Diagnosis, Treatment and Prevention of Hepatitis B Virus Infection in Kilifi, Kenya.

Louise Downs

Exeter College

University of Oxford

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List of Abbreviations

aFP	Alfa-fetoprotein
AHB	Acute hepatitis B
AHF	AIDS healthcare foundation
AHRI	African Health Research Institute
AIDS	Acquired immunodeficiency syndrome
ALP	Alanine phosphatase
ALT	Alanine aminotransferase
ANC	Antenatal care
Anti-HBc	Antibodies against hepatitis B core antigen
Anti-HBe	Antibodies against hepatitis B ‘e’ antigen
Anti-HBs	Antibodies against hepatitis B surface antigen
APRI	Aspartate transaminase to platelet ratio index
AST	Aspartate transaminase
CBC	Complete blood count

CCC	Comprehensive care clinic
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
CHP	Community health promotors
CLEIA	Chemiluminescent immunosorbent assay
CME	Continuing medical education
DNA	Deoxyribose nucleic acid
DRC	Democratic Republic of Congo
dsDNA	Double stranded DNA
EAC	East African Community
EASL	European Association for the Study of the Liver
ELISA	Enzyme linked immunosorbent assay
EPI	Expanded programme for immunisation
ETV	Entecavir
FDA	Food and drug administration
FGD	Focus group discussions
FIB-4	Fibrosis 4 score
FNA	Fine needle aspiration
GAVI	Global Alliance for Vaccines and Immunisations
GGT	Gamma glutyl transferase
GPC	General population cohort, Uganda.
GVAP	Global Vaccine Action Plan
HBcAg	Hepatitis B core antigen
HBcrAg	Hepatitis B core related antigen
HBeAg	Hepatitis B ‘e’ antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBx	Hepatitis B x-gene
HCC	Hepatocellular carcinoma
HCW	Healthcare worker
HepB-BD	Hepatitis B birth dose vaccine
HIV	Human immunodeficiency virus
INR	International normalised ratio

KCRH	Kilifi county referral hospital
KEMRI	Kenya medical research institute
KEMSA	Kenya Medical Supplies Authority
KES	Kenyan shillings
KHDSS	Kilifi Health and Demographic Surveillance System
KNBTS	Kenya national blood transfusion service
KWTRP	KEMRI-Wellcome Trust Research Programme
LFT	Liver function tests
LMICs	Low- and middle-income countries
MAFFT	Multiple alignment
MCH	Maternal child health
MRC	Medical research council
mRNA	Messenger RNA
MSM	Men who have sex with men
MV	Multivariable
NA	Nucleoside analogue
NAAT	Nucleic acid amplification test
NGS	Next generation sequencing
NHIF	National health insurance fund
NIHR	National institute for health research
PPV	Positive predictive value
NSI	Needlestick injury
NTCP	Sodium taurocholate co-transporting polypeptide
OBI	Occult hepatitis B infection
ONT	Oxford Nanopore Technologies
OUHFT	Oxford University Hospitals NHS Foundation Trust
OxTREC	Oxford tropical network ethics committee
pgRNA	Pre-genomic RNA
PLWHB	People living with HBV
PLWHIV	People living with HIV
PMTCT	Prevention of mother to child transmission
POCT	Point of care test
Pol	Polymerase gene

PPV	Positive predictive value
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PWID	People who inject drugs
qPCR	Quantitative polymerase chain reaction
RAM	Resistance association mutation
rcDNA	Relaxed circular DNA
RDT	Rapid diagnostic test
RNA	Ribonucleic acid
RPHA	Reverse passive haemagglutination
RT	Reverse transcriptase
RT-PCR	Real time polymerase chain reaction
SDGs	Sustainable development goals
SERU	Scientific ethical review unit
SNP	Single nucleotide polymorphism
SOP	Standard operation procedure
SRQR	Standards for Reporting Qualitative Research
StaRI	Standards for Reporting Implementation Studies
TDF	Tenofovir disoproxil
TE	Transient elastography
TWG	Technical working group
UKRI	UK Research and Innovation
ULN	Upper limit of normal
UN	United nations
USA	United states of America
USAID	United states agency for international development
USD	United States dollars
UV	Univariable
VL	Viral load
WHO	World Health Organisation
WHO-AFR	WHO African region

Declaration of Contribution

I hereby declare that the work undertaken and described in this thesis is my own work. I designed, set up and undertook both the STRIKE-HBV and the HepB-Boost study myself. I provided education and training for all those involved in the studies. I designed focus group discussions including the interview guides and trained those undertaking the discussions. Study recruitment for STRIKE-HBV along with the focus group discussions themselves were primarily undertaken by STRIKE-HBV staff due to the need to speak fluent Kiswahili, however I was present for all activities, answered questions as they arose and provided clinical input where needed. With training from Dorcas Okanda and other members of the KWTRP laboratories, I undertook all laboratory assays including whole genome sequencing and data analysis myself. None of the material presented in this thesis has been published or written by another person, nor submitted for the award of any other degree or diploma at the University of Oxford or any other institution. Where the work has been undertaken in collaboration with others, this has been clearly stated throughout and all sources have been acknowledged as references in the text.

Peer reviewed publications arising from this work

Downs LO 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 Health. 2023;3(1):e0001165.

Downs LO et al. Where do those data go? Reuse of screening results from clinical trials to estimate population prevalence of HBV infection in adults in Kilifi, Kenya. J Virus Erad. 2023;9(4):100355.

Downs LO et al. STRIKE-HBV: establishing an HBV screening programme in Kilifi, Kenya-challenges, successes and lessons learnt. Sex Transm Infect 2024; Available from: http://dx.doi.org/10.1136/sextrans-2024-056163.

Downs LO et al. Hepatitis B core-related antigen (HBcrAg) point-of-care tests as a risk stratification tool for treatment eligibility: experience from Kenya, Open Forum Infectious Diseases, 2025;, ofaf125, https://doi.org/10.1093/ofid/ofaf125.

Downs LO. Is a test better than no test when there is no treatment? J Clin Invest. 2023 Jul 17;133(14):e173286. doi: 10.1172/JCI173286. PMID: 37463452; PMCID: PMC10348760.

A list of publications not directly resulting from the work in this thesis but closely related to my PhD is presented in chapter 6.5.

Abstract

Hepatitis B virus (HBV) infection is a global health disaster with an estimated 254 million people chronically infected worldwide leading to over 1 million deaths annually, despite cheap, effective treatment and prevention measures being available. The WHO African Region (WHO-AFR) is particularly affected with over 700,000 new HBV infections occurring in 2022. Unfortunately HBV research in WHO-AFR has historically been poorly funded, and many countries lack access to even basic diagnostics and treatment. Kenya has no viral hepatitis national strategic plan, minimal routine HBV testing, and no guidelines in line with the World Health Organisation. This thesis presents analyses undertaken to understand the burden of HBV infection in Kenya, along with several real world studies implementing HBV testing, vaccination and qualitative work in a County Hospital in Kenya.

HBV prevalence was estimated using systematic review and meta-analyses of existing studies reporting HBV prevalence in Kenya, along with analysing clinical trial data from KEMRI-Wellcome Trust Research Programme. This showed HBV prevalence varies dramatically depending on the population screened, and that there is a large amount of information that could be gleaned from clinical trials, but is not routinely collated or reported.

The STRIKE-HBV study tested adults attending Kilifi County Referral Hospital (KCRH) for HBV, and undertook liver health assessment on 200 individuals testing HBsAg positive and negative. A family history of liver disease and body scarification were identified as risk factors for HBsAg positivity. Next generation HBV sequencing showed closely related familial HBV sequences along with potential tenofovir resistance mutations. Focus group discussions illustrated minimal HBV knowledge prior to the STRIKE-HBV study, along with significant confusion between HBV and HIV. Stigma and economic factors were identified as the biggest barriers to accessing HBV care.

The HepB-Boost study showed around two thirds of healthcare workers at KCRH were unvaccinated against HBV and that implementing free HBV vaccination at KCRH was feasible and well received.

This work has clinical relevance both in Kenya and further afield. It has generated the most comprehensive description of a population living with HBV in WHO-AFR and has demonstrated ways in which clinical care pathways can be improved. It highlights avenues for novel research into HBV

genomics and transmission pathways and illustrates the urgent need for better investment in HBV clinical studies in WHO-AFR.

1. BACKGROUND AND INTRODUCTION

My DPhil focusses on Hepatitis B virus infection (HBV), a significant global health problem that is under-researched and under-funded. I began my DPhil in Oxford in October 2021, then relocated to Kilifi, Kenya with my husband and children, to work at Kenya Medical Research Institution (KEMRI)-Wellcome Trust Research Programme (KWTRP) in August 2022. My DPhil started out to be a deep dive into the biomarkers and genomics associated with poor outcomes in HBV, and whilst this is still the overriding theme, on beginning work at Kilifi County Referral Hospital (KCRH), I realised far bigger challenges were being faced on the ground. There was minimal knowledge and understanding of HBV, considerable stigma attached to a diagnosis and often a lack of access to even basic diagnostics and vaccines. Alongside this, World Health Organization (WHO) Guidelines around treatment eligibility for HBV changed during my DPhil. This work therefore evolved to be a much broader evaluation of where the challenges lie and how programmes can be implemented to improve care. Throughout chapter one, I will first describe the broad background of HBV and then hone down into the situation in the WHO-African Region (WHO-AFR) and Kenya more specifically. I aim to highlight where the challenges lie and where the evidence gaps are, to justify the different sections of work I have undertaken.

1.1 Broad Overview

Hepatitis B virus (HBV) is a hepatotropic virus, infecting hepatocytes and is responsible for causing acute and chronic hepatitis B infection (AHB and CHB respectively) in humans. It was first discovered by Baruch Blumberg in 1967, although the symptoms of HBV were described in literature for decades before this (1). In chronic infection, HBV establishes a persistent viral reservoir in the liver and can cause progressive liver disease and primary liver cancer (hepatocellular carcinoma, HCC).

HBV is transmitted through contact with blood or bodily fluids from an infected person, most commonly from mother to child at birth. The age of primary infection usually determines whether infection is acute or chronic, with the risk of development of chronic infection being inversely

proportional to the age of exposure. Most people living with CHB as adults were infected as babies or young children.

In 2022, an estimated 254 million people were living with CHB worldwide (PLWHB) (2), disproportionally in low to middle income countries (LMICs). CHB has been identified by the WHO Sustainable Development Goals (SDGs) for elimination as a public health threat by 2030 (3), however the Global Hepatitis Report 2024 illustrates that most specific targets are off track in many settings. Deaths from HBV are continuing to rise globally, increasing from approximately 800,000 deaths in 2019 to one million deaths in 2022 (2). There is also a considerable mismatch between numbers living with CHB, and numbers diagnosed, estimating in the WHO-AFR only around 4.3% of people are aware of their infection, and 0.2% are on treatment (2).

HBV is a vaccine preventable disease and there is effective antiviral treatment in the form of nucleos(t)ide analogue (NA) therapy. NA treatment suppresses viral replication rather than curing infection, meaning long term therapy is needed to reduce the risk of liver disease and cancer, and to reduce the likelihood of transmission.

The WHO currently recommends universal infant HBV monovalent vaccination within the first 24 hours of life to prevent mother to child transmission (PMTCT) (4,5) followed by the routine childhood pentavalent or hexavalent vaccine immunisation series at 6, 10 and 14 weeks of age, which include HBV, delivered through the WHO Expanded Programme for Immunization (EPI). Along with childhood vaccination, WHO also recommends vaccination of at-risk adults, such as close household or sexual contacts of PLWHB and healthcare workers (HCWs).

HBV is still a major contributor to liver related morbidity and mortality worldwide, being the leading cause of HCC globally (2,6) despite the existence of effective treatment and vaccination. There are many crucial evidence gaps around HBV infection including: what is the epidemiology of infection (e.g. what ages, geographical areas and population groups are most significantly affected?), of those infected, who will develop severe disease, what are the roles of new blood markers in helping to predict severe disease and how can HBV screening and vaccine programmes be introduced effectively into communities, particularly where resources are constrained, where knowledge around HBV is limited and PLWHB are often stigmatised. Without understanding these questions and bringing HBV to the forefront of national public health policies, we will not make significant advances towards elimination.

1.2 Virology and lifecycle of HBV

HBV is part of the Hepadnaviridae family, and the Orthohepadnavirus genus. These are small, enveloped viruses with icosahedral symmetry and a genome made up of partially double stranded (ds) genomic deoxyribonucleic acid (DNA) (7). The HBV genome is only 3.2Kb in length and uses a reverse transcriptase (RT) to replicate through a pre-genomic ribonucleic acid (pgRNA) intermediate. It is therefore classified into group VII of the Baltimore virus classification system (sometimes called pararetroviruses), which underlines its biological homology with HIV (8). HBV genomic material exists in the peripheral blood as relaxed circular DNA (rcDNA) within HBV virions. The lifecycle of HBV is shown in figure 1.1.

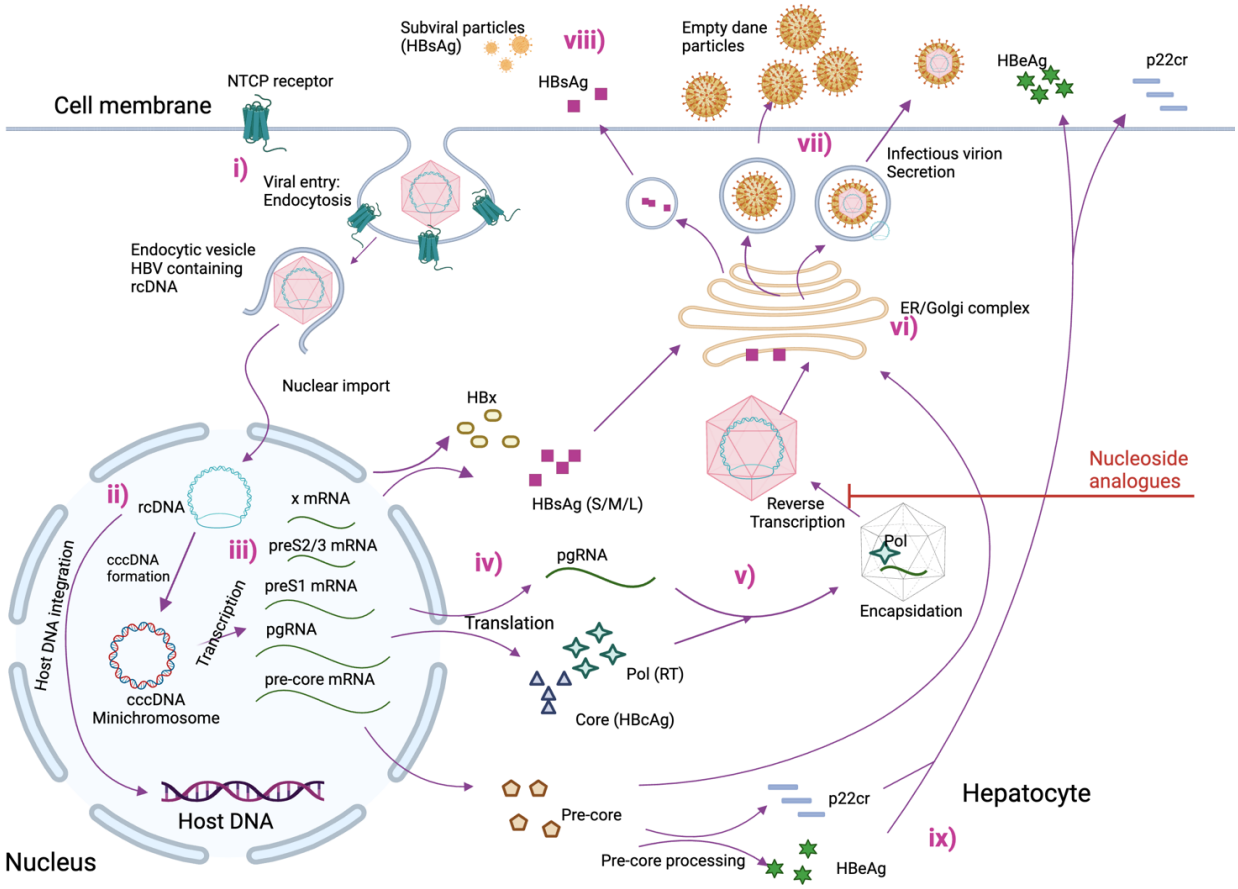


Figure 1-1: Lifecycle of HBV. i) HBV is endocytosed by hepatocytes using NTCP receptor; ii) In the hepatocyte nucleus, rcDNA is 'repaired' by host machinery producing a stable covalently closed circular DNA (cccDNA) molecule. Some HBV DNA also integrates into the host cell DNA; iii) From cccDNA, 5 viral transcripts are produced, including pgRNA; iv) these encode seven proteins – Hepatitis core antigen (HBcAg), hepatitis pre-core protein, X-protein (HBx), polymerase (Pol) and three sizes of hepatitis B surface antigen (large, medium and small – collectively known as HBsAg) (7,9); v) In the hepatocyte cytoplasm, HBcAg packages pgRNA and viral Pol into the nucleocapsid; vi) To egress from the cell, the nucleocapsid binds to HBsAg in the endoplasmic reticulum for encapsidation and vii) exits the cell through multivesicular bodies (9) forming new infectious virions. viii)

Along with secretion of these infectious virions, empty viral shells called Dane particles and smaller subviral particles made of HBsAg are secreted into the blood. In the cytoplasm, pre-core protein undergoes post translational modification to produce HBeAg and a small protein p22cr which are secreted into the blood (ix). (adapted from McNaughton et al. 2019 (7)

The circular nature of the HBV genome makes numbering a challenge. HBV genomic numbering tends to start from a restriction enzyme site in the Pol and Surface genes referred to as EcoR1. There are several overlapping reading frames within the four mRNA transcripts which go onto produce seven proteins. Most sections of the HBV genome therefore encode at least two proteins, meaning any genomic mutations may have significant consequences on HBV and its fitness. The structure of the HBV genome is shown in figure 1.2.

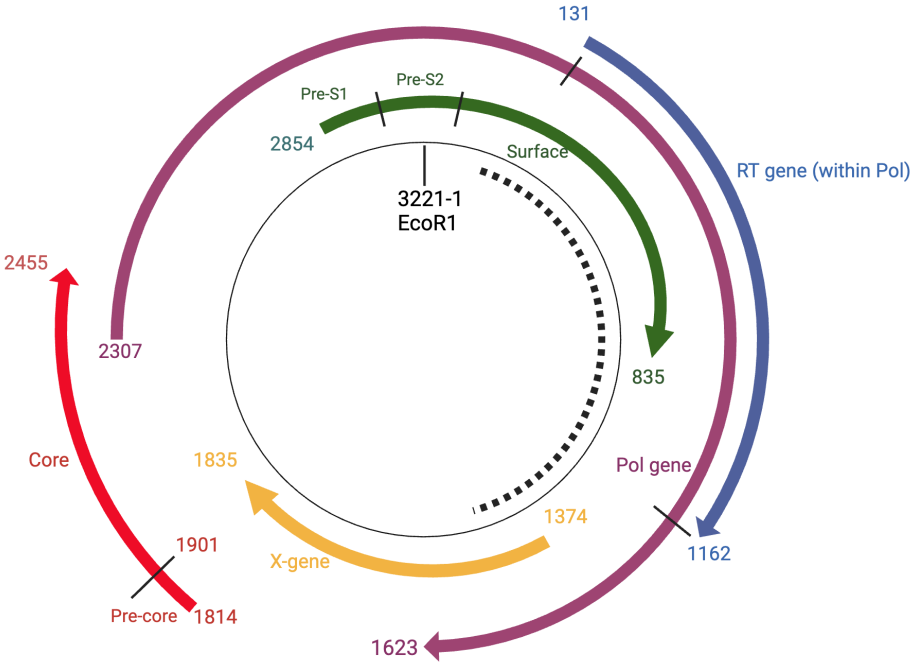


Figure 1-2: The structure of the partially dsDNA HBV genome. The four main mRNA transcripts are shown along with their nucleotide positions within the genome (Pol, Surface, X and Core). The location of the RT gene within Pol is also shown. The location of the restriction enzyme site EcoR1 is illustrated to show where numbering begins. The exact length of the HBV genome along with the start and finish of each gene varies slightly depending on genotype. The genome structure above is based on genotype A and is based on numbering from HBVdb (10). Created in Biorender.com.

Due to HBV replication relying on viral RT to produce new viral DNA from an RNA template, HBV is susceptible to nucleoside analogue reverse transcriptase inhibitors (NAs) which inhibit the growing DNA chain (location of action shown in figure 1.1), discussed further in chapter 1.1.4. However, the

cccDNA minichromosome is extremely stable, survives for the lifespan of the infected hepatocytes (11) and is not affected by NAs. Viral RNA transcription continues even when on NA therapy which contributes to viral persistence, risk of HBV reactivation on NA cessation and HCC development (12). Monitoring of cccDNA levels currently requires invasive sampling such as fine needle aspiration (FNA) or liver biopsy which is not possible as part of routine clinic practice. Presence of cccDNA remains one of the biggest stumbling blocks in development of HBV cure.

HBV Integration into the host DNA appears to occur early in infection and can be at multiple integration sites (13) with oncogenic consequences. Several major integration hotspots have been identified relating to HCC development, some within oncogenes, and some related to disrupted cell cycle regulation. Along with this, host genomic architectural changes have been seen which lead to loss of tumour suppressor genes such as p53 (13–15).

1.3 HBV infection diagnosis

The primary method of diagnosing CHB infection is by the detection of HBsAg in peripheral blood on two occasions ≥ 6 months apart (16). The WHO recommends using a validated *in vitro* laboratory test to do this, such as an enzyme linked immunoassay (ELISA) or chemiluminescence immunoassay (CLEIA) or a rapid diagnostic test (RDT) that meets minimum diagnostic standards (4). Given HBsAg is produced from viral RNA, and from HBV DNA integrated into the hepatocyte genome without the need for reverse transcription, HBsAg levels decline slightly over time as the cellular reservoir of HBV declines, but they are largely unaffected by NA therapy and so remain detectable despite treatment (17). Ideally those with a positive RDT should have their result confirmed using a validated ELISA, but in many resource-limited settings, this is not feasible. Currently in Kenya, there are many RDTs on the market to detect HBsAg, but most are not WHO pre-accredited and the performance of these tests has not been well validated. Additionally, a confirmatory ELISA is not routinely done, particularly in the public sector where it is expensive and usually unavailable. HBV in Kenya is therefore primarily diagnosed on RDT for HBsAg alone, and testing is often not repeated after six months to confirm chronicity.

International guidelines recommend HBsAg testing for all those at higher risk of HBV infection such as men who have sex with men (MSM), people living with HIV (PLWHIV) and healthcare workers (HCWs). Testing is also recommended for all antenatal women as part of routine antenatal care (ANC) and this should be done on their first interaction with healthcare services. In Kenya however, there is very little routine HBV testing except in blood donors, despite this being part of national guidelines on the diagnosis and management of HBV (18). Even HBV testing as part of ANC is not done universally due

to a combination of lack of education and awareness of both the general population and HCWs around the need for testing and often testing not being offered free of charge. There are currently efforts ongoing to sensitise HCWs and mothers towards the need for testing, along with encouraging national provision of testing kits to allow testing for free but this is a challenge both in Kenya and in many countries globally.

1.4 HBV progression

The spectrum of illness caused by HBV is diverse ranging from asymptomatic infection to severe liver cirrhosis and HCC. There are several internationally recognised phases of HBV infection largely dependent on HBeAg status and HBV DNA levels along with presence of liver inflammation, a summary of which are shown in table 1.1 (4).

Biomarker or characteristic	HBeAg positive infection	HBeAg positive disease	HBeAg negative infection	HBeAg negative disease
HBeAg	Positive	Positive	Negative	Negative
Anti-HBe	Negative	Negative	Positive	Positive
HBV DNA	Very high, usually $>10^7$IU/ml	High, around 10^5 - 10^7 IU/ml	Low, usually $<10^3$ IU/ml	Moderate, around 10^3 - 10^5 IU/ml
ALT	Around ULN	Raised >ULN	Around ULN	Raised >ULN
Histology				
• **Necroinflammation**	Minimal	Moderate/severe	Minimal	Moderate/severe
• **Fibrosis**	Minimal	Varying	Minimal	Moderate/severe

Table 1-1: Biomarker and histological characteristics of different stages of chronic hepatitis B virus progression (adapted from the WHO Guidelines (4)).

Studies of untreated individuals show a 8-20% cumulative risk of developing cirrhosis over five years (19–21), those with cirrhosis have a 20% annual risk of decompensation (22,23) and a variable risk of HCC from <1% - 5% (24,25). Most people who develop HCC have cirrhosis as the precursor lesion, however an estimated 0.1% of patients with HBV without cirrhosis develop HCC annually (figure 1.3) (26). Patients with CHB account for 50-60% of all HCC cases in the WHO-AFR with 43% of cases occurring in adults younger than 40 years (27). The incidence of HCC is higher in those from WHO-AFR compared with Caucasian populations (20) and black ethnicity is an independent risk factor for development of HCC in the absence of cirrhosis (26,28).

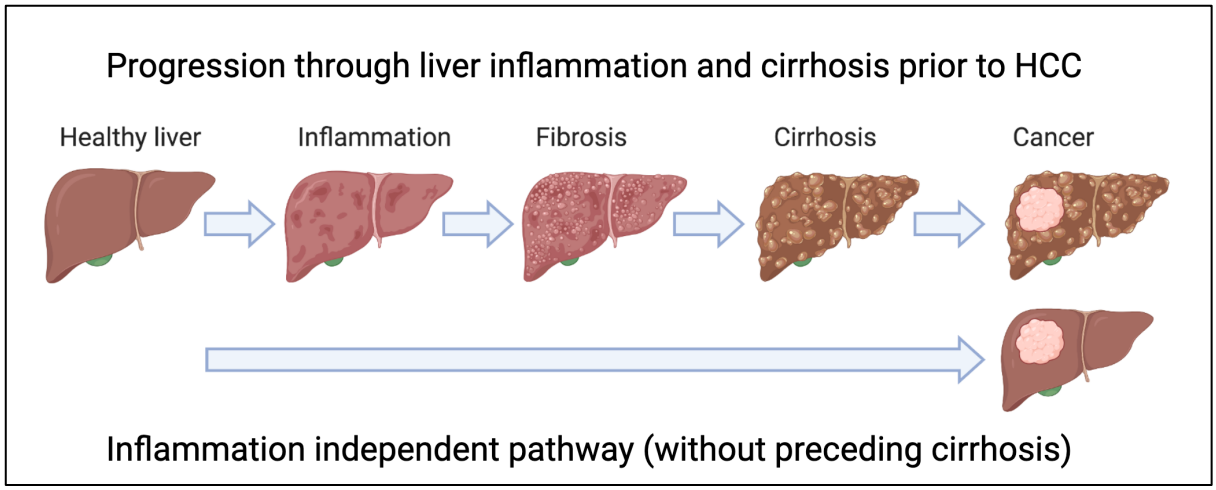


Figure 1-3: Progression of liver disease either through the more common inflammation dependent pathway with proceeding fibrosis then cirrhosis prior to HCC development, or through the inflammation independent pathway where HCC develops from a seemingly healthy liver (adapted from a figure produced by Professor Philippa Matthews (2022)).

The single biggest predictor of liver disease progression and long-term HBV outcome is the level of HBV viral replication. Reducing viral replication has been shown to eliminate liver necroinflammation and reverse or stall the progression of liver fibrosis in the vast majority of PLWHB (25,29). HBeAg is an accessory protein to HBV being not required for viral replication or infection. HBeAg positivity is however associated with high levels of HBV replication and lack of immune control, and it is required for development of chronic infection through immunomodulation of host immune responses (30). It is translated from the pre-core mRNA as shown in figure 1.1 and secreted into the blood stream, tending to be present in the early phases of HBV infection, therefore particularly in children. It is lost as HBV progresses when anti-HBe antibodies develop. HBeAg loss can be part of the natural course of infection, or induced by NA therapy, and infers partial immune control of the virus, and outcomes are improved (31). HBeAg measurement in clinical practice gives an indication of infectivity and positivity indicates a highly active virus without immune control, although it should be borne in mind that a minority of patients possess a mutant virus which does not express HBeAg despite high replication rates.

The predictive value of ALT is uncertain with some studies showing this marker had a high sensitivity for predicting liver fibrosis (32), whilst in others it was a very poor predictor of any fibrosis grade (33). There are many reasons ALT could be elevated unrelated to HBV; hence serial quantification is essential to give a long-term estimate of liver inflammation.

1.5 HBV staging

Once an individual has received an HBsAg positive test result, they need further assessment to guide management. This includes taking a comprehensive medical, lifestyle and family history, measuring blood biomarkers, undertaking imaging and possibly a liver tissue sample as described below:

i) Blood markers

Biomarkers in blood to be measured include HBV VL, HBeAg status anti-core IgM and quantitative HBsAg (qHBsAg). Assessment of host liver inflammation and liver function is also required, including platelet count (measured as part of complete blood count (CBC)) and liver function tests (LFTs) (including albumin, ALT, aspartate transaminase (AST), bilirubin and alanine aminophosphatase (ALP)) (16,34). Renal and bone profile are also recommended if NA treatment is to be offered.

ii) Imaging

The most commonly used method worldwide in most settings to assess the health of liver parenchyma is transient elastography (TE) measurement, using Fibroscan® (Echosens, Paris). TE measures the velocity of a low frequency shear wave through the liver which is directly related to tissue stiffness (35) and can be performed in under 10 minutes in outpatient settings. While training is required, the procedure can easily be learnt and does not require clinically trained personnel. The stiffer the tissue, the faster the shear wave propagates. TE gives a measurement in kilopascals, with higher scores being indicative of a stiffer liver. Along with TE, more direct abdominal imaging in the form of ultrasound is required to check for masses that could indicate HCC.

iii) Tissue Diagnosis

The gold standard to stage liver disease in HBV is liver biopsy, and liver health is divided into METAVIR stages shown in table 1.2:

METAVIR STAGE	F0	F1	F2	F3	F4
Definition	No fibrosis	Portal fibrosis without septa	Portal fibrosis with septa	Numerous septa without cirrhosis	Cirrhosis

Table 1-2: Different METAVIR stages of liver disease as defined by liver biopsy characteristics. Adapted from 2024 WHO Guidelines (4).

There are however considerable issues with liver biopsy. It only samples a small section of the liver, it is costly and invasive requiring significant physician training, as well as expert histopathological interpretation, and can lead to rare but potentially life-threatening complications as a result of bleeding.

iv) Non-invasive scoring systems

Although the assessment methods above are detailed in most international guidelines, they are often not feasible in resource limited settings. HBV VL quantification and HBeAg assessment for example are often only available at a considerable cost to the patient and are not universally accessible. The hardware for TE is prohibitively expensive and requires regular maintenance, and issues with liver biopsy are described above.

Simpler, non-invasive liver health scores using routinely available blood markers have been developed to try and fill this gap - the aspartate transaminase (AST) to platelet ratio index (APRI) and Fibrosis-4 (FIB-4) score. Two optimal thresholds for these scores have been established corresponding to either significant fibrosis (METAVIR ≥F2) or cirrhosis (METAVIR F4) (4). There are however significant evidence gaps around the use of these non-invasive scores, particularly in under-studied population such as those from the WHO-African Region including Kenya.

All current serum biomarkers are imperfect predictors of outcome, and whilst many risk models for prediction of HBV progression to liver disease and cancer based on the biomarkers outlined above have been explored in Caucasian and Asian populations, there has been a lack of validation in African populations. There is an urgent need to develop affordable methods to assess replication and presence of liver disease in the WHO-AFR, and to properly validate current simpler liver health scoring systems. There is also a need to develop biomarkers that can support a mechanistic understanding of liver pathology, aid monitoring and facilitate safe treatment cessation, discussed further in chapter 1.2.

1.6 HBV treatment and changing eligibility criteria

The mainstay of treatment for HBV is NA therapy, primarily tenofovir (TFV) which is most commonly prescribed as tenofovir disoproxil fumarate, (TDF) and entecavir (ETV). These are both competitive NAs, binding to the RT active site and competitively inhibiting ongoing production of the new HBV DNA chain as discussed in chapter 1.1.1 (figure 1.1). Once started, NA therapy is generally continued long term due to the persistent cccDNA reservoir. Treatment interruptions can be associated with risks of liver flares (36), and may lead to drug resistance, although there is little evidence of this at present with TFV therapy (37). There are some situations where NA therapy is recommended for short periods such as during pregnancy for PMTCT or during immunosuppressive therapy in those who are HBsAg negative but have antibody to HBV core antigen (anti-HBc) to reduce the chance of HBV reactivation.

PLWHB are usually followed up every 6-12 months in outpatient clinics to monitor HBV DNA levels, LFTs and to undergo liver imaging if indicated and available. For those not on NA therapy, this monitoring helps identify when treatment is necessary, and for those already on NA therapy, this can identify emergence of potential drug resistance and allow for assessment of treatment compliance (16). Liver ultrasound can also assess for the presence of HCC and routine 6-monthly surveillance is recommended for groups at highest risk, with or without measurement of alphafetoprotein (aFP) in settings where this can be accessed.

Until March 2024, treatment guidelines for HBV were complex and relied on a combination of demographics, viral and host markers to determine treatment eligibility. This led to only around 20% of those living with CHB being eligible for NA therapy by international guidelines (4,16,34). Treatment was restricted to only those at highest risk of severe liver disease or cancer, those with highly active HBV replication, or those with signs of active liver inflammation. This was despite increasing knowledge that even those not meeting any of the above criteria have a substantial risk of liver related death and HCC compared to individuals not living with HBV (38). Furthermore, most of the markers used in these guidelines were not available in LMICs and hence their relevance was limited in these settings. There was an urgent need for more simplified guidance with more global relevance in countries with limited resources.

In March 2024, the WHO released new guidelines, significantly expanding treatment eligibility and reducing the dependence on unavailable markers when making treatment decisions (4) (figure 1.4). Although HBV DNA measurement is still recommended, there is acknowledgement that in many LMICs this is not available, and they give the option to treat with much broader criteria e.g with measurement of alanine aminotransferase (ALT) alone; with the presence of other co-morbidities or risk factors; if there are particular concerns about onward transmission; based on patient wishes to be treated. Along with this, the new guidelines lower the non-invasive liver scores required to be treatment eligible and expand treatment to all HBsAg pregnant mothers if HBV DNA quantification is unavailable. At least 50-60% of PLWHB should be eligible for treatment based on these alterations. It should also be noted that in some LMICs no method of disease stratification is possible, even ALT measurement, and in these cases, NA therapy can be offered regardless of any other risk-stratification.

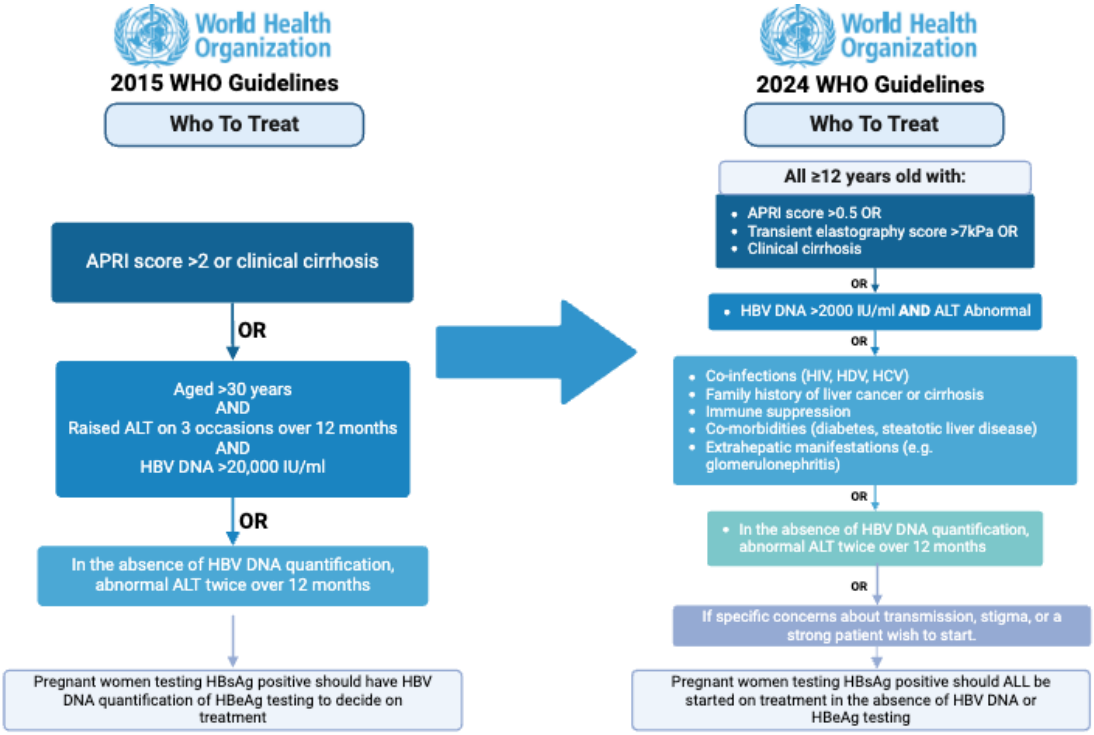


Figure 1-4: How WHO HBV management guidelines have changed over the last 10 years from 2015 on the left to 2024 on the right (Figure created using Biorender.com adapted from the 2015 and 2024 WHO Guidelines (4,39)).

The most desirable treatment endpoint in CHB infection is HBsAg loss – this is known as 'functional cure'. This can happen spontaneously in around 1% of PLWHB per year, and can also be associated with NA therapy, albeit at a lower rate of <1% of PLWHB per year (40). The biggest predictor of HBsAg loss either spontaneously or on treatment, is baseline qHBsAg levels – the lower the baseline level the more likely HBsAg loss becomes (17,41). When HBsAg loss does occur, it is generally associated with both undetectable HBV DNA in serum and improvement in clinical outcomes (42,43). There is ongoing research going towards developing a 'sterilising cure' for HBV, being defined as elimination of cccDNA and integrated DNA from hepatocytes, however there is no way to determine sterilising cure, so this currently remains a hypothetical endpoint

1.7 HBV prevention

Immunisation against HBV is one of the cornerstones of prevention of infection. The first HBV vaccine was developing using purified plasma from PLWHB to induce antibodies against the HBsAg protein (anti-HBs). This vaccine was licensed in 1981 in the United States of America (USA) and became commercially available in 1982 (44). This production method was expensive however, so a new HBV vaccine was produced using HBsAg purified from recombinant yeast cells and went onto be approved

in 1986 by the USA food and drug administration body (FDA). Vaccines containing recombinant HBsAg are now used globally produced from either yeast or HBV transfected mammalian cells (45).

HBV vaccination strategies were initially targeted towards those with higher risk of HBV infection, such as HCWs, MSM, people working in transactional sex and people who inject drugs (PWID) (45). This had little effect on reducing HBV burden at a population level, so in 1992 (46), the WHO's Global Advisory Group for the EPI recommended all countries introduce universal HBV vaccination into their childhood immunisation series.

