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Hepatitis B prevalence using residual sera from febrile rash surveillance in South Africa in 2018

Cuamba, Inocência Augusto

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HEPATITIS B PREVALENCE USING RESIDUAL SERA FROM FEBRILE RASH SURVEILLANCE IN SOUTH AFRICA IN 2018

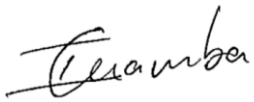
Inocência Augusto Cuamba

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Masters of Science in Medicine in the field of Vaccinology.

Johannesburg, 2021

DECLARATION

I Inocência Augusto Cuamba declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine in the field of Vaccinology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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08th day of April 2021 in Johannesburg

ABSTRACT

Hepatitis B is a significant public health issue caused by the hepatitis B virus. In 2017 there were 1.1 million new hepatitis B infections, most of them occurring in low and middle-income countries. A viral hepatitis strategy has been developed whereby countries are required to have updated information on the prevalence of hepatitis B and this data may be collected by ongoing surveillance. In South Africa, there is no active surveillance for hepatitis B amongst asymptomatic individuals in the general community. Therefore, in order to obtain estimates of the hepatitis B burden in South African children we have used residual samples from the active febrile rash surveillance conducted in 2018. We measured serological markers for hepatitis B. Of the included children, 0.2% (2/1010) were positive for hepatitis B surface antigen. Immunity due to vaccination was observed in 76% (770/1010) of the children. Of the analyzed samples 3% (30/1010) had immunity due to previous exposure, 1.1% (11/1010) had evidence of previous exposure by the presence of antibodies to hepatitis B core antigen and 20% (197/1010) had no evidence of previous exposure to hepatitis B. The results suggest that the current prevalence of hepatitis B infection is low in comparison to the period before implementation of hepatitis B immunization in South Africa.

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ACRONYMS

- **anti-HBc:** Antibodies against hepatitis B core antigen
- **anti-HBs:** Antibodies against hepatitis B surface antigen
- **DNA:** Deoxyribonucleic acid
- **EPI:** Expanded program on immunization
- **HBcAg:** Hepatitis B core antigen
- **HBeAg:** Hepatitis B e antigen
- **HBsAg:** Hepatitis B surface antigen
- **HBV:** Hepatitis B virus
- **HIV:** Human immunodeficiency virus
- **IgM:** Immunoglobulin M
- **NICD:** National Institute For Communicable Diseases
- **OBI:** Occult hepatitis B infection
- **WHO:** World health organization

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CHAPTER ONE

1.1. INTRODUCTION

Viral hepatitis, comprising hepatitis A, B, C, D, and E, is an international public health problem that is responsible for an estimated 1.4 million deaths per year, of which approximately 47% are due to hepatitis B (1). Viral hepatitis is also an increasing cause of mortality in people living with HIV, where 2.6 million people living with HIV are coinfected with the hepatitis B virus (1). According to the World Health Organization, in 2015, 257 million people were living with chronic hepatitis B infection which resulted in 887 000 deaths, Africa and Asia being the most affected continents (Figure 1.1.). As of 2016, only 10.5% of the individuals estimated to be infected with the hepatitis B virus knew that they were infected (2). The lack of knowledge of infection status may influence future disease incidence because of sustained transmission due to a lack of treatment of infected individuals.

