Seroprevalence of Hepatitis B virus infection in a household-based representative sample of African men and women in KwaZulu-Natal, South Africa

by

Natasha Samsunder

Student number: 891101429

Submitted in partial fulfilment of the requirements for the degree of
Master of Medical Sciences

CAPRISA Laboratory
Department of Medical Microbiology,
School of Laboratory Medicine and Medical Sciences,
Nelson R. Mandela School of Medicine, College of Health Sciences,
University of KwaZulu-Natal

2018

Supervisor: Professor Ayesha B Kharsany

Co-supervisor: Dr Sinaye Ngcapu

This dissertation is dedicated to my parents, Noharpershad Ramjith and Soorajmani Ramjith,

who sacrificed so that I could be educated.

I love you both dearly.

DECLARATION

This research represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. The original protocol for all the research in this dissertation was written by the author. The routine laboratory tests were performed by a contracted laboratory, Global Clinical and Virology Laboratory. The data was collated, checked and analysed by the author. A statistician at CAPRISA was consulted for advice on the more complex statistical tests.

Where use has been made of the work of others it is duly acknowledged in the text.

The research described in this dissertation was carried out in central KwaZulu–Natal (KZN) in Vulindlela and greater Edendale, two sub-districts of uMgungundlovu municipality, KZN, South Africa. This dissertation was supervised by Professor Ayesha B Kharsany and co-supervised by Dr Sinaye Ngcapu.

Signed: Natasha Samsunder (candidate)	Date
Signed: Ayesha BM Kharsany (supervisor)	Date
Signed: Sinaye Ngcapu (co-supervisor)	Date

ACKNOWLEDGEMENTS

A warm and sincere thank you to all who contributed, in their own individual way to the completion of this dissertation.

To my supervisors; Professor Ayesha Kharsany, thank you for always encouraging me and for your unlimited support and to Dr Sinaye Ngcapu, thank you for your resilience with me and for your support and guidance through my writing phase. Your mentorship has moulded me into a better person.

My sincere thanks to Dr Cheryl Baxter for her guidance on my dissertation and for reviewing and providing constructive comments to enhance the documents.

My sincere thanks to all household members and individual study participants for their participation in the HIV Provincial Surveillance System- the population-based household survey that has been undertaken to understand the evolving HIV epidemic in the region. The participant's consent for this study on the testing of samples for HBV infection is sincerely appreciated. My sincere thanks for the ongoing support of District Manager the uMgungundlovu Health District, members of the Provincial Department of Health, members of the uMgungundlovu district municipality, Provincial Health Research and Knowledge Management, local traditional leadership and community members for their support throughout the primary HIPSS study. A special thanks to the study staff for the field work and Primary Health Care clinic staff in the district.

My sincere thanks to CAPRISA for providing the research infrastructure support and the staff at CAPRISA for encouraging me to pursue this degree.

My thanks to the HIPSS study staff who contributed to the meticulous fieldwork, questionnaire administration, collection of the data that was used in this dissertation. Special thanks to the Epicentre and Global Laboratory teams.

Sincere thanks to all the laboratory staff for their meticulous assistance with sample processing.

In particular, I would like to thank:

My husband Vinesh, for his constant support and unwavering faith in me and ensuring the family was fed and taken care of while I spent the evenings working. To my children, Darian and Keirra, thank you also for being understanding and supporting me through this process. This degree is also for both of you and remember, you are never too old to study.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
Anti-HBc (HBcAb)	Hepatitis B core antibody
Anti-HBe (HBeAb)	Hepatitis B envelope antibody
Anti-HBs (HBsAb)	Hepatitis B surface antibody
AST	Aspartate aminotransferase
ART	Antiretroviral therapy
ARV	Antiretroviral drug
BREC	Biomedical Research Ethics Committee
CAPRISA	Centre for the AIDS Program of Research in South Africa
cART	combined Antiretroviral Therapy
DNA	Deoxyribonucleic acid
EPI	National Expanded Programme of immunisation
EFV	Efavirenz
FDC	Fixed dose combination
FTC	Emtricitabine
HB	Hepatitis B
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus deoxyribonucleic acid
HIPSS	HIV Incidence Provincial Surveillance System

HIV	Human Immunodeficiency Virus
kb	Kilobase
KZN	KwaZulu-Natal
pgRNA	Pregenome RNA
RNA	Ribonucleic Acid
RT	Reverse transcriptase
STI	sexually transmitted infections
TAF	Tenofovir alafenamide fumarate
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
WHO	World Health Organisation

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: In South Africa, hepatitis B virus (HBV) infection remains a major cause of morbidity and mortality, however, little is known about the prevalence and distribution of HBV in some regions and populations.

Methods: This secondary analysis is based on 9791 participants (15-49 years old) enrolled in the HIV incidence Provincial Surveillance System (HIPSS); a population-based household study undertaken from June 2014 to June 2015 in the Vulindlela (rural) and Greater Edendale (periurban) areas of the uMgungundlovu district, KwaZulu-Natal (KZN), South Africa. Interviewer administered questionnaires were completed to obtain demographic, psycho-social, behavioural and clinical information. Peripheral blood samples were collected and sera were tested for hepatitis B surface antigen (HBsAg) and all samples testing positive were further tested for hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe). The estimated weighted seroprevalence of HBV markers was calculated and the association of HBsAg with sociodemographic and behavioural factors measured.

Results: The overall HBsAg prevalence was 4.0% (95% confidence interval (CI) 3.4-4.5); 4.8% (95% CI 3.8- 5.8) in men and 3.2% (95% CI 2.5-3.9) in women, P=0.01. Among HBsAg positive participants, 35.2% (95% CI 29.2-41.2) were HBeAg positive and 66.3% (95% CI 60.1-72.4) were anti-HBe positive. Among men 15-19 years old HBeAg seroprevalence was 92.2% (95% CI 75.8-100) compared to 4.4% (95% CI 0-13.7) in women in the same age group; P <0.01. HBsAg prevalence was 6.4% (95% CI 5.3-7.5) among HIV positive participants compared to 2.6% (95% CI 1.9-3.2) among HIV negative participants, (P<0.01) and was higher among HIV positive men 8.7% (95% CI 6.3-11.2) compared to HIV positive women 5.0% (95% CI 3.8-6.2), P<0.01.

Conclusion: HBV infection, particularly among HIV positive men remains an important public health problem in rural and periurban communities in KwaZulu-Natal, South Africa. The prevalence of HBsAg and HBeAg highlight the importance of surveillance and an important missed opportunity for the scale up of programmes to achieve the goal of controlling HBV for public health benefit.

CHAPTER 1

INTRODUCTION

1.0 CHAPTER 1: INTRODUCTION

1.1 Overview

Almost eight percent of the 257 million people globally infected with Hepatitis B virus (HBV) reside in sub-Saharan Africa, with over 80 000 new infections occurring each year. HBV is a common cause of viral hepatitis, which affects the liver resulting in acute or chronic liver disease and complications of cirrhosis and hepatocellular carcinoma contributing to high rates of morbidity and mortality (WHO, 2017).

In 1995, the South African Department of Health introduced the HBV vaccine into the Expanded Programme on Immunisation at 6, 10 and 14 weeks of age. However, there was no catch-up immunization programme for older children and adults to ensure complete vaccine coverage (Tsebe et al., 2001). Despite the widespread implementation of HBV immunization, recent data indicate that HBV prevalence ranges between 0.0% and 13.2% in infants (Vardas et al., 1999, Tsebe et al., 2001, Mphahlele et al., 2002, Mdlalose et al., 2016), 7.0% and 10.0% in adults (Burnett et al., 2012, Matthews et al., 2015), and 0.0% and 5.3% in pregnant women (Thumbiran et al., 2014). Compared to any other racial or ethnic groups, black South Africans represent a population most affected by HBV infection, with the prevalence between 5.0% and 16.0% in rural males, 8.0% to 9.0% in urban males, 4.0% to 12.0% in rural females and 3.0% to 4.0% in urban females (Vos et al., 1980, Kew et al., 1976, Prozesky et al., 1983, Kew et al., 1987, Abdool Karim et al., 1988, Dusheiko et al., 1989).

South Africa contributes a disproportionate 70% of all the world's human immunodeficiency virus (HIV) infection, with over seven million people living with HIV in 2017 (UNAIDS, 2017). Similar to heterosexual transmission of HIV-1, HBV infection through the genital mucosa in women is a potential route of infection (Shattock and Moore, 2003, Abdool Karim et al., 2010, UNAIDS, 2017). Most HBV-HIV co-infected individuals may have acquired HBV

horizontally in childhood and HIV sexually in adulthood. Therefore, it is not unexpected that co-infection with HBV and HIV is common (Soriano et al., 2007). Unlike HIV, routine HBV screening is not offered in South Africa, yet local studies have reported HBV and HIV coinfections rates between 0.4% and 22.9% (Firnhaber et al., 2008, Firnhaber, 2008, Lukhwareni et al., 2009, Boyles and Cohen, 2011, Mayaphi et al., 2012, Bell et al., 2012, Ayuk et al., 2013, Thumbiran et al., 2014, Matthews et al., 2015, Mdlalose et al., 2016, King, 2016). In addition, studies from South African have shown variability in HBsAg positivity rates of 19.8% in an HIV care and treatment cohort (Hoffmann et al., 2008), 4.8% (Firnhaber et al., 2008) and 6.5% (Mayaphi et al., 2012) in urban cohorts, 22.9% in a periurban (Lukhwareni et al., 2009) and 7.1% (Boyles and Cohen, 2011) in rural settings. Consequently, HBV is a growing cause of liver cirrhosis, end-stage liver disease, and death among people living with HIV. HIV treatment guidelines by the Southern African HIV Clinicians Society recommend that all HIV infected patients attending the antiretroviral therapy (ART) clinics to start treatment must be screened for HBsAg (Meintjes et al., 2014). There are several anti-HIV agents approved for treating HBV, two of them being lamivudine (3TC) and tenofovir disoproxil fumarate (TDF) (Keeffe and Marcellin, 2007, Palumbo, 2008, United States Food and Drug Administration., 2017).Our anti-retroviral regimen at present is a fixed drug combination of Efavirenz/ Emtricitabine/ Tenofovir and is highly effective against both HBV and HIV. HIV-HBV co-infected individuals are by default effectively treated for both infections with the present antiretroviral regimen. Due to lack of screening in South Africa, HBV mono -infected individuals are usually not diagnosed and are often only identified when they present with complications of chronic liver disease. Routine screening, immunization and treatment for HBV and HIV may assist to achieve the World Health Organisation (WHO) goal of eliminating viral hepatitis as a major public health threat by year 2030.

Given that the WHO strategy is to eliminate HBV as a public health threat by the year 2030, (World Health Organisation., 2017, World Health Organisation., 2016) it is important to establish what the seroprevalence is through surveillance. The purpose of this study was to assess the HBV seroprevalence as part of a household survey in rural and periurban areas in KwaZulu-Natal, South Africa.

1.2 Aims and Objectives

1.2.1 Aim of the study

- To determine the prevalence and distribution of HBV infection markers in a population-based sample of men and women 15-49 years old in Vulindlela (rural) and Greater Edendale (periurban) KwaZulu-Natal, South Africa.

1.2.2 Objectives:

- To determine the prevalence of markers of active HBV infection (serum HBV surface antigen; HBsAg positive), active replication of virus (serum HBV envelope antigen; HBeAg positive), and recovery from infection (serum anti-HBe (HBeAb) positive) in the population-based sample of men and women 15-49 years old.
- To determine the association of prevalence and distribution of HBV by sex and age.
- To determine the prevalence and distribution of HBV-HIV co-infection.

1.3 Hypothesis:

We hypothesised that the prevalence of HBV is high in this rural and periurban communities and the imperative for improved surveillance to guide the scale up of HBV control programmes.

1.4 Significance of the study:

This study will inform current clinical screening recommendations and enhance our understanding of the distribution of HBV and HBV-HIV co-infection in the study sample.

CHAPTER 2

LITERATURE REVIEW

2.0 CHAPTER 2: LITERATURE REVIEW

2.1 Composition of hepatitis B virus structure

Hepatitis B infection is caused by the hepatitis B virus, a small DNA virus that belongs to the Hepadnaviridae family (Ott et al., 2012). The virus has features similar to retroviruses and humans are its only known host (Centres for Disease Control and Prevention., 2017). The complete infectious virion known as Dane particle, has a 42 nm (diameter) double shelled structure made up of surface proteins and lipids from the hepatocyte membrane, outer envelope of viral S proteins, the outer protein nucleocapsid (27nm) and an inner protein core (27nm), (Figure 2.1), (Dane et al., 1970). The outer protein nucleocapsid structure contains the hepatitis B surface antigen (HBsAg) made up of spheres and filaments (Gavilanes et al., 1982). The inner protein core contains the hepatitis B core antigen (HBcAg), a partially double stranded DNA molecule and a soluble non- nucleocapsid hepatitis B e antigen (HBeAg). The complete HBV genome is about 3.2 kilobase (kb) pairs sequence length (Gerlich and Robinson, 1980, Gitlin, 1997).

Although similar to HIV reverse transcriptase (RT) enzyme, the trademark HBV replication is protein-primed reverse transcription, the mechanism is distinct from that of HIV RT replication (Liang, 2009). HBV establish infection through attaching to the host cell membrane using the pre-S domain of the surface protein. Once the viral genome is inside the cell, the single stranded gap region in the viral genome is repaired and circularized by the *pol* protein of the virus to serve as a template for reverse transcription of several species of genomic RNAs (Kang et al., 2006, Kock and Schlicht, 1993). Replication of HBV starts with the encapsidation of the genome, followed by the reverse transcription of the pregenome RNA (pgRNA) to minus strand DNA, subsequently positive strand synthesis and several steps of strand transfer. After the replication steps, the immature viral particles migrate to the endoplasmic reticulum to be

assembled into mature viruses, which are the secreted into the blood stream (Will et al., 1987, Pollack and Ganem, 1993). Once the HBV infection has been established, the body produces antibodies to its antigens. The antibodies involved are Hepatitis B surface antibody (anti-HBs/HBsAb), Hepatitis B core antibody (anti-HBc/HBcAb) and Hepatitis B e antibody (anti-HBe/HBeAb). Multiple number of viral antigens and their corresponding antibodies can be detected in serum samples during or after HBV infection but the isolated measure of HBsAg in serum may not always indicate infectivity or viral replication since HBsAg can circulate in blood free of viral particles and is not infectious (Gitlin, 1997). HBV DNA levels determine the degree of replication as HBV-infected individuals can be HBsAg positive, HBeAg negative and actively replicating in the setting of precore/basal core promotor mutations.

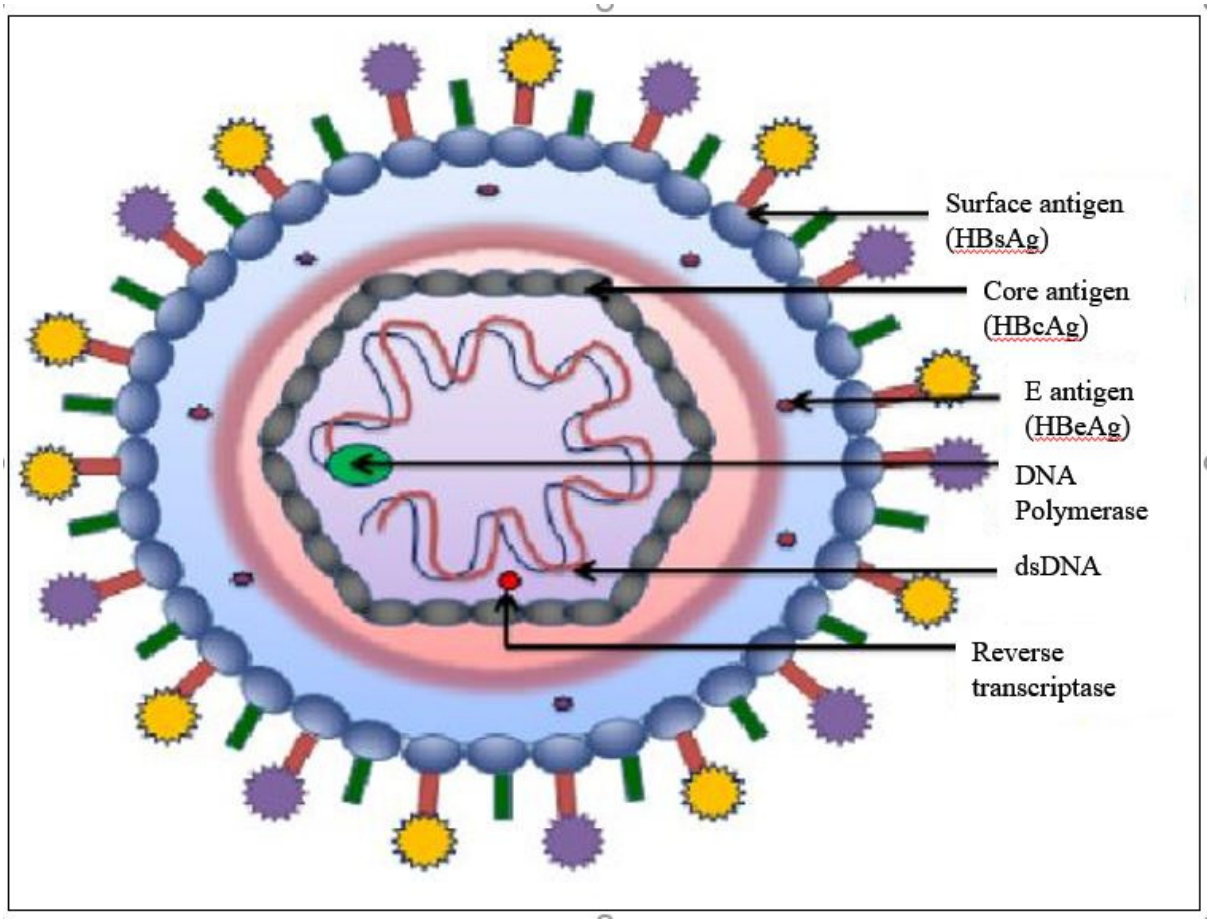


Figure 2.1. *Hepatitis B virion 42nm in diameter and an inner core 27nm in diameter which contains the partially double stranded DNA genome (Figure adapted from Jagtar Singh, Department of Biotechnology, Panjab University, Chandigarh, India.*

2.2 Natural history of Hepatitis and distribution of genotypes

Hepatitis or liver inflammation is the infection of the liver tissue caused by the hepatitis virus (WHO, 2016). Excessive alcohol intake, some medications or medical conditions, toxic substances, parasitic infections and autoimmune conditions are some of the factors also known to cause hepatitis (WHO, 2016). The condition is often acute, self-limiting and short lived but may progress as well. In some instances, the condition may persist and result in chronic hepatitis that can cause scarring of the liver, liver failure or liver cancer. Some individuals experiencing this condition may have no symptoms in early stages while others may present with yellow discolouration of the skin and eyes (jaundice), passing of dark urine, vomiting, nausea, loss of appetite, fatigue and abdominal pain (WHO, 2016).