HBV vaccine was introduced into the childhood EPI schedule in Kenya in 2001, given as part of the pentavalent vaccine along with diphtheria, pertussis, tetanus and haemophilus influenzae at 6, 10 and 14 weeks of life. The WHO's Global Vaccine Action Plan (GVAP) set a target for pentavalent vaccination coverage of 90% nationally by 2020 (47), and according to the Global Alliance for Vaccines and Immunisations (GAVI), Kenya achieved this coverage in 2022 (48). There are however spatial heterogeneities masked by this country average, with rural communities having lower coverage (49,50). Given the first dose of HBV vaccine is not routinely received until 6 weeks of life as part of the EPI schedule, until that point babies remain unprotected against HBV infection. To close the window of peripartum vulnerability, in 2009, WHO recommended universal administration of HBV birth dose vaccine (HepB-BD) within 24 hours of life (51). There is however limited evidence on the effectiveness of this strategy as most studies compare HepB-BD with no vaccination so the benefits of adding HepB-BD to multivalent vaccine at 6, 10 and 14 weeks are not well documented. Studies are ongoing to try and generate evidence around this question (52). There are also implementation challenges to HepB-BD, particularly in countries where a large proportion of births occur outside of health facilities such as Kenya. In Kilifi in 2014, it was estimated just over 50% of births were attended by a skilled birth attendant at a health facility (53). To date only 15/47 (32%) countries in the WHO-AFR have introduced HepB-BD and only Algeria and Cape Verde have reached >90% coverage (54).

Antenatal testing is crucial to inform interventions for HBV PMTCT, but despite recommendations is poorly implemented, so often women are only diagnosed at delivery or remain undiagnosed throughout their childbearing years. For women who are diagnosed antenatally, NA therapy can be given to reduce HBV viral load (VL), lowering the risk of HBV transmission at delivery or postpartum. Previous WHO guidelines suggested that NA therapy was only indicated in pregnancy for those testing HBeAg positive or with HBV VL >200,000 IU/ml. 2024 WHO guidelines have readdressed this with the recognition that these markers are unavailable in many countries, and have now advised antenatal

NA therapy can be offered to all who test HBsAg positive if there is no way of undertaking further risk-stratification (4).

Whilst significant focus around HBV testing and vaccination has been on pregnancy, guidelines also specify that other groups at increased risk should be tested and vaccinated if negative. This includes MSM, PLWHIV and HCWs. PLWHIV tend to have testing and vaccination provided free of charge in Kenya through the Comprehensive Care Clinics (CCC's) which are usually funded by external organisations such as USAID and the AIDS Healthcare Foundation (AHF). For other groups, this funding is not available and testing and vaccination is not routinely provided. HCWs are at significant ongoing risk of exposure to HBV infection due to contact with blood and bodily fluids. Studies in Kenya have shown needlestick injury (NSI) rates of up 14% annually with some studies indicating 40% of HCWs will sustain an NSI during their career (55,56). Other studies investigating attitudes of HCWs towards PLWHB indicates a significant concern regarding infection, and therefore PLWHB can receive substandard care and stigmatisation due to these worries (57). Despite this, HBV vaccination rates in HCWs are variable throughout the country with studies based in healthcare settings indicating only around 50% of their staff have received all three doses of HBV vaccine (58,59). Improved implementation of HBV vaccination in at risk groups, particularly healthcare workers is urgently needed.

1.8 Novel approaches to liver disease stratification

WHO 2024 guidelines have moved more towards a 'treat all' strategy globally for HBV, recognising global limitations in diagnostics, but there are still a proportion of PLWHB who do not meet treatment criteria yet are still at risk of liver disease progression. New, more accurate serum markers of liver disease are needed to improve stratification of all PLWHB, along with a focus on developing more affordable diagnostics for use in LMICs.

1.8.1 Hepatitis B core related antigen

One serum marker that is a focus of current interest is hepatitis B core related antigen (HBcrAg). This is a circulating biomarker that combines HBeAg, HBcAg and a small 22kd protein called p22cr (60,61), the generation of which is shown in figure 1.5.

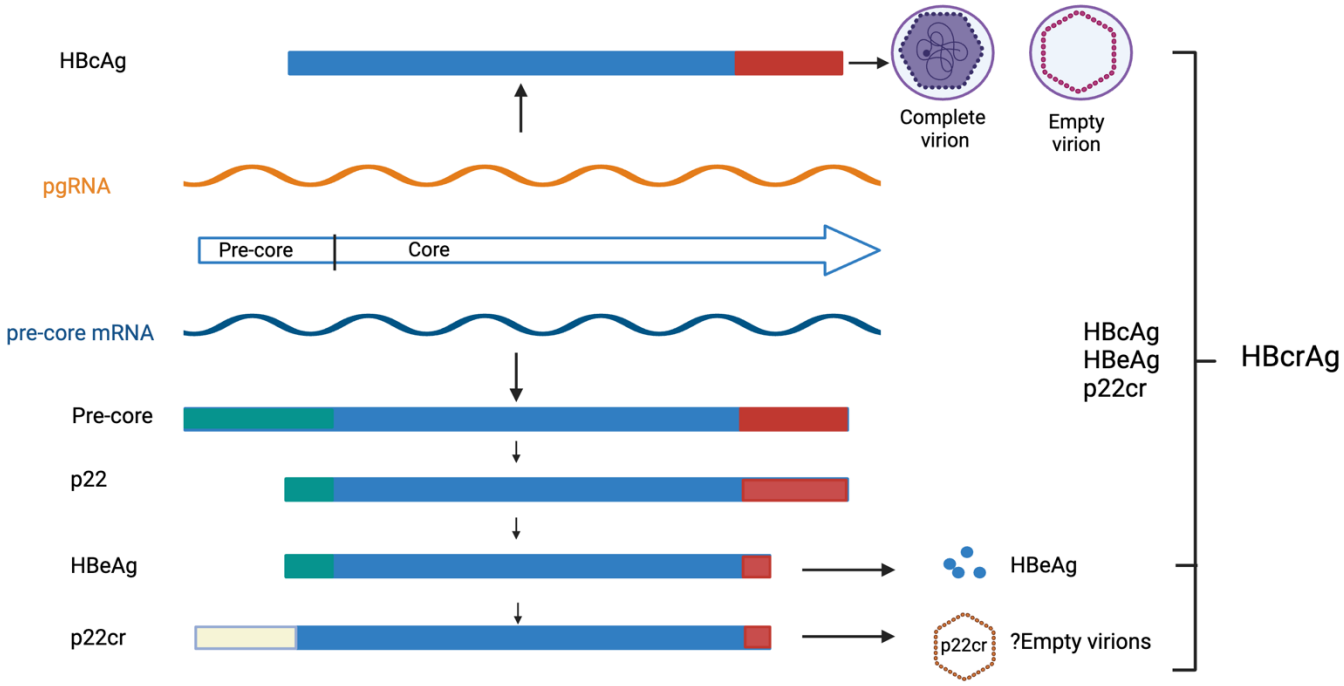


Figure 1-5: Generation of HBcAg, HBeAg and p22cr which together form the HBcrAg biomarker are all measured by the HBcrAg assay as they share the same 149 amino acid sequence shown in blue. HBcAg is translated directly from pgRNA and forms the icosahedral viral capsid. The pre-core protein is translated from pre-core mRNA and undergoes post translational modification to produce HBeAg and a small protein called p22r which may form empty virions. Adapted from Jer-Wei Wu *et al,* 2021 (60).

HBcrAg has been shown to correlate with quantification of cccDNA through liver biopsy (60,62,63), with peripheral HBV VL and HBeAg status (64,65), with APRI and FIB-4 scores, ALT levels (66,67), hepatic necroinflammation (63,64), HCC (68) and liver flares after treatment cessation (69). One study of >400 Asian treatment-naïve patients reports significant changes in HBcrAg associated with the different disease 'stages' (shown in table 1.1) (70). Over 10 years of follow up, HBcrAg was a better predictor of HCC than HBV DNA (71).

HBcrAg could also be used as a biomarker of viral reservoir or replication when HBV VL is low or tools for VL quantification are not available, as in much of the world. In one study after 7 years of follow up, over 50% of HBeAg-negative patients (primarily with undetectable VL) still had detectable HBcrAg (72). WHO guidelines highlight HBcrAg assays as a potential approach for improving risk stratification (4), although HBcrAg is currently only available as a laboratory based CLEIA in a few specialist centres around the world.

To date, there are limited data on HBcrAg representing diverse populations, with heterogeneity of results, especially depending on HBeAg status. Most existing studies investigating HBcrAg are in Asian populations (therefore representing HBV genotypes B and C), or in Europe, thus neglecting representation of individuals of African descent (73,74). To date, there is only one study of HBcrAg in an African population (conducted through the PROLIFICA cohort in the Gambia), which showed that HBcrAg could predict clinically important HBV VL thresholds (75).

To move towards more accessible HBcrAg measurement, a point of care test (POCT) has been developed and validated in the PROLIFICA cohort (76) to identify highly viraemic patients, corresponding with a laboratory HBcrAg CLEIA value of 4.3 log IU/mL. The POCT was shown to have 100% sensitivity and 87.5% specificity for identifying women of reproductive age with an HBV viral load of >200,000 IU/ml, an important threshold when defining the need for antenatal prophylaxis with TFV. An accurate POCT could enhance timely clinical assessment, support early treatment decisions, and promote linkage to care and continuity of follow-up (77). This POCT has not yet been evaluated alongside ALT or liver fibrosis scores or trialled elsewhere in the WHO-AFR.

1.8.2 Role of viral sequence

Along with biomarkers, viral sequencing has shown promise in understanding different clinical HBV disease phenotypes. HBV genotypes and sub-genotypes differ in their risk of progression to severe disease and cancer, indicating this could be an important factor to consider when determining patient management plans. To be defined as a separate genotype, HBV sequences are required to have >8% divergence at the nucleotide level, and >4% divergence for a subgenotype. There are currently nine different HBV genotypes identified (A-I), with a putative 10^{th} genotype (J) identified from one individual (78,79) and at least 35 sub-genotypes (figure 1.6). HBV genotypes vary by geographic distribution and clinical outcome, including risks of liver disease progression and response to therapy.

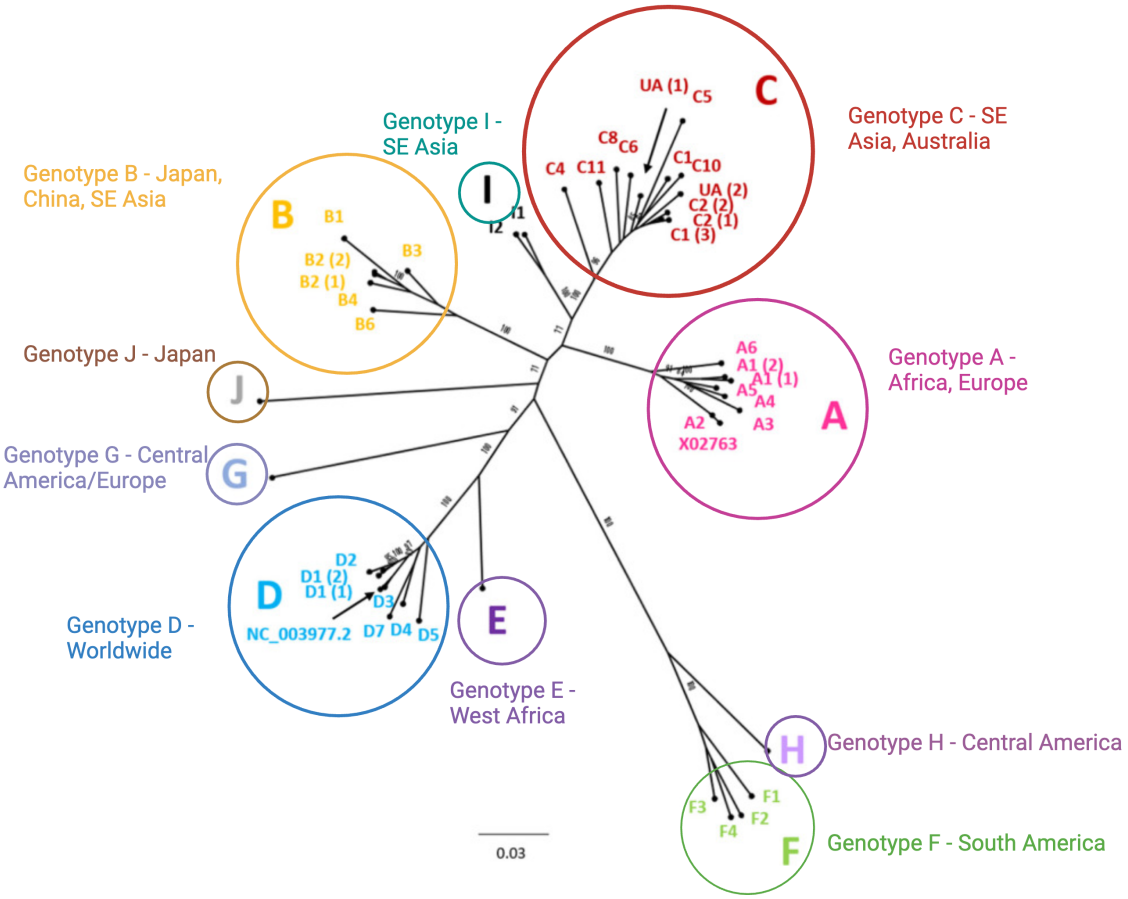


Figure 1-6: Maximum likelihood phylogenetic tree of HBV showing genotype, sub-genotype and clade reference strains where possible, along with their predominant geographic locations. Bootstrap support for branches ≥70 after 100 replicates is shown. X02763 and NC_003977.2 are also shown as these are commonly used as reference sequences within genotype A and D respectively. The scale bar indicates estimated nucleotide substitutions per site. Adapted from McNaughton *et al.* 2020 (80). SE – South East, UA – unidentified clade.

In East and Southern Africa, genotype A predominates which has been associated with horizontal transmission and lower rates of HBeAg positivity (81,82), but increased incidence of progression to chronic disease after acute infection and faster progression to HCC (83,84) compared with genotypes B, C and D. Along with determining genotype, next generation sequencing (NGS) of HBV has been shown to help differentiate acute infection from a flare of chronic disease (85) and has been used to investigate presence of drug resistance associated mutations (RAMs), particularly in patients who are not suppressed on NA therapy (86). HBV NGS has been challenging to date for a variety of reasons including the low HBV VLs that tend to be seen in chronic infection (87) and the unusual structure of the partially dsDNA HBV genome (figure 2) making amplification prior to library preparation challenging (88). Studies using HBV NGS data to date have been small in the WHO-AFR and many retrospectively test sera from blood donations, without access to clinical meta-data (89,90). Sanger

sequencing has been done in some HBV cohorts focussing on individual genes and a minority of studies have correlated polymorphisms with disease state.

1.9 HBV disease burden

1.9.1 Global burden and Epidemiology

Estimating the prevalence of HBV infection globally has been challenging given many studies give point prevalence figures from low quality or non-representative groups at different time points. The Polaris group in 2022 attempted to model the prevalence of HBV globally by combining traditional meta-analysis, national expert interviews and dynamic modelling considering the effects of vaccination and treatment programmes on HBV prevalence (91). This data then fed into the 2024 Global Hepatitis report to estimate around 254 million people worldwide are living with HBV, giving a global prevalence of 3.2% (2). It was also estimated that 17 countries accounted for over 75% of HBV infections in the general population, of which Kenya is one, and China, India, Indonesia, Nigeria and Ethiopia account for 57% of all infections (91). Deaths from HBV infection are increasing year on year. In 2019 it was estimated around 800,000 people were dying annually from HBV related disease and in 2022 this had increased to 1.1 million (2). HBV is the biggest viral killer globally after COVID-19 and it is one of the only communicable diseases for which deaths are still rising (2).

In 2022 there were 1.2 million new HBV infections and 63% of these occurred in the WHO-AFR. In 2015 the United Nations (UN) highlighted viral hepatitis in sustainable development goal 3 (SDG3) to eliminate HBV as a global health threat by 2030. Specific targets have been set around increasing the number of PLWHB who are diagnosed, and on treatment, reducing numbers of children <5 years who are infected and increasing HBV vaccination coverage including provision of a timely birth dose vaccine. Currently however, we are well off track for meeting most of these targets, especially in LMIC settings. In the WHO-AFR, it is estimated only 4.2% of PLWHB are diagnosed, and 0.2% on treatment, and this region is the only one with an HBV prevalence of >1% in children <5 years.

To begin heading towards international elimination targets, a huge public health effort is required to improve access to diagnostics, treatment and prevention strategies in WHO-AFR.

1.9.2 Burden in Kenya

Estimates around the prevalence of HBV in Kenya varies between sources. In 2019, the Global Burden of Disease Study estimated prevalence to be around 3% (92), then in 2022, the Polaris Observatory

Collaborators increased this to 4.4% (91) . This is reflective of the limited and skewed evidence around HBV prevalence in Kenya with most studies focussing on specific populations such as PLWHIV (93,94), MSM (95), people who use intravenous drugs (PWIDs) (96) and those presenting to hospital with jaundice (97,98). There are some studies that focus on blood donors (99,100) or antenatal screening data (101), but to date there has only been one national survey on the prevalence of HBV in Kenya in 2007, and even this excluded PLWHIV (102).

To advocate for provision of improved HBV care in Kenya, high quality seroepidemiological data is needed to describe the burden of disease. This includes screening not only of those in high-risk groups, but also of the general population and antenatal mothers. These data can then be used as evidence to present to both national government and international funding bodies such as the Global Fund, to demonstrate the need for enhanced viral hepatitis elimination efforts.

1.9.3 Challenges of HBV care in Kenya

Healthcare in Kenya is decentralised, meaning individual counties have responsibility for their own healthcare budget and priorities. Communicable diseases including tuberculosis, HIV/AIDS and lower respiratory tract infections are the leading causes of mortality in Kenya (103), so there are many competing healthcare interests, in addition to viral hepatitis.

There is no Viral Hepatitis National Strategic Plan, no consistent HBV testing algorithm, treatment strategies vary depending on location, and HepB-BD is not currently part of the routine immunisation schedule for infants. Education around HBV within both HCW and the general population is vastly limited. The last Kenyan national HBV management guidelines were released in 2014, but these are now outdated and advocate tests that are routinely unavailable in much of the country, or unaffordable to Kenyan citizens.

Along with lack of availability and high costs of HBV testing and care, there are ongoing problems with access to HBV clinics, particularly in larger Kenyan counties where many people live some distance away from central hospitals. Kilifi County is on the Kenyan Coast with a population of around 1.5 million people (figure 1.7) (104). KCRH is the main referral hospital for Kilifi County, with around 300 inpatient beds and sees approximately 193,000 outpatients every year.

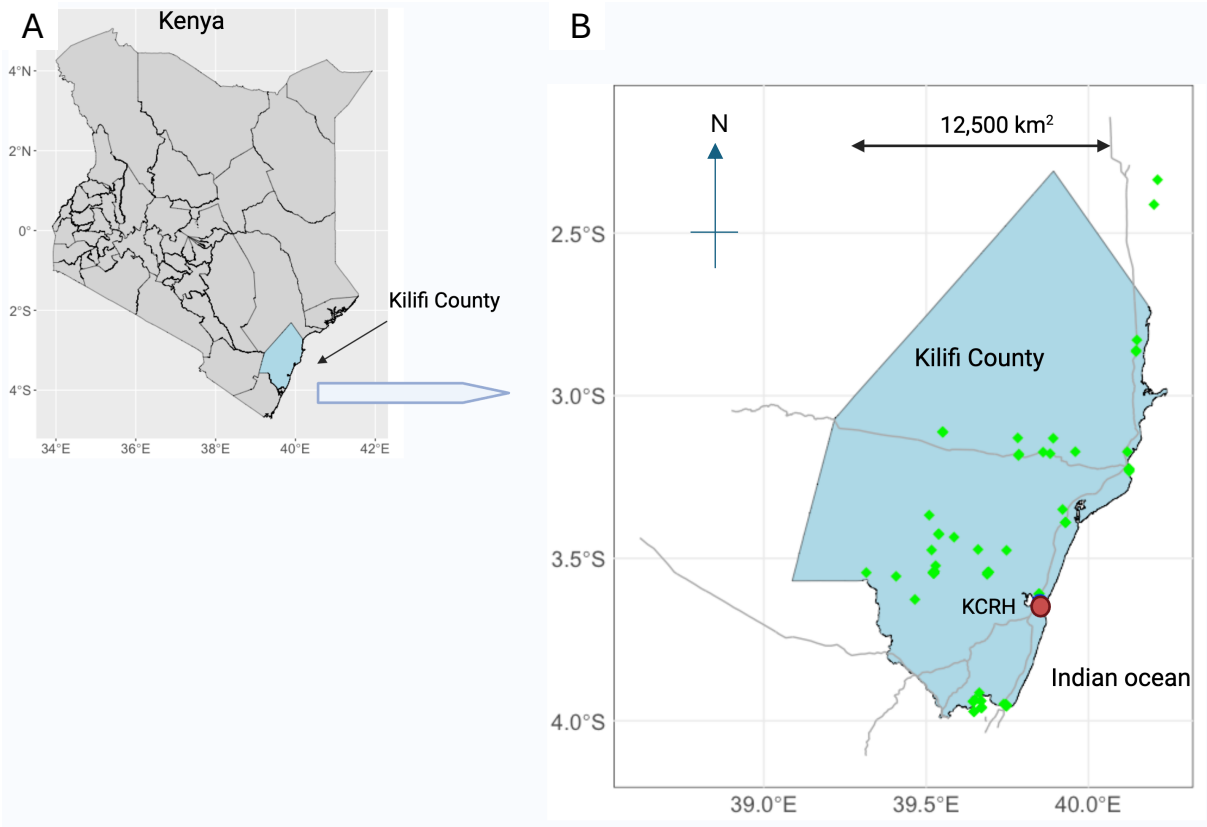


Figure 1-7: A: Map of Kenya showing the location of Kilifi County. B: Detailed map of Kilifi County with the land area of 12,500 km^2 indicated. Major road networks are shown in grey, along with the location of smaller health centres in green (ranging from dispensaries to sub-county hospitals). Kilifi County Referral Hospital (KCRH) is shown in red. The Indian Ocean is to the East. Figure created using R version 4.4.1 using osmdata and naturalearth packages.

Poverty levels in Kilifi are above the national average for Kenya (105), with 53% of the population living in poverty in 2022 compared to the national average of 40% (105). In Kilifi, the majority of HBV care is provided in County or Sub-County hospitals, although only around 35% of the Kilifi population live within 2km of an all-weather road meaning in some seasons, access to hospital care is unmanageable due to annual rainfall. Decentralised HBV care has been set up in a few rural clinics where individual patients have specifically asked for this, but only on a case-by-case basis. Scale up of this provision may help people access care more effectively but needs government investment along with HCW and community education.

1.9.4 Knowledge and understanding of HBV in Kenya

Throughout the WHO-AFR including Kenya, most studies reviewing attitudes towards HBV and investigating knowledge gaps have primarily been done in HCW, and show a considerable lack of

understanding of the infection with many HCWs being unvaccinated against HBV, and unaware of their HBV serostatus (57,106,107). One general population study in Southwest Uganda showed there was no local language word for HBV infection and many people had not heard of HBV infection (108).

There has been very little documentation of the impact of an HBV diagnosis on those living with the virus in the WHO-AFR. One study from Ghana showed people suffering psychological and social problems when initially informed about their HBV status, along with denial, fear, shock and shame (109). Another study from Burkina Faso showed that less than 25% of those diagnosed with HBV were linked to care due to a combination of factors (110). In this study there was a lot of influence from family members to consult traditional healers rather than going to official medical facilities, information provided from trained medical practitioners was inaccurate, and the costs of care were prohibitively expensive. There have been no studies to date in Kenya outlining the impact of HBV on those living with the infection, and no investigation of barriers to care. This highlights the urgent need for inclusion of the patient voice to improve representation and advocacy and drive progress towards improved clinical care and global elimination targets.

1.10 DPhil aims

1.10.1 STRIKE-HBV study

1.10.1.1 Background

Prior to beginning my DPhil, I began discussions with KWTRP in Kilifi, Kenya regarding establishing HBV research in Kilifi. There was no such work being undertaken at that time and certainly no implementation work or active recruitment of people living with HBV to take part in a research study.

Prior to relocating to Kilifi in August 2022, I had established a relationship with Dr Nadia Aliyan, the consultant physician based at KCRH. She had extensive knowledge regarding current HBV care at KCRH and the inner workings of the hospital. She was paramount in helping to design STRIKE-HBV in a way that was feasible and acceptable to both the hospital staff and community.

In KCRH prior to STRIKE-HBV, there was no routine testing for HBV infection, even in antenatal women. HBsAg tests were costed at around $2, there was little education given to patients regarding the need for testing, and HBsAg tests were often out of stock. People would often present to hospital and be diagnosed at a late stage when they had jaundice, ascites and sometimes encephalopathy. Treatment would then be only symptomatic management and end-of-life care.

There were a small group of around 25 PLWHB being cared for in the CCC who had mostly been diagnosed outside of KCRH, often privately prior to travelling abroad. The staff at the CCC had little training specific on HBV infection, there was very minimal HBV knowledge in the community, and no understanding of the prevalence of HBV in Kilifi.

The main aim of this study was to undertake HBV testing in hospital attenders at KCRH and investigate demographic, viral and host factors that are associated with poor liver health. This involved establishing HBV NGS at KWTRP, reviewing the HBcrAg blood test, and assessing the potential use of the novel HBcrAg POCT.

1.10.1.2 STRIKE-HBV Objectives:

OBJECTIVE 1:

Research question 1: What are the demographic and lifestyle characteristics of adults living with HBV infection in Kilifi, Kenya?

Research question 2: How do patient demographics, lifestyle factors and HBV biomarkers correlate with severity of liver disease in this population?

OBJECTIVE 2:

Research question 3: What is the estimated prevalence of HBV infection in Kilifi County based on existing data from the Kilifi Health and Demographic Surveillance System (KHDSS)?

Research question 4: What is the prevalence of HBV infection among outpatients at KCRH as determined by the STRIKE-HBV screening programme using point of care HBsAg testing?

OBJECTIVE 3:

Research question 5: What are the circulating HBV genotypes and subgenotypes amongst adults living with HBV in Kilifi, Kenya?

Research question 6: What is the extent of inter and intra-host HBV genetic diversity in the Kilifi Cohort?

Research question 7: What is the prevalence of mutations associated with drug resistance and vaccine escape in this population?

OBJECTIVE 4

Research question 8: How does HBcrAg level in PLWHB in Kilifi correlate with laboratory and clinical markers of disease progression, viral genotype and specific sequence polymorphisms?

Research question 9: How does the performance of a new POCT for HBcrAg compare to both laboratory HBcrAg CLEIA and HBV DNA qPCR quantification?

1.10.2 HEP-B-BOOST study

1.10.2.1 Background

Whilst undertaking the STRIKE-HBV study, I was approached by the management team at KCRH regarding introduction of HBV vaccination for HCWs. As described in chapter 1.1.5, most healthcare workers in Kenya are incompletely vaccinated against HBV, and there was growing concern from the staff at KCRH regarding the numbers of patients living with HBV that had not been diagnosed. The KCRH team were keen for the vaccination programme to be primarily implemented and funded internally, with me providing consultancy advice, education and troubleshooting as the programme progressed. To document the process of implementing a vaccination program such as this, I worked together with colleagues at KWTRP and KCRH to design the HepB-Boost study. The main aims of this study were to evaluate the feasibility of healthcare worker HBV testing and vaccination at Kilifi County Hospital using the process evaluation framework from the Medical Research Council/NIHR as a structure (111).

1.10.2.2 HepB-Boost Objectives

OBJECTIVE 1:

Research question 1: What is the current self-reported rate of HBV vaccination among HCWs at KCRH?

OBJECTIVE 2:

Research question 2: What proportion of HCWs accept a free HBsAg test, and what is the rate of HBsAg positivity among those tested?

OBJECTIVE 3:

Research question 3: What is the uptake of HBV top-up vaccination at KCRH and how many HCWs complete the recommended vaccination course?

OBJECTIVE 4: What are the summary demographics and employment details of those attending the vaccination clinics at KCRH?

2. DETERMINING HBV PREVALENCE AND GENOTYPES IN KENYA

2.1 Introduction

As discussed in chapter 1.3.2, HBV seroprevalence estimates in Kenya vary depending on the population screened, with most studies focusing on specific groups such as PLWHIV, MSM, PWIDs and those undertaking transactional sex (112,113). There are limited studies screening broader populations such as blood donors (100) and antenatal women (114), and to date only one national survey of HBV infection from 2007, but even that excluded PLWHIV so was not fully inclusive (102). Accurate HBV seroprevalence estimates are essential to underpin an evidence base for local strategies for interventions in Kenya and to highlight knowledge gaps to inform research.

Along with a lack of seroprevalence data, there is also limited understanding of circulating HBV genotypes in Kenya and the WHO African region more broadly. As already discussed in chapter 1.2.2, there are currently 9 established HBV genotypes which vary by geographical location, with genotype A being thought to predominate in East Africa, although data supporting this is limited, and again primarily from isolated populations and from Sanger sequencing of one or two genes. Genotype potentially affects both the progression of HBV disease and response to treatment, however most studies of the impact of HBV genotype have been in Asia and Europe and there is a paucity of data on circulating genotypes and subgenotypes in Africa, including Kenya. Before beginning new genomic research in Kenya, I set out to understand what data is currently available regarding HBV genotypes in Kenya

In this chapter I take two approaches to addressing these data gaps, first presenting a systematic review and meta-analysis of existing studies reviewing HBV prevalence and circulating HBV genotypes and sub-genotypes in Kenya, and secondly summarising data from clinical trials previously done at KWTRP which screened for HBV.

2.2 Systematic review & meta-analysis of HBV epidemiology & genomic data in Kenya

The work in this chapter has been published in PLoS Global Public Health:

Downs LO, 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 Health. 2023;3(1):e0001165.

I undertook the online search, quality-assessed the papers, extracted the relevant data, undertook statistical analysis (with assistance from Dr Cori Campbell), produced all the figures, and wrote the first draft of the manuscript and subsequently took oversight of the final document incorporating revisions from other authors. This work has been included in my thesis with permission from all the other authors.

2.2.1 Methods

2.2.1.1 Search Strategy

I reviewed the literature on HBV prevalence and genetic data in Kenya using the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) 2020 checklist (115). I searched the online databases PubMed, Embase, African Journals Online (AJOL) and Scopus on 6th December 2021 using the terms in Table 2.1. I included studies published in English, from 2000 to December 2021 (from 2003 for AJOL) that investigated prevalence, genotype and sequencing of HBV infection in Kenya. I only included data for adults from studies for which the full text was available. There was no minimum number of participants for studies included. I initially screened using a thorough review of the title and abstract and subsequently reviewed the full manuscripts of eligible articles. Articles that did not meet the inclusion criteria were excluded. I discussed any uncertainty regarding the inclusion of papers with another reviewer to reach a consensus.

Database searched	Search terms
PubMed	(("Hepatitis B"[Mesh]) OR ("hepatitis b"[Title/Abstract] OR HBV[Title/Abstract] OR "hepatitis type b"[Title/Abstract] OR "type b hepatitis"[Title/Abstract] OR "hep b"[Title/Abstract])) AND (("Kenya"[Mesh]) OR (kenya*[Title/Abstract] OR nairobi*[Title/Abstract] OR mombasa*[Title/Abstract]))
Embase	1 exp hepatitis B/ (109266) 2 ("hepatitis b" or HBV or "hepatitis type b" or "type b hepatitis" or "hep b").ti,ab. (135330) 3 1 or 2 (161216) 4 Kenya/ (22999) 5 (Kenya* or nairobi* or mombasa*).ti,ab. (26384) 6 4 or 5 (29072) 7 3 and 6 (227) 8 limit 7 to yr="2000 -Current" (173)
AJOL	(("Hepatitis B" OR ("hepatitis b" OR HBV OR "hepatitis type b" OR "type b hepatitis" OR "hep b" AND (("Kenya") OR (Kenya* OR nairobi* OR mombasa*))
Scopus	(("Hepatitis B" OR ("hepatitis b" OR HBV OR "hepatitis type b" OR "type b hepatitis" OR "hep b" AND (("Kenya") OR (Kenya* OR nairobi* OR mombasa*))

Table 2-1: Terms used to search online databases for a systematic review reporting prevalence and genetic data for HBV in Kenya. I also modified search terms to include the 5 largest cities in Kenya (Nairobi, Mombasa, Eldoret, Nakuru and Kisumu with no change to the results).

From each study, I extracted:

- Total number of individuals tested for HBV;
- Number of individuals found to be infected with HBV (either HBsAg positive or HBV DNA positive);
- Study location (city or geographical region);
- Participant selection criteria;
- Laboratory methods for confirmation of HBV infection.

2.2.1.2 Study Heterogeneity and HBV risk groups

During the initial screening steps, the study populations reported were found to be so heterogenous, that pooling them to make an overall prevalence estimate would not be informative. Therefore, I divided the studies into four groups representing populations at differing risk of HBV acquisition. The low-risk group included studies likely to be most representative of the general population (antenatal women, healthcare workers, blood donors and the national survey). The moderate risk group consisted of studies of PLWHIV. High-risk groups were defined as people with risk factors for acquisition of blood-borne virus infection, including PWID, MSM and those undertaking transactional sex. Those presenting to hospital with hepatitis or jaundice were defined as a very high-risk group, as HBV infection is enriched in populations presenting with established liver disease, particularly if the background population has medium or high HBV prevalence. I used this risk stratification system throughout the systematic review and analysis as the most pragmatic way to approach highly heterogenous literature.

2.2.1.3 Quality appraisal

I undertook a thorough assessment of the study quality using the PRISMA guidelines (115) and Joanna Briggs Institute critical appraisal checklist for prevalence studies (116) (table 2.2). I discussed any dispute surrounding study quality with another reviewer and we reached a consensus.

First Author	Was the sample frame appropriate to address the target population?	Were the participants sampled in an appropriate way	Was the sample size adequate	Study subjects and setting described in detail?	Was the data analysis conducted with sufficient coverage of the identified sample?	Were valid methods used for the identification of the condition?	Was the condition measured in a standard, reliable way for all participants?	Was there appropriate statistical analysis?	Was the response rate adequate, and if not, was the low response rate managed appropriately?
Ngaira JA (1)	Yes	Yes	Yes	Yes	Yes	Yes ELISA	Yes	Yes	Yes
Awili HO (2)	Yes	Yes	No calculation	Yes	Yes	Yes ELISA	Yes	Yes	Yes
Onyango CG (3)	Yes	Yes	No calculation	No – looks like recruited from schools	Yes	Yes - ELISA	Yes	Yes	Yes
Wamamba D (4)	Yes	Yes	No calculation	Yes	Yes	Yes ELISA	Yes	Yes	Yes
Aluora PO (5)	Yes	Yes	Yes	Yes	Yes	Yes – CMIA	Yes	Yes	Mostly young men
Bartonjo G (6)	Yes	Yes	? Intuitive sample size	Yes	Yes	Yes - ELISA	Yes	Yes	Over-represented one area (Nakuru)
Kisangau EN (7)	Yes	Yes	Yes	Yes	Yes	Yes - ELISA	Yes	Yes	Mostly female nurses <35 years
Ly KN (8)	No – only HIV -ve patients included	Yes	No calculation	Yes	Yes	Yes – ELISA	Yes	Yes	Yes
Maina DN (9)	No – only small part of Kenya sampled	Yes	No calculation	Yes	Yes	Yes - CLEIA	Yes	Yes	Yes
Kim HN (10)	No – only in Nairobi	Randomisation technique not described	No calculation	No clear inclusion/exclusion	Yes	Yes – ELISA	Yes	Yes	Yes
EN Njuguna (11)	Yes	No – purposive sampling	No calculation	Yes	Yes	Yes – ELISA	Yes	Yes	Yes

Muriuki BM (12)	Yes	Yes	No calculation	Yes	Yes	Yes - ELISA	Yes	Yes	Yes
Harania RS (13)	No – only done in Nairobi	No description of sampling technique	No calculation	Minimal detail	Yes	Yes – ELISA	Yes	Yes	Yes
Greer AE (14)	Yes	Yes	No calculation	No clear inclusion/exclusion	Yes	Yes – CLIA	Yes	Yes	Yes
Oyaro M (15)	Yes	Yes	No calculation	Yes	Yes	Yes - ELISA	Yes	Yes	Many more males than females
Webale MK (16)	yes	Yes	No calculation	Yes	Yes	Unclear – 5 panel rapid test	Yes	Yes	HIV population over-represented
Day SL (17)	Yes	Yes	No calculation	Yes	Yes	Yes – CLIA	Yes	Yes	Yes
Ochwoto M (18)	Yes	Yes	No calculation	Yes	Yes	Yes - CLEIA	Yes	Yes	Many more from Nairobi
Muchiri I (19)	Yes	No description of sampling	No calculation	No clear inclusion/exclusion	Yes	Reverse passive haemagglutination	Yes	Yes	Yes
Atina JO (20)	Yes	Yes	No calculation	Yes	Yes	Reverse passive haemagglutination	Yes	Yes	Yes
Wahome E (21)	Yes	Yes	No calculation	Yes	Yes	Yes ELISA	Yes	Yes	High proportion of sex workers
Jepkemei (22)	No – only done in Nairobi	Yes	No calculation	Yes	Yes	Yes ELISA and DNA PCR	Yes	Yes	Yes
Salyani (23)	Yes	Yes	Yes	Yes	Yes	Yes ELISA and DNA PCR	Yes	Yes	Yes

Table 2-2: Joanna Briggs Checklist for Prevalence Studies (Available from https://jbi.global/critical-appraisal-tools).

2.2.1.4 Identifying and analysing full-length HBV sequences from Kenya

I accessed GenBank on 1st December 2021 and downloaded all full genome HBV sequences from Kenya to assimilate a reference set. I aligned these sequences with available HBV reference sequences for each genotype (80) using MAFFT (117) and generated a maximum likelihood phylogenetic tree with bootstrap replicates of 1000 using NGPhylogeny.fr (118).

2.2.1.5 Statistical analysis

I generated pooled prevalence estimates for CHB infection (i.e. HBsAg seroprevalence) via meta-analysis using logit transformed proportions of standard errors. I conducted meta-analysis in R (version 4.1.1) using the 'meta' package (version 5.2–1), calculating standard errors for prevalence using the formula $\sqrt{-p(1-p)/n}$, with the total sample size represented by n and the proportion of the sample seropositive for HBV represented by p. I stratified meta-analysis by risk group as described above. I used the I2 statistic to quantify study heterogeneity within each risk group, calculated using $I^2 = 100\%\, x\, (Q - df)/Q$ where Q– Cochran's coefficient and df = degrees of freedom. I then presented pooled prevalence estimates from the random effects models for each risk group. I identified study site locations using latitudinal and longitudinal data from Googlemaps and using R (version 4.1.1) packages 'rworldmap' (version 1.3–6) 'ggmap' (version 3) and'ggplot2' (version 3.3.5) packages I created a map plot to visualise this.

2.2.1.6 Occult HBV infection

Some studies identified in this systematic review reported on occult HBV infection prevalence. Occult HBV infection (OBI) is defined as detectable HBV DNA in the absence of HBsAg. To ensure data sets were comparable between studies, I excluded those study participants identified as having OBI from the meta-analysis, although did report the prevalence of OBI found in these studies separately.