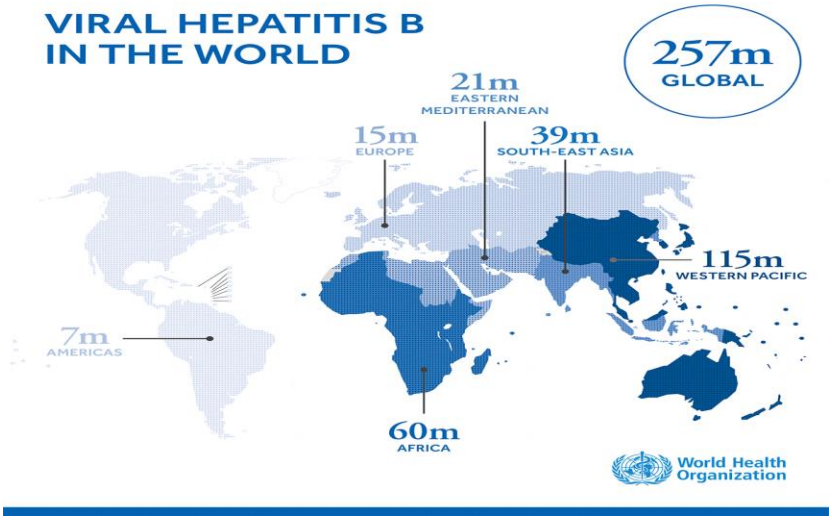

Figure 1.1. Chronic viral hepatitis B in the world in 2015 (3)

Hepatitis B is caused by the hepatitis B virus. The hepatitis B virus is a double-stranded DNA virus, belonging to the *Hepadnaviridae* family. Hepatitis B virus is mainly a hepatotropic virus that replicates in the liver and causes liver damage and dysfunction (4–6). The hepatitis B virus particle has an outer lipid envelope that contains proteins that are involved in viral binding and entry into susceptible cells. The virus also has an icosahedral nucleocapsid that encloses the viral DNA and DNA polymerase that has reverse transcriptase activity important for replication (7,8).

Hepatitis B virions are internalized by the host hepatocytes through endocytosis where the virions replicate and interfere with liver functions.

During infection, the host's immune system responds resulting in viral clearance, however, the immune system's response also results in hepatocellular damage (9). The virus-specific T helper cells and cytotoxic T lymphocytes eliminate the HBV infection either by killing infected cells or by producing cytokines that contribute to the inflammatory process or inhibit viral replication (10).

HBV has various antigenic components including, the hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), and hepatitis B e antigen (HBeAg) (11). The presence of hepatitis B surface antigen in the blood is indicative of acute or chronic HBV infection (12) and the presence of its respective antibody (anti-HBs), indicates recovery from natural infection or successful vaccination against HBV (13). Hepatitis B core antigen disappears from the blood early in the course of infection, however, the antibody against hepatitis B core antigen persists for life post-infection and the antibodies are not protective (14). Hepatitis B e antigen is only found in the blood when the hepatitis B virus is present (15) and is associated with active HBV replication and transmission of infection (16).

Blood and body fluids are the main vehicles of transmission of the Hepatitis B virus. Transmission can occur from mother to child transplacentally, during birth, or breastfeeding (17,18). Mother to child transmission accounts for 35%-40% of new hepatitis B cases worldwide (19) and it is considered to be one of the major causes of chronic hepatitis B infections as 80-90% of infants infected in the first year of life develop chronic infection (20). HBV may also be transmitted through transfusion of infected blood, through the use of unsafe injection practices especially amongst drug users and through sexual contact (21–23). Additionally, some articles also report the occurrence of transmission from health care workers to patients during medical procedures (24,25).

Infection by the hepatitis B virus may cause symptomatic or an asymptomatic infection and may result in acute hepatitis, chronic hepatitis, cirrhosis, and eventually, may progress to hepatocellular carcinoma (26). An acute infection normally lasts for 2 to 4 months and the case fatality rate is around 1% where the highest rates occur mainly in individuals aged 60 years or more (27). On the other hand, HBV chronic hepatitis is responsible for the majority of cases of HBV related

morbidity and mortality (28). A chronic hepatitis B viral infection may progress through various phases. There is an immune tolerant phase, where there is no liver disease, an immune clearance or immune active phase, where there is active liver inflammation, an inactive or non-replicative phase and in some individuals reactivation can occur (29). Hepatitis B reactivation is the appearance of HBV DNA in patients with past or chronic HBV infection, usually occurring in individuals with immunosuppression due to disease or immunosuppressive therapy (30,31).

