East Africa, along with their respective institution review boards.

4.1.Informed consent

During the consent process, all potential participants were informed that their participation was voluntary and that they were not obligated to share any personal information they were uncomfortable revealing. They were also informed that they could discontinue their participation at any time.

For illiterate participants, an unbiased witness was involved. Those who were not proficient in any of the survey languages were disqualified from participating in the study. Respondents who agreed to be interviewed were also asked for permission to do biomarker testing. Subsequently, participants between the ages of 15 and 64 provided written consent to be interviewed and to take part in the biomarker survey of the study, which included HIV test result return and home-based testing and counselling. Participants had to receive their test results to participate in the biomarker component.

4.2.Privacy and Confidentiality

Preserving individual data privacy was implemented by putting in place strong measures to protect personal information from unauthorized access, breaches, and misuse. This includes using secure data storage systems, encryption technologies, and access controls to limit data availability to authorized personnel only. Confidentiality is the ethical duty to safeguard participants' identities and sensitive health details. In the PHIA dataset, rigorous protocols are followed to anonymize data by either removing or obscuring identifying information to prevent individuals from being re-identified. This process is crucial for maintaining participant's trust and upholding ethical research standards.

4.3.Risk and benefits

The parent study, which was conducted by the Public Health Institute of the respective countries in this study, ICAP at Columbia University, and the Ministry of Health, has produced a dataset with comprehensive information on HIV prevalence, incidence, and viral load suppression. This rich dataset enables researchers to evaluate the effectiveness of HIV programs, identify gaps in service delivery, and develop evidence-based interventions to reduce HIV transmission and enhance health outcomes. Despite its potential, the use of such sensitive data presents some risks associated with the confidentiality and privacy of participants. Mismanagement or breaches of data could lead to the identification of individuals, potentially causing stigma, discrimination, or other social harm. Therefore, the PHIA team implemented robust data protection measures and ethical standards to ensure the anonymity and integrity of the participants. Balancing these benefits and risks is vital to maximize the positive impact of the parent study's dataset, contributing to the global fight against HIV/AIDS responsibly and ethically.

This study will also contribute to the existing literature on the prevalence of HBV among PLWH and will intend to create awareness about the seriousness of the health issue, in addition to suggesting interventions to create a healthier and a productive society.

4.4.Dissemination

The information in this dissertation is vital for understanding and addressing the HBV epidemic in East Africa. Findings from this study will be shared through scientific publications and presentations at public health conferences. This data also has the potential to be used by stakeholders to plan targeted interventions, allocate resources effectively, and monitor progress towards global targets. Making the data available to researchers and policymakers will allow for further analysis to better understand the epidemic and the effectiveness of ongoing programs. This dissemination process will ensure that the information reaches a wide audience, including government agencies, non-governmental organizations, and the international community, fostering collaboration to combat HBV in East Africa and beyond.

4.5.Timeline

Tasks	January	February	March	April	May	June	July	August	September	October	November	December	Jan -Feb 2025	Aug-Sep 2025
Finding a Topic														
Develooping a research question														
Developing protocol														
Ethics clearance														
Clean dataset														
Data analysis														
writing up discussion														
Thesis completion														
Final Submission														
Dissemination of information														

Figure 1: Timeline for MSc dissertation

5. References

1. Wang M, Yan L, Wang J, Jin Y, Zheng ZJ. Global burden of hepatitis B attributable to modifiable risk factors from 1990 to 2019: a growing contribution and its association with socioeconomic status. Global Health [Internet]. 2023 Dec 1 [cited 2024 Apr 1];19(1). Available from: /pmc/articles/PMC10064596/

2. Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z, et al. Global, regional, and national burden of hepatitis B, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. lancet Gastroenterol Hepatol [Internet]. 2022 Sep 1 [cited 2024 Apr 2];7(9):796–829. Available from: https://pubmed.ncbi.nlm.nih.gov/35738290/

3. Ginzberg D, Wong RJ, Gish R. Global HBV burden: guesstimates and facts. Hepatol Int [Internet]. 2018 Jul 1 [cited 2024 Apr 2];12(4):315–29. Available from: https://link.springer.com/article/10.1007/s12072-018-9884-8

4. Maepa MB, Ely A, Kramvis A, Bloom K, Naidoo K, Simani OE, et al. Hepatitis B Virus Research in South Africa. Viruses 2022, Vol 14, Page 1939 [Internet]. 2022 Aug 31 [cited 2024 Apr 2];14(9):1939. Available from: https://www.mdpi.com/1999-4915/14/9/1939/htm

5. Nguyen MH, Wong G, Gane E, Kao JH, Dusheiko G. Hepatitis B virus: Advances in prevention, diagnosis, and therapy. Clin Microbiol Rev [Internet]. 2020 Apr 1 [cited 2024 Apr 2];33(2). Available from: https://journals.asm.org/journal/cmr

6. Riches N, Vinikoor M, Guingane A, Johannessen A, Lemoine M, Matthews P, et al. Hepatitis B in Africa Collaborative Network: cohort profile and analysis of baseline data. Epidemiol Infect [Internet]. 2023 Apr 3 [cited 2024 Apr 2];151. Available from: https://pubmed.ncbi.nlm.nih.gov/37009881/

7. Sonderup MW, Dusheiko G, Desalegn H, Lemoine M, Tzeuton C, Taylor-Robinson SD, et al. Hepatitis B in sub-Saharan Africa—How many patients need therapy? J Viral Hepat. 2020 Jun 1;27(6):560–7.

8. Kitandwe PK, Muyanja E, Nakaweesa T, Nanvubya A, Ssetaala A, Mpendo J, et al.

Hepatitis B prevalence and incidence in the fishing communities of Lake Victoria, Uganda: a retrospective cohort study. BMC Public Health [Internet]. 2021 Dec 1 [cited 2024 Apr 18];21(1):1–8. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-021-10428-1

9. Belyhun Y, Maier M, Mulu A, Diro E, Liebert UG. Hepatitis viruses in Ethiopia: A systematic review and meta-analysis. BMC Infect Dis [Internet]. 2016 Dec 19 [cited 2024 Apr 2];16(1):1–14. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-016-2090-1

10. Makokha GN, Zhang P, Hayes CN, Songok E, Chayama K. The burden of Hepatitis B virus infection in Kenya: A systematic review and meta-analysis. Front Public Heal [Internet]. 2023 Jan 26 [cited 2024 Apr 14];11. Available from: /pmc/articles/PMC9909240/

11. Makuza JD, Rwema JOT, Ntihabose CK, Dushimiyimana D, Umutesi J, Nisingizwe MP, et al. Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. BMC Infect Dis [Internet]. 2019 May 3 [cited 2024 Apr 18];19(1). Available from: https://pubmed.ncbi.nlm.nih.gov/31053097/

12. Umutesi J, Klett-Tammen C, Nsanzimana S, Krause G, Ott JJ. Original research: Cross-sectional study of chronic hepatitis B virus infection in Rwandan high-risk groups: unexpected findings on prevalence and its determinants. BMJ Open [Internet]. 2021 Dec 30 [cited 2024 Apr 18];11(12). Available from: /pmc/articles/PMC8719204/

13. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, et al. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. Lancet Gastroenterol Hepatol [Internet]. 2017 Dec 1 [cited 2024 Apr 14];2(12):900. Available from: http://www.thelancet.com/article/S2468125317302959/fulltext

14. Kilonzo SB, Gunda DW, Mpondo BCT, Bakshi FA, Jaka H. Hepatitis B Virus Infection in Tanzania: Current Status and Challenges. J Trop Med [Internet]. 2018 [cited 2024 Apr 17];2018. Available from: /pmc/articles/PMC5831599/

15. Mangowi I, Mirambo MM, Kilonzo SB, Mlewa M, Nyawale H, Majinge D, et al.

Hepatitis B virus infection, associated factors, knowledge and vaccination status among household contacts of hepatitis B index cases in Mwanza, Tanzania. IJID Reg. 2024 Mar 1;10:168–73.

16. Umutesi J, Simmons B, Makuza JD, Dushimiyimana D, Mbituyumuremyi A, Uwimana JM, et al. Prevalence of hepatitis B and C infection in persons living with HIV enrolled in care in Rwanda. BMC Infect Dis [Internet]. 2017 May 2 [cited 2024 Apr 18];17(1):1–7. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-017-2422-9

17. Mutagoma M, Balisanga H, Malamba SS, Sebuhoro D, Remera E, Riedel DJ, et al. Hepatitis B virus and HIV co-infection among pregnant women in Rwanda. BMC Infect Dis [Internet]. 2017 Sep 11 [cited 2024 Apr 18];17(1). Available from: /pmc/articles/PMC5594460/

18. Weldebrhan D, Berhe H, Tesfay Y. Risk Factors for Hepatitis B Virus Infection in North Ethiopia: A Case–Control Study. Hepatic Med Evid Res [Internet]. 2023 Jul 19 [cited 2024 Apr 18];15:79–91. Available from: https://www.dovepress.com/risk-factors-for-hepatitis-b-virus-infection-in-north-ethiopia-a-casec-peer-reviewed-fulltext-article-HMER

19. Jentsch U, Vermeulen M, van den Berg K, Swanevelder R, Creel D, Jacobs G, et al. A case-control study of risk factors for incident hepatitis B virus infection in South African blood donors. Int J Infect Dis [Internet]. 2024 Apr 1 [cited 2024 Apr 18];141:106958. Available from: http://www.ijidonline.com/article/S1201971224000262/fulltext

20. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2024 Apr 18];18(4):e0284646. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0284646

21. Kafeero HM, Ndagire D, Ocama P, Kudamba A, Walusansa A, Sendagire H. Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. Arch Public Heal [Internet]. 2021 Dec 1 [cited 2024 Apr 18];79(1):1–19. Available

from: https://archpublichealth.biomedcentral.com/articles/10.1186/s13690-021-00686-1

22. Bollinger RC, Thio CL, Sulkowski MS, Mckenzie-White J, Thomas DL, Flexner C. Addressing the global burden of hepatitis B virus while developing long-acting injectables for the prevention and treatment of HIV.

23. Corcorran MA, Kim HN. Strategies for Hepatitis B Virus Prevention in People Living with HIV. Curr HIV/AIDS Rep [Internet]. 2023 Dec 1 [cited 2024 Aug 13];20(6):451–7. Available from: https://pubmed.ncbi.nlm.nih.gov/37837570/

24. Worldometer. Population > Eastern Africa - Worldometer [Internet]. 2024 [cited 2024 Jun 19]. Available from: https://www.worldometers.info/population/africa/eastern-africa/

PART B: LITERATURE REVIEW

1. Introduction

Hepatitis B virus (HBV) is a major global health concern, causing acute and chronic liver infections that can lead to severe complications, which include cirrhosis and hepatocellular carcinoma (1–3) HBV as well as hepatitis C virus account for 96% of the deaths from viral hepatitis (4). According to WHO estimates, 316 million people worldwide, across all age groups, had a chronic HBV infection in 2019 (1). In the same year, infections linked to HBV caused 550,000 deaths globally. Between 2015 and 2019, as well as between 1990 and 2019, the number of deaths associated with HBV increased (1). According to WHO Interim Guidance, in 2019, only 68 out of the 194 countries had already met the 2030 objective of f 4 deaths per 100,000 people from HBV-related causes (1). In 2021, the WHO African Region had a 17% coverage rate for the HBV vaccination birth dose, while the global coverage rate was 42%. However, only 2% of people living with the hepatitis B infection in this region have been diagnosed (5). Overtaking TB and malaria, globally, hepatitis B and hepatitis C viruses are the world's leading causes of liver cancer and overall mortality (6). Access to care is thought to be extremely low in both developing and high-income countries, including the United States, Western Europe, and Japan. According to Centers for Disease Control and Prevention (CDC) estimates, less than one-third of people in the United States who have been infected with HBV are aware that they have the virus (6).

The geographical incidence of this infection varies greatly because of the different transmission mechanisms. Since the 1990s and 2000s, the vaccine has been widely accessible in the majority of African nations as part of the childhood expanded immunization program (7–10). Nevertheless, the vaccine does not always provide protection against HBV for various reasons, such as host immunological, genetic, viral, socio/demographic/economic, cultural, and health system factors (7–12). According to a multicenter prospective cohort study there were noticeable differences between cases and controls in risk factors for HBV infection (13). Having a history of jaundice, sharing sharp objects, having several sexual partners, body tattooing, blood transfusions, sexually transmitted illnesses (STDs), and a family history of HBV were more common in cases than controls (13). Clinical findings have also indicated that HIV encourages hepatitis B replication. More importantly mothers with HIV/HBV co-infection are also more likely to be HBsAg-positive which makes it more contagious. This could raise the odds of transmission of HBV to the newborn by more than 2 times (5).

HIV/HBV co-infection poses clinical and epidemiological difficulties, particularly in areas with scarce resources like East Africa (7–9,14–17). People living with HIV (PLWH) are more prone to contract HBV because of the two viruses' common modes of transmission and develop chronic HBV infection, adding to its being endemic in SSA region (3). Additionally, compared to HBV mono-infection, hepatitis B antigen (HBeAg) positivity a sign of active viral replication is more prevalent in HIV/HBV coinfection (3). Globally, there are roughly 37 million HIV-positive individuals, and 5–20% of them also have HBV (18). The prevalence of chronic HBV in people with HIV varies greatly by region and risk-based category, indicating distinct transmission routes (18). Furthermore, PLWH have a 40% greater risk of contracting HBV infection compared to people without HIV infection (19). Co-infection with HBV increases death and morbidity among PLWH. Furthermore, many HIV-positive individuals are not aware that they have an HBV infection, putting them at substantial risk of liver-related illnesses. Numerous studies revealed that people with HIV/HBV co-infection had lower CD4 cell counts than those with HIV alone (20). According to a large retrospective cohort study, HBV/HIV co-infected people who had HIV viral suppression at 6 months or longer and were on an HBV active antiretroviral therapy (ART) regimen had a lower risk of liver-related complications following ART treatment (20). Although comprehensive screening for HBV co-infection is strongly advised, especially in settings with limited resources, it is not frequently done before initiating ART (19). Despite the fact that ART's present effectiveness has drastically lowered opportunistic infection and increased life expectancy of PLWH, the chronic HBV infection that was previously unreported in these populations is once again present being a major public health issue (10). Significantly, initial ART can conceal undetected HBV co-infection; however, a hepatitis outbreak could occur when the ART regimen is changed (10). Although there is a lot of research published regarding HBV and HIV mono-infection prevalence and risk factors in different subgroups, there is limited research regarding coinfection. Given high prevalence of the two diseases in the African continent, especially in Sub-Saharan Africa (SSA), it is crucial to explore the burden of coinfection.

2. Aim

The aim of this literature review is to examine, evaluate, and synthesize the available research on the burden of Hepatitis B and its risk factors among PLWH in Sub-Saharan Africa. In order to

find gaps in the literature that need more investigation, this review will concentrate on a few studies, based on the inclusion criteria.

3. Literature search strategy and Methodology

Electronic medical databases such as PubMed, BMC and Embase were used to search for relevant and existing literature on HBV, HIV and coinfection. Titles, abstracts and reference lists of published papers were also reviewed.

3.1.Search methods

Keywords used for searching in databases include prevalence, Hepatitis B, risk factors, coinfection, sub-Saharan Africa, East Africa, HBsAg and Predictors. Using the Boolean operators "OR" and "AND" the key words were used either separately or in combination together with their MeSH terms as shown below.

("hiv infections"[MeSH Terms] OR ("hiv"[All Fields] AND "infections"[All Fields]) OR "hiv infections"[All Fields] OR ("hiv"[All Fields] AND "infection"[All Fields]) OR "hiv infection"[All Fields]) AND HBV[All Fields] AND ("infections"[MeSH Terms] OR "infections"[All Fields] OR "infection"[All Fields]) AND ("coinfection"[MeSH Terms] OR "coinfection"[All Fields] OR ("co"[All Fields] AND "infection"[All Fields]) OR "co infection"[All Fields]) AND ("africa south of the Sahara"[MeSH Terms] OR ("africa"[All Fields] AND "south"[All Fields] AND "Sahara"[All Fields]) OR "africa south of the Sahara"[All Fields] OR ("sub"[All Fields] AND "Saharan"[All Fields] AND "africa"[All Fields]) OR "Saharan africa"[All Fields]) AND ("africa, eastern"[MeSH Terms] OR ("africa"[All Fields] AND "eastern"[All Fields]) OR "eastern africa"[All Fields] OR ("east"[All Fields] AND "africa"[All Fields]) OR "east africa"[All Fields]) AND ("hepatitis b"[MeSH Terms] OR "hepatitis b"[All Fields]) AND ("epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "prevalence"[All Fields] OR "prevalence"[MeSH Terms]) AND ("hepatitis b surface antigens"[MeSH Terms] OR "hepatitis b surface antigens"[All Fields] OR "hbsag"[All Fields]) AND Predictors[All Fields]

In addition to the key words our search method was based on the population, exposure, comparison, and outcome (PECO) elements. In terms of the population, data extraction was done by searching all the literature on the status of HIV/HBV coinfection that included study

participants in SSA. The total number of people exposed and unexposed to HBV in the study population pool used for the individual studies revealed the prevalence of HBV. In terms of comparison, information on infection risk factors was retrieved from the research article's main body and compared between those who had been exposed to and those who had not. And Our Outcome was HBV infection.

3.2.Inclusion and exclusion criteria

The search was limited to English-language publications only. For this review, there were limited publications in East Africa, thus we extended our search to SSA. There were no limitations on the years of publication because of the number of publications that could be retrieved. The population of interest was Individuals living with both HIV and HBV (≥15 years old) with HIV-HBV coinfection, and all study designs were included. Only studies with access to the full-text studies were retained for review from we identified and excluded duplicated studies. This review did not include research that looked into viral hepatitis types A, C, D, or E. Below Table 1 provides a summary of all studies included in this review.

4. Quality and comparability of studies

A total of 1176 articles were retrieved from the electronic database search of PubMed (117), BMC (183) and Embase (876). Before the screening 614 articles were deemed irrelevant for the review because they were not in the context of our study and didn't assess risk factors and prevalence. After screening for full text review, title and abstract, 603 of them were excluded for various reasons such as population group(children), outcome of interest(Hepatitis c and syphilis), not conducted in SSA (Figure 1), leaving a total of 19 articles to assess for eligibility criteria and 4 of them deemed irrelevant because they were either duplicates or doesn't focus on HIV/HBV coinfection leaving 15 articles for our review from the initial search conducted (21–35). 4 additional articles were obtained from review of reference list of included publications (13,36–38). This makes the total number of reviewed studies 18. The quality and comparability of all 18 studies were assessed based on study design, sample size and outcome assessment as summarized in Table 1.

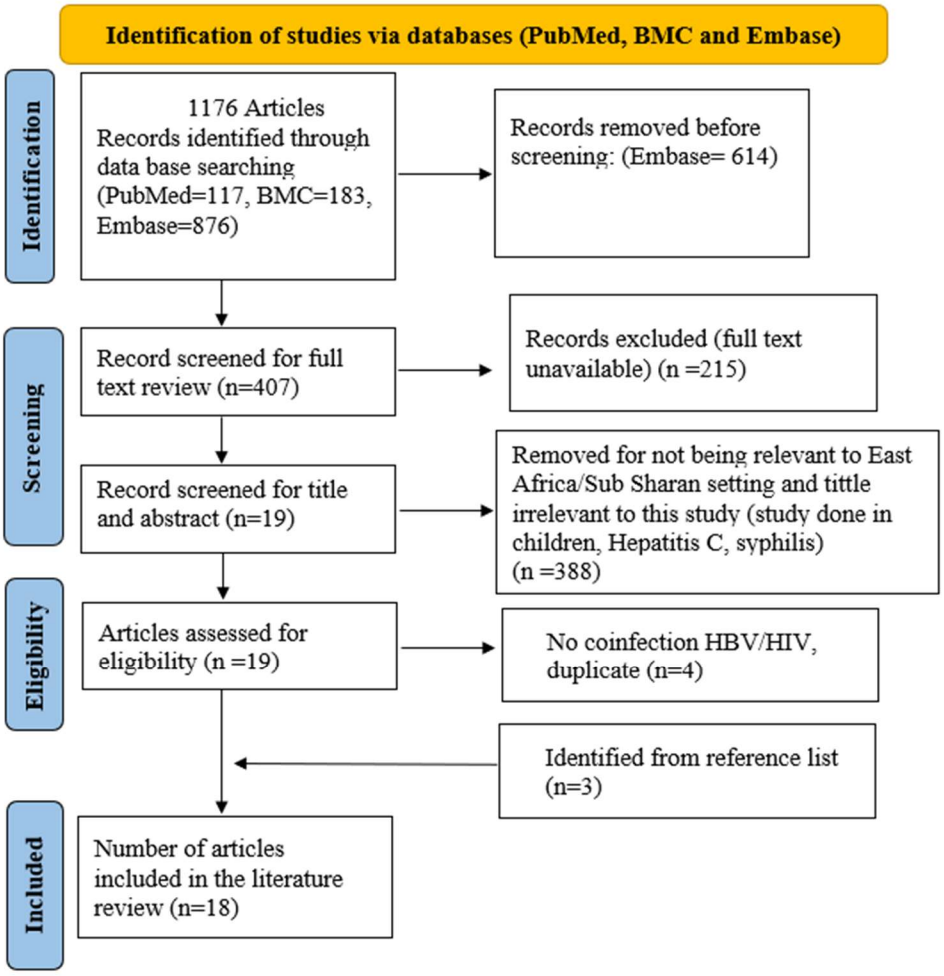

Figure 1: Flow diagram for the literature review, study eligibility following PRISMA criterion.

4.1.Study design

Of the 18 reviewed studies, 17 were observational, with 15 being cross-sectional studies (21,23,24,27–29,31,33–35,37–39) and two being retrospective cohort studies (30)(22). One being a systematic review and meta-analysis (25).

The 15 cross-sectional studies primarily focused on estimating the seroprevalence of HBV and identifying associated risk factors in different populations, including HIV-positive individuals attending ART clinics (30)(38)(29), pregnant women (33) (36) (28), men who have sex with men (MSM) (34), people who inject drugs (PWID)(24), and the general population in high-endemic

regions (26,30–32). These studies employed structured questionnaires, medical records, and laboratory tests such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) to detect hepatitis B surface antigen (HBsAg) and quantify viral load.

The 2 retrospective observational studies Matthews et al. 22; Wandeler et al. 30 utilized hospital and ART clinic records to analyze past HBV screening results, disease progression, and liver function markers among HIV-positive patient sided valuable insights into long-term outcomes, co-infection patterns, and treatment responses, particularly in resource-limited settings.