2.2.2 Results

2.2.2.1 Identification of studies

I identified 272 published studies, of which 23 studies met the inclusion criteria for prevalence assessment, representing a total of 11,467 people (Figure 2.1 and Table 2.3). Three of these studies also screened individuals for occult HBV infection (OBI) in a total of 666 people using HBV DNA polymerase chain reaction (PCR) in addition to testing for HBsAg. Two studies screened initially with HBsAg, then undertook HBV DNA PCR on those testing HBsAg negative (100,119). A further study included two different populations: a) those attending a clinic for sex workers, whom they screened

initially for HBsAg, then HBV PCR in those who were HBsAg negative and b) known HBsAg negative, jaundiced participants whom they screened with HBV DNA PCR to detect OBI (120). I identified nine studies reporting HBV sequence data (full or partial genome), including seven studies from among the 23 seroprevalence studies described above (Table 3), and an additional two studies that only included HBsAg positive participants so were not included in prevalence analysis (121,122). One study did not clearly report how many HBV samples were sequenced or the genotyping results, and this study was excluded from further analysis (123). Eight studies remained for analysis representing 247 individuals (Table 3). I identified eight studies reporting HBsAg prevalence in low-risk populations (total number of individuals = 6828), seven studies in people living with HIV (medium risk, total number of individuals = 1861), five studies in high-risk groups (total number of individuals = 2221) and three studies in people presenting to clinical services with established liver disease (defined here as very high-risk for HBV infection; total number of individuals = 492).

2.2.2.2 Geographical distribution of HBV seroprevalence data

Of the 23 studies included, 14 (61%) were in Nairobi or Mombasa, Kenya's most populous cities (Table 3), and all studies were done in the South of the country along the infrastructure routes between Mombasa, Nairobi and Kisumu. These are also the most densely populated Kenyan counties (124). Kisumu was the city most represented in the studies by overall sample size (Figure 2.2). The mean cohort sample size was 599 participants (IQR 434). 14 studies recruited participants for cohort inclusion at outpatient clinics (8 in HIV clinics, 4 in blood donor clinics, 1 in a health clinic and 1 in antenatal clinic), one captured data through the blood donor registry, three undertook community outreach screening, three recruited hospital inpatients, one recruited HCWs and one was a national survey of urban and rural population groups (Table 2.3).

2.2.2.3 Quality assessment of the literature

Overall, the quality of studies investigating HBV prevalence in Kenya was low (Figure 2.3 and Table 2.2). 17/23 studies were cross sectional, reporting HBV population prevalence at a single time point only. Most cohort sampling methods were non-randomised and only 4/21 studies detailed their sample size calculation (58,100,114,119). Several studies sampled people only from small geographical locations or from a subset of the general population e.g. HIV negative individuals. 21/23 studies used either ELISA or CLEIA for HBsAg diagnosis. Two studies used reverse passive haemagglutination for diagnosis of CHB, a method previously demonstrated to have poor sensitivity (98,125,126) (Figure 2.3). 2/23 studies went on to screen the HBsAg negative population for HBV DNA

via PCR (100,119) and one study included a known HBsAg negative population which was screened for HBV DNA (120).

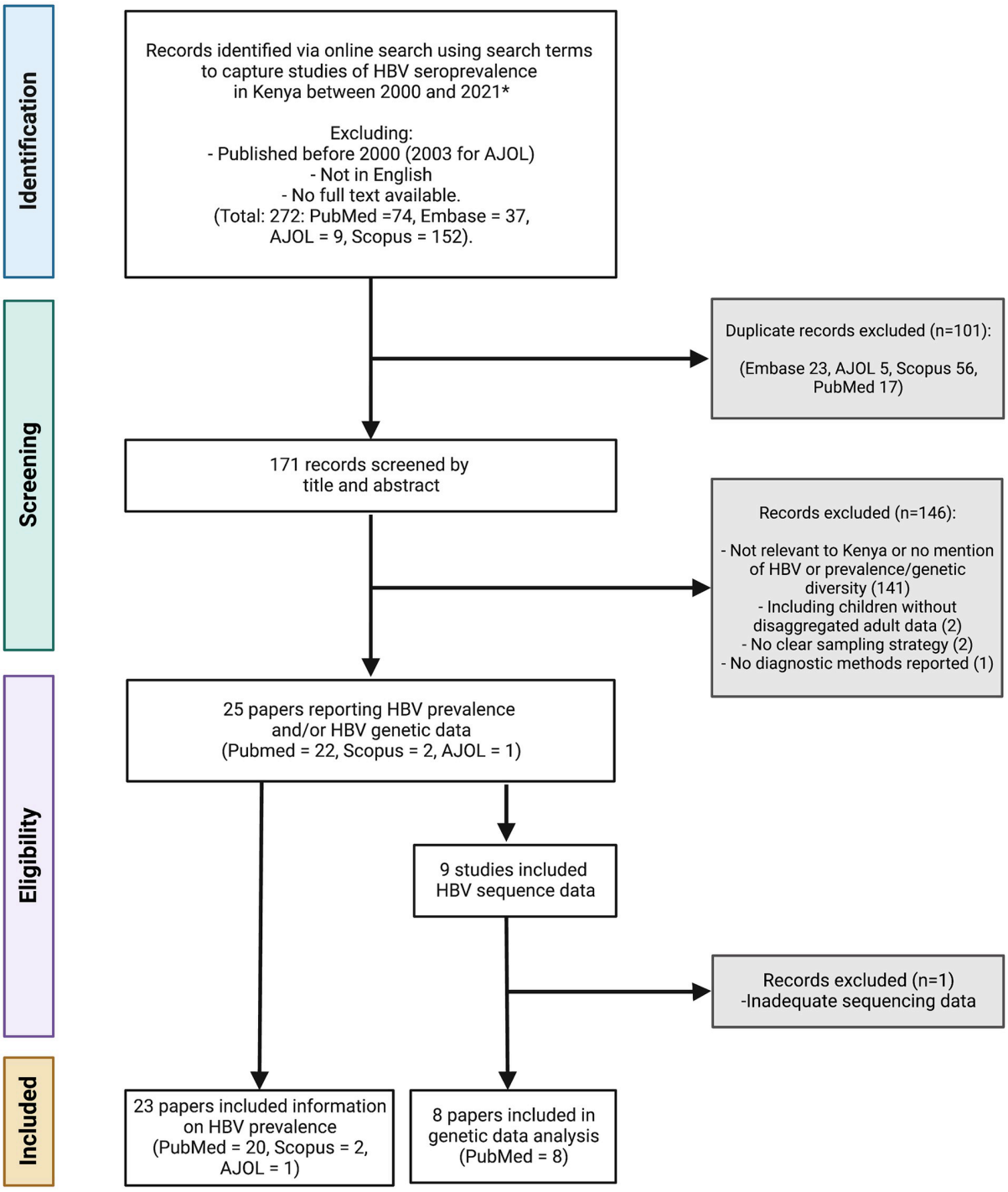


Figure 2-1: Flow chart for eligibility of studies included in a systematic review reporting prevalence and genetic data for HBV in Kenya between 2000–2021. (AJOL: African Journal Online). All eight studies included for genetic analysis contain information on HBV genotype. Figure created in Biorender.com with licence to publish.

First Author	Year	N	Location	HBV +ve (%)	Data collection method	Study design	Sampling type	Population recruitment location	Population Investigated	Detection Method
Very high-risk populations (total 3 studies representing 492 individuals)										
Atina JO (98)	2004	60	Nairobi	30	Prospective	Cross sectional	Non-random	Hospital	Hepatitis	RPHA
Muchiri I (125)	2013	100	Nairobi	13	Prospective	Cross sectional	Non-random	Hospital	Hepatitis	RPHA
Ochwoto M* (127)	2016	332	Nairobi, Mombasa, Kisumu, Eldoret	50.6	Prospective	Cross sectional	Non-random	Hospital	Jaundiced	CLEIA
High risk populations (total 5 studies representing 2221 individuals)										
Day SL (128)	2013	159	Mombasa	7	Prospective	Cohort	Non-random	HIV clinic	Female sex workers	CLEIA
Webale MK* (112)	2015	752	Mombasa	5.2	Retrospective	Cross sectional	Non-random	Community	Drug users	5 panel rapid test
Wahome E (95)	2017	525	Coastal Kenya	8	Retrospective	Cohort	Non-random	Community	HIV neg MSM	ELISA
Oyaro M* (113)	2018	673	Nairobi, Mombasa, Kisumu	4.3	Prospective	Cross sectional	Non-random	Community	Drug users	ELISA
Jepkemei (120)	2020	112	Nairobi	9.8	Retrospective	Cohort	Non-random	Health clinic	MSM	CLEIA
Moderate risk populations (total 7 studies representing 1861 individuals)										
Harania RS (129)	2008	357	Nairobi	6.2	Prospective	Cross sectional	Non-random	HIV clinic	Adults HIV	ELISA
Kim HN* (130)	2011	389	Nairobi	6.9	Retrospective	RCT	Random	HIV clinic	Adults HIV	ELISA
Muriuki BM (93)	2013	290	Nairobi	6.2	Prospective	Cross sectional	Non-random	HIV clinic	Adults HIV	ELISA
EN Njuguna (131)	2015	322	Nairobi, Thika	1	Prospective	Cross sectional	Random	HIV clinic	HIV discordant couples	ELISA
Maina DN (94)	2017	190	Kajiado	5.8	Prospective	Cross sectional	Non-random	HIV clinic	Adults HIV	CLEIA
Greer AE (123)	2017	105	Kisumu	11	Prospective	RCT	Non-random	HIV clinic	HIV discordant couples	CLEIA
Salyani A (119)	2021	208	Nairobi, Kijabe, Mbagathi	5.8	Prospective	Cross sectional	Non-random	HIV clinic	Adults HIV	CLEIA
Low risk populations (total 8 studies representing 6828 individuals)										
Ngaira JA (114)	2016	287	Mbagathi	3.8	Prospective	Cross sectional	Random	Antenatal clinic	Pregnant Women	ELISA
Ly KN (102)	2016	1091	Countrywide	2.1	Retrospective	2 stage cluster	Random	National survey	Urban and rural clusters	ELISA
Wamamba D (132)	2017	2046	Kisumu	3.1	Retrospective	Cross sectional	Non-random	Blood donor clinic	Blood Donors	ELISA
Onyango CG (133)	2018	1215	Kisumu, Siaya, Homa Bay	3.5	Prospective	Cross sectional	Random	Blood donor clinic	Blood Donors	ELISA
Bartonjo G (134)	2019	594	Nakuru, Tenwek	5.6	Prospective	Cross sectional	Random	Blood donor registry	Blood Donors	ELISA
Kisangau EN (58)	2019	295	Makueni	4.5	Prospective	Cross sectional	Random	Hospital rota	Healthcare workers	ELISA
Awili HO (99)	2020	1000	Kisumu, Siaya, Homa Bay	3.4	Prospective	Cross sectional	Non-random	Blood donor clinic	Blood Donors	ELISA
Aluora PO (100)	2020	300	Nairobi	2.3	Prospective	Cross sectional	Random	Blood donor clinic	Blood Donors	CLEIA
Studies of known HBsAg positive people included for genetic data analysis (total 2 studies representing 90 individuals)										
Kwange SO* (121)	2013	32	Nairobi	100	Retrospective	Cross sectional		Blood donor registry	Blood Donors	ELISA
Ochwoto* (122)	2013	58	Nairobi	100	Prospective	Cross sectional		Blood donor registry	Blood Donors	ELISA
Studies of occult HBV infection (OBI) prevalence in HBsAg negative individuals (total 3 studies representing 666 individuals)										
Jepkemei* (120)	2020	166	Nairobi	18.7	Retrospective on HBsAg -ve	Cohort	Non-random	Health clinic	MSM/Jaundice	HBV DNA PCR
Salyani A (119)	2021	196	Nairobi, Kijabe, Mbagathi	5.3	Retrospective on HBsAg -ve	Cross sectional	Non-random	HIV clinic	Adults HIV	HBV DNA PCR
Aluora PO* (100)	2020	293	Nairobi	2.4	Retrospective on HBsAg -ve only	Cross sectional	Random	Blood donor clinic	Blood Donors	HBV DNA PCR

Table 2-3: Description of studies included in a systematic review and meta-analysis of HBV prevalence and available HBV genomic data in Kenya between 2000 – 2021. This includes two studies on solely HBsAg +ve patients used only for analysis of genetic data [23,24] and three studies of Occult HBV infection (OBI) prevalence. Studies are ranked as very high / high / moderate / low risk groups (presented in order) and within these categories ordered by date of publication. Studies marked with an asterisk (*) are those which presented HBV genetic information including genotype. N – number in the study; RCT – randomised controlled trial; RPHA – Reverse passive haemagglutination; CLEIA – Chemiluminescent immunoassay; ELISA – enzyme linked immunosorbent assay; PCR – polymerase chain reaction; HBsAg – Hepatitis B surface antigen; MSM – men who have sex with men.

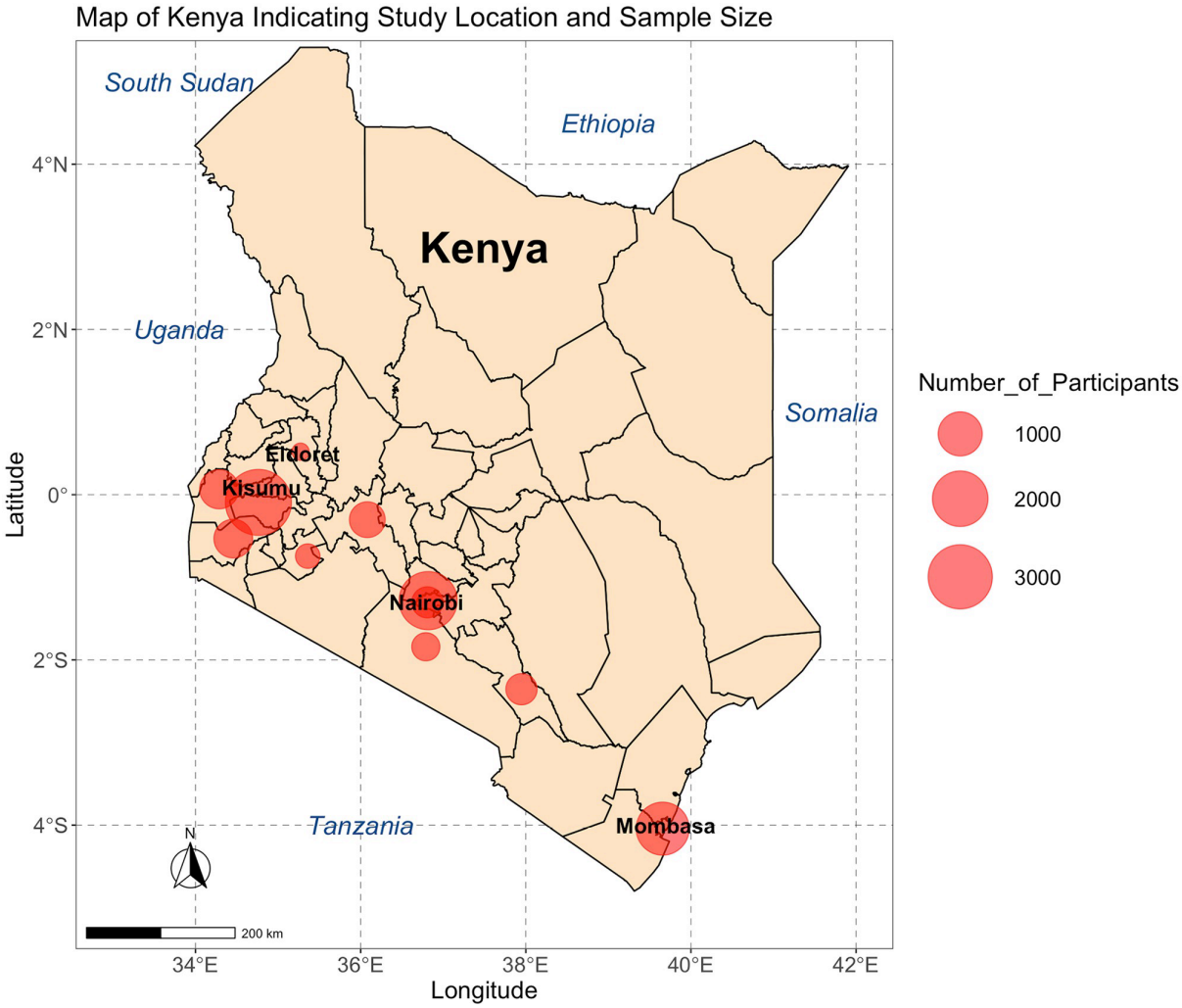


Figure 2-2. Map of Kenya indicating the locations and size of populations from which seroprevalence of HBV infection is reported. Data from a systematic review of papers reporting prevalence and genetic data for HBV in Kenya between 2000 and 2021. The size of the red circle indicates numbers screened in each location, studies in the same location are grouped together. n = number of individuals reported. Figure created using R version 4.2.0, packages ggmaps version 3.0.0, ggplot2version 3.3.6 and sf version 1.0–7. The Kenyan County shapefiles were obtained from the Humanitarian Data Exchange, available open source from https://data.humdata.org/dataset/geoboundaries-admin-boundaries-for-kenya.

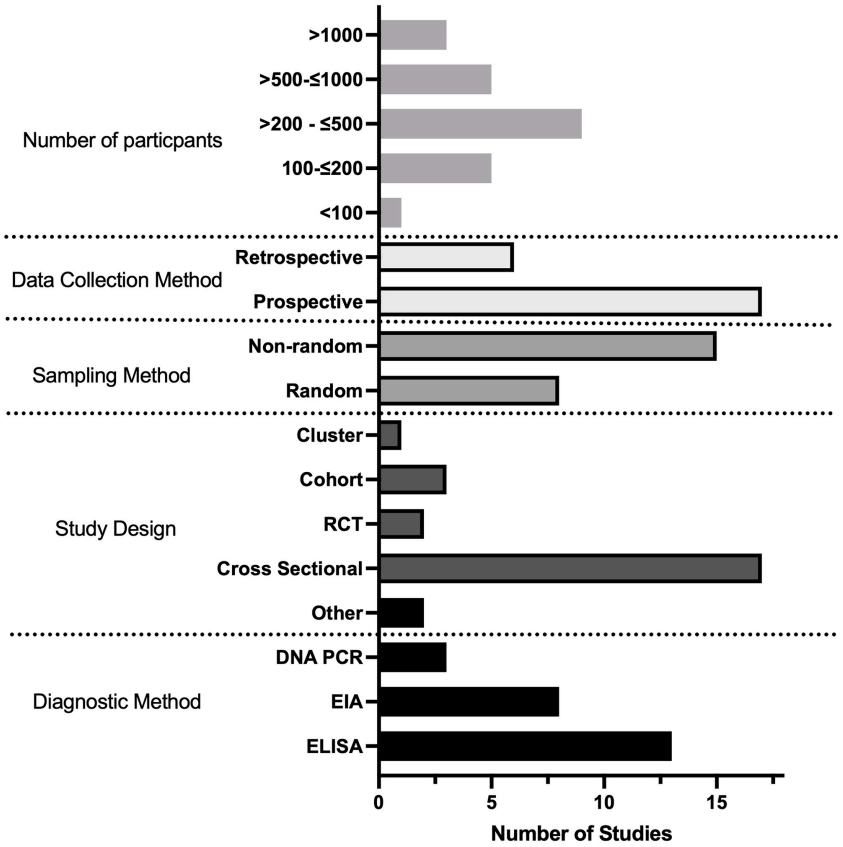


Figure 2-3. Bar chart showing characteristics of studies identified for a systematic review reporting prevalence and genetic data for HBV in Kenya. This is stratified by number of participants, study design, sampling method, data collection and diagnostic methods. RCT: Randomised controlled trial; EIA: Chemiluminescent enzyme immunoassay; ELISA: Enzyme linked immunosorbent assay.

2.2.2.4 HBV prevalence estimates in different risk groups

The pooled estimate for HBV prevalence using a random effects model in the low-risk group was 3.36% (95% CI 2.67–4.21%) compared with 6.14% in the moderate risk group (95% CI 5.08–7.41%), 6.18% (95% CI 4.6–8.19%) in the high-risk group and 29.19% (95% CI 12.15–55.14%) in the very high-risk group, however the confidence interval of this estimate was very wide (Figure 2.4). Heterogeneity was significant (I^2 > 50%) within each subgroup, and highest in the very high-risk sub-group (I^2 = 95%, p < 0.01). Three studies screened for OBI using HBV DNA PCR. These were in populations known to be HBsAg negative and from different HBV risk groups: blood donors, those living with HIV and those presenting to hospital with jaundice. OBI prevalence estimates in these studies were 2.4%, 5.3% and 18.7% respectively (100,119,120).

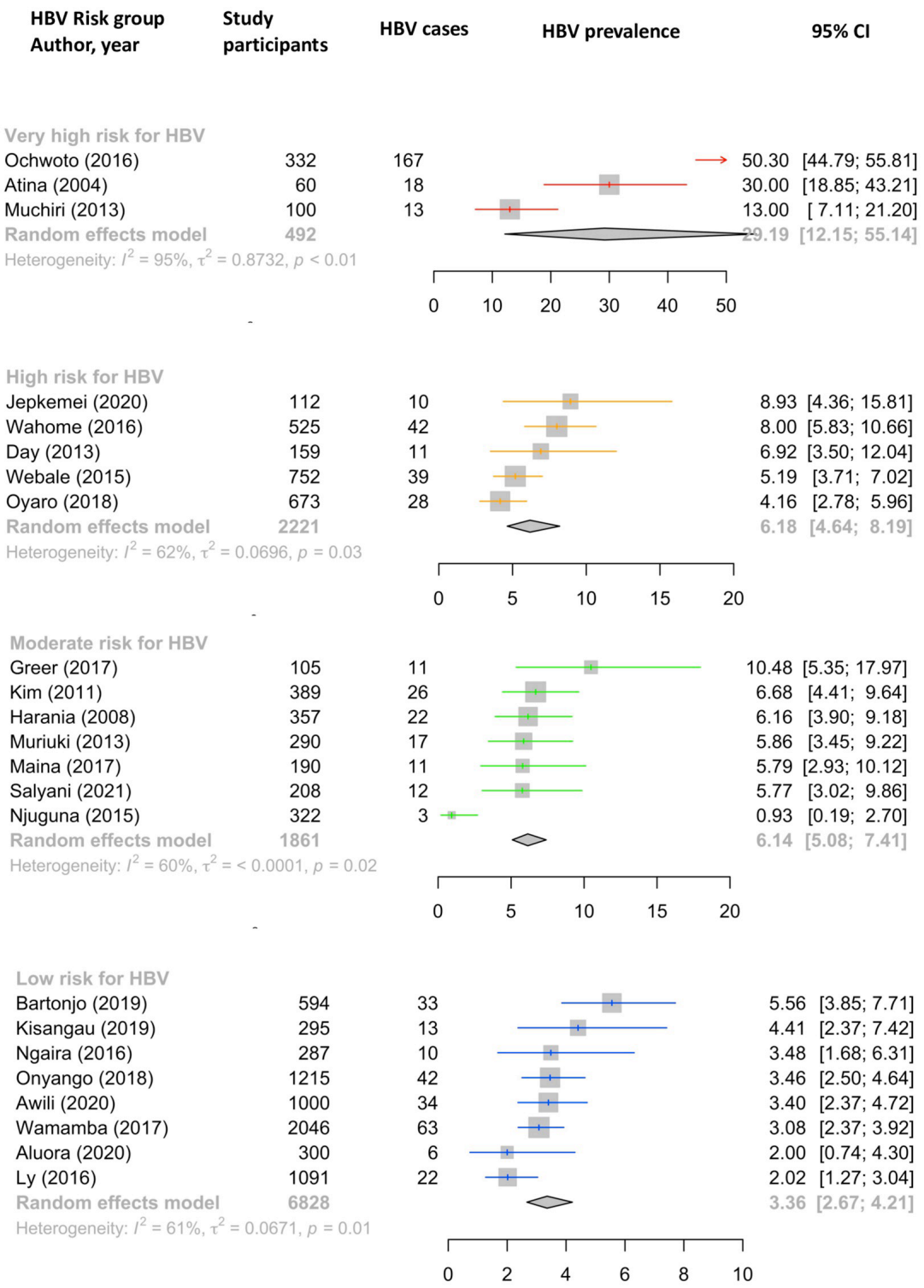


Figure 2-4: Forest plot showing pooled HBV prevalence for populations in different risk groups by random effects model. Data generated through a systematic review reporting prevalence and genetic data for HBV in Kenya between 2000–2021. In each case, the size of the population included is represented by the size of the square. Point prevalence and 95% Confidence Interval (CI) is indicated for each study. Studies are ordered by HBV prevalence in each risk group.

2.2.2.5 Identification of HBV sequences

All eight studies including HBV genetic information used PCR of the HBV basal core promotor, Pol or S genes for amplification, followed by Sanger sequencing to determine genotype. Two studies looked for known drug resistance-associated mutations (RAMs) (121,122). Two studies undertook whole genome HBV sequencing in a total of 22 patients (121,127). 228/247 (92%) of participants were infected with HBV genotype A, 15/247 (6%) with genotype D infection, whilst the remaining were either mixed genotype populations (2/247) or genotype D/E recombinants (2/247) (Table 4). Sub genotype was determined in 146/247 (59%) participants. This was most commonly sub-genotype A1 (134/146, 92%) in keeping with previous regional data (135). To provide further background context for HBV sequences in Kenya, I identified 25 full length HBV sequences from GenBank (Figure 2.5). These were generated from three studies, published in 2013, 2015 and 2016 (112,122,127). They primarily represented individuals presenting to hospital with jaundice (21/25 sequences), infected with genotypes A1 and D. 5/8 studies provided a detailed analysis of either amino acid or nucleotide substitutions found in the sequenced region of HBV (100,114,121,122,130). 2/5 studies correlated these with known drug resistance mutations to lamivudine and other NAs (Table 2.4) (100,130). One study reported the emergence of drug resistance mutations during lamivudine treatment associated with breakthrough HBV viraemia (130). Multiple other mutations were described in the five studies, some of which were in the major hydrophilic region of the surface gene, and thus potentially important in influencing both natural and vaccine-mediated immunity (136,137).

First Author	***Publication Year and number of participants***	***Mutations Identified***	***Number of individuals with mutation***	***Resistance conferred***
Kim HN (130)	2011 (n=389) 21 participants had HBV sequenced	rt1233V rtA181S rtV207I	3/21 had rt1233V, rtA181S or rtV207I prior to treatment	Adefovir + lamivudine
		A200V M204I	1/21 developed A200V + M204I during lamivudine treatment	Lamivudine
		M204I	1/21 developed M204I during lamivudine treatment	Lamivudine
Patrick Okoti Aluora (100)	2020 (n=300) 9 participants had HBV sequenced (2 HBsAg +ve, 7 HBV DNA +ve only)	rt181T	1/9	Lamivudine, Adefovir
		M129L V163I	9/9	Lamivudine

Table 2-4: Details of HBV mutations found in two studies in Kenya which reported presence of drug resistance mutations, as part of a systematic review on prevalence and genetic data for HBV in Kenya from 2000 – 2021. In Kim HN, two patients developed HBV breakthrough viraemia during lamivudine treatment and developed either RT M204I mutation alone or both A200V and M204I mutations during treatment.

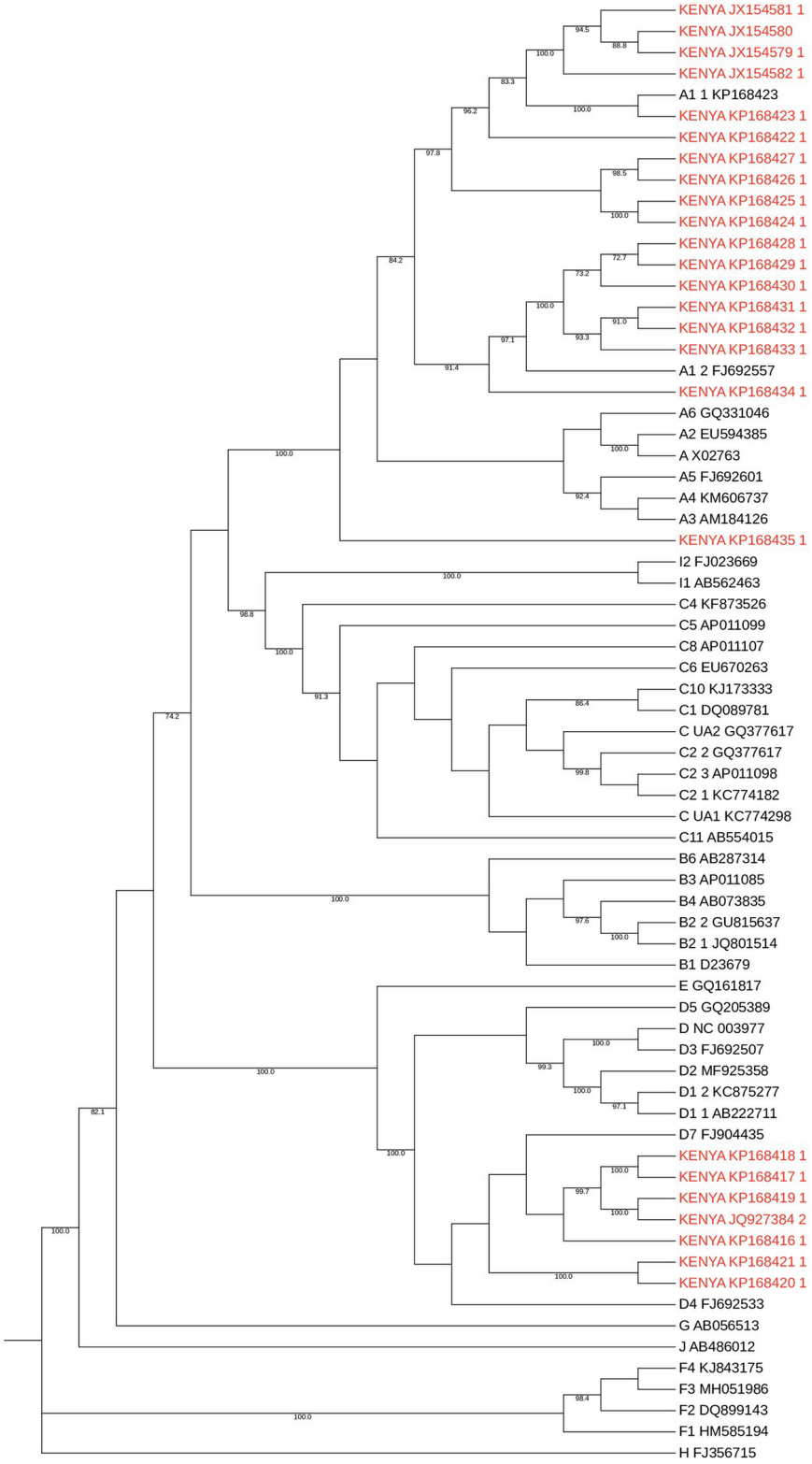


Figure 2-5. Maximum likelihood phylogenetic tree of full-length consensus HBV sequences from Kenya. Kenyan sequences are those published in GenBank (downloaded 1st Dec 2021) and are shown in red alongside genotype reference sequences in black (1000 bootstrap replicates were performed, and bootstrap support of ≥70% are indicated. Reference sequences from McNaughton et al. (2020) (80).

2.2.3 Discussion

This was the first systematic review and meta-analysis covering HBV prevalence and genotypes in Kenya. The pooled prevalence estimate in the low-risk group was 3.4%, in keeping with data from the 2019 Global Burden of Disease study which estimated the HBV prevalence in Kenya to be around 3% (92). Blood donation in Kenya is voluntary without financial compensation and often done to help family members in need. Routine screening through the Kenyan National Blood Transfusion Service (KNBTS) consists of ELISA for HBsAg only however - there is no nucleic acid amplification testing (NAAT); some OBI may therefore go unidentified.

It is notable that only one study here focussed on pregnant women (114) and one on HCWs (58). HBV screening in pregnancy is recommended in all international and Kenyan guidelines as described in chapter 1.1.2 (4,18), and HBsAg negative HCWs should receive HBV vaccination. These are accessible groups with frequent interactions with healthcare, are likely to come for follow up visits, and have the potential to continue transmission events without intervention. Testing and treatment of pregnant mothers could interrupt the transmission cycle, vaccination of babies at birth, and healthcare workers would decrease the overall burden of infection. More general population HBV screening is lacking in Kenya and is not routinely done when presenting to healthcare facilities (138) - even the one nationwide study excluded PLWHIV (102). Some areas of Kenya have been more rigorous in their diagnostic approaches, but this is sporadic and may be increased only when there is a known outbreak of HBV in the local community, as has been the case in other African countries (139,140). This may give a skewed view on population prevalence but also leads to missed opportunities for diagnosis and intervention.

No studies have been done on the burden of HBV in northern Kenya given the low population density and hostility of the environment. The majority of Kenya's population live along the major highways between Kisumu, Nairobi and Mombasa. It should be noted however that the estimated HBV prevalence in neighbouring Somalia and South Sudan is very high (19% and 12% respectively, (141,142)), and there is the potential for migration into Kenya through these borders. These borders tend to be fraught with conflict so the only screening that maybe possible here is likely through humanitarian organisations.

I classified those living with HIV to be in the moderate risk group and PWIDs, MSM and people who undertake transactional sex in the high-risk group, however on analysis, the prevalence in the two groups was very similar (both ~6%). This is likely as these activities are also risk factors for HIV

acquisition, and therefore the two groups overlap. These groups could have been incorporated into one larger group, however I decided on these groups *a priori* on a first look through the data before undertaking the analysis in detail. I decided to continue with the initial allocations throughout the work as I felt demonstrating PLWHIV had a higher HBV prevalence than the general population helped justify the need for enhanced screening in this group. PLWHIV are better represented than other at-risk groups in this systematic review, being more accessible to offer HBV screening, tending to be more engaged with healthcare and returning for repeat medication and monitoring. This highlights the potential use of established HIV infrastructure (including clinics with staff, laboratory support, blood monitoring and drug distribution services), to support clinical care pathways for HBV.

It was striking from this work that there was a very high prevalence of HBV infection in those presenting to hospital with symptoms of jaundice or hepatitis (around 30%), although noting the very wide confidence intervals (12-55%) and high heterogeneity (I^2 = 95%). In Kenya, given there is no routine screening for HBV infection, many people do not get diagnosed until presenting to hospital with manifestations of liver disease. Although this high prevalence cannot be extrapolated to the general population, it is worrying that HBV accounts for such a large percentage of end stage liver disease and highlights the need for increased access to testing to enable diagnosis and treatment before catastrophic consequences.

Three studies reviewed here screened for OBI using PCR. OBI prevalence was comparable to estimated pooled HBsAg prevalence in the associated risk group (2.4%, 5.3% and 18.7% OBI prevalence in low, medium and high/very-risk groups compared with 3.36%, 6.14% and 6.18/29.19% pooled HBsAg positivity estimates in the equivalent groups). This indicates that many HBV cases are being missed due to screening only using HBsAg ELISA or POCT, however the cost and poor availability of HBV DNA testing means it is not currently feasible to use as a universal screening test in Kenya. It is worth noting that of those presenting to hospital with jaundice who tested HBsAg negative, nearly 20% were HBV DNA positive. It is not known whether the jaundice was due to acute HBV infection, or reactivation of chronic disease, but it seems to be an important indicator of HBV infection and screening of all those presenting to hospital with jaundice or hepatitis for OBI with HBV DNA PCR would be optimal. Few studies had characterised HBV exposure and vaccination status using anti-HBc and anti-HBs respectively. This highlights a broader issue around funding and access to laboratory tests needed for complete epidemiological assessment of populations.

Along with minimal general population screening, there is also a paucity of HBV sequencing from Kenya. Of the 25 papers I reviewed for sequencing data, only two reported whole genome sequencing (121,127), and none did NGS. There were only 25 whole genomes on GenBank, all from these two papers. The rest of the data was single gene PCR and Sanger sequencing of surface and pol genes to determine genotype. Expansion of HBV genomic data from Kenya, especially NGS data will enable early detection of minority variant mutations that may be relevant in the emergence of either drug resistance or vaccine escape mutations, and investigation of how viral quasispecies diversity relates to disease phenotype and other biomarkers.

2.2.4 Limitations

The HBV prevalence estimates generated here vary considerably between different risk groups. I established these risk groups *a priori* based on my understanding of risks for HBV acquisition, and the study numbers were too small to break down risk groups further.

The quality of the studies included in this systematic review was low. Many were cross sectional, did not use random sampling, several used unreliable diagnostic methods, and only one study reported its sample size calculations. Even those studies seemingly more inclusive had limitations, for example the national survey excluded PLWHIV, and the study screening HCWs only included women. I did consider restricting the inclusion criteria for this review to only those studies meeting a specific quality threshold, however this would have substantially reduced the amount of available data.

Children were not included in this review. As described in chapter 1.1.5, HBV vaccination has been available as part of the expanded programme of immunisation since 2001 with an estimated coverage of 91% (although this is with considerable geographical variation). Most children should therefore have been vaccinated against HBV, and most of the studies included in this review were done >5 years ago when those vaccinated would still be <18 years old. This review therefore primarily focusses on HBV prevalence in adults PRIOR to the introduction of routine HBV vaccination. Determining HBV prevalence in those <5 years in Kenya will however be of utmost importance to determine vaccine coverage, and to identify groups or geographical areas being missed.

There are no data for the North of Kenya where there is the potential for high HBV rates given the prevalence in neighbouring countries. This highlights the fact that HBV prevalence will be very regional throughout depending on multiple factors, and this should be considered when interpreting these data.

2.2.5 Conclusions

These data suggest that the population prevalence of HBV in sampled regions of southern and coastal Kenya fall into the 'intermediate' HBV prevalence group (2–5%, as defined by the WHO). A sparse literature highlights the pressing need for clinical and research enterprise, to provide an evidence base for realistic and practical strategies that support country-specific scale-up of screening and treatment. Along with this, the paucity of HBV genomic data indicates a pressing need for a better understanding of circulating genotypes in Kenya, including HBV diversity and presence of drug resistance mutations which informs the work I have generated in chapter 3.

2.3 Review of existing data from KEMRI-Wellcome Trust Clinical Trials

The work in this section has been published: ***Downs LO,*** *et al. Where do those data go? Reuse of screening results from clinical trials to estimate population prevalence of HBV infection in adults in Kilifi, Kenya. J Virus Erad. 2023;9(4):100355.*

I developed the concept for this paper, contacted the data governance committee at KWTRP and all relevant primary investigators to consent for use of their data. I undertook data analysis with the assistance of Dr Mark Otiende at KWTRP, produced all the figures, wrote the firsts draft of the manuscript and subsequent revisions after comments from co-authors. This work has been included in my thesis with permission from all authors.

2.3.1 Background

The number of clinical trials conducted in the WHO-AFR has increased over time (143) and many involve screening for infections of public health importance as part of determining eligibility for participation. One such infection widely tested for is HBV. However, as people testing positive for HBV are typically excluded from trial participation at screening, these data are not routinely presented as part of the study output and not accessible in published data sets for reuse, collation or analysis. I therefore postulate that screening data from clinical trials may be an important untapped source of information about HBV prevalence in African populations.