There are 5 different types of hepatitis, namely A, B, C, D, and E. Hepatitis A and E can be acquired by the intake of contaminated food or water while hepatitis B can be contracted through contact with open sores, wounds, blood and other bodily fluids of an infected person, sharing of needles by injectable drug users, transfusion of infected blood products, or from mother to baby at birth (NIAID, 2016). There are nine key genotypes of hepatitis B (named A to I), which display a unique pattern of geographical distribution worldwide (Figure 2.2.). Hepatitis B genotypes A to H have long been accepted as individual genotypes while less characterised hepatitis I has recently been described (Kramvis et al., 2005, Kurbanov et al., 2008, Tran et al., 2008). In Africa, hepatitis A, D and E are the most common genotypes observed. Hepatitis A is highly prevalent in Southern and Eastern Africa while hepatitis B and C are mostly concentrated in Asia (Kramvis et al., 2005, Kramvis and Kew, 2007). Hepatitis A, D and G are associated with frequent injection drug use and blood transfusion, while B and C are linked to vertical transmission from mother to child, sexual intercourse, blood transfusion and sharing of needles (Krekulova et al., 2003, Liu and Kao, 2013, Komatsu et al., 2015,

NIAID, 2016). All hepatitis genotypes respond to treatment with reverse transcriptase inhibitors, and interferon alpha, but Hepatitis A, and B can be preventable by effective vaccines. Prevention of Hepatitis B by HBV vaccination prevents Hepatitis D acquisition (WHO, 2016).

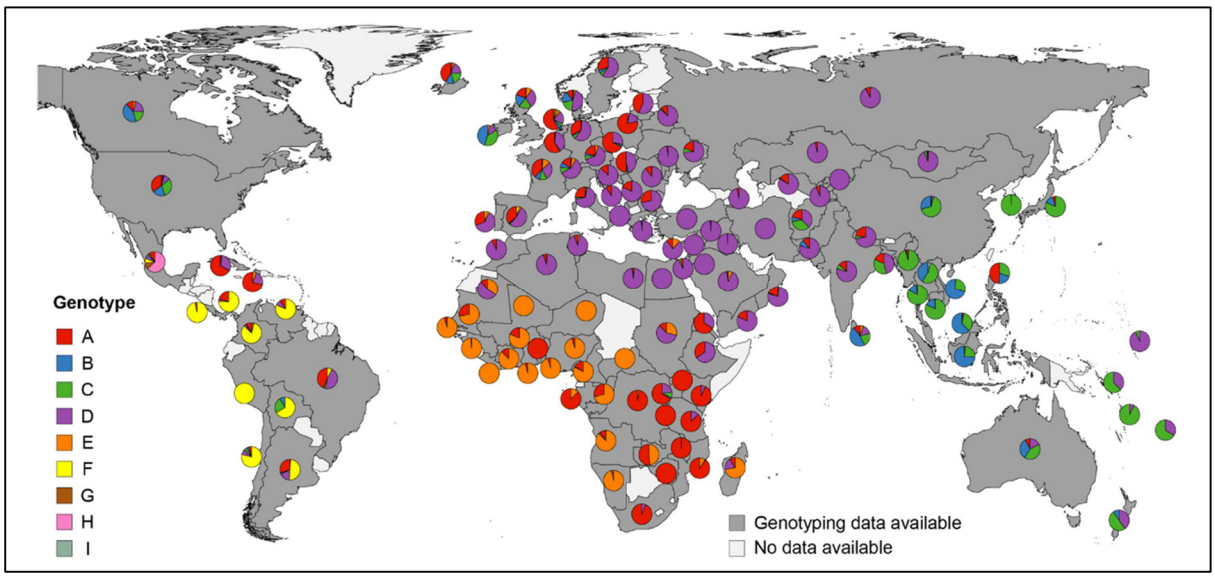


Figure 2.2. *Geographical distribution of hepatitis genotypes worldwide. Pie chart and colour represent proportion of the genotype distribution by country. (Figure adapted from (Velkov et al., 2018).*

2.3 Acute and chronic HBV infection

HBV infection is a self-limiting disease that can lead to a wide spectrum of liver disease which can clear within 6 months of acquisition. If acquired in adulthood and the individual is immunocompetent, <5% would develop chronic infection. However, in vertical transmission, 70-90% would develop chronic infection and 30-50% if acquired during childhood. HBV acquisition perinatally or in early childhood, which is common occurrence in South Africa is usually asymptomatic. If the infection is acquired in adolescents or during adulthood, an incubation period ranges from of 45 to 150 days (Gitlin, 1997), followed by evidence of HBV DNA and HBsAg in the serum. The serum of an infected patient would normally have increased levels of liver enzyme such as aspartate aminotransferase (AST) and alanine aminotransferase

(ALT) that are released during the episode of inflammation in the liver. During this time, infected individuals may show varied symptoms such as jaundice that's accompanied by fever, tiredness, nausea, vomiting and rash (Previsani, 2002). This infection may persist for a week or two, thereafter the patient recovers with HBsAg clearance from circulation but HBV DNA still detectable (Liang, 2009). During this period, antibodies to HBsAg, anti-HBs (HBsAb) can be measured serve as an indication of immunity to HBV infection (Shepard et al., 2006), (Figure 2.3, a).

If HBsAg positivity persists for more than 6 months the patient could be regarded as a chronically infected with HBV (Levy and Grant, 2006). In chronically HBV infected patients, HBsAg and HBeAg can be detected with high levels of HBV DNA (McMahon, 2009). Chronic HBV infection is progressive and has multiple phases that include immune tolerant phase, immune clearance phase, immune control phase, immune escape phase and inactive carrier phase (Figure 2.3, b). During the immune tolerant phase, the virus cannot be cleared by the host immunity and persist with high levels of viral load, high levels of HBeAg and HBsAg. However, ALT levels are normal with little or no symptoms and little evidence of impact on the liver (Merican et al., 2000). This phase is followed by the immune clearance phase, in which HBeAg clearance occurs and HBV DNA levels decrease but ALT levels increase with evidence of anti-HBe (HBeAb) antibodies. This phase can last a few months to years (Gitlin, 1997, Pan and Zhang, 2005). The following phase is the inactive HBsAg carrier phase (or the non-replicative).

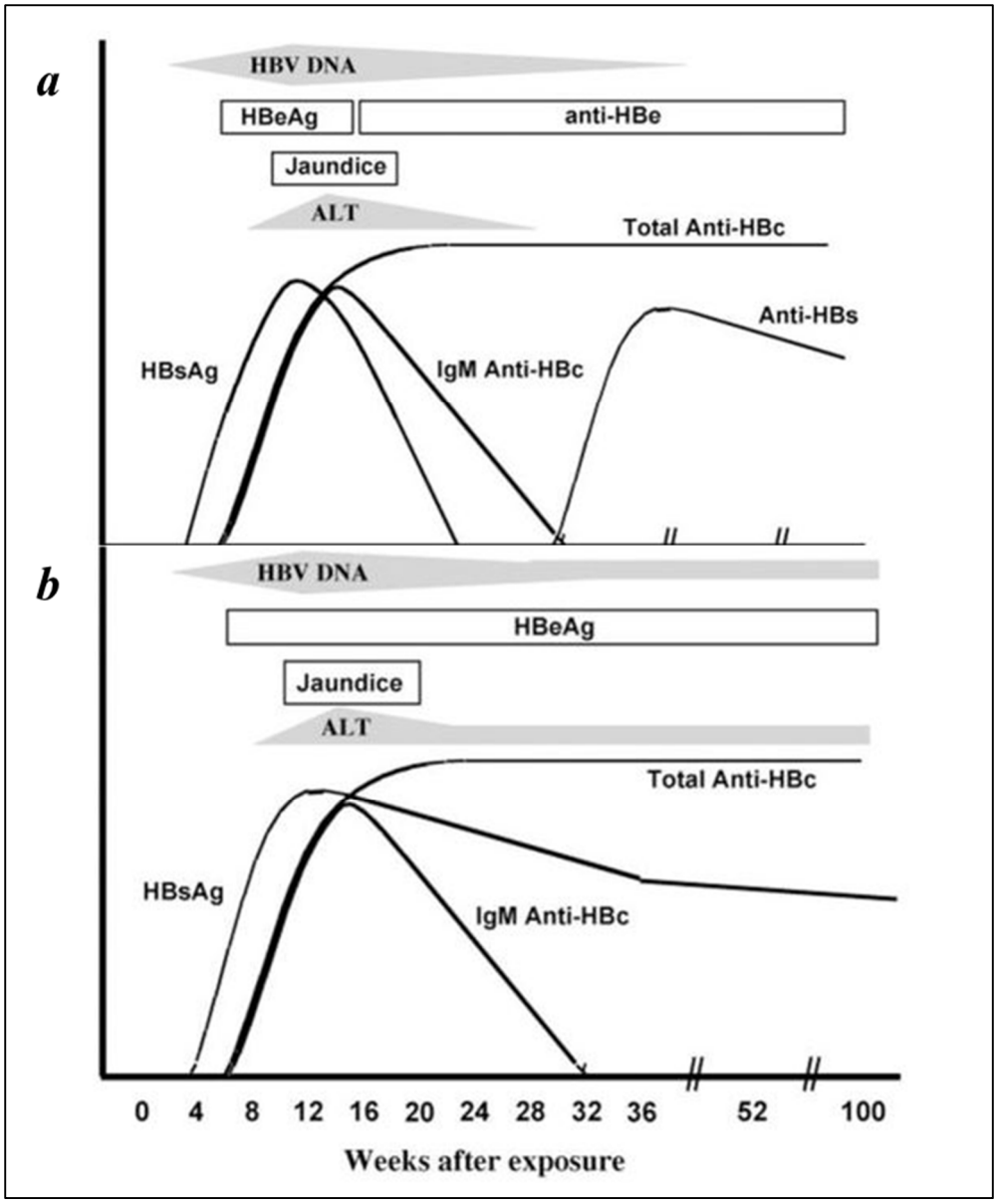


***Figure 2.3**. The clinical course and serological profiles of a) acute and b) chronic hepatitis B (Liang, 2009)*

phase) characterized by the loss of HBeAg and the presence of anti-HBe (HBeAb), persistently low levels of HBV DNA titers and normal ALT levels. In this phase, there is very little or no injury to the liver and can last for years or for life (Gitlin, 1997, Pan and Zhang, 2005, McMahon, 2009, Liang, 2009). In some cases, the patient clear HBsAg but later has reactivation due to immunosuppression of the patient with reversion to the HBeAg-positive phase or emergence of pre-core mutant strains of HBV which are unable to produce HBeAg

(Spearman et al., 2013). This phase is called the immune escape phase, with evidence of anti-HBs (HBsAb) and anti- HBc (HBcAb) but still has detectable HBV DNA with no liver damage observed (Spearman et al., 2013).

2.5 Diagnosis and serology of hepatitis B virus infections

The diagnosis of HBV infection is established on a collection of clinical, biochemical, histological, and serologic findings. At the onset of infection, HBsAg is produced in the liver and released into the blood and detection of this antigen is evidence of infection and can be detected using serological tests (Krajden et al., 2005). Table 2.1. shows HBV serological and viral markers used to establish HBV infection and staging of infection. These include HBsAg, HBeAg, anti-HBc (Immunoglobulin M -IgM), anti-HBc (IgG), anti-HBe, anti-HBc (IgG) and anti-HBs, anti- HBc (IgG) and HBsAg, anti-HBc (IgG) and/or anti-HBs and HBV DNA (PCR) (Krugman et al., 1979, Will et al., 1987). During acute infection, presence of HBV DNA is followed by HBsAg and HBeAg as first viral markers present in serum within 1 to 2 weeks or as late as 11 to 12 weeks after infection. The presence of HBeAg in the blood is an indication of high levels of HBV replication and infectivity while persistence of HBsAg is a marker of chronicity.

The viral markers can be accompanied by increased ALT, AST and jaundice (Krugman et al., 1979, Will et al., 1987). HBeAg is the first marker that is cleared at the peak of clinical illness while HBsAg and HBV DNA usually continue to be present in the serum until cleared with recovery. Clearance of HBeAg is usually accompanied by a rise in anti-HBe (HBeAb), a common serological antibody during acute hepatitis B. Although anti-HBe appears during the acute phase, it is not the first antibody during this stage (Tabor et al., 1981). IgM is the first antibody to appear during the acute stage and its appearance is triggered by the appearance of

anti-HBc (HBcAb), but this antibody decline as levels of IgG anti-HBc rise. The HBeAg negative reactivation phase is HBsAg positive, anti-HBs negative, anti-HBc positive, HBeAg negative and anti-HBe positive with HBV DNA levels >2000 IU/ ml. Anti-HBs tend to arise and persist during recovery or after clearance of HBsAg. Some individuals do not develop detectable levels of anti-HBs, but develop anti-HBc, a marker of previous infection. Consequently, in South Africa, anti-HBc is the most reliable and widely used marker for assessing previous infection and response to HBV vaccine (Tabor et al., 1981).

Table 2.1. *The serological tests used to establish hepatitis B infection and staging of infection (Liang, 2009)*

Test	***Interpretation***
HBsAg	HBV infection, both acute and chronic
HBeAg	High-level HBV replication and infectivity; marker for infectivity
HBV DNA	Level of HBV replication; primary virologic marker for treatment response
Anti-HBc (IgM)	Acute HBV infection; could be seen in flare of chronic hepatitis B
Anti-HBc (IgG)	Is the best marker of previous HBV exposure and is always positive in chronic HBV infection.
Anti-HBe	Indicates the individual has cleared HBeAg; marker for treatment response.
Anti-HBc (IgG) and anti-HBs	Past HBV infection; could lose anti-HBs
Anti- HBc (IgG) and HBsAg	Chronis HBV infection
Anti-HBc (IgG) and/or anti-HBs and HBV DNA (PCR)	Latent or occult HBV infection

2.6 Epidemiology of HBV with emphasis on Africa

Almost two billion people alive today have been infected with HBV globally, with 257 million infected cases and 887 000 deaths reported in 2015 (WHO, 2017). Eighteen percent of the global HBV infections are found in Africa, with the sub-Saharan Africa region accounting for 8% of the burden of disease. Zimbabwe has the highest prevalence (25%), followed by Mali (15.5%), Burkina Faso (14.5%), Ghana and Senegal (13.8%), Nigeria (13.6%), Mauritania (10.9%), Cameroon (10.1%), Gabon (9.5%) and Zambia (6.5%).

In South Africa, local epidemiological studies show varying prevalence of HBV infection according to the region and population under investigation. Several studies have shown that HBV infection in South Africa is more common in rural than in urban areas (Vos, Rose, &

Marimuthu, 1980; Di Bisceglie et al., 1986; WHO, 2008) and black South Africans represent a population most affected by HBV infection. Studies conducted prior to widespread immunization programmes in 1995 showed that the HBV prevalence was 10%, 18.5% and 25.1% among black children aged 6-14 years old from urban areas, rural areas and residential care facilities respectively (Abdool Karim et al., 1988). In addition, the prevalence was between 5.0% and 16.0% in rural males, 8.0% to 9.0% in urban males, 4.0% and 12.0% in rural females and 3.0% and 4.0% in urban females (Kew et al., 1976, Vos et al., 1980, Prozesky et al., 1983, Kew et al., 1987, Abdool Karim et al., 1988, Dusheiko et al., 1989). Although HBV infection occurs predominantly in the black population, a small community of Chinese residents of South Africa are also disproportionately affected. One study by Song and colleagues (1988) demonstrated that the HBV carrier rate among the South African Chinese population was 5.3%. Whites and Indians had a HBV carrier rate of 0.2% and an exposure rate of 5.0%, whilst whites with European origin had a carrier rate of 0.4-3.0% and a total exposure rate of 18.0-25.0%.

Despite the widespread implementation of HBV immunization, recent data indicate that HBV prevalence ranges between 0.5% and 13.2% in infants (Vardas et al., 1999, Tsebe et al., 2001, Mphahlele et al., 2002, Mdlalose et al., 2016), 7.0% and 10.0% in adults (Burnett et al., 2012, Matthews et al., 2015), and 0.0% and 5.3% in pregnant women (Thumbiran et al., 2014). In addition, recent studies showed a high prevalence of HBV infection (between 5.3% and 10.0%) in KwaZulu Natal compare to other South African provinces (Thumbiran et al., 2014, Matthews et al., 2015, Mdlalose et al., 2016). This is no surprise considering that the province of KwaZulu-Natal is the epicentre of HIV infection worldwide (Kharsany et al., 2018) and HBV share a common transmission route with HIV. Table 2.2 highlights the geographic, age and sex variation with South Africa.