The studies were conducted in sub-Saharan Africa (Ethiopia (2,36,38,40), Nigeria (26,28), Uganda (31), Ghana (29), Mozambique (30), South Africa (35), Rwanda (41), and Cameroon (23,27)), with most being hospital-based (institution based cross-sectional studies), A few studies were community-based, involving random sampling from high-risk populations. Standardized diagnostic criteria and data collection protocols were followed, ensuring comparability despite differences in sample sizes, geographical coverage, and research objectives.

4.2.Sample size

Some observational studies had a sample size of below 500, with 7 studies reporting sample sizes ranging from 310 to 500 participants (21,24,26,29,36,37,39). However, the largest observational study, conducted in Rwanda (29), had 13,121 participants, significantly exceeding the sample sizes of other studies. Among the cross-sectional, community-based surveillance study, sample sizes varied from 310 to 761 participants, with the largest study conducted in Cameroon (27). The systematic review and meta-analysis (42) synthesized data from multiple sources, covering a sample size of 44,114 participants from different sub-Saharan African regions (43). The retrospective observational study, conducted in South Africa and Botswana, had a sample size of 1022 participants assessing the influence of HBV co-infection on CD4+ T-cell counts and HIV-specific CD8+ T-cell response. In multicenter studies, sample sizes were generally larger, ranging from 1,002 to 13,212 participants (28,30,33).

4.3.Outcome assessment

The studies reviewed assessed a range of clinical and epidemiological outcomes related to HBV prevalence, risk factors, co-infections, and disease progression. Fourteen studies reported HBV prevalence and risk factors as an outcome, using serological markers such as HBsAg detection via enzyme-linked immunosorbent assay (ELISA) or rapid diagnostic tests (34–36,39). Among these, two studies also assessed liver enzyme levels and CD4 counts (27,37). One study focused on the virological determinants of HBV (44). Studies conducted in ART clinics focused on HBV co-infection rates among HIV-positive individuals, with some assessing HBV sero-prevalence post-ART initiation (29,39). Three studies specifically analyzed sociodemographic and behavioral risk factors such as unsafe sex, intravenous drug use, history of blood transfusions, and previous surgical procedures (27,34,45). Multivariate logistic regression was used to determine independent predictors of HBV infection across different populations.

Eleven studies reported using structured questionnaires to collect sociodemographic and behavioral data (21,23,28,31,34–39) of which two of them used face to face interviews (24,36), one of them used cross-sectional survey (33) and thirteen studies used trained clinicians for direct patient assessment and blood sample collection (21–23,28,30,31,33–39). Two studies reported outcomes and risk factors based on retrospective medical record reviews for data abstraction rather than direct patient follow-up (22,28). One study used a systematic review and meta-analysis PRISMA standards for data collection (25).

4.4.Summary of study quality appraisal

The aim of this study was to assess the prevalence of Hepatitis B Virus (HBV) co-infection among people living with HIV (PLWH) in SSA and identify associated sociodemographic and clinical factors. Therefore, the quality of studies synthesized in this review was determined based on clear definitions of HBV co-infection, inclusion of HIV-positive individuals, use of validated diagnostic methods (HBsAg, HBV DNA testing), and adequate sample sizes to detect differences in prevalence and risk factors. Additionally, studies that accounted for potential confounders such as ART status, CD4 count, and history of liver disease were considered of higher quality. While most studies met all the criteria comprehensively, the systematic review and meta-analysis by (43) the most rigorous in methodology and data reporting and hierarchy of evidence.

5. Results from the studies reviewed

The results of the review of the included studies focus on the definitions of HBV co-infection and the association between sociodemographic and clinical factors with HBV prevalence among PLWH. The prevalence of HBV/HIV co-infection varied across SSA, with estimates ranging from 1.16% to 16.1%, depending on the population studied and diagnostic methods used. Risk factors consistently associated with co-infection included male sex, lower CD4 count, high HIV viral load, history of sexually transmitted infections (STIs), and lack of HBV vaccination. Several studies also highlighted regional differences, with West Africa showing the highest burden of co-infection (23,43). The overview of studies included in this study, as well as key findings, are summarized in Tables 1 and 2.

5.1.Exposure Assessment

Across the 18 studies reviewed, HIV status and HBV status were commonly determined using rapid diagnostic tests and laboratory confirmation. The primary method for HIV testing in most studies involved rapid antibody tests, with confirmatory ELISA or PCR-based assays where available (39,43). For HBV status, the majority of studies utilized Hepatitis B surface antigen (HBsAg) testing as the primary screening tool, with the Determine HBsAg rapid test being the most frequently used method for initial detection (26,35). Some studies included additional confirmatory tests such as HBV DNA quantification, anti-HBc IgM/IgG antibodies, and HBeAg testing to differentiate between chronic and acute HBV infections (22,30,34,38). In certain studies, CD4 count, and HIV viral load were also assessed alongside HBV markers to evaluate disease progression among co-infected individuals (28,37). The studies varied in their approach to determining HBV/HIV co-infection prevalence, with some using hospital-based samples from antiretroviral therapy (ART) clinics, while others analyzed community-based populations, leading to variations in prevalence estimates (30,34,43).

5.2.Risk factors for HBV among PLWH

The studies reviewed provided extensive insights into the existing demographic, behavioral, and clinical risk factors associated with HBV/HIV co-infection, with some findings demonstrating consistency across multiple studies, while others varied due to differences in study populations, geographical regions, and methodological approaches. One of the most commonly reported demographic risk factors was male sex, with studies from South Africa, Nigeria, and Ethiopia

consistently showing that men had a higher prevalence of HBV/HIV co-infection compared to women (26,35,37). This trend may be attributed to higher rates of high-risk sexual behaviors, lower healthcare-seeking behavior, and reduced vaccination coverage among men (46). Similarly, older age (>30 years) was significantly associated with HBV/HIV co-infection in studies from West and Central Africa, possibly reflecting longer exposure to infection risks and lower likelihood of childhood HBV vaccination (24,43). However, studies from Mozambique and Cameroon suggested that younger age groups (20–29 years) were also at significant risk, particularly among populations with high rates of early sexual debut and this can also be explained by limited access to HBV vaccination programs (23,24,47).

Behavioral risk factors were also widely examined across studies, with findings indicating multiple sexual partners, history of sexually transmitted infections (STIs), and injection drug use (IDU) as major contributors to HBV/HIV co-infection (24,27,33). Several studies highlighted a strong correlation between high-risk sexual behavior and co-infection, particularly among populations in West and Southern Africa, where low condom use and high rates of transactional sex were reported (34,43). The role of injection drug use (IDU) was more pronounced in studies conducted in Mozambique, where needle-sharing practices significantly increased the risk of both HBV and HIV transmission (24). In contrast, studies from Ethiopia and Nigeria reported lower IDU prevalence but found that body modifications such as tattooing, scarification, and traditional bloodletting practices were key contributors to HBV transmission, especially in rural populations (26,39). These differences in risk factors suggest that cultural and behavioral patterns play a significant role in shaping HBV/HIV epidemiology across regions (48).

Clinical risk factors also varied across studies, with low CD4+ T-cell count and high HIV viral load emerging as consistent predictors of HBV/HIV co-infection severity (37). Several studies reported that co-infected individuals had significantly lower CD4 counts (<350 cells/mm^3) compared to HIV mono-infected individuals, suggesting that HBV accelerates immune suppression in PLWH (37) (38). Additionally, ART regimen type was found to influence HBV progression, with studies from Cameroon and South Africa indicating that individuals on non-tenofovir-based ART regimens (e.g., lamivudine-based therapy) were more likely to develop HBV drug resistance mutations, leading to worsened disease outcomes(26,38) However, studies from Ethiopia and Cameroon reported better HBV suppression in individuals on tenofovir-based ART

regimens, reinforcing the importance of integrating HBV active antiretrovirals into HIV treatment programs (27,31).

The variability in findings across studies can be attributed to differences in sample populations, regional healthcare policies, and accessibility to HBV vaccination programs. Countries with longstanding HBV vaccination policies, such as South Africa (49), reported lower HBV/HIV co-infection rates in younger age groups, whereas regions with weaker vaccination programs, such as West and Central Africa, showed persistently high HBV co-infection rates across all age groups (43). Additionally, variations in sociocultural practices, such as traditional medical procedures, bloodletting rituals, and high-risk sexual behaviors, contribute to different transmission dynamics across SSA (31,50,51). These differences highlight the need for region-specific intervention strategies, including enhanced HBV vaccination coverage, targeted harm reduction programs, and optimized ART regimens to mitigate HBV/HIV co-infection in SSA.

5.3.Methodological differences in literature

The studies reviewed present different conclusions regarding the prevalence and risk factors of HBV/HIV co-infection in SSA. These differences could be attributed to variations in study populations, diagnostic methods, data collection approaches, and study designs. Some studies relied on hospital-based samples, while others used community-based sampling, leading to differences in reported prevalence. Additionally, the choice of HBV diagnostic tests (e.g., HBsAg rapid tests vs. HBV DNA testing) and variations in the definition of HBV infection status contributed to inconsistencies in findings. Differences in sociodemographic characteristics, ART regimens, and healthcare access across study sites further influenced the variability in results.

5.3.1. Study population

The studies reviewed were conducted across multiple countries in SSA, each with distinct sociodemographic profiles, healthcare infrastructure, and HBV/HIV burden. These regional differences influenced the prevalence and risk factors for HBV/HIV co-infection across studies. For instance, some studies were conducted in urban hospital settings, where healthcare access, ART coverage, and HBV vaccination rates were generally higher, potentially leading to lower

HBV co-infection rates (24,30,36,43). In contrast, studies conducted in rural communities often reported higher HBV prevalence, likely due to lower vaccination rates, limited healthcare access, and increased exposure to traditional medical practices such as scarification and bloodletting (30,31,35,39).

Beyond geographical factors, studying population characteristics also varied. Some studies focused on pregnant women living with HIV and attending antenatal clinics (ANCs), while others included general PLWH or high-risk groups such as people who inject drugs (PWID) and MSM (22,28,33,34,43). The inclusion of key populations, such as sex workers and PWID, contributed to higher HBV co-infection rates in some studies compared to those focusing on general HIV clinic attendees. Additionally, studies conducted in regions with longstanding HBV vaccination programs, such as South Africa, reported lower HBV prevalence, whereas studies in West and Central Africa, where vaccination coverage is lower, showed significantly higher HBV/HIV co-infection rates (43). These methodological and demographic differences in study populations influenced the findings, highlighting the need for standardized screening and prevention strategies across different regions.

5.3.2. Study design and exposure group.

More than half of the studies were cross-sectional, a design which is effective for estimating HBV/HIV co-infection prevalence at a single point in time but do not provide insights into disease progression or causal relationships (52). These studies relied on one-time HBV screening, making it difficult to differentiate between acute, chronic, and resolved infections, especially when HBV DNA and anti-HBc markers were not tested (30). The discrepancies in the findings could be explained by the study group's selection and the sensitivity of the diagnostic techniques employed (21).

Differences in exposure group definitions also contributed to variations in findings. While most studies defined HBV exposure using HBsAg positivity, others included HBV DNA quantification, an alanine aminotransferase (ALT) levels, or liver fibrosis assessments, leading to discrepancies in defining chronic HBV infection (30). Additionally, most of the risk factors and outcomes of earlier hepatitis B testing were self-reported, they may be subjective and prone to recall bias (29).

Furthermore, some studies compared ART experienced vs. ART naïve individuals, showing that tenofovir-based ART regimens reduced HBV viral loads, whereas non-tenofovir based regimens were linked to higher HBV persistence and drug resistance mutations (31). Other limitation on some of the cross-sectional studies was the absence of randomness in the selection of the survey sites (33).

We also had retrospective observational studies and prospective observational(cross-sectional), which is used to assess HBV/HIV co-infection trends and associated risk factors. These designs rely on pre-existing clinical records or follow-up data, making them prone to missing data issues and inconsistencies in exposure classification and Responses to questionnaires might be subject to recall bias (35)(30). For example, some of these studies had to exclude participants due to incomplete medical histories or refusal of participation by participants, leading to a reduced sample size and potential bias in prevalence estimates (37) (38) (21) (28).This has also resulted in the results being limited by missing data (30).

6. Summary

The studies reviewed highlight significant variability in HBV/HIV co-infection prevalence, risk factors, and study methodologies across different regions in SSA. Many of the studies focused on HBV prevalence in HIV-infected populations, but variations in study design, exposure classification, and diagnostic methods impacted the comparability of findings. The majority of studies utilized cross-sectional study designs, which, while effective for estimating prevalence, limited the ability to assess disease progression and long-term clinical outcomes and risk factors.

Differences in risk factor assessment also contributed to variations in findings. Some studies identified low CD4 count, high HIV viral load, and ART regimen type as key risk factors for HBV persistence in PLWH, while others emphasized sociocultural factors such as traditional medical practices, low vaccination rates, and healthcare access disparities (22,23,27,30,31,38). Furthermore, one systematic review and meta-analysis provided a broader synthesis of HBV-HIV burden across SSA, reinforcing that HBV prevalence remains high in HIV-infected populations, despite expanded vaccination programs and ART rollout (43).

Overall, the methodological inconsistencies across studies underscore the need for standardized HBV screening protocols, and with a consistent exposure definition to better understand the

trajectory of HBV/HIV co-infection in SSA. While current research provides valuable epidemiological insights, further investigations are needed to evaluate the effectiveness of HBV prevention strategies, treatment approaches, and policy interventions in PLWH population group. However, findings from East Africa remain limited, with inconsistent prevalence estimates and insufficient data on region-specific risk factors such as sociocultural practices, ART regimen effects, and healthcare infrastructure. Given these gaps, there is a critical need for focused studies in East Africa to accurately assess HBV burden, identify risk factors, and evaluate the existing HBV/HIV coinfection burden.

7. Clinical implication

In addition to the overlapping HIV and HBV epidemics, the use of antiretroviral medication has increased globally, which has led to the evolution of novel HBV mutant strains(53). Additionally, HBV genetic diversity may be influenced by the degree of immunosuppression experienced by HIV/HBV co-infected individuals, resulting in less effective antibody reactions to HBV immunization (53–55). The death and disability rates from HIV/HBV coinfection are higher than those from the mono-infections alone. HIV coinfected individuals have greater hepatitis B viremia levels, proceed to chronic hepatitis B at a rate that is roughly five times faster than that of HBV mono-infected individuals, and are more likely to develop cirrhosis and hepatocellular carcinoma (53,56).

8. Recommendation

Effective prevention and treatment of hepatitis B virus infection is a public health priority, and effective ART has been linked to a lower incidence of hepatitis B infection (57). Although our study was limited to PLWH, there is vast evidence that indicated co-infection rates with HBV are higher rates among individuals with a compromised immune system than those who are HIV-negative population groups. As a result, ongoing monitoring of these illnesses is necessary, which presents a problem for the development of vaccines and therapeutic alternatives (58). Clinicians should be aware of the possibility of hepatitis B in PLWH, as well as the importance of early detection and treatment to ensure optimal case management and follow-up (57)(58).

The following markers are recommended for hepatitis B screening tests by the American Association for the Study of Liver Diseases (AASLD): hepatitis B surface antigen (HBsAg) to identify active infection, hepatitis B core antibody (anti-HBc) to identify previous or current exposure, and hepatitis B surface antibody (anti-HBs) to evaluate immunity status (55,59,60). PLWH who test positive for HBsAg should also be tested for hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe), as well as for an HBV DNA level to assess the extent of HBV replication (55,59,60).

Routine HBV testing should be incorporated into HIV care programs, prenatal care, and blood donor screens to ensure early detection and treatment (35). Research has indicated that a large number of HBV-positive individuals in Africa are not aware of their virus status, which can result in treatment delays and problems such hepatocellular carcinoma and liver cirrhosis (35,61,62). Early detection rates may be increased by task-shifting tactics in which additional community health workers receive training to perform HBV screenings. In rural and underdeveloped areas, point-of-care diagnostics should also be expanded to reduce dependency on centralized labs, which frequently cause delays in diagnosis (61–63).

Many countries in Africa still have poor HBV vaccination rates despite the availability of an effective vaccine because of a lack of funding, ineffective health systems, and vaccine shortages (64,65). In order to prevent mother-to-child transmission (MTCT), governments should bolster national vaccination programs and make sure that HBV birth-dose immunizations are accessible and given within 24 hours of delivery (66). In order to lower transmission, catch-up immunization efforts should also give priority to teenagers, healthcare professionals, and persons who inject drugs (PWID) (23,43,66).

In many African nations, access to tenofovir-based antiviral regimens for the treatment of Hepatitis B Virus (HBV) continues to be scarce and very costly (24,26,67). These challenges might put an additional burden on SSA, especially after recent development in the politics, such as the suspension of foreign aid, including USAID financing, by the U.S. administration. Important health services, including HIV and HBV treatment programs throughout sub-Saharan Africa,

will be severely disrupted because of this suspension. Foreign aid withdrawals pose serious problems, but they also highlight the significance it is for African countries to strengthen their healthcare systems. The continent can mitigate the adverse consequences of shifts in external funding and ensure their citizens' affordable access to necessary antiviral treatments by bolstering their own healthcare policies, making investments in regional pharmaceutical production, promoting regional cooperation (68–71).

Table 1: Overview of studies included in this review.

Study	Year published	Country	Time period	Setting	Study population	Sample size	Study design	Data collection
Zenebe et al. 2015	2014	Ethiopia	March 2013 to April 2013	Bahir Dar administrative city	pregnant women	318	cross-sectional	Study measures
Wondimeneh et al. 2013	2013	Ethiopia	March-May, 2011	University of Gondar Teaching Hospital	HIV positive adult individuals who visited the ART clinics	400	cross-sectional	Study measures
Weldemhret et al. 2016	2016	Ethiopia	August to October 2014	Mekelle hospital, Tigray	HIV positive individuals attending ART clinic	508	cross-sectional	Study measures
Balew et al. 2014	2014	Ethiopia	February to April, 2012	Debre Tabor hospital	HIV/AIDS patients	395	cross-sectional	Study measure
Matthews et al. 2015	2015	South Africa and Botswana	between 2004 and 2013	antenatal and pediatric clinics	72 HIV negative (comparison group) and 950 HIV-positive. All attending antenatal clinics in South Africa and Botswana.	1,022	Retrospective observational	Data abstraction
Ezechi et al. 2014	2014	Nigeria	between January 2006 and December 2011	PMTCT clinic in Lagos	HIV Positive pregnant women	2391	cross sectional	Study measure
Kye-Duodu et al. 2016	2016	Ghana	March to June 2012	antiretroviral clinics in the Eastern Region	PLHIV	320	cross sectional	Study measure

Wandeler et al. 2016	2016	Mozambique and Zambia	May 2013 and August 2014	two urban clinics in Zambia and four rural clinics in Northern Mozambique	HIV-infected adult patients initiating antiretroviral therapy (ART)	1829	Retrospective observational study and cross-sectional	Study measure, data abstraction
Chiesa et al. 2020	2020	Uganda	April to June 2016	outpatients' clinic of St. Mary's Hospital, Gulu,	PLWH attending the outpatients' clinic	1000	cross-sectional	Study measure
Mutagoma et al. 2017	2017	Rwanda	May to November 2011	30 sentinel sites national representative in Rwanda in 2011	pregnant women attending antenatal clinic and prevention of mother-to-child transmission (ANC-PMTCT) services	13,121	cross-sectional survey	Study measure
Adeyemi et al. 2021	2021	Nigeria	between March 2013 and February 2020 in Abuja, and between April 2014 and May 2018 in Lagos	Abuja and Lagos	men who have sex with men (MSM) and transgender women (TGW)	717	Cross-sectional	Study measure
Wasihun, Asnake, and Kebede 2024	2024	Ethiopia	from April to May 2022	ART clinic, South Wollo zone, northeast Ethiopia	HIV-positive patients attending ART clinics	350	institutional-based cross-sectional study	Study measure
Shivakumar et al. 2024	2024	South Africa	from 2013–2017	Umlazi township	Patients newly diagnosed with HIV(PLWH)	3105	cross-sectional	Study measure

Shevell et al. 2015	2015	Cameroon	June to July 2011	Five hospitals, Fako division	Participants over the age of 18 attended a free screening in one of five hospitals	761	Cross-sectional	Study measure
Saing et al. 2020	2020	Mozambique	2013–2014	Urban cities (Maputo and Nampula/Nacala)	people who inject drugs	492	cross-sectional	Study measure
Kafeero et al. 2021c	2020	Sub-Saharan Africa	2004 to 2019	Sub-Saharan Africa	pregnant women attending antenatal care (ANC)	38 articles with sample size of 44,114	a systematic review and meta-analysis	Data abstraction
Opaleye et al. 2021	2021	Nigeria	June 2017 and August 2017	HIV clinic in three south-western states	HIV-positive individuals	310	Cross-sectional	Study measure
Luma et al. 2016	2016	Cameroon	December 2014 to March 2015	HIV treatment centers	HIV-infected Individuals	833	Cross-sectional	Study measure

Table 2: Key findings from included studies.