Hepatitis B virus infection in children less than five years tends to be asymptomatic however many infant infections progress to chronic infection (32,33) and may result in premature death due to complications (34). Approximately 90% percent of HBV exposed infants may develop a chronic infection, as opposed to 5% to 10% of HBV exposed adults (35). Furthermore, individuals with chronic infections are the main reservoir for hepatitis B virus transmission (36). Hepatitis B signs and symptoms may occur in 30% to 50% of hepatitis B virus infected people who are more than 5 years of age (37). Symptomatic acute infection is characterized by non-specific symptoms such as myalgia, fever, headache, jaundice, fatigue, loss of appetite, nausea, vomiting, abdominal pain, dark urine, and joint pain (38). Symptoms may begin on average 90 days after infection with HBV and may last for weeks or up to 6 months (34).

Hepatitis B is a disease difficult to diagnose based only on clinical symptoms, therefore laboratory analysis is crucial to confirm the diagnosis. Diagnosis of hepatitis B is made by the biochemical assessment of the liver function and confirmed through detection in sera of specific viral antigens or antibodies. The specific viral antigens and antibodies that can be detected in serum are HBsAg, HBeAg, anti-HBsAg, anti-HBcAg, and antibody to HBeAg. Within 10 weeks after exposure to the hepatitis B virus, HBsAg can be detected in serum and persistence of the antigen in serum for periods longer than 6 months may be indicative of chronic infection (16). As shown in Table 1.1., these hepatitis B serological markers can be used to distinguish an acute infection, chronic infection, immunity due to natural exposure and immunity due to vaccination (39,40). Additionally, molecular tests are also available to detect and measure hepatitis B virus DNA (41), however, these are mainly used for research purposes.

Table 1.1. Interpretation of serological markers (39)

HBsAg	IgM anti-HBc	Total anti-HBc	anti-HBs	Interpretation
Negative	Negative	Negative	Negative	Never infected, Susceptible
Negative	Negative	Negative	Positive (≥10mlU/ml)	Immune due to immunization
Negative	Negative	Positive	Positive	Immune due to natural infection
Positive	Negative	Negative	Negative	Early acute infection
Positive	Positive	Positive	Negative	Acute infection
Positive	Negative	Positive	Negative	Chronic infection

Depending on symptoms, individuals with acute infection are provided with supportive treatment and individuals with chronic infection can be provided antiviral therapy (34,42). Individuals with acute infection clear the infection within weeks to months, however, children compared to adults are less likely to clear the infection. Only a proportion of individuals with chronic infection require therapy, however, in most people the treatment does not cure the infection, it only suppresses viral replication thus the medication must be continued for life (20). Individuals who resolve the HBV infection develop specific antibodiesagainst the virus and thus acquire natural immunity.

Geographically, before the widespread global use of the hepatitis B vaccine, the prevalence of chronic hepatitis B virus infection could vary from high (>8%) or intermediate (2-8%) to low (<2%) (43). Prior to 1995, which was when the hepatitis B vaccine was introduced into the Expanded Program on Immunisation (EPI) schedule (44), South Africa was classified as having a high prevalence (45). A study conducted in the Eastern Cape during the period before the introduction of the hepatitis B vaccine showed that 10.4% of study participants (who were children below six years of age), were positive for HBsAg (46). In rural black populations, hepatitis B infection was reported to be acquired early in childhood with a slight increase in infection rates occurring at school-going age (47).

Since 1982, safe and effective vaccines against hepatitis B have been available (48). The available hepatitis B vaccine contains recombinant HBsAg (49). The vaccine is administered in 3 doses and immunity may last for twenty years or more (50). In South Africa, hepatitis B vaccine is administered at six, ten, and fourteen weeks. The World Health Organisation recommends a hepatitis B vaccine birth dose within twenty four hours of birth, however, South Africa has not yet started administering a birth dose for hepatitis B. A hepatitis B vaccine birth dose helps prevent mother to child HBV transmission (51) and when given at birth followed by at least two subsequent doses, the hepatitis B vaccine is 90% effective in preventing perinatal transmission of HBV (52).

The known marker of immunity to the hepatitis B virus is anti-HBs (53), and an individual having an anti-HBs concentration of ≥10mlU/ml is considered immune. Immunity against hepatitis B can give protection against infection and disease. Protection against infection is associated with antibody persistence, and protection against disease is associated with immune memory which is maintained even after antibodies wane (54).