Table 2.2: *Comparison of studies discussed in the literature review*

Author/Citation	Population	Location	Sample size (n)	HBV prevalence (%)
(Burnett et al., 2005)	General population	Rural population in South Africa	unknown	10.0%
(Kew, 1996b)	In hospital patients	Johannesburg	231	8.6%
(Kew et al., 1976)	adults	Gauteng	1050	0.2% White Diabetics 4.6% Black diabetics 1.4% White Hospital patients 4.3% Black hospital patients
(Dibisceglie et al., 1986)	Urban-children 3-19 year old	Soweto	2364	1.0%
(Kew et al., 1987)	Urban and rural population	Soweto	1234	1.3% urban; 4.0% rural
(Tsebe et al., 2001)	Under 5 year olds	Northern province	598	13.2%
(Prozesky et al., 1983)	Rural adult	Mpumalanga (formally Kangwane)	1495	14.6% in men; 4.6% in women
(Mphahlele et al., 2002)	Infants	Limpopo	186	0.0%
(Jooste et al., 2016)	<17 year olds	Kimberly	625	0.8%
(Vardas et al., 1999)	0-6 year old.	Eastern Cape	2299	10.4%
(Chotun et al., 2015)	infants	Western Cape	1000	0.4%
(Vos et al., 1980)	Black adults	Durban and Transkei	unknown	7.4% Durban; 15.5% Transkei
(Thumbiran et al., 2014)	Pregnant women	KwaZulu Natal	570	5.3%

(Mdlalose et al., 2016)	infants	KwaZulu Natal	322	10.0% overall (13.0% in HIV + and 7.5% in HIV-)
(Abdool Karim et al., 1989)	Adult men and women	Northern KwaZulu Natal	441	5.0%
(Abdool Karim et al., 1988)	School children	Mseleni	238	18.4%
(Dusheiko et al., 1989)	Mine workers	18 geographic locations in South Africa	29312	9.9%
(Matthews et al., 2015)	Adult women	South Africa	1022	9.7%

2.6.1 HBV-HIV co-infection

In Europe, sexual transmission among men who have sex with men is the most common mode of acquisition of HIV while heterosexual contact and injecting drug use were the main modes of transmission in eastern Europe. In Southern Africa the main mode of HIV acquisitions is via mother to child transmission at birth or via heterosexual transmission among adolescents and adults. Due to similar routes of transmission, co-infection with HBV and HIV is common (Soriano, et al., 2007). In addition, HIV coinfection is known to impact the course of HBV infection. HIV-HBV coinfection has a potential to increase the risk of perinatal HBV transmission and is linked to a more aggressive disease course of chronic hepatitis B (Puoti et al., 2006, Hoffmann and Thio, 2007, Sangare et al., 2009, Kourtis et al., 2012). Chronicity of HBV is dependent on the age of acquisition. A person coinfected with HBV-HIV and not on treatment are likely to become chronically infected. Co-acquisition of HBV and HIV in adulthood may results in an increased probability of progressing to fulminant liver failure. This is indicated by HBV replicating to high levels, resulting in of cirrhosis and HBV-induced hepatocellular carcinoma (Thio et al., 2002, Levy and Grant, 2006, Puoti et al., 2006). In areas with low HIV prevalence, chronic HBV prevalence is ten times higher in people living with HIV (Thio et al., 2002, Soriano et al., 2005, Levy and Grant, 2006).

HBV-HIV co-infected patients have also been observed to have a higher prevalence of HBeAg, which is the indicator of infectivity, higher HBV DNA titers and more occult HBV infections (Gilson et al., 1997, Colin et al., 1999, Puoti et al., 2006). It has also been reported that co-infected patients are more unlikely to produce anti-HBs (HBsAb) after HBV vaccination (Shire et al., 2006, Kim et al., 2009). In HBV-HIV coinfection patients, there is a 3- fold increase in observing liver related mortality compared to patients who were mono- infected with either disease (Bonacini et al., 2004, Weber et al., 2006). However, there is no evidence that active

HBV disease may create an immune active environment and enhance HIV disease progression (Soriano et al., 2018). High prevalence of HIV and HBV co-infection also has implications for treatment (Rouet et al., 2004) and could affect the choice of treatment as some of the antiretroviral agents are active against both HIV and HBV.

2.6.2 HBV-HIV co-infection prevalence

Approximately 3.3 million (10%) people infected with HIV are also co-infected with HBV globally and 2.6 million of these reside in sub-Saharan Africa. West and Southern Africa are the most affected African regions, with up to 36% of chronic HIV-HBV coinfection in 2014 (Matthews et al., 2014)). Many African countries report high HBV-HIV co-infection rates with prevalence ranging from 4.0% to 28.0% (Figure 2.4.) (Barth et al., 2010). Nigeria has the highest prevalence of HIV-HBV coinfection (ranging between 10% and 70%) and Zambia has the lowest prevalence (7%) compared to other African countries (Barth et al., 2010).

In South Africa, HIV and HBV co-infection rates range from 0.4% to 22.9% (Lukhwareni, et al., 2009; Barth, et al., 2011), with 3.1% found in pregnant women in KwaZulu Natal, 4.8% in urban cohorts and 22.9% in a cohort in Limpopo (Firnhaber, 2008, Firnhaber et al., 2009, Lukhwareni et al., 2009, Thumbiran et al., 2014). Another study of 100 consecutive AIDS patients in Johannesburg, South Africa, showed that 6% of patients were co-infected with HBV, and 35% had markers of previous exposure to HBV (Lodenyo, Schoub, Ally, Kairu, & Segal, 2000).

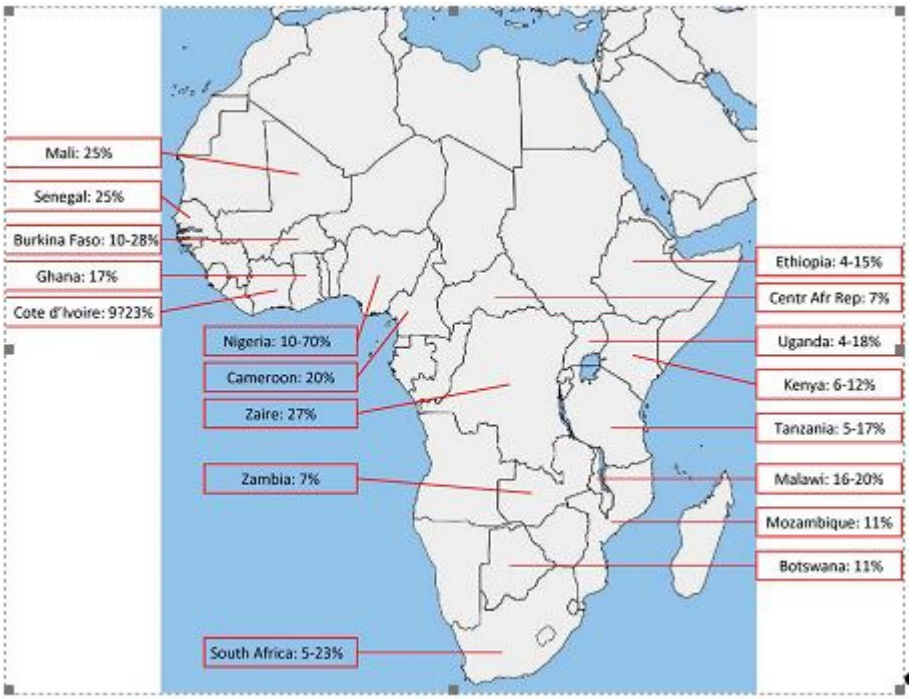


Figure 2.4. *HBV-HIV co-infection prevalence in selected African countries (Barth et al., 2010)*

2.7 Treatment of HBV and HIV infections

In most instances acute hepatitis infection is self-resolving but if the infection worsens or chronic hepatitis infection is suspected, treatment is required to reduce the risk of liver cirrhosis and liver cancer development. Therapies like tenofovir can also be used for the treatment of HBV infection (Lok and McMahon, 2007) as first line therapy. Treatment of chronic hepatitis mono-infection depends on the phase of the chronic infection or if the individual requires immunosuppressive therapy. The HBV infection can be successfully treated with lamivudine which acts on the reverse transcriptase path of the virus replication (Gitlin, 1997). Lamivudine should not be used except in certain clinical situations such as fulminant liver failure as it has low genetic barrier to resistance

In South Africa, current ART guidelines recommend that HIV-infected individuals being initiated on ART treatment, should be tested for HBV as antivirals can lead to potentially life-threatening flares of hepatitis B if HIV treatment is stopped. In addition, patients coinfected

with HIV-HBV should be treated with antiretroviral therapy (ART) that is active against both HBV and HIV. Emtricitabine (FTC), lamivudine (3TC), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF) are all active against HBV and HIV. Due to its superior resistance profile, a first-line ART regimen that contains tenofovir is recommended for treating HIV-HBV co-infection individuals (Hamers et al., 2013) combined with emtricitabine and lamivudine, which have excellent safety profiles. A fixed-dose combination (FDC) antiretroviral (ARV) regimen consisting of tenofovir (TDF), emtricitabine (FTC) and efavirenz (EFV) is currently recommended for all HIV-infected individuals in South Africa (Department of Health, 2013).

2.8 Hepatitis immunization programme in South Africa

With the use of the HBV vaccine in most countries, there is substantial evidence of the impact on the reduction of the HBV prevalence globally. Taiwan, the first country to initiate a HBV vaccine programme in 1984, reduced HBsAg prevalence to 0.7% in children younger than 15 years by 1999 (Ni et al., 2001, Hou et al., 2005). In 1995, South Africa became one of the first countries in Africa to introduce the HBV vaccine as part of the National Expanded Programme of Immunisation (EPI) (Tsebe et al., 2001). The hexavalent vaccine is given to infants at 6, 10 and 14 weeks of age and again at 18 months (Tsebe et al., 2001) and the dose is according to age. The monovalent vaccine given at birth or as part of the adult catch up program is a genetically engineered recombinant HB vaccine that stimulates the immune response to produce antibodies against the disease (Department of Health, 2009). Numerous studies have shown that the HBV vaccine is highly immunogenic and effective (Burnett et al., 2012). Since its introduction, it has resulted in robust immunity against HBV among South Africans aged 1-25 years old (Burnett et al., 2012). The HBV vaccine has led to an increase in anti-HBs (HBsAb) from 13% to 57% and steady decline in HBsAg carriage from 4.2% to 1.4% in the

post-vaccine era (Amponsah-Dacosta et al., 2014). However, there is no catch-up vaccination programme for older children and adults to ensure complete vaccination coverage (Tsebe et al., 2001). Between 1995 and 1999, 31.0% of mothers of 8 to 72 month–old children in South Africa had evidence of current or past exposure to HBV (Tsebe et al., 2001). A recent study showed a 10% HBV prevalence in infants attending one of the KwaZulu Natal hospitals (Mdlalose et al., 2016). It was also shown that only 10.0% of black African babies were receiving the vaccine (Kew, 2008b). There could be reasons for the low coverage amongst which are difficulty in accessing the vaccine and dealing with low supply chain issues, financial constraints of individuals preventing accessing the vaccine, other illnesses including HIV, AIDS and TB, political issues, other communicable diseases being prioritized by government, the lack of understanding as to the extent of the HBV epidemic in South Africa (Kew, 2008b).

CHAPTER 3

MANUSCRIPT

3.0 CHAPTER 3: MANUSCRIPT

Submitted to International Journal of Epidemiology

Seroprevalence of hepatitis B virus: a population-based household study in KwaZulu Natal, South Africa

Authors: Natasha Samsunder[1], Sinaye Ngcapu[1], Lara Lewis[1], Cheryl Baxter[1], Cherie Cawood [2], David Khanyile [2], Ayesha BM Kharsany[1] *

[1]Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa Durban, South Africa

[2]Epicentre AIDs Risk Management (Pty) Limited, Cape Town, South Africa.

Corresponding Author: Ayesha BM Kharsany, Centre for the AIDS Programme of Research in South Africa (CAPRISA), 2nd Floor, Doris Duke Medical Research Institute, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road (Private Bag X7), Congella, 4013 Durban, South Africa. Phone: +27 31 260 4969, Fax: +27 31 260 4566, Email: Ayesha.kharsany@caprisa.org

Keywords: hepatitis B virus (HBV) prevalence, HBsAg, HBeAg, anti-HBe, HBV-HIV co-infection, South Africa

Running title: Seroprevalence of hepatitis B virus (HBV)

Word count: Abstract: 318; Main text :3250

Key Messages

- Hepatitis B virus (HBV) infection in some populations has not been well described and remains an important cause of morbidity and mortality.
- Surveillance for hepatitis B virus surface antigen (HBsAg), a marker of HBV infection, is an important measure of the burden of infection.
- Hepatitis B virus nucleocapsid antigen (HBeAg), is an important marker of acute infection and identifies individuals who have high levels of virus and are at high risk of transmitting the HBV; therefore, an important measure of sustained transmission.
- Scale up of HBV prevention programmes to include immunisation and treatment is an important missed opportunity to reduce the spread of HBV

Research in context

Evidence before this study

Despite the availability of a highly effective vaccine in South Africa, hepatitis B virus (HBV) infection is an important cause of morbidity and mortality. Apart from studies on seroprevalence of hepatitis B surface antigen (HBsAg), a marker of HBV infection, among pregnant women attending public sector facilities, there are no large scale population-based studies that provide reliable estimates on the HBV burden in the general population.

Added value of this study

This study determined the seroprevalence of HBV infection in the general population HBsAg, HBeAg and anti-HBe markers were measured among 9791 men and women 15-49 years old in rural and periurban areas of the uMgungundlovu district in KwaZulu-Natal, South Africa. Thus, we provide critical data on the population seroprevalence of HBV infection to guide the strategic prioritisation of HBV immunisation and treatment programmes.

Implications of all the available evidence

The overall seroprevalence of HBsAg was 4.0%; 4.8% in men and 3.2% in women. Furthermore, an estimated 35.1% of HBsAg positive participants were HBeAg positive; 36.9% of men and 32.6% of women. These findings highlight an important missed opportunity to establish HBV surveillance, testing for linkage to immunisation or treatment programmes for men and women in this community to achieve the World Health Organization's (WHO) goal of eliminating viral hepatitis as a major public health threat by year 2030.

Abstract

Background: In South Africa, hepatitis B virus (HBV) infection remains a major cause of morbidity and mortality, however, little is known about the prevalence and distribution of HBV in some regions and populations.

Methods: This secondary analysis is based on 9791 participants (15-49 years old) enrolled in the HIV incidence Provincial Surveillance System (HIPSS); a population-based household study undertaken from June 2014 to June 2015 in the Vulindlela (rural) and Greater Edendale (periurban) areas of the uMgungundlovu district, KwaZulu-Natal (KZN), South Africa. Interviewer administered questionnaires were completed to obtain demographic, psycho-social, behavioural and clinical information. Peripheral blood samples were collected and sera were tested for hepatitis B surface antigen (HBsAg) and all samples testing positive were further tested for hepatitis B e antigen (HBeAg) and hepatitis B e antibody (anti-HBe). The estimated weighted seroprevalence of HBV markers was calculated and the association of HBsAg with sociodemographic and behavioural factors measured.

Results: The overall HBsAg prevalence was 4.0% (95% confidence interval (CI) 3.4-4.5); 4.8% (95% CI 3.8-5.8) in men and 3.2% (95% CI 2.5-3.9) in women, P=0.01. Among HBsAg positive participants, 35.2% (95% CI 29.2-41.2) were HBeAg positive and 66.3% (95% CI 60.1-72.4) were anti-HBe positive. Among men 15-19 years old HBeAg seroprevalence was 92.2% (95% CI 75.8-100) compared to 4.4% (95% CI 0-13.7) in women in the same age group; P<0.01. HBsAg prevalence was 6.4% (95% CI 5.3-7.5) among HIV positive participants compared to 2.6% (95% CI 1.9-3.2) among HIV negative participants, (P<0.01) and was higher among HIV positive men 8.7% (95% CI 6.3-11.2) compared to HIV positive women 5.0% (95% CI 3.8-6.2), P<0.01.

Conclusion: HBV infection, particularly among HIV positive men remains an important public health problem in rural and periurban communities in KwaZulu-Natal, South Africa. The prevalence of HBsAg and HBeAg highlight the importance of surveillance and an important missed opportunity for the scale up of programmes to achieve the goal of controlling HBV for public health benefit.

Introduction

Hepatitis B virus (HBV) infection is a common cause of viral hepatitis and affects more than 257 million people worldwide. Almost eight percent of this global burden is in sub-Saharan Africa, with over 80 000 new infections occurring each year (World Health Organisation., 2017). In South Africa, HBV infections accounts for an estimated 3.5 million infected individuals (Schweitzer et al., 2015). HBV remains endemic in the region. Viral hepatitis from HBV infections is a potentially life-threathening liver infection resulting in acute hepatitis and chronic liver disease, leading to complications of cirrhosis and hepatocellular carcinoma; contributing to high rates of morbidity and mortality (World Health Organisation., 2017).

The transmission of HBV is known to occur through multiple routes. The most common being mother-to-child at birth or through exposure to infected blood (Kiire, 1996). Other routes of transmission include sexual transmission, percutaneous or mucosal exposure to infected blood and various body fluids. Transmission can also occur through saliva, menstrual, vaginal, and seminal fluids, and iatrogenic spread through re-use of contaminated needles. Equipment in health-care settings, or among persons who inject drugs and use of razors or similar objects during ritual scarification may be routes of transmission (Kew, 1996a, Mphahlele et al., 2002, Heiberg et al., 2010).