As discussed in chapter 2.1.1, studies estimating CHB prevalence in Africa most often focus on key populations with underlying risk factors, and thus do not represent general population epidemiology (144). Furthermore, most studies do not account for age and sex structure of the local population, which can influence the accuracy of overall prevalence estimates. HBsAg prevalence estimates are therefore likely to be biased, particularly in the WHO-AFR (145). This lack of robust data contributes to the neglect of funding, advocacy, public health interventions and clinical care pathways for CHB. KEMRI and its collaborators generate significant research outputs, including clinical trial data. I set out to collate data from studies previously conducted at KWTRP, Kilifi, Kenya which screened study volunteers for HBsAg, aiming to i) determine the scale of the potential available data source from clinical studies, and ii) derive both a crude and age/sex standardised HBsAg prevalence estimate.

2.3.2 Methods

2.3.2.1 Eligible studies

I determined which studies at KWTRP (enrolling adult participants (≥18 years) since January 2010 and completed by January 2023 had screened for HBsAg (± measured alanine aminotransferase, ALT levels)), through review of laboratory data generated by the local research departments and laboratories over this time. HBsAg data was only accessible from 2016 onwards, as prior to this, routine HBsAg testing was not widely available through the central laboratory at KWTRP.

2.3.2.2 Study populations

All study screening was done in community groups from the Kilifi Health and Demographic Surveillance System (KHDSS), other than SERU (Scientific Ethics Review Unit) number 4024 which recruited frontline staff (Table 5). All studies screened healthy adults (age ≥18 years) with no known chronic disease prior to initial testing. Studies typically restricted the ages of screened participants, with only two studies allowing a broad range of ages from 18 to 64 years. All studies excluded pregnant or breastfeeding women, along with any women planning to become pregnant during the trial. Women were also required to take birth control during the trial, for which most were referred to a local family planning clinic.

In addition to the data from KWTRP, I also searched Clinicaltrials. gov for other completed vaccine trials conducted in Kenya since 2010 that had recruited adults aged ≥18 years and screened for HBsAg prior to enrolment to estimate the amount of data that may be available over a larger geographical area.

2.3.2.3 Ethics and governance

Ethical approval from both the KEMRI SERU in Kenya and the Oxford Tropical Research Ethics Committee (OxTREC) for the collection and collation of existing KWTRP data was obtained (SERU approval number 4565, OxTREC approval number 22–23). Principal investigators for eligible studies were asked to approve data use, and an application was submitted to the KWTRP data governance committee for data access. When necessary, permission from study sponsors was also sought.

2.3.2.4 Data collection

Data managers were contacted to provide study protocols to identify characteristics of the target study population. Data on ALT and HBsAg from the initial trial screening were extracted from the Kilifi Integrated Data Management System (KIDMS) at KWTRP and demographic data including age at time

of screening and sex for each individual participant were extracted from the study database. All data collected were anonymised.

2.3.2.5 Generation of laboratory data

For these studies, Ilab Aries (Instrumentation Laboratory, USA) had been used for alanine transferase (ALT) measurement and point of care HBsAg testing kits were used for HBsAg screening (QuickProfile HBsAg test (Lumiquick Diagnostics Inc, USA) or Hexagon HBsAg test (Human Diagnostics Worldwide, Wiesbaden, Germany)). The upper limit of normal (ULN) for ALT in the Kilifi population was taken as 35 U/L for women and 57 U/L for men, as per 2021 locally derived reference ranges (146). Where electronic data were not available, paper copies of patient screening forms were reviewed.

2.3.2.6 Statistical analysis

Crude and age-sex stratified HBsAg prevalence estimates were calculated for the period covered by the studies, from 2016 to 2022. I adjusted for bias in the age-sex structure of the clinical trial samples by calculating age-sex standardized estimates using the 2019 KHDSS data as the reference population (147). Wilcoxon rank sum test was used to analyse differences in ALT between different sexes and those who were HBsAg positive and negative. Analyses were conducted using R (version 4.2.0) and STATA/IC version 15.1 (StataCorp College Station, Texas, USA, RRID:SCR_012763).

2.3.3 Results

2.3.3.1 KWTRP study data

I identified nine studies which tested potential participants for HBsAg, representing 2839 individuals. Of these studies, 3 were excluded due to data being unavailable (n = 1108) leaving six studies representing 1727 individuals (Supplementary Fig. 1). 1641 individuals had age and sex data recorded alongside HBsAg. Most participants were male (1263/1641, 77%) and median age was 28 years (IQR 23–36 years). Study recruitment and characteristics are shown in table 5.

2.3.3.2 HBsAg prevalence

Of 1727 individuals tested, 60 were HBsAg positive, giving a crude period prevalence estimate of 3.5% (95% CI 2.6–4.5%, prevalence range between studies 1.9 %–3.9%). Standardised HBsAg positivity was highest in those aged >55 years (6.3% CI 2.9–9.7, Table 6). Crude prevalence was higher in men compared with women (3.9% vs 2.1% respectively, Table 6), however when standardised, confidence

intervals for HBsAg prevalence in women were too wide to make any conclusions. Overall standardised HBsAg population prevalence was estimated at 5.0% (CI 3.4–6.6), higher than the 3.5% crude prevalence estimate based on data for 1641 individuals for whom age and sex data were available.

2.3.3.3 ALT data

Of the 1727 individuals screened for HBsAg, 1462 also had ALT measured (85%). ALT ranged from 7 to 333 U/L, with a median of 24 U/ L (IQR 19–33 U/L). Median ALT was higher in those who were HBsAg positive (24 vs 28 U/L, Wilcoxon rank sum p = 0.017), although was not over the ULN for either group. This median difference in ALT between HBsAg positive and negative adults was significant among males but not females (figure 2.6).

	SERU study number, topic and phase (where applicable)	Age restrictions	Recruitment dates	N (adults) screened for HBsAg	Groups excluded	Target population Recruitment strategy
1	SERU 4024. Covid vaccine study. Phase 1b/2.	18-64	2020 - 2022	680	Pregnant women Bf women Those planning pregnancy	Healthy adults - Frontline staff as defined by the government of Kenya*. Recruited through posters advertising the study – individuals had to get in touch with study team.
2	SERU 4106. Use of Unithiol for snakebite. Phase 1 (5).	18-64	2019 - 2023	156	Pregnant women Bf women	Healthy adult volunteers recruited from the KHDSS using established strategies e.g. health talks, barazas, community health volunteers and fieldworkers
3	SERU 3150. Shigella vaccine study (6). Phase 2a	18-45	2016 - 2017	132	Pregnant women Bf women Those planning pregnancy	Healthy adult volunteers recruited from Kilifi town.
4	SERU 3190. Malaria inoculation study (7)	18-45	2016 - 2020	638	Pregnant women Bf women Those planning pregnancy	Healthy adult volunteers** living in Ahero, Kilifi North and South locations with high, low and moderate malaria transmission respectively through community wide meetings.
5	SERU 3711. Malaria vaccine study (8). Phase 1b	18-45	2018 - 2022	40	Pregnant women Bf women Those planning pregnancy	Healthy adult volunteers recruited from the whole KHDSS using established strategies e.g. health talks, barazas, community health volunteers and fieldworkers
6	SERU 3145 *Streptococcus pneumoniae* Vaccine study (9) Phase 1/2	18-40	2016 - 2018	85	Pregnant women Bf women Those planning pregnancy	Healthy adult volunteers recruited from the whole KHDSS using established strategies e.g. health talks, barazas, community health volunteers and fieldworkers

Table 2-5: Characteristics of studies previously conducted at KWTRP which screened potential participants for HBsAg. Bf = Breastfeeding. SERU = Scientific ethics review unit. References given are for the study protocol, study location on ClinicalTrials.gov, or a completed study manuscript. *Frontline staff included healthcare workers, allied health professionals, truckers, security personnel, banking personnel, supermarket staff, police, security personnel, prison workers, laboratory technicians, scientists, logistics personnel, public transport workers including aviation industry amongst others. KHDSS – Kilifi Health Demographic Surveillance System. **Of the 638 volunteers, 134 volunteers were repeat screens over a period of more than 120 days

Group	Total Tested	Proportion of the tested population	Crude HBsAg Seroprevalence (%)	Total in KHDSS population	Proportion of the KHDSS population	Standardised HBsAg Prevalence (%)	95% CI of the standardised prevalence
Overall							
Crude	1727		3.5				
Standardised	1641		3.5	139303		5.0	3.4 – 6.6
Sex							
Male	1263	1263/1641	3.9	61006	61006/139303	3.3	2.4 – 4.2
Female	378	378/1641	2.1	78298	78298/139303	5.0	0.0 – 14.2
Age Group in Years							
18-25	632	632/1641	3.6	40624	40624/139303	2.2	1.0 – 3.4
26-35	588	588/1641	2.4	36686	36686/139303	2.1	0.9 – 3.4
36-45	338	338/1641	5.6	26314	26314/139303	4.3	1.7 – 6.9
46-55	65	65/1641	0	16732	16732/139303	0.0	NA
>55	18	18/1641	5.6	18946	18946/139303	6.3	2.9 – 9.7

Table 2-6: Crude and standardised Hepatitis B surface antigen (HBsAg) prevalence rates derived from six clinical trials and standardised by age and sex based on the structure of the Kilifi Health and Demographic Surveillance System (KHDSS). CI = Confidence Interval.

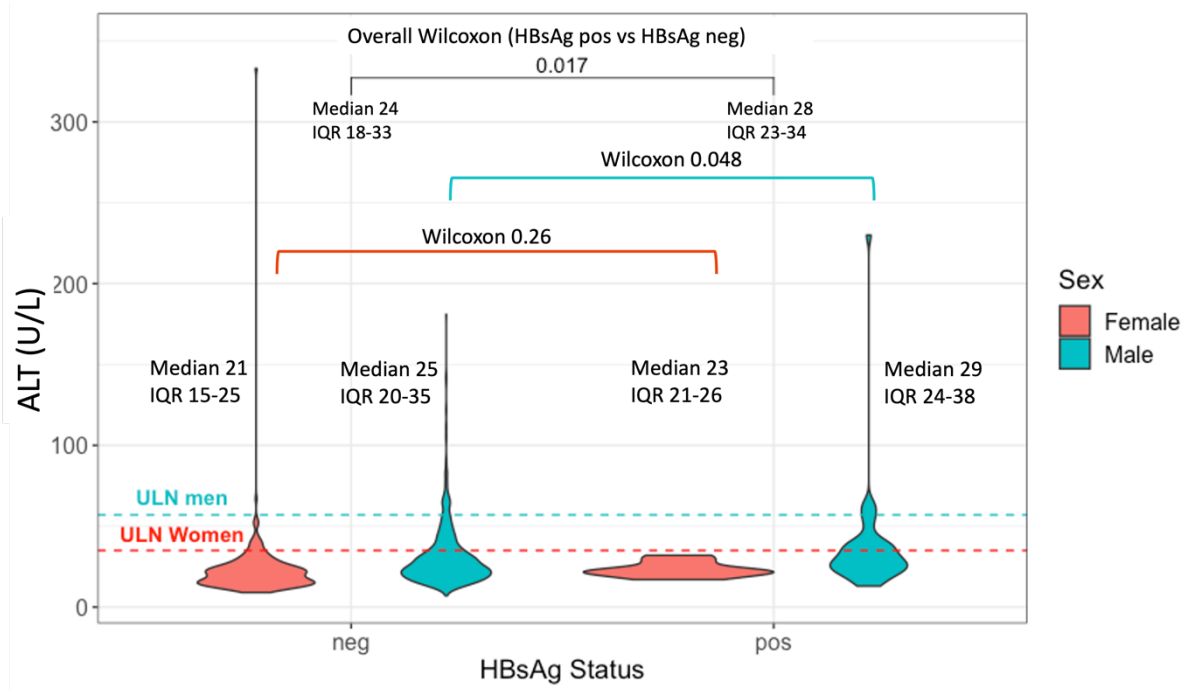


Figure 2-6: Violin plots showing distribution of alanine transaminase (ALT) in adults by HBsAg status and sex in studies conducted at KEMRI-Wellcome Trust Research Programme. ALT reference ranges are shown with dashed horizontal lines showing upper limit of normal (ULN) based on 2021 thresholds derived locally at KWTRP (male ULN = 57 U/L, women ULN = 35 U/L). Wilcoxon p-values are shown comparing ALT in those who were HBsAg positive and negative within each sex and overall between all those who were HBsAg positive and negative.

2.3.4 Discussion

I have demonstrated that there are valuable HBsAg prevalence data in Kilifi, Kenya that have not been previously collated or reported. Studies currently ongoing will, in due course, collect data for a further >1000 participants. The dataset of >1700 adults collated here is comparable in size to the largest pre-existing HBsAg seroprevalence studies in Kenya (reviewed in Ref 45, and although the crude seroprevalence estimates here are similar to those in the national survey or previous studies screening blood donors (102,132,133), the standardised estimate is considerably higher. The populations included in the studies reviewed here were not intended to be representative of the KHDSS population structure or designed to assess HBsAg seroprevalence. Standardization by age and sex is therefore necessary to obtain an estimate that is locally representative and will also be required when using other clinical trials data to estimate infection seroprevalence.

In addition to studies reviewed here, I identified ten studies from Clinicaltrials.gov that had screened for HBsAg, enrolling a total of 1253 participants. Based on an assessment of KWTRP studies, the ratio

tends to be at least 2 people screened for every participant enrolled to a study (although this does vary between studies), indicating there could be potential information on HBsAg status from around another 2500 people in Kenya if data from other studies on Clinicaltrials.gov could be accessed

Large seroprevalence studies are expensive and require substantial infrastructure and resources to deliver. In contrast, the results we have generated here capitalise on the use of pre-existing data, with relatively low additional expenditure required, although time and effort are needed to manage data governance, collation and analysis. HBsAg status was associated with a small but significant elevation in ALT, suggesting HBV infection is a driver of liver inflammation in this population, highlighting the opportunity to identify those with CHB infection for closer monitoring and treatment. This is in line with previous studies in East Africa identifying CHB as a significant cause of deranged LFTs in different populations (148,149). The male excess in clinical trials shown here is striking and highlights the underrepresentation of women. All trials had strict exclusion criteria around pregnancy and excluded women not receiving contraception for the trial duration. Given the age criteria of many of these studies, most eligible women were likely to be of childbearing age, leading to exclusion even from screening. It is also possible that fewer women presented for screening due to other responsibilities such as work, childcare and household management preventing trial participation. The need to include pregnant and breastfeeding women in clinical trials has previously been highlighted (150,151), and although clearly there should be some stratification regarding inclusion of this group, broad exclusion of all women of reproductive age leaves clinicians and women with little or no high-quality data on which to base intervention decisions.

2.3.5 Limitations

Individuals who volunteer to take part in clinical trials are not completely representative of the general population, being a group who are usually healthier, more educated and engaged in research. HBV prevalence is likely to be lower in this group than in the general population. When studies are discussed with community groups to invite study volunteers, exclusion criteria will be made clear. Therefore, those known to have HBV or liver disease may chose not to attend screening, leading to a falsely lower HBV population prevalence estimate. When using clinical trials more widely for prevalence estimates, the population included in the trial must be considered prior to assessment of disease prevalence and any application to a more general population.

Point of care tests (POCT) were used in these trials to screen for HBsAg. These are not as accurate as laboratory-based enzyme-linked immunoassay (ELISA), with published specificity ranging from 97%

for Hexagon to 99.5% for QuickProfile (152,153), so some positive results here are likely to be false. Sensitivity is however also variable, so there will be some false negative cases. Newer HBsAg POCTs have improved sensitivity and specificity, so false positive results will be less of a concern going forwards. The ALT measurement and HBsAg testing were done at the same visit and no longitudinal ALT data was reviewed to evaluate longer term liver function. Many other factors can influence ALT (such as medications, alcohol, aflatoxin consumption, a range of acute and chronic infections) and these may contribute to any derangement at a single time point. Triple HBV vaccination at birth was introduced into Kenya in 2001 as part of the routine childhood immunisation programme, so those born after this date are likely to have a lower HBV seroprevalence than older adults who were born prior to widespread vaccination. This should be born in mind when applying this prevalence estimate to older age groups. So few women were included in these studies that useful estimates of HBsAg prevalence in women are not possible, even when standardised by sex.

2.3.6 Conclusions

I generated a new HBsAg seroprevalence estimate, which is higher than previous figures but likely to be representative of the general Kilifi population due to age-sex standardization to the local population structure. This estimate has been obtained without any extra laboratory or fieldwork and is therefore a highly efficient surveillance exercise, providing a prevalence estimate in a setting where such data are relatively rare and often poorly representative of the general population. I document a small significant increase in ALT in those who are HBsAg positive and also highlight the under-representation of women in clinical trials. The secondary value of clinical trials screening data is sufficiently high to justify the archiving of all hepatitis serology and liver enzyme data, and more broadly hepatitis C and HIV data. This could then provide the substrate for modelling not only HBsAg prevalence by age, sex and geography, and estimating the associated burden of liver disease, across the WHO African region, but also prevalence of other chronic blood borne viral infections.

3. THE STRIKE-HBV STUDY

3.1 Introduction

The STRIKE-HBV study took place at Kilifi County Referral Hospital between March 2023 – June 2024. This constitutes a large body of diverse work.

The overall aim of STRIKE-HBV was to investigate the characteristics of CHB in adults in Kilifi, and identify lifestyle factors, demographics, HBV biomarkers and sequencing characteristics associated with liver disease. More specific objectives are described below:

Objective 1: To establish HBV screening at KCRH to diagnose and recruit a cohort of adults living with CHB. Following this, to develop a detailed cross-sectional description of CHB infection in this group and identify correlations between demographics, lifestyle factors, HBV biomarkers and severity of liver disease.

Objective 2: To obtain an overall HBV prevalence estimate for HBV in Kilifi County in two ways:

i) Using data from existing clinical trials at KWTRP which tested for HBsAg as part of their trial inclusion screening as described in chapter 2.2.
ii) Using data from the new STRIKE-HBV screening programme at KCRH as above.

Objective 3: To characterise how quantitative HBcrAg co-varies with laboratory and clinical markers of disease progression, viral genotype and specific sequence polymorphisms. To trial the new HBcrAg POCT and compare this with laboratory-quantified HBcrAg, other HBV biomarkers and disease phenotype.

Objective 4: To optimise and undertake HBV NGS using Oxford Nanopore Technology (ONT) , to describe:

- Circulating HBV genotypes and subgenotypes
- Inter-host diversity
- Prevalence of mutations associated with tenofovir resistance

The methods, results and discussion have been set out to represent the objectives of the study. Some of this work has already been published in peer reviewed journals and as conference posters.

i) Study implementation, successes and challenges:

Downs, et al. STRIKE-HBV: establishing an HBV screening programme in Kilifi, Kenya-challenges, successes and lessons learnt. Sex Transm Infect 2024; Available from: http://dx.doi.org/10.1136/sextrans-2024-056163.

I conceptualised this piece, undertook the analyses, designed the figures and wrote the manuscript. The final published paper was reviewed and edited by all the authors.

ii) Analysis of the HBcrAg POCT:

Downs, et al. Hepatitis B core-related antigen (HBcrAg) point-of-care tests as a risk stratification tool for treatment eligibility: experience from Kenya, Open Forum Infectious Diseases, 2025;, ofaf125, https://doi.org/10.1093/ofid/ofaf125.

I also developed the concept for this paper, undertook the data analysis, produced the figures and wrote the manuscript. The final published paper was reviewed and edited by all the authors.

iii) Analysis of the HBcrAg POCT - Poster

Downs, et al. (2024). Use of HBcrAg point of care tests in people living with Hepatitis B Virus infection in Kilifi, Kenya to identify those at highest risk of liver disease. figshare. Poster. https://doi.org/10.6084/m9.figshare.27251769.v1 Presented at the Conference on Liver Disease in Africa (COLDA, Cairo, Egypt, September 2024).

As above, I developed the concept for this poster, undertook the data analysis, designed and presented the poster. The final poster was reviewed and edited by all authors. I won best poster for this work.

3.2 Methods

3.2.1 Establishing HBV Screening at KCRH

3.2.1.1 Study Site and timeline

KCRH is described in chapter 1.2.5. We initially undertook HBV screening once per week at KCRH general outpatient clinics where, at the time of the study, people with chronic non-communicable diseases such as diabetes and hypertension were routinely seen. From June 2023, I negotiated access to an extra tent space enabling us to add extra screening days, allowing screening three days a week. Data were collected into a REDCap database (154).

3.2.1.2 Ethics, education and training.

This study was approved by KEMRI SERU – approval ref. SERU 4656 and Oxford Tropical Research Ethics Network (OxTREC) – approval ref. 22-23. Along with this, I gained a NACOSTI research licence and presented STRIKE-HBV to KCRH, subcounty and county management teams for sensitisation and approval. I employed and trained study staff and conducted hospital healthcare worker sensitisation sessions about both HBV and the study specifically.

Working with the Hepatitis B Foundation (155) I developed educational resources in English and Kiswahili (https://doi.org/10.6084/m9.figshare.c.7114813.v2) to support information sessions led by fieldworkers in outpatient waiting areas during screening clinics.

3.2.1.3 Study Design

STRIKE-HBV was a cross-sectional study recruiting a group of non-pregnant adults with CHB and collecting data at a single time-point. We recruited PLWHB in several ways aiming for a total of 100-150 people:

i) By consecutively screening up to 3000 adults at KCRH using a fingerprick point of care test in KCRH outpatients as described by convenience sampling.

ii) By inviting those already known to be living with HBV to participate. These individuals were either already managed by KCRH or presented to our clinic having heard about the study from the community after being diagnosed elsewhere.

iii) By testing close family contacts of PLWHB.

The flow of the study recruiting those testing HBsAg positive is shown in figure 3.1. Alongside this, we recruited 200 people testing HBsAg negative as a control group. These people were recruited in the same clinics as PLWHB in a consecutive manner by convenience sampling. From all recruited to the study (both HBsAg positive and HBsAg negative) we collected:

- Demographic information including sex, age and ethnicity.
- A detailed questionnaire on factors which may influence liver health (e.g. alcohol consumption, diet, use of prescribed/over the counter/recreational drugs, nature of employment, family history. (10.6084/m9.figshare.25714347)
- Liver stiffness scores using transient elastography (Fibroscan® Echosens, Paris).

Blood samples as described in section 3.1.3.

(i) Inclusion criteria

- Non-pregnant adults ≥ 18 years attending outpatient clinics at KCRH or those already known to be living with HBV.
- Able to provide valid informed consent for the study

(ii) Exclusion criteria

- Pregnant
- Unable to provide valid informed consent for the study.

I excluded pregnant women, as at the time of the study liver elastography was not validated in pregnancy, plus there are other reasons why pregnant individuals may have deranged LFTs.

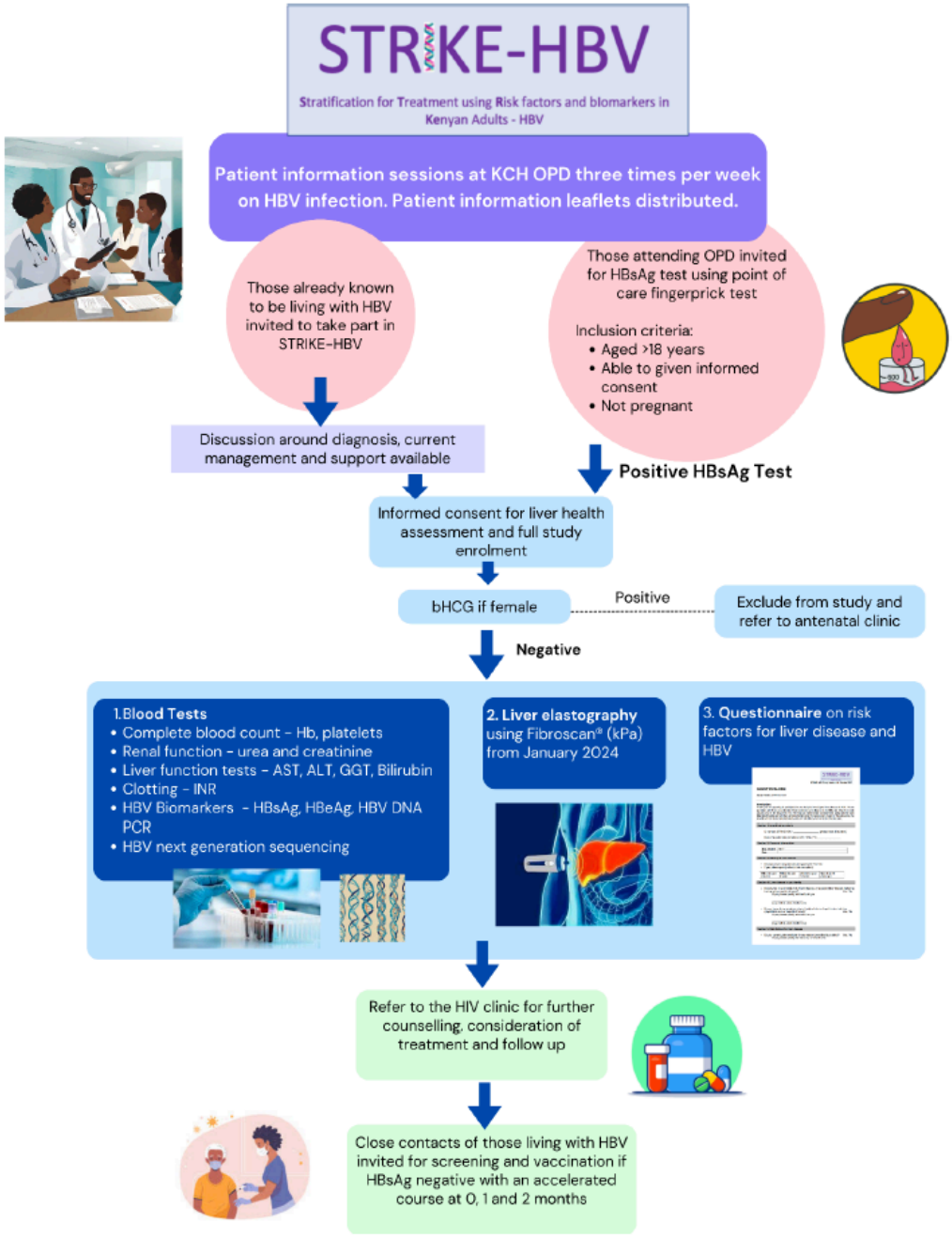


Figure 3-1: Flow diagram illustrating screening and selection of study participants for the STRIKE-HBV study including the planned samples to be collected. Figure created using Biorender.com.

3.2.1.4 Informed Consent.

Informed consent was undertaken separately for the initial screening test and for full study enrolment. All consent forms were available in English and Kiswahili and consent was primarily done by study staff, although I was present in nearly all recruitment clinics to answer questions.

- Screening consent – All eligible adults attending the general medical outpatient clinic on screening days were given verbal information about the study in a group, along with leaflets and invited to participate. We undertook group consent for HBV screening supported by individual discussions if needed.
- Full study consent **–** All those testing HBsAg positive along with 200 people testing HBsAg negative were individually consented for the study.

Study staff were already familiar with the procedure of informed consent, but I trained them in information giving specific to this study, including how to break the news of a new diagnosis of HBV which is a chronic infection requiring lifelong therapy.

3.2.1.5 HBsAg screening

Testing was done using a POCT for HBsAg (first using QuickProfile HBsAg kits (LumiQuick Diagnostics, CA, USA) and subsequently Determine HBsAg 2 (Abbott, USA)) due to variable kit availability. All those who had been diagnosed outside of our clinic had a repeat HBsAg POCT to confirm positivity. Prior to using each new HBsAg POCT kit, positive and negative controls were undertaken to confirm validity.

Although having one positive HBsAg test does not necessarily mean a diagnosis of CHB, given HBV infection in Kenya is primarily acquired in childhood, most adults testing HBsAg positive will have chronic infection. I did consider also testing for hepatitis C virus, however the prevalence is low in the general population in Kenya (156) and at the time of the study there was no treatment available locally.

3.2.1.6 Liver health assessment

All people enrolled in STRIKE-HBV (both HBsAg positive and negative) had a full liver health assessment including liver stiffness measurement using elastography (Fibroscan® Mini + 430 (Echosens, Paris)) and ALT quantification. Fibroscan results were explained to participants and results were passed onto the

CCC with a written interpretation. For those testing HBsAg positive, we also calculated an APRI score using the formula: $\frac{AST/AST\ (ULN)}{Platelets}\ x\ 100$. AST upper limit of normal (ULN) was taken as 42 IU/L for women and 47 IU/L for men as per local Kilifi reference ranges (146). Elastography scores >7kPa and APRI scores >0.5 were considered significant fibrosis as per 2024 WHO HBV guidelines (4).

3.2.2 Sample Size Determination

3.2.2.1 Sample size calculation for prevalence estimates:

In my previous systematic review and meta-analysis described in chapter 2, the estimated prevalence of HBsAg positivity in the general population in Kenya is between 2-5%, I used the formula below to show a variety of sample sizes required to power the prevalence estimate to 95% confidence (table 3.1):

$$\frac{n=z^2\ P(1-P)}{d^2}$$

Where p = suspected prevalence (2-4%)

Where d = precision (usually ¼ of p)

Where z = 95% confidence (standard value of 1.96)

Prevalence (*p*)	Precision (*d*)	1.96^2 (Z^2)	Sample size (*n*)
0.02 (2%)	0.005	3.84	3010
0.03 (3%)	0.0075	3.84	1987
0.04 (4%)	0.01	3.84	1474

Table 3-1: Sample sizes required to power an estimate of HBV prevalence to 80% in the STRIKE-HBV study, depending on the population prevalence.

We therefore planned to screen 2000-3000 people for HBV in Kilifi to power our prevalence estimate to 95%, and to allow recruitment of the required number of PLWHB to detect associations between fibrosis scores and biomarkers as described below.

3.2.2.2 Sample size calculations for associations between fibrosis scores and biomarkers

It was difficult to do a power calculation a priori in the absence of existing data on liver fibrosis and biomarkers in this cohort. However, I determined the range of sample sizes needed to provide 80% power (with α=0.05) to detect a range of correlations, such as between HBcrAg and HBV DNA (table 3.2).

Calculated using https://www.sample-size.net/correlation-sample-size/.

r (correlation coefficient)	Required sample size
0.2	194
0.3	85
0.4	47

Table 3-2: Sample sizes required to detect a range of correlations between liver fibrosis and different HBV biomarkers, with 80% power.

I therefore aimed to recruit a sample size of 100 – 150 adults which will detect a correlation coefficient of between 0.2-0.3 and is in keeping with other studies looking at HBV infection in West, East and Southern Africa. This number was also felt to be feasible with the planned screening numbers and including the people already known to be living with HBV and managed at KCRH.

3.2.3 Laboratory Analysis

Blood samples were drawn and transported to the research or clinical laboratories within 2-3 hours of collection. Routine laboratory markers were measured immediately in the validated diagnostic clinical laboratory at KWTRP. Samples at the research lab were spun to separate serum, and frozen in aliquots at -80°C. The platforms used for analysing HBV biomarkers including where these were done are shown in table 3.3. Those done at KWTRP were performed by me with assistance from Dorcas Okanda. The assays were undertaken retrospectively on defrosted serum.

Biomarker	Platform	Location
Full blood count Urea and electrolytes Liver function tests HIV Ab/Ag	AcT 5diff CP, Beckman Coulter Ilab Aries (Instrumentation Laboratory, MA, USA) Ilab Aries Determine HIV kits (Abbott, Illionois USA)	KWTRP clinical diagnostic lab
HBsAg Detection Semi-quantitative HBsAg	ELISA Murex HBsAg Version 3 (Diasorin, Italy) Abbott Architect (Abbott, US)	KWTRP OUHFT Department of Microbiology, Oxford, UK.
HBeAg	CLEIA (Ig Biotechnology, CA, US)	KWTRP
HBV DNA quantification	Inhouse qPCR developed by Dr Marion Delphin at the Francis Crick Institute Abbott Alinity m HBV Assay (Illinois, USA)	KWTRP OUHFT, Department of Microbiology, Oxford, UK.
HBcrAg HBcrAg POCT	Lumipulse G HBcrAg CLEIA (Fujirebio, Japan) ESPLINE^{TM} (RUO), (Fujirebio, Japan) shipped from Kumamoto University, Japan.	King's College Hospital NHS Foundation Trust, London, UK KWTRP.
HBV NGS	Oxford Nanopore Technologies. Protocol developed by Dr Sheila Lumley at Oxford University.	KWTRP.

Table 3-3: Platforms used to undertake measurement of routine blood tests and HBV biomarkers in PLWHB along with where these were performed for the STRIKE-HBV study done in Kilifi. Kenya. Liver function tests included alanine aminotransferase, aspartate transaminase and gamma-glutyl transferase. HBsAg – hepatitis B surface antigen, ELISA – Enzyme linked immunosorbent assay, KWTRP – KEMRI-Wellcome Trust Research Programme, OUHFT – Oxford University Hospitals Foundation Trust, UK – United Kingdom, US – United States, HBeAg – hepatitis B 'e' antigen, CLEIA – chemiluminescent assay, HBV – hepatitis B virus, DNA – deoxyribonucleic acid, qPCR – quantitative polymerase chain reaction, HBcrAg – hepatitis B core related antigen, POCT – point of care test, NGS – next generation sequencing.

We sent serum to Oxford University Hospitals Foundation Trust (OUHFT), UK for measurement of HBV DNA in the clinical diagnostic laboratory to ensure accuracy, given the in-house HBV DNA qPCR assay had not been validated in this population.

The HBcrAg CLEIA (Fujirebio, Japan) has a linear measurement range of 3-7 $\log_{10}$ U/mL, using 3 $\log_{10}$ U/ml as the lower limit of detection (as recommended by the manufacturer). Samples generating HBcrAg measurements >7 $\log_{10}$IU/ml were diluted (1:5, 1:100 or 1:1000 in Tris Buffered Saline) before repeat testing. My team and I performed HBcrAg-POCT in three batches and were blinded to patient details including HBV VL and HBeAg status. Positive and negative controls were provided and undertaken once prior to each batch testing.

HBV NGS was undertaken on the ONT platform using a HBV tiled amplicon scheme (HEP-TILE) (157). This is a pan-genotypic HBV scheme developed using PrimalScheme3, generating overlapping amplicons to cover the whole HBV circular genome using several discrete primers at each position to handle intraspecies diversity. The ONT pipeline (including wet lab and data analysis) was developed through collaboration with Dr Sheila Lumley (Oxford University), the University of Birmingham (Dr Josh Quick and Chris Kent) and the Francis Crick Institute (Hep B Elimination Laboratory). The full protocol is online at protocols.io (158) and reported in a peer reviewed paper (157).

3.2.4 Statistical Analysis

Objectives 1, 3 and 5: I used multivariable regression analysis to investigate factors associated with:

i) HBsAg positivity
ii) Liver health (liver stiffness scores, APRI scores and ALT) in individuals testing HBsAg positive.
iii) Quantitative HBcrAg

I first did univariable (UV) analysis, and any significant associations were included in multivariable (MV) analysis, along with any parameter of particular interest. In the case of a significant parameter being present in different formats (e.g. HBcrAg CLEIA result as a continuous variable along with the HBcrAg POCT) I only included one of these in the MV analysis. When assessing ALT, I excluded other closely related liver parameters from the MV analysis (GGT). When assessing predictors of APRI score, I also excluded parameters used to derive this score (platelets).

Prior to regression analysis, I performed Multiple Imputation by Chained Equations (MICE) to account for missing variables. Analysis was undertaken using R version 4.1.1.

Assessment of new HBcrAg POCT

WHO guidelines published in 2024 give the option to treat all PLWHB age 12+ who have an abnormal ALT in the absence of other tests (4). Therefore, to assess the potential use of the HBcrAg POCT in clinical practice in settings where most HBV biomarkers are unavailable, I compared the HBcrAg POCT with ALT and calculated sensitivity, specificity, positive predictive value and negative predictive value for how well these tests identify those eligible for treatment based on WHO guidelines either individually or together (figure 3.2). Treatment eligibility criteria, based on the 2024 WHO HBV guidelines (2) were defined as follows:

- Elastography score >7.0kPa = significant fibrosis, >12.5kPa = cirrhosis.
- APRI >0.5 = significant fibrosis, >1.0 = cirrhosis.
- Those with i) elastography score >7kPa; OR ii) APRI score >0.5; OR iii) HBV DNA >2000 IU/ml AND ALT > upper limit of normal (ULN, 19 IU/L for women, 30 IU/L for men)
- HIV co-infection

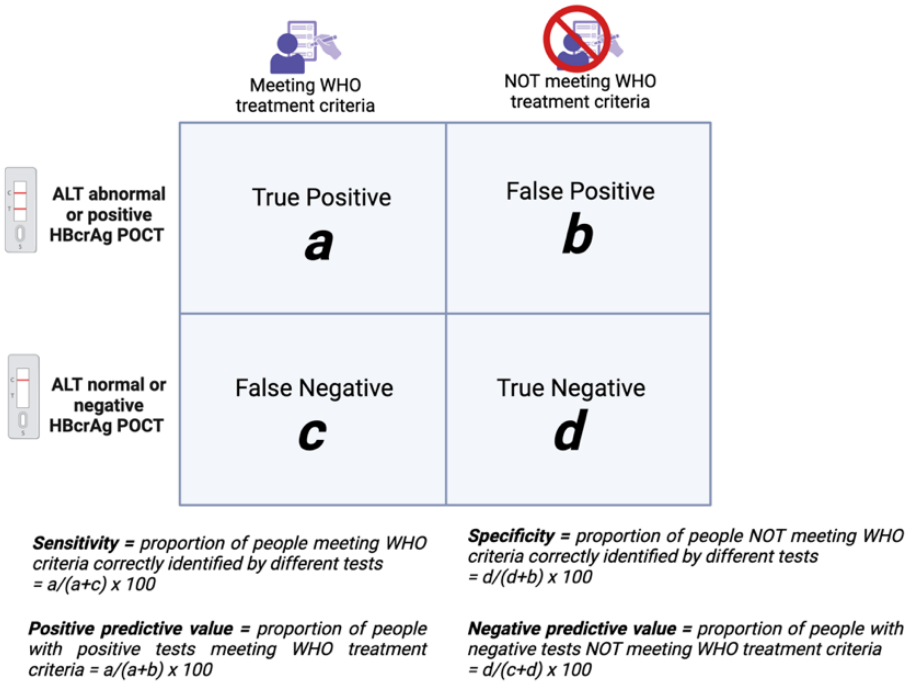


Figure 3-2: Calculation of sensitivity, specificity, positive and negative predictive values of i) Abnormal ALT alone, ii) Positive HBcrAg POCT alone, and iii) Either abnormal ALT OR positive HBcrAg POCT in a population of adults living with chronic hepatitis B infection in Kilifi, Kenya. Image created with BioRender.com; exported with a license to publish under a paid subscription. WHO – World health Organisation; ALT – alanine aminotransferase, HBcrAg – hepatitis B core related antigen, POCT – point of care test. **Figure created using Biorender.com.**

I also assessed (a) whether the previously defined laboratory HBcrAg threshold of 4.3 $\log_{10}$U/L for a positive HBcrAg POCT held true for this population (76) using AUROC analysis, and (b) the extent to which the HBcrAg POCT is associated with clinically important thresholds of HBV DNA (>2000 IU/ml, >20,000 IU/ml and >200,000 IU/ml). All analyses were conducted using R, version 4.4.1.