Information on the impact of hepatitis B immunization in South Africa suggests a decline in hepatitis B virus infection when comparing the period before and after the introduction of immunization against hepatitis B (55,56). In an outbreak report from an academic hospital in South Africa, 12% of the children tested from 2011 to 2013 were confirmed to have a hepatitis B virus infection (57). This study was hospital-based therefore might not represent the prevalence in the community. A prevalence of 0.4% was reported from samples collected in 2013 from children below 15 years of age (58), which is more indicative of the prevalence in the community. Although there has been a reduction in the prevalence of hepatitis B, the above data suggest that the hepatitis B virus is still prevalent amongst South African children.

The burden of HIV infections in South Africa reported as 2.7% in children 0-14 years and 20.6% in individuals 15-49 years of age (59), also creating concern regarding the prevalence and effect of coinfection of HIV and hepatitis B virus (60,61). There is evidence suggesting that HIV infected individuals, in comparison to non-HIV infected individuals, are more likely to be infected with the hepatitis B virus and more likely to become hepatitis B virus chronic carriers (62). Although the global and local implementation of the vaccine assisted in reducing the burden of disease (63,64), in 2017 there were 1.1 million hepatitis B new infections globally (65), most of them occurring in

low and middle-income countries (66), indicating that hepatitis B virus is still a public health concern.

Concerns regarding the impact of hepatitis on public health led to the development of a viral hepatitis strategy by the WHO, as part of the 2030 Agenda for Sustainable Development. The goal of this strategy is to eliminate viral hepatitis as a major public health threat by 2030. The set targets, amongst others, are to reduce new infections by 90%, reduce deaths by 65%, and have a prevalence equivalent to or lower than 0.1% HBsAg in children (1). As part of the strategy, countries are required to have updated data on the prevalence of hepatitis B and this data may be collected by ongoing surveillance. Conducting hepatitis B surveillance is also important for obtaining accurate prevalence data (67) which can be used to guide different public health interventions. Conducting seroprevalence surveys at certain intervals is also essential in order to understand the impact of vaccination over the years (68).

In South Africa, hepatitis B is a notifiable medical condition with a passive surveillance system that allows the collation of diagnostic hepatitis B laboratory test results. There is no active surveillance for hepatitis B in South Africa amongst asymptomatic individuals in the general community. Regarding active surveillance, febrile rash surveillance for measles is done in South Africa, whereby blood is collected from patients who present at a health facility with fever, morbilliform rash, and, either cough, coryza or conjunctivitis. The samples are sent to the National Institute for Communicable Diseases (NICD) where they are tested for measles and rubella.

In order to estimate the hepatitis B burden in South African children below the age of 15 years, we used residual negative samples from the febrile rash surveillance conducted in 2018. These samples represent a cross-section of otherwise healthy individuals with acute infectious illness and are thus largely representative of the general population.

1.2. STUDY OBJECTIVES

Main objective

- To estimate the prevalence of hepatitis B in children ≤ 15 years of age in South Africa.

Secondary objective

- To determine the proportion of immune children ≤ 15 years of age through natural exposure or immunization

CHAPTER TWO

2.1. METHODS

2.1.1. Study population

The samples used in this project were collected from children less than or equal to 15 years of age as part of the community based febrile rash surveillance for measles. The clinical case definition for febrile rash illness is any person in whom a clinician suspects measles infection, or any person with fever and maculopapular rash and cough, coryza, or conjunctivitis. The febrile rash surveillance for measles is conducted in hospitals and clinics of all nine provinces (namely, Eastern Cape, Free State, Gauteng, Kwazulu Natal, Limpopo, Mpumalanga, North West, Northern Cape, and Western Cape) of South Africa.

2.1.2. Sample size and selection

Through the community-based febrile rash surveillance conducted for measles in 2018, 1911 samples were collected from different provinces and sent to NICD. Of the collected samples some were excluded due to positivity to either measles or rubella and due to insufficient residual sample, leaving for analysis 1010 samples. The samples were stored following analysis for the detection of measles and rubella. Those which tested negative for both measles and rubella were analyzed to determine the burden of hepatitis B. Estimating a hepatitis B prevalence of approximately 0.4% in children under 15 years of age (58), a minimum sample size of 265 was calculated to be necessary to estimate the burden of disease at a 99% confidence level with a margin of error of +/- 1% (calculation done using Open epi online). However, to increase statistical power no sampling was conducted, and all available specimens were tested.