South Africa has been in the forefront of introducing new vaccines and as early as 1995, the country introduced the HBV vaccine as part of the Expanded Programme on Immunisation. By 2015 South Africa had become the first African country to phase out the pentavalent vaccine and replaced it with the more baby-friendly hexavalent vaccine (Dlamini and Maja, 2016). Despite the introduction of these vaccines, there are no nation-wide surveillance programmes that measure and monitor trends in HBV (Mayaphi et al., 2012). Thus, data on burden of HBV including clinical manifestation of advancing disease are limited and are based on cohort studies undertaken more than a decade ago. Prior to the immunisation programme, approximately 6.5 million South Africans were HBV carriers, with prevalence being highest among school-going children, people residing in rural areas and among sexually active individuals (Schneider et al., 1977, Botha et al., 1984, Dibisceglie et al., 1986, Burnett et al., 2005). The prevalence of HBV infection in rural areas was estimated to be between 5% and 16% among men and 4% and 12% among women, while in urban areas, rates were between 8% and 9% among men and 3% and 4% among women (Kew et al., 1976, Vos et al., 1980, Prozesky et al., 1983, Kew et al., 1987, Abdool Karim et al., 1988, Dusheiko et al., 1989).

Currently, there are an estimated 3 to 4 million hepatitis B carriers in South Africa (Kew, 2008a).

Given that the World Health Organisation strategy is to eliminate HBV as a public health threat by the year 2030, (Perz et al., 2006, World Health Organisation., 2016, World Health Organisation., 2017) it is important to establish what the seroprevalence is through surveillance. The purpose of this study was to assess the HBV seroprevalence as part of a household survey in rural and periurban areas in KwaZulu-Natal, South Africa.

Materials and Method

Study design, setting and population

This secondary analysis is based on testing of samples from participants enrolled in the HIV incidence Provincial Surveillance System (HIPSS). The HIPSS study design, source population, recruitment and enrollment procedures have been previously described (Kharsany et al., 2015, Kharsany et al., 2018). Briefly, HIPSS was a population-based household study conducted in the Vulindlela (rural) and the Greater Edendale (periurban) areas in the uMgungundlovu district of KwaZulu-Natal, South Africa. The study used a multi-stage cluster sampling method to randomly select enumeration areas, households, and individuals, resulting in a total of 9812 men and women (15-49 years old) enrolled between June 2014 and June 2015. All enrolled participants provided written informed consent and/or parental consent/child assent for those participants below the age of 18 years for study participation and for long-term storage of clinical samples for future testing; completed interviewer administered questionnaires; had peripheral blood samples collected and allocated a unique identification number with a linking number to link the household, participants' questionnaire and laboratory data.

The Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal reviewed and approved the HBV seroprevalence protocol (BE057/17), the HIPSS study protocol, informed consent and questionnaire forms (BF269/13). The Centers for Disease Control and Prevention from the United States of America and the Provincial Department of Health (KwaZulu- Natal (HRKM 08/14) approved the HIPSS study protocol. All participants were referred to their local primary health care clinics to access their study related laboratory test results and for individualised HIV testing services. Prior to any interaction with household members and participants, community engagement processes were established with key

community stakeholders including health-service providers and traditional leadership and key members from community structures.

Laboratory tests

Peripheral blood samples were shipped to the laboratory within six hours of collection. Of the 9812 participants enrolled in HIPSS, blood samples from 21 (0.2%) were insufficient for any HBV testing and were excluded

Testing for HBV

For the detection of markers of HBV infection, testing was performed using the automated ADVIA Centaur System (Siemens, Tarry Town, USA) which utilizes magnetic particle separation technology with direct chemiluminescence. The HBV assay panel included the qualitative detection of HBsAg, HBeAg and anti-HBe. HBsAg positive samples were further tested for HBeAg and anti-HBe. All results were reported as positive or negative. The diagnostic specificity are reported to be 99.4% for the HBsAg assay, 99.5% for the HBeAg assay and 98.2% for the anti-HBe assay (van Helden et al., 2004, van Helden et al., 2008).

Interpretation of HBV test results

In this study we defined HBV status (North Carolina Hepatitis B Public Health Program Manual., 2012) as:

HBsAg positive: the presence of HBsAg in serum indicates that the individual has a current infection.

HBeAg positive: the presence of HBV nucleocapsid gene (HBeAg) in serum indicates active viral replication in individuals with acute or recently acquired infection, have high levels of viraemia and more likely to transmit the virus.

Anti-HBe positive: the presence of antibodies to HBV nucleocapsid gene (HBeAb) indicates that the individual is recovering as a result of an immune system response to the HBeAg and has a reduced level of infectivity.

HBV and HIV positive: this indicates that the individual is co-infected with HBV (HBsAg positive) and HIV-1 infection.

Testing for HIV

HIV testing was performed using the 4th generation HIV enzyme Biomeriuex Vironostika Uniform 11 Antigen/Antibody Microelisa system (BioMerieux, Marcy I ‘Etoile, France) and

HIV 1/2 Combi Roche Elecys (Roche Diagnostics, Penzberg, Germany). Samples testing indeterminate for HIV were further tested using ADVIA Centaur HIV Antigen/Antibody Combo (CHIV) Assay (Siemens, Tarry Town, USA).

Statistical Analysis

Participants laboratory and questionnaire data were merged using a unique linking number. All analyses were performed using SAS 9.4 (SAS Institute Inc. Cary, NC) survey procedures. Sampling weights were calculated taking into account the probabilities of selecting the enumeration area, the household in the enumeration area, and the individual in the household, weighted for sampling, participation bias, and non-response; and rescaled to the size of the population in the survey area with the StatsSA 2011 Census population (Kharsany et al., 2018). Descriptive statistics with counts and population-weighted percentages with 95% CIs are presented for key demographic, psychosocial and behavioural factors. Taylor series linearization method was used to estimate standard errors of estimates, from which Wald confidence limits were derived. The Rao-Scott Chi-Square test is used to test for the association between prevalence and sociodemographic, behavioural and clinical characteristics.

Results

Demographic characteristics of the study participants

Among the 9791 participants tested for HBV infection, the median age of the 3541 (36.2%) men was 27 years [Interquartile range (IQR) 21-35 years] and of the 6250 (63.8%) women was 28 years (IQR 21-37). The majority of participants were single (92.1% of men and 85.8% of women), reported having 1 to 5 lifetime number of sexual partners (39.0% men and 51.3% women), and lived in rural areas (57.3% men and 58.7% women). In addition, prevalence of HIV (28.0% men and 44.0% women) and sexually transmitted infections (STIs) (52.3% men and 78.3% women) was high. Over a third of participants had incomplete secondary education (38.1% of men and 41.3% of women) and earned a monthly income of between South African Rand (ZAR) 501-ZAR2500 (44.5% men and 46.3% women). Overall, consistent condom use during sex in the last 12 months was low (Table 3.1).

Seroprevalence of HBsAg

Among the 9791 participants, 361 were HBsAg positive with an overall weighted seroprevalence of 4.0% (95% confidence interval (CI) 3.4-4.5) (Table 3.2). Seroprevalence in women was 3.2% (95% CI 2.5-3.9) and was higher in men at 4.8% (95% CI 3.8-5.8); P=0.01.

Seroprevalence varied with age among men (P <0.01) and women (P <0.01). Seroprevalence was 1.1% (95% CI 0-2.3) among men in the 15-19 year old age group and steadily increased to reach a peak of 9.6 % (95% CI 5.0-14.1) in the 40-44 year old age group. In contrast, among women seroprevalence was consistently lower in each of the age groups and was 0.9% (95% CI 0-1.8) among women 15-19 years old and peaked at 6.0% (95% CI 3.1-8.9) in the 30-34 year old age group. Seroprevalence increased with an increasing number of lifetime sex partners in women (P <0.01), with HIV positive status among men (P <0.01) and women (P <0.01) and with positive STIs (other than HIV) among men (P<0.01). There were no differences in seroprevalence among rural and periurban men (P =0.69) and rural and periurban women (P =0.92).

Seroprevalence of HBeAg

Of the 361 HBsAg positive samples, 357 had sufficient sample for HBeAg testing. Of these, 133 were HBeAg positive with an overall weighted seroprevalence of 35.2% (95% CI 29.2-41.2) (Table 3.3). HBeAg seroprevalence in men was 36.9% (95% CI 27.5-46.2) and in women was 32.8% (95% CI 32.3-41.9); P=0.58. Among men 15-19 years old HBeAg seroprevalence was 92.2% (95% CI 75.8-100) compared to 4.4% (95% CI 0-13.7) in women in the same age group; P <0.01. Similarly, HBeAg seroprevalence was higher among men aged 40-44 years old 55.5% (95% CI 30.6-80.4) compared to 27.7% (95% CI 10.4-45.0) in women in the same group. However, seroprevalence of HBeAg among men and women across the other age groups was similar. There were no differences in seroprevalence of HBeAg among men in rural 34.0% (95% CI 24.0-44.0) and periurban areas 38.9% (95% CI 24.6-53.2), P = 0.58, or women in rural 36.7% (95% CI 26.9-45.9) and periurban areas 30.0% (95% CI 16.1-43.9), P=0.43.

Seroprevalence of anti-HBe

Of the 361 HBsAg positive samples, 356 had sufficient sample for anti-HBe testing. Of these, 237 samples were positive for anti-HBe with an overall weighted seroprevalence of 66.3% (95% CI 60.1-72.4) (Table 3.3). Seroprevalence in men was 64.5% (95% CI 54.7-74.3) and was similar in women at 68.8% (95% CI 59.3-78.3), P = 0.51. Anti-HBe seroprevalence was high among men and women across most age groups. More women than men in the age group 15-19 years old were anti-HBe positive 81.0% (95% CI 45.5-100) vs 7.8% (95% CI 0-24.2), P <0.01. Similarly, among women in the age group 40-44 years old seroprevalence was 76.8% (95% CI 60.9-92.7) compared to 47.1% (95% CI 21.6-72.6) in men in the same age group, P=0.03. There were no differences in seroprevalence of anti-HBe among men in rural (61.4%,

95% CI 46.7-76.1) and periurban areas (68.9%, 95% CI 57.4-80.3), *P*=0.43 and among women in rural (69.3%, 95% CI 54.4-84.2) and periurban areas (68.1%, 95% CI 58.6-77.6), *P*=0.89.

HBV-HIV co-infection

Of the 3955 HIV positive participants, 229 were also infected with HBV, of which 91were men and 138 were women. HBV seroprevalence was 6.4% (95% CI 5.3-7.5) among HIV positive participants compared to 2.6% (95% CI 1.9-3.2) among HIV negative (n=5836) participants; *P*<0.01. Among HIV positive men, seroprevalence of HBsAg was 8.8% (95% CI 6.3-11.2) compared to 3.3% (95% CI 2.3-4.3) among HIV negative men; *P*<0.01. Among HIV positive women, seroprevalence of HBsAg was 5.1% (95% CI 3.8-6.3) compared to 1.7% (95% CI 1-2.3) among HIV negative women; *P*<0.01. Differences in HBsAg prevalence among HIV positive and negative individuals was greatest in age groups 20-24 and 25-29. Overall, seroprevalence of HBsAg was higher among HIV positive participants in the 20-24 and 25-29 years old age groups, *P*<0.01 (Figure 3.1). Among HIV positive men in the age group 20-24 years old HBsAg seroprevalence was 11% (95% CI 2.8-19.2) compared to 2.7 % (95% CI 1.0-4.5) among HIV negative men in the same age group; *P*<0.01. Among HIV positive women in the age group 20-24 years old, HBsAg seroprevalence was 6.2% (95% CI 2.8-9.7) compared to 1.0% (95% CI 0.3-1.6) among HIV negative women in the same age group; *P*<0.01. Similarly, among HIV positive women in the 25-29 year old age group HBsAg seroprevalence was 6.4% (95% CI 3.3-9.5) compared to 1.3% (95% CI 0.4-2.1) among HIV negative women in the same age group; *P*<0.01.

Discussion

In this population-based study in the rural and periurban areas of the uMgungundlovu district of KwaZulu-Natal, South Africa, the overall HBsAg seroprevalence among men and women in the age group 15-49 years old was 4.0%, with a seroprevalence of 4.8% in men and 3.2% in women. While lower than the HBsAg seroprevalence of 6.1% reported for sub-Saharan Africa, (Spearman et al., 2017) and between 5.3% and 9.7% in recent cohort studies among pregnant women (Thumbiran et al., 2014, Matthews et al., 2015), this still represents a substantial HBV burden. South Africa has been reported to be at the forefront on the implementation of the Expanded Programme on Immunisation which includes HBV immunisation, (Dlamini and Maja, 2016) however, the prevalence of HBV infection remains moderate to high, especially in the age group 15-19 years old as this age group should have benefitted from the immunisation programme. In the absence of an active HBV surveillance programme it is

unlikely that HBV immunisation and treatment programmes are easily accessible and reach individuals where the needs are the greatest. These findings emphasise that HBV remains an important public health problem and all efforts to achieve the WHO targets for the elimination of viral hepatitis are urgently needed (World Health Organisation., 2016). WHO in 2016 set ambitious targets of a 90% reduction in new cases of HBV infection and an associated 65% decline in HBV related mortality to be achieved by the year 2030. These measures rely on 80% of treatment eligible individuals with chronic HBV infection to be on treatment (World Health Organisation., 2016).

Whilst vertical transmission of HBV is the commonest route of transmission, the age specific HBV data highlighted several important findings. Firstly, men had higher seroprevalence compared to women in every age group. Secondly, the data showed a bimodal distribution of seroprevalence of HBsAg among men in the 30-34 and 40-44 year old age groups whilst in women, peak seroprevalence occurred in the 30-34 year old age group. These findings show that the peak prevalence occurred among men and women in the age groups that are likely to sustain the transmission of HBV. Thirdly, overall estimates are useful to understand the burden of HBV infection in the general population, however, disaggregation by sex and age provides a more precise understanding of the age group that might be affected and allows for prioritisation and targeted interventions to further reduce this burden. Several studies have shown urban-rural differences with the highest prevalence of HBsAg infection found in men residing in rural areas, placing a greater burden on rural health care services (Vos et al., 1980, Abdool Karim et al., 1988, Abdool Karim et al., 1991, Lodenyo et al., 2000, Hoffmann et al., 2008, Firnhaber et al., 2008, Di Bisceglie et al., 2010). However, this study found no differences in the seroprevalence of HBsAg among men and women, irrespective of their area of residence being rural or periurban. It is possible that extensive in- and out- migration in the study community and cultural practices of scarification (Abdool Karim et al., 1988) may not clearly delineate urban-rural differences.

This study found that at least a third of men (36.9%) and women (32.6%) were HBeAg positive and therefore more likely to be infectious. These prevalence values are higher than the 16.7% -30.0% observed in cohorts of pregnant women in South Africa (Thumbiran et al., 2014). Whilst men in the 15 to 19 year age group and women in the 20 to 24 year old age groups had low seroprevalence of HBsAg, more than 90% of these men and about 60% of these women were positive for HBeAg, suggesting that these individuals were in the acute stage of infection and have a high HBV viral load (Spearman et al., 2017) and therefore highly infectious. The high prevalence of HBeAg among both men and women highlights the sustained transmission

in this community. Reaching out to men and women at high risk of transmitting HBV with messaging on immunisation and treatment options could further guide and scale-up targeted programming and strategies to reduce the further spread of HBV and the opportunity for a catch-up immunisation programme targeting young boys.

An overall seroprevalence of HBsAg of 6.4% among HIV positive individuals was observed, with a higher seroprevalence amongst HIV-positive men (8.8%) compared to HIV-positive women (5.1%). Since, HBV-HIV share similar routes of transmission, co-infections are likely to be common and are reported to range from 5% to 30% (Abdool Karim et al., 1991). Although co-infections were low at 0.8% in the 15 to 19 year old age group, co-infections were high in all other age groups, ranging from 6.4% to 11.9%. Identifying co-infections is important as HBV infections are associated with an increased risk of liver disease. ART use in HIV positive individuals may be compromised by drug associated hepatotoxicity leading to the use of complex regimens (Di Bisceglie et al., 2010). In addition, our study showed that among women, HBV-HIV co-infections were above six percent in age groups that women are in their reproductive years and are already disproportionately burdened with HIV, (Kharsany et al., 2015). This could potentially increase the risk of HBV and HIV transmission. Expanding treatment for HBV and HIV positive individuals could limit onwards transmission of both infections. Even initiating ART in the absence of HBV, reduces the risk of new HBV cases and reduces the risk of mother to child transmission of HBV (Di Bisceglie et al., 2010). Therefore, public sector ART and antenatal care programmes are missed opportunities to screen for HBV at the time of testing for HIV and when initiating ART. These individuals would benefit from immunisation if HBV negative or if positive to receive treatment to prevent the onward and mother-to-child transmission of HBV (Cui et al., 2018).

This study has several strengths and limitations. A key strength was our measurement of HBV seroprevalence in a large randomly selected study sample from a population-based household survey. Furthermore, the sex and age disaggregated data provides a nuanced understanding of the segments of population that might be affected, though our sample was not representative of all age groups. Our results are from cross sectional data and are limited to the Greater Edendale and Vulindela areas of KwaZulu-Natal, South Africa and not necessarily generalizable to other settings. It is important to note that this study was not able to fully assess the impact of HBV immunisation programme on seroprevalence of HBV markers, as the benefit would be in the<15 year old age groups rather than those included in our sample (Tsebe et al., 2001). Our testing strategy did not include the full HBV testing panel to comprehensively classify individuals; for example, we did not test for occult HBV infection, HBV viral load and

for hepatitis B core antibody (anti-HBc) or hepatitis B surface antibody (anti-HBs) to determine recovery and/or immunity from HBV infection. Lastly, prevalence of HBeAg may be unreliable due to small sample sizes in the age specific strata. Nevertheless, the HBeAg data provide a reasonable signal of the sustained HBV transmission in this community.