Objective 2: To estimate HBV seroprevalence from this population, I included only those who were recruited through the random screening programme at KCRH, using the total number screened as the denominator (excluding those who already knew they had a diagnosis of HBV, or close family contacts, which would artificially inflate the prevalence estimate). Initially I generated crude prevalence estimates which were then standardised for age and sex using the Kilifi population structure data from

the KHDSS with the help of Nelson Ouma at KWTRP. Analyses were conducted using STATA/IC version 15.1 (StataCorp College Station, Texas, USA, RRID:SCR_012763).

Objective 4: ONT data were analysed using a bioinformatics pipeline (159) to generate consensus genomes. HBV reads were mapped against representative genomes from a previously defined panel of reference genomes (80) and the reference genome with the most mapped reads was selected as the primary reference. For phylogenetics, Kilifi sequences were aligned to the panel of global reference sequences using mafft online (117) and trees were constructed using R version 4.2.1 packages 'ape' version 5.8-1 and 'treeio' version 1.30.0.

I then compared HBV sequence data from Kilifi with data generated using the same pipeline from both Uganda and the Democratic Republic of Congo (DRC). These sequences were aligned, and phylogenetic trees were created as above. Ugandan samples were from the Kyamulibwa sub-county general population cohort (GPC) in Southwest Uganda whilst DRC samples were from the capital city Kinshasa.

Shannon entropy was calculated across Kilifi consensus sequences with an entropy of >0.5 being classed as high variation. When comparing entropy in Kilifi with DRC and Uganda, the same number of sequences were used from each location (22 sequences), chosen by random selection using base R, version 4.2.1 to ensure any differences were not due to sequence numbers. SNPs associated with drug resistance were taken from a combination of EASL guidance and other SNPs described in the literature as per our systematic review and meta-analysis (160).

3.3 Results

3.3.1 Screening Numbers

Between March 2023 – June 2024, we screened 2003 people for HBV infection. Screening was scaled up over time and peak numbers tested were 257 in one month and 84 in one week (figure 3.3).

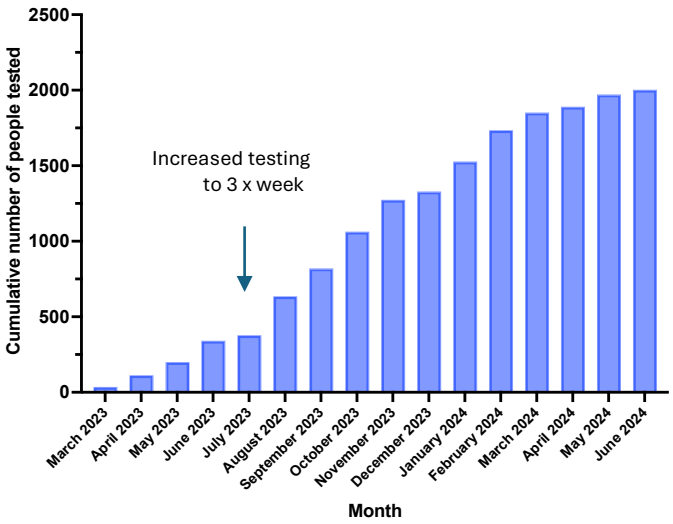


Figure 3-3: The cumulative number of people tested for HBV per month between March 2023 – June 2024 as part of the STRIKE-HBV study in Kilifi, Kenya.

Many people we recruited into the study were waiting at post-natal care clinics for infant checks and vaccinations. Therefore, we had a predominance of women (1530/2003 female (76%)) and the median age was 38 years (range 18-98 years) (figure 3.4).

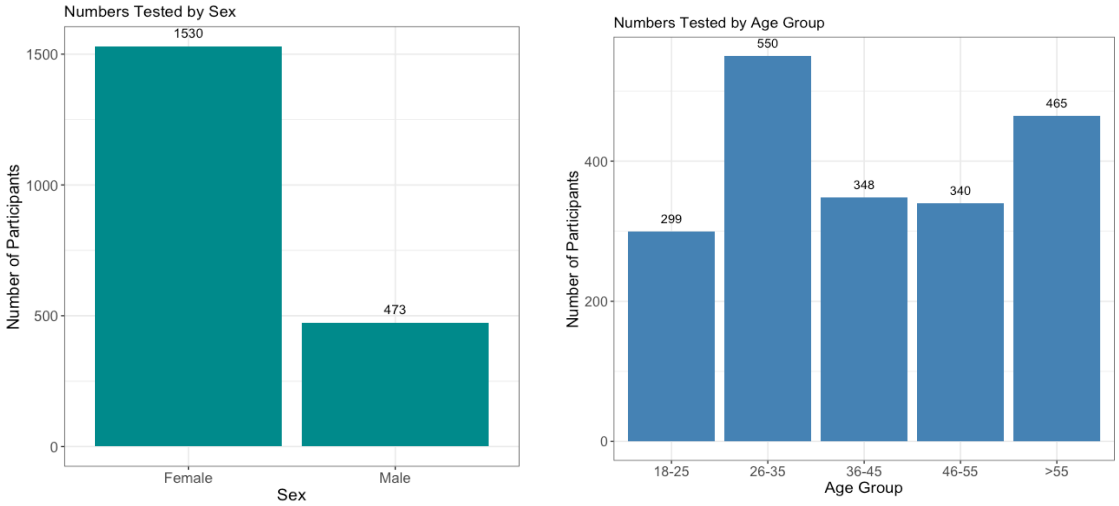


Figure 3-4: Numbers of people tested for HBsAg as part of the STRIKE-HBV study in Kilifi, Kenya, split by sex (left) and age (right).

Along with this, we tested 179 close family contacts of PLWHB. Most contacts we screened were siblings (31%), adult children of the HBV positive index case (23%) or spouses (15%), with the remainder including cousins, aunts, nieces and nephews, stepsiblings, in-laws, and half-siblings.

3.3.2 HBV seroprevalence

38/2003 people tested HBsAg positive using the POCT, however when confirmed using the HBsAg ELISA, four of these were false positive results. Therefore 34/2003 tested truly positive, giving a crude seroprevalence of 1.7%. When adjusted for age and sex using the KHDSS population structure as the reference, seroprevalence increased to 2.2% overall and was higher in men than women (3.2% in men, 1.4% in women). Peak positivity was in the 46-55 years age group at 3.5% when corrected (table 3.4).

	Total Samples	HBsAg Positive	Crude Prevalence	95% CI	Reference Population	Adjusted Prevalence	95% CI
Overall	2003	34	1.7	1.3 - 2.5	153264	2.2	1.6 - 2.7
Sex							
Males	473	14	3	1.6 - 4.9	68354	3.2	1.1 - 5.3
Females	1530	22	1.4	0.9 - 2.2	84910	1.4	0.8 - 2.0
Age group							
18-25y	363	2	0.6	0.1 - 2.0	42322	0.9	0.3 - 1.5
26-35y	526	11	2.1	1.1 - 3.7	38243	3.3	2.1 - 4.5
36-45y	364	10	2.8	1.3 - 5.0	27814	2.5	0.8 - 4.2
46-55y	316	8	2.5	1.1 - 4.9	19622	3.5	1.7 - 5.2
56+y	434	5	1.2	0.4 - 2.7	25263	1.2	0.3 - 2.1

Table 3-4: Crude and standardised HBV seroprevalence split by age and sex of those testing HBsAg positive when screened for HBV as part of the STRIKE-HBV study in Kilifi, Kenya. Standardised prevalence is corrected for age and sex using the structure of the KHDSS reference population. 95% confidence intervals (CI) are shown.

Out of 179 close contacts of PLWHB screened, 25/179 (14%) also tested HBsAg positive, giving a much higher prevalence than in the general population (as expected). HBsAg positivity was most common in siblings with 17/56 (29%) also testing HBsAg positive, followed by parents (3/17 tested HBsAg positive, (18%)) and half-siblings (1/7 (14%)). Only one spouse tested positive (1/23, 4%) **(figure 3.5).**

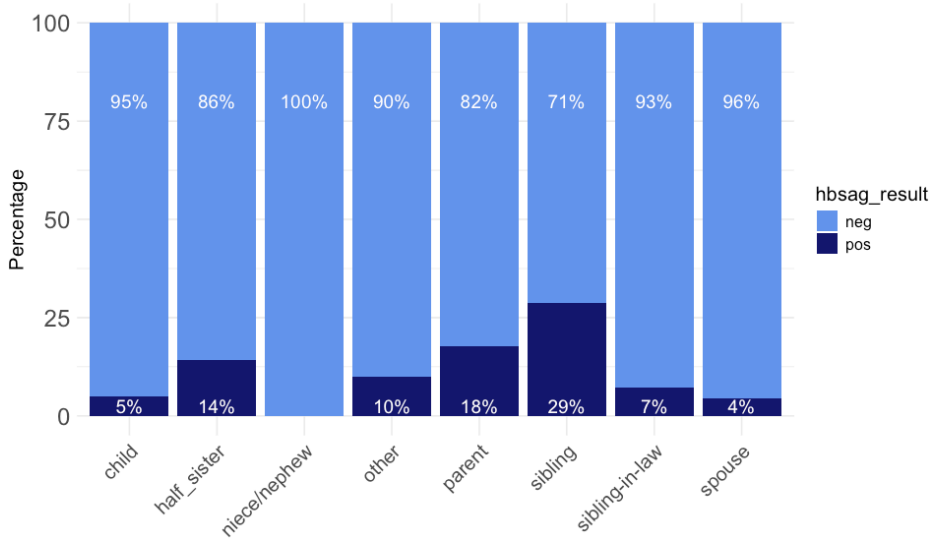


Figure 3-5: Relationships of contacts of PLWHB screened and percentages testing positive and negative for HBsAg. 'Other' includes two aunts, four cousins, one granddaughter, two children-in-law and one stepdaughter.

3.3.3 Study recruitment

Overall, we recruited 102 people testing HBsAg positive and 200 people testing HBsAg negative to STRIKE-HBV. Of the 34 people positive on random screening, three did not want to be recruited to the study. 24/25 HBsAg positive close family contacts were recruited to the study. 47 people already known to be living with HBV were also recruited (figure 3.6). Many of these people had been tested before travelling abroad for work, whilst others were tested at a routine health checkup or whilst admitted to hospital (figure 3.7). 75/102 (74%) PLWHB were not currently taking TFV therapy at the time of recruitment.

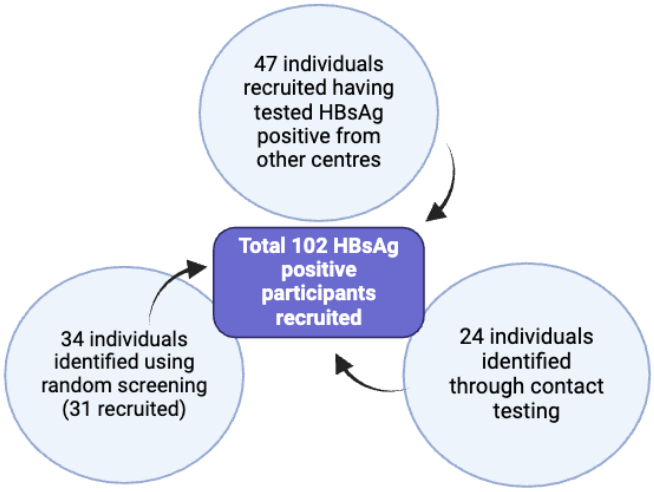


Figure 3-6: Numbers of people living with HBV recruited by different methods into STRIKE-HBV. Figure created using Biorender.com

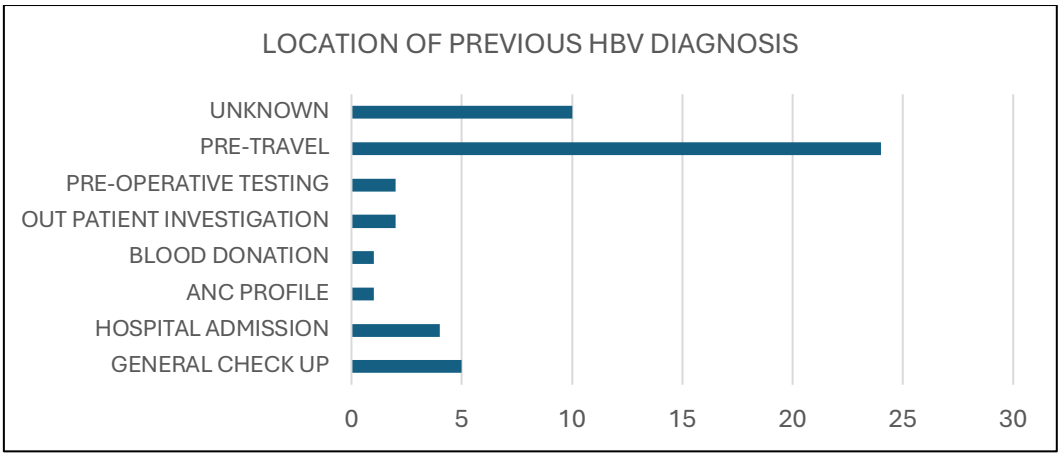


Figure 3-7: The locations of previous HBV diagnoses from those 47 people recruited to STRIKE-HBV who were already known to be living with the infection. ANC – antenatal clinic.

3.3.4 Comparison of HBsAg positive and negative groups

There was no significant difference in age or sex of those recruited to the HBsAg positive or negative groups (p>0.1 in both cases). On multivariable analysis, a family history of liver disease (both p<0.01) and tattoos or body scarification were risk factors for being HBsAg positive (p=0.03). Piercings were associated with a lower risk of HBsAg positivity (p=0.002) (table 3.5).

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor	Odds Ratio	95% lower CI	95% upper CI	P-value	Odds Ratio	95% lower CI	95% upper CI	P-value
Sex (male)	1.074	-0.419	0.561	0.775				
Age	0.992	-0.025	0.01	0.389				
History of jaundice	1.224	-0.716	1.121	0.665				
Family history liver disease	**2.913**	**0.442**	**1.696**	**0.001**	**2.9828**	**1.5024**	**5.9217**	**0.0019**
Taking herbal medication	0.188	-3.748	0.407	0.115				
Freshwater exposure	0.853	-0.675	0.358	0.546				
Tattoos/scarification	**3.298**	**0.209**	**2.177**	**0.018**	**3.3528**	**1.1514**	**9.7634**	**0.0267**
Piercings	**0.561**	**-1.104**	**-0.05**	**0.032**	**0.3927**	**0.2173**	**0.7098**	**0.0021**
Ever injected drugs	0	-1800.752	1768.896	0.986				
History of transfusion	0.557	-1.311	0.14	0.113				
History of surgery	0.97	-0.633	0.571	0.919				
Eats maize	0.295	-2.678	0.239	0.101				
Eats beans	1.433	-0.546	1.265	0.435				
Eats groundnuts	0.83	-0.746	0.373	0.512				
Eats matooke	0.609	-1.058	0.066	0.083				
Drinks alcohol	1.234	-0.319	0.74	0.435				
History of transactional sex	1.408	-0.183	0.868	0.201				
Transgender	0	-1242.318	1214.522	0.982				
ALT	1.03	0.014	0.046	<0.001				
ALT > ULN	**3.208**	**0.665**	**1.667**	**<0.001**	**3.5064**	**2.0469**	**6.0067**	**<0.001**
Elastography score (kPa)	1.014	-0.072	0.099	0.754				
Abnormal elastography	2.214	-0.008	1.598	0.052	1.5029	0.6403	3.5273	0.3481

Table 3-5: Univariable and multivariable analysis comparing characteristics and lifestyle factors in those testing HBsAg positive and HBsAg negative and recruited to the STRIKE-HBV study in Kilifi Kenya. Demographics were compared along with risk factors for HBsAg positivity and liver health. Factors associated with HBsAg positivity are highlighted in blue, and factors associated with a lower risk of HBsAg positivity are highlighted in orange.

Those testing HBsAg positive had higher median ALT measurements on univariable analysis (p<0.001), and being HBsAg positive was associated with 3.5 times higher odds of having an ALT > ULN on multivariable analysis. Elastography scores did not significantly differ between the two groups. 36% of those testing HBsAg negative had ALT > ULN and 7% had an elastography score >7kPa corresponding to liver fibrosis or cirrhosis. This indicates that there are other factors influencing liver health in this population other than HBV and will be explored further in chapter 3.2.9.

3.3.5 HBV biomarkers and liver health in those testing HBsAg positive

A summary of HBV biomarkers in PLWHB are shown in table 3.6. More women were diagnosed with HBV than men, despite the higher standardised prevalence in men, due to the skewed population

tested. 17/101 people had abnormal APRI scores (17%), 13/91 (14%) people had abnormal elastography scores and 66/102 (65%) had ALT > ULN. Median HBV DNA in those not taking NA therapy was 2.8 $\log_{10}$ IU/ml which is lower than what has been found previously as set point for HBV DNA in chronic infection (87) probably due to the low prevalence of HBeAg positivity in this population. Most people had an HBV DNA of 50-2000 IU/ml with only seven people being at very high risk of ongoing HBV transmission with an HBV DNA >200,000 IU/ml, all of whom were HBeAg positive.

Characteristic	Number for which the result is available (Total = 102)	Number or median (% or IQR)
Sex	102	
Female		61 (60%)
Male		41 (40%)
Age (years)	101	37 (28, 49)
HIV status	102	
Positive		2 (2%)
Negative		100 (98%)
Treatment status	102	
On tenofovir		27 (26%)
Off tenofovir		75 (74%)
Quantitative HBsAg	102	4,517 (3,691, 5,021)
HBcrAg CLEIA ($\log_{10}$U/ml)	102	2.65 (2.30, 3.50)
HBcrAg POCT result	102	
Negative		88 (86%)
Positive		14 (14%)
HBeAg result	101	
Negative		94 (93%)
Positive		7 (6.9%)
APRI score	101	0.31 (0.23, 0.42)
APRI severity	101	
Normal		84 (83%)
Fibrosis		12 (12%)
Cirrhosis		5 (5.0%)
ALT (U/L)	102	28 (21, 36)
ALT > ULN	102	66 (65%)
Elastography score (kPa)	91	4.50 (3.50, 6.20)
Elastography severity	91	
Normal		78 (86%)
Fibrosis		11 (12%)
Cirrhosis		2 (2.2%)
Untreated group n = 75		
HBV DNA ($\log_{10}$ IU/ml)	74	2.8 (1.8, 3.7)
HBV DNA group (IU/ml)	74	
Undetectable		17 (23%)
50-2000		32 (43%)
2000-200,000		18 (24%)
>200,000		7 (9.5%)

Table 3-6: Demographics, hepatitis B virus (HBV) biomarkers and liver health in 102 people testing HBsAg positive as part of the STRIKE-HBV study in Kilifi, Kenya. HBV DNA quantification is only shown for those not currently taking tenofovir therapy. HBsAg – hepatitis B surface antigen, HBcrAg – hepatitis B core related antigen, CLEIA – chemiluminescent assay, HBeAg – hepatitis B 'e' antigen, APRI score – aspartate to platelet ratio index, ALT – alanine aminotransferase, kPa – kilopascals.

3.3.6 Univariable (UV) and multivariable (MV) analysis

On MV analysis to assess for factors influencing liver health in the HBsAg positive group, I assessed predictors of ALT, elastography score and APRI score. HBcrAg result was consistently significantly associated with all markers of liver health, whilst HBeAg status and HBV DNA were both significantly associated with ALT and APRI score but not liver stiffness result (table 3.7, 3.8 and 3.9).

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Sex (male)	12.752	-4.402	29.906	0.143				
Age	-0.638	-1.321	0.045	0.067				
On tenofovir	-10.726	-29.878	8.426	0.269				
History of jaundice	-16.752	-48.198	14.695	0.293				
Family history of liver disease	-9.215	-28.398	9.968	0.343				
Freshwater exposure	2.909	-15.403	21.222	0.753				
Tattoos/scarification	18.755	-8.399	45.909	0.174				
Piercings	-11.683	-30.813	7.447	0.228				
History of transfusion	-17.011	-44.210	10.188	0.218				
History of surgery	-14.078	-35.308	7.151	0.191				
Eats maize	12.422	-26.876	51.719	0.532				
Eats beans	-42.228	-74.794	-9.662	0.012				
Eats groundnuts	-7.060	-26.517	12.397	0.473				
Eats matooke	-3.412	-24.071	17.246	0.744				
Drinks alcohol	-2.419	-20.896	16.058	0.796				
History of transactional sex	-1.963	-20.131	16.205	0.831				
HBeAg positive	**33.792**	**0.841**	**66.743**	**0.045**	**-48.002**	**-80.798**	**-15.206**	**0.005**
HBcrAg result	**16.907**	**10.478**	**23.336**	**<0.001**	**15.553**	**8.381**	**22.725**	**<0.001**
Positive HBcrAg POCT	51.879	29.421	74.338	**<0.001**				
APRI score	**6.877**	**4.476**	**9.277**	**<0.001**	**9.409**	**4.633**	**14.185**	**<0.001**
Liver stiffness	**5.512**	**2.399**	**8.624**	**0.001**	**3.131**	**0.223**	**6.039**	**0.035**
INR	4.808	-15.272	24.888	0.636				
Bilirubin	0.582	0.293	0.872	**<0.001**	-0.437	-0.964	0.091	0.103
Albumin	-0.207	-1.288	0.874	0.705				
Platelets	-0.104	-0.204	-0.004	0.041	0.011	-0.079	0.101	0.810
HBV DNA (log10IU/ml)	**9.689**	**5.741**	**13.637**	**0.000**	**4.591**	**0.746**	**8.435**	**0.020**
qHBsAg (log10 IU/ml)	2.501	-17.504	22.507	0.805				

Table 3-7: UV and MV analysis of factors influencing ALT result in PLWHB recruited to the STRIKE-HBV study in Kilifi Kenya.

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Sex (male)	**1.165**	**0.226**	**2.105**	**0.016**	0.903	-0.039	1.846	0.060
Age	-0.016	-0.054	0.023	0.427				
On tenofovir	0.760	-0.305	1.825	0.160				
History of jaundice	-0.133	-1.897	1.632	0.881				
Family history of liver disease	-0.293	-1.366	0.781	0.590				
Freshwater exposure	0.218	-0.804	1.239	0.674				
Tattoos/scarification	0.413	-1.114	1.940	0.593				
Piercings	-0.696	-1.762	0.371	0.199				
History of transfusion	-0.576	-2.101	0.950	0.456				
History of surgery	-0.407	-1.600	0.785	0.499				
Eats maize	0.676	-1.518	2.869	0.542				
Eats beans	1.052	-0.813	2.917	0.266				
Eats groundnuts	0.655	-0.426	1.736	0.232				
Eats matooke	-0.098	-1.252	1.055	0.866				
Drinks alcohol	0.107	-0.925	1.138	0.838				
History of transactional sex	0.034	-0.980	1.048	0.947				
HBeAg positive	0.942	-0.925	2.809	0.319				
HBcrAg result	**0.567**	**0.179**	**0.956**	**0.005**	**0.481**	**0.087**	**0.876**	**0.017**
Positive HBcrAg POCT	**1.621**	**0.281**	**2.962**	**0.018**				
ALT	0.009	-0.002	0.020	0.108				
APRI score	-0.054	-0.208	0.100	0.486				
INR	-0.469	-1.591	0.652	0.408				
Bilirubin	0.000	-0.018	0.017	0.982				
Albumin	-0.021	-0.082	0.039	0.483				
Platelets	-0.004	-0.010	0.001	0.142				
Gamma-GT	0.014	-0.001	0.029	0.072				
HBV DNA (log10IU/ml)	0.151	-0.093	0.396	0.223				
qHBsAg (log10 IU/ml)	-0.907	-2.009	0.196	0.106				

Table 3-8: UV and MV analysis of factors influencing liver stiffness scores in PLWHB recruited to the STRIKE-HBV study in Kilifi Kenya.

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**	**Estimate**	**Lower 95% CI**	**Upper 95% CI**	**P-value**
Sex (male)	0.889	-0.344	2.122	0.156				
Age	-0.025	-0.075	0.024	0.317				
On tenofovir	-0.428	-1.810	0.954	0.540				
History of jaundice	-0.283	-2.555	1.988	0.805				
Family history of liver disease	-0.475	-1.856	0.907	0.497				
Freshwater exposure	-0.424	-1.737	0.890	0.524				
Tattoos/scarification	0.028	-1.942	1.997	0.978				
Piercings	-0.573	-1.953	0.807	0.412				
History of transfusion	-0.488	-2.455	1.479	0.624				
History of surgery	-0.417	-1.953	1.120	0.592				
Eats maize	0.268	-2.560	3.097	0.851				
Eats beans	-0.021	-2.437	2.395	0.986				
Eats groundnuts	0.390	-1.010	1.790	0.582				
Eats matooke	-0.431	-1.914	1.052	0.565				
Drinks alcohol	-0.452	-1.777	0.873	0.500				
History of transactional sex	-0.507	-1.809	0.795	0.442				
HBeAg positive	**4.513**	**2.269**	**6.757**	**<0.001**	**-44.143**	**-79.449**	**-8.837**	**0.015**
HBcrAg result	**0.696**	**0.193**	**1.198**	**0.007**	**14.485**	**6.712**	**22.258**	**<0.001**
Positive HBcrAg POCT	2.616	0.919	4.314	0.003				
Liver stiffness	-0.090	-0.345	0.165	0.486				
INR	1.870	0.469	3.270	0.009	-8.313	-26.435	9.809	0.365
Bilirubin	**0.101**	**0.091**	**0.111**	**<0.001**	**0.565**	**0.256**	**0.875**	**<0.001**
Albumin	-0.122	-0.195	-0.048	0.002	0.308	-0.608	1.223	0.506
HBV DNA (log10IU/ml)	**0.454**	**0.150**	**0.758**	**0.004**	**6.392**	**2.352**	**10.432**	**0.002**
qHBsAg (log10 IU/ml)	0.342	-1.094	1.778	0.638				

Table 3-9: UV and MV analysis of factors influencing APRI score scores in PLWHB recruited to the STRIKE-HBV study in Kilifi Kenya.

3.3.7 Assessment of the HBcrAg POCT

HBcrAg-POCT was positive in 14/102 PLWHB (14%) – with a positivity rate of 4/61 (7%) in women and 10/41 (24%) in men (p=0.01). Those with a positive-POCT were younger (median age 28 years compared to 38 years with a negative-POCT, p=0.03). There was no difference in current antiviral treatment status between those testing POCT-positive or negative (table 3.10). We found the POCT was easy to perform with a turnaround time of approximately 40 minutes.

Analysis in the untreated population

We analysed the POCT in the 75 individuals not taking NA therapy (42 female and 33 male) of whom 11/75 (15%) had a positive test. Within this group, none was HIV co-infected, and the median age was 40 years (IQR 27 - 49). Those with a positive POCT had a significantly higher median HBV DNA level than those with a negative POCT (6.05 $\log_{10}$IU/ml vs 2.7 $\log_{10}$IU/ml respectively; p <0.001, table 3.10). To identify those with HBV DNA >200,000 IU/ml, HBcrAg-POCT had a sensitivity of 100% (7/7) and a specificity of 94% (64/68). All those who had HBV DNA >200,000 IU/ml also tested HBeAg-positive (table 3.10), and all those HBeAg positive were also HBcrAg-POCT positive.

Comparison with liver health markers

A positive HBcrAg-POCT was significantly associated with a higher median ALT than a negative test (58 IU/L vs 26 IU/L, p=0.006, table 3.10). Those with a positive POCT also had higher median APRI scores (0.57 vs 0.29, p=0.003), and were more likely to meet APRI criteria for significant fibrosis or cirrhosis (8/11(73%) vs 6/63, (10%), respectively; p<0.001 (table 3.10)). Individuals with a positive HBcrAg-POCT were more likely to meet elastography criteria for significant fibrosis or cirrhosis than those with a negative POCT (3/11, (27%) vs 6/64 (9%) with a negative test, p=0.03), but there was no significant difference in median elastography score between those with a positive and negative HBcrAg-POCT (table 3.10).

Review of treatment criteria

In the untreated group, 27/75 (36%) people met treatment criteria as defined by WHO guidelines described in section 3.1.4. The sensitivity of abnormal ALT alone for identifying treatment eligibility was 85% (23/27), compared with 33% (9/27) for a positive HBcrAg-POCT alone. Defining treatment eligibility as either an abnormal ALT OR a positive HBcrAg-POCT increased the sensitivity to 89% (24/27), identifying one extra person as treatment eligible compared to either test alone (table 3.11). The extra individual identified had a high HBV VL of 6.1 $\log_{10}$IU/ml and fibroscan score of 10.1kPa, suggesting some existing liver fibrosis, a risk of progressive chronic liver disease, and a potential

transmission risk. The improved sensitivity was at the detriment of specificity, but as per 2024 WHO guidelines, overtreating is preferential to missing those who need treatment (4).

Characteristic (all participants)	N=102	Negative HBcrAg POCT n = 88[1]	Positive HBcrAg POCT n = 14[1]	p-value[2]
Sex	**102**			**0.010**
Female	61	57/61 (93%)	4/61 (7%)	
Male	41	31/41 (76%)	10/41 (24%)	
Age in years, median (IQR)	**102**	**38 (30, 49)**	**28 (27, 39)**	**0.03**
Unknown	1	1/1 (100%)	0/1 (0%)	
On Antiviral Therapy	**102**			**0.8**
Yes	27	24/27 (89%)	3/27 (11%)	
No	75	64/75 (85%)	11/75 (15%)	
Characteristic (untreated group)	**N=75**	**Negative HBcrAg POCT n = 64[1]**	**Positive HBcrAg POCT n = 11[1]**	**p-value[2]**
HBV DNA (log_{10} IU/ml), median (IQR)	**74**	**2.68 (1.56, 3.45)**	**6.05 (3.19, 7.03)**	**<0.001**
HBV DNA Group (log_{10} IU/ml)	**75**			**<0.001**
<20	13	12/13 (92%)	1/13 (8%)	
20 - 2000	36	34/36 (94%)	2/36 (6%)	
2000 - 20,000	15	14/15 (93%)	1/15 (7%)	
20,000 - 200,000	3	3/3 (100%)	0/3 (0%)	
>200,000	7	0/7 (0%)	7/7 (100%)	
Unknown	1	1/1 (100%)	0/1 (0%)	
ALT U/L, median (IQR)	**75**	**26 (19, 35)**	**58 (31, 67)**	**0.006**
ALT >ULN	**75**			**0.7**
Yes	48	40/48 (83%)	8/48 (17%)	
No	27	24/27 (89%)	3/27 (11%)	
Elastography score (kPa), median (IQR)	**66**	**4.25 (3.50, 5.55)**	**6.65 (3.50, 8.98)**	**0.2**
Unknown	9	6/9 (67%)	3/9 (33%)	
Liver Health Fibroscan	**75**			**0.03**
Normal (≤7kPa)	57	52/57 (91%)	5/57 (9%)	
Significant fibrosis (>7 - ≤12.5kPa)	8	6/8 (75%)	2/8 (25%)	
Cirrhosis (>12.5kPa)	1	0/1 (0%)	1/1 (100%)	
Unknown	9	6/9 (67%)	3/9 (33%)	
APRI Score, median (IQR)	**74**	**0.29 (0.22. 0.39)**	**0.57 (0.34, 0.86)**	**0.003**
Unknown	1	1/1 (100%)	0/1 (0%)	
Liver health APRI	**75**			**<0.001**
Normal (<0.5)	60	57/60 (95%)	3/60 (5%)	
Significant Fibrosis (0.5 - 1)	11	5/60 (45%)	6/60 (55%)	
Cirrhosis (>1)	3	1/3 (33%)	2/3 (67%)	
Unknown	1	1/1 (100%)	0/1 (0%)	
HBeAg	**75**			**<0.001**
Positive	7	0/7 (0%)	7/7 (100%)	
Negative	68	64/68 (94%)	4/68 (6%)	

[1]n (%); Median (IQR), [2]Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

Table 3-10: Characteristics of 102 adults with chronic HBV infection in Kilifi, Kenya, assessed with a point of care test (POCT) for Hepatitis B core-related antigen (HBcrAg). Data are presented to show the percentage in each category, adding up to 100% in each row to allow comparison in characteristics between those testing HBcrAg-POCT positive vs negative.

The positive predictive value (PPV) of the HBcrAg-POCT alone for treatment eligibility was 82% but the negative predictive value (NPV) was only 28%. Defining treatment eligibility as a positive HBcrAg-POCT OR an abnormal ALT reduced the PPV to 47% but improved the NPV to 88%, indicating an 88% certainty that the individual did not meet treatment criteria if both tests were normal (table 3.11).

	Identified by abnormal ALT alone	Identified by positive HBcrAg-POCT alone	Identified by abnormal ALT OR positive HBcrAg-POCT
Sensitivity	85%	33%	89%
Specificity	48%	96%	44%
PPV	48%	82%	47%
NPV	85%	28%	88%

Table 3-11: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for ALT alone, HBcrAg-POCT alone or by either test being positive at identifying individuals living with chronic hepatitis B virus infection in Kilifi, Kenya meeting treatment eligibility criteria (defined as Fibroscan score >7kPa, or APRI score >0.5, or the combination of raised ALT AND HBV DNA >2000 IU/ml). kPa – kilopascals; APRI – Aspartate transaminase-to-platelet ratio index, ALT – Alanine aminotransferase, HBcrAg-POCT – Hepatitis B core-related antigen point-of-care test; WHO – World Health Organisation. (Calculated as shown in figure 3.2).

3.3.8 HBV genomic data analysis

3.3.8.1 Summary statistics

Of 102 samples that underwent library prep and sequencing using ONT, 40 had >98% coverage at consensus level and 51 had >80% coverage at consensus level and could be used for phylogenetics and genotyping. A summary of the data generated during sequencing is shown in table 3.12, along with comparisons of other sequencing data generated by our team from other East African sites (Dr Sheila Lumley, Dr Camille Morgan and Elizabeth Waddilove).

Sequencing characteristic	**Kenya (n = 102)**	**Uganda (n=80)**	**DRC (n = 33)**
Mean total reads (IQR) per sample	436,303 (187,441 – 504,849)	266,832 (57,588 – 347,953)	305,973 (64,105 – 369,538)
Mean total mapped reads (IQR)	251,760 (2289 – 325,398)	225,491 (16842 - 265,864)	191,037 (33,179 – 213,049)
Mean % of reads mapped to HBV (IQR)	42% (1 – 79)	65% (30-98)	61% (32 – 89)
Mean % of genome covered (IQR)	95% (18 – 100)	80% (69 – 100)	81% (76 – 100)
Number of samples with >98% coverage at consensus	40 (39%)	42 (53%)	15 (45%)
Samples with >80% coverage for phylogenetics	51 (50%)	58 (73%)	23 (70%)
Median viral load all samples (log_{10} IU/ml) (IQR)	2.2 (0.9-3.0)	2.78 (2.2 – 3.7)	3.78 (2.5 – 5.2)
Median viral load of those with >80% coverage (log_{10} IU/ml) (IQR)	3.5 (2.9-4.0)	3.0 (2.4 – 4.0)	4.7 (3.6 – 6.9)

Table 3-12: Summary of next generation sequencing statistics from 102 samples from people living with HBV infection enrolled in the STRIKE-HBV study in Kilifi, Kenya compared with those from Uganda and the Democratic Republic of Congo (DRC) using the same sequencing pipeline. 12ul of HBV DNA library was used for each run.

As expected, genome coverage from the Kilifi sequences had a significant positive correlation with HBV DNA level (figure 3.8).

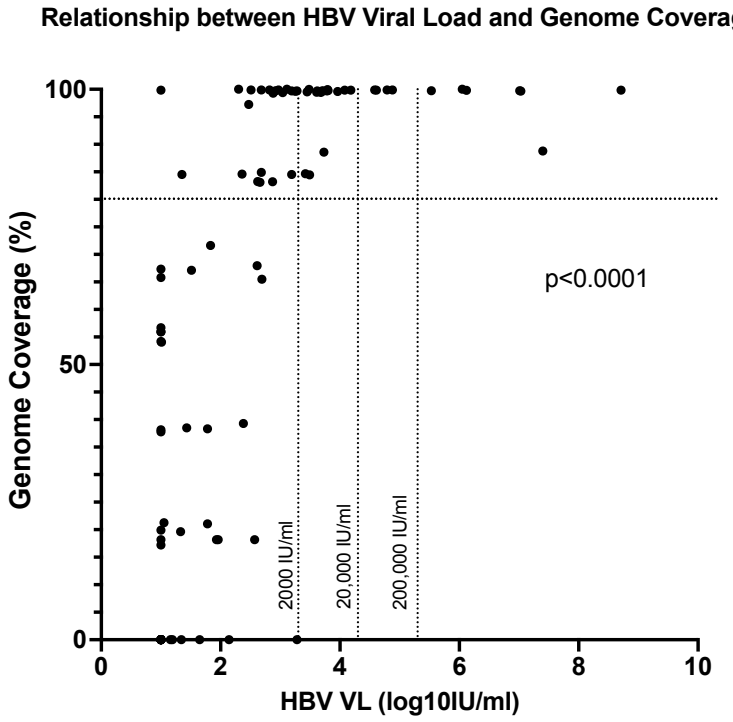
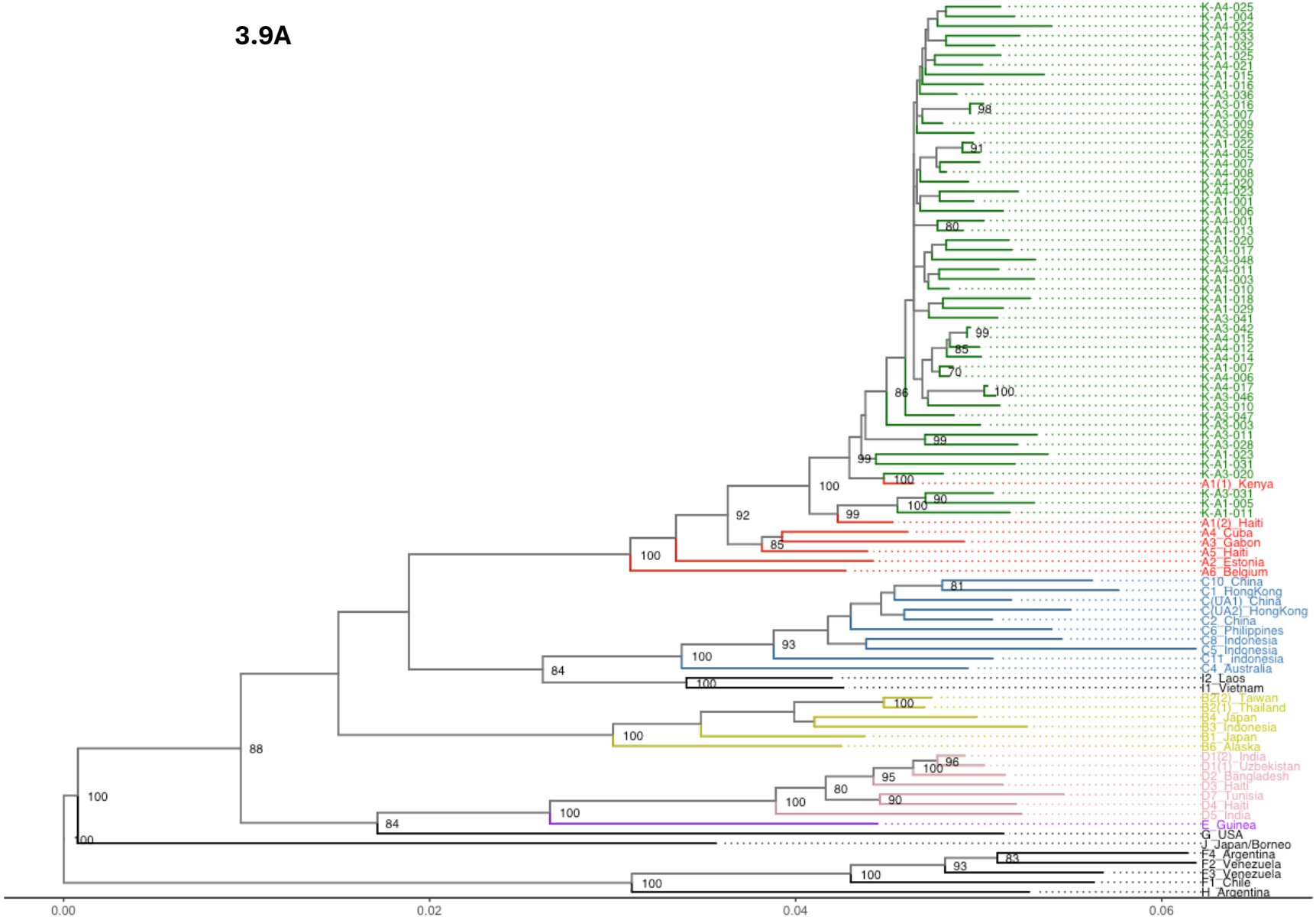


Figure 3-8: Pearsons correlation between HBV genome coverage and HBV VL during ONT sequencing as part of the STRIKE-HBV study in Kilifi, Kenya. Clinically significant VL thresholds are shown as dashed vertical lines from the x-axis, and the threshold of 80% genome coverage that can be used for phylogenetics is shown by the dashed horizontal line on the y-axis.