2.1.3. Laboratory assays

Samples were stored at -20°C. In order to test the samples for hepatitis B serological markers, the samples were thawed at room temperature and vortexed before testing. Serological markers for hepatitis B (HBsAg, anti-HBs and anti-HBc) were measured using the ARCHITECT instrument (Abbott, Germany).

For quality control calibration procedures were conducted between lots. In order to verify the calibration, controls for each assay were processed daily before running samples.

Detection of hepatitis B surface antigen was done using commercial assays (ARCHITECT HBsAg Qualitative assay, Abbot). This is an immunoassay for the qualitative detection of HBsAg in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. The HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate.

For the detection of antibodies against hepatitis B surface antigen and core antigen the ARCHITECT anti-HBs and anti-HBc assay (Abbot) were used. These are also immunoassays and they use chemiluminescent microparticle immunoassay technology for the quantitative determination of anti-HBs and anti-HBc in human serum and plasma. Here the sample and recombinant HBsAg or recombinant HBcAg coated paramagnetic microparticles are combined. The specific antibodies present in the samples bind to the recombinant coated microparticles. After washing, the acridinium-labeled conjugate is added.

These assays result in a chemiluminescent reaction which is measured as relative light units. The chemiluminescent signal in the reaction is compared to the cutoff signal determined from an active calibration curve.

HBsAg and anti-HBc values equal to or greater than 1.00 Sample/Cutoff (S/CO) were considered positive. Anti-HBs titres were reported as milli-international units per ml (mIU/ml) and samples with values equal to or greater than 10mIU/ml were considered positive.

2.1.4. Data analyses

Data entry and analyses were performed using Microsoft Excel and Stata. The list of samples and their respective details were obtained from the NICD database. Serum samples with positive hepatitis B surface antigen were considered infected. Individuals with antibodies against hepatitis B surface antigen only were considered immune due to vaccination, individuals with antibodies against both hepatitis B surface antigen and hepatitis B core antigen were considered immune due to natural exposure.

After obtaining the number of samples that were positive for hepatitis B, we calculated the lower and upper confidence intervals with a binomial exact calculation using an online calculator (https//.sample-size.net, University of California San Francisco Clinical and Translational Science Institute) (69). We considered our obtained number of positives (2) as the numerator, the total number of samples (1010) as the denominator and a confidence interval of 99%.

2.1.5. Ethics

Ethical clearance for the purpose of this research project was obtained from the University of Witwatersrand Human Research Ethics Committee. Certificate number: M200169 (Appendix A)

CHAPTER THREE

3.1. RESULTS

3.1.1. Demographics

A total of 1010 samples were analyzed. Of these 429 were female, 555 were male and 26 did not have their gender registered. Samples were from all provinces of South Africa being most from 5 provinces (80%[803/1010]) namely, Western Cape, Mpumalanga, Eastern Cape, Gauteng and Kwazulu Natal. Majority of the patients from whom these samples were taken were aged between 0-5 years (62% [624/1010]) (Table 3.1.).

Table 3.1. Description of the study population

Gender distribution		
	n	**%**
Females	429	42
Males	555	55
Unknown	26	3
Total	**1010**	**100**
Provincial distribution		
	n	**%**
Unknown	1	0
Free State	43	4
Limpopo	50	5
Northern Cape	56	6
North West	57	6
Western Cape	123	12
Mpumalanga	138	14
Eastern Cape	152	15
Gauteng	191	19
Kwazulu Natal	199	20
Total	**1010**	**100**
Age distribution		
	n	**%**
0 - 11 months	91	9
1 - 5 years of age	533	52.8
6 - 10 years of age	304	30.1