Study	Key findings
Zenebe et al. 2015	• 6.6% of the 318 pregnant women tested positive for HIV and 3.8% tested positive for Hepatitis B surface antigen (HBsAg). • The co-infection rate of HBV/HIV was 19.0%. • risk factors for HBV infection included blood transfusion, body tattooing, surgery, and unsafe injection practices. • risk factors for HIV infection included piercing with sharp materials and the history of abortion.
Wondimeneh et al. 2013	• The overall prevalence of HIV-viral hepatitis co-infection was 11.7%. (HIV-HBV (5.6%), HIV-HCV (5.0%) • Higher prevalence of HIV/HBV co-infection in males (9.4%) compared to females (3.4%). • Study participants with co-infections had higher mean liver enzyme levels (ALT, AST, ALP) and lower mean CD4 levels compared to HIV mono-infected individuals.
Weldemhret et al. 2016	• The prevalence of coinfection was 5.9%. • Male gender, having multiple sexual partners, and CD4 count <200 cells/µl were significantly associated with HIV-HBsAg coinfection.
Balew et al. 2014	• The prevalence of HBsAg (hepatitis B surface antigen) was 6.1%. • Multiple sexual partners (AOR=8.1) and history of opportunistic infections (AOR=3.17) were significantly associated with HBsAg positivity.
Matthews et al. 2015	• HBV/HIV coinfected individuals in South Africa had significantly lower CD4+ T cell counts compared to those with only HIV, suggesting an impact of HBV on immune status. This finding was not replicated in the Botswana cohort. • Approximately 27% of HBsAg-positive individuals were also positive for HBeAg, indicating active HBV replication. • The overall prevalence of HBV among the study cohort was 7%, with higher prevalence observed in South Africa (9.7%) compared to Botswana (3.8%).
Ezechi et al. 2014	• HBV/HIV coinfection was 4.2% in this population, History of blood transfusion: increased risk (aOR: 2.3; 95% CI:1.1 - 4.6) • History of induced abortion: increased risk (aOR: 2.2;95% CI:1.3 - 3.6), Elevated baseline ALT levels: increased risk (aOR: 2.2; 95%CI:2.2 - 4.2)
Kye-Duodu et al. 2016	• 8.8% of PLHIV tested positive for HBV

Wandeler et al. 2016	• 7.6% of participants in Mozambique and 11.3% in Zambia tested positive for HBV. • Among HBsAg-positive patients, 49.4% had high HBV viral loads (>20,000 IU/mL). • Men and patients with low CD4 counts were more likely to have high HBV DNA levels.
Chiesa et al. 2020	• Overall HBV prevalence among PLWH was 7.9%. and Males exhibited a higher prevalence of HBV infection compared to females. • Coinfection with HBV was linked to lower CD4+ T cell counts among PLWH. • A poorer CD4+ T cell response to antiretroviral therapy (ART) was observed in coinfected individuals.
Mutagoma et al. 2017	• The proportion of HIV-infected pregnant women who were HBsAg-positive was 4.1%. • HBV-HIV co-infection was more common among women aged 15-24 years, those living in urban areas, those with more than two pregnancies, and those who tested positive for syphilis.
Adeyemi et al. 2021	• 6% were co-infected with HIV and HBV. • Condomless sex at last anal intercourse was independently associated with higher odds of HBV infection. • HBV mono infection prevalence was moderately high (10%) but did not differ significantly by HIV status
Wasihun, Asnake, and Kebede 2024	• The prevalence of Hepatitis B Surface Antigen (HBsAg) among the study population was 7.14%. • Significant factors influencing HBV co-infection included gender (females less likely), educational status (lower education associated with higher risk), marital status (single individuals more likely), higher viral load, and CD4 count.
Shivakumar et al. 2024	• 6% of the participants were co-infected with HBV. Males had a higher prevalence (10.4%) compared to females (5.2%). Risk factors: • Males: Alcohol use decreased HBV risk (aPR = 0.36) while smoking increased it. • Females: Smoking history significantly increased HBV risk (aPR = 2.58).
Shevell et al. 2015	• HIV/HBV co-infection was 1.16% in this study, no association was found between HIV and HBV infection. • Significant associations were found between HIV and high-risk sexual behaviors such as condom use, number of lifetime sexual partners, and age at first sexual intercourse. • HBV was not associated with high-risk sexual behaviors.
Saing et al. 2020	• Older age, history of needle/syringe sharing, and injecting with used needles were linked to HIV/HBV • Living in Maputo was specifically associated with co-infection.

Kafeero et al. 2021c	• The pooled prevalence of HBV/HIV co-infection among pregnant women was 3.302%. • West Africa had the highest prevalence at 5.2%, significantly higher than other regions. • Articles from 2004–2010 reported a higher prevalence of 6.4% compared to more recent studies from 2011–2019. • The prevalence of HBV/HIV co-infection among HIV-positive pregnant women ranged from 1.661% (Cameroon) to 27.3% (Nigeria). • Married women had a higher risk compared to unmarried women. • No significant association was found between HBV/HIV co-infection and education level
Opaleye et al. 2021	• 16.1% of HIV-positive individuals were HBsAg positive, with 72.0% of these being HBV DNA positive. • 32.2% of HBV DNA-positive samples had DRMs associated with lamivudine resistance, mainly in ART-experienced individuals. • HBV DRMs were detected in 32.3%
Luma et al. 2016	• HBV/HIV co-infection in the study was 6.1%. • Individuals with a history of surgical procedures had a 1.82 times higher risk of co-infection. • Individuals with a history of STIs had a 2.20 higher risk of co-infection. • Having multiple sexual partners Increased risk of HBV/HIV co-infection (OR 2.46, 95% CI: 1.34-4.50)

9. References

1. Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Gastroenterol Hepatol. 2022 Sep 1;7(9):796–829.

2. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2025 Jan 23];18(4):e0284646. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0284646

3. Chihota B V., Wandeler G, Chilengi R, Mulenga L, Chung RT, Bhattacharya D, et al. High Rates of Hepatitis B Virus (HBV) Functional Cure Among Human Immunodeficiency Virus-HBV Coinfected Patients on Antiretroviral Therapy in Zambia. J Infect Dis [Internet]. 2019 Dec 1 [cited 2025 Jan 28];221(2):218. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7184905/

4. Yirsaw BG, Agimas MC, Alemu GG, Tesfie TK, Derseh NM, Abuhay HW, et al. Prevalence of Hepatitis B virus infection and its determinants among pregnant women in East Africa: Systematic review and Meta-analysis. PLoS One [Internet]. 2024 Jul 1 [cited 2025 Feb 3];19(7):e0307102. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11244773/

5. WHO. Analytical Fact Sheet. In 2023 [cited 2025 Jan 23]. p. 2018–22. Available from: https://files.aho.afro.who.int/afahobckpcontainer/production/files/iAHO_Hepatitis_Regional_Factsheet_ENG.pdf

6. Nguyen MH, Wong G, Gane E, Kao JH, Dusheiko G. Hepatitis B Virus: Advances in Prevention, Diagnosis, and Therapy. Clin Microbiol Rev [Internet]. 2020 Apr 1 [cited 2025 Feb 2];33(2):e00046-19. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7048015/

7. Umutesi J, Klett-Tammen C, Nsanzimana S, Krause G, Ott JJ. Cross-sectional study of chronic hepatitis B virus infection in Rwandan high-risk groups: unexpected findings on

prevalence and its determinants. BMJ Open [Internet]. 2021 Dec 30 [cited 2025 Feb 3];11(12):e054039. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8719204/

8. Makokha GN, Zhang P, Hayes CN, Songok E, Chayama K. The burden of Hepatitis B virus infection in Kenya: A systematic review and meta-analysis. Front Public Heal [Internet]. 2023 Jan 26 [cited 2025 Feb 3];11:986020. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9909240/

9. Adugna A, Demeke G, Toru M, Tsehay D, Esmael A, Mihret A, et al. Reduced protective efficacy of hepatitis B vaccine among fully vaccinated children in Ethiopia. PLoS One [Internet]. 2023 Jul 1 [cited 2025 Feb 3];18(7):e0288355. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10328307/

10. Kaswa R, de Villiers M. Prevalence of hepatitis-B virus co-infection among people living with HIV in Mthatha region of South Africa. Afr Health Sci. 2023;23(1):149–56.

11. Frambo AAB, Atashili J, Fon PN, Ndumbe PM. Prevalence of HBsAg and knowledge about hepatitis B in pregnancy in the Buea Health District, Cameroon: a cross-sectional study. BMC Res Notes [Internet]. 2014 Jun 25 [cited 2025 Feb 5];7(1). Available from: https://pubmed.ncbi.nlm.nih.gov/24965844/

12. Kafeero HM, Ndagire D, Ocama P, Kudamba A, Walusansa A, Sendagire H. Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. Arch Public Heal [Internet]. 2021 Dec 1 [cited 2025 Feb 5];79(1):1–19. Available from: https://archpublichealth.biomedcentral.com/articles/10.1186/s13690-021-00686-1

13. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2025 Feb 2];18(4):e0284646. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0284646

14. Wandera BO, Onyango DM, Musyoki SK. Hepatitis B virus genetic multiplicity and the associated HBV lamivudine resistance mutations in HBV/HIV co-infection in Western Kenya: A review article. Infect Genet Evol. 2022 Mar 1;98:105197.

15. Ndegwa Maina D, Kimanga Nyerere A, Wambui Gicho R, Maina Mwangi J, Wekesa Lihana R. Prevalence and Factors Associated With Hepatitis B and C Co-Infection Among HIV-1-Infected Patients in Kenya. East African Heal Res J. 2017;1(2):73–9.

16. Maponga TG, Andersson MI, van Rensburg CJ, Arends JE, Taljaard J, Preiser W, et al. HBV and HIV viral load but not microbial translocation or immune activation are associated with liver fibrosis among patients in South Africa. BMC Infect Dis [Internet]. 2018 May 8 [cited 2024 Dec 17];18(1):1–11. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-018-3115-8

17. Makuza JD, Rwema JOT, Ntihabose CK, Dushimiyimana D, Umutesi J, Nisingizwe MP, et al. Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. BMC Infect Dis [Internet]. 2019 May 3 [cited 2024 Dec 17];19(1):381. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6499977/

18. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-hepatitis B virus coinfection: Epidemiology, pathogenesis, and treatment. AIDS [Internet]. 2017 Sep 24 [cited 2025 Jan 28];31(15):2035–52. Available from: https://journals.lww.com/aidsonline/fulltext/2017/09240/hiv_hepatitis_b_virus_coinfection__epidemiology,.1.aspx

19. Weldemhret L. Epidemiology and challenges of hbv/hiv co-infection amongst hiv-infected patients in endemic areas: Review. HIV/AIDS - Res Palliat Care [Internet]. 2021 [cited 2025 Jan 28];13:485–90. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=dhiv20

20. Lo Re V, Newcomb CW, Carbonari DM, Roy JA, Althoff KN, Kitahata MM, et al. Determinants of Liver Complications among HIV/Hepatitis B Virus-Coinfected Patients. J Acquir Immune Defic Syndr [Internet]. 2019 Sep 1 [cited 2025 Jan 28];82(1):71–80. Available from: https://journals.lww.com/jaids/fulltext/2019/09010/determinants_of_liver_complications_among.11.aspx

21. Balew M, Moges F, Yismaw G, Unakal C. Assessment of hepatitis B virus and hepatitis C virus infections and associated risk factors in HIV infected patients at Debretabor hospital,

South Gondar, Northwest Ethiopia. Asian Pacific J Trop Dis. 2014;4(1):1–7.

22. Matthews PC, Beloukas A, Malik A, Carlson JM, Jooste P, Ogwu A, et al. Prevalence and characteristics of hepatitis B virus (HBV) coinfection among HIV-Positive women in South Africa and Botswana. PLoS One. 2015;10(7):1–11.

23. Shevell L, Meriki HD, Cho-Ngwa F, Fuller C. Epidemiology of human immunodeficiency virus-1 and hepatitis B virus co-infection and risk factors for acquiring these infections in the Fako division of Southwest Cameroon Infectious Disease epidemiology. BMC Public Health [Internet]. 2015;15(1):1–9. Available from: http://dx.doi.org/10.1186/s12889-015-2386-x

24. Saing CH, Prem K, Uk P, Chhoun P, Chann N, Tuot S, et al. Risk factors associated with HIV and hepatitis C virus co-infection among people who inject drugs in Cambodia. Int J Drug Policy. 2020;86:1–11.

25. Kafeero HM, Ndagire D, Ocama P, Walusansa A, Sendagire H. Sero-prevalence of human immunodeficiency virus–hepatitis B virus (HIV–HBV) co-infection among pregnant women attending antenatal care (ANC) in sub-Saharan Africa (SSA) and the associated risk factors: a systematic review and meta-analysis. Virol J [Internet]. 2020;17(1):1–19. Available from: https://doi.org/10.1186/s12985-020-01443-6

26. Opaleye OO, Akanbi OA, Osundare FA, Wang B, Adesina O, Oluremi AS, et al. Prevalence and characteristics of hepatitis B and D virus infections among HIV-positive individuals in Southwestern Nigeria. Virol J [Internet]. 2021;18(1):1–10. Available from: https://doi.org/10.1186/s12985-021-01493-4

27. Luma HN, Eloumou SAFB, Ekaney DSM, Lekpa FK, Donfack-Sontsa O, Ngahane BHM, et al. Sero-prevalence and Correlates of Hepatitis B and C Co-infection Among HIV-infected Individuals in Two Regional Hospitals in Cameroon. Open AIDS J. 2016;10(1):199–208.

28. Ezechi OC hukwujekw., Kalejaiye OO lufel., Gab-Okafor CV ivia., Oladele DA yol., Oke BO ludar., Musa ZA desol., et al. Sero-prevalence and factors associated with Hepatitis B and C co-infection in pregnant Nigerian women living with HIV infection. Pan Afr Med J.

2014;17:197.

29. Kye-Duodu G, Nortey P, Malm K, Nyarko KM, Sackey SO, Ofori S, et al. Prevalence of hepatitis B virus co-infection among HIV-seropositive persons attending antiretroviral clinics in the Eastern Region of Ghana. Pan Afr Med J. 2016;25(Supp 1):1–6.

30. Wandeler G, Musukuma K, Zürcher S, Vinikoor MJ, Llenas-García J, Aly MM, et al. Hepatitis B infection, viral load and resistance in HIV-infected patients in Mozambique and Zambia. PLoS One. 2016;11(3):1–11.

31. Chiesa A, Ochola E, Oreni L, Vassalini P, Rizzardini G, Galli M. Hepatitis B and HIV coinfection in Northern Uganda: Is a decline in HBV prevalence on the horizon? PLoS One [Internet]. 2020;15(11 November):1–14. Available from: http://dx.doi.org/10.1371/journal.pone.0242278

32. Yang R, Yuan R, Gui X, Ke H, Zhuang K, Hu H, et al. Characteristics of Hepatitis B Virus, Hepatitis C Virus, and Syphilis Coinfection in People With HIV/AIDS Contracted Through Different Sources: Retrospective Study. JMIR Public Heal Surveill. 2024;10(1):1–12.

33. Mutagoma M, Balisanga H, Malamba SS, Sebuhoro D, Remera E, Riedel DJ, et al. Hepatitis B virus and HIV co-infection among pregnant women in Rwanda. BMC Infect Dis. 2017;17(1):1–7.

34. Adeyemi OA, Mitchell A, Shutt A, Crowell TA, Ndembi N, Kokogho A, et al. Hepatitis B virus infection among men who have sex with men and transgender women living with or at risk for HIV: a cross sectional study in Abuja and Lagos, Nigeria. BMC Infect Dis. 2021;21(1):4–11.

35. Shivakumar M, Moe CA, Bardon A, Krows M, Govere S, Moosa MYS, et al. Hepatitis B prevalence and risk factors among adults living with HIV in South Africa: a clinic-based cohort study. BMC Infect Dis [Internet]. 2024;24(1). Available from: https://doi.org/10.1186/s12879-024-09746-7

36. Zenebe Y, Mulu W, Yimer M, Abera B. Sero-prevalence and risk factors of hepatitis C virus infection among pregnant women in Bahir Dar city, Northwest Ethiopia: Cross

sectional study. Pan Afr Med J. 2015;21:1–7.

37. Wondimeneh Y, Alem M, Asfaw F, Belyhun Y. HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia. Virol J. 2013;10:1–8.

38. Weldemhret L, Asmelash T, Belodu R, Gebreegziabiher D. Sero-prevalence of HBV and associated risk factors among HIV positive individuals attending ART clinic at Mekelle hospital, Tigray, Northern Ethiopia. AIDS Res Ther. 2016;13(1):1–7.

39. Wasihun Y, Asnake D, Kebede N. Investigating factors associated to HBV/HIV co-infected patients in antiretroviral treatment clinic, in Northeast Ethiopia. BMC Infect Dis [Internet]. 2024 Dec 1 [cited 2024 Dec 17];24(1). Available from: https://pubmed.ncbi.nlm.nih.gov/38693473/

40. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2024 Dec 17];18(4):e0284646. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10132657/

41. Makuza JD, Rwema JOT, Ntihabose CK, Dushimiyimana D, Umutesi J, Nisingizwe MP, et al. Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. BMC Infect Dis [Internet]. 2019 May 3 [cited 2024 Apr 18];19(1). Available from: https://pubmed.ncbi.nlm.nih.gov/31053097/

42. Kafeero HM, Ndagire D, Ocama P, Kudamba A, Walusansa A, Sendagire H. Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. Arch Public Heal [Internet]. 2021 Dec 1 [cited 2024 Apr 18];79(1):1–19. Available from: https://archpublichealth.biomedcentral.com/articles/10.1186/s13690-021-00686-1

43. Kafeero HM, Ndagire D, Ocama P, Kudamba A, Walusansa A, Sendagire H. Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. Arch Public Heal [Internet]. 2021 Dec 1 [cited 2024 Dec 17];79(1):167. Available

from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8449462/

44. Riches N, Vinikoor M, Guingane A, Johannessen A, Lemoine M, Matthews P, et al. Hepatitis B in Africa Collaborative Network: cohort profile and analysis of baseline data. Epidemiol Infect [Internet]. 2023 Apr 3 [cited 2024 Apr 2];151. Available from: https://pubmed.ncbi.nlm.nih.gov/37009881/

45. Jentsch U, Vermeulen M, van den Berg K, Swanevelder R, Creel D, Jacobs G, et al. A case-control study of risk factors for incident hepatitis B virus infection in South African blood donors. Int J Infect Dis [Internet]. 2024 Apr 1 [cited 2024 Apr 18];141:106958. Available from: http://www.ijidonline.com/article/S1201971224000262/fulltext

46. Awoke N, Mulgeta H, Lolaso T, Tekalign T, Samuel S, Obsa MS, et al. Full-dose hepatitis B virus vaccination coverage and associated factors among health care workers in Ethiopia: A systematic review and meta-analysis. PLoS One [Internet]. 2020 Oct 1 [cited 2025 Feb 4];15(10):e0241226. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7591041/

47. Dos Muchangos V, Chambal L, Nilsson C, Sevene E. HIV and Hepatitis B virus co-infection in Mozambique: policy review and health professionals' knowledge and practices. [cited 2025 Feb 4]; Available from: https://doi.org/10.1101/2024.03.23.24304747

48. Tanga AT, Teshome MA, Hiko D, Fikru C, Jilo GK. Sero-prevalence of hepatitis B virus and associated factors among pregnant women in Gambella hospital, South Western Ethiopia: Facility based cross-sectional study. BMC Infect Dis [Internet]. 2019 Jul 10 [cited 2025 Feb 4];19(1):1–7. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-019-4220-z

49. van Zyl GU, Maponga T, Rabie H, Taljaard J. The role of new hepatitis B vaccines in South Africa. South African Med J. 2024;114(2):60–3.

50. Ansari JA, Salman M, Safdar RM, Ikram N, Mahmood T, Zaheer HA, et al. HIV/AIDS outbreak investigation in Jalalpur Jattan (JPJ), Gujrat, Pakistan. J Epidemiol Glob Health [Internet]. 2013 [cited 2025 Feb 4];3(4):261–8. Available from:

https://pubmed.ncbi.nlm.nih.gov/24206797/

51. Makuza JD, Nisingizwe MP, Rwema JOT, Dushimiyimana D, Habimana DS, Umuraza S, et al. Role of unsafe medical practices and sexual behaviours in the hepatitis B and C syndemic and HIV co-infection in Rwanda: a cross-sectional study. BMJ Open [Internet]. 2020 Jul 12 [cited 2025 Feb 4];10(7). Available from: https://pubmed.ncbi.nlm.nih.gov/32660951/

52. Setia MS. Methodology Series Module 3: Cross-sectional Studies. Indian J Dermatol [Internet]. 2016 May 1 [cited 2025 Feb 4];61(3):261. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4885177/

53. Singh KP, Crane M, Audsley J, Avihingsanon A, Sasadeusz J, Lewin SR. HIV-Hepatitis B virus co-infection: epidemiology, pathogenesis and treatment. AIDS [Internet]. 2017 Sep 24 [cited 2025 Feb 5];31(15):2035. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5661989/

54. Chan HLY, Fung S, Seto WK, Chuang WL, Chen CY, Kim HJ, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of HBeAg-positive chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol [Internet]. 2016 Nov 1 [cited 2025 Feb 5];1(3):185–95. Available from: http://www.thelancet.com/article/S2468125316300243/fulltext

55. Corcorran MA, Kim HN. Chronic Hepatitis B and HIV Coinfection. Top Antivir Med [Internet]. 2023 Feb 1 [cited 2025 Feb 5];31(1):14. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10089291/

56. Kourtis AP, Bulterys M, Hu DJ, Jamieson DJ. HIV–HBV Coinfection — A Global Challenge. N Engl J Med [Internet]. 2012 May 10 [cited 2025 Feb 5];366(19):1749–52. Available from: https://www.nejm.org/doi/full/10.1056/NEJMp1201796

57. Rachel M, Barbara C, Murphy C, Komujuni C, Nyakato P, Ocama P, et al. Uptake of hepatitis B-HIV co-infection screening and management in a resource limited setting. Hepatol Med Policy 2018 31 [Internet]. 2018 Jan 6 [cited 2025 Feb 5];3(1):1–7. Available from: https://hmap.biomedcentral.com/articles/10.1186/s41124-017-0030-3

58. Muriuki BM, Gicheru MM, Wachira D, Nyamache AK, Khamadi SA. Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals in Nairobi, Kenya. BMC Res Notes [Internet]. 2013 Sep 9 [cited 2025 Feb 5];6(1):1–6. Available from: https://link.springer.com/articles/10.1186/1756-0500-6-363

59. Conners EE, Panagiotakopoulos L, Hofmeister MG, Spradling PR, Hagan LM, Harris AM, et al. Screening and Testing for Hepatitis B Virus Infection: CDC Recommendations — United States, 2023. MMWR Recomm Reports [Internet]. 2023 Mar 10 [cited 2025 Feb 5];72(1):1–25. Available from: https://www.cdc.gov/mmwr/volumes/72/rr/rr7201a1.htm

60. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on Prevention, Diagnosis, and Treatment and of Chronic Hepatitis B: AASLD 2018 Hepatitis B Guidance. Hepatology [Internet]. 2018 Apr 1 [cited 2025 Feb 5];67(4):1560. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5975958/

61. Lingala S, Ghany MG. Hepatitis B: Screening, Awareness, and the Need to Treat. Fed Pract [Internet]. 2016 Apr [cited 2025 Feb 5];33(Suppl 3):19S. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6375415/

62. Minier N, Guingané AN, Okeke E, Sinkala E, Johannessen A, Andersson MI, et al. Development of a simple treatment eligibility algorithm to decentralise hepatitis B care in Africa. lancet Gastroenterol Hepatol [Internet]. 2024 Apr 1 [cited 2025 Feb 5];9(4):323. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7616035/

63. Ajuwon BI, Richardson A, Roper K, Sheel M, Audu R, Salako BL, et al. The development of a machine learning algorithm for early detection of viral hepatitis B infection in Nigerian patients. Sci Reports 2023 131 [Internet]. 2023 Feb 24 [cited 2025 Feb 5];13(1):1–11. Available from: https://www.nature.com/articles/s41598-023-30440-2

64. Shah SM, Rodin H, Pogemiller H, Magbagbeola O, Ssebambulidde K, Zewde A, et al. Hepatitis B Awareness and Vaccination Patterns among Healthcare Workers in Africa. Am J Trop Med Hyg [Internet]. 2020 Dec 1 [cited 2025 Feb 5];103(6):2460. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7695068/

65. Solomon-Rakiep T, Olivier J, Amponsah-Dacosta E. Weak Adoption and Performance of Hepatitis B Birth-Dose Vaccination Programs in Africa: Time to Consider Systems Complexity?—A Scoping Review. Trop Med Infect Dis [Internet]. 2023 Oct 1 [cited 2025 Feb 5];8(10):474. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10611266/

66. Borch A, Kolster C, Gluud C, Gluud LL. Vaccines for preventing hepatitis B in healthcare workers (an updated protocol). Cochrane Database Syst Rev [Internet]. 2017 Jun 22 [cited 2025 Feb 5];2017(6). Available from: https://immunisationhandbook.health.gov.au/contents/vaccine-preventable-diseases/hepatitis-b

67. Bendavid E, Grant P, Talbot A, Owens DK, Zolopa A. Cost-effectiveness of antiretroviral regimens in the World Health Organization's treatment guidelines: A South African analysis. AIDS [Internet]. 2011 Jan 14 [cited 2025 Feb 5];25(2):211–20. Available from: https://journals.lww.com/aidsonline/fulltext/2011/01140/cost_effectiveness_of_antiretroviral_regimens_in.12.aspx

68. Zimbabwe fears US withdrawal from WHO will hit HIV/AIDS programmes | Reuters [Internet]. [cited 2025 Feb 5]. Available from: https://www.reuters.com/business/healthcare-pharmaceuticals/zimbabwe-fears-us-withdrawal-who-will-hit-hivaids-programmes-2025-01-22/

69. PEPFAR freeze risks HIV treatment for millions in South Africa and around the world - Health-e News [Internet]. [cited 2025 Feb 5]. Available from: https://health-e.org.za/2025/01/28/pepfar-freeze-risks-hiv-treatment-for-millions-in-south-africa-and-around-the-world/

70. Trump's foreign aid freeze leaves panic in Africa over PEPFAR and more | AP News [Internet]. [cited 2025 Feb 5]. Available from: https://apnews.com/article/trump-africa-aid-freeze-pepfar-usaid-hiv-d1c34ac35af30e8f680f580f7d1b3029

71. Spearman CW, Andersson MI, Bright B, Davwar PM, Desalegn H, Guingane AN, et al. A new approach to prevent, diagnose, and treat hepatitis B in Africa. BMC Glob Public Heal [Internet]. 2023 Nov 2 [cited 2025 Feb 5];1(1):24. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11116268/

Part C: MANUSCRIPT

Title: The Burden of Hepatitis B Among People Living with HIV in East Africa: Evidence from 2016–2019 Population-Based HIV Impact Assessment Surveys

Mr. Mitiku Tamre Genamo[1]

Author Affiliations:

[1]Division of Epidemiology and Biostatistics, School of Public Health, University of Cape Town, Western Cape, South Africa.