3.3.8.2 Phylogenetics and genotyping

All Kilifi sequences mapped to sub-genotype A1. 48/51 mapped to the Kenyan A1 reference sequence KP168423.1. The remaining 3/51 mapped to the Haiti reference sequence FJ692557.1 (figure 3.9A and 3.9B). When comparing ONT sequences generated in Uganda and DRC with those from Kilifi, Ugandan sequences also primarily mapped to genotype A1 with a small number mapping to genotype D. Sequences from the DRC were more spread-out mapping across genotypes A1, A6, D4 and E (figure 3.9C).

3.9A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K-A1-004
K-A4-022
K-A1-033
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K-A1-025
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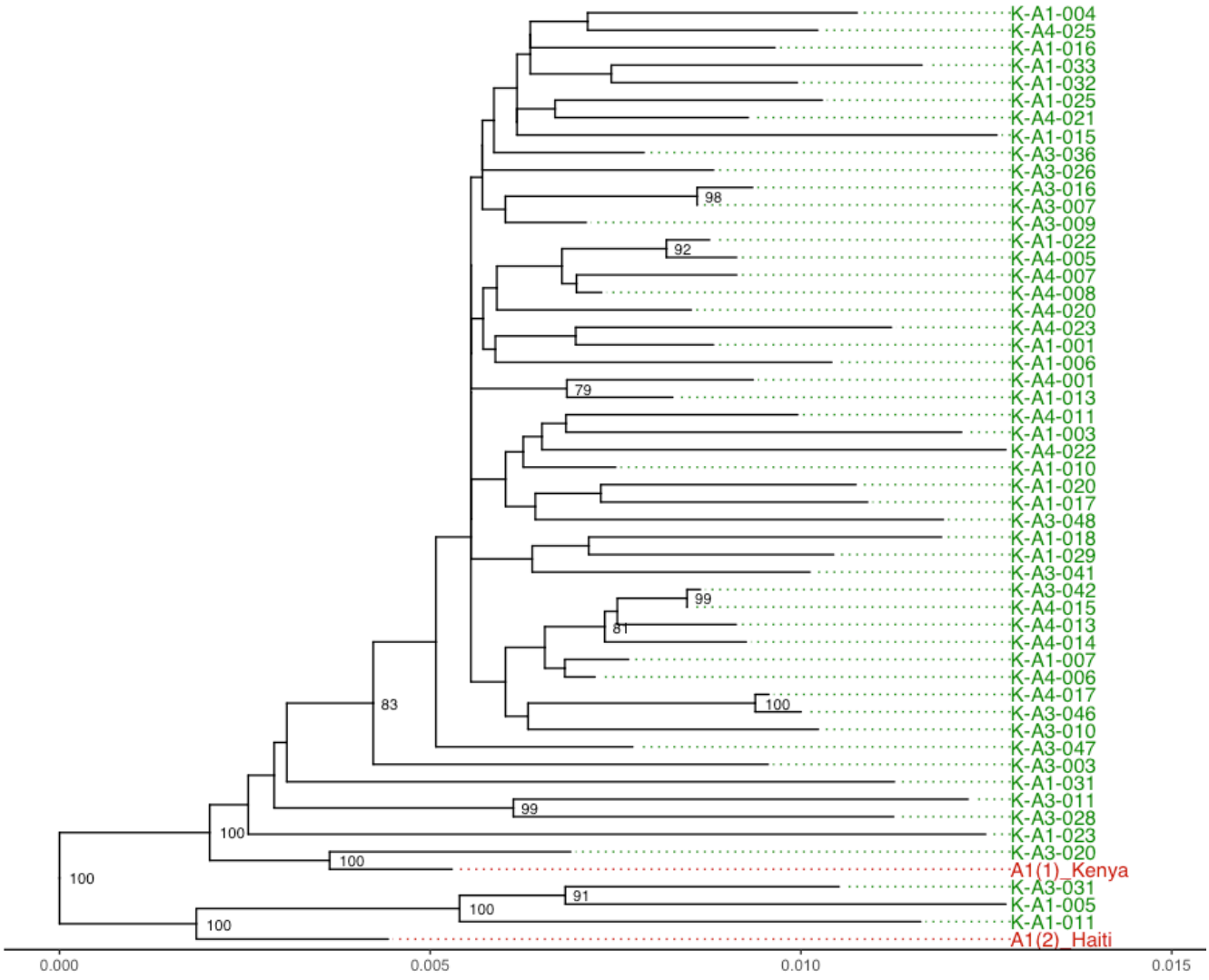
K-A3-036
K-A3-016
K-A3-007
K-A3-009
K-A3-026
K-A1-022
K-A4-005
K-A4-007
K-A4-008
K-A4-020
K-A4-023
K-A1-001
K-A1-006
K-A4-001
K-A1-013
K-A1-020
K-A1-017
K-A3-048
K-A4-011
K-A1-003
K-A1-010
K-A1-018
K-A1-029
K-A3-041
K-A3-042
K-A4-015
K-A4-012
K-A4-014
K-A1-007
K-A4-006
K-A4-017
K-A3-046
K-A3-010
K-A3-047
K-A3-003
K-A3-011
K-A3-028
K-A1-023
K-A1-031
K-A3-020
A1(1)_Kenya
K-A3-031
K-A1-005
K-A1-011
A1(2)_Haiti
A4_Cuba
A3_Gabon
A5_Haiti
A2_Estonia
A6_Belgium
C10_China
C1_HongKong
C(UA1)_China
C(UA2)_HongKong
C2_China
C6_Philippines
C8_Indonesia
C5_Indonesia
C11_Indonesia
C4_Australia
I2_Laos
I1_Vietnam
B2(2)_Taiwan
B2(1)_Thailand
B4_Japan
B3_Indonesia
B1_Japan
B6_Alaska
D1(2)_India
D1(1)_Uzbekistan
D2_Bangladesh
D3_Haiti
D7_Tunisia
D4_Haiti
D5_India
E_Guinea
G_USA
J_Japan/Borneo
F4_Argentina
F2_Venezuela
F3_Venezuela
F1_Chile
H_Argentina
0.00
0.02
0.04
0.06

3.9B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K-A1-016
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K-A1-025
K-A4-021
K-A1-015
K-A3-036
K-A3-026
K-A3-016
K-A3-007
K-A3-009
K-A1-022
K-A4-005
K-A4-007
K-A4-008
K-A4-020
K-A4-023
K-A1-001
K-A1-006
K-A4-001
K-A1-013
K-A4-011
K-A1-003
K-A4-022
K-A1-010
K-A1-020
K-A1-017
K-A3-048
K-A1-018
K-A1-029
K-A3-041
K-A3-042
K-A4-015
K-A4-013
K-A4-014
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K-A4-017
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K-A3-047
K-A3-003
K-A1-031
K-A3-011
K-A3-028
K-A1-023
K-A3-020
A1(1)_Kenya
K-A3-031
K-A1-005
K-A1-011
A1(2)_Haiti
98
92
79
99
81
83
100
99
100
100
100
91
100
100
0.000
0.005
0.010
0.015

3.9C

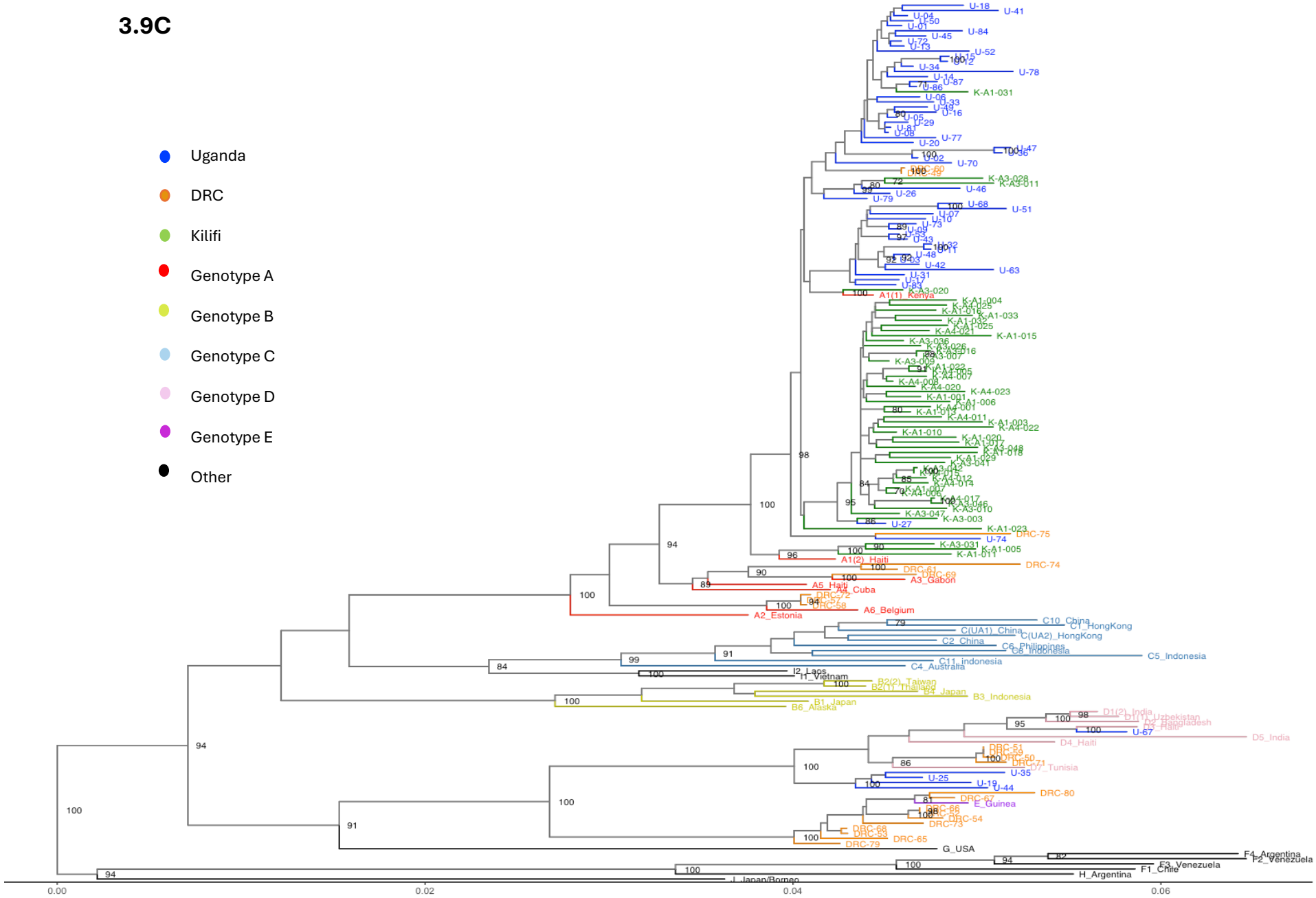
Uganda
DRC
Kilifi
Genotype A
Genotype B
Genotype C
Genotype D
Genotype E
Other
0.00
0.02
0.04
0.06

Figure 3-9: Maximum likelihood phylogenetic trees showing consensus sequences from Kilifi ONT data (green) along with A) representative global reference genomes; B) against nearest A1 reference sequences from Kenya and Haiti (red) and C) against global reference sequences and sequences generated using the same ONT pipeline in Uganda and Democratic Republic of Congo (DRC). Bootstrap replicates of 1000 were generated for each tree. Bootstrap values of >70 are shown. Scale bar shows substitutions per site per year.

3.3.8.3 Interhost diversity

A plot of Shannon entropy for the Kilifi sequences is shown in figure 3.9. Entropy was greatest in the non-overlapping sections of the genome (mean entropy 0.3 in non-overlapping vs 0.17 in the overlapping sections). Entropy also varied by gene, being highest in the core gene (mean entropy 0.44 compared to 0.19, 0.17 and 0.15 in pol, x and surface genes respectively). The five most variable sites were all located in non-overlapping sections of the genome and 3/5 were in the core gene. One of the most variable sites in Kilifi was site 1764, located in the basal core promotor section of the X-gene with an entropy of 1.5.

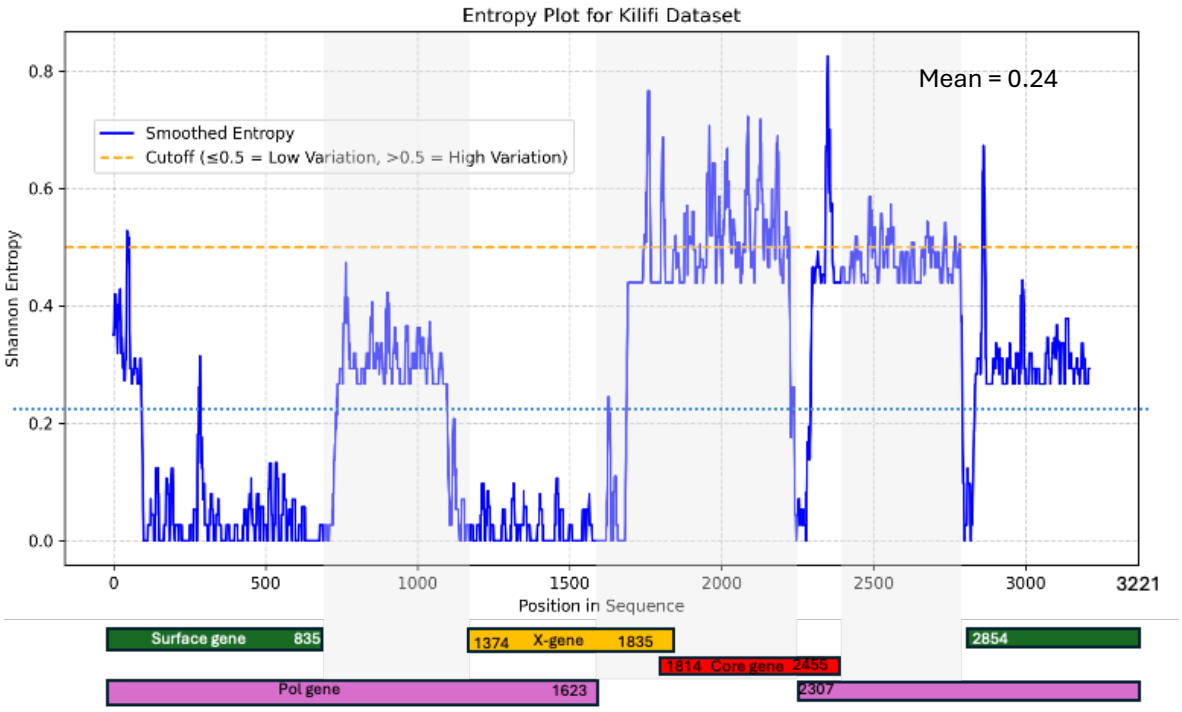

Figure 3-10: Smoothed Shannon entropy across 52 consensus HBV sequences derived using next generation sequencing with Oxford Nanopore technologies in Kilifi, Kenya, along with locations of the four HBV mRNA transcripts shown below. Sections in light grey represent non-overlapping areas of the genome. The cut off 0.5 is applied to indicate high vs low entropy. Mean entropy is shown.

22 sequences from each of Kenya, Uganda and DRC were included in Shannon entropy comparisons. DRC had the highest mean entropy at 0.29 (IQR 0 – 0.52) compared to 0.28 (IQR 0 – 0.46) and 0.24 (IQR 0 – 0.31) for Uganda and Kilifi respectively (figure 3.11). DRC sequences also had the highest proportion of the genome above and entropy of 0.5 (25% of genome) whilst Kilifi had the lowest (9% of genome).

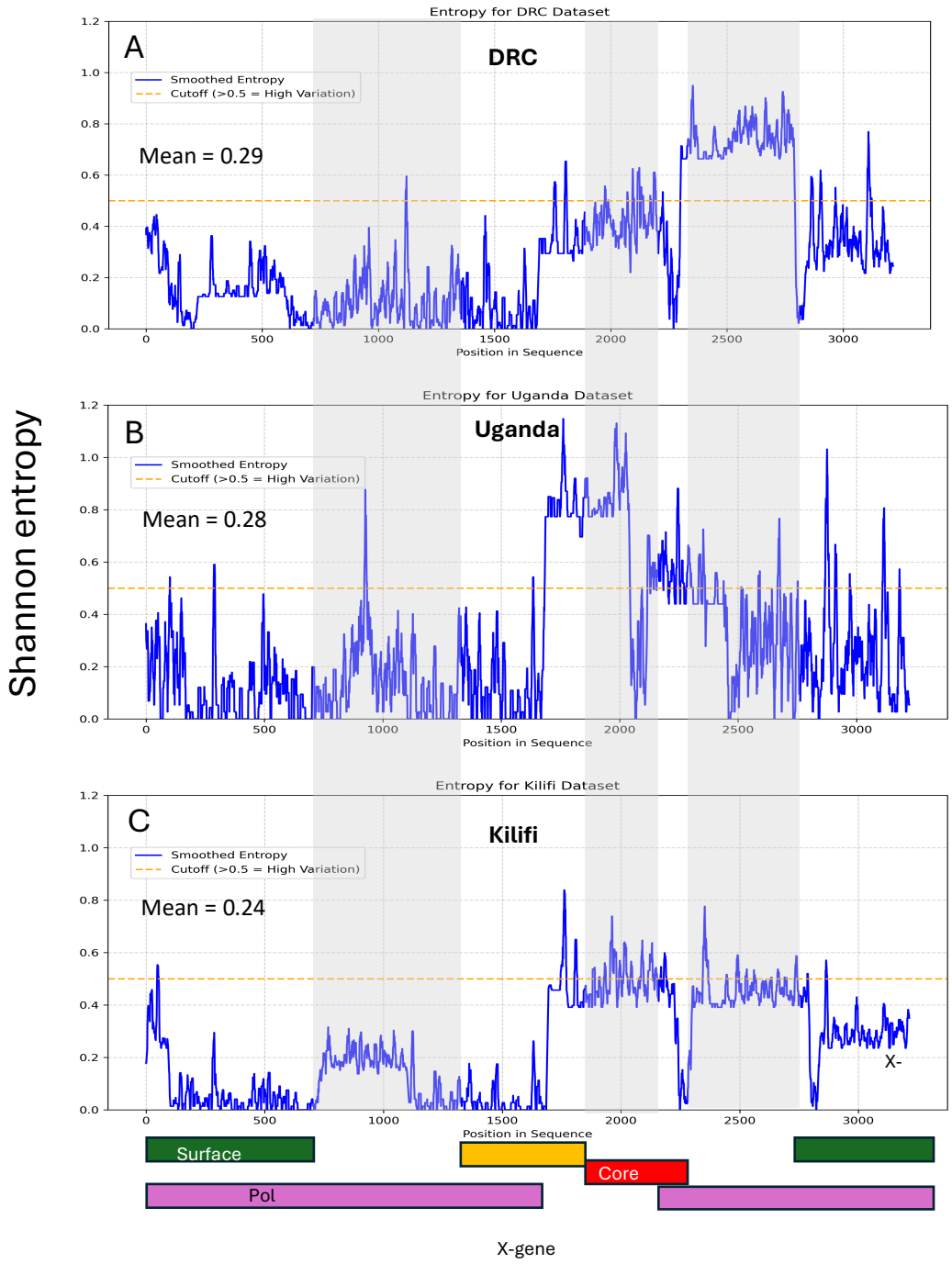

Figure 3-11: Smoothed Shannon entropy across 22 consensus genomes from DRC (3.11a) Uganda (3.11b) and Kilifi (3.11c). Locations of the four HBV mRNA transcripts is shown below the plots. Sections in light grey represent non-overlapping areas of the genome. The cut off 0.5 is applied to indicate high vs low entropy. Mean entropy is shown.

3.3.8.4 Family groups

Family groups were closely related on the phylogenetic tree with several sibling groups being on adjacent branches, along with the group including a mother and two children (figure 3.12)

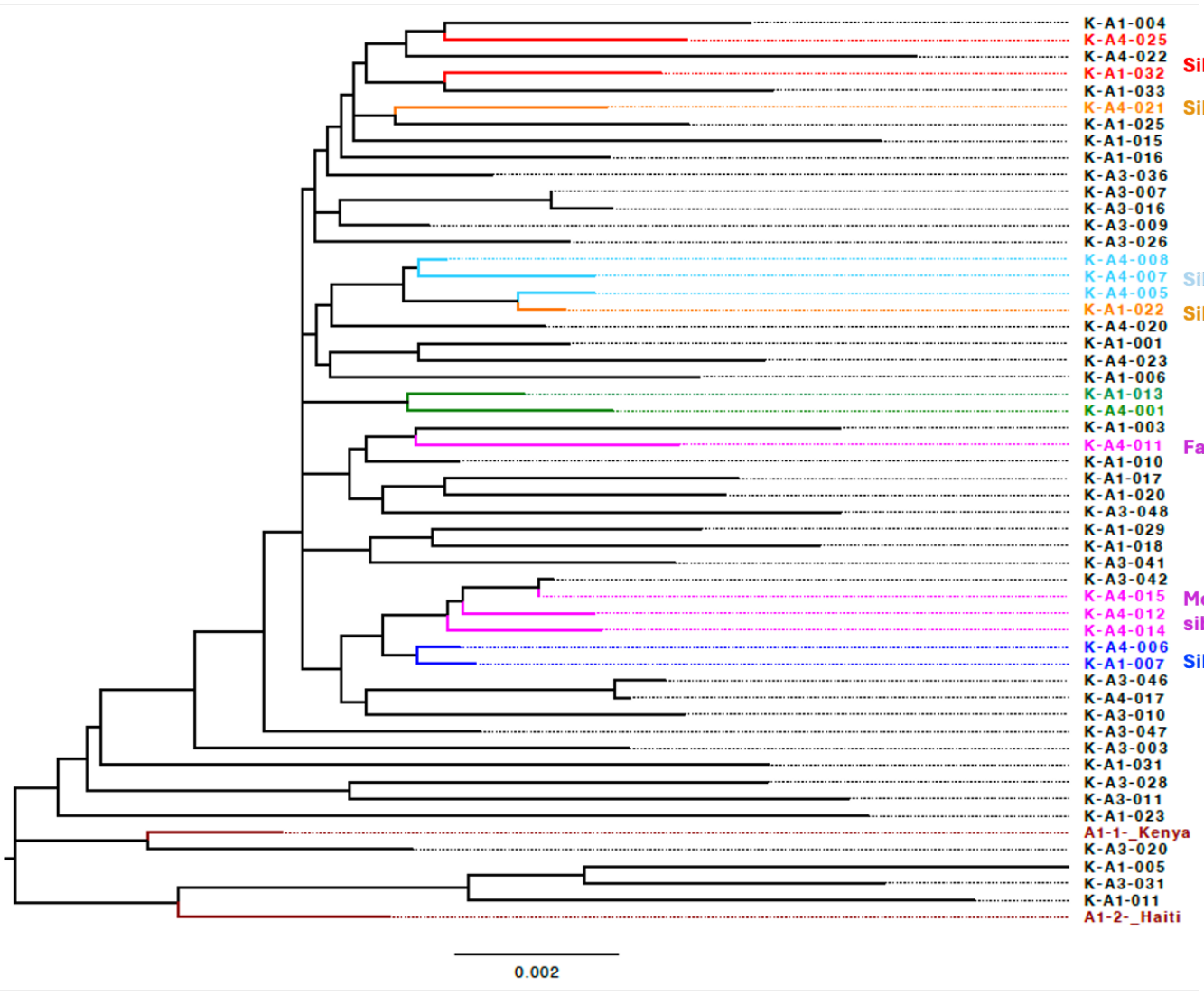

Figure 3-12: Maximum likelihood phylogenetic trees showing consensus Kilifi sequences derived using next generation sequencing with Oxford Nanopore technologies highlighting family groups, along with A1 reference sequences from Kenya and Haiti. Scale bar shows substitutions per site per year.

3.3.8.5 Resistance associated mutations

No TFV RAMs listed in the EASL guidelines were present in the Kilifi ONT consensus amino acid sequences. However, when reviewing the more diverse literature around TFV drug resistance, several mutations which have been associated with TFV resistance when present in a group were identified in both the Kenyan reference sequence KP168423 and Kilifi sequences (table 3.13).

HBV variant	TFV susceptability	Evidence	Present in Kilifi genomes
Wild-type	S	EASL guidelines	0
M204V	S	EASL guidelines	0
M204I	S	EASL guidelines	0
L180M + M204V	S	EASL guidelines	0
A181T/V	I	EASL guidelines	0
N236T	I	EASL guidelines	0
L180M + M204V/I +/- I169T +/- V173L +/- M250V	S	EASL guidelines	0
L180M + M204V/I +/- T184G +/- S202I/G	S	EASL guidelines	0
A194T	Conflicting evidence		0
S106C + H126Y + D134E +/- L269I	Conflicting evidence (161,162)		Kenya reference KP168423 has H126Y. 5 Kilifi genomes also have H126Y. Kenya reference KP168423 has L269I. All Kilifi genomes have L269I except 2.
L180M + M204I/V + R153W/Q + I163V + V173L	Conflicting evidence (163)		Kenya reference KP168423 has R153W. All Kilifi genomes have R153W except 3.

Table 3-13: Prevalence of tenofovir (TFV) resistance mutations in Kilifi genomes described both in the EASL guidelines (34) and in more diverse literature. The amino acid substitution profiles are shown on the left along with the documented susceptibility of TFV with S = sensitive, I = intermediate/reduced susceptibility. The additional mutations outside of EASL guidelines have been described in limited literature to be related to reduced TFV susceptibility.

3.4 Discussion

3.4.1 Prevalence

The overall prevalence found in those randomly screened as part of the STRIKE-HBV study was lower than what has been reported previously for both Kilifi and Kenya at 2.2% when adjusted for age and sex (144). As discussed in chapter 2, most published HBV prevalence estimates only include specific populations, and would not have access to local population structure data through demographic health surveillance so would be unable to correct estimates for age and sex. Men had a higher HBV prevalence than women at 3.2% (95% CI 1.1 - 5.3%) compared to 1.4% (95% CI 0.8 – 2.2%) Globally and in other studies from WHO-AFR, CHB is more common in men (2,164) and they are more likely to develop complications. It is unclear whether men are more likely to be exposed to HBV, or have a higher incidence of chronic infection when exposed, this is hard to disaggregate given much of the epidemiological literature around HBV prevalence focuses on populations subject to sex or gender bias (144).

3.4.2 Risk factors for HBsAg positivity

A family history of liver disease and tattoos or body scarification were shown here as risk factors for HBsAg positivity. Studies have shown that HBV accounts for up to 50% of people presenting to hospital with liver disease in Kenya (97,127). Therefore, HBV is likely to have been the causative factor in many reporting liver disease in the family. Risk factors for HBV are dependent on the population studied, and the questions asked within any risk assessment. Many studies tend to focus on sexual risk factors, with higher parity and a history of multiple sexual partners being associated with HBsAg positivity (99,114). One study in Western Kenya did show body scarification was a significant risk factor for HBV in adolescent blood donors (99), and several studies in WHO-AFR have shown living in close contact with someone living with HBV is a risk factor for infection (165,166).

In STRIKE-HBV I found HBsAg positivity rate in close family contacts of PLWHB was much higher than in the general population (14% vs 2.2%), with siblings of the index case living with HBV being the most likely to test positive (29% of siblings tested were also HBsAg positive). These siblings were all adults who mostly no longer lived together indicating horizontal transmission within family groups at a young age. HBV is very infectious – around 50 – 100 times more infectious than HIV (167), spreading easily between children who have small percutaneous injuries from cuts or abrasions, sharing blood or serous fluid. A 2023 systematic review and meta-analysis showed risks of HBV horizontal transmission to children of uninfected mothers in WHO-AFR increased at the age children generally become mobile, allowing interaction with the wider family group and more opportunity for small skin breaks (166). Along with transmission risks being high, children infected before the age of 5 years are much more likely to develop chronic infection than if infected as an adult (168).

Interestingly here I showed that those with piercings were at lower risk of being HBsAg positive. A systematic review and meta-analysis on HBV risk factors in Africa in 2024 showed no significant association between piercings and HBV infection, providing piercing devices are not shared, however a negative association has not been previously reported. Reasons for this are unclear. It is possible those who have piercings in Kilifi are aware of the dangers of blood borne virus transmission during this practice and hence also take precautions in other aspects of their life.

3.4.3 Liver health in those testing HBsAg positive

A higher proportion of those testing HBsAg positive had an ALT > ULN compared to those testing HBsAg negative, although there was no difference in liver stiffness measurements between the two

groups. This is concerning as it indicates that even in this primarily asymptomatic group living with HBV, liver inflammation is still ongoing with a risk of liver fibrosis and cirrhosis over time.

ALT and liver elastography assess liver health in different ways, with elastography assessing liver stiffness and indicating fibrosis grade, and ALT being released from damaged hepatocytes indicating inflammation. Whilst many studies have shown CHB causes liver damage over time, few have assessed how liver stiffness and ALT compare through the course of CHB. ALT tends to be influenced by more temporary factors such as recent alcohol consumption or medication. Along with this the ALT upper limit of normal defined by WHO is applicable globally, however many studies have shown that the normal range for ALT in the WHO-AFR may be higher than that in European populations (148,169). Therefore although ALT is defined here as high using WHO reference ranges, in fact were region specific ranges used, more people would have a 'normal' ALT (146).

3.4.4 HBcrAg POCT

Here I undertook the first comparison of the HBcrAg POCT with ALT, markers of liver fibrosis, and HBeAg status. I compared how well a positive HBcrAg POCT identified those meeting WHO treatment criteria as defined in 3.1.4, compared with using raised ALT alone which is the other method suggested by WHO in the absence of other diagnostic tests. A positive HBcrAg-POCT was strongly associated with liver inflammation by ALT, and fibrosis/cirrhosis by both Fibroscan and APRI scores. However, as expected, the HBcrAg-POCT alone had a lower sensitivity for identifying those meeting WHO treatment criteria than ALT alone (33% vs 85%). Addition of the HBcrAg POCT to ALT did identify one extra person as treatment eligible and the combination of tests improved the sensitivity of either test alone, however further assessment is needed to see if this benefit translates to larger populations. Although the NPV of both tests combined was 88%, using this approach to make treatment decisions would still miss 12% people at risk of liver disease progression in this population.

A positive HBcrAg-POCT was 100% sensitive at identifying a sample that was laboratory-positive for HBeAg, performing much better than currently available HBeAg-POCTs which have sensitivities ranging from 30-62% (170,171). A positive HBcrAg-POCT was 100% sensitive at identifying those with HBV VL >200,000 IU/ml, which is higher than reported from the Gambia (76). This POCT is therefore of potential clinical utility to identify individuals eligible for perinatal antiviral prophylaxis. This is the first time this POCT has been evaluated in East Africa, where circulating HBV genotypes differ from those in West Africa.

The HBcrAg-POCT was quick and we did not need any specific training outside of reading the package insert, although we are a team familiar with undertaking laboratory tests. It has relatively low production costs (<$5 per test), compared to HBV VL which varies in price across WHO-AFR up to $62/test (2), and HBeAg up to $40/test for the laboratory ELISA (172,173) assuming these tests are actually available. ALT is relatively low cost (<$10) and available in much of WHO-AFR, so continues to be a good method of liver health assessment where other tests are not affordable. Further implementation research is needed to determine whether inclusion of the HBcrAg-POCT alongside ALT could enhance linkage to care and improve decentralisation of clinical assessment, and how its impact and cost-effectiveness varies by setting.

3.4.5 HBV genomics

All samples here were genotype A1 which I have previously shown to be common in Kenya as described in chapter 2.1.2.5 (144). All Kilifi sequences were very closely related phylogenetically (figure 3.9a and 3.9b), and more closely related to Ugandan sequences than those from DRC. This is in keeping with migration patterns, as Uganda and Kenya share a border and historically have had considerable inter-country migration. They are both member states of the East African Community (EAC) allowing easy movement. DRC is not part of the EAC and migrants tend to come from more central African countries including Central African Republic and Angola where genotype E is prevalent (174,175). Higher Shannon entropy in the non-overlapping sections of the HBV genome emphasises restrictions on HBV variability due to the small genomic structure, and the five most variable sites identified may highlight possible locations for emergence of immune escape. Site 1764 was one of the most variable sites in Kilifi and plays a central role in HBV replication. It has previously been shown to be associated with high risk of progression to severe liver disease and HCC in the United States and Asia (176,177), but has not to date been demonstrated in African populations.

There was little interhost variation between Kilifi sequences (mean Shannon entropy 0.24), whereas sequences from both Uganda and DRC had greater interhost variation (mean Shannon entropy 0.28 and 0.29 respectively). A much lower proportion of Kilifi genomes were also over the 0.5 high entropy threshold compared to DRC and Uganda. This may be due to the population dynamics of these different areas. Kyamulibwa in Uganda has significant migration both from within Uganda and other surrounding countries including Burundi, Rwanda and Tanzania adding to sequence diversity (178). The population of Kinshasa has doubled from 6.1 million people in 2000 to 12.6 million in 2017, according to the World Development Indicators (WDI) (179). Urban-urban migration from other cities in DRC to Kinshasa accounts for the highest proportion of migration as people search for work and

better living conditions or are displaced due to conflict. Kilifi however is an emigration hotspot, with high unemployment rates and poor education. Many workers leave Kilifi to work in the Middle East or to elsewhere in Kenya seeking employment or education (180).

Consensus sequences from members within family groups were very closely related, particularly older siblings who no longer live together. This likely indicates significant intra-familial horizontal transmission in early childhood. This has been previously described, but most studies are old and mechanisms are not well understood (181–184). Only one study from WHO-AFR has previously used phylogenetic analysis of the core region to look for intra-familial transmission in the Gambia (181). This also showed strong phylogenetic clustering within family groups, and the wider village community. They did however also identify some family members who had completely different HBV sequences to each other, and some people from distant villages who had identical sequences. This indicates some family members can become infected from different sources, likely due to movement of infected individuals between villages. A systematic review and meta-analysis analysing horizontal transmission in WHO-AFR (166) showed that in unvaccinated children born to HBsAg negative mothers, the risk of being exposed to HBV increased significantly with age. This likely coincides with the age at which the child becomes mobile, representing a high likelihood of small percutaneous injuries and blood or serous fluid transmission and increased interaction with other family members and older siblings.

No TFV mutations described in EASL guidance (34) were seen within the consensus genomes of the Kilifi sequences. This is in keeping with other data on TFV resistance in the WHO-AFR (144,160). However, when reviewing other literature on TFV resistance, several mutations were identified in Kilifi sequences that may confer reduced susceptibility to TFV when part of a constellation of mutations. The uncertain evidence around how these mutation groups relate to TFV resistance is primarily due to insufficient data from WHO-AFR, and a lack of data correlating genomic sequence with clinical outcome (e.g. relating sequence to a lack of HBV VL suppression or VL resurgence). It is therefore possible that these SNPs may have more of an association with TFV resistance than is currently recognised and emphasizes the need for improved genomic surveillance from currently underrepresented regions.

Previous data has shown that combining two NAs can increase the genetic barrier to treatment resistance in HBV, however this has only been demonstrated in older NAs such as lamivudine combined with adefovir (185). It is possible this could also be true when using dual therapy with TFV

and lamivudine as is the case in Kenya, but this has not been demonstrated to date. It has been poorly studied particularly in WHO-AFR however, and it may be that as genomic data from this region increases, this pattern will emerge. Along with this, until recently, WHO HBV treatment guidelines were very restrictive only recommending TFV therapy for a small proportion of those living with HBV based on a host of markers such as HBV DNA levels, HBeAg status and liver health (39). Due to the lack of diagnostics in many WHO-AFR countries however, assessment for treatment eligibility was often not possible, and treatment was therefore not given. With recent changes to WHO guidelines simplifying eligibility criteria, and recommending treatment more broadly, it is likely those PLWHB who need treatment, will be given treatment (4). It is therefore important to continually monitor for TFV resistance in populations where adherence can be difficult due to poor access to healthcare services and financial instability along with concerns about stigma.

There is still ongoing data analysis from STRIKE-HBV which is out of the scope of my DPhil. This includes enhanced genomic analysis of different groups identified in STRIKE-HBV, such as those with high liver stiffness scores, those testing HBeAg positive and those within family groups. I am also analysing data from those testing negative for HBsAg to understand other factors influencing liver disease in this population.

3.5 Conclusions

The STRIKE-HBV study was the first study focussed on HBV in Kilifi and KWTRP. We found the population was willing to be tested with the appropriate sensitisation and many people came for assessment having heard about the study through the community. HBV prevalence was moderate, especially in men, although we exemplified the difficulties accurately estimating prevalence as skewed populations tend to present for testing. We showed likely horizontal transmission within Kilifi, with much higher HBV prevalence in close family contacts of PLWHB particularly siblings even if they no longer live together. Family testing should therefore be prioritised to identify those living with HBV for onward care. This was supported a family history of liver disease being a significant risk factor for HBsAg positivity.

The HBcrAg POCT was correlated strongly with several markers of liver disease, although did not perform better than ALT at identifying those with abnormal elastography or APRI scores. It has potential as an affordable option to identify antenatal individuals at highest risk of vertical transmission but needs further evaluation in WHO-AFR.