11 - 15 years of age	82	8.1
Total	**1010**	**100**

3.1.2. Hepatitis B serological results

Of the included children, 0.2% (2/1010, 99% CI: 0.1%-0.9%) were positive for HBsAg. One was a 12-year-old female from the North West (also positive to anti-HBc) and the other was a 2-month-old male from the Western Cape. Immunity due to vaccination, where samples were anti-HBs positive and anti-HBc negative, was observed in 76% (770/1010) of the children. Of the analyzed samples 3% (30/1010) were positive for both Anti-HBs and Anti-HBc, indicating immunity due to previous natural infection. The analyzed samples also included 1.1% (11/1010) of individuals positive only to Anti-HBc and 20% (197/1010) without any hepatitis B serological marker (Table 3.2).

Table 3.2. Hepatitis B prevalence and immunity results stratified by age

	Stratified by age (n)								**Total**	
	0 -11 months		**1 - 5 years**		**6 - 10 years**		**11 - 15 years**			
	n	%	n	%	n	%	n	%	n	%
HBsAg positive (infected)	1	1.1	0	0.0	0	0.0	1	1.2	2	0.2
Immune due to vaccination (anti-HBs positive)	70	77	465	87	195	64	40	49	770	76
Immune due to exposure (anti-HBs positive and anti-HBc positive)	5	5.5	12	2.3	12	3.9	1	1.2	30	3
Evidence of previous exposure (only anti-HBc positive)	0	0	4	0.8	6	2.0	1	1.2	11	1.1
No evidence of exposure or vaccination (negative to all serological markers)	15	16	52	9.8	91	30	39	48	197	20
Total	**91**	**100**	**533**	**100**	**304**	**100**	**82**	**100**	**1010**	**100**

3.2. DISCUSSION

Our study showed a prevalence of 0.2% (99% CI: 0.1%-0.9%) for hepatitis B surface antigen amongst children aged 0 to 15 years. This result is comparable to the 0.4% previously reported from children under 15 years of age in South Africa using residual samples from community-based febrile rash surveillance (58). Our findings add an estimate of the current hepatitis B burden in South African children and indicate that the prevalence is low in this age group. The low prevalence may be attributed to the implementation of hepatitis B vaccination in the South African

expanded program for Immunisation. Previous studies have reported that giving HBV vaccine as routine immunization is an effective tool for reducing the burden of hepatitis B amongst the population (70,71) and our figures likely reflect the declining hepatitis B incidence in women of childbearing age due to vaccination as children.

The use of the hepatitis B vaccine in the expanded program for immunization results in protective anti-HBs antibody titers in more than 95% of vaccinees, but with time the antibody prevalence reduces (54). Previous studies that looked at long term immunity after infant hepatitis B vaccination show that anti-HBs results vary greatly between 7% to 78% (72). In our study population, vaccine-induced immunity (anti-HBs levels ≥10 mIU/ml) was 76%. Vaccine-induced immunity levels in the community can be influenced by waning immunity, the presence of vaccine non-responders due to various factors (age, obesity, chronic illnesses), the existence of people that have not been vaccinated (58), and non-completion of vaccine doses. In this study we observed that the number of individuals immune due to vaccination reduced after the age of 5 which could be due to waning immunity since children in South Africa receive the hepatitis B vaccine in their first year of life. According to the WHO (73), hepatitis B vaccine coverage was 82% in 2018 and 88% in 2000, showing that there probably was a considerable number of people in the population that was unvaccinated. Waning immunity does not indicate a lack of protection since protection can occur due to immune memory even after anti-HBs levels decrease to below 10 mIU/ml (54). A study showed that despite being seronegative 15 years after vaccination, upon administration of a booster dose, 70% of the children presented an anamnestic response (74), thus showing that being seronegative does not necessarily mean that the individual is susceptible.

In our analysis, we did not compare the prevalence in the different provinces because of the number of samples available per province. Due to the high prevalence of HIV in South Africa, it would have also been of interest to compare the hepatitis B results of the children with their HIV status, however, we did not have ethical clearance to test for HIV. There is evidence that chronic HBV and HIV coinfection is associated with long term morbidity and mortality that exceeds the impact of infection with either one of these viruses (75).