Corresponding Author (*)

Mr. Mitiku Tamre Genamo

Division of Epidemiology and Biostatistics, School of Public Health, University of Cape Town, South Africa, Tel: +27633080186; Email: gnmmit001@uct.ac.za

Word Count Main text: 5480

Abstract: 417

Tables: 8

Figures: 2

ABSTRACT

Background:

Hepatitis B Virus (HBV) infection is a significant global health issue among people living with HIV (PLWH). Africa ranks second only to Asia in chronic HBV prevalence, with approximately 60 million cases reported in 2017 and nearly 74% of global HBV/HIV co-infections occurring in sub-Saharan Africa. In East Africa, hepatitis B surface antigen (HBsAg) prevalence rates are reported as Ethiopia (6.03%), Uganda (9.19%), Kenya (5.16%), Rwanda (6.67%), and Tanzania (7.17%). Co-infections of HBV and HIV are linked to severe liver disease, increased morbidity, and higher mortality rates. While the HIV epidemic is well documented in Africa, limited data exists on HBV co-infections among PLWH.

Objectives:

To assess the prevalence of Hepatitis B Virus (HBV) co-infection among people living with HIV (PLWH) in East Africa and identify associated sociodemographic and clinical factors.

Methods:

This study used a cross-sectional design to analyze secondary data from the PHIA dataset collected in five East African countries (Ethiopia, Kenya, Rwanda, Tanzania, and Uganda) between 2016 and 2019. A stratified multistage probability sampling approach was applied to select enumeration areas, households, and eligible adults aged 15–64 years living with HIV. Data was collected using mobile tablets and included demographic, clinical, and HIV-related information. HBV status was determined via rapid diagnostic tests. Ethical approvals were obtained from relevant institutions, adhering to the Helsinki Declaration. Data were analyzed in R using descriptive statistics, logistic regression, and tests for statistical significance ($P < 0.05$).

Results:

A total of 4,944 PLWH were included in this analysis, of the total 4,944 PLWH screened for HBV 248 (5.02%) were HBV-positive. The median age of PLWH participants was 36.5 years (IQR: 30–45), while HBV-negative participants had a median age of 38 years (IQR: 30–47). Males accounted for 42% of HBV-positive cases, compared to 29% among HBV-negative individuals. The overall HBV prevalence among PLWH was 5.02% (95% [CI]: 4.42% – 5.66%), with different country-

specific prevalence: Rwanda 3.49% (95% CI: 2.40% – 4.89%), Tanzania 4.29% (95% CI: 0.89% – 12.02%), Kenya (4.99%), Ethiopia 5.54% (95% CI: 3.86% – 7.65%), and Uganda 5.67% (95% CI: 4.65% – 6.84%). Females had higher odds of HBV infection than males (aOR 1.78, P=0.003). Additionally, those who are HBV positive exhibited higher HIV viral loads (≥1,000 copies/mL; 39% vs. 31%) and lower median CD4 counts (455 vs. 488.5 cells/μL). Participants with viral suppression had 1.4 times higher odds of HBV infection than those without viral suppression (OR = 1.40; 95% CI: 1.08–1.82; P = 0.01). Active syphilis infections were more common among HBV-positive individuals (5%) than HBV-negative participants (3%).

Conclusion:

HBV co-infection poses a significant burden among PLWH in East Africa, with variations in prevalence and associated factors across countries. These findings highlight the need for targeted interventions, including enhanced screening, vaccination, and treatment strategies to address this dual health challenge.

1. INTRODUCTION

Hepatitis B Virus (HBV) infection is a significant global health issue among people living with HIV (PLWH) (1). The developing region of Africa ranks second only to Asia in terms of the prevalence of chronic HBV infections, with around 60 million cases reported in 2017 (2). In countries like Kenya, the infection rates surpass 5%, and Africa accounts for 70% of all new cases of HBV infections globally (2). The decline in morbidity and mortality associated with HIV in resource-limited nations has been largely driven by the extensive implementation of antiretroviral therapy (3). Nevertheless, the co-infection of HBV and hepatitis C virus (HCV) has surfaced as a significant clinical and public health concern (3). Understanding the prevalence and regional distribution of these infections is crucial. The global distribution of HBV infection is notably uneven, with the highest prevalence observed in the WHO Western Pacific and African regions, at 6.2% and 6.1%, respectively. In 2013, a systematic review assessed the prevalence of the hepatitis B virus (HBV) in the general population, focusing on the hepatitis B surface antigen (HBsAg). The findings showed HBsAg prevalence rates in East African countries: Ethiopia 6.03%, Uganda 9.19%, Kenya 5.16%, Rwanda 6.67%, and Tanzania 7.17% (4). Systematic reviews conducted in Ethiopia in 2019 revealed that the prevalence of co-infection between HIV and HBV is approximately 5% (95% CI: 4–7%) (5). According to the 2017 UNAIDS report, it is estimated that more than 3.1 million individuals worldwide are co-infected with HBV and HIV, with a substantial concentration in regions such as Africa and Asia. Nearly 74% of cases are found in sub-Saharan Africa, highlighting the urgent need for targeted interventions and public health strategies to mitigate the impact of these co-infections (1).

Individuals at high risk for HIV also face greater risks from other viral infections, including HBV and HCV. Co-infections with HBV or HCV have become more prevalent among PLWH (3). Globally, it is estimated that between 5% and 10% of PLWH are also co-infected with HBV. Also, researches indicate that HBV is a significant contributor to non-AIDS-related mortality among those with HIV (6). This occurs due to similar modes of transmission. These co-infections are linked to increased morbidity and complications, such as severe liver disease, as well as higher mortality rates (1). Additionally, a compromised liver condition heightens the risk of hepatotoxicity associated with antiretroviral therapy (ART) (3). HBV, HIV, and HBV/HIV co-infections can be transmitted from mother to child during childbirth, as well as through contact with blood or other body fluids during unprotected sex with an infected partner, unsafe injections,

or exposure to sharp instruments (7). In a study conducted in Kenya, cases of HBV were associated with condom use and HIV viral load (3).

In many African countries, the HIV epidemic is well documented; however, there is limited data on HBV coinfections among both HIV-infected patients and the general population (3). It remains a burden that poses an additional challenge to the national health system, which is already battling various infectious and non-infectious diseases (5). This study was carried out among PLWH, to determine the prevalence of HBV coinfections and their associated factors.

2. METHODS AND MATERIALS

2.1. Study design, setting and study population

This is a household-based cross-sectional study which analyses secondary data collected from the PHIA dataset between April 2024 – August 2024.

There are thirteen countries in East Africa: Burundi, Comoros, Djibouti, Ethiopia, Eritrea, Kenya, Rwanda, Seychelles, Somalia, South Sudan, Sudan, Tanzania, and Uganda. The PHIA data is available for only five of these countries, so this study was focused on the available data. These five countries include Ethiopia, Kenya, Rwanda, Tanzania and Uganda. In 2023, it is estimated that Ethiopia had a population of 123 million. In 2024, the latest population figures, based on Worldometer's elaboration of the most recent United Nations data, are as follows: Kenya's population stands at 56,153,297; Rwanda has a population of 14,400,058; the United Republic of Tanzania's population has reached 69,321,072; and Uganda's population is 49,858,955 (Worldometer, 2024). Data collection was conducted in urban and rural areas of Kenya, Uganda, Rwanda, and Tanzania. In Ethiopia, data collection was conducted only in urban areas, including large and small urban areas.

The national survey population included women and men 0–64 years of age, and this analysis was only focused on adult individuals living with HIV aged 15–64 years.

2.2. Sampling

The sampling strategy used for the Population-based HIV Impact Assessment (PHIA) in East Africa employed a stratified multistage probability sample design. This entailed defining strata as the urban areas in 11 regions of Ethiopia, subdividing the 47 counties by urban-rural status for Kenya, and categorizing the five provinces of Rwanda, the 31 regions of Tanzania, and the 10 regions of Uganda. The sample design was structured into three stages of sampling units: enumeration areas (EAs) within the strata as the first-stage units, households within EAs as the second-stage units, and eligible persons within households as the third sampling units.

Within each sampling stratum, the first-stage sampling units, also referred to as "primary sampling units" or PSUs were selected with probabilities proportionate to the number of households in the PSU. These probabilities were based on data from the Population and Housing Census of the respective countries. The allocation of the sample PSUs to the EA sampling strata aimed to achieve specified precision levels for regional estimates of viral load suppression (VLS) rates among people living with HIV (PLWH) adults aged 15 and older. Furthermore, the second-stage sampling units were selected from lists of dwelling units/households compiled by trained staff for each of the sampled PSUs. Following the listing process, random systematic samples of specified numbers of dwelling units/households were chosen from each PSU. Finally, eligible individuals within households were sampled in the PHIA dataset. For this study, all eligible adults living with HIV aged 15 to 64 within the sampled households are included in the analysis.

2.3. Eligibility

2.3.1. Inclusion criteria:

The eligible participants for this secondary analysis were filtered out from the main PHIA biomarker survey dataset based on their HIV status (Figure 1).

- Women and men aged 15 – 64 years who were living with HIV(PLWH).

- Individuals with the outcome of interest (positive and negative cases of HBV)

2.3.2. Exclusion

- HIV-negative individuals and individuals below the age of 15 years

- Individuals with missing outcome and missing personal ID (unique identifier)

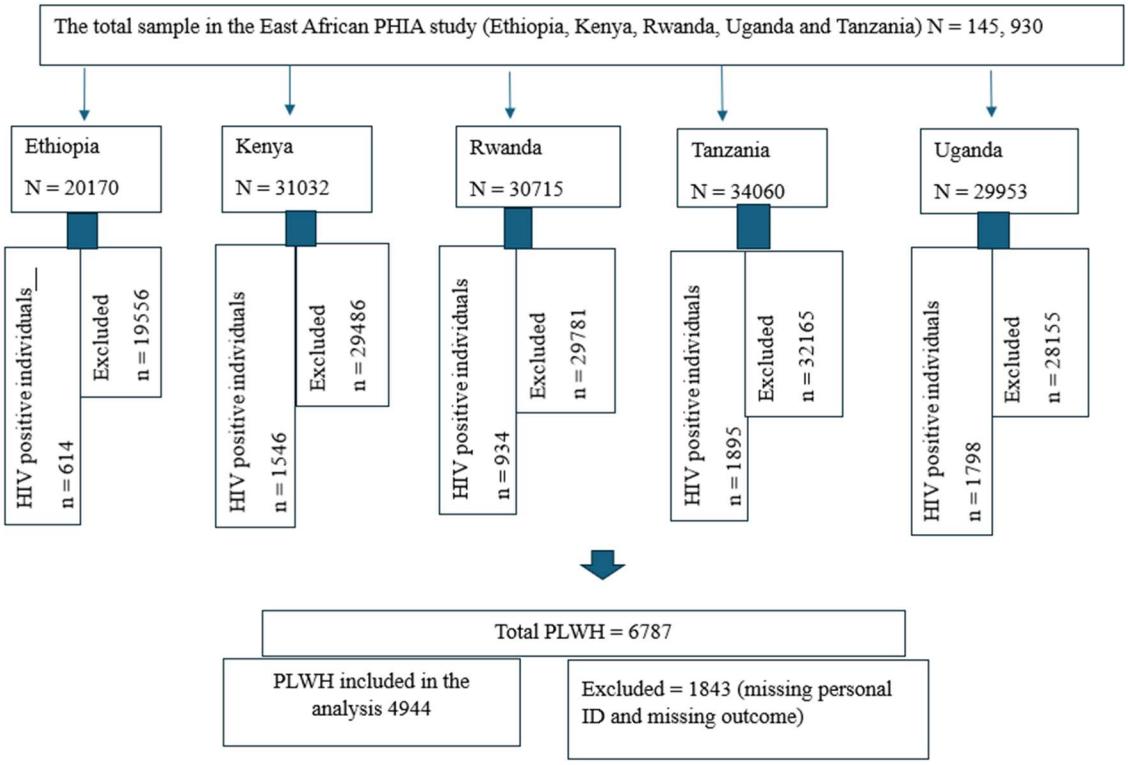

Figure 1: Flow Diagram of Inclusion and Exclusion Criteria

2.4. Data collection

Data was collected between October 2017 and April 2018 for Ethiopia, in 2018 for Kenya, between 2018 and 2019 for Rwanda, and from 2016 to 2017 for Tanzania and Uganda. The PHIA team conducted HIV testing using the national rapid diagnostic testing protocol and confirmed the results in the laboratory. In-person interviews were conducted using the adult questionnaire to eligible participants aged 15 and older. The adult questionnaires were composed of two sections, including demographic/social, and clinical information (Appendix 1). Data was collected electronically using mobile tablets. The data collection application used was Open Data Kit (ODK) which is programmed for mobile tablet devices.

2.5. Ethical consideration

This study involved secondary data analysis using publicly available PHIA datasets. Permission was granted by the PHIA team (Appendix 2). Additionally, Ethical approval was obtained from

the University of Cape Town Human Research Ethics Committee (HREC REF 788/2024) (Appendix 3). For the main study, ethical clearance for the study protocol, screening forms, primary data collection materials including adult questionnaires and biomarker tests, consent forms, and digital documentation of consent was granted by the Centers for Disease Control and Prevention (CDC), Columbia University, and all respective Public Health Institutes in East Africa, along with their respective institution review boards. All survey staff, including laboratory technologists, nurse interviewers, and supervisors, underwent training on good clinical and laboratory practices, and ethical protection of survey respondents, and signed a data confidentiality agreement. Additionally, staff members from the Institutional Review Boards or Ethics Committees oversee the conduct of these surveys to ensure the protection of the rights of participants. The PHIA study adheres to the ethical principles outlined in the Helsinki Declaration, emphasizing respect for individuals, informed consent, and the protection of participants in medical research.

The institutional approval reference numbers and their corresponding dates are as follows: EPHIA – 7044 (September 6, 2017); KENPHIA – 7094 (March 30, 2018); RPHIA – 7157 (August 24, 2018); THIS – 6880 (July 27, 2016); and UPHIA 2016 – 6830 (March 9, 2016) (34).

2.6. Data management and Statistical analysis

The data was cleaned and analyzed using the Windows version of R statistical software. We summarized the study population's characteristics using descriptive statistics. This includes reporting measures of central tendency (such as median) and dispersion (such as interquartile range) for continuous variables. For categorical variables, we provided the frequencies and percentages to address objectives 1 and 2. To address objective 3 we performed univariable and multivariable analysis to examine the relationships between specific risk factors and HBV status. We applied the Shapiro-Wilk test to check the distribution of continuous variables, which will then guide the selection of the right parametric or non-parametric statistical tests. Pearson’s chi-square test or Fisher’s exact tests for categorical variables and the Wilcoxon signed-rank test for non-normally distributed continuous variables, were used to assess associations between the outcome. The logistic regression model was used to pinpoint the critical factors contributing to HBV prevalence, taking into account potential confounding variables. We regarded findings as statistically significant if they were within a 95% confidence interval ($P < 0.05$).

3. RESULTS

3.1. Sociodemographic characteristics of the study participants

A total of 4,944 PLWH were included in this analysis, of the total 4,944 PLWH screened for 248 (5.02%) were HBV-positive, while 4,696 were HBV-negative (Table 1).

The median age of HBV-positive participants was 36.5 years (IQR: 30–45), comparable to 38 years (IQR: 30–47) for HBV-negative participants. Gender distribution revealed a higher proportion of males among HBV-positive participants (42%) compared to 29% in HIV-negative group. Educational status was similar across the groups, with 86% of HBV-positive and 88% of HBV-negative participants having attended school. Among those who attended school, primary education was the most common level achieved (67% HBV-positive vs. 70% HBV-negative), followed by secondary education (29% HBV-positive vs. 26% HBV-negative) and tertiary education (4% in both groups).

In terms of nationality, Ugandans accounted for the largest proportion of HBV-positive cases (41%), followed by Kenyans (31%), Ethiopians (14%), Rwandans (13%), and Tanzanians (1%). Marital status was similar between groups, with most participants being married (51% HBV-positive vs. 47% HBV-negative). The number of sexual partners in the past year was also comparable, with 87% of HBV-positive and 85% of HBV-negative individuals reporting one or no sexual partners. Male circumcision prevalence was nearly equal in both groups, at 49% HBV-positive and 50% HBV-negative, as was condom use in the past year (35% HBV-positive vs. 34% HBV-negative). 14% of participants reported having a beneficiary sexual relationship in both groups.

3.2. Biomarker characteristics of the study participants

A total of 4,944 PLWH were included in this analysis, of the total 4,944 PLWH screened for 248 (5.02%, (95% [CI]: 4.42% – 5.66%) were HBV-positive, while 4,696 were HBV-negative (Table 1).

Biomarker comparisons showed notable differences by HBV status. Active syphilis infections were more common among HBV-positive individuals (5%) than HBV-negative participants (3%).

A higher proportion of HBV-positive participants had viral loads ≥1,000 copies/mL (39% vs. 31%). Viral suppression was less frequently achieved among HBV-positive participants (61%) compared to HBV-negative individuals (69%). HBV-positive individuals reported slightly lower ART use (91%) compared to HBV-negative participants (93%). Median CD4 count was lower in HBV-positive participants (455 cells/μL) than in HBV-negative individuals (488.5 cells/μL). Additionally, a larger proportion of HBV-positive participants fell into lower CD4 count categories (<350 cells/μL) (Table 1).

No significant difference was observed in age distribution between HBV groups (P = 0.29), while CD4 count differed significantly (P = 0.02). Gender was significantly associated with HBV status (P < 0.0001), whereas variables such as education, marital status, male circumcision, and condom use showed no significant associations (all P > 0.05). No associations were found between HBV status and HIV testing (as a couple or ever tested), tuberculosis treatment history or clinic visits, or pregnancy status (all P > 0.05). Wealth quintile showed a borderline association (P = 0.06); urban/rural residence did not (P = 0.88). Among clinical indicators, viral load suppression (P= 0.014) and viral load category (P < 0.001) were significantly associated with HBV status. No significant associations were found with CD4 category, ART duration, self-reported ART use, ARV detection, or efavirenz presence.