Conclusion

Our findings show that HBV infection is an important public health problem. Men across all age groups and women in the age group 20 to 24 and 25 to 29 years old had the highest seroprevalence of HBsAg. Furthermore, more than a third of these men and women were HBeAg positive underscoring the risk of sustained onward HBV transmission in this community. Ensuring that HIV positive individuals with HBV infection are initiated on treatment to reduce viral loads of both HIV and HBV is essential. These findings highlight the importance of surveillance and an important missed opportunity for the scale up of programmes to achieve the overall goal of controlling HBV for public health benefit.

Author contributions

ABMK is the principal investigator of the HIPSS study; ABMK, NS an SN designed the study; NS wrote the first draft of the manuscript; LL for statistical analysis; NS, ABMK, SN, LL and CB contributed to analysis and interpretation of the data; NS, SN were responsible for laboratory measurements and quality assurance; ABMK, CC and DK were responsible for the field work, quality assurance and were responsible for community and stakeholder engagement activities. All authors critically reviewed and approved the final version of the manuscript.

Acknowledgements

Our sincere thanks to all household members and individual study participants for their participation in the HIV Incidence Provincial Surveillance System – the population based household based survey to understand the evolving HIV epidemic in the region. We sincerely acknowledge the ongoing support of the District Manager the uMgungundlovu Health District, members of the Provincial Department of Health, members of the uMgungundlovu district municipality, Provincial Health Research and Knowledge Management, local traditional leadership and community members for their support throughout the study. A special thanks to the study staff for the field work, laboratory and Primary Health Care clinic staff in the district.

Disclosure of Interest Statement:

All authors declare no competing interests.

Study sponsorship and funding Statement:

This work was supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of operative agreement 3U2GGH000372-02W1, the Joint South Africa–US Program for Collaborative Biomedical Research from the National Institutes of Health (R01HD083343 to ABMK) and the South African Department of Science and Technology and the National Research Foundation's Centre of Excellence in HIV Prevention (Grant # UID: 96354). The Centre for the AIDS Program of Research in South Africa (CAPRISA) provided research infrastructure support.

Disclaimer:

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

Role of the funding source:

The funders of the survey contributed to the survey design, study monitoring and reviewed the manuscript. ABMK, NS, SN and LL had full access to all the data. ABMK, NS, SN and CC had final responsibility for the decision to submit for publication.

References

1. World Health Organisation.: **Global Hepatitis Report**. *Geneva, Switzerland* 2017, **http://www.who.int/iris/handle/10665/255016** Date accessed 21 July 2018.

2. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ: **Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013**. *Lancet* 2015, **386**(10003):1546-1555.

3. Kiire CF: **The epidemioloogy and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa**. *Gut* 1996, **38**(2):S5-S12.

4. Kew MC: **Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa**. *Gut* 1996, **38 Suppl 2**:S31-36.

5. Mphahlele MJ, Francois G, Kew MC, Van Damme P, Hoosen AA, Meheus A: **Epidemiology and control of hepatitis B: implications for eastern and southern Africa**. *S AFR J Epidemiol Infect* 2002, **17**(1.2):12-17.

6. Heiberg IL, Hoegh M, Ladelund S, Niesters HG, Hogh B: **Hepatitis B virus DNA in saliva from children with chronic hepatitis B infection: implications for saliva as a potential mode of horizontal transmission**. *Pediatr Infect Dis J* 2010, **29**(5):465-467.

7. Dlamini NR, Maja P: **The Expanded Programme on Immunisation in South Africa: A story yet to be told**. *S Afr Med J* 2016, **106**(7):675-677.

8. Mayaphi SH, Roussow TM, Masemola DP, Olorunju SA, Mphahlele MJ, Martin DJ: **HBV/HIV co-infection: the dynamics of HBV in South African patients with AIDS**. *S Afr Med J* 2012, **102**(3 Pt 1):157-162.

9. Schneider J, King J, Macnab GM, Kew MC: **Hepatitis-B surface antigen and antibody in Black and White patients with venereal diseases**. *Br J Vener Dis* 1977, **53**(6):372-374.

10. Botha JF, Dusheiko GM, Ritchie MJJ, Mouton HWK, Kew MC: **Hepatitis-B virus carrier state in black-children in Ovamboland - role of perinatal and horizontal infection**. *Lancet* 1984, **1**(8388):1210-1212.

11. Burnett RJ, Francois G, Kew MC, Leroux-Roels G, Meheus A, Hoosen AA, Mphahlele MJ: **Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation**. *Liver international : official journal of the International Association for the Study of the Liver* 2005, **25**(2):201-213.

12. Dibisceglie AM, Kew MC, Dusheiko GM, Berger EL, Song E, Paterson AC, Hodkinson HJ: **Prevalence of hepatitis B virus infection among black children in Soweto**. *Br Med J (Clin Res Ed)* 1986, **292**(6533):1440-1442.

13. Vos GH, Rose EF, Marimuthu T: **Hepatitis B antigen and antibodies in rural and urban Southern African blacks**. *S Afr Med J* 1980, **57**(21):868-870.

14. Kew MC, MacKay ME, Mindel A, Joffe BI, Kusman B, MacNab GM, Seftel HC: **Prevalence of hepatitis B surface antigen and antibody in white and black patients with diabetes mellitus**. *J Clin Microbiol* 1976, **4**(6):467-469.

15. Prozesky OW, Szmuness W, Stevens CE, Kew MC, Harley EJ, Hoyland JA, Scholtz JE, Mitchell AD, Shabangu A, Kunene E *et al*: **Baseline epidemiological studies for a hepatitis B vaccine trial in Kangwane**. *S Afr Med J* 1983, **64**(23):891-893.

16. Kew MC, Kassianides C, Berger EL, Song E, Dusheiko GM: **Prevalence of chronic hepatitis B virus infection in pregnant black women living in Soweto**. *J Med Virol* 1987, **22**(3):263-268.

17. Abdool Karim SS, Coovadia HM, Windsor IM, Thejpal R, van den Ende J, Fouche A: **The prevalence and transmission of hepatitis B virus infection in urban, rural and institutionalized black children of Natal/KwaZulu, South Africa**. *Int J Epidemiol* 1988, **17**(1):168-173.

18. Dusheiko GM, Conradie JD, Brink BA, Marimuthu T, Sher R: **Differences in the regional prevalence of chronic hepatitis B in southern Africa--implications for vaccination**. *S Afr Med J* 1989, **75**(10):473-478.

19. Kew MC: **Hepatitis B virus infection: the burden of disease in South Africa**. *South Afr J Epidemiol Infect* 2008, **23**(1):4-8.

20. Thio CL: **Hepatitis B in the human immunodeficiency virus-infected patient: epidemiology, natural history, and treatment**. *Semin Liver Dis* 2003, **23**(2):125-136.

21. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP: **The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide**. *J Hepatol* 2006, **45**(4):529-538.

22. World Health Organisation.: **Global Health Sector Strategy on Viral Hepatitis 2016-2021**. *Geneva, Switzerland* 2016, **http://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf;jsessionid=161DC25381F15DFA568362716A5B4874?sequence=1**:Date accessed 16 July 2018.

23. Kharsany AB, Cawood C, Khanyile D, Grobler A, McKinnon LR, Samsunder N, Frohlich JA, Abdool Karim Q, Puren A, Welte A *et al*: **Strengthening HIV**

surveillance in the antiretroviral therapy era: rationale and design of a longitudinal study to monitor HIV prevalence and incidence in the uMgungundlovu District, KwaZulu-Natal, South Africa. *BMC Public Health* 2015, **15**:1149.

24. Kharsany ABM, Cawood C, Khanyile D, Lewis L, Grobler A, Puren A, Govender K, George G, Beckett S, Samsunder N *et al*: **Community-based HIV prevalence in KwaZulu-Natal, South Africa: results of a cross-sectional household survey**. *Lancet HIV* 2018, **5**(8):e427-e437.

25. van Helden J, Cornely C, Dati F, Levy HR, Bal T, Seeger M, Wright T, Baker L: **Performance evaluation of the ADVIA Centaur anti-HBe and HBeAg assays**. *J Clin Virol* 2008, **43**(2):169-175.

26. van Helden J, Denoyel G, Karwowska S, Reamer R, Schmalz J, Wright T, Preisel-Simmons B: **Performance of hepatitis B assays on the Bayer ADVIA Centaur Immunoassay System**. *Clinical Laboratory* 2004, **50**(1-2):63-73.

27. North Carolina Hepatitis B Public Health Program Manual.: **Hepatitis B Serology** 2012, **https://epi.publichealth.nc.gov/cd/lhds/manuals/hepB/docs/hbv_serology.pdf**:Date accessed 14 July 2018

28. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, Dusheiko G, Gogela N, Kassianides C, Kew M *et al*: **Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets**. *Lancet Gastroenterol Hepatol* 2017, **2**(12):900-909.

29. Thumbiran NV, Moodley D, Parboosing R, Moodley P: **Hepatitis B and HIV co-infection in pregnant women: indication for routine antenatal hepatitis B virus screening in a high HIV prevalence setting**. *S Afr Med J* 2014, **104**(4):307-309.

30. Matthews PC, Beloukas A, Malik A, Carlson JM, Jooste P, Ogwu A, Shapiro R, Riddell L, Chen F, Luzzi G *et al*: **Prevalence and Characteristics of Hepatitis B Virus (HBV) Coinfection among HIV-Positive Women in South Africa and Botswana**. *PLoS One* 2015, **10**(7):e0134037.

31. Hoffmann CJ, Charalambous S, Martin DJ, Innes C, Churchyard GJ, Chaisson RE, Grant AD, Fielding KL, Thio CL: **Hepatitis B virus infection and response to antiretroviral therapy (ART) in a South African ART program**. *Clin Infect Dis* 2008, **47**(11):1479-1485.

32. Lodenyo H, Schoub B, Ally R, Kairu S, Segal I: **Hepatitis B and C virus infections and liver function in AIDS patients at Chris Hani Baragwanath Hospital, Johannesburg**. *East Afr Med J* 2000, **77**(1):13-15.

33. Firnhaber C, Reyneke A, Schulze D, Malope B, Maskew M, MacPhail P, Sanne I, Di Bisceglie A: **The prevalence of hepatitis B co-infection in a South African urban government HIV clinic**. *S Afr Med J* 2008, **98**(7):541-544.

34. Abdool Karim SS, Thejpal R, Coovadia HM: **Household clustering and intra-household transmission patterns of hepatitis B virus infection in South Africa**. *Int J Epidemiol* 1991, **20**(2):495-503.

35. Di Bisceglie AM, Maskew M, Schulze D, Reyneke A, McNamara L, Firnhaber C: **HIV-HBV coinfection among South African patients receiving antiretroviral therapy**. *Antivir Ther* 2010, **15**(3 Pt B):499-503.

36. Cui F, Woodring J, Chan P, Xu F: **Considerations of antiviral treatment to interrupt mother-to-child transmission of hepatitis B virus in China**. *International Journal of Epidemiology* 2018, **47**(5):1529-1537.

37. Tsebe KV, Burnett RJ, Hlungwani NP, Sibara MM, Venter PA, Mphahlele MJ: **The first five years of universal hepatitis B vaccination in South Africa: evidence for elimination of HBsAg carriage in under 5-year-olds**. *Vaccine* 2001, **19**(28-29):3919-3926.

Table 3.1: *Sociodemographic, behavioural and clinical characteristics of participants 15-49 years, enrolled between June 2014 and June 2015 in rural and periurban areas of KwaZulu-Natal, South Africa*

	Men (n=3541)	**Women (n=6250)**
Median (IQR) age	**27 (21-35)**	**28 (21-37)**
Age groups in years, *number in sample, (weighted %)*		
15-19	657(19.6)	956 (18.1)
20-24	813 (20.9)	1262 (19.5)
25-29	602 (18.3)	1085 (17.9)
30-34	459 (13.9)	831 (13.7)
35-39	404 (12.3)	757 (12.2)
40-44	319 (8.6)	660 (9.6)
45-49	287 (6.5)	699 (8.9)
Relationship status, *number in samples, (weighted %)*		
Single (includes divorced, separated, widowed)	3300 (92.1)	5288 (85.8)
Married	180 (5.9)	681 (11.7)
Living with someone	61 (2.0)	175 (2.5)
Education, *number in samples, (weighted %)*[b]		
Incomplete secondary or less	1929 (56.5)	3306 (53.4)
Complete secondary	1403 (38.1)	2597 (41.3)
Tertiary	207 (5.4)	344 (5.3)
Income per household per month, *number in samples, (weighted %)*[c]		
No income	523 (13.2)	764 (10.4)
<ZAR500	293 (6.2)	610 (7.3)
ZAR501-2500	1434 (44.5)	2713 (46.3)
ZAR2501-6000	914 (35.5)	1558 (34.6)
>ZAR6000	30 (0.9)	70 (1.5)
Geographic location, *number in samples, (weighted %)*		
Urban	2296 (42.8)	4047 (41.3)
Rural	1245 (57.3)	2203 (58.7)
Lifetime # sexual partners, *number in samples, (weighted %)*[d]		
0 partners	691 (21.2)	816 (15.6)
1 partner	420 (13.8)	1543 (28.9)
1-5 partners	1196 (39.0)	2848 (51.3)
>5 partners	758 (26.1)	280 (4.3)
HIV status, *number in samples, (weighted %)*		
Negative	2531 (72.1)	3305 (56.0)
Positive	1010 (28.0)	2945 (44.0)
Self-reported to be on ART (HIV positive only), *number in samples, (weighted %)*		
No	669 (63.1)	1695 (54.3)
Yes	341 (36.9)	1250 (45.7)
STI[a] present, *number in samples, (weighted %)*		
No	1689 (47.7)	1354 (21.7)
Yes	1852 (52.3)	4896 (78.3)
Condom use during sex in the last 12 months for those sexually active in the last 12 months , *number in samples, (weighted %)*		
Always	593 (25.2)	992 (22.8)
Sometimes	1290 (54.9)	2317 (53.4)
Never	468 (19.9)	1032 (23.8)

% = Population weighted percentage; IQR= Interquartile range, ZAR= South African Rand; a =any laboratory diagnosis of Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis and/or Mycoplasma genitalium DNA from self-collected swabs (women) and first-pass urine (men) samples and antibodies to herpes simplex virus type 2 and Treponema pallidum (syphilis). (Kharsany et al., 2015) b=2 men and 3 women were missing education data; c=347 men and 535 women were missing income data; d=476 men and 763 women did not provide their number of lifetime partners

Table 3.2: *Seroprevalence of HBsAg and association with sociodemographic, behavioural and clinical characteristics of 9791 participants (15-49 years), enrolled between June 2014 and June 2015 in rural and periurban KwaZulu-Natal, South Africa*

Variable	Men		Women	
	n/N	HBsAg seroprevalence Weighted % (95% CI)	n/N	HBsAg seroprevalence Weighted % (95% CI)
Seroprevalence of HBsAg				
Overall	**165/3541**	**4.8 (3.8-5.8)**	**196/6250**	**3.2 (2.5-3.9)**
By Age group in years				
15-19	7/657	**1.1** (0-2.3)	8/956	**0.9** (0-1.8)
20-24	26/813	**3.6** (1.7-5.4)	32/1262	**2.7** (1.5-3.8)
25-29	30/602	**4.5** (2.4-6.6)	39/1085	**3.8** (2.2-5.4)
30-34	40/459	**7.8** (4.5-11)	40/831	**6.0** (3.1-9)
35-39	23/404	**5.6** (3.1-8.1)	27/757	**2.9** (1.4-4.5)
40-44	24/319	**9.6** (5-14.1)	26/660	**4.1** (1.9-6.3)
45-49	15/287	**6.6** (2.3-10.8)	24/699	**2.5** (1.3-3.8)
		***P value* <0.01**[c]		***P value* <0.01**[c]
Relationship status				
Single (includes divorced, separated, widowed)	150/330	**4.7** (3.7-5.7)	173/5394	**3.1** (2.4-3.9)
Married	12/180	**5.7** (2.3-9.2)	16/681	**2.9** (1.1-4.7)
Living with someone	3/61	**8.6** (0-20.3)	7/175	**4.3** (0.6-8.1)
Other (divorced, separated, widowed)	0/13	**0** (0-0)	5/106	**5** (0-10.4)
		P value= 0.52 [c]		***P value= 0.79*** [c]
Education[d]				
Incomplete secondary or less	96/1929	**5.3** (4-6.7)	116/3306	**3.3** (2.6-4.0)
Complete secondary	60/1403	**4.1** (2.6-5.6)	75/2597	**3.3** (2.1-4.5)
Tertiary	9/207	**4.3** (0.4-8.3)	5/344	**1** (0-2.1)
		***P value*=0.49**[c]		***P* value=0.16**[c]
Income per household per month [a,e]				
No income	28/523	**5.5** (2.9-8.1)	23/764	**3** (1.2-4.8)
<ZAR500	19/293	**5.5** (2.6-8.3)	24/610	**4.5** (2.2-6.8)
ZAR501-2500	66/1434	**4.9** (3.2-6.5)	96/2713	**3.3** (2.5-4.1)
ZAR2501-6000	37/914	**4.2** (2.6-5.9)	39/1558	**2.8** (1.3-4.3)
>ZAR6000	1/30	**5.4** (0-15.9)	3/70	**7** (0-15.5)
		***P value*=0.91**[c]		***P value*=0.48**[c]
Geographic location				
Urban	104/2296	**4.6** (3.5-5.7)	133/4047	**3.2** (2.6-3.8)
Rural	61/1245	**5.0** (3.4-6.5)	63/2203	**3.1** (2-4.2)
		***P value*=0.69**[c]		***P value*=0.92**[c]
Lifetime # sexual partners[f]				
0 partners	16/691	**3.2** (1.1-5.2)	10/816	**1.7** (0.3-3.1)
1 partner	18/420	**3.4** (1.6-5.2)	30/1543	**1.9** (1-2.9)
1-5 partners	64/1196	**5.2** (3.4-7.1)	103/2848	**3.7** (2.7-4.8)
>5 partners	28/758	**4.5** (2.4-6.6)	17/280	**6.8** (2.8-10.9)
		***P value*=0.41**[c]		***P value*<0.01**[c]
HIV status				
Negative	74/2531	**3.3** (2.3-4.3)	58/3305	**1.7** (1.1-2.3)
Positive	91/1010	**8.7** (6.3-11.2)	138/2945	**5.0** (3.8-6.2)
		***P value*<0.01**[c]		***P value*<0.01**[c]
Self-reported to be on ART (HIV positive only)				
No	57/669	**6.9** (4.5-9.4)	78/1695	**4.9** (3.5-6.4)
Yes	34/341	**11.9** (6.8-16.9)	60/1250	**5.2** (3.6-6.8)
		***P value*=0.05**[c]		***P value*=0.78**[c]
STI[b] ***present***				
No	49/1689	**2.8** (1.7-3.8)	20/1354	**1.9** (0.7-3.1)
Yes	116/1852	**6.7** (5.2-8.3)	176/4896	**3.6** (2.8-4.4)
		***P value*<0.01**[c]		***P value*=0.06**[c]
Condom Use in the last 12 months(sexually active in last 12 months only)				
Always	24/593	**3.6** (1.9-5.4)	34/992	**2.9** (1.5-4.3)

Sometimes	66/1290	**5.3**	(3.5-7.1)	73/2317	**3.7** (2.3-5.1)
Never	27/468	**6.1**	(3.1-9.0)	35/1032	**3.5** (2.1-4.9)
		P value=0.33[c]			***P value=0.72[c]***

%=Population weighted percentages; a=ZAR= South African Rand, ZAR20 ~ GBP1; b=any laboratory diagnosis of Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis and/or Mycoplasma genitalium DNA from self-collected swabs (women) and first-pass urine (men) samples and antibodies to herpes simplex virus type 2 and Treponema pallidum (syphilis)(Kharsany et al., 2015); c=p value for the association for variable with HBsAg status; d=2 men and 3 women were missing education data; e=347 men and 535 women were missing income data; f= 476 men and 763 women did not provide their number of lifetime partners; CI= 95% confidence interval.