A small percentage (1.1%) of our study population was positive only to anti-HBc, indicating that they had previous exposure to the hepatitis B virus and 20% of our study population had no evidence of previous exposure to HBV. Individuals positive only to anti-HBc can be infectious if they have hepatitis B viral DNA in their serum and the presence of anti-HBc only can be an indicator of occult hepatitis B viral infection (76). We did not look for hepatitis B viral DNA, however, a previous study conducted in South Africa found that 6.5% of unvaccinated infants that were serologically negative to HBV infection had HBV DNA in their serum. Thus, performing detection of HBV DNA could be of benefit for the identification of cases of occult hepatitis B infection in our population and therefore providing more accurate data on the burden of hepatitis B.

3.3. CONCLUSION

Twenty-three years after the introduction of the hepatitis B vaccine in the expanded program on immunization, we report a prevalence of HBsAg of 0.2% in South African children aged 15 years or less. We also report that 76% of the children included in the study had immunity to hepatitis B as a result of immunization, 3% had immunity to HBV due to previous exposure and 1.1% had evidence of previous exposure to HBV.

We also show that it is possible to obtain disease burden estimates based on residual sera from febrile rash surveillance especially in a context where active surveillance for hepatitis B is not being conducted. Febrile rash surveillance is routine in most African countries and thus may provide a convenience sample for estimating hepatitis B prevalence in children in other countries. Expansion of hepatitis B surveillance will assist in verifying the achievement of hepatitis B control goals.

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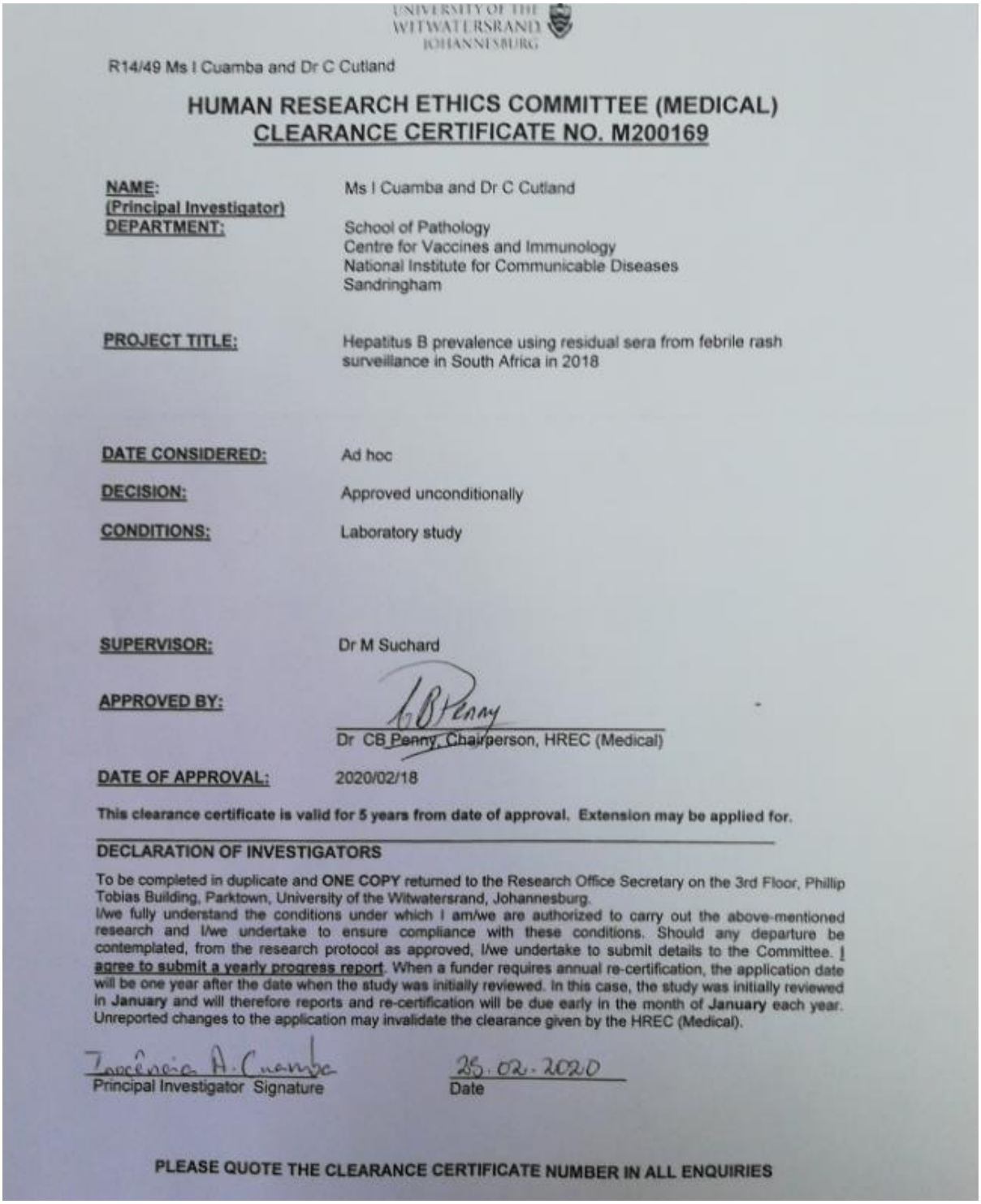
5. APPENDICES