Table 1: Socio-demographic and Biomarkers characteristics of people living with HIV by Hepatitis B outcome

Characteristics	Total, N = 4944	HBV status Positive, N= 248	Negative, N = 4696	P value
STUDY PARTICIPANTS DEMOGRAPHICS				
Age (years) (median (IQR)	38(30-47)	36.50(30-45)	38(30-47)	0.29
Gender				<0.0001*
Male	1,488(30%)	104(42%)	1,384(29%)	
Female	3,456(70%)	144(58%)	3,312(71%)	
Ever attended school				0.49
Yes	4,336(88%)	214(86%)	4,122(88%)	
No	606(12%)	34(14%)	572(12%)	
(Missing)	2	0	2	
Educational level				0.50
Primary	2,982(70%)	140(67%)	2,842(70%)	
Secondary	1,092(26%)	61(29%)	1,031(26%)	
Tertiary	177(4%)	8(4%)	169(4%)	
(Missing)	693	39	654	
Nationality				0.14

Ethiopian	614(12%)	34(14%)	580(12.35%)	
Kenyan	1,544(31%)	77(31%)	1,467(31.24%)	
Rwandan	918(19%)	32(13%)	886(18.87%)	
Ugandan	1,798(36%)	102(41%)	1,696(36.12%)	
Tanzanian	70(2%)	3(1%)	67(1.43%)	
Currently married				0.64
Married	2,052(47%)	115(51%)	1,937(47%)	
Living together	566(13%)	31(14%)	535(13%)	
Widowed	805(19%)	39(17%)	766(19%)	
Divorced	249(6%)	11(5%)	238(6%)	
Separated	660(15%)	28(13%)	632(15%)	
(Missing)	612	24	588	
Number of sexual partners (past 12 months)				0.61
More than one	152(14%)	10(13%)	142(15%)	
One or none	899(86%)	65(87%)	834(85%)	
(Missing)	3893	173	3720	
Male circumcision				0.91
Yes	743(50%)	51(49%)	692(50%)	
No	741(50%)	53(51%)	688(50%)	
(Missing)	3,460	144	3,316	
Condom use (past 12 months)				0.92
Yes	1,147(34%)	60(35%)	1,087(34%)	
No	2,206(66%)	113(65%)	2,093(66%)	
(Missing)	1,591	75	1516	
Beneficiary sexual relationships				0.91
Yes	469(14%)	23(14%)	446(14%)	
No	2,856(86%)	147(86%)	2,709(86%)	
(Missing)	1,619	78	1,541	
HIV tested as a couple				0.65
Yes	1,742(53%)	93(55%)	1,649(53%)	
No	1,562(47%)	77(45%)	1,485(47%)	
Missing	1,640	78	1,562	
Ever tested for HIV				0.93
Yes	4,619(93%)	231(93%)	4,388(93%)	
No	323(7%)	17(7%)	306(7%)	
(Missing)	2	0	2	
Currently pregnant				0.66
Yes	158(5%)	8(6%)	150(5%)	
No	3,179(95%)	128(94%)	3,051(95%)	
(Missing)	1,607	112	1,495	
Taking isoniazid (INH)				1.00
Yes	181(18%)	10(19%)	171(18%)	
No	826(82%)	43(81%)	783(82%)	
(Missing)	3,937	195	3,742	
TB clinic visit				0.37
Yes	1,500(30%)	82(33%)	1,418(30%)	
No	3,431(70%)	165(67%)	3,266(70%)	
(Missing)	13	1	12	
Currently on TB medication				1.00
Yes	43(9%)	3(9%)	40(9%)	
No	455(91%)	29(91%)	426(91%)	

(Missing)	4,446	216	4,230	
Ever treated for TB				1.00
Yes	355(93%)	19(95%)	336(93%)	
No	25(7%)	1(5%)	24(7%)	
Missing	4,564	228	4,336	
Urban area indicator				0.88
Urban	2,239(45%)	114(46%)	2,125(45%)	
Rural	2,705(55%)	134(54%)	2,571(55%)	
Wealth quintile Status				0.06
Lowest	959(20%)	64(26%)	895(19%)	
Second	927(19%)	41(17%)	886(19%)	
Middle	1,051(21%)	41(17%)	1,010(22%)	
Fourth	1,104(22%)	58(23%)	1,046(22%)	
Highest	901(18%)	44(17%)	857(18%)	
Missing	2	0	2	
STUDY PARTICIPANTS BIOMARKER				
Active syphilis infection				0.16
Yes	129(3%)	11(5%)	118(3%)	
No	3,896(97%)	205(95%)	3,691(97%)	
Missing	919	32	887	
ARVs detected				0.63
Yes	3,377(69%)	166(67%)	3,211(69%)	
No	1,513(31%)	80(33%)	1,433(31%)	
Missing	54	2	52	
Viral load category (copies/mL)				<0.001*
≤ LLOQ 200	1465(30%)	77(31%)	1388(30%)	
< LLOQ1000	228(5%)	17(7%)	211(4%)	
≥LLOQ 1000	1554(31%)	96(39%)	1458(31%)	
TND - TARGET NOT DETECTED	1697(34%)	58(23%)	1639(35%)	
Efavirenz In Blood				0.74
Positive	2,067(42%)	107(43%)	1,960(42%)	
Negative	2,823(58%)	139(57%)	2,684(58%)	
Missing	54	2	52	
An Adult Is On ART self-reported				0.43
Yes	3,237(93%)	158(91%)	3,079(93%)	
No	240(7%)	15(9%)	225(7%)	
Missing	1,467	75	1,392	
Duration Of Time On ART				0.08
≥24months	2,478(52%)	124(51%)	2,354(52%)	
12-23 months	270(6%)	7(3%)	263(6%)	
< 12 months	352(7%)	25(10%)	327(7%)	
Not on ART	1,644(35%)	87(36%)	1,557(35%)	
Missing	200	5	195	
Viral Load Suppressed				0.014*
Yes	3,390(69%)	152(61%)	3,238(69%)	
No	1,554(31%)	96(39%)	1,458(31%)	
Cd4 count (cells per microliter (µL))	485(320-666)	455(268.5-619)	488.5(324-669)	0.02*
Missing	2,487	109	2,378	
Cd4 Categorical (cells per microliter (µL))				0.46
<100	84(3%)	7(5%)	77(3%)	

≥100 and <200	172(7%)	11(8%)	161(7%)
≥200 and <350	455(19%)	31(22%)	424(18%)
≥350 and <500	572(23%)	32(23%)	540(23%)
≥ 500	1,174(48%)	58(42%)	1,116(48%)
Missing	2,487	109	2378

n (%) for all the categorical variables, Median (IQR) for not normally distributed continuous(numeric) variables (age and cd4 count)

The overall prevalence across all countries surveyed was 5.02% (95% [CI]: 4.42% – 5.66%), reflecting the burden of HBV among PLHIV in the region (Table 2).

Country-specific prevalence of HBV among PLWH across East Africa is presented in Table 2 and figure 1, Uganda exhibited the highest prevalence at 5.67% (95% CI: 4.65% – 6.84%), followed closely by Ethiopia with a prevalence of 5.54% (95% CI: 3.86% – 7.65%). Kenya's prevalence was 4.99% (95% CI: 3.96% – 6.19%), which is slightly below the overall average of 5.02%. Tanzania's prevalence was 4.29% (95% CI: 0.89% – 12.02%), reflecting a mid-range level of HBV infection among the surveyed populations. Rwanda had the lowest prevalence at 3.49% (95% CI: 2.40% – 4.89%), marking it as the country with the least HBV burden in the dataset (Figure 2).

Table 2: HBV prevalence (objective 1) of overall sample and 95% Confidence Intervals by Country

Country	HBV Positive Count	Total PLWH	country-level HBV Prevalence (%) and (95%CI)
Overall study PLWH	248	4,944	5.02% (4.42-5.66)
Ethiopia	34	614	5.54% (3.86 – 7.65)
Kenya	77	1,544	4.99% (3.96 – 6.19)
Rwanda	32	918	3.49% (2.40 – 4.89)
Uganda	102	1,798	5.67% (4.65 – 6.84)
Tanzania	3	70	4.29% (0.89 – 12.02)

When comparing these findings with HBV prevalence estimates from the general population, the confidence intervals of our estimates largely overlap with those reported in previous studies. This

overlap suggests that there is no clear evidence that people living with HIV (PLHIV) in this cohort are at significantly higher risk of HBV infection compared to the general population.

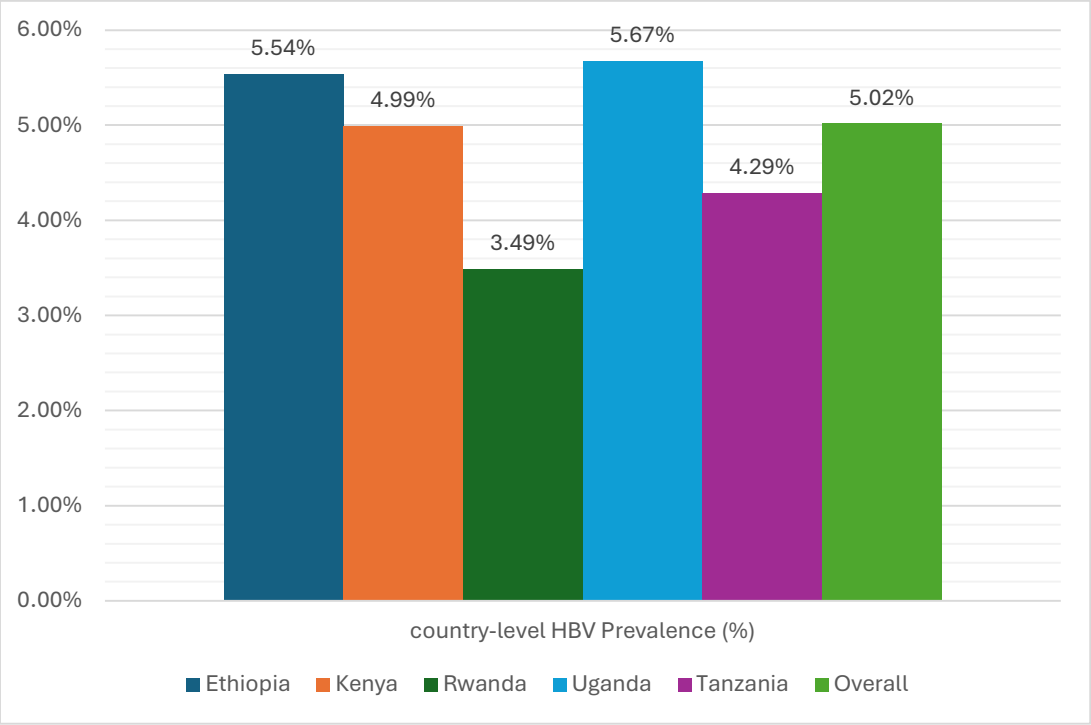

Figure 2: Bar Graph of Hepatitis B Virus Prevalence Among PLWH in East Africa (PHIA 2016-2019)

3.3. Risk factors associated with HBV infection among study participants

Table 3 provides the results of univariable and multivariable logistic regression models for factors associated with HBV infection in the overall sample. Univariate logistic regression analysis identified several variables associated with HBV infection. Female participants had significantly higher odds of HBV positivity compared to males (OR = 1.73; 95% CI: 1.33–2.24; P < 0.001). The lowest wealth quintile status was associated with a decreased risk of HBV infection (OR = 0.57; 95% CI: 0.38-0.85; P = 0.005) compared to the middle wealth quintile status (reference group). Active syphilis infection showed a non-significant trend towards association (OR: 0.60, 95% CI: 0.33–1.19). Participants with viral suppression had 1.4 times higher odds of HBV infection than those without viral suppression (OR = 1.40; 95% CI: 1.08–1.82; P = 0.01). Other sociodemographic variables, including age (OR = 1.01; 95% CI: 0.99–1.02; P = 0.33) and

educational level (OR for secondary or higher vs. primary = 0.86; 95% CI: 0.64–1.16; P = 0.31), were not significantly associated with HBV infection in univariate models.

In multivariable model female participants remained at higher odds of HBV positivity compared to males (aOR = 1.78; 95% CI: 1.21–2.62; P = 0.003). Wealth quintiles showed a strong gradient effect, with participants in the lowest quintile having lower odds of HBV infection (aOR = 0.35; 95% CI: 0.20–0.60; p < 0.001), followed by the second quintile (aOR = 0.54; 95% CI: 0.30-0.94; P = 0.03) compared to middle wealth quintiles. Viral suppression continued to be significantly associated with HBV infection in the adjusted model (aOR = 4.07; 95% CI: 1.93–8.60; P < 0.001) (Table 3).

Similar to univariate models, other variables, such as educational attainment, marital status, and urban vs. rural residence, were not significantly associated with HBV positivity in the adjusted model. For example, for marital status, being widowed, divorced, separated or living together did not significantly affect HBV odds compared to being married (aOR for widowed = 1.42; 95% CI: 0.67-3.52; P = 0.40) or living together (aOR =1.06; 95% CI: 00.68-1.72; P=0.79). Similarly, condom use(past 12 month)(aOR =0.94; 95% CI:0.66-1.35; p=0.74), active syphilis infection)(aOR =0.92; 95% CI:0.36-3.12; P=0.89), and CD4 count (aOR =1.00; 95% CI:1.00-1.00; P=0.30), CD4 Categorical (cells per microliter (μL)) and number of sexual partners(past 12 months) did not show significant associations with HBV infection after adjusting for confounders.

3.4. Country subgroup analysis of risk factors associated with HBV infection

In Ethiopia (Table 4), gender was not a significant factor in the multivariable model (aOR: 3.11, 95% CI: 0.73–13.55, P=0.12), contrasting with the overall analysis. Educational level showed an inverse association in the univariable model, with secondary education associated with lower odds of HBV infection (OR: 0.39, 95% CI: 0.17–0.89, P=0.02), but this was not retained in the multivariable analysis. Other variables, including those who had viral load suppressed had higher odd of infections (aOR: 4.31; 95% CI: 0.89–20.18, P=0.06), and wealth quintile, but it did not show significant associations in this subgroup.

In Kenya (Table 5), male circumcision emerged as a significant protective factor in the univariable model (OR: 2.42, 95% CI: 1.08–5.43, P=0.03), a finding not observed in the overall analysis.

Wealth quintile (lowest) showed a significant association with decreased odds of HBV infection (aOR: 0.46, 95% CI: 0.23-0.90, P=0.03) compared to middle quintile. Viral load suppression (aOR: 1.12, 95% CI: 0.36-3.03, P=0.83) was not significantly associated with HBV infection in this subgroup.

In Uganda (Table 6), females had higher odds of HBV infection in the univariable model (OR: 1.55, 95% CI: 1.03–2.32, P=0.03), but this association was not significant in the multivariable model. The lowest quintile lowest (aOR: 0.30, 95% CI: 0.12-0.68, P=0.005) remained significantly associated with decreased odds of HBV infection compared to middle quintile in the multivariable model. Suppressed viral load showed a strong association and increased odds of HBV infection in the multivariable model (aOR: 4.49, 95% CI: 1.86–10.99, P<0.001), which was consistent with the overall analysis.

In Rwanda (Table 7), gender showed a significant association, with females having higher odds of HBV infection (aOR: 4.12, 95% CI: 1.18–17.16, P=0.03). Educational attainment also emerged as significant, with individuals who attended school showing increased odds of HBV infection (aOR: 4.43, 95% CI: 1.34–13.92, P=0.01). Viral load suppression had lower odds of infection, forth and highest wealth quintile had lower odds of infection, lowest and second wealth quintile had highest odds of infection but did not show statistically significant associations in this subgroup.

Table 3:Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Hepatitis B Virus (HBV) Infection

	Univariable logistic regression models			Multivariable logistic regression models		
	Intercept	OR (95% CI)	P value	Intercept	aOR (95% CI)	P value
DEMOGRAPHIC PREDICTORS						
Age (years)	15.17	1.01(0.99,1.02)	0.33	9.88	1.01(0.99, 1.03)	0.17
Gender						
Male (reference)						
Female	13.31	1.73(1.33, 2.24)	< 0.001*		1.78 (1.21, 2.62)	0.003*
Ever attended school						
Yes	16.82	1.14 (0.78, 1.64)	0.48		1.47(0.88, 2.33)	0.12
No(reference)						
Educational level						
Primary (reference)						
Secondary and above	20.30	0.86(0.64, 1.16)	0.31			
Currently married						
Married (reference)						
Living together	16.84	1.02(0.69, 1.57)	0.91		1.06(0.68, 1.72)	0.79
Widowed		1.17(0.81,1.71)	0.42		1.42(0.67, 3.52)	0.40
Divorced		1.28(0.71,2.56)	0.42		0.98(0.42, 2.87)	0.96
Separated		1.34(0.89,2.08)	0.18		0.97 (0.57, 1.71)	0.91
Number of sexual partners (past 12 months)						
≤1(reference)						
>1	18.68	1.14(0.76, 1.76)	0.56		1.64(1.00, 2.82)	0.06
Male circumcision						
Yes	13.57	0.96(0.64, 1.43)	0.83			
No (reference)						
Number of wives						
≤ 1 (reference)						
>1	12.83	1.11(0.58, 2.24)	0.77			
Condom use (past 12 months)						
Yes	18.52	0.98(0.71, 1.36)	0.89		0.94(0.66, 1.35)	0.74
No (reference)						
Beneficiary sexual relationships						
Yes	18.43	1.05(0.68, 1.69)	0.83		0.85(0.51, 1.46)	0.53
No (reference)						
HIV tested as a couple						
Yes	19.29	0.92(0.67, 1.25)	0.59		0.97(0.67, 1.40)	0.88
No (reference)						
Ever tested for HIV						
Yes	18.00	1.06(0.61, 1.70)	0.84		1.07(0.50, 2.10)	0.85
No (reference)						
Currently pregnant						

Yes (reference)	23.84	0.79(0.40, 1.78)	0.52			
No						
Taking isoniazid (INH)						
Yes	18.21	0.94(0.48, 2.01)	0.86			
No(reference)						
Currently on TB treatment						
Yes	14.69	0.91(0.30, 3.91)	0.88			
No (reference)						
TB clinic visit						
Yes	19.79	0.87(0.67, 1.15)	0.33		0.85 (0.59, 1.23)	0.37
No(reference)						
Ever treated for TB						
Yes	24.00	0.74(0.04, 3.80)	0.77			
No(reference)						
Urban area indicator						
Urban (reference)						
Rural	18.64	1.03(0.80, 1.33)	0.83		1.10(0.75, 1.60)	0.62
Wealth quintile Status						
Lowest		0.57(0.38, 0.85)	0.005*		0.35(0.20, 0.60)	<0.001*
Second	24.63	0.88(0.56, 1.37)	0.56		0.54(0.30, 0.94)	0.03*
Middle (reference)						
Fourth		0.73(0.48, 1.10)	0.14		0.60(0.33,1.05)	0.08
Highest		0.79(0.51, 1.22)	0.30		0.68(0.35, 1.32)	0.25
BIOMARKER PREDICTORS						
Active syphilis infection						
Yes	18.00	0.60(0.33, 1.19)	0.11	24.78	0.92(0.36, 3.12)	0.89
No (reference)						
ARVs detected						
Yes	17.91	1.08(0.82, 1.42)	0.58		0.47(0.17, 1.18)	0.12
No (reference)						
Viral load (copies/mL)						
TND – Target not detected (reference)	28.26					
≤ LLOQ 200		0.64 (0.45, 0.90)	0.01*		0.51(0.28, 0.92)	0.18
< LLOQ: 1000		0.44 (0.26, 0.79)	0.003*		0.33(0.14, 0.89)	0.11
>=LLOQ 1000		0.54(0.38, 0.75)	<0.001*		NA	NA
Efavirenz In Blood						
Positive	19.31	0.95(0.73, 1.23)	0.69		0.97(0.58, 1.62)	0.92
Negative (reference)						
An Adult Is On ART						
Yes	15.00	1.3(0.72, 2.17)	0.35		0.75 (0.25, 2.21)	0.59
No (reference)						
Duration Of Time On ART						
Not on ART (reference)						
≥24 months	17.90	1.06(0.80, 1.40)	0.68		1.36(0.66, 2.60)	0.38
12-23 months		2.10(1.03, 5.04)	0.06		1.65(0.61, 4.90)	0.33
< 12 months		0.73(0.47, 1.18)	0.18		NA	NA
Viral Load Suppressed						
Yes	15.19	1.40(1.08, 1.82)	0.01*		4.07(1.93, 8.60)	<0.001*
No(reference)						
Cd4 count (cells per microliter (µL))	10.09	1.00(1.00, 1.00)	0.003*		1.00(1.00, 1.00)	0.30
Cd4 Categorical (cells per microliter (µL))						
<100 (reference)						
≥100 and <200	11	1.33(0.47, 3.51)	0.57		0.51(0.10, 2.01)	0.36
≥200 and <350		1.24(0.49, 2.77)	0.62		0.52(0.11, 1.83)	0.35

≥350 and <500	1.53(0.60, 3.41)	0.33	0.37(0.07, 1.43)	0.18
≥500	1.75(0.71, 3.72)	0.18	0.34(0.05, 1.84)	0.23

Meaningful statistical analysis was not possible because there were only 3 outcome (HBV) events for Tanzania in the sample. A minimum of 10–20 events per variable (EPV) is often advised for logistic regression and related techniques in order to provide reliable and significant estimates (8). This rule was broken in this instance due to the limited information, which also made multivariable modeling prone to overfitting, a phenomenon in which the model records noise instead of real patterns, producing inconsistent and untrustworthy findings (9). Furthermore, the small number of events led to reduced statistical power and wide confidence intervals, which hindered the detection of significant relationships (8).

4. DISCUSSION

The findings from the 2016–2019 Population-Based HIV Impact Assessment (PHIA) surveys underscore the burden of Hepatitis B Virus (HBV) co-infection among people living with HIV (PLHIV) in East Africa. This study revealed an overall prevalence of 5.02% for HBV among PLHIV, with substantial variations across different countries in the region. According to the World Health Organization (WHO), In regions where 2–7% of the population tests positive for the hepatitis B surface antigen (HBsAg), it classifies this as an intermediate prevalence of HBV (10). In this study Uganda had the highest HBV prevalence (5.67%), and this is higher than what's seen in the general population of PHIA Uganda (4.3%) (11), similarly another study in Uganda's Victoria fishing community also had higher prevalence of 7% (12). This increase in prevalence is explained by delayed vaccination rollout and lower screening uptake in the area. In Ethiopia the prevalence was (5.54%), which was lower than a finding from meta-analysis and systematic review conducted in Ethiopia that showed 6.3% of overall HBV prevalence in the general population over the last five decades (13). Our finding from Kenya (4.99%) this finding obtained from this analysis was higher than the most reflective of the general population, The recent pooled prevalence estimate for HBV infection was 3.4% (14). Tanzania is regarded to be a higher-endemic country. A seroprevalence of HBV infection in the country was reported to be 6% in the general population

of Dar es Salaam (15). This rate has increased from older studies that reported the prevalence of 4.4% in the same population (15). The results from this analysis also indicate a prevalence of (4.29%). While Rwanda reported the lowest (3.49%) from this study, another national study estimated a higher prevalence of 4.3% among PLWH(16), Previous national statistics from 24 of 30 Rwandan administrative districts revealed that 3.9% of the general population had chronic HBV (16), and 2% in a most recent finding that covered all districts of the country (17). This highlights the diverse HBV epidemiology found in East Africa. These variations most likely reflect variances in public health infrastructure, treatment, monitoring, and immunization programs (18).

Given the overlapping confidence intervals, it remains uncertain whether HIV infection increases susceptibility to HBV in these settings. Further studies with larger sample sizes and longitudinal designs may help clarify this relationship.