Table 3.3: *Seroprevalence of hepatitis B virus makers (HBsAg, HBeAg and anti-HBe) among 9791 participants (15-49 years), enrolled between June 2014 and June 2015 in rural and periurban KwaZulu-Natal, South Africa*

Variable	Men			Women			*P value*[a]
	n/N	Weighted % (95% CI)		n/N	Weighted % (95% CI)		
Seroprevalence of HBsAg							
Overall	**165/3541**	**4.8**	**(3.8-5.8)**	**196/6250**	**3.2**	**(2.5-3.9)**	**0.01**
By Age group in years							
15-19	7/657	**1.1**	(0-2.3)	8/956	**0.9**	(0-1.8)	**0.66**
20-24	26/813	**3.6**	(1.7-5.4)	32/1262	**2.7**	(1.5-3.8)	**0.40**
25-29	30/602	**4.5**	(2.4-6.6)	39/1085	**3.8**	(2.2-5.4)	**0.59**
30-34	40/459	**7.8**	(4.5-11)	40/831	**6.0**	(3.1-9.0)	**0.47**
35-39	23/404	**5.6**	(3.1-8.1)	27/757	**2.9**	(1.4-4.5)	**0.08**
40-44	24/319	**9.6**	(5-14.1)	26/660	**4.1**	(1.9-6.3)	**<0.01**
45-49	15/287	**6.6**	(2.3-10.8)	24/699	**2.5**	(1.3-3.8)	**0.02**
		***P value* <0.01**[b]			***P value* <0.01**[b]		
Seroprevalence of HBeAg[c,d]							
Overall	**65/164**	**36.9**	**(27.5-46.2)**	**68/193**	**32.8**	**(23.7-41.9)**	**0.58**
By Age group in years							
15-19	6/7	**92.2**	(75.8-100)	1/8	**4.4**	(0-13.7)	**<0.01**
20-24	10/26	**43.3**	(18.3-68.4)	17/32	**56.7**	(32.6-80.8)	**0.45**
25-29	11/30	**29.8**	(11.1-48.5)	12/39	**30.2**	(9.6-50.7)	**0.98**
30-34	15/39	**24.8**	(9.4-40.1)	14/39	**31.0**	(11.5-50.5)	**0.62**
35-39	7/23	**24.7**	(6.2-43.1)	8/27	**24.6**	(7.0-9-42.3)	**1.00**
40-44	11/24	**55.5**	(30.6-80.4)	9/25	**27.7**	(10.4-45.0)	**0.05**
45-49	5/15	**23.3**	(0.7-45.9)	7/23	**34.0**	(12.4-55.5)	**0.51**
		P value=0.09 [b]			***P value=0.18*** [b]		
Seroprevalence of anti-HBe[c,e]							
Overall	**104/163**	**64.5**	**(54.7-74.3)**	**133/193**	**68.8**	**(59.3-78.3)**	**0.51**
By age group in years							
15-19	1/7	**7.8**	(0-24.2)	7/8	**81.0**	(45.5-100)	**<0.01**
20-24	17/26	**64.7**	(40.3-89.2)	20/32	**65.9**	(44.1-87.8)	**0.94**
25-29	20/30	**69.7**	(50.5-88.8)	27/39	**62.9**	(42.9-82.9)	**0.63**
30-34	23/39	**70.0**	(52-87.9)	25/39	**65.4**	(40.2-90.6)	**0.77**
35-39	18/22	**83.0**	(66.4-99.5)	20/27	**78.5**	(60.0-97.1)	**0.72**
40-44	15/24	**47.1**	(21.6-72.6)	17/25	**76.8**	(60.9-92.7)	**0.03**
45-49	10/15	**75.2**	(51.5-98.8)	17/23	**68.3**	(46.7-90.0)	**0.68**
		P value=0.11[b]			***P value=0.91***[b]		

%=Population weighted percentages; a= p value for the association of HBsAg/HBeAg/HBeAb status and sex by age category;;b=p value for the association of age with HBsAg/HBeAg/ anti-HBe *status by sex; c =based on HBsAg positive samples; d=4 samples insufficient for HBeAg testing; e=5 samples insufficient for HBeAb testing; CI= confidence interval*

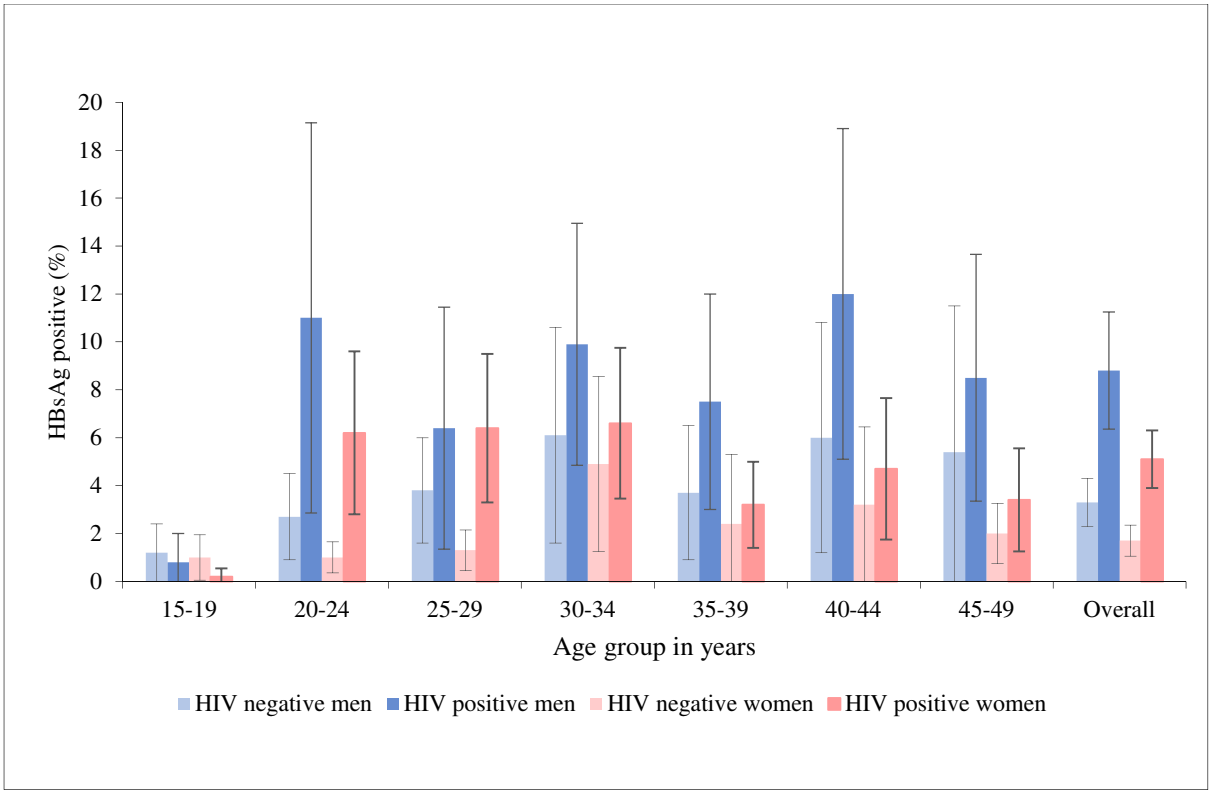


Figure 3.1. *Seroprevalence HBV (HBsAg)-HIV co-infection by sex and age groups*

REFERENCES FOR DISSERTATION

ABDOOL KARIM, Q., SIBEKO, S. & BAXTER, C. 2010. Preventing HIV infection in women: a global health imperative. *Clin Infect Dis,* 50 Suppl 3**,** S122-9.

ABDOOL KARIM, S. S., COOVADIA, H. M., WINDSOR, I. M., THEJPAL, R., VAN DEN ENDE, J. & FOUCHE, A. 1988. The prevalence and transmission of hepatitis B virus infection in urban, rural and institutionalized black children of Natal/KwaZulu, South Africa. *Int J Epidemiol,* 17**,** 168-73.

ABDOOL KARIM, S. S., THEJPAL, R. & COOVADIA, H. M. 1991. Household clustering and intra-household transmission patterns of hepatitis B virus infection in South Africa. *Int J Epidemiol,* 20**,** 495-503.

ABDOOL KARIM, S. S., THEJPAL, R. & SINGH, B. 1989. High prevalence of hepatitis B virus infection in rural black adults in Mseleni, South Africa. *Am J Public Health,* 79**,** 893-4.

AMPONSAH-DACOSTA, E., LEBELO, R. L., RAKGOLE, J. N., BURNETT, R. J., SELABE, S. G. & MPHAHLELE, M. J. 2014. Evidence for a change in the epidemiology of hepatitis B virus infection after nearly two decades of universal hepatitis B vaccination in South Africa. *J Med Virol,* 86**,** 918-24.

AYUK, J., MPHAHLELE, J. & BESSONG, P. 2013. Hepatitis B virus in HIV-infected patients in northeastern South Africa: prevalence, exposure, protection and response to HAART. *S Afr Med J,* 103**,** 330-3.

BARTH, R. E., HUIJGEN, Q., TALJAARD, J. & HOEPELMAN, A. I. 2010. Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and meta-analysis. *Int J Infect Dis,* 14**,** e1024-31.

BELL, T. G., MAKONDO, E., MARTINSON, N. A. & KRAMVIS, A. 2012. Hepatitis B virus infection in human immunodeficiency virus infected southern African adults: occult or overt--that is the question. *PLoS One,* 7**,** e45750.

BONACINI, M., LOUIE, S., BZOWEJ, N. & WOHL, A. R. 2004. Survival in patients with HIV infection and viral hepatitis B or C: a cohort study. *Aids,* 18**,** 2039-45.

BOTHA, J. F., DUSHEIKO, G. M., RITCHIE, M. J. J., MOUTON, H. W. K. & KEW, M. C. 1984. Hepatitis-B virus carrier state in black-children in Ovamboland - role of perinatal and horizontal infection. *Lancet,* 1**,** 1210-1212.

BOYLES, T. H. & COHEN, K. 2011. The prevalence of hepatitis B infection in a rural South African HIV clinic. *S Afr Med J,* 101**,** 470-1.

BURNETT, R. J., FRANCOIS, G., KEW, M. C., LEROUX-ROELS, G., MEHEUS, A., HOOSEN, A. A. & MPHAHLELE, M. J. 2005. Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation. *Liver Int,* 25**,** 201-13.

BURNETT, R. J., KRAMVIS, A., DOCHEZ, C. & MEHEUS, A. 2012. An update after 16 years of hepatitis B vaccination in South Africa. *Vaccine,* 30 C45-51.

CENTRES FOR DISEASE CONTROL AND PREVENTION. 2017. The 13th Edition Epidemiology and Prevention of Vaccine-Preventable Diseases,. https://www.cdc.gov/vaccines/pubs/pinkbook/index.html**,** Date accessed 30 November 2018.

CHOTUN, N., NEL, E., COTTON, M. F., PREISER, W. & ANDERSSON, M. I. 2015. Hepatitis B virus infection in HIV-exposed infants in the Western Cape, South Africa. *Vaccine,* 33**,** 4618-22.

COLIN, J. F., CAZALS-HATEM, D., LORIOT, M. A., MARTINOT-PEIGNOUX, M., PHAM, B. N., AUPERIN, A., DEGOTT, C., BENHAMOU, J. P., ERLINGER, S., VALLA, D. & MARCELLIN, P. 1999. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. *Hepatology,* 29**,** 1306-10.

CUI, F., WOODRING, J., CHAN, P. & XU, F. 2018. Considerations of antiviral treatment to interrupt mother-to-child transmission of hepatitis B virus in China. *International Journal of Epidemiology,* 47**,** 1529-1537.

DANE, D. S., CAMERON, C. H. & BRIGGS, M. 1970. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet,* 1**,** 695-8.

DEPARTMENT OF HEALTH, S. A. 2009. South African Department of Health. Expanded Programme on Immunisation-EPI(SA) revised Childhood Immunisation Schedule from April 2009. *In:* SOUTH AFRICAN DEPARTMENT OF HEALTH (ed.).

DEPARTMENT OF HEALTH, S. A. 2013. The South African Antiretroviral Treatment Guidelines 2013. *In:* DEPARTMENT OF HEALTH, S. A. (ed.).

DI BISCEGLIE, A. M., MASKEW, M., SCHULZE, D., REYNEKE, A., MCNAMARA, L. & FIRNHABER, C. 2010. HIV-HBV coinfection among South African patients receiving antiretroviral therapy. *Antivir Ther,* 15**,** 499-503.

DIBISCEGLIE, A. M., KEW, M. C., DUSHEIKO, G. M., BERGER, E. L., SONG, E., PATERSON, A. C. & HODKINSON, H. J. 1986. Prevalence of hepatitis B virus infection among black children in Soweto. *Br Med J (Clin Ed),* 292**,** 1440-2.

DLAMINI, N. R. & MAJA, P. 2016. The Expanded Programme on Immunisation in South Africa: A story yet to be told. *S Afr Med J,* 106**,** 675-7.

DUSHEIKO, G. M., CONRADIE, J. D., BRINK, B. A., MARIMUTHU, T. & SHER, R. 1989. Differences in the regional prevalence of chronic hepatitis B in southern Africa--implications for vaccination. *S Afr Med J,* 75**,** 473-8.

FIRNHABER, C., REYNEKE, A., SCHULZE, D., MALOPE, B., MASKEW, M., MACPHAIL, P., SANNE, I. & DI BISCEGLIE, A. 2008. The prevalence of hepatitis B co-infection in a South African urban government HIV clinic. *S Afr Med J,* 98**,** 541-4.

FIRNHABER, C., VIANA, R., REYNEKE, A., SCHULTZE, D., MALOPE, B., MASKEW, M., DI BISCEGLIE, A., MACPHAIL, P., SANNE, I. & KEW, M. 2009. Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa. *Int J Infect Dis,* 13**,** 488-92.

FIRNHABER, W. 2008. Article of 30 years ago. *Dtsch Arztebl Int,* 105**,** 318; author reply 318.

GAVILANES, F., GONZALEZ-ROS, J. M. & PETERSON, D. L. 1982. Structure of hepatitis B surface antigen. Characterization of the lipid components and their association with the viral proteins. *J Biol Chem,* 257**,** 7770-7.

GERLICH, W. H. & ROBINSON, W. S. 1980. Hepatitis B virus contains protein attached to the 5' terminus of its complete DNA strand. *Cell,* 21**,** 801-9.

GILSON, R. J., HAWKINS, A. E., BEECHAM, M. R., ROSS, E., WAITE, J., BRIGGS, M., MCNALLY, T., KELLY, G. E., TEDDER, R. S. & WELLER, I. V. 1997. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. *Aids,* 11**,** 597-606.

GITLIN, N. 1997. Hepatitis B: diagnosis, prevention, and treatment. *Clin Chem,* 43**,** 1500-6.

HAMERS, R. L., ZAAIJER, H. L., WALLIS, C. L., SIWALE, M., IVE, P., BOTES, M. E., SIGALOFF, K. C., HOEPELMAN, A. I., STEVENS, W. S., RINKE DE WIT, T. F. & PHARMACCESS AFRICAN STUDIES TO EVALUATE, R. 2013. HIV-HBV coinfection in Southern Africa and the effect of lamivudine- versus tenofovir-containing cART on HBV outcomes. *J Acquir Immune Defic Syndr,* 64**,** 174-82.