The lack of TFV resistance mutations identified here is reassuring, however the presence of some SNPs that may reduce the barrier for resistance in future is concerning, particularly with the increase in treatment eligibility following 2024 WHO guidelines. Ongoing resistance monitoring is required, particularly correlating with phenotypic resistance to ensure it is identified early.

4. INSIGHTS FROM PEOPLE LIVING WITH HBV AND HEALTHCARE WORKERS PROVIDING CARE IN KENYA.

"I think it would be better for those of us who have the disease not to be ashamed".

4.1 Introduction

This chapter covers social sciences work done in parallel with the STRIKE-HBV study. I undertook this work after attending a social science course on qualitative data interpretation at Oxford University and with the assistance of Dr Nancy Kagwanja at KWTRP who has a background in implementation and social sciences. This work has been submitted to PLoS Global Public Health, and we are awaiting a decision regarding publication. It is available as a pre-print on MedRxiv:

Louise O Downs et al. *"It would be better for those of us who have the disease not to be ashamed". Insights from people living with chronic hepatitis B virus infection and healthcare workers providing HBV care in Kilifi, Kenya.*
medRxiv 2024.11.28.24317957; doi: https://doi.org/10.1101/2024.11.28.24317957

Throughout the WHO-AFR region, reports are emerging of the impact of an HBV diagnosis on those living with the infection, and a new HBV stigma survey has been piloted in selected countries by the World Hepatitis Alliance (186). It is widely agreed that representation of those with lived experience is urgently needed to improve representation and advocacy, and inform improved service provision, which in turn will drive progress towards global elimination targets (187). The positive role of peer support workers in HBV care is being increasingly recognised, to encourage testing, improve treatment adherence and reduce stigma (188). HCWs providing care for PLWHB also have unique insights into issues including misinformation, stigma and barriers to long-term follow-up and medication. They also understand the current healthcare infrastructure for PLWHB, have insights into gaps and challenges, and can advise how access could be improved.

In this work, I set out to engage people living with HBV, and HCWs caring for PLWHB in KCRH to generate evidence to inform care delivery, by establishing a platform for scale up of qualitative data collection in this setting and elsewhere. We convened focus group discussions (FGDs), aiming to i) explore understanding of HBV infection on an individual and community level, ii) discuss how the lives of PLWHB have changed since their diagnosis and iii) investigate barriers to receiving care and consider

how these could be reduced. The methods and results of this study are reported in compliance with the Standards for Reporting Qualitative Research (SRQR)(189).

4.2 Methods

The individuals who took part in these FGDs had all been recruited to the STRIKE-HBV study. When this work was undertaken, HBV care at KCRH was provided at the CCC, which is a secondary care outpatient clinic primarily designed for people living with HIV. The CCC provides adherence counselling, medication and follow up for both adults and children living with HBV. At KCRH, there is no routine mechanism for offering further laboratory or imaging assessment to determine HBV treatment eligibility, therefore standard care is that all those testing HBsAg positive receive NA therapy using tenofovir/lamivudine combination therapy. This approach is in line with conditional recommendations for 'treat all' which are incorporated into the 2024 WHO guidelines (4).

4.2.1 Data collection

We conducted four FGDs, two with PLWHB (consisting of six men and seven women) on 18th June 2024, and two with HCWs (consisting of seven men and seven women) on 14th November 2024. PLWHB were invited to participate in FGDs during peer support educational sessions initiated by the CCC matron when coming to pick up medication, and HCWs were invited via a group messaging platform for CCC staff. Participants were purposively sampled to create groups with diverse ages and experience, and in the case of HCWs to ensure a spread of cadres involved in HBV service delivery.

FGDs were held in private rooms in the CCC to promote participant privacy. Participants were given a number from 1-6 or 1-7 within their group to enhance anonymity of the respondents. FGDs with PLWHB and HCWs were conducted primarily in Kiswahili by fieldworkers from STRIKE-HBV and facilitated by me and Juliet Odhiambo, a member of the KWTRP community liason group (CLG) experienced in qualitative data collection. Topic guides were used to direct FGDs, informed by study objectives and literature about patient experiences of accessing and receiving HBV care (190). The topic guides covered the understanding of HBV, reactions to diagnosis, the impact on the lives of PLWHB, barriers to care and how these could be reduced (190). FGDs took around 1.5 hours each, were audio recorded using an encrypted dictation device from KWTRP, and those participating were given refreshments. PLWHB attending specifically for the FGDs were also compensated for lost earnings of around USD $5, as is standard for community engagement activities at KWTRP.

4.2.2 Data Analysis

Following FGDs, audio files were transcribed verbatim and translated into English and analysed in Nvivo version 14. I used both inductive and deductive thematic analysis (191,192) to scrutinise the data on a line-by-line basis, identifying and analysing patterns and drawing out themes using constant comparison technique (193). Deductive analysis was primarily based on the themes and questions I had developed in the topic guides as described above. I identified sections within the transcripts relating to these questions, reported and combined them with inductive analysis to give a summary of concepts running through the data. Inductive analysis involved identifying new themes 'a priori' by reading and re-reading transcript data looking for concepts and ideas. After this I generated initial codes covering meaningful features of the data. I then began to group these initial codes into broader themes related to the initial research questions, creating mind maps to help with organisation. I re-reviewed themes to check they were distinct, sometimes splitting or merging themes to ensure they accurately represented the data. I went onto summarise each theme, combined with deductive analysis, chosing representative quotes from the focus groups to enhance clarity. A summary of the themes and sub-themes emerging are shown in table 4.1.

THEMES	SUBTHEMES
UNDERSTANDING OF HBV	Hepatitis B as a death sentence
	Treatment only for six months
	HBV referred to as 'pregnancy'
	Hepatitis B as a 'curse'
COMPARISON WITH HIV	Dislike of care being in the same clinic
	Dislike of medication being the same
	Having to 'prove' they don't have HIV
LIVES CHANGING SINCE DIAGNOSIS	Varied reactions by loved ones
	Unable to pursue employment plans
	Reduced freedom
	Improved self-care
	Improved non-specific symptoms
IMPROVED HBV CARE	STRIKE-HBV has been beneficial
	Feel healthcare workers are better informed
	Peer support sessions were helpful
CARE BARRIERS	Difficult with work
	Economic difficulties with travel to clinic
	Need for decentralised clinics
STIGMA	Social isolation
	Dislike of integration with HIV
	Little education and community understanding
REDUCING TRANSMISSION	Requirement for education
	Vaccination provision is needed
	Campaigns by trusted society members
	Expansion of free testing into villages

4-1: Themes and sub-themes emerging from thematic analysis of focus group discussions with PLWHB and healthcare workers at Kilifi County Hospital, Kilifi, Kenya.

Sections with unclear themes were examined collaboratively with the research team, and differences in interpretation were resolved through group consensus to ensure consistency and strengthen the rigour of the analysis (108,194).

4.2.3 Ethics and governance

This study was approved by SERU (SERU 3416). Written consent was obtained from all FGD participants prior to participation in FGDs, including consent for audio recording.

4.3 Results

Focus groups with PLWHB included six men and seven women with a median age of 37 years (range 24 – 61). Five participants had been newly diagnosed with HBV in the STRIKE-HBV study, eight already knew their diagnosis prior to enrolment in STRIKE-HBV. Two of those participants who were newly diagnosed had family members already known to be living with HBV.

HCW focus groups consisted of nine women and five men, with a median age of 31 years (range 18 – 61). The range of cadres included three clinical officers, three community health promotors (CHPs), two nurses and then one of each: counsellor, adherence supervisor, peer educator, nutritionist, records officer, and laboratory technician.

4.3.1 Knowledge and understanding of HBV

Both PLWHB and HCWs knew that HBV is a virus causing liver disease and is transmitted sexually or by contact with infected blood. Sexual transmission was mentioned most by both groups, including men and women, and still considered the most common route of transmission, especially by PLWHB, although other routes were mentioned. As participating PLWHB were those already receiving treatment, they were aware of HBV medication, and some HCWs specifically mentioned the use of tenofovir/lamivudine combination therapy. In both groups however, there was still some confusion around what happens after taking treatment for 6 months - some PLWHB thought they would be cured; however, others knew this was not the case:

> *"In addition, if you have liver disease, you can, if you use the medicine properly for six months and if you are tested, it may be that the virus is gone", (participant 5, PLWHB, male, FGD 1).*

Within the HCW groups, although there was discussion around poor community HBV knowledge, they also noted a significant knowledge gap amongst HCWs prior to the STRIKE-HBV study. They felt STRIKE-HBV had given them confidence in HBV management, and that many healthcare providers in other facilities were still providing incorrect information:

"At first, I didn't know how to give the treatment. I used to just guess. I didn't know how long I should be giving this medication. I didn't even have the knowledge of package information to give the patient but right now I can stand comfortably with confidence and tell the patient what he or she is going to take and for how long the treatment should be and what he or she should do." (participant 7, HCW, male, FGD 2)

When discussing a local language description of HBV, participants from both groups stated it was an unknown disease, or only described by the symptoms:

"But this hepatitis B virus is unknown, let's say it has no name. People don't know, they only know AIDS", (participant 1, PLWHB, female, FGD 2).

"In Kilifi they just describe the complications, when you have jaundice they say "yellow", when you have ascites they say "mwamimba" [pregnancy], only that." (participant 1, HCW, female, FGD 2)

Others reported HBV infection was called *'cancer'*. Several people mentioned that they had heard of HBV as it was causing illness in their village, and perceived it to be a death sentence:

"*I knew it [HBV] was killing people in the village. You don't know if he is starting [to get unwell] but you will only know that something has reached. That is, they don't get medicine, that is, there is no nursing, it is only when he is taken to the hospital, when he begins to be treated, then death has already arrived" (participant 6, PLWHB, male, FGD 1).*

Several respondents in both HCW and PLWHB groups mentioned that PLWHB were sometimes thought to be bewitched, and they would go to traditional healers to be cleansed and take herbal medications instead of tenofovir, or to church to pray to be cured:

"…those [with HBV] who have complications, or have ascites, in local language or native language we call it "mwamimba" [pregnant] and so they say that they have been bewitched and somebody

has thrown something in their stomach and now like you are like a man but you are "pregnant". (participant 5, HCW, female, FGD 2).

4.3.2 Comparison with HIV

Both PLWHB and HCWs discussed the confusion between HBV and HIV, particularly given that care is received in the same clinic, and the medication is the same. Many PLWHB found it difficult to accept they did not have HIV, and friends and family sometimes accused them of lying. This was common to both men and women. Many PLWHB felt they needed to 'prove' they didn't have HIV by going with family or partners to be tested:

"…one day my wife came and explained to me, "I heard some people say that you have AIDS." I told her, "No, I don't have AIDS and if you want to know, let's go, let's all go and be tested" (participant 5, PLWHB, male, FGD 2).

"… [many] people who don't understand and then once they get the results and then they are being escorted to this building CCC, majority don't get to understand clearly the relationship like we are telling them it's not HIV and we are giving them ARV's (anti-retroviral therapy)." (participant 1, HCW, female, FGD 2).

Several times it was mentioned that HIV was better funded than HBV, and there was not the same level of resources for the two infections.

4.3.3 How lives have changed since HBV diagnosis

We initially discussed reactions to diagnosis, both individually and those of friends and family. Many PLWHB were shocked at their diagnosis and felt anxious as they had little knowledge of HBV. HCWs reported many newly diagnosed people thought they were dying, and associated HBV only with end stage disease. Counselling helped alleviate anxiety and was felt to be very important by both PLWHB and HCWs. Peer support sessions were motivating and HCWs thought these should be given more routinely to allow PLWHB to discuss their concerns. Several PLWHB mentioned that once they got used to their diagnosis, they realised they could continue to take the medication and live just like anyone else.

Many women diagnosed with HBV had been tested as a condition for travelling abroad for work, and their diagnosis had not been properly explained to them which caused a lot of distress. They felt lost and abandoned, unable to pursue their plans, whilst peers and colleagues went off on their travels.

> *"He [employment agent] refused to tell me because he said [the problem was] either liver or kidney. I told him, "Now there is no disease that is good and there is no disease which is bad, you can just explain it to me." He said, "Just go to the doctor and get tested or go and get an x-ray and he [the other doctor] will explain more, but I won't explain it to you."" (participant 3, PLWHB, female, FGD 2).*

Reactions of friends and family varied. Some were very supportive, went to get tested themselves and encouraged participants to continue medication, but some partners left after the diagnosis. Sometimes after an initial lack of understanding, relatives came to the CCC to seek more information about HBV and its consequences.

Almost all participants commented that their lives had changed since HBV diagnosis, sometimes in beneficial ways, some less so. One male participant had considerably cut his alcohol consumption due to concerns about his liver:

> "*Even that time I came to be tested, I came already drunk…I have been told "The liver's job is this and this and this and when you eat, the food is processed like this and this and this in the stomach"... I saw that there is a very important knowledge…. I stopped drinking alcohol; I continue to take medicine. So I see there is a big change. That is, since I have been drunk all the time and this time, I can be sober, I'm grateful", (participant 1, PLWHB, male, FGD 1).*

Many PLWHB had experienced non-specific symptoms that had improved with treatment such as tiredness, stomach pains and swelling, nausea, itching, heartburn and constipation. Symptom improvement with treatment encouraged them to continue:

> *"I was given about five days [of medication]. I came back, I came and was given for two weeks, I came back. But since then, I see changes myself even in my body. I don't get hurt a lot and I don't get a lot of fatigue". (participant 7, PLWHB, female, FGD 1).*

"I was diagnosed with this disease, and I just saw that I was already dying. But when I got this treatment, I am fine now because even when I was sleeping and I could feel my stomach like water going this way, when I turned over, I could feel like water coming back this way and I said, "My stomach has started to swell too." But this time when I started this treatment, that issue stopped and the anxiety of saying that I was dying because I had not received [education]. This time I have received [education]. Now I know that if I use these drugs properly, I can live normally." (participant 5, PLWHB, male, FGD 1).

Many participants perceived that they had less freedom as they felt they needed to ensure they were home from work at a particular time and sometimes decided not to engage in social activities due to concerns about taking their medication:

"But since I use these drugs, I always tell my friends that I can't walk at night. Because when it reaches eight o'clock, I have to take medicine" (participant 7, PLWHB, female, FGD 1).

4.3.4 Current provision of HBV care in Kilifi

We discussed with HCWs what care was available for HBV both in KCRH and further afield, and how this had changed since the STRIKE-HBV study. Adherence counselling and medication had always been available at KCRH free of charge for HBV, however as described above, HCWs reported inadequate knowledge about medication and a lack of confidence when prescribing. In Kilifi County, diagnosis was most often made in private facilities prior to travel abroad for work, and treatment was provided primarily at KCRH. Public peripheral facilities and private hospitals often referred patients to KCRH as it was known to have an established hepatitis clinic. Occasionally people would be able to pick up their medication from subcounty hospitals and smaller health centres, but only after education and service establishment by HCWs from KCRH:

"So, there were no services [no hepatitis clinic] there, but we had to recreate the services for her to get the drugs. So, some have opted to go to a smaller health centre, and some to two sub-county hospitals, and so we have some sites which are offering [HBV treatment] but the diagnosis was done here." (Participant 7, HCWs, male, Group 2).

The HCWs however still perceived that peripheral facilities require more support to increase their confidence in providing HBV care. Many health centres and dispensaries had HBV medication available

along with adherence counsellors as they cared for those living with HIV, yet patients who desired to get their treatment from their nearby facilities were sometimes still referred upwards to KCRH:

> *"…as much as they wish to go there, some facilities have limited knowledge on client follow up and now it becomes a challenge, but we have clients who would prefer to take medication from a nearby facility. I have had two who have been returned [to KCRH from peripheral facilities] for further management because the dispensary is not well equipped to manage hepatitis clients." (FGD 2, HCWs, female, participant 1).*

Since STRIKE-HBV, HCWs reported they were able to provide better informed psychosocial support for PLWHB. They also encouraged PLWHB to talk to partners and family about testing to try to reduce stigma. They reported encouraging PLWHB to be ambassadors and educate the community about HBV and some HCWs were sharing their improved HBV knowledge with colleagues in peripheral facilities to support them in providing HBV care.

4.3.5 Barriers to HBV care and how these can be reduced

4.3.5.1 Economic Factors

Economic factors seemed to be the biggest barrier to receiving care. Firstly, the cost of transport to attend a distant hospital-based clinic was mentioned by both HCWs and PLWHB. To counter this cost, many people felt HBV medication should be available at the local dispensary:

> *"Because the rest of us are far away [from the hospital], but if we find our local dispensaries nearby, these local medicines, the way we are known, they are placed there. It will be easy even for us because we won't have to pay fare", (participant 2, PLWHB, female, FGD 1).*

> *"…they come from far and first they have the transport issues and secondly they are living in abject poverty and so most of them they cannot make it to Kilifi and so at least if we have the resources [medication] and we dispense the resources [medication] nearer to them then it will be better." (participant 7, HCWs, male, FGD 2).*

Several PLWHB mentioned the need to plan to make sure they had fare to attend clinic. Along with fare, the timing of clinic appointments had little flexibility, and often the patients experienced long waiting times. This was difficult with employment, with men in particular mentioning they struggled to get days off work leading to lost earnings:

"So the challenge always arises because maybe your boss refuses to agree with you, maybe it [the clinic return date] can be balanced until you get the chance to come take medicine or a meeting like this. You may find that your boss telling you, "Now you might not get paid today, or we will remove this day from your rest days, we will cut it there". (Participant 5, PLWHB, male, FGD 2).

The wish for decentralised clinics however was not universal. Several people in both the patient and HCW groups mentioned that (despite the potential higher cost) some PLWHB would prefer to go to a distant clinic (where they are not known) to reduce stigma, and sometimes asking for money for transport caused problems with questions about where they were going:

"…many of them still have stigma so they are thinking like if I am seen there, I will be isolated, so many prefer to go to those far away hospitals that no one knows, very few said they were referred to go to a nearby hospital." (Participant 4, HCW, female, FGD 1).

"[If I ask a friend]: Help me with 200 shillings ($1.5) so that I can go somewhere and then I will give you back." He asks, "Where do you want to go?" What do you want to do with it?" Now that's where I see the difficulty." (Participant 5, PLWHB, male, FGD 1).

4.3.5.2 Stigma and lack of education

Stigma was raised as an issue by all focus groups, both men and women, with participants highlighting instances of self-stigma and community stigma. Concerning self-stigma, PLWHB felt they were being talked about, and others were distancing themselves for fear of infection:

"And if the food itself I can't eat with them at the same table, it is as if I will infect them, I should have my own bowl and sit aside", (participant 3, PLWHB, female, FGD 1).

As noted earlier, HIV and HBV services are currently integrated in KCRH and all clients are seen together in the same clinic. PLWHB revealed tensions around this, with some participants feeling they should have a separate clinic from those living with HIV, partly due to stigma when seen entering the HIV clinic, but also due to long wait times when being seen together. Some people felt those living with HIV were prioritised, although there was also agreement that separating those with HBV and HIV

could increase stigma, and they should all be together because they had more in common than differences:

> *"I see they should just be together because we cannot separate them [HIV patients]. We all say we are birds of the same colour, we are going in the same direction. It means that if we separate them, it is like we are bringing that stigma and we don't want it to be like that", (participant 2, PLWHB, male, FGD 2).*

Education was seen by both PLWHB and HCWs as the best way to combat stigma, including continuing education for PLWHB through expert led peer support sessions, along with informing the immediate family and community. Several people mentioned initiatives on television or the radio to spread information about HBV, and respected community members such as pastors and chiefs to encourage testing and treatment. Several people in the HCW group felt the community health promotors (CHPs) could play a pivotal role in HBV sensitisation and treatment adherence. They mentioned how CHPs are funded to visit people living with HIV at home but not those living with HBV:

> *"I feel the CHP should also be brought on board on issues of Hepatitis B sensitisation and treatment adherence. Because you will get CHP bringing a referral or a patient who had defaulted treatment for [HIV treatment] but they will never do follow up for a client with Hepatitis." (Participant 1, HCW, female, FGD 2).*

4.3.5.3 Reducing transmission

When asking PLWHB how to reduce HBV transmission, practical ways were discussed to reduce contact with the virus including safe sexual practices, and one participant mentioned wearing gloves before helping anyone who was injured. Reducing the sharing of instruments that spread HBV was felt to be important, particularly needles, razors and toothbrushes, and a few participants mentioned vaccination, but did not distinguish groups they felt could benefit from vaccination, or how vaccination should be offered.

Both PLWHB and HCWs felt that community awareness is paramount in stopping the spread of HBV, including reducing fears of testing by educating people that HBV can be managed well with medication, which is available free of charge, and is not a death sentence. Participants suggested testing could be promoted through television and radio campaigns, by religious leaders and the Ministry of Health:

"So you find when people already know that [HBV can be dangerous], if the pastors also intervene, after explaining, now you will get a large group that say, "Uh, let me look at myself." And there I think we will find many people will volunteer [for HBV testing]. You can't go to someone's house and grab his shirt and bring him here, no, it's up to him to decide and say, "At my own free will, let me check."", (participant 6, PLWHB, male, FGD 2).

Access to testing was also mentioned by several PLWHB, ensuring the community knows this is available, where to be tested and ideally testing being offered for free. Many HCWs and PLWHB discussed that HBV testing should be part of the routine screening that everyone has when they go to a hospital, like HIV. There was also discussion of the benefits of antenatal testing for HBV, and how this should be routine:

"… There should be free testing, random or all over, everyone to be reached. I can say here in Kilifi, the people of Kilifi are lucky because even I am lucky because of your research and the services that are provided here." (participant 3, PLWHB, female, FDG 1).

"*…perhaps if the pregnant mother it is her first time, she must come with her husband so that they can be tested for AIDS, and the likes. Now I was thinking it would also be good if this hepatitis B was placed in that category, when they come to be tested, if she has come to the clinic, being tested for the HIV virus, they should also test the Hepatitis B virus…", (participant 6, PLWHB, male, FGD 2)*

Reducing stigma around an HBV diagnosis was felt to be key in reducing transmission. If those living with HBV felt able to reveal and discuss their diagnosis, they could also be the ones to spread awareness and educate their communities:

"I think it would be better for those of us who have the disease to not be ashamed. I explain that I was diagnosed with that disease [HBV] and that I use those drugs, I tell the people there to go to the hospital, not to wait until the organisation reaches the grassroots, it will be too late", (participant 4, PLWHB, male, FGD 2).

A common theme that ran through all the discussion questions was lack of education. Improved HBV awareness and knowledge was seen as crucial in all areas including reducing stigma, reducing

transmission, improving testing and diagnoses and improving access to care. Both PLWHB and HCWs highlighted this throughout as paramount to improve the lives of PLWHB in Kenya.

Knowledge and understanding	How lives have changed	Current care available	Barriers to care	How to reduce transmission and improve care
- Ongoing confusion about medication duration after the initial 6-month period. - Poor community knowledge with no local language word for HBV - HCWs poorly informed prior to STRIKE-HBV and incorrect information still given in other facilities - PLWHB concerns about being 'bewitched' and visiting traditional healers to be 'cured'. - Confusion with HIV. Many PLWHB having to 'justify' they don't have HIV.	- Unable to travel abroad due to HBV diagnosis. - Reduced alcohol intake, better self-care. - Reduced freedom in the evenings due to medication timing. - Concerns about dying.	**Benefits** - Adherence counselling and psychosocial support provided at KCRH. - Staff at KCRH educate partners and family. **Concerns** - Testing not free of charge outside of STRIKE-HBV. - No vaccination other than routine infant schedule. - Smaller facilities have the correct provisions but feel unable to provide HBV care due to lack of knowledge	**Economic factors** - Cost of transport - Lack of decentralised clinics - Living in poverty - Loss of work - Difficulties getting time off work to attend **Stigma and lack of education** - Self-stigma and community stigma - Concerns from others about transmission - Unhappy about HIV/HBV service integration due to increased stigma. **Others** - Lack of freely available testing and vaccination.	- Education about safe sexual practices. - Reduce sharing of household implements (razors/toothbrushes) - Provision of vaccination including birth dose vaccine. - Community educational campaigns. - TV and radio campaigns, advocacy by prominent society members and the ministry of health. - Better availability of free testing, particularly in antenatal women. Testing available in communities. - Decentralised clinics for those who want them, and education of more HCWs. - Specific hepatitis clinics, separate from HIV.

Table 4-2: Summary of the different themes arising from focus group discussions (FGDs) with people living with hepatitis B virus infection (PLWHB) and healthcare workers (HCWs) caring for PLWHB at Kilifi County Referral Hospital (KCRH) in Kilifi, Kenya. HBV – hepatitis B virus.

4.4 Discussion

I set out to explore the experiences of both PLWHB and HCWs providing HBV care in Kilifi Kenya aiming to understand HBV knowledge, impact of HBV on people's lives and barriers to accessing care. These findings, summarised in Table 4.1, add to the limited literature exploring the lives of PLWHB in WHO-AFR. Several studies have evaluated different mechanisms to try and improve HBV care, however very few have included the patient voice, or discussed with HCWs involved with patient care when planning interventions, making this a valuable addition to HBV literature (195,196).

Financial barriers have also been highlighted elsewhere (109,110,197), and can lead to catastrophic costs for individuals and families living with HBV (198). In this study, travel costs to the hospital clinic were seen as most prohibitive, whereas in studies from Burkina Faso and Ghana, costs of tests, monitoring and medications were mentioned more commonly (110,199). This may be because in our setting, testing and NA therapy were provided free at the point of care in KCRH which is not the case in many other countries. Many studies both from within WHO-AFR and elsewhere have raised concerns around lost employment (200–202) as in our study, and this was particularly common amongst men. Lack of childcare was mentioned as a significant barrier to care in some studies outside of WHO-AFR (201). This was not mentioned at all in our study or others from Ghana or Burkina Faso (109,110) possibly due to the different family structures in countries in WHO-AFR compared to those in the Global North, with less reliance on parents being the sole care givers.

The concerns raised by our participants around the integration of HBV and HIV care particularly related to increased stigma has been discussed previously (203). The utilisation of existing HIV services is one of the key components hypothesised to enable decentralisation of HBV care. However, I suggest that caution is needed, and extensive community education and sensitisation should be done before this is implemented. I also found that there is often confusion between HIV and HBV, which was also the case in Burkina Faso where HBV was closely associated with HIV when explained to patients (110). Although this was seen as a negative association, the good understanding of HIV transmission routes did help explain HBV transmission. In Burkina Faso, those diagnosed with HBV outside of a hospital setting were often given poor information around HBV, similar to several of our PLWHB who were diagnosed pre-travel.

Stigma has previously been identified as a barrier to accessing HBV care, however there is a lack of available instruments to accurately measure HBV related stigma, particularly in WHO-AFR. The Berger

HIV stigma scale has been readapted in some settings to be relevant to HBV (204), however this needs further geographical exploration and validation.

An interesting finding in our study was that many PLWHB felt they were restricted in the evenings due to taking medication, having to be home from work at a particular time or being unable to meet friends. This has been described in HIV literature as people are more likely to take their medication at home and do not want to carry tablets with them due to fear of discovery (205). It may also be that the HBV clinic has given instructions around when to take medication related to food to reduce side effects, and this is most convenient in the evenings. Education from the HBV clinic around the flexibility of medication timing could be helpful, so PLWHB understand it can take be taken at any time of day as long as this is consistent and does not need to interfere with any other social, domestic or work commitments.

Several suggestions from participants on how to improve care are already included in national Kenyan guidelines on the management of HBV such as antenatal HBV testing and provision of vaccination to close family contacts (206), however these are not universally incorporated into care. To continue strengthening HBV care at KCRH and across the County, ensuring education is a funding priority is crucial. Education was seen as one of the most important initiatives to increase diagnoses, drive down stigma and improve HBV care pathways. As HCWs frequently transfer between facilities, even localised educational initiatives have the potential to disseminate knowledge more broadly.

4.5 Caveats and limitations

PLWHB included in this study were already linked to care and receiving treatment, and both HCWs and PLWHB had been involved in previous research and have engaged with a peer support group. These groups are likely to have better knowledge and PLWHB be more engaged in care than those not involved in such a study. Here we included people from a limited geographical area and only a small number of HCWs and PLHWB. To fully understand care barriers, we need to expand these data to ensure themes were saturated and undertake similar discussions with PLWHB who have been lost to follow up, those not offered treatment and include community groups not tested or living with HBV; ideally a future study would expand to include PLWHB and HCWs accessing healthcare services both in Kilifi and further afield.

4.6 Conclusions

Community education and decentralisation of care for PLWHB should be prioritised to reduce stigma, enable easier access to care, reduce cost of travel and address health inequities to support wider roll-out of treatment. In future research, I would like to expand this data to cover a wider geographical area, to increase the number of PLWHB included in FGDs and to ensure other groups were represented such as antenatal women. I would like to conduct individual interviews with these groups and with local and national policy makers to understand systemic barriers to improving HBV care. Moving forwards, new interventions could be co-designed with the community to ensure they are appropriate, accessible, acceptable and affordable, and can be sustained over time.

5. HEPB-BOOST STUDY

5.1 Introduction

As discussed in chapter 1.1.6, HCWs have a considerable risk of HBV exposure. However, despite HBV vaccination being included in Kenyan National Immunisation guidelines since 2007, there are low rates of HBV vaccination amongst HCWs in Kenya. HBV vaccine is not mandatory, and routine HCW occupational health assessment and vaccine access is inconsistent across hospitals in Kenya. Some studies from Kenyan healthcare facilities have reported <50% of their staff are fully vaccinated (58,59). Few HCWs know their HBV serostatus, and HBsAg positivity rates in HCWs can be as high as 18% (207). At KCRH there is no formal HCW assessment of HBV serostatus or vaccination upon employment, and following the start of STRIKE-HBV, HCWs became increasingly concerned regarding their HBV vaccination status.

We established an HBV technical working group (TWG) AT KCRH consisting of representatives from various hospital departments including management, clinicians, pharmacists, laboratory staff and nurses, aiming to implement free of charge HBV testing and vaccination for all HCWs employed at KCRH. To evaluate the implementation and success of this programme we set up a research study based at KWTRP to run alongside this clinical programme called HepB-Boost. HepB-Boost and the vaccine implementation programme had separate funding, management and governance structures.

While many studies in WHO-AFR have assessed HBV vaccination coverage among HCWs, there is a notable lack of research detailing the processes and outcomes of programme implementation. Such studies could provide valuable insights into overcoming logistical challenges, improving vaccine accessibility, and enhancing awareness and education efforts tailored to healthcare settings. To my knowledge HepB-Boost is the first study in WHO-AFR to evaluate the planning and implementation of HCW HBV vaccination in a structured way in a government hospital.

5.2 Objectives

The overall aim of HepB-Boost was to understand the current HBV infection and vaccination status of HCWs at KCRH and evaluate the feasibility of an HBV vaccination programme existing hospital infrastructure and budget.

Specific objectives:

Objective 1: What is the current self-reported rate of HCW HBV vaccination at KCRH?

Objective 2: What proportion of HCWs accept an HBsAg test free of charge and how many test HBsAg positive?

Objective 3: What is the uptake of HBV top-up vaccination at KCRH and how many complete the recommended vaccination course?

Objective 4: What are the summary demographics and employment details of those attending the vaccination clinics?

Objective 5: What was the success of the implementation programme? This will be assessed considering the following questions:

- Did sensitisation reach all target groups?
- Was vaccine procured at a reasonable cost and in a timely manner?
- Was vaccine schedule acceptable and feasible for staff delivering vaccination? Vaccination must not interfere with the usual work of the MCH clinics, and the workload must be manageable for staff delivering vaccine.

This study protocol was developed and undertaken through strong and consistent collaboration between me and the KCRH team and is testament to their determination and tenacity. The data for this paper was collected by the KCRH team during vaccination clinics, and deidentified data was provided by the hospital team for analysis in HepB-Boost.

5.3 Methods

I developed methods for this study using the UK Research and Innovation (UKRI) process evaluation framework: https://www.ukri.org/wp-content/uploads/2021/11/MRC-291121-PHSRN-ProcessEvaluationSummaryGuidance.pdf. The study has been reported in line with the Standards for Reporting Implementation Studies (StaRI) checklist (208). The definition of HCW in HepB-Boost is broad, referring to anyone at KCRH who may encounter patients or bodily fluids including clinicians, nurses, pharmacists, laboratory staff and support staff such as cleaners and porters. At the time of implementation, awareness of HBV at KCRH had been heightened due STRIKE-HBV and should be considered when evaluating outcomes. Many members of the HBV TWG had been involved in the research study.

Hospital Department	Services Offered
Administration	Managing day to day hospital operations, ensuring smooth functionality. Oversees data analysis, financial and human resource management.
Emergency Services	Management of all types of acute illness and accident. Includes ambulance drivers and the emergency medicine services.
Intensive and critical care	Life support services for children and adults with life threatening injuries or illness. Providing support to patients.
Internal Medicine	Medical care for adult patients encompassing all medical disciplines. Caring for both those with acute and chronic disease. Includes managing those with blood borne virus infections.
Laboratory	Haematology and biochemistry analysis for inpatients and outpatients. Some point of care diagnostics for infections such as HIV and HBV.
Outpatients	Manages many acute and chronic diseases for which people do not need hospital admission including ophthalmology, ENT, acute outpatients, diabetes, hypertension and other specialist services.
Paediatrics	Diagnosis and treatment of acute and chronic illness in children <14 years. Includes growth monitoring, nutritional counselling, and specialised care such as delayed development and congenital abnormalities.
Pharmacy	Procurement, prescription dispensing and medicine counselling.
Physiotherapy	Providing rehabilitation services after illness including physical strength and training exercises, equipment to aid mobility, patient education and promoting self-management.
Public Health	Focussed on disease surveillance and response, environmental health services and promoting staff health and wellbeing.
Radiology	Providing diagnostic services including x-ray, ultrasound and CT.
Reproductive Health	Mother and child health including family planning, antenatal and post-natal clinics.
Social Work	Evaluation of the socio-economic status of patients, developing care plans and connecting patients with community services like community health volunteers.
Surgery	General surgery, orthopaedics, neurosurgery, obstetric surgery, paediatric surgery.

Table 5-1: Hospital departments and services provided within each department at Kilifi County Referral Hospital (KCRH), Kenya involved in a drive to provide hepatitis B (HBV) vaccination to healthcare workers (HCWs). ENT – ear nose and throat, CT – computed tomography.

5.3.1 Targeted Sites and Resources

HBV vaccination was aimed at all departments in KCRH (table 5.1), with vaccines being delivered in MCH clinics where there is cold chain storage, and staff experienced in vaccine administration. HBsAg testing was made available at the hospital laboratory, or at the vaccination clinic prior to vaccine administration. A custom password protected database domain was purchased by KCRH from TrueHost (https://truehost.co.ke/) including identification protection to collect information on vaccinees and to enable vaccine reminders to be sent. The database was designed by the TWG, and the hospital data manager set it up. Information collected from each vaccinee included:

- Demographics (age, sex, national ID number, contact details)
- Number of vaccines previously received, and number required
- Numbers attending for HBsAg testing and results of this test
- Employment information (role in KCRH, number of years worked in KCH, any risky exposures at work in the last 12 months – needlestick or splash injury)
- Whether HCWs complete their recommended vaccination course.

5.3.2 Study Design – Implementation Plan

The implementation programme had three main stages: i) HCW sensitisation, development of vaccination guidelines and other promotional materials; ii) implementation of HBV testing and vaccination free of charge and iii) programme evaluation, identifying where processes were altered, challenges faced and how these were addressed.

5.3.2.1 Planning and sensitisation (Dec 2023 – Feb 2024)

I delivered educational sessions covering the basics of HBV biology, transmission and the importance of vaccination. These were both through continuing medication education (CME) sessions and in individual departments tailored to specific groups. These were delivered in English with translation to Kiswahili where required. I developed a standard operating procedure (SOP) covering vaccination procedure, delivery and storage and data collection (209) which was shared with and approved by the TWG. I made posters and leaflets (210) detailing information about HBV and the importance of vaccination and these were given to staff as physical copies and circulated on WhatsApp (Facebook, Inc., 2020).

Printed notices were distributed around KCRH informing staff of the vaccination programme. Self-reported vaccination status was collected by departmental heads to guide procurement, and KCRH pharmacy team procured rDNA hepatitis B vaccine (Serum Institute of India, Pune, India) privately

through Sai Pharmaceuticals (https://saipharm.com/) in 10 dose vials. Monovalent multidose vaccine was not available through the state corporation Kenya Medical Supplies Authority (KEMSA). Self-reported vaccination status data was collected for 353 staff, 269/353 (76%) staff reporting being unvaccinated, 41/353 (12%) reported one previous vaccine, 20/353 (6%) two previous vaccines and 25/353 (7%) were fully vaccinated, giving a total of around 900 vaccines being required to fully vaccinate all those eligible. Initially 600 vaccines were ordered to ensure vaccines did not expire prior to use.

5.3.2.2 Vaccination implementation (Feb 2024 – August 2024)

The planned vaccine schedule was 0, 1 and 6 months. HCWs were invited to sign up for vaccination on an electronic sheet or manually through departmental managers. Vaccine clinics began on 6th February 2024 and ran for two hours, twice weekly until May 2024, aiming to deliver first and second doses to those who were unvaccinated. Third dose clinics then ran through August 2024. Those who had received one or two previous vaccines could attend any clinic to complete their course. Each vaccine clinic required five staff - two nurses, one administrator, one data manager and one supervisor.

Voluntary HBsAg testing was offered prior to vaccination using a POCT (ACON Laboratories, CA, USA). Those testing HBsAg negative or choosing not to be tested were offered HBV vaccination based on their self-reported vaccine status as per the schedule in figure 5.1. Those testing HBsAg positive were referred to the CCC for counselling and ongoing care.

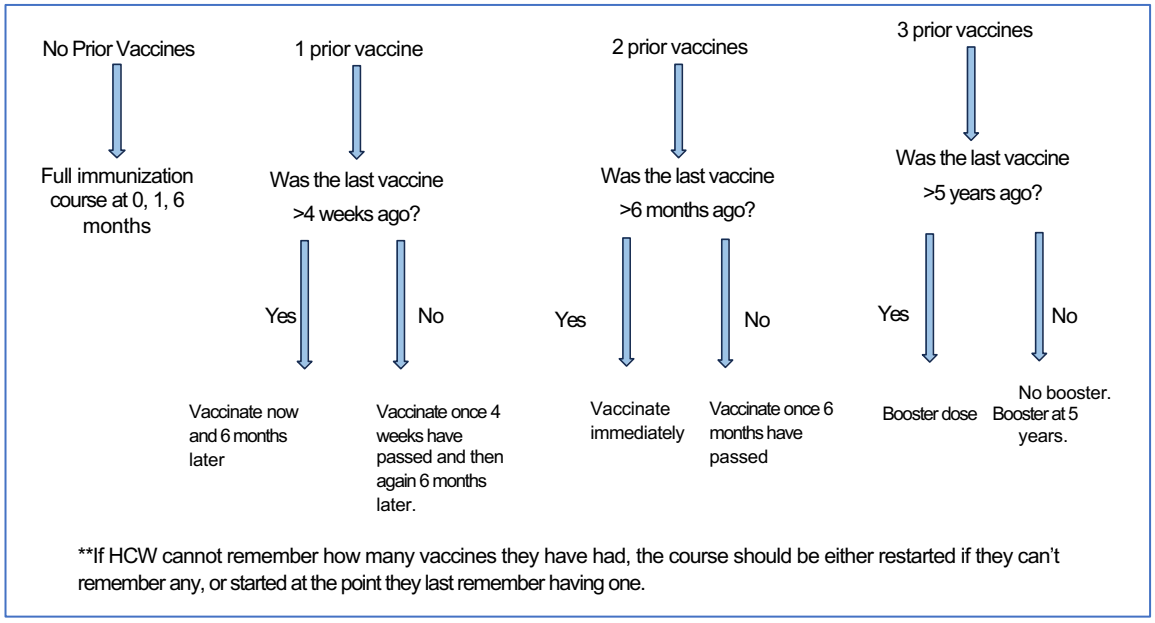

Figure 5-1: Planned vaccination schedule against hepatitis B virus (HBV) depending on previous vaccination status during an HBV vaccination drive for healthcare workers (HCWs) at Kilifi County Referral Hospital (KCRH), Kenya.