Sociodemographic characteristics revealed that female gender had a higher proportion of HBV-positive cases compared to males (Table 1). Similarly, in the regression analysis female participants had significantly 1.73 times higher odds of HBV positivity compared to males. However, in subgroup analysis, Ethiopia (Table 4), female gender was associated with a decreased risk of HBV and this result was not a significant factor in the multivariable model, compared with the overall analysis. These gender disparity echoes findings from studies like that conducted by Wasihun et al(19), which highlight behavioral and sociocultural factors, including differential access to healthcare and vaccination(19). This is in contrary with study conducted in Uganda identified male gender having a higher likelihood of hepatitis B infection compared to female sex (11). Similar study conducted in Rwanda also identified male gender as one of demographic characteristics associated with HBsAg positivity in this study(16). The reasons behind this association may be that males may be more prone to high-risk behaviors like sexual contact, violence and conflicts in which blood contact may occur.

We found that lower economic status protects against HBV infection, a paradox that could be caused from variations in healthcare-seeking behavior or community transmission dynamics (20) (21). Similarly, educational attainment and condom usage was not significantly associated with HBV infection, contradicting studies in other regions that report an inverse relationship between literacy and infection risks. In a clinic-based cohort study conducted in South Africa, individuals aged 40 to 49 found that increased condom use was significantly associated with a lower

prevalence of HBV infection(29). Furthermore, regular condom use was linked to a threefold reduction in the prevalence of anti-HBV antibodies (30). A different study conducted in Kenya found contradictory findings among which reported the odds of being infected with HBV were over six times higher for individuals with more than one sexual partner compared to those who had one or zero sexual partners (22), the odds of infections were over five times higher in the lowest quintile of wealth and the odds of being infected with HBV were over four times higher for individuals with lack of formal education compared to those with some level of education (22), this associations were significantly associated with HBV infection (22). Our unique finding from Rwanda, where education attainment correlated with increased HBV infection, call for more research to identify the underlying sociocultural causes. These findings support the need for localized strategies to address heterogeneous risk profiles. Even though our analysis did not find any significant association between number of sexual partners and HBV infection, a study conducted on pregnant women in Ethiopia, A history of having multiple sexual partners was another significant predictor of HBV infection (13). Pregnant women with a history of multiple sexual partners were more than two times more likely to develop HBV infection than their counterparts (13). This is similar to a cross-sectional study conducted in Kenya (23).

Biomarker data underscore the complex interplay between HBV and HIV co-infection, indicated that HBV-positive individuals had higher HIV viral loads and lower CD4 counts compared to HBV-negative individuals. This highlights the immunosuppressive interaction between HBV and HIV. Individuals who had lower viral loads (less than 1000 copies/mL) had lower odds of HBV infections. In both our univariate and multivariable model Cd4 Categorical (cells per microliter (μL)) did not show significant associations with HBV infection after adjusting for confounding. This is in contrast to the findings in a study conducted in Ethiopia, participants with a baseline CD4 count of less than 200 cells/ul had significantly higher odds of having HBV infection than the other groups (24). In accordance with our finding a study in South Africa finds co-infected individuals had lower CD4 cell counts than HIV-only patients, even if they were taking ART. Additionally, patients with co-infection had a higher proportion of patients with plasma HIV viral load than patients with HIV alone (25). The low CD4 cell counts among co-infected patients may possibly be explained by this since there was a negative association found between CD4 cell

counts and HIV viral load (25). In subgroup analysis, in Kenya, viral load suppression was not significantly associated with HBV infection.

Although the study's cross-sectional design limits causal inference, its nationally representative biomarker confirmed data, and multivariable adjustments are among its strengths. The findings, however, have clear clinical implications as they demonstrate that viral suppression offers dual protection(31), reducing both HIV and HBV, and that higher education does not eliminate HBV risk, underscoring the necessity of inclusive screening across viral load and educational strata.

In both our models, viral suppression was associated with increased odds of infection of HBV (Table 3). Clinically this doesn't make sense, as viral suppression through antiretroviral therapy (ART) is generally protective against HBV progression and related complications, not a risk factor. A possible explanation for the association observed can be frequency of viral suppression in this study, we observed virally suppressed individuals less frequently in HBV-positive participants compared to HBV-negative individuals. HBV-positive individuals reported slightly lower ART use compared to HBV-negative participants. Emphasizing the critical role of effective ART in mitigating HBV transmission risks. This was supported by study conducted in urban Uganda, In the last 2 decades of HIV management in Uganda, there has been introduction of better treatment guidelines and use of more efficacious ART regimens that have antiviral activity against HBV infection as well which ultimately resulted in viral load suppression (26). In a multi-country West African study conducted by Coffie et al, HBsAg positivity was significantly higher among those not on ART (45.5 %) compared to ART users (12.0 %), reinforcing the protective association between ART use and lower HBV infection (32). In contrast, an Ethiopian study highlighted that HIV/HBV co-infected patients on ART containing lamivudine often developed drug resistance, suggesting that ART regimens lacking potent anti-HBV activity may be less protective(33)

Active syphilis infections were more common among HBV-positive individuals than HBV-negative participants, highlighting overlapping risk behaviors such as unprotected sexual activity since both share the same routes of transmission as HBV. But Active syphilis infection showed a non-significant trend towards association and a decreased odds of infection, this was contradictory to what have been observed in a study conducted in SSA region, participants with a history of sexually transmitted infections were more likely to be infected with either HBV or HIV(27). Country-specific analyses added further contradiction, with male circumcision offering a positive association in Kenya, those who are circumcised are 2.5 times high likely to have HBV infection, which is not consistent with its documented efficacy in reducing viral transmission(27).

5. CONCLUSION

This study highlights the intermediate burden of HBV co-infection among PLWH in East Africa, with substantial variation in prevalence across the region. The findings underscore the importance of addressing HBV as a critical public health concern, especially in resource-limited settings. Sociodemographic disparities, such as gender and economic status, and biomarker characteristics, including viral suppression and CD4 counts, play a significant role in shaping HBV epidemiology among PLWH. These results call for integrated and localized strategies that prioritize comprehensive HBV screening, vaccination, and treatment programs. Strengthening healthcare systems to manage co-infections effectively is essential in mitigating the dual burden of HIV and HBV in East Africa. Socio demographic factors such as age, education attainment, level of education, marital status, number of sexual partners, male circumcision and condom use did not show statistical significance, thus further research is needed to explore the underlying factors driving regional differences and to develop targeted interventions.

6. LIMITATIONS /RECOMMENDATION

There were few variables removed from our statistical analysis for two main reasons, missingness of data was very high (more than 50%) in variables such as number of wives, male circumcision status, taking INH medication, Currently on TB treatment, TB Treated. Additionally, some

variables had unbalanced categories such as pregnancy status which resulted in our model not performing well. Furthermore, some countries did not include some variables in their questioner design phase, which ultimately contributed to missing data in the overall dataset.

The analysis did not account for multistage sampling and applied weighting because sampling was proportional to size of the sampling units. Thus, we acknowledge that omitting weight sampling and design corrections limits the precision and generalizability of the HBV prevalence estimates. The reported prevalence may not fully reflect the national burden, particularly if certain subgroups (e.g., by age, gender, or geographic region) were over or underrepresented in the sample. As such, the prevalence figures presented should be interpreted as unweighted, descriptive estimates, and not definitive population-level metrics. Future analyses aimed at nationally representative prevalence estimates should incorporate appropriate survey design adjustments to ensure accuracy.

There are very few studies that are published that look at the prevalence of HBV at the national and regional levels. It also suggests that because certain population groups may exhibit unique predictors of infection, the risk factors for HBV infection have not been thoroughly assessed at a country or regional level (28). Most of the literature identified was from subgroup analysis, therefore, identifying the epidemiological nature of HBV at a broad geographic region would provide a number of potential risk factors linked to HBV infection at the regional level in addition to evidence-based data on HBV prevalence.

Competing Interests

The authors declare that there are no conflicting interests.

Acknowledgements

None.

Funding Information

None.

7. REFERENCES

1. Leumi S, Bigna JJ, Amougou MA, Ngouo A, Nyaga UF, Noubiap JJ. Global Burden of Hepatitis B Infection in People Living With Human Immunodeficiency Virus: A Systematic Review and Meta-analysis. Clin Infect Dis [Internet]. 2020 Dec 1 [cited 2024 Nov 4];71(11):2799–806. Available from: https://pubmed.ncbi.nlm.nih.gov/31813969/
2. Makokha GN, Zhang P, Hayes CN, Songok E, Chayama K. The burden of Hepatitis B virus infection in Kenya: A systematic review and meta-analysis. Front Public Heal. 2023;11(1).
3. Ndegwa Maina D, Kimanga Nyerere A, Wambui Gicho R, Maina Mwangi J, Wekesa Lihana R. Prevalence and Factors Associated With Hepatitis B and C Co-Infection Among HIV-1-Infected Patients in Kenya. East African Heal Res J. 2017;1(2):73–9.
4. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between 1965 and 2013. Lancet [Internet]. 2015 Oct 17 [cited 2024 Nov 4];386(10003):1546–55. Available from: http://www.thelancet.com/article/S014067361561412X/fulltext
5. Yazie TD, Tebeje MG. An updated systematic review and meta-analysis of the prevalence of hepatitis B virus in Ethiopia. BMC Infect Dis [Internet]. 2019 Oct 29 [cited 2024 Nov 4];19(1):917. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6820955/
6. Stabinski L, O'Connor S, Barnhart M, Kahn RJ, Hamm TE. Prevalence of HIV and Hepatitis B virus co-infection in Sub-Saharan Africa and the potential impact and program feasibility of Hepatitis B surface antigen screening in resource-limited settings. J Acquir Immune Defic Syndr. 2015;68(Suppl 3):S274–85.
7. Wandera BO, Onyango DM, Musyoki SK. Hepatitis B virus genetic multiplicity and the associated HBV lamivudine resistance mutations in HBV/HIV co-infection in Western Kenya: A review article. Infect Genet Evol. 2022 Mar 1;98:105197.
8. Van Smeden M, De Groot JAH, Moons KGM, Collins GS, Altman DG, Eijkemans MJC, et al. No rationale for 1 variable per 10 events criterion for binary logistic regression analysis. BMC Med Res Methodol [Internet]. 2016;16(1):1–12. Available from: http://dx.doi.org/10.1186/s12874-016-0267-3
9. Grant SW, Hickey GL, Head SJ. Statistical primer: multivariable regression considerations and pitfalls. Eur J Cardio-Thoracic Surg [Internet]. 2019 Feb 1 [cited 2024 Dec 12];55(2):179–85. Available from: https://dx.doi.org/10.1093/ejcts/ezy403
10. Nelson NP, Easterbrook PJ, McMahon BJ. Epidemiology of Hepatitis B Virus Infection and Impact of Vaccination on Disease. Clin Liver Dis. 2016 Nov 1;20(4):607–28.
11. Rugaatwa Ndibarema E, Olum R, Ayebare D, Kabakyenga J. Prevalence and Factors Associated with Hepatitis B Infection Among Outpatient Adults in South-Western Uganda. Hepat Med [Internet]. 2022 Oct [cited 2024 Dec 17];14:163–72. Available from: https://pubmed.ncbi.nlm.nih.gov/36304917/

12. Kitandwe PK, Muyanja E, Nakaweesa T, Nanvubya A, Ssetaala A, Mpendo J, et al. Hepatitis B prevalence and incidence in the fishing communities of Lake Victoria, Uganda: a retrospective cohort study. BMC Public Health [Internet]. 2021 Dec 1 [cited 2024 Dec 17];21(1):1–8. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-021-10428-1

13. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2024 Dec 17];18(4):e0284646. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10132657/

14. Downs LO, Campbell C, Yonga P, Githinji G, Azim Ansari M, Matthews PC, et al. A systematic review of Hepatitis B virus (HBV) prevalence and genotypes in Kenya: Data to inform clinical care and health policy. PLOS Glob Public Heal [Internet]. 2023 Jan 1 [cited 2024 Dec 17];3(1):e0001165. Available from: https://journals.plos.org/globalpublichealth/article?id=10.1371/journal.pgph.0001165

15. Kilonzo SB, Gunda DW, Mpondo BCT, Bakshi FA, Jaka H. Hepatitis B Virus Infection in Tanzania: Current Status and Challenges. J Trop Med [Internet]. 2018 [cited 2024 Dec 17];2018:4239646. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5831599/

16. Makuza JD, Rwema JOT, Ntihabose CK, Dushimiyimana D, Umutesi J, Nisingizwe MP, et al. Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. BMC Infect Dis [Internet]. 2019 May 3 [cited 2024 Dec 17];19(1):381. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6499977/

17. Umutesi J, Klett-Tammen C, Nsanzimana S, Krause G, Ott JJ. Cross-sectional study of chronic hepatitis B virus infection in Rwandan high-risk groups: unexpected findings on prevalence and its determinants. BMJ Open [Internet]. 2021 Dec 30 [cited 2024 Dec 17];11(12):e054039. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8719204/

18. Spearman CW, Andersson MI, Bright B, Davwar PM, Desalegn H, Guingane AN, et al. A new approach to prevent, diagnose, and treat hepatitis B in Africa. BMC Glob Public Heal 2023 11 [Internet]. 2023 Nov 2 [cited 2024 Dec 17];1(1):1–11. Available from: https://link.springer.com/article/10.1186/s44263-023-00026-1

19. Wasihun Y, Asnake D, Kebede N. Investigating factors associated to HBV/HIV co-infected patients in antiretroviral treatment clinic, in Northeast Ethiopia. BMC Infect Dis [Internet]. 2024 Dec 1 [cited 2024 Dec 17];24(1). Available from: https://pubmed.ncbi.nlm.nih.gov/38693473/

20. Wang X, Liu J, Wang Q, Qiao Y, Jin X, Li Z, et al. Economic-related inequalities in hepatitis B virus infection among 115.8 million pregnant women in China from 2013 to 2020. eClinicalMedicine [Internet]. 2022 Jul 1 [cited 2024 Dec 17];49:101465. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9124701/

21. Morgan CE, Ngimbi P, Boisson-Walsh AJ, Ntambua S, Matondo J, Tabala M, et al. Hepatitis B virus prevalence and transmission in the households of pregnant women in Kinshasa, Democratic Republic of Congo. medRxiv [Internet]. 2023 Nov 27 [cited 2024 Dec 17];2023.11.27.23298863. Available from:

https://pmc.ncbi.nlm.nih.gov/articles/PMC10705650/

22. Ondondo RO, Muthusi J, Oramisi V, Kimani D, Ochwoto M, Young P, et al. Prevalence of hepatitis B virus infection in Kenya: A study nested in the Kenya Population-based HIV Impact Assessment 2018. Blackard JT, editor. PLoS One [Internet]. 2024 Nov 14 [cited 2024 Dec 17];19(11):e0310923. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11563396/

23. Merali Z. Stephen Hawking: “There are no black holes.” Nature. 2014 Jan 24;

24. Teame G, Gebreyesus A, Tsegay E, Gebretsadik M, Adane K. Hepatitis B and C viral coinfections and their association with HIV viral load suppression among HIV-1 infected patients on ART at Mekelle hospital, northern Ethiopia. AIDS Res Ther [Internet]. 2022 Dec 1 [cited 2024 Dec 17];19(1):1–10. Available from: https://aidsrestherapy.biomedcentral.com/articles/10.1186/s12981-022-00479-8

25. Maponga TG, Andersson MI, van Rensburg CJ, Arends JE, Taljaard J, Preiser W, et al. HBV and HIV viral load but not microbial translocation or immune activation are associated with liver fibrosis among patients in South Africa. BMC Infect Dis [Internet]. 2018 May 8 [cited 2024 Dec 17];18(1):1–11. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-018-3115-8

26. Wekesa C, Ocama P, Parkes-Ratanshi R, Kirk GD. Burden and correlates of significant liver fibrosis among HIV-infected and uninfected adults in urban Uganda. Dialogues Heal. 2022 Dec 1;1:100027.

27. Getaneh Y, Getnet F, Amogne MD, Liao L, Yi F, Shao Y. Burden of hepatitis B virus and syphilis co-infections and its impact on HIV treatment outcome in Ethiopia: nationwide community-based study. Ann Med [Internet]. 2023 Dec 12 [cited 2024 Dec 17];55(2). Available from: https://www.tandfonline.com/doi/abs/10.1080/07853890.2023.2239828

28. Kafeero HM, Ndagire D, Ocama P, Kudamba A, Walusansa A, Sendagire H. Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. Arch Public Heal [Internet]. 2021 Dec 1 [cited 2024 Dec 17];79(1):167. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8449462/

29. Shivakumar M, Moe CA, Bardon A, Krows M, Govere S, Yunus M, et al. Hepatitis B prevalence and risk factors among adults living with HIV in South Africa: a clinic-based cohort study. [cited 2025 Jun 19]; Available from: http://creativecommons.org/licenses/by/4.0/.

30. Bernabe-Ortiz A, Carcamo CP, Scott JD, Hughes JP, Garcia PJ, Holmes KK. HBV Infection in Relation to Consistent Condom Use: A Population-Based Study in Peru. PLoS One [Internet]. 2011 Sep 13 [cited 2025 Jun 19];6(9):e24721. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0024721

31. Shilaih M, Studya TSHC, Marzel A, Studya TSHC, Scherrer AU, Studya TSHC, et al. Dually Active HIV/HBV Antiretrovirals as Protection Against Incident Hepatitis B Infections: Potential for Prophylaxis. J Infect Dis [Internet]. 2016 Aug 15 [cited 2025 Jun 19];214(4):599–606. Available from: https://dx.doi.org/10.1093/infdis/jiw195

32. Coffie PA, Tchounga BK, Bado G, Kabran M, Minta DK, Wandeler G, et al. Prevalence of hepatitis B and delta according to HIV-type: A multi-country cross-sectional survey in West Africa. BMC Infect Dis [Internet]. 2017 Jul 4 [cited 2025 Jun 19];17(1):1–8. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-017-2568-5

33. Teame G, Gebreyesus A, Tsegay E, Gebretsadik M, Adane K. Hepatitis B and C viral coinfections and their association with HIV viral load suppression among HIV-1 infected patients on ART at Mekelle hospital, northern Ethiopia. AIDS Res Ther [Internet]. 2022 Dec 1 [cited 2025 Jun 19];19(1):57. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9714192/

34. Metz M, Among VH, Dzinamarira T, Ussery F, Nkurunziza P, Bahizi J, et al. People Who Self-Reported Testing HIV-Positive but Tested HIV-Negative: A Multi-Country Puzzle of Data, Serology, and Ethics, 2015–2021. Trop Med Infect Dis 2024, Vol 9, Page 220 [Internet]. 2024 Sep 19 [cited 2025 Jun 25];9(9):220. Available from: https://www.mdpi.com/2414-6366/9/9/220/htm

PART D: APPENDICES

Appendix 1: QUESTIONERS AND DATA ABSTRACTION FORMS

Adult Questionnaire Codebook		
DEMOGRAPHIC CHARACTERISTICS	**SCALE OF MEASUREMENT**	**CATEGORIES**
Gender	Categorical	1 -Male 2 - Female
Age	Continuous	15, 16, 17, ..., 80
Ever Attended School	Categorical	1-YES, 2- NO, -8 -DON'T KNOW, -9 - REFUSED
Highest Level of School Attended	Categorical	1 - NONE 2 – PRIMARY/PREPRIMARY 3 – SECONDARY 4 – TERTIARY 5 – MISSING -
Nationality		1 - KENYAN 2 - UGANDAN 3 – TANZANIAN 4 – ETHIOPIAN 5 - RWANDA
Currently Married	Categorical	1 - MARRIED 2 - LIVING TOGETHER 3 - WIDOWED 4 - DIVORCED 5 - SEPARATED -8 - DON'T KNOW -9 - REFUSED
Number Of Partners	Continuous	integer
Male Circumcision	Categorical	1 - YES 2 - NO -8 - DON'T KNOW -9 - REFUSED
Number Of People They Had Sex Within the Last 12 Months	Continuous	INTEGER
Condom Use (Past 12 Months)	Categorical	1 - ALWAYS 2 - MOST OF THE TIME 3 - SOMETIMES
Beneficiary Sexual Relationships	Categorical	1 – YES 2 – NO -8 - DON'T KNOW -9 - REFUSED
HIV Tests as A Couple	Categorical	1 - YES 2 - NO -8 - DON'T KNOW

		-9 - REFUSED
Ever Tested for HIV	Categorical	1 - YES 2 - NO -8 - DON'T KNOW -9 - REFUSED
Awareness Of HIV (If They Know Their Status)	Categorical	1 - POSITIVE 2 - NEGATIVE 3 - UNCERTAIN/INDETERMINATE 4 - DID NOT RECEIVE THE RESULT -8 - DON'T KNOW -9 - REFUSED
Pregnancy	Categorical	1 - YES 2 – NO -8 - DON'T KNOW/UNSURE -9 - REFUSED
Taking Medicine Called Isoniazid (INH)	Categorical	1 – YES 2 - NO -8 - DON'T KNOW -9 - REFUSED
TB Clinic Visit	Categorical	1 - YES 2 – NO -8 - DON'T KNOW -9 - REFUSED
Ever Was Treated For TB	Categorical	1 - YES 2 – NO -8 - DON'T KNOW -9 - REFUSED
Urban Area Indicator	Categorical	1 - SMALL (50,000)/RURAL 2 - LARGE (>50,000)/URBAN
Wealth Quintile	Categorical	1 - LOWEST 2 - SECOND 3 - MIDDLE 4 - FOURTH 5 - HIGHEST 99 – MISSING
BIOMARKERS		
Active Syphilis Infection	Categorical	1 - HAS ACTIVE SYPHILIS INFECTION 2 - DOES NOT HAVE ACTIVE SYPHILIS INFECTION 99 - MISSING
HIV Status	Categorical	1 - HIV POSITIVE 2 - HIV NEGATIVE 99 - MISSING
Arvs Detected	Categorical	1 - ARVS DETECTED 2 - ARVS NOT DETECTED 99 - MISSING
Viral Load	Categorical	< LLOQ: 400 - LESS THAN LOWER LIMIT OF DETECTION OF 400

		< LLOQ: 40 - LESS THAN LOWER LIMIT OF DETECTION OF 40 < LLOQ: 20 - LESS THAN LOWER LIMIT OF DETECTION OF 20 TND - TARGET NOT DETECTED VALUES <= 10000000
Efavirenz In Blood	Categorical	1 - POSITIVE 2 - NEGATIVE
Hepatitis B Status	Categorical	1 – POSITIVE 2 - NEGATIVE 99 - MISSING
An Adult Is On ART	Categorical	1 - ON ART 2 - NOT ON ART 99 - MISSING
Duration Of Time On ART	Categorical	1 - ON ART 24 MONTHS OR MORE 2 - ON ART 12-23 MONTHS 3 - ON ART < 12 MONTHS 4 - NOT ON ART 99 - MISSING
Individual Has Their Viral Load Suppressed	Categorical	1 - VIRAL LOAD SUPPRESSED (ML) 2 - NOT VIRAL LOAD SUPPRESSED 99 - MISSING
Cd4 count	Continuous	CD4 COUNT: INSTRUMENT READING PREFERRED OVER ODK TRANSCRIPTION
Cd4 Categorical	Categorical	1 - CD4 LESS THAN 100 2 - CD4 GREATER THAN OR EQUAL TO 100 AND LESS THAN 200 3 - CD4 GREATER THAN OR EQUAL TO 200 AND LESS THAN 350 4 - CD4 GREATER THAN OR EQUAL TO 350 AND LESS THAN 500 5 - CD4 GREATER THAN OR EQUAL TO 500 99 - CD4 MISSING

Appendix 2: PHIA ACCESS TO DATA APPROVAL

Appendix 2: Files Request Approval

PHIA Data Manager <no-reply@mg.icap.columbia.edu> Fri, Mar 22, 2024 at 12:38 PM
To: mitikuhermanng@gmail.com

PHIA PROJECT | POPULATION-BASED HIV IMPACT ASSESSMENT
GUIDING THE GLOBAL HIV RESPONSE

We have approved your request to access files.