HEIBERG, I. L., HOEGH, M., LADELUND, S., NIESTERS, H. G. & HOGH, B. 2010. Hepatitis B virus DNA in saliva from children with chronic hepatitis B infection: implications for saliva as a potential mode of horizontal transmission. *Pediatr Infect Dis J,* 29**,** 465-7.

HOFFMANN, C. J., CHARALAMBOUS, S., MARTIN, D. J., INNES, C., CHURCHYARD, G. J., CHAISSON, R. E., GRANT, A. D., FIELDING, K. L. & THIO, C. L. 2008. Hepatitis B virus infection and response to antiretroviral therapy (ART) in a South African ART program. *Clin Infect Dis,* 47**,** 1479-85.

HOFFMANN, C. J. & THIO, C. L. 2007. Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. *Lancet Infect Dis,* 7**,** 402-9.

HOU, J., LIU, Z. & GU, F. 2005. Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci,* 2**,** 50-57.

JOOSTE, P., VAN ZYL, A., ADLAND, E., DANIELS, S., HATTINGH, L., BRITS, A., WAREING, S., GOEDHALS, D., JEFFERY, K., ANDERSSON, M., GOULDER, P. & MATTHEWS, P. C. 2016. Screening, characterisation and prevention of Hepatitis B virus (HBV) co-infection in HIV-positive children in South Africa. *J Clin Virol,* 85**,** 71-74.

KANG, H. Y., LEE, S., PARK, S. G., YU, J., KIM, Y. & JUNG, G. 2006. Phosphorylation of hepatitis B virus Cp at Ser87 facilitates core assembly. *Biochem J,* 398**,** 311-7.

KEEFFE, E. B. & MARCELLIN, P. 2007. New and emerging treatment of chronic hepatitis B. *Clin Gastroenterol Hepatol,* 5**,** 285-94.

KEW, M. C. 1996a. Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa. *Gut,* 38 Suppl 2**,** S31-6.

KEW, M. C. 1996b. Progress towards the comprehensive control of hepatitis B in Africa: a view from South Africa. *Gut,* 38 S31-6.

KEW, M. C. 2008a. Hepatitis B virus infection: the burden of disease in South Africa. *South Afr J Epidemiol Infect,* 23**,** 4-8.

KEW, M. C. 2008b. Hepatitis B virus infection: the burden of disease in South Africa. *S Afr J Epidemiol infect.,* 23**,** 4-8.

KEW, M. C., KASSIANIDES, C., BERGER, E. L., SONG, E. & DUSHEIKO, G. M. 1987. Prevalence of chronic hepatitis B virus infection in pregnant black women living in Soweto. *J Med Virol,* 22**,** 263-8.

KEW, M. C., MACKAY, M. E., MINDEL, A., JOFFE, B. I., KUSMAN, B., MACNAB, G. M. & SEFTEL, H. C. 1976. Prevalence of hepatitis B surface antigen and antibody in white and black patients with diabetes mellitus. *J Clin Microbiol,* 4**,** 467-9.

KHARSANY, A. B., CAWOOD, C., KHANYILE, D., GROBLER, A., MCKINNON, L. R., SAMSUNDER, N., FROHLICH, J. A., ABDOOL KARIM, Q., PUREN, A., WELTE, A., GEORGE, G., GOVENDER, K., TOLEDO, C., CHIPETA, Z., ZEMBE, L., GLENSHAW, M. T., MADURAI, L., DEYDE, V. M. & BERE, A. 2015. Strengthening HIV surveillance in the antiretroviral therapy era: rationale and design of a longitudinal study to monitor HIV prevalence and incidence in the uMgungundlovu District, KwaZulu-Natal, South Africa. *BMC Public Health,* 15**,** 1149.

KHARSANY, A. B. M., CAWOOD, C., KHANYILE, D., LEWIS, L., GROBLER, A., PUREN, A., GOVENDER, K., GEORGE, G., BECKETT, S., SAMSUNDER, N., MADURAI, S., TOLEDO, C., CHIPETA, Z., GLENSHAW, M., HERSEY, S. & ABDOOL KARIM, Q. 2018. Community-based HIV prevalence in KwaZulu-Natal, South Africa: results of a cross-sectional household survey. *Lancet HIV,* 5**,** e427-e437.

KIIRE, C. F. 1996. The epidemioloogy and prophylaxis of hepatitis B in sub-Saharan Africa: a view from tropical and subtropical Africa. *Gut,* 38**,** S5-S12.

KIM, H. N., HARRINGTON, R. D., CRANE, H. M., DHANIREDDY, S., DELLIT, T. H. & SPACH, D. H. 2009. Hepatitis B vaccination in HIV-infected adults: current evidence, recommendations and practical considerations. *International Journal of Std & Aids,* 20**,** 595-600.

KING, J. 2016. Hepatitis B co-infection in HIV- infected patients receiving antiretroviral therapy at the TC Newman Anti Retriviral Treatment Clinic in Paarl, Western Cape. *Southern African Journal of HIV Medicine,* 17**,** 3.

KOCK, J. & SCHLICHT, H. J. 1993. Analysis of the earliest steps of hepadnavirus replication: genome repair after infectious entry into hepatocytes does not depend on viral polymerase activity. *J Virol,* 67**,** 4867-74.

KOMATSU, H., INUI, A., FUJISAWA, T., TAKANO, T., TAJIRI, H., MURAKAMI, J. & SUZUKI, M. 2015. Transmission route and genotype of chronic hepatitis B virus infection in children in Japan between 1976 and 2010: A retrospective, multicenter study. *Hepatol Res,* 45**,** 629-37.

KOURTIS, A. P., BULTERYS, M., HU, D. J. & JAMIESON, D. J. 2012. HIV-HBV coinfection--a global challenge. *N Engl J Med,* 366**,** 1749-52.

KRAJDEN, M., MCNABB, G. & PETRIC, M. 2005. The laboratory diagnosis of hepatitis B virus. *Can J Infect Dis Med Microbiol,* 16**,** 65-72.

KRAMVIS, A., KEW, M. & FRANCOIS, G. 2005. Hepatitis B virus genotypes. *Vaccine,* 23**,** 2409-2423.

KRAMVIS, A. & KEW, M. C. 2007. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatol Res,* 37**,** S9-S19.

KREKULOVA, L., REHAK, V., DA SILVA FILHO, H. P., ZAVORAL, M. & RILEY, L. W. 2003. Genotypic distribution of hepatitis B virus in the Czech Republic: a possible association with modes of transmission and clinical outcome. *Eur J Gastroenterol Hepatol,* 15**,** 1183-8.

KRUGMAN, S., OVERBY, L. R., MUSHAHWAR, I. K., LING, C. M., FROSNER, G. G. & DEINHARDT, F. 1979. Viral hepatitis, type B. Studies on natural history and prevention re-examined. *N Engl J Med,* 300**,** 101-6.

KURBANOV, F., TANAKA, Y., KRAMVIS, A., SIMMONDS, P. & MIZOKAMI, M. 2008. When should "I" consider a new hepatitis B virus genotype? *J Virol,* 82**,** 8241-2.

LEVY, V. & GRANT, R. M. 2006. Antiretroviral therapy for hepatitis B virus-HIV-coinfected patients: promises and pitfalls. *Clin Infect Dis,* 43**,** 904-10.

LIANG, T. J. 2009. Hepatitis B: the virus and disease. *Hepatology,* 49**,** S13-21.

LIU, C. J. & KAO, J. H. 2013. Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. *Semin Liver Dis,* 33**,** 97-102.

LODENYO, H., SCHOUB, B., ALLY, R., KAIRU, S. & SEGAL, I. 2000. Hepatitis B and C virus infections and liver function in AIDS patients at Chris Hani Baragwanath Hospital, Johannesburg. *East Afr Med J,* 77**,** 13-5.

LOK, A. S. & MCMAHON, B. J. 2007. Chronic hepatitis B. *Hepatology,* 45**,** 507-39.

LUKHWARENI, A., BURNETT, R. J., SELABE, S. G., MZILENI, M. O. & MPHAHLELE, M. J. 2009. Increased detection of HBV DNA in HBsAg-positive and HBsAg-negative South African HIV/AIDS patients enrolling for highly active antiretroviral therapy at a Tertiary Hospital. *J Med Virol,* 81**,** 406-12.

MATTHEWS, P. C., BELOUKAS, A., MALIK, A., CARLSON, J. M., JOOSTE, P., OGWU, A., SHAPIRO, R., RIDDELL, L., CHEN, F., LUZZI, G., JAGGERNATH, M., JESUTHASAN, G., JEFFERY, K., NDUNG'U, T., GOULDER, P. J., GERETTI, A. M. & KLENERMAN, P. 2015. Prevalence and Characteristics of Hepatitis B Virus (HBV) Coinfection among HIV-Positive Women in South Africa and Botswana. *PLoS One,* 10**,** e0134037.

MATTHEWS, P. C., GERETTI, A. M., GOULDER, P. J. & KLENERMAN, P. 2014. Epidemiology and impact of HIV coinfection with hepatitis B and hepatitis C viruses in Sub-Saharan Africa. *J Clin Virol,* 61**,** 20-33.

MAYAPHI, S. H., ROUSSOW, T. M., MASEMOLA, D. P., OLORUNJU, S. A., MPHAHLELE, M. J. & MARTIN, D. J. 2012. HBV/HIV co-infection: the dynamics of HBV in South African patients with AIDS. *S Afr Med J,* 102**,** 157-62.

MCMAHON, B. J. 2009. The natural history of chronic hepatitis B virus infection. *Hepatology,* 49**,** S45-55.

MDLALOSE, N., PARBOOSING, R. & MOODLEY, P. 2016. The prevalence of hepatitis B virus infection in HIV-positive and HIV-negative infants: KwaZulu-Natal, South Africa. *Afr J Lab Med,* 5**,** 283.

MEINTJES, G., BLACK, J. & F., C. 2014. Guidelines- Adult antiretrovirals therapy guidelines 2014- By the Southern African Clinicians Society. *S Afr J HIV Med,* 15**,** 121-143.

MERICAN, I., GUAN, R., AMARAPUKA, D., ALEXANDER, M. J., CHUTAPUTTI, A., CHIEN, R. N., HASNIAN, S. S., LEUNG, N., LESMANA, L., PHIET, P. H., SJALFOELLAH NOER, H. M., SOLLANO, J., SUN, H. S. & XU, D. Z. 2000. Chronic hepatitis B virus infection in Asian countries. *J Gastroenterol Hepatol,* 15**,** 1356-61.

MPHAHLELE, M. J., FRANCOIS, G., KEW, M. C., VAN DAMME, P., HOOSEN, A. A. & MEHEUS, A. 2002. Epidemiology and control of hepatitis B: implications for eastern and southern Africa. *S aFR J Epidemiol infect.,* 17**,** 12-17.

NI, Y. H., CHANG, M. H., HUANG, L. M., CHEN, H. L., HSU, H. Y., CHIU, T. Y., TSAI, K. S. & CHEN, D. S. 2001. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med,* 135**,** 796-800.

NIAID 2016. Hepatitis. Available on: https://www.niaid.nih.gov/diseases-conditions/hepatitis. [Accessed on: 17 September 2018].

NORTH CAROLINA HEPATITIS B PUBLIC HEALTH PROGRAM MANUAL. 2012. Hepatitis B Serology

https://epi.publichealth.nc.gov/cd/lhds/manuals/hepB/docs/hbv_serology.pdf**,** Date accessed 14 July 2018

OTT, J. J., STEVENS, G. A., GROEGER, J. & WIERSMA, S. T. 2012. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine,* 30**,** 2212-9.

PALUMBO, E. 2008. New drugs for chronic hepatitis B: a review. *Am J Ther,* 15**,** 167-72.

PAN, C. Q. & ZHANG, J. X. 2005. Natural History and Clinical Consequences of Hepatitis B Virus Infection. *Int J Med Sci,* 2**,** 36-40.

PERZ, J. F., ARMSTRONG, G. L., FARRINGTON, L. A., HUTIN, Y. J. & BELL, B. P. 2006. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol,* 45**,** 529-38.

POLLACK, J. R. & GANEM, D. 1993. An RNA stem-loop structure directs hepatitis B virus genomic RNA encapsidation. *J Virol,* 67**,** 3254-63.

PREVISANI, N. L., D. 2002. World Health Organization. Hepatitis B (WHO/CDS/CSR/LYO/2002.2). Available on: :http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf [Accessed on: 24 August 2012.

PROZESKY, O. W., SZMUNESS, W., STEVENS, C. E., KEW, M. C., HARLEY, E. J., HOYLAND, J. A., SCHOLTZ, J. E., MITCHELL, A. D., SHABANGU, A., KUNENE, E. & ET AL. 1983. Baseline epidemiological studies for a hepatitis B vaccine trial in Kangwane. *S Afr Med J,* 64**,** 891-3.

PUOTI, M., TORTI, C., BRUNO, R., FILICE, G. & CAROSI, G. 2006. Natural history of chronic hepatitis B in co-infected patients. *Journal of Hepatology,* 44**,** S65-S70.

SANGARE, L., SOMBIE, R., COMBASSERE, A. W., KOUANDA, A., KANIA, D., ZERBO, O. & LANKOANDE, J. 2009. [Antenatal transmission of hepatitis B virus in an area of HIV moderate prevalence, Burkina Faso]. *Bull Soc Pathol Exot,* 102**,** 226-9.

SCHNEIDER, J., KING, J., MACNAB, G. M. & KEW, M. C. 1977. Hepatitis-B surface antigen and antibody in Black and White patients with venereal diseases. *Br J Vener Dis,* 53**,** 372-4.

SCHWEITZER, A., HORN, J., MIKOLAJCZYK, R. T., KRAUSE, G. & OTT, J. J. 2015. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet,* 386**,** 1546-55.

SHATTOCK, R. J. & MOORE, J. P. 2003. Inhibiting sexual transmission of HIV-1 infection. *Nature Reviews Microbiology,* 1**,** 25-34.

SHEPARD, C. W., SIMARD, E. P., FINELLI, L., FIORE, A. E. & BELL, B. P. 2006. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev,* 28**,** 112-25.

SHIRE, N. J., WELGE, J. A. & SHERMAN, K. E. 2006. Efficacy of inactivated hepatitis A vaccine in HIV-infected patients: a hierarchical bayesian meta-analysis. *Vaccine,* 24**,** 272-9.

SORIANO, V., BARREIRO, P., MARTIN-CARBONERO, L., CASTELLARES, C., RUIZ-SANCHO, A., LABARGA, P., RAMOS, B. & GONZALEZ-LAHOZ, J. 2007. Treatment of chronic hepatitis B or C in HIV-infected patients with dual viral hepatitis. *J Infect Dis,* 195**,** 1181-3.

SORIANO, V., PUOTI, M., BONACINI, M., BROOK, G., CARGNEL, A., ROCKSTROH, J., THIO, C. & BENHAMOU, Y. 2005. Care of patients with chronic hepatitis B and HIV co-infection: recommendations from an HIV-HBV International Panel. *AIDS,* 19**,** 221-40.

SORIANO, V., YOUNG, B. & REAU, N. 2018. Report from the International Conference on Viral Hepatitis - 2017. *AIDS Rev,* 20**,** 58-70.

SPEARMAN, C. W., AFIHENE, M., ALLY, R., APICA, B., AWUKU, Y., CUNHA, L., DUSHEIKO, G., GOGELA, N., KASSIANIDES, C., KEW, M., LAM, P., LESI, O., LOHOUES-KOUACOU, M. J., MBAYE, P. S., MUSABEYEZU, E., MUSAU, B., OJO, O., RWEGASHA, J., SCHOLZ, B., SHEWAYE, A. B., TZEUTON, C., SONDERUP, M. W., GASTROENTEROLOGY & HEPATOLOGY ASSOCIATION OF SUB-SAHARAN, A. 2017. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. *Lancet Gastroenterol Hepatol,* 2**,** 900-909.

SPEARMAN, C. W., SONDERUP, M. W., BOTHA, J. F., VAN DER MERWE, S. W., SONG, E., KASSIANIDES, C., NEWTON, K. A., HAIRWADZI, H. N. & DIVISION OF HEPATOLOGY, D. O. M. U. O. C. T. S. A. 2013. South African guideline for the management of chronic hepatitis B: 2013. *S Afr Med J,* 103**,** 337-49.

TABOR, E., HOOFNAGLE, J. H., BARKER, L. F., PINEDA-TAMONDONG, G., NATH, N., SMALLWOOD, L. A. & GERETY, R. J. 1981. Antibody to hepatitis B core antigen in blood donors with a history of hepatitis. *Transfusion,* 21**,** 366-71.

THIO, C. L., SEABERG, E. C., SKOLASKY, R., PHAIR, J., VISSCHER, B., MUNOZ, A. & THOMAS, D. L. 2002. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet,* 360**,** 1921-1926.

THUMBIRAN, N. V., MOODLEY, D., PARBOOSING, R. & MOODLEY, P. 2014. Hepatitis B and HIV co-infection in pregnant women: indication for routine antenatal hepatitis B virus screening in a high HIV prevalence setting. *S Afr Med J,* 104**,** 307-9.

TRAN, T. T., TRINH, T. N. & ABE, K. 2008. New complex recombinant genotype of hepatitis B virus identified in Vietnam. *J Virol,* 82**,** 5657-63.

TSEBE, K. V., BURNETT, R. J., HLUNGWANI, N. P., SIBARA, M. M., VENTER, P. A. & MPHAHLELE, M. J. 2001. The first five years of universal hepatitis B vaccination in South Africa: evidence for elimination of HBsAg carriage in under 5-year-olds. *Vaccine,* 19**,** 3919-26.