Demographics and employment data were collected from vaccinees as described in 5.2.1 and entered into the TrueHost database. Future vaccine dates were recorded both on the database and on physical vaccination cards given to vaccinees (211). Text reminders were sent manually prior to the next vaccination dose, avoiding the costs of automatic reminders through a mobile phone provider. In some cases, personal phone calls were made if vaccinees did not attend for their scheduled dose.

5.3.2.3 Evaluation

The primary outcome for the HBV vaccination programme evaluation was the number of HCWs tested for and vaccinated against HBV, and whether there were any factors associated with these outcomes. Further process evaluation objectives are described in section 5.0.1

- Did sensitisation reach all target groups?
- Was vaccine procured at a reasonable cost and in a timely manner?
- Was vaccine schedule acceptable and feasible for staff delivering vaccination? Vaccination must not interfere with the usual work of the MCH clinics, and the workload must be manageable for staff delivering vaccine.

I aimed to assess the process of implementation by meeting as a TWG every two weeks during the programme to identify challenges and discuss how these could be addressed.

Univariable analysis was undertaken to identify individual factors associated with vaccination completion and opting for HBsAg testing. Any factor reaching statistical significance was taken forward to multivariable analysis. Odds ratios were calculated, with reference categories being the largest group. For continuous predictor variables, the odds ratio represents the odds of the outcome with each increasing year of age or employment at KCRH. Analysis was undertaken using R version 4.2.0.

Finally, I evaluated the relevance of this implementation in the broader context of providing HBV vaccination to at risk populations in Kenya and further afield.

5.3.3 Ethics

This implementation programme was undertaken by the hospital in line with existing Kenyan immunisation guidelines (206) so ethical approval was not required. The research initiative (HepB-Boost) undertaken alongside this programme to collate and report summary information to help inform development of future similar programmes was approved by KEMRI SERU (4949) and OxTREC (23-24).

5.4 Results

5.4.1 Planning and sensitisation

I undertook one hospital wide CME prior to initiation of the vaccination programme attended by 40 staff members – most of these were clinicians, nurses and pharmacists. Of the 13 attendees who gave feedback, all knew about the HBV vaccine, but 4/13 (30%) did not realise three doses were needed. 12/13 (94%) responders felt the session helped them understand more about the HBV vaccine and all respondents felt the session would encourage people be vaccinated. I provided a smaller, targeted session to the MCH team who were providing vaccination which gave more chance for discussion around how the running of the clinics would work and to answer any questions they might have.

Later in the programme, a tailored session was organised specifically for support staff to encourage their attendance and given in Kiswahili by one of the pharmacists at KCRH. Moving forwards further sessions outside of the CME setting should be organised to reach more staff groups as CME's tend to be dominated by clinical staff.

5.4.2 Vaccine implementation

Of the estimated 574 people working at KCRH, 366 (64%) received at least one vaccine. Most people who participated were women (222/366, 61%). Age was available for 315/366 people with a median of 34 years (IQR 30-41 years). It is difficult to know how representative these demographics are of the whole hospital workforce, as there is no unified database collating demographics of hospital employees.

A summary of vaccinations received by those attending the vaccine clinic is shown in figure 5.2. The number of staff fully vaccinated against HBV increased from 18/366 (5%) prior to the programme to 164/366 (45%) at the end of our assessment period, with 289/366 (79%) of people receiving at least two vaccines (table 5.2 and figure 5.2). Anyone due for their second or third vaccine after August 2024 could not complete their required schedule due to lack of vaccine availability.

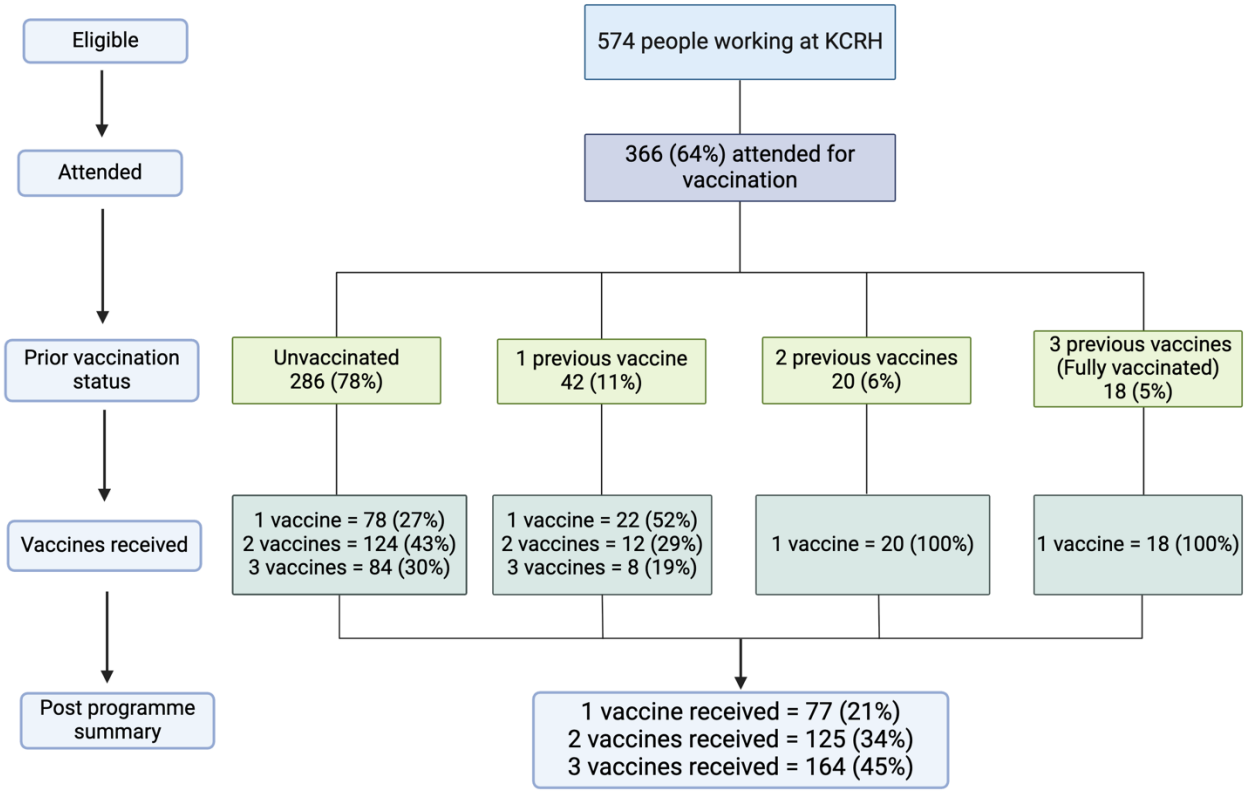

Figure 5-2: Summary of HCW numbers attending for HBV vaccination, and number of vaccinations received as part of a staff implementation programme at Kilifi County Referral Hospital in Kenya. **Figure created using Biorender.com.**

Most staff attending for vaccination were nurses (141/366, 39%) or support staff (72/366, 20%). The median length of service at KCRH was three years (IQR 1-7 years) and 41 people (11%) reported a needlestick or splash injury in the previous 12 months. 125/366 (34%) of those attending opted for HBsAg testing of whom 4/125 were positive (3.2%) (table 5.2) and referred on for care.

Characteristic	N	N = 366[1]
Age (years)	315	34 (30, 41)
Sex	366	
Female		222 (61%)
Male		144 (39%)
Hospital Role	366	
Accountant		12 (3.3%)
Administration		17 (4.6%)
Clinical Officer		53 (14%)
Laboratory Worker		20 (5.5%)
Nurse		141 (39%)
Pharmacy		11 (3.0%)
Support Staff		72 (20%)
Other*		40 (11%)
Needlestick last 12 months	366	41 (11%)
Years of Service at KCRH	366	3 (1, 7)
Number of previous vaccines (self-reported)	366	
0		286 (78%)
1		42 (11%)
2		20 (6%)
3		18 (5%)
Tested for HBV	366	
Yes		125 (34%)
No		241 (66%)
HBsAg result	125	
Positive	4	(3%)
Negative	121	(97%)
Completed vaccination schedule	366	
Yes		164 (45%)
No		202 (65%)
Vaccination status following the programme	366	
3 doses (Fully vaccinated)		164 (45%)
2 doses of vaccine received		125 (34%)
1 dose of vaccine received		77 (21%)

[1]Median (Q1, Q3); n (%)

Table 5-2: Characteristics of those attending hospital vaccination clinics at Kilifi County Referral Hospital as part of a hospital drive to vaccinate employees against HBV infection. Administration includes accountants, IT officers, procurement staff. *Any role containing <10 people was included in 'Other', including engineers, drivers, nutritionists, doctors, drivers, social workers, public health officers, security guards, dental officers, physiotherapists and radiographers.

5.4.3 Multivariable analysis

There was no association with age, sex or hospital role with completion of vaccination schedule. Those who had at least one previous vaccine and those who opted for an HBV test were more likely to complete their vaccination schedule on both univariable and multivariable analysis (table 5.3).

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor (reference group)	**Odds Ratio**	**95% CI Lower**	**95% CI Upper**	**P-value**	**Odds Ratio**	**95% CI Lower**	**95% CI Upper**	**P-value**
Sex (female)	0.93	-0.49	0.35	0.74				
Age	1.02	-0.01	0.04	0.18				
Hospital role (nurse)	0.75	-1.55	0.89	0.63				
Years of service	0.99	-0.04	0.02	0.58				
Previous needlestick (no)	1.20	-0.48	0.83	0.59				
Previous Vaccine (none)	**97.00**	**3.06**	**7.46**	**<0.01**	**93.02**	**20.15**	**1655.7**	**<0.01**
HBV test (no)	**2.20**	**0.35**	**1.24**	**<0.01**	**1.91**	**1.12**	**3.27**	**0.02**

Table 5-3: Univariable and multivariable analysis of factors associated with completion of hepatitis B vaccination at Kilifi County Referral Hospital as part of a hospital drive to vaccinate staff. Rows in bold indicate significant associations. Reference groups are shown in brackets. CI – confidence interval. P-value <0.05 was taken as significant.

	UNIVARIABLE ANALYSIS				MULTIVARIABLE ANALYSIS			
Predictor (reference group)	**Odds Ratio**	**95% CI Lower**	**95% CI Upper**	**P-value**	**Odds Ratio**	**95% CI Lower**	**95% CI Upper**	**P-value**
Sex (female)	0.83	-0.64	0.26	0.41				
Age	1.02	-0.002	0.05	0.08	1.00	0.97	1.04	0.91
Hospital role (nurse)	0.61	-2.04	0.76	0.47				
Years of service	1.04	0.01	0.07	0.01	1.04	1.00	1.08	0.07
Previous needlestick (no)	1.69	-0.15	1.18	0.12				
Previous vaccines (none)	**1.39**	**0.07**	**0.59**	**0.01**	**1.38**	**1.03**	**1.84**	**0.03**

Table 5-4: Univariable and multivariable analysis of factors associated with being tested for hepatitis B at Kilifi County Referral Hospital as part of a hospital drive to test and vaccinate staff. Rows in bold indicate significant associations. Reference groups are shown in brackets.

When investigating factors associated with being tested for HBV, on univariable analysis, age, years of service at KCRH and having been previously vaccinated were significantly associated with having a test, however only a history of vaccination was significant on multivariable analysis (table 5.4).

5.4.4 Vaccine access and delivery feasibility

Following completion of second-dose clinics (in July 2024), vaccine supplies ran out and we were unable to procure more multi-dose vials. Single dose vials were prohibitively expensive (492 Kenyan Shillings (KES) ($3.80)/dose as opposed to 223 KES ($1.70)/dose). I organised a donation of 200 near-expiry vaccines from KWTRP allowing the programme to continue through August 2024, where those still unvaccinated were encouraged to attend, along with who had presented late and required second and third doses. The donated vaccines expired on 31st August 2024, so at that point the vaccination programme stopped.

Clinics were labour-intensive, especially in the first few weeks when many people attended and were frequently opened outside of scheduled hours. Significant time was dedicated to following up those who missed their appointments. Use of automatic mobile phone reminders could have reduced this burden and ensured accuracy despite the added cost. Vaccine clinics did not interfere with the day to day running of the MCH department.

5.4.5 Fidelity and Adaptation

We made some alterations to the original implementation plan as the programme progressed:

- Non patient facing staff were vaccinated. We opted not to turn anyone away for fear of negative repercussions on the programme, however this meant more vaccines were used than anticipated.
- Several staff from peripheral hospitals attended for vaccination. Staff did not have official identification badges at the time of this programme, however running an inclusive programme was felt to be better than turning people away.
- Those who were fully vaccinated many years ago wanted to restart an HBV vaccine course. To reinforce WHO vaccination schedule, we implemented a clinical supervisor at each clinic to ensure those incompletely vaccinated were prioritised.

We would suggest in future programmes, vaccination provision should be stratified depending on risk, and history of vaccination to ensure those in greatest need are prioritised.

5.4.6 Contextual Changes

During this implementation there was a change in hospital management meaning repeat sensitisation, and differing priorities for the use of hospital resources. Along with this, HBV multi-dose monovalent vaccine became nationally unavailable over the course of the study. Despite donations from KWTRP, this limited the number of staff who could be completely vaccinated.

5.5 Discussion

Here KCRH delivered an HBV vaccine programme, and I evaluated the process and feasibility of implementation through HepB-Boost. There was excellent engagement from the TWG and maternal child health teams throughout.

Of the 64% of KCRH staff who attended a vaccination clinic, 76% were unvaccinated prior to this programme, and following this implementation, 79% had received at least two vaccines. This was a self-selecting population and staff who chose not to attend may have been already fully vaccinated or disengaged with healthcare and not vaccinated at all.

Studies from other hospitals in Kenya have reported varying vaccination levels. One study covering a sub selection of HCWs from eight county hospitals around Nairobi showed 80% of staff had received at least one vaccine, and 48% were fully vaccinated (58), whilst another study assessing all HCWs from two hospitals in Thika District in Central Kenya showed 87% HCWs had never been previously vaccinated (212).

Sensitisation efforts during the vaccination programme reached many hospital groups; however, support staff were initially underrepresented due to limited internet access. These groups are at high risk of NSI as they handle hospital waste, which may contain improperly discarded sharps. Targeted sensitisation sessions increased attendance later in the programme, however these strategies should have been employed from the outset. Doing this programme again, I would recommend early, dedicated engagement sessions for support staff organised independently of digital messaging through managers.

Those who had received at least one previous HBV vaccine and those who opted for HBsAg testing were significantly more likely to complete their vaccination schedule, and those previously vaccinated were more likely to opt for HBsAg testing than those unvaccinated. These people are likely to be more

engaged in healthcare and perhaps have a greater understanding of the need for testing and vaccination.

Only 34% of those attending for vaccination accepted free HBsAg testing. We intentionally made this optional to avoid deterring individuals from seeking vaccination. While testing was mentioned during sensitisation sessions, the primary focus was on highlighting vaccination benefits. Moving forward, we recommend placing greater emphasis on the advantages of HBsAg testing and suggest having a clinical advisor on hand at the vaccination clinic dedicated to encouraging individuals to get tested.

The most significant barrier to vaccination schedule completion was unavailability of multi-dose vials. Single dose vials were over twice the price per dose and therefore not feasible. We did not consider using combination vaccines which might be more readily available, but this could be considered in future programmes. Cuts to global health funding by international governments makes future vaccine access even more uncertain. The US administration recently terminated a $2.6 billion grant to the Global Vaccine Alliance (GAVI) (213) which will severely impact the availability of vaccines to low and middle income countries like Kenya. Steps must be made by vaccine suppliers to ensure equitable, affordable access, whilst efforts to fill funding gaps are consolidated.

Whilst undertaking this programme was labour intensive, the direct costs involved were relatively small and represent outgoings for initial set up. Moving forwards, I would like to undertake a full cost effectiveness analysis of the implementation with the assistance of a health economist to include not only the costs of vaccinating HCWs but also accounting for costs saved later in life from sequelae due to chronic HBV infection. One study from Ethiopia modelled the costs of increasing HBV HCW vaccination from 14% (as is the current situation) to 80% as recommended by WHO (214). They found increasing HCW HBV vaccination had higher life year gains with lower investment and that mandatory vaccination was cost effective. Another study from Ethiopia assessed how likely HCWs were to pay for their own HBV vaccination and found they were willing to pay a lot more than the market price. It could therefore be an option for healthcare facilities to make HBV vaccine available at low cost if they are not able to front the entire costs of vaccination.

The end goal of programmes such as this is provision of routine hospital occupational health services for HCWs, involving blood borne virus testing and review of vaccination history. Allocation of an occupational health specialist may not be possible for many County hospitals but should be considered in future health budget planning.

5.6 Conclusions

Implementing HBV testing and vaccination for HCWs in KCRH using existing infrastructure was feasible, and is scalable to other hospitals, however, requires ongoing sensitisation and a dedicated team. Ensuring consistent access to affordable HBV vaccines must be a priority WHO-AFR regions, to enable protection of at-risk groups and make headway towards global elimination targets.

6. DISCUSSION AND FINAL CONCLUSIONS

6.1 Summary of work and findings

The work I have presented here is the first of its kind throughout much of the WHO-AFR region and provides one of the most comprehensive descriptions of an African cohort of PLWHB. I aimed to enhance the understanding of HBV epidemiology, HBV related liver disease and factors influencing diagnosis, treatment and prevention of HBV in Kenya.

The global HBV landscape has changed dramatically from the start to the end of my DPhil with updated WHO guidelines on the management of CHB being released in March 2024 (4). These acknowledged the difficulties with implementing previous recommendations in resource limited settings and moved to a more flexible approach, expanding treatment eligibility criteria and relying less on often unavailable or unaffordable diagnostics. These guidelines were welcomed by the HBV community; however the global response is still off track towards reaching the 2030 SDGs. Viral hepatitis is one of the few communicable disease for which deaths are still rising, with around 1 million deaths in 2022, compared to 800,000 in 2019 (2). The global coverage of HBV prevention, diagnosis and treatment is too low, with only 4.3% of PLWHB in WHO-AFR being diagnosed and only 18% of infants receiving a timely HepB-BD vaccine (2). Kenya's efforts towards viral hepatitis elimination have been lagging behind other East African countries such as Uganda and Rwanda, with no national treatment programme, no HepB-BD vaccine implementation and no HBV treatment guidelines in line with WHO recommendations (215). Antenatal HBV testing is still not routine in Kenya and the infection is still largely unknown in the general population.

Evidence gaps prior to this work are presented in **chapter 1** and I aimed to fill these gaps in different ways. During my DPhil, whilst the overall direction of the project remained the same, new challenges and opportunities arose which I used for my clinical and academic development. My work started

setting the scene around HBV in Kenya, with a systematic review and meta-analysis covering HBV prevalence and available genomics **(chapter 2).** This work illustrated a paucity of epidemiological data, and how prevalence estimates can be skewed by screening specific populations. Available HBV genomics were primarily from two studies and only included people presenting to hospital with liver disease. This highlighted the need for generation of high-quality HBV genomic data from a range of population groups to inform transmission dynamics, identify potential drug resistance and therapeutic targets. Moving on from this, using data from clinical trials done at KWTRP, I highlighted the extent of data on HBV prevalence that is generated globally but not routinely collated or shared **(chapter 2).**

The clinical work I have done through my DPhil (STRIKE-HBV and HepB-Boost) was developed through discussion with international collaborators and I have used many other studies throughout WHO-AFR to help design and implement this work.

The PROLIFICA study run by the Medical Research Council (MRC) unit in the Gambia was the first study to show a screen and treat programme for HBV was feasible and acceptable (164). I used this as a base for STRIKE-HBV presented in **chapter 3** and had multiple discussions with Dr Gibril Ndow, the post-doctoral clinician running PROLIFICA, around the practicalities of undertaking a study such as this. Dr Ndow is now leading the COR-HEPB research coordination centre, which aims to catalyse investment and inform strategies to improve access to HBV care in LMICs (216).

Through STRIKE-HBV I gained experience in implementing clinical studies. I navigated the ethical approval process, managed a field team and trained my team in Fibroscan use. In the lab I gained experience in ELISA, CLEIA and NGS wet lab work. I am now proficient in using R and genomic data analysis tools. The clinical nature of STRIKE-HBV required a lot of lateral thinking along with ensuring a good, productive relationship was maintained with KCRH.

Prior to STRIKE-HBV, there was very little understanding of HBV both in the Kilifi community and healthcare workers. I provided many educational and sensitisation sessions and my relationship with KCRH clinical team culminated in the formation of a technical working group for improving HBV care. In STRIKE-HBV I showed that those with a family history of liver disease are more likely to be living with HBV. This fits with previous data showing up to 50% of acute hospital presentations with liver disease in Kenya are due to HBV (97,127), and evidence from STRIKE-HBV showing much higher HBsAg positivity rates within families than in the general population.

We predominantly tested women due to the demographic of those attending the hospital. My findings showing a higher standardised HBV prevalence in men along with worse liver stiffness scores (although not quite to the 95% significance level on multivariable analysis) are concerning, however concordant with previous literature investigating sex differences in HBV outcome (217,218). Men are less likely to engage in healthcare and mechanisms are needed to improve their access to HBV testing and treatment. I have shown that the biomarker HBcrAg is significantly associated with liver health irrespective of the assessment mechanism (ALT, APRI and elastography), and that the HBcrAg POCT may be a useful tool particularly to identify women at highest risk of vertical transmission to their babies (219). Whilst the practicalities and costs of rolling out this POCT need further assessment, given HBV DNA and HBeAg testing are not realistically going to be widely available or affordable in the near future in Kenya, this test could aid decentralisation of care and prioritisation of people for treatment (or prophylaxis) and vaccination. This POCT was donated to my study from Kumamoto University, Japan after a collaboration with Professor Yusuke Shimakawa at the Pasteur Institute. I am now a member of the HBcrAg-RDT study group, and we are developing evidence supporting this test as a viable alternative particularly for identifying pregnant women at highest risk of vertical transmission (220).

During my DPhil studies, the EVOLVE-HBV study was established in KwaZulu-Natal, South Africa, run by Professor Matthews and the wider HBV elimination group based at the Africa Health Research Institute (AHRI) (221). I shared many of the promotional materials I developed for STRIKE-HBV with the AHRI team to assist with study development (222). EVOLVE-HBV differed from STRIKE-HBV as it tested banked samples for HBV and then invited individuals selected by specific characteristics for clinical review and study enrolment. Many aspects were then similar including demographic profiling, liver health assessment and NGS using ONT. The HBV prevalence in EVOLVE-HBV was much higher at around 10% compared to 2-5% in Kilifi (223,224) and work is still ongoing.

Undertaking HBV NGS as part of STRIKE-HBV has established a new ONT protocol in Kilifi (HEP-TILE as described in **chapter 3** (157)), trained scientists in both wet lab work and data analysis and built up a network of collaborators also undertaking HBV NGS in Africa. Dr Camille Morgan based in the DRC under the supervision or Prof. Peyton Thompson came to Oxford for HBV NGS training with Dr Sheila Lumley, and Sheila along with Elizabeth Waddilove from the Francis Crick Institute used the HEP-TILE protocol in Uganda at the MRC unit in Kyamulibwa. This generated the data I have presented in **chapter 3** and is the first work comparing HBV NGS across different African sites.

Variations in HBV entropy I have shown across the HBV genome and between geographical regions is completely novel data from the African continent and may have future applications including assessment of transmission patterns and identification of therapeutic targets in universally conserved regions. The current goal for HBV therapy is functional cure, meaning the sustained loss of HBsAg. In this scenario cccDNA may still reside in hepatocytes at low levels, but the host immune response is sufficient to prevent ongoing viral replication without treatment. T-cells are however dysfunctional in CHB (225,226) so functional cure is rare and requires restoration of a functional immune response. Several immunotherapies are currently under development targeting different stages of the HBV lifecycle, from preventing the production of HBV RNAs through RNA interference (227), to causing aberrant capsule assembly (228) and preventing degradation of pgRNA needed for new DNA strand synthesis (229). We have shown a stark lack of HBV clinical trials done in the WHO-AFR (74), and no work on HBV cure has been undertaken in these populations. To make progress towards clinical trials being viable in the region, establishing consistent access to existing NA therapies is crucial, as most new agents are used on top of an established suppressive NA backbone. Demonstrating the ability to establish high quality HBV clinical cohorts in WHO-AFR may encourage new interest in clinical trials.

In STRIKE-HBV I showed the presence of some SNPs associated with TFV resistance. This is concerning and has not been well described previously in WHO-AFR. TFV resistance is traditionally thought to be very rare, however in 2025 we published a systematic review and meta-analysis exploring data on TFV resistance globally and only two papers were available from the whole of the African continent (230). This illustrates a huge paucity of data, and a potential for drug resistance that has not yet been described. Ongoing surveillance is therefore crucial, particularly with changes to WHO treatment guidelines expanding treatment eligibility.

To add to the limited information evaluating understanding of HBV in WHO-AFR, I proceeded to conduct FGDs with both HCWs and PLWHB in Kilifi **(chapter 4).** I used some of the qualitative work previously done at the Uganda MRC unit (108), with advice from Prof Janet Seeley to feed into designing these FGDs (190)**.** Similar to findings in Uganda, no local language word for HBV was known in Kilifi and people had variable knowledge about the infection. We did however find concerns about stigma which did not emerge in Uganda, primarily due to HBV being mistaken for HIV in Kilifi, as clinics are often run in the same location. It is possible in Uganda this is not the case, so such stigma has not developed.

Those who took part in FGDs were all involved or enrolled in the STRIKE-HBV study, so whilst this group may not be completely representative, this work still gave important insights into barriers to care in a very underrepresented group, and how these could be improved. Key observations from these FGDs included: i) There is significant confusion between HIV and HBV; ii) Transport cost are prohibitive to access care at the County Hospital and decentralisation is important; iii) PLWHB face stigma from friends and family, and also self-stigma with concerns about transmission and poor outcome; iv) HBV education is seen as critical for improving lives for PLWHB. I have published the topic guides for these FDGs on Figshare to allow accessibility for others doing similar work (190).

During STRIKE-HBV, it emerged that many HCWs at KCRH were unvaccinated against HBV. This was causing increasing concern given the number of individuals we were diagnosing with the infection. The KCRH management team were keen to introduce HBV vaccination and, to formally assess this implementation, we set up the HepB-Boost study to run alongside this vaccination programme as described in **chapter 5** (231)**.** From this study I developed a whole range of materials aiming to help others set up a similar vaccination programme, including an SOP, information leaflets, posters and vaccination record cards (209–211)**.** This was my first experience of an implementation study and through constructive feedback from the scientific committee at KWTRP I gained an understanding of assessment structures that can be used for such programmes.

HepB-Boost not only highlighted gaps in HBV vaccination that are prevalent throughout WHO-AFR but also illustrated the lack of documented analysis of how such programmes can be undertaken. To my knowledge, HepB-Boost is the first study from WHO-AFR that has formally assessed implementation of HBV vaccination for HCWs. Here, KCRH provided the staff, the funds and the data systems to undertake this programme and my findings showing this was feasible, and this model could be rolled out to other government hospitals in Kenya. This experience also highlighted to me how crucial it is to have engagement from local partners as without the enthusiasm and dedication of the staff running the vaccination clinics, this project would not have been possible.

6.2 Conclusions from this work

Conclusions I have drawn from my DPhil studies include those informed by my data and recommendations around Kenya's priorities to improve their national hepatitis elimination progress:

i) Conclusions drawn from my data

- CHB is prevalent but underrepresented in Kenya. Accurate epidemiological estimates of population prevalence are difficult even when aiming for general screening, and age/sex standardisation to the local population structure is necessary.
- Men are underrepresented in hospital settings due to women attending more frequently with babies and children, however, are at risk of worse liver disease outcomes.
- Those living with CHB in Kilifi have worse liver health than those without CHB.
- HBcrAg could be a useful biomarker for assessing liver disease however is not yet widely accessible. The new HBcrAg POCT shows promise as a tool for decentralising care and supports the agenda for elimination of vertical transmission.
- One family member with HBV increases the odds of other family members also having the infection. Intra-familial spread seems common and HBV genomic sequences within families are closely related.
- HBV genomics are poorly represented in WHO-AFR however have the potential to shed light on transmission dynamics and emergence of clinically relevant HBV polymorphisms. Some of these polymorphisms are present in Kilifi although their full relevance is not known.
- Travel costs and lost workdays when accessing HBV care place financial burden on PLHWB, and many members of the community are not well educated about the infection.
- Vaccination of HCWs against HBV is not routine in Kenya, however with a dedicated team, implementation of a vaccination programme is feasible in Kenyan government hospitals. This vaccine is however only readily available as high-cost single dose vials which creates a cost barrier for routine hospital purchase.

ii) Recommended interventions in Kenya.

- Screening of close family members of PLWHB should be prioritised to increase numbers diagnosed and treated.
- Consistent access to well validated, affordable diagnostic tests is needed and should be coordinated nationally, along with access to HBV vaccine both for at risk adults and infants.
- Educational campaigns should be led by local and national government to encourage testing, reduce stigma and improve follow up. Establishment of patient advocacy groups, including those with lived experience, will advocate for and empower PLWHB.
- HBV care should be decentralised by leveraging existing HIV service infrastructure. All dispensaries in Kenya are authorised to dispense tenofovir, however many do not currently feel empowered to do so for HBV management. With targeted education and training, this

gap could be readily addressed, facilitating broader access to NA therapy at the primary care level.

The work I have done aligns with the current global strategy for HBV and the summary recommendations above feed into many of the gaps highlighted in the 2024 Global Hepatitis Report – 'Action for access in low and middle income countries'(2). I have developed relationships with the Kenyan Ministry of Health through my DPhil and have been part of their Technical Working Group advising on the development of new Kenyan HBV guidelines. I have been in discussions with the Clinton Health Access Initiative (CHAI) regarding procurement issues I have faced during STRIKE-HBV and how to establish consistent access to HBV diagnostics, vaccines and treatment. I have published my work at many international conferences, was invited for an oral presentation at the Conference on Liver Disease in Africa (COLDA) 2023 and won best poster at COLDA 2024. Below is a list of first author conference contributions I have made along with where they were presented:

1. Use of HBcrAg point of care tests in people living with Hepatitis B Virus infection in Kilifi, Kenya to identify those at highest risk of liver disease. **Downs** et al. Conference on Liver Disease in Africa (Cairo, Sept 2024). Winner of best poster. *https://doi.org/10.6084/m9.figshare.27251769.v1*

2. Insights from implementation of healthcare worker HBV vaccination in Kilifi, Kenya. **Downs** *et al*. Conference on Liver Disease in Africa (Cairo, Sept 2024). *https://doi.org/10.6084/m9.figshare.27251793.v1*

3. Hepatitis B core related antigen in South Africa and the United Kingdom - does one size fit all? **Downs** *et al*. (International Liver Conference, Milan, June 2024). *https://doi.org/10.6084/m9.figshare.26022472.v1*

4. Real world implementation of a healthcare worker hepatitis B vaccination programme in Kilifi, Kenya. **Downs** *et al*. (World Hepatitis Summit, Lisbon, April 2024). *https://doi.org/10.6084/m9.figshare.25718268.v1*

5. **Downs LO** (2024). Implementation of HBV screening in Kilifi, Kenya: Real world insights to inform clinical service development and translational studies. figshare. Presentation. https://doi.org/10.6084/m9.figshare.25399687.v1

6. Serum hepatitis B core-related antigen in a diverse UK cohort of patients with chronic hepatitis B infection. **Downs** *et al.* (International HBV Meeting, Paris, Sept 2022). *https://doi.org/10.6084/m9.figshare.21455874.v1*

6.3 Future recommendations and next steps

Although many of the gaps highlighted in the first chapter of my thesis have been explored during my doctoral studies, even more gaps have emerged during my work. There are still many unknowns around HBV infection in Kenya and potential next research steps are as follows:

- Improved epidemiological estimates. Given prospective screening studies take time and are expensive, I would suggest using retrospective data or existing serum samples to gather this information. This could include expanding the use of clinical trial screening data as I did in chapter 2, or testing serum samples from the many studies on SARS-CoV-2 that have been done throughout the African continent over the last 5 years.
- Implementation studies to assess strategies for improving access to care for PLWHB, such as decentralisation of care and peer support workers. Educational programmes could draw on trusted members of society including religious leaders and village chiefs, and advertising campaigns led by the Ministry of Health to encourage testing.
- Development of improved, affordable, accessible non-invasive methods to assess liver disease related to HBV. Currently liver stiffness measurement using elastography is the gold standard outside of liver biopsy, however the hardware for this is prohibitively expensive and not widely available in the WHO-AFR region (232).
- Expansion of HBV genomic data. HBV response to TFV therapy varies between individuals. Mechanisms underpinning these variations are not understood but likely include polymorphisms in the HBV genomic sequence particularly the RT gene influencing TFV susceptibility. Along with this, a better understanding of the prevalence of TFV resistance mutations in WHO-AFR will help determine the potential for emergence of drug resistance.
- Cost-effectiveness studies to evaluate the impact of adopting new interventions for HBV care such as expanding treatment provision, particularly universal TFV prophylaxis for all pregnant women living with HBV.
- Social sciences work throughout the whole cascade of care for HBV. We need better insight into the impact of an HBV diagnosis on people's lives and barriers experienced to consistently accessing care. We need to investigate systemic barriers to accessing affordable high-quality consumables at the local and national level by discussing with healthcare workers and policy makers.

6.4 Reflections on initiating a research programme abroad

I have been very privileged to undertake my doctoral studies at KWTRP in Kilifi. Setting up a prospective research study from scratch in Kenya has been a steep learning curve but an invaluable experience. I feel the work I have done has been impactful, not just in the research world being one of a minority of such studies in WHO-AFR, but also at a local level. I have consistently had feedback from both KWTRP and KCRH that my work has raised the profile of HBV in Kilifi, made people enthused about researching a virus that was previously poorly understood, and drastically improved care for PLWHB at KCRH. Along with this, being part of the MoH TWG for HBV has given me insights into how national policies and guidelines are developed, highlighted how few people have expertise in HBV, and how valuable my knowledge and understanding of the local context can be.

The project of course has not been without its challenges. Ensuring I gained ethical approval to start the study in a workable time frame took dedication and rapid turnaround of many comments at each level of the approval process. On some days our study clinic was overrun with people wanting HBV testing and maintaining confidentiality of those testing positive and ensuring they were given the time to discuss their diagnosis was challenging, not to mention running the clinics in a tent which often reached extremely high temperatures and humidity. We had a long delay obtaining the Fibroscanner to measure liver stiffness and due to this delay, we had to recall all those who had tested HBV positive from the first 9 months of the study to return to have their scan, leading to some loss to follow up, and meant we did not start recruiting the HBV negative control group until the study had nearly completed.

Overall, I hugely enjoyed my time in Kilifi, I have learnt a lot and have emerged as a more well-rounded researcher. I hope the foundations I have laid will make the implementation of new HBV research projects much smoother, and my strong connections both at KWTRP and KCRH will keep me striving to access more funding to continue this work in Kilifi. I am planning to apply for a Wellcome Early Career Award in September 2025. This work will expand on what I have achieved here but will focus on mechanisms that influence how individuals respond to TFV therapy particularly in pregnancy. I will expand the NGS work looking for SNPs associated with reduced susceptibility and resistance to TFV and measure TFV levels throughout pregnancy to understand how they correspond with HBV VL.

6.5 Research output outside of this thesis

Along with the work described in my results chapters, I have contributed to a variety of other studies and investigations outside the scope of this thesis but informed by and closely linked to my doctoral work and experiences in Kilifi. These have been wide ranging and have allowed me to expand my clinical and data analysis skills, along with obtaining new skills in health economics and social sciences. I was also one of the winners of the Laskar essay prize in 2023, for which I was awarded $5000 towards my ongoing studies. Below is a list of citations I have contributed to over the course of my DPhil over and above those described in my thesis:

- Lumley SF, Mokaya J, Maponga TG, Kramvis A, Dusheiko G, Irving W, Delphin M, Said Mohammed K, **Downs LO** et al. Hepatitis B virus resistance to nucleos(t)ide analogue therapy: WHO consultation on questions, challenges, and a roadmap for the field. Lancet Microbe. 2025 Apr 9;(101076):101076.
- Wang S, Harrington M, Graham CS, **Downs LO,** Kagwanja N, Etyang AO, et al. Pathogens don't respect politicians: US federal disruption poses a new threat to global public health. Lancet Gastroenterol Hepatol. 2025 Feb 6 Available from: https://pubmed.ncbi.nlm.nih.gov/39923777/
- Jemutai J, **Downs L** et al. Elimination of hepatitis B requires recognition of catastrophic costs for patients and their families. Lancet Gastroenterol Hepatol. 2025 Feb;10(2):100–3.
- Lumley SF, Delphin M, Mokaya JF, Tan CCS, Martyn E, Anderson M, **Downs LO** et al. A systematic review and meta-analysis of the risk of hepatitis B virus (HBV) resistance in people treated with entecavir or tenofovir. J Clin Virol. 2024 Oct;174(105711):105711.
- **Downs LO** et al. Peer support for people living with hepatitis B virus-A foundation for treatment expansion. Journal of Viral Hepatitis . 2024 May 26; (10.1111/jvh.13952)
- **Downs LO.** "Is a test better than no test when there is no treatment?" Journal of Clinical Investigation. 2023 Jul 17;133(14). (10.1172/JCI173286)

6.6 Conclusion

I am hugely grateful to have had the experience I have through my doctoral project. I have begun to develop a large, collaborative global network of multidisciplinary individuals including clinicians, field staff, patients and advocates, social scientists, health economists, implementation scientists, data scientists and biostatisticians. I am confident that with continued efforts on the African continent to

understand from the ground up which processes are effective in improving HBV care, we can make meaningful progress toward achieving the HBV 2030 SDGs.

Recent changes to the global health funding landscape have been concerning. Given the close links between HIV and HBV infrastructure, consequences from the sabotage of USAID (the US Agency for International Development) and PEPFAR (the President's Emergency Plan for AIDS Relief) funding will significantly impact access to HBV diagnostics and medication (233). In Kenya, PEPFAR supplies of TFV will run out at the end of September and KCRH are already rationing prescriptions to give only small quantities at a time. This is the time for countries such as Kenya, traditionally supported by international aid, to take responsibility for the health of their own people. If they can rise to meet that challenge, they can truly design a National Viral Hepatitis Plan for the people, by the people.

48,901 Words

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