Your request:

Name: MITIKU TAMRE GENAMO
Email: mitikuhermanng@gmail.com
Country: MITIKU TAMRE GENAMO
Institution Name: University of Cape Town
Institution Type: University Faculty

Title: The burden of Hepatitis B among people living with HIV(PLWH) in East Afrika. The aim of this research project is to investigate the burden of Hepatitis B among individuals living with HIV in East Africa.

Description:
The objectives of the study are as follows: 1. To access the prevalence of Hepatitis B stratified by gender. 2. To investigate the risk factors associated with Hepatitis B 3. To examine whether the burden of hepatitis B differs by country. By looking at population-based HIV impact assessment datasets, I want to gain insight into the prevalence, risk factors, and geographical disparities in Hepatitis B among East African PLWH. Furthermore, I plan to use demographic data and biomarkers such as CD4 count to investigate potential associations with Hepatitis B infection. The study's findings will not only add to the existing information base but will also guide health initiatives aimed at reducing the burden of Hepatitis B among East African HIV patients. I assure you that all data accessible will be maintained at strict confidentiality and used only for the Academic purpose stated above. Future publications produced by this research will appropriately acknowledge the source of the data. I sincerely request your cooperation in granting access to the necessary datasets for my Master of Science in Epidemiology and Biostatistics dissertation project. If there are any procedures or protocols that must be followed

in order to gain access, please notify me, and I will make sure that all requirements are satisfied. Thank you for considering my request. I eagerly await your positive response and the opportunity to get involved in this important research project. I have looked at the data from Ethiopia which I requested previously, Initially, my focus was primarily on Ethiopia; however, after thorough consideration and reflection on the broader context of the topic(hepatitis B), I believe it is helpful for the analysis phase to include other East African countries (Ethiopia, Kenya, Tanzania, Rwanda, and Uganda) in the study. Kind regards, Mitiku Tamre
+27633080186

Files requested

KENPHIA 2018 Household Interview and Biomarker Datasets v1.1 (CSV).zip

RPHIA 2018-2019 Household Interview and Biomarker Datasets v1.1 (CSV).zip

THIS 2016-2017 Household Interview and Biomarker Datasets v2.0 (CSV).zip

UPHIA 2016-2017 Household Interview and Biomarker Datasets v1.1 (CSV).zip

EPHIA 2017-2018 Household Interview and Biomarker Datasets v1.1 (CSV).zip

EPHIA 2017-2018 Intermediary Weights v1.1 (CSV).zip

Thank you
PHIA Data Manager
ICAP at Columbia University

Appendix 3: UCT ETHICS APPROVAL

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

16 October 2024

HREC REF: 788/2024

Dr H Madlala
Division of Epidemiology & Biostatistics
Public Health FHS
Email: mdlhle004@myuct.ac.za
Student: mitikulermanng@gmail.com/gnmmit001@myuct.ac.za

Dear Dr Madlala

PROJECT TITLE: THE BURDEN OF HEPATITIS B AMONG PEOPLE LIVING WITH HIV IN EAST AFRICA: EVIDENCE FROM 2016–2019 POPULATION-BASED HIV IMPACT ASSESSMENT SURVEYS-
(MASTERS CANDIDATE-EPIDEMIOLOGY & BIOSTATISTICS-MR MITIKU TAMRE GENAMO)

Thank you for your response letter dated 10 September 2024, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 October 2025.

Please submit a progress form, using the standardised Annual Report Form (FHS016) or FHS017 if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Mr Mitiku Tamre Genamo will also be involved in this study.

Please quote HREC REF 788/2024 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007

HREC/ref 788/2024

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

TABLES FOR SUBGROUP ANALYSIS

Ethiopia

Table 4: Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Hepatitis B Virus (HBV) Infection, Subgroup analysis: Ethiopia

	Univariable logistic regression models			Multivariable logistic regression models		
	Intercept	OR (95% CI)	P value	Intercept	aOR (95% CI)	P value
DEMOGRAPHICS PREDICTORS						
Age (years)	6.91	1.03(0.99, 1.06)	0.15	3,23	1.06(0.99, 1.15)	0.13
Gender						
Male (reference)						
Female	11.75	1.70(0.80, 3.46)	0.15		3.11(0.73, 13.55)	0.12
Ever attended school						
Yes	16.29	1.06(0.41, 2.36)	0.90		0.68(0.15, 3.65)	0.62
No (reference)						
Educational level						
Primary (reference)						
Secondary	25.45	0.39(0.17, 0.89)	0.02*			
Tertiary		0.72(0.22, 3.26)	0.62			
Currently married						
Married (reference)						
Living together	22.27	0.61(0.15, 4.05)	0.53		1.25(0.20, 24.66)	0.84
Widowed		0.58(0.23, 1.55)	0.26		6760664	0.99
Divorced		0.96(0.35, 3.17)	0.97		0.85(0.15, 6.91)	0.87
Separated		0.63(0.16, 4.19)	0.56		8372632	0.99
Number of sexual partners (past 12 months)						
≤1(reference)						
>1	17.32	2456195.35	0.99			
Male circumcision						
Yes	3.00	4.43(0.60, 22.39)	0.09			
No (reference)						
Number of wives						
≤1 (reference						
>1	13.00	1203951	0.99			
Condom use (past 12 months)						
Yes	19.40	1.07(0.35, 3.99)	0.92		0.76(0.21, 3.08)	0.68
No(reference)						
Beneficiary sexual relationships						
Yes	18.86	2256164.29	0.99			
No (reference)						
HIV tested as a couple						
Yes	15.86	1.52(0.51, 4.56)	0.44		1.93(0.51, 7.14)	0.32
No(reference)						
Ever tested for HIV						
Yes	24.50	0.67(0.11, 2.32)	0.60		0.23(0.01, 2.06)	0.25
No(reference)						
Currently pregnant						
Yes	23.53	0.64(0.12, 11.90)	0.67			
No(reference)						
Taking isoniazid (INH)						
Yes	14.00	8260628	0.99			
No(reference)						
Currently on TB treatment						
Yes	14.5	7975778	0.99			
No (reference)						
TB clinic visit						

Yes	17.95	0.85(0.42, 1.81)	0.67		1.22(0.36, 4.90)	0.76
No(reference)						
Ever treated for TB						
Yes	4.00	3.91(0.19, 30.64)	0.25			
No(reference)						
Urban area indicator						
small(reference)						
Large	22.73	0.63(0.29, 1.29)	0.22		0.85 (0.24, 2.94)	0.80
Wealth quintile Status						
Lowest		0.61(0.21, 1.76)	0.35		0.30(0.05, 1.47)	0.14
Second	19.43	1.80(0.49, 8.51)	0.40		0.62(0.10, 3.69)	0.58
Middle(reference)						
Fourth		0.71(0.25, 1.89)	0.49		0.84(0.12, 7.11	0.76
Highest		0.92(0.30,2.93)	0.88		1.26(0.14, 27.22)	0.80
BIOMARKER PREDICTORS						
Active syphilis infection				11.85		
Yes	17.78	0.31(0.08, 2.05)	0.14		0.53(0.06, 11.74)	0.60
No (reference)						
ARVs detected						
Yes	13.09	1.45(0.66, 2.98)	0.33		2.20(0.32, 13.27)	0.40
No(reference)						
Viral load (copies/mL)						
≤ LLOQ: 200		0.68(0.26, 1.77)	0.42		0.48(0.16, 1.35)	0.17
< LLOQ: 1000	26.00	0.81(0.14, 15.25)	0.84		0.33(0.05, 6.61)	0.33
≥ LLOQ: 1000		0.43(0.18, 0.99)	0.05		NA	NA
TND – Target not detected (reference)						
Efavirenz In Blood						
Positive	17.94	0.90(0.45, 1.81)	0.77		0.51(0.16, 1.42)	0.22
Negative (reference)						
An Adult Is On ART						
Yes	20.00	0.88(0.05, 4.55)	0.90		0.52(0.02, 16.70)	0.68
No (reference)						
Duration Of Time On ART						
Not on ART (reference)						
≥24 months	17.67	1.05(0.44, 2.33)	0.91		0.63(0.03, 3.40)	0.66
12-23 months		0.28(0.07, 1.38)	0.08		0.21(0.01, 2.04)	0.21
< 12 months		1.30(0.23, 24.46)	0.81		NA	NA
Viral Load Suppressed						
Yes	11.13	1.95(0.96, 3.92)	0.06		4.31(0.89, 20.18)	0.06
No(reference)						
Cd4 count (cells per microliter (µL))	13.63	1.00(1.00, 1.00)	0.50		1.00(1.00, 1.01)	0.42
Cd4 Categorical (cells per microliter (µL))						
<100 (reference)						
≥100 and <200	9.00	3.33(0.38, 29.43)	0.24		1.44.(0.06, 19.91)	0.79
≥200 and <350		1.60(0.23, 6.87)	0.56		0.86(0.04, 8.12)	0.90
≥350 and <500		2.78(0.39, 13.14)	0.23		0.63(0.02, 9.08)	0.76
≥500		1.64(0.25, 6.47)	0.53		0.37(0.01, 10.47)	0.59

*p-value less than 0.05

Kenya

Table 5: Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Hepatitis B Virus (HBV) Infection, Subgroup analysis: Kenya

	Univariable logistic regression models			Multivariable logistic regression models		
	Intercept	OR (95% CI)	P value	Intercept	aOR (95% CI)	P value
DEMOGRAPHICS PREDICTORS						
Age (years)	22.28	1.00(0.98,1.02)	0.70	64.75	1.00(0.97, 1.04)	0.94
Gender						
Male (reference)						
Female	15.58	1.34(0.81, 2.15)	0.24		1.34(0.64, 2.77)	0.43
Ever attended school						
Yes	12.44	1.60(0.73, 3.14)	0.20		1.78(0.58,4.51)	0.26
No(reference)						
Educational level						
Primary (reference)						
Secondary		0.85(0.50, 1.49)	0.56			
Tertiary	21.44	NA	NA			
Currently married						
Married (reference)						
Living together	16.15	1.42(0.42, 8.89)	0.63		2.29(0.46, 41.68)	0.42
Widowed		1.22(0.68, 2.33)	0.52		1.63(0.57, 5.92)	0.41
Divorced		1.49(0.44, 9.27)	0.59		2628167	0.99
Separated		1.22(0.58, 3.01)	0.63		0.69(0.28, 1.98)	0.45
Number of sexual partners (past 12 months)						
≤1(reference)						
>1	18.81	0.91(0.45, 2.09)	0.81		1.06(0.46, 2.76)	0.90
Male circumcision						
Yes	9.08	2.42(1.08, 5.43)	0.03			
No(reference)						
Number of wives						
≤1(reference group)						
>1	13.84	0.77(0.25, 3.41)	0.69			
Condom use (past 12 months)						
Yes	20.52	0.79(0.45, 1.36)	0.39		0.84(0.45, 1.54)	0.57
No(reference)						
Beneficiary sexual relationships						
Yes	19.60	0.63(0.34, 1.28)	0.18		0.59(0.27, 1.37)	0.20
No (reference)						
HIV tested as a couple						
Yes	21.22	0.75(0.43, 1.30)	0.31		0.79 (0.42, 1.45)	0.45
No (reference)						
Ever tested for HIV						
Yes	24.67	0.76(0.18, 2.11)	0.65		0.43(0.02, 2.34	0.43
No (reference)						
Currently pregnant						
Yes	20.24	1.88(0.39, 33.66)	0.54			
No (reference)						
Taking isoniazid (INH)						
Yes	20.96	0.67(0.33, 1.49)	0.30			
No(reference)						
Currently on TB treatment						
Yes	16.36	7067426	0.99			
No (reference)						

TB clinic visit						
Yes	18.35	1.10(0.69, 1.82)	0.69		0.77(0.42, 1.45)	0.41
No(reference)						
Ever treated for TB						
Yes		0	0.99			
No(reference)						
Urban area indicator						
Urban (reference)						
Rural	22.58	0.76(0.46, 1.23)	0.28		0.74(0.35, 1.47)	0.40
Wealth quintile Status						
Lowest		0.46(0.23, 0.90)	0.03*		0.29(0.11,0.70)	0.008*
Second	28.08	0.72(0.34, 1.47)	0.37		0.48(0.18, 1.20)	0.13
Middle(reference)						
Fourth		0.75(0.34, 1.65)	0.46		0.58(0.19,1.75)	0.33
Highest		0.66(0.26, 1.78)	0.38		0.42(0.11,1.84)	0.22
BIOMARKER PREDICTORS						
Active syphilis infection				13.56		
Yes	19.39	0.31(0.08, 2.01)	0.13		0.30(0.08, 2.01)	0.13
No (reference)						
ARVs detected						
Yes	20.24	0.90(0.53, 1.48)	0.69		0.98(0.30, 2.75)	0.97
No(reference)						
Viral load (copies/mL)						
≤ LLOQ: 200	20.54	0.87(0.50, 1.53)	0.64		0.84(0.47, 1.51)	0.57
< LLOQ: 1000		0.72(0.29, 2.19)	0.52		0.60(0.22, 2.13)	0.37
≥ LLOQ: 1000		0.97(0.53, 1.79)	0.91		NA	NA
TND – Target not detected (reference)						
Efavirenz In Blood						
Positive	19.90	0.88(0.55, 1.39)	0.57		0.76(0.41, 1.37)	0.37
Negative (reference)						
An Adult Is On ART						
Yes	12.50	1.44(0.42, 3.67)	0.50		1.53(0.30, 7.44)	0.60
No (reference)						
Duration Of Time On ART						
Not on ART (reference)						
≥24 months	21.85	0.73(0.42, 1.23)	0.25		0.91(0.38, 1.90)	0.81
12-23 months		4.49(0.92, 80.97)	0.15		5.90(1.06, 110.39)	0.10
< 12 months		0.73(0.33, 1.80)	0.47		NA	NA
Viral Load Suppressed						
Yes	19.85	0.95(0.55, 1.57)	0.83		1.12(0.36, 3.03)	0.83
No(reference)						

*p-value less than 0.05

Uganda

Table 6: Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Hepatitis B Virus (HBV) Infection, Subgroup analysis: Uganda

	Univariable logistic regression models			Multivariable logistic regression models		
	Intercept	OR (95% CI)	P value	Intercept	aOR (95% CI)	P value
DEMOGRAPHICS PREDICTORS						
Age (years)	10.17	1.01(1.00, 1.03)	0.15	6.36	1.01(0.99, 1.04)	0.33
Gender						
Male (reference)						
Female	12.63	1.55(1.03, 2.32)	0.03*		1.56(0.88, 2.76)	0.13
Ever attended school						
Yes	21.22	0.76(0.35, 1.46)	0.45		1.06(0.45, 2.22)	0.88
No (reference)						
Educational level						
Primary (reference)						
Secondary	15.28	1.18(0.73, 2.00)	0.51			
Tertiary		1.20(0.52, 3.50)	0.70			
Currently married						
Married (reference)						
Living together	14.43	0.89(0.52, 1.58)	0.68		0.90(0.49, 1.73)	0.75
Widowed		1.49(0.79, 3.06)	0.25		1.05(0.35, 4.60)	0.93
Divorced		1.20(0.47, 4.05)	0.74		0.85(0.28, 3.74)	0.81
Separated		1.51(0.85, 2.83)	0.17		0.91(0.44, 1.99)	0.80
Number of sexual partners (past 12 months)						
≤1(reference)						
>1	16.23	1.23(0.69, 2.40)	0.51		1.54(0.79, 3.27)	0.23
Male circumcision						
Yes	15.57	0.59(0.32, 1.11)	0.10			
No (reference)						
Number of wives						
≤1 (reference)						
>1	11.68	1.15(0.51, 2.96)	0.75			
Condom use (past 12 months)						
Yes	15.17	1.24(0.72, 2.25)	0.45		1.21(0.68, 2.30)	0.54
No (reference)						
Beneficiary sexual relationships						
Yes	15.06	1.50(0.8, 3.14)	0.24		1.36(0.67, 3.08)	0.43
No (reference)						
HIV tested as a couple						
Yes	17.84	0.80(0.51, 1.25)	0.33		0.81(0.47, 1.39)	0.46
No (reference)						

Ever tested for HIV						
Yes	12.40	1.38(0.66, 2.59)	0.35		1.48(0.56, 3.43)	0.39
No (reference)						
Currently pregnant						
Yes	20.86	0.53(0.23, 1.41)	0.16			
No (reference)						
Currently on TB treatment						
Yes	12.40	0.16(0.04, 0.85)	0.02			
No (reference)						
TB clinic visit						
Yes	18.28	0.72(0.47, 1.14)	0.15		0.78(0.45, 1.41)	0.39
No(reference)						
Urban area indicator						
Urban (reference)						
Rural	15.13	1.16(0.76, 1.74)	0.48		1.77(0.95, 3.23)	0.07
Wealth quintile Status						
Lowest		0.42(0.21, 0.80)	0.01*		0.30(0.12, 0.68)	0.005*
Second	26.69	0.59(0.28, 1.21)	0.15		0.44(0.17, 1.06)	0.07
Middle(reference)						
Fourth		0.59(0.29, 1.15)	0.13		0.65(0.26, 1.52)	0.34
Highest		0.76(0.35, 1.57)	0.46		0.81(0.29, 2.17)	0.69
BIOMARKER PREDICTORS						
Active syphilis infection				34.22		
Yes	16.88	0.78(0.38, 1.89)	0.54		1.05(0.36, 4.46)	0.93
No (reference)						
ARVs detected						
Yes	16.95	0.98(0.64, 1.47)	0.91		0.27(0.08, 0.83)	0.03
No (reference)						
Viral load (copies/mL)						
≤ LLOQ: 200	31.24	0.50(0.27, 0.93)	0.03*		0.53(0.25, 1.07)	0.08
< LLOQ: 1000		0.41(0.17, 1.08)	0.05		0.33(0.12, 1.01)	0.04*
≥LLOQ: 1000		0.41(0.23, 0.70)	0.001		NA	NA
TND – Target not detected (reference)						
Efavirenz In Blood						
Positive	17.02	0.95(0.63, 1.45)	0.81		1.22(0.65, 2.26)	0.53
Negative (reference)						
An Adult Is On ART						
Yes	15.87	1.14(0.49, 2.32)	0.74		0.87(0.26, 2.98)	0.82
No (reference)						
Duration Of Time On ART						
Not on ART (reference)						
≥ 24 months	14.70	1.16(0.75, 1.80)	0.50		1.61(0.73, 3.35)	0.21

12-23 months		2.38(0.85, 9.93)	0.15	3.23(0.96,14.74)	0.08
< 12 months		0.80(0.42, 1.66)	0.52	NA	NA
Viral Load Suppressed					
Yes	12.78	1.60(1.07, 2.39)	0.02*	4.49(1.86, 10.99)	<0.001
No(reference)					
Cd4 count (cells per microliter (µL))	9.22	1.00(1.00, 1.00)	0.006*	1.00(1.00, 1.00)	0.44
Cd4 Categorical (cells per microliter (µL))					
<100 (reference)					
≥ 100 and <200	11.60	1.00(0.29, 3.15)	0.99	0.34(0.05, 1.76)	0.22
≥ 200 and <350		1.15(0.37, 2.96)	0.79	0.41(0.06, 1.86)	0.30
≥ 350 and <500		1.30(0.43, 3.27)	0.61	0.26(0.03, 1.26)	0.13
≥ 500		1.73(0.58, 4.1	0.27	0.28(0.03, 1.94)	0.23

*p-value less than 0.05

Rwanda

Table 7: Univariate and Multivariate Logistic Regression Analysis of Factors Associated with Hepatitis B Virus (HBV) Infection, Subgroup analysis: Rwanda

	Univariable logistic regression models			Multivariable logistic regression models		
	Intercept	OR (95% CI)	P value	Intercept	aOR (95% CI)	P value
DEMOGRAPHICS PREDICTORS						
Age (years)	54.46	0.98(0.95, 1.01)	0.28	14.85	0.99(0.93, 1.05)	0.74
Gender						
Male (reference)						
Female	13.95	3.63(1.77, 7.74)	< 0.001*		4.12(1.18, 17.16)	0.03
Ever attended school						
Yes	17.87	1.73(0.72, 3.78)	0.19		4.43(1.34, 13.92)	0.01*
No(reference)						
Educational level						
Primary (reference)						
Secondary	33.47	0.64(0.27, 1.69)	0.34			
Tertiary		1271110.39	0.99			
Currently married						
Married (reference)						
Living together	28.12	0.92(0.33, 2.53)	0.86		0.57(0.16, 1.99)	0.37
Widowed		0.85(0.29, 2.64)	0.77		2071051	0.99
Divorced		556492	0.99		68218616	0.99
Separated		1.16	0.82		0.86(0.10, 11.05)	0.90
Number of sexual partners (past 12 months)						
≤ 1(reference)						
>1	27.58	1.01(0.41, 3.02)	0.99		5.51(0.92, 107.98)	0.12
Male circumcision						
Yes	15.54	0.71(0.28, 1.94)	0.48			
No(reference)						
Number of wives						
≤ 1 (reference)						
>1	15.17	2805152.46	0.99			
Condom use (past 12 months)						
Yes	31.42	0.71(0.30, 1.71)	0.43		1.00(0.32, 3.36)	0.99
No (reference)						
Beneficiary sexual relationships						
Yes	26.80	2.09(0.42, 37.85)	0.48		0.53(0.04, 15.50)	0.66
No (reference)						
HIV tested as a couple						
Yes	22.60	1.38(0.57, 3.26)	0.46		1.81(0.43, 6.57)	0.39
No (reference)						
Ever tested for HIV						
Yes	26.50	1.05(0.17,3.60)	0.95		1.01(0.04, 10.18)	0.99
No (reference)						
Currently pregnant						
Yes	48.17	2401013	0.99			
No (reference)						
TB clinic visit						
Yes	32.28	0.66(0.33, 1.38)	0.26		0.97(0.32, 3.11)	0.96
No(reference)						
Urban area indicator						

Urban (reference)						
Rural	26.46	1.03(0.51, 2.19)	0.84		0.52(0.12, 1.99)	0.36
Wealth quintile Status						
Lowest		2.33(0.49, 16.45)	0.32		2.23(0.19, 51.34)	0.53
Second	29.20	2.07(0.44, 14.65)	0.39		1.58(0.14, 35.93)	0.72
Middle(reference)						
Fourth		0.80(0.24, 2.37)	0.70		0.33(0.05,1.56)	0.20
Highest		0.66(0.21, 1.77)	0.44		0.38(0.04, 2.33)	0.31
BIOMARKER PREDICTORS						
ARVs detected						
Yes	27.29	1.02(0.40, 2.27)	0.97	13.36	0.52(0.07, 3.03)	0.49
No (reference)						
Viral load (copies/mL)						
≤ LLOQ: 200	45.00	0.50(0.20, 1.20)	0.13		0.44(0.15, 1.22)	0.12
< LLOQ: 1000		0.14(0.04, 0.57)	0.003		0.09(0.02, 0.43)	0.001
≥ LLOQ: 1000		0.66(0.23, 1.90)	0.42		NA	NA
TND – Target not detected (reference)						
Efavirenz In Blood						
Positive	29.75	0.86(0.42, 1.76)	0.68		0.99(0.37, 2.56)	0.98
Negative(reference)						
An Adult Is On ART						
Yes	12.00	2.36(0.37, 8.67)	0.26		1.02(0.10, 9.34)	0.98
No (reference)						
Duration Of Time On ART						
Not on ART (reference)						
≥ 24 months	25.80	1.21(0.53, 2.63)	0.64		3.00(0.87, 8,92)	0.06
12-23 months		4482511.36	0.98		9838559	0.99
< 12 months		0.35(0.12, 1.16)	0.07		NA	NA
Viral Load Suppressed						
Yes	29.57	0.92(0.36, 2.05)	0.85		2.66(0.51, 11.55)	0.21
No(reference)						

*p-value less than 0.05

PLOS ONE Journal instructions to authors

Submission Guidelines

Related information for authors

- PLOS Writing Center
- Submission system
- Journal scope and publication criteria
- Getting started guide
- Guidelines for revisions
- Publication fees
- APC Support

PLOS encourages all authors to post a preprint of their submitted manuscript.