UNAIDS 2017. WHO Global HIV and AIDS report 2017. Available from: http://www.unaids.org/en/resources/documents/2017/2017_data_book (Date accessed 21 August 2018).

UNITED STATES FOOD AND DRUG ADMINISTRATION. 2017. Hepatitis B and C Treatments. https://www.fda.gov/forpatients/illness/hepatitisbc/ucm408658.htm, Date accessed 30 November 2018.

VAN HELDEN, J., CORNELY, C., DATI, F., LEVY, H. R., BAL, T., SEEGER, M., WRIGHT, T. & BAKER, L. 2008. Performance evaluation of the ADVIA Centaur anti-HBe and HBeAg assays. *J Clin Virol,* 43**,** 169-75.

VAN HELDEN, J., DENOYEL, G., KARWOWSKA, S., REAMER, R., SCHMALZ, J., WRIGHT, T. & PREISEL-SIMMONS, B. 2004. Performance of hepatitis B assays on the Bayer ADVIA Centaur Immunoassay System. *Clin Lab,* 50**,** 63-73.

VARDAS, E., MATHAI, M., BLAAUW, D., MCANERNEY, J., COPPIN, A. & SIM, J. 1999. Preimmunization epidemiology of hepatitis B virus infection in South African children. *J Med Virol,* 58**,** 111-5.

VELKOV, S., OTT, J. J., PROTZER, U. & MICHLER, T. 2018. The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. *Genes (Basel),* 9.

VOS, G. H., ROSE, E. F. & MARIMUTHU, T. 1980. Hepatitis B antigen and antibodies in rural and urban Southern African blacks. *S Afr Med J,* 57**,** 868-70.

WEBER, R., SABIN, C. A., FRIIS-MOLLER, N., REISS, P., EL-SADR, W. M., KIRK, O., DABIS, F., LAW, M. G., PRADIER, C., DE WIT, S., AKERLUND, B., CALVO, G., MONFORTE, A., RICKENBACH, M., LEDERGERBER, B., PHILLIPS, A. N. & LUNDGREN, J. D. 2006. Liver-related deaths in persons infected with the human immunodeficiency virus: the D:A:D study. *Arch Intern Med,* 166**,** 1632-41.

WHO 2016. Global health sector strategy on viral hepatitis 2016-2021. Geneva, Switzerland: World Health Organization.

WHO 2017. Global Hepatitis report, 2017. Available from: https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/ [Date accessed 19 September 2018]. Geneva, Switzerland: World Health Organization.

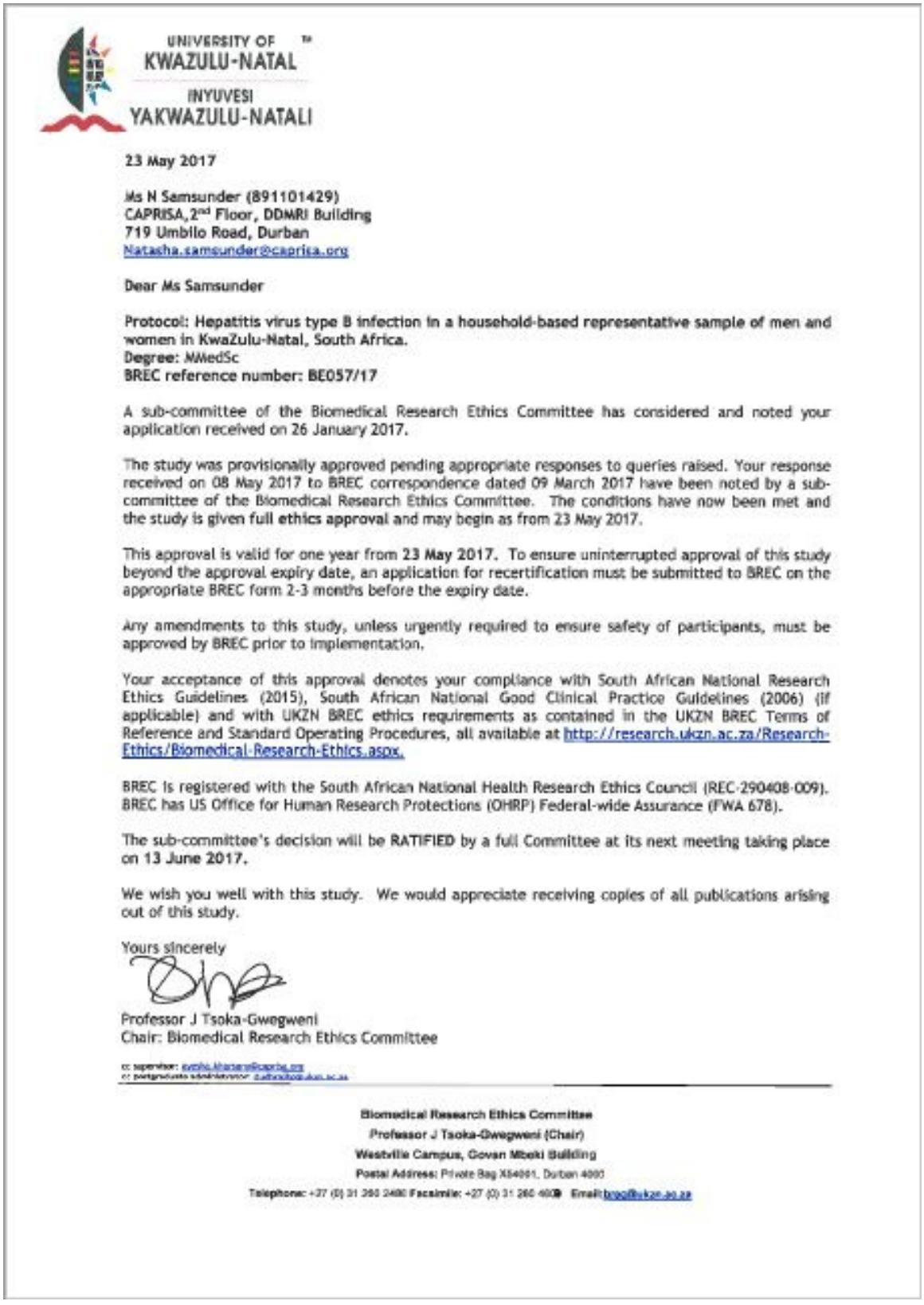
WILL, H., REISER, W., WEIMER, T., PFAFF, E., BUSCHER, M., SPRENGEL, R., CATTANEO, R. & SCHALLER, H. 1987. Replication strategy of human hepatitis B virus. *J Virol,* 61**,** 904-11.

WORLD HEALTH ORGANISATION. 2016. Global Health Sector Strategy on Viral Hepatitis 2016-2021. *Geneva, Switzerland* http://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf;jsessionid=161DC25381F15DFA568362716A5B4874?sequence=1**,** Date accessed 16 July 2018.

WORLD HEALTH ORGANISATION. 2017. Global Hepatitis Report. *Geneva, Switzerland,* http://www.who.int/iris/handle/10665/255016 Date accessed 21 July 2018.

APPENDICES

Appendix A: Initial ethics approval for the study



UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

23 May 2017

Ms N Samsunder (891101429)
CAPRISA, 2nd Floor, DDMRI Building
719 Umbilo Road, Durban
Natasha.samsunder@caprisa.org

Dear Ms Samsunder

Protocol: Hepatitis virus type B infection in a household-based representative sample of men and women in KwaZulu-Natal, South Africa.
Degree: MMedSc
BREC reference number: BE057/17

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 26 January 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 08 May 2017 to BREC correspondence dated 09 March 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 23 May 2017.

This approval is valid for one year from **23 May 2017.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **13 June 2017.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc supervisor:
cc postgraduate administrator:

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2480 Facsimile: +27 (0) 31 260 4609 Email:

Appendix B: Annual ethics recertification for the study

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

19 July 2018

Ms N Samsunder (891101429)
CAPRISA, 2nd Floor, DDMRI Building
719 Umbilo Road, Durban
Natasha.samsunder@caprisa.org

Dear Ms Samsunder

Protocol: Hepatitis virus type B infection in a household-based representative sample of men and women in KwaZulu-Natal, South Africa.
Degree: MMedSc
BREC reference number: BE057/17

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	23 May 2018
Expiration of Ethical Approval:	22 May 2019

I wish to advise you that your application for Recertification received on 24 May 2018 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its meeting taking place on 14 August 2018.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc supervisor: ayesha.kharsany@caprisa.org
cc postgraduate administrator: dudhrajhp@ukzn.ac.za

Appendix C: Turnitin report

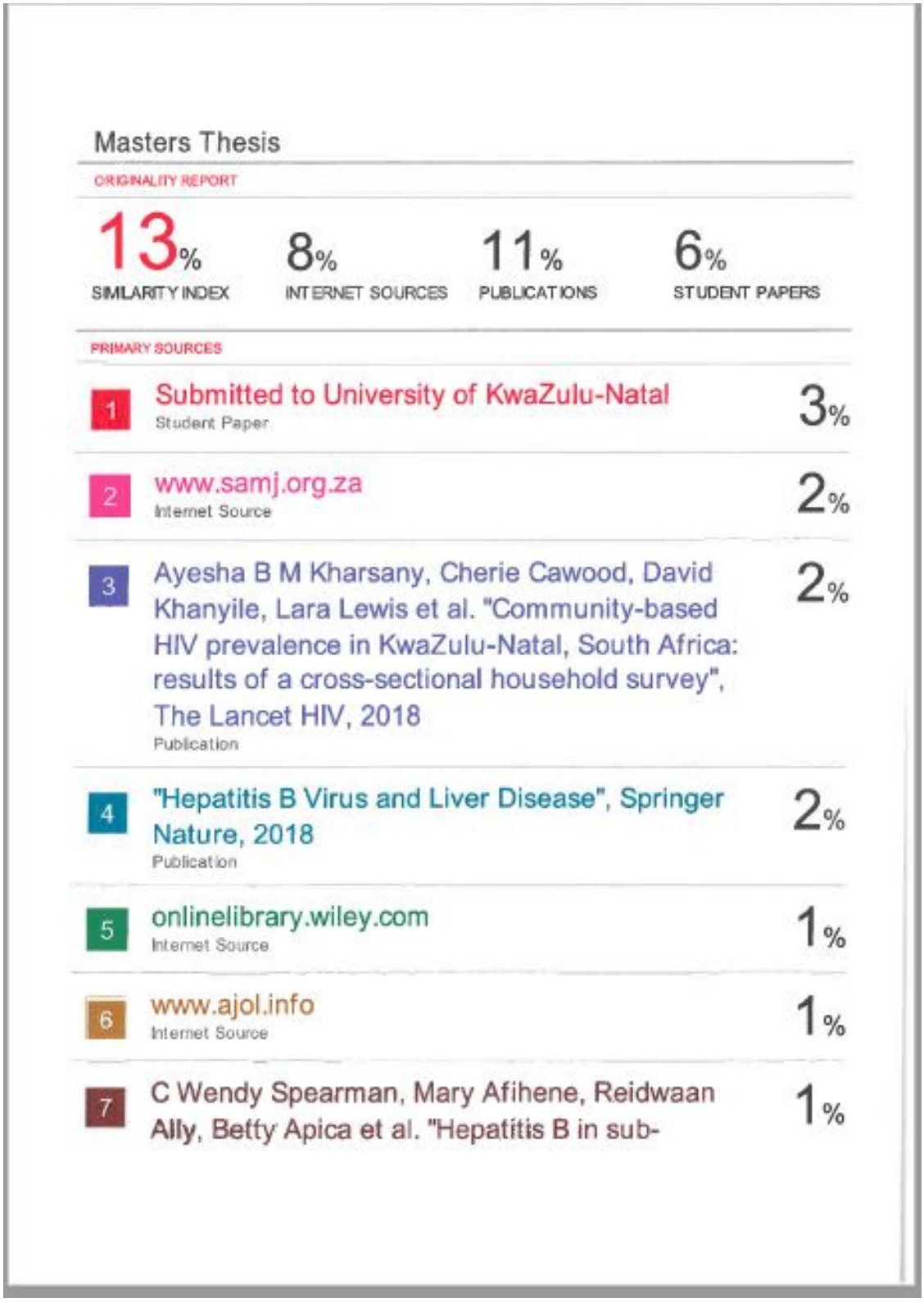
turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Natasha Samsunder
Assignment title:	Post graduate students
Submission title:	Masters Thesis
File name:	Thesis__Turnitin_format_20_Nov_...
File size:	1.38M
Page count:	43
Word count:	6,799
Character count:	36,820
Submission date:	20-Nov-2018 04:04PM (UTC+0200)
Submission ID:	1042586373



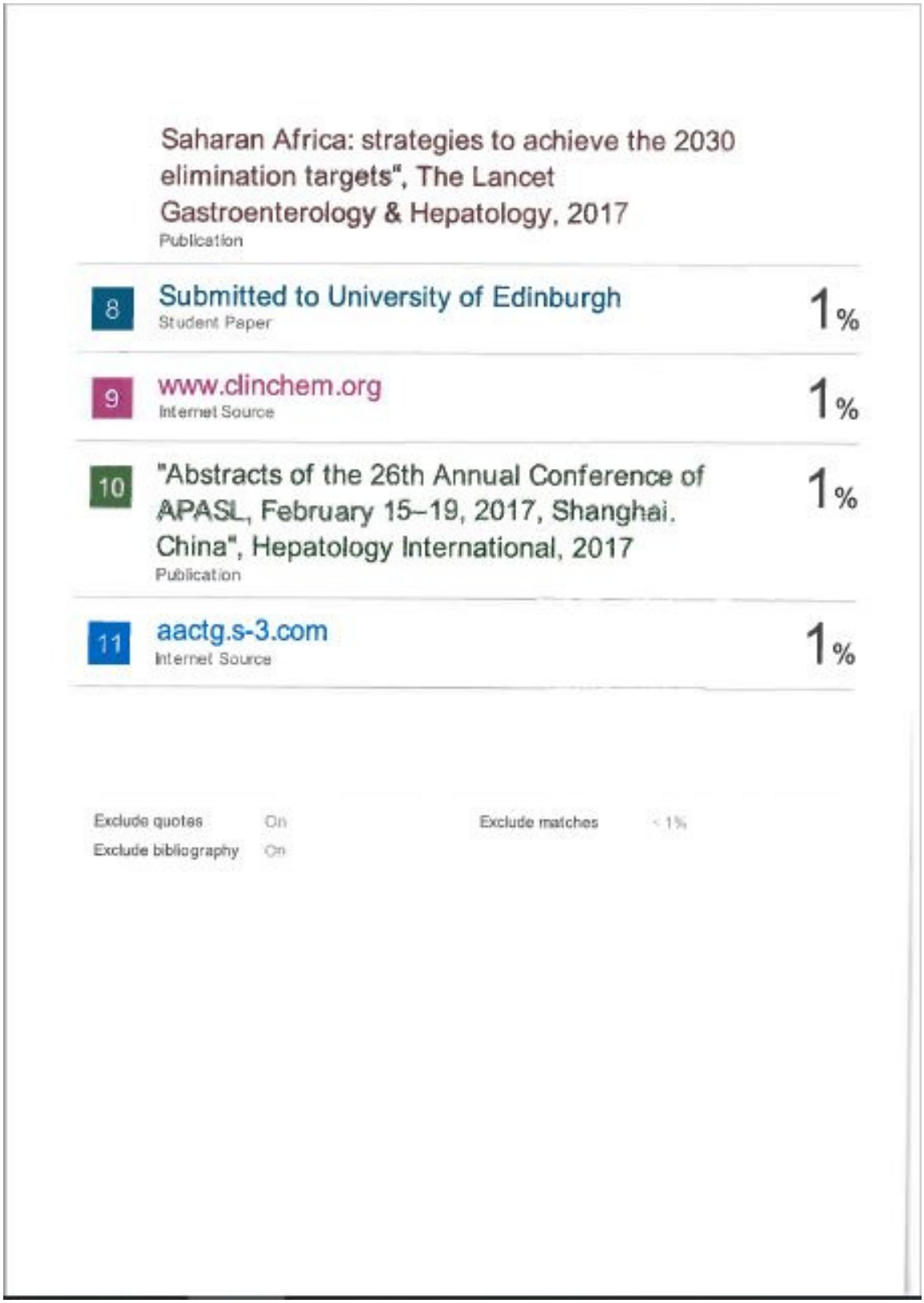
Masters Thesis

ORIGINALITY REPORT

13%	8%	11%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of KwaZulu-Natal Student Paper	3%
2	www.samj.org.za Internet Source	2%
3	Ayesha B M Kharsany, Cherie Cawood, David Khanyile, Lara Lewis et al. "Community-based HIV prevalence in KwaZulu-Natal, South Africa: results of a cross-sectional household survey", The Lancet HIV, 2018 Publication	2%
4	"Hepatitis B Virus and Liver Disease", Springer Nature, 2018 Publication	2%
5	onlinelibrary.wiley.com Internet Source	1%
6	www.ajol.info Internet Source	1%
7	C Wendy Spearman, Mary Afihene, Reidwaan Ally, Betty Apica et al. "Hepatitis B in sub-	1%



Saharan Africa: strategies to achieve the 2030 elimination targets", The Lancet Gastroenterology & Hepatology, 2017
Publication

8	Submitted to University of Edinburgh Student Paper	1%
9	www.clinchem.org Internet Source	1%
10	"Abstracts of the 26th Annual Conference of APASL, February 15–19, 2017, Shanghai, China", Hepatology International, 2017 Publication	1%
11	aactg.s-3.com Internet Source	1%

Exclude quotes On
Exclude bibliography On
Exclude matches < 1%