File format	Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use a standard font size and any standard font, except for the font named “Symbol”. To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses “Vancouver” style, as outlined in the ICMJE sample references. See reference formatting examples and additional instructions below.
Equations	We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable. Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., “$a^2 + b^2 = c^2$”), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values. Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid “hybrid” inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.
Nomenclature	Use correct and established nomenclature wherever possible. *Units of measurement*: Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units. *Drugs*: Provide the Recommended International Non-Proprietary Name (rINN).

Species names	mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HGNC for human genes; we strongly recommend using this tool to check against previously approved names). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior to manuscript publication. Examples of the systematic allergen nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

Copyediting manuscripts

Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like “scientific editing service” or “manuscript editing service.”

Submissions are not copyedited before publication.

Submissions that do not meet the *PLOS ONE* publication criterion for language standards may be rejected.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* › Title page: List title, authors, and affiliations as first page of the manuscript › Abstract › Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* › Materials and Methods › Results › Discussion › Conclusions (optional)
Ending section	*The following elements are required, in order:* › Acknowledgments › References › Supporting information captions (if applicable)

- Tables are inserted immediately after the first paragraph in which they are cited.
- Supporting information files are uploaded separately.

Refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

- Download sample title, author list, and affiliations page (PDF)
- Download sample manuscript body (PDF)

Viewing Figures and Supporting Information in the compiled submission PDF
The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

PLOS encourages all authors to post a preprint of their submitted manuscript.

- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled "current address." At a minimum, the address must include the author's current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

Corresponding author

The submitting author is automatically designated as the corresponding author in the submission system. The corresponding author is the primary contact for the journal office and the only author able to view or change the manuscript while it is under editorial consideration.

The corresponding author role may be transferred to another coauthor. However, note that transferring the corresponding author role also transfers access to the manuscript. (To designate a new corresponding author while the manuscript is still under consideration, watch the video tutorial below.)

Only one corresponding author can be designated in the submission system, but this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication. Include an email address for each corresponding author listed on the title page of the manuscript.

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include its name in the manuscript byline. Do not add it to the author list in the submission system. You may include the full list of members in the Acknowledgments or in a supporting information file.

PubMed only indexes individual consortium or group author members listed in the article byline. If included, these individuals must qualify for authorship according to our criteria.

Read the group authorship policy.

Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

- Summarize the study's contribution to the scientific literature
- Relate the study to previously published work
- Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)
- Describe any prior interactions with PLOS regarding the submitted manuscript
- Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)

PLOS encourages all authors to post a preprint of their submitted manuscript.

IMPORTANT: Do not include requests to reduce or waive publication fees in the cover letter. This information will be entered separately in the online submission system.

Read about publication fee assistance.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Download our sample title, author list, and affiliations page (PDF)

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Supporting reproducibility with protocols

To enhance the reproducibility of your results, we recommend and encourage you to make your protocols public. There are several options:

Protocols associated with Research Articles

Protocol documents may be uploaded as Supporting Information or linked from the Methods section of the article. For laboratory protocols, we recommend protocols.io. Include the DOI link in the Methods section of your manuscript using the following format: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]. This allows editors and reviewers to consult the detailed step-by-step protocol when evaluating your manuscript. You can choose to keep the protocol private on the protocols.io platform until your article is published—at which time it will be published automatically.

Protocols published in their own right

PLOS encourages all authors to post a preprint of their submitted manuscript.

projects. Specific guidelines apply to the submission of Lab Protocol and Study Protocol manuscripts. Read the detailed instructions for submitting Lab Protocols and Study Protocols.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
- Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article's retracted status in your article's text and reference list.

Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article's submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09

Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an “S” and number. For example, “S1 Appendix” and “S2 Appendix,” “S1 Table” and “S2 Table,” and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

Read the guidelines for figures and requirements for reporting blot and gel results.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and “Figure” abbreviated to “Fig” (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of “Fig 1” must refer to a figure file named “Fig1.tif”).
- A concise, descriptive title

The caption may also include a legend as needed.

PLOS encourages all authors to post a preprint of their submitted manuscript.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., “Table 1”) and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

Statistical reporting

Manuscripts submitted to *PLOS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- SAMPL guidelines, for general guidance on statistical reporting
- *PLOS ONE* guidelines, for clinical trials requirements
- *PLOS ONE* guidelines, for systematic review and meta-analysis requirements
- EQUATOR, for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
- Describe technical details or procedures required to reproduce the analysis
- Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
- For pre-processed data:
  - Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).
  - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
- Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).
- For step-wise multiple regression analyses:
  - Report the alpha level used
  - Discuss whether the variables were assessed for collinearity and interaction

- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

- **Units of measurement.** Clearly define measurement units in all tables and figures.
- **Properties of distribution.** It should be clear from the text which measures of variance (standard deviation, standard error of the mean, confidence intervals) and central tendency (mean, median) are being presented.
- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.
- **Reporting parameters.** Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.
- **P-values.** Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.
- **Displaying data in plots.** Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.
- **Open data.** As explained in PLOS's Data Policy, be sure to make individual data points, underlying graphs and summary statistics available at the time of publication. Data can be deposited in a repository or included within the Supporting Information files.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

See instructions on providing underlying data to support blot and gel results.

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a “Striking Image” that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

- Specific grant numbers
- Initials of authors who received each award
- Full names of commercial companies that funded the study or authors
- Initials of authors who received salary or other funding from commercial companies
- URLs to sponsors’ websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

- Study design
- Data collection and analysis
- Decision to publish
- Preparation of the manuscript

PLOS encourages all authors to post a preprint of their submitted manuscript.

If the study was unfunded, include this sentence as the Financial Disclosure statement: “The author(s) received no specific funding for this work."

Read our policy on disclosure of funding sources.

Competing interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Read our policy on competing interests.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into “parts.” Each submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Read our policies on related manuscripts.

Preprints

PLOS is committed to accelerating the dissemination of research, and encourages authors to support this important goal by posting a preprint of their manuscript. Posting a manuscript on a preprint server does not impact its consideration at any PLOS journal.

Authors submitting manuscripts in the life and health sciences to PLOS ONE may choose to have PLOS forward their submission to bioRxiv or medRxiv for consideration for posting as a preprint. This free and convenient service is offered during the submission process to authors who have not already posted a preprint. Please visit bioRxiv or medRxiv for information about the scope of each server.

Authors posting preprints on bioRxiv or medRxiv can also choose to concurrently submit their manuscripts to relevant PLOS journals through the direct transfer service.

Read more about preprints.

Learn how to post a preprint to bioRxiv or medRxiv at *PLOS ONE*.

Study design, reporting, and analyses are assessed against all relevant research and methodological technique standards held by the community. Guidelines for specific study types are outlined below.

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the PLOS Data policy
- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form here. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s)**. If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:

- How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Manuscripts describing observational clinical studies are subject to all policies regarding human research and community standards for reporting observational research as outlined by the STROBE statement. Furthermore, authors submitting work of this nature should pay special attention to the following requirements:

- If the submitted manuscript is very similar to previous work, authors must provide a sound scientific rationale for the submitted work and clearly reference and discuss the existing literature.
- The sampling strategy and eligibility criteria of enrolled subjects should be described in sufficient detail.
- Sample size calculations should be justified with relevant inputs defined.
- Independent and dependent variables considered for statistical analysis should be clearly defined and justified.
- The validity and reliability testing of self-developed data collection tools should be reported.
- Conclusions should be appropriate for the study design, with indications on how the study results will contribute to the base of academic knowledge.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

> *A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.*

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- The name of the registry and the registration number must be included in the Abstract.
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

Lab Protocols consist of two interlinked components: a step-by-step protocol hosted on protocols.io, and a peer-reviewed article in *PLOS ONE* that contextualises the protocol.

protocols.io is a secure platform for developing and sharing reproducible methods. It enables scientists to make, exchange, improve, and discuss protocols for specific experimental procedures. The platform provides specialized tools for communicating technical details, including reagents, measurements, and formulae.

The *PLOS ONE* article component must comply with the general *PLOS ONE* submission guidelines (detailed above) and criteria for publication. In addition, the *PLOS ONE* article component should:

- Describe the value that the protocol adds to the published literature. **Lab Protocols describing routine methods or extensions and modifications of routine methods that add little value to the published literature will not be considered for publication.**
- Provide evidence that the protocol works, by either:
  - Linking, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.
    or
  - Providing validation or benchmarking data, which demonstrates that the underlying method achieves its intended purpose.
- Provide the step-by-step protocol as a supporting information (S1) file.

Download a Lab Protocol article template

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to *PLOS ONE*. **Posting your protocol prior to submission is not considered prior publication by *PLOS ONE* and will not affect your eligibility to publish a Lab Protocol.**

Authors submitting a Lab Protocol can also use protocols.io's protocol entry service at no cost: the team at protocols.io will enter your protocol for you and format it in a way that takes advantage of the platform's features. You will have an opportunity to review and make further changes before your protocol is shared with anyone else.

If you would like to use protocols.io's protocol entry service in connection with a Lab Protocol submission, please contact plosone@plos.org to request the customer code.

If you prefer to submit your manuscript to *PLOS ONE* before posting your protocol to protocols.io, then you must still provide your step-by-step protocol as a supporting information (S1) file in a format of your choosing. You will be expected to replace this file with a protocols.io PDF later in the editorial process.

Study Protocols

Study Protocols describe plans for conducting research projects and consist of a single article on *PLOS ONE*.

PLOS encourages all authors to post a preprint of their submitted manuscript.

- Relate to a research study that has not yet generated results.
- Be submitted before recruitment of participants or collection of data for the study is complete.
- Meet the same standards for ethics of experimentation and research integrity as the research study. If it involves human or animal subjects, cell lines or field sampling, or has potential biosafety implications, prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

- Clinical trials:
  - The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors).
  - The name of the registry and the trial or study registration number must be included in the Abstract.
  - A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an additional English translation if the original document is not in English. Please note that the protocol will be published with the manuscript if accepted.
  - A SPIRIT schedule of enrollment, interventions, and assessments must be included as the manuscript's Figure 1, and a completed SPIRIT checklist must be uploaded as Supporting Information file S1.
- Systematic reviews and meta-analyses:
  - A completed PRISMA-P checklist must be provided as a supporting information (SI) file. See PRISMA-P Explanation and Elaboration for more information on completing your checklist.

Study Protocols must also comply with general *PLOS ONE* criteria for publication and in addition you should:

- include the word "Protocol" in your Title.
- include a detailed description of the planned study in the Materials and Methods section.This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:
  - the aim, design, and settling
  - the sample size calculation
  - how data saturation will be determined (for qualitative studies)
  - the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained
  - how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured
  - the data management plan
  - safety considerations
  - the type of data and statistical analyses to be used
  - the status and timeline of the study, including whether participant recruitment or data collection has begun
  - where and when the data will be made available. See our Data Availability policy for more.
- include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.
- we encourage authors you to register with OSF and provide the your registration number in the Materials and Methods section. This is optional.
- optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the supporting information guidelines for more details about adding SI files.

Download our sample Study Protocol template or an OSF discipline or study-specific template.

Study Protocols are subject to the same editorial and peer review process as all other articles, and are eligible for both signed and published peer review.

You can expedite the review process by providing:

- proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved by this body.

The proof of external funding and approval or waiver letter are used for internal purposes and do not form part of the published Study Protocol.
Expedited review is conducted by an internal Staff Editor only and bypasses the external review process.

If the Study Protocol describes a replication study or involves re-analysis of published work, we will invite the author of the initial or replicated study to provide a signed review.

We encourage you to share your Study Protocol with other researchers, either before or after submission. You can publish it on your website or protocols.io, or submit it for posting on medRxiv or another preprint server.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research*, including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. Please see the NC3Rs guidelines for more information. Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-Neves, I. Anna S. Olsson (2012) How "Humane" Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behavior were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLOS ONE*'s data availability criterion.
- **Ethics.** *PLOS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses should include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

See our reporting guidelines for human subjects research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLOS ONE's* requirements for reporting blot and gel results and providing the underlying raw images.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.

PLOS encourages all authors to post a preprint of their submitted manuscript.

We encourage authors to consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to initial submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility
The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation
Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability
If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

hosting site from within the paper.

Read the PLOS policy on sharing materials, software and code.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called “Nomenclatural Acts”:

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: LOCKSS [author to insert any additional repositories].

All PLOS articles are deposited in LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

PLOS encourages all authors to post a preprint of their submitted manuscript.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

> The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.
>
> In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (LOCKSS etc)].

All PLOS articles are deposited in LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

> ***Hymenogaster huthii.*** Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording. Note that this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum using the prefix http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=.

> The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.
>
> In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (LOCKSS etc)].

your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist or Standards for reporting qualitative research (SRQR) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

You may be eligible for APC support

Many institutional partners globally have publishing agreements with PLOS to allow their corresponding authors to publish with reduced or no APCs. To determine if your corresponding author is eligible, please visit our institutional partners page to determine what kind of agreement your institution has with PLOS.

If your corresponding author is affiliated with a participating institution, they must follow the instructions below to demonstrate eligibility.

Read the full instructions for submitting to a journal with the Flat Fee Agreement.

If your corresponding author is not from a participating institution and requires assistance paying publishing fees, please consider applying for a fee waiver at submission.

PART E: POLICY BRIEF

PART E: POLICY BRIEF

© University of Cape Town, School of Public Health, Epidemiology and Biostatistics Unit
E-mail: mitikuhermanng@gmail.com , gnnmit001@myuct.ac.za

Addressing the Dual Burden of Hepatitis B and HIV Co-Infection in East Africa: Implications for National Health Strategies

Prepared for: Department of Health / National Government
Date: 05/02/2025
Prepared by: Mitiku Tamre Genamo

This policy brief examines the burden of coinfections in East Africa.

The policy brief:

(i) Contextualizes the burden of HIV_HBV coinfections in East Africa;

(ii) Maps out the cascade from exposure and diagnosis to treatment outcomes;

(iii) Assesses the role of demographic and clinical risk factors of coinfection prevalence and management; and

(iv) Recommends strategies for strengthening integrated care and prevention programs.

What's at Stake?

Hepatitis B Virus (HBV) infection is a significant global health issue among people living with HIV (PLWH) (1). The developing region of Africa ranks second only to Asia in terms of the prevalence of chronic HBV infections, with around 60 million cases reported in 2017 (2). In countries like Kenya, the infection rates surpass 5%, and Africa accounts for 70% of all new cases of HBV infections globally. Despite substantial improvements in HIV treatment, HBV co-infection is still underdiagnosed and undertreated (2). In East Africa, HBV-HIV coinfection is a serious public health concern. The co-occurrence of chronic Hepatitis B virus (HBV) infection could accelerate the evolution of liver disease, resulting in cirrhosis and hepatocellular malignancies, regardless of how HIV affects immune function (3–5). Despite similar transmission channels, standard HBV screening in HIV patients is inconsistent, resulting in delayed diagnoses and inadequate treatment outcomes. Coinfection also makes antiretroviral therapy (ART) decisions

more difficult because of possible drug-drug interactions and hepatotoxicity issues, which puts more strain on already overburdened health systems. The ramifications go beyond personal health. Coinfection between HBV and HIV raises morbidity and mortality rates, lowers quality of life, and raises healthcare expenses. This policy brief highlights the need to implement integrated healthcare measures for dealing with the combined burden of HIV and HBV in East Africa.

Key Messages/findings:

1. **High Prevalence of HBV-HIV Co-Infection:** 5.02% of people living with HIV (PLWH) in East Africa are co-infected with Hepatitis B Virus (HBV), with variation across countries (3.49% in Rwanda. 4.29% in Tanzania, 4.99% in Kenya, 5.54% in Ethiopia and 5.67% in Uganda)
2. **Vulnerable Populations:** Females exhibit 1.78 times higher odds of HBV positivity compared to males. Poorer households have paradoxically lower odds of HBV infection, potentially due to variations in healthcare access and community transmission dynamics.
3. **Health Complications:** Individuals living with both HIV and HBV face increased risks of severe liver disease, higher HIV viral loads, and reduced CD4 counts, contributing to elevated morbidity and mortality.
4. **Protective Role of ART:** Viral suppression through antiretroviral therapy (ART) significantly reduces the risk of HBV infection, underscoring the importance of integrated HIV-HBV treatment protocols.
5. **Policy and research Gaps:** There is limited data, screening and vaccination for HBV among PLWH in East Africa, contributing to the high prevalence and burden of disease.

Research overview

This study was approved by the Human Research Ethics committee (HREC) of University of Cape Town (HREC REF 788/2024). It is based on a synthesis of secondary data from nationally representative health surveys (PHIA) and cross-sectional studies conducted in the East African region (Ethiopia, Kenya, Rwanda, Tanzania and Uganda). Data for the primary study was collected from 2016 – 2019 and for the secondary data analysis it was extracted from the PHIA dataset between April 2024 – August 2024. HIV testing was done using the national rapid diagnostic testing protocol and confirmed the results in the laboratory. Structured in-person interviews were conducted using the adult questionnaire to eligible participants aged 15 and older.

Policy Recommendations:

1. **Integrate HBV Screening with HIV Services:**

    - Require all PLWH to undergo routine HBV screening at ART clinics.
    - Utilize rapid diagnostic tests for efficient HBV detection.

2. **Expand HBV Vaccination Programs:**

    - Implement universal HBV vaccination for PLWH, focusing on high-prevalence areas.
    - Introduce catch-up vaccination programs for adults at risk.

3. **Strengthening ART Programs for Dual Management:**

    - Ensure ART regimens include HBV-active agents (e.g., Tenofovir).
    - Monitor viral load suppression as a key performance indicator for HBV-HIV co-management.

4. **Enhance Public Health Education:**

    - Develop targeted campaigns addressing gender disparities and promoting safe practices to prevent HBV transmission.
    - Raise awareness about the importance of early screening and treatment adherence.

5. **Invest in Data and Research:**

    - Conduct nationwide HBV prevalence studies among PLWH to identify regional disparities.
    - Investigate socioeconomic factors influencing HBV transmission dynamics.

Conclusion:

The dual burden of HBV and HIV co-infection in East Africa is a serious public health concern, affecting 5.02% of PLWH. Clinical and demographic indicators such as viral suppression, gender, and economic condition often overlap, which emphasizes the necessity of integrated healthcare approaches. To improve health outcomes for vulnerable groups, public health systems may significantly reduce the effect of HBV-HIV co-infection by giving priority to dual treatment protocols, screening, national vaccination programs and national level research on coinfection.

Reference

1. Leumi S, Bigna JJ, Amougou MA, Ngouo A, Nyaga UF, Noubiap JJ. Global Burden of Hepatitis B Infection in People Living With Human Immunodeficiency Virus: A Systematic Review and Meta-analysis. Clin Infect Dis [Internet]. 2020 Dec 1 [cited 2024 Nov 4];71(11):2799–806. Available from: https://pubmed.ncbi.nlm.nih.gov/31813969/

2. Makokha GN, Zhang P, Hayes CN, Songok E, Chayama K. The burden of Hepatitis B virus infection in Kenya: A systematic review and meta-analysis. Front Public Heal. 2023;11(1).

3. Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Gastroenterol Hepatol. 2022 Sep 1;7(9):796–829.

4. Tesfu MA, Habtemariam TT, Berhe Belay N. Risk factors associated with Hepatitis B virus infection among pregnant women attending public hospitals in Addis Ababa, Ethiopia. PLoS One [Internet]. 2023 Apr 1 [cited 2025 Jan 23];18(4):e0284646. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0284646

5. Chihota B V., Wandeler G, Chilengi R, Mulenga L, Chung RT, Bhattacharya D, et al. High Rates of Hepatitis B Virus (HBV) Functional Cure Among Human Immunodeficiency Virus-HBV Coinfected Patients on Antiretroviral Therapy in Zambia. J Infect Dis [Internet]. 2019 Dec 1 [cited 2025 Jan 28];221(2):218. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7184905/