APPENDIX A: Ethics clearance certificate



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Ms I Cuamba and Dr C Cutland

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200169

NAME: (Principal Investigator)	Ms I Cuamba and Dr C Cutland
DEPARTMENT:	School of Pathology Centre for Vaccines and Immunology National Institute for Communicable Diseases Sandringham
PROJECT TITLE:	Hepatitus B prevalence using residual sera from febrile rash surveillance in South Africa in 2018
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Laboratory study
SUPERVISOR:	Dr M Suchard
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/02/18

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **January** and will therefore reports and re-certification will be due early in the month of **January** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Inocência A. Cuamba
Principal Investigator Signature

25.02.2020
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

APPENDIX B: Raw data

Table 5.1. Samples with evidence of exposure to hepatitis B virus

Sample number	ASSAY	RESULT	Repeat HBsAg	INTERPRETATION	HBsAg positive	Immune due to vaccination	Immune due to Infection	No marker
CVI00045/18-SE	Anti-HBs 3	2.89 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00045/18-SE	Anti-HBcII	1.89 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00045/18-SE	HBsAgQ2	0.30 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI00088/18-SE	Anti-HBs 3	32.21 mIU/mL		Reactive	No	No	Yes	No
CVI00088/18-SE	Anti-HBcII	1.06 S/CO		Reactive	No	No	Yes	No
CVI00088/18-SE	HBsAgQ2	0.42 S/CO		Nonreactive	No	No	Yes	No
CVI00107/18-SE	Anti-HBs 3	669.10 mIU/mL		Reactive	No	No	Yes	No
CVI00107/18-SE	Anti-HBcII	1.23 S/CO		Reactive	No	No	Yes	No
CVI00107/18-SE	HBsAgQ2	0.17 S/CO		Nonreactive	No	No	Yes	No
CVI00151/18-SE	Anti-HBs 3	0.84 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00151/18-SE	Anti-HBcII	1.08 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00151/18-SE	HBsAgQ2	0.24 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI00237/18-SE	Anti-HBs 3	433.64 mIU/mL		Reactive	No	No	Yes	No
CVI00237/18-SE	Anti-HBcII	3.99 S/CO		Reactive	No	No	Yes	No
CVI00237/18-SE	HBsAgQ2	0.29 S/CO		Nonreactive	No	No	Yes	No
CVI00325/18-SE	Anti-HBs 3	0.00 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00325/18-SE	Anti-HBcII	1.30 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00325/18-SE	HBsAgQ2	0.25 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI00345/18-SE	Anti-HBs 3	0.31 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00345/18-SE	Anti-HBcII	1.39 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00345/18-SE	HBsAgQ2	0.23 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI00407/18-SE	Anti-HBs 3	22.84 mIU/mL		Reactive	No	No	Yes	No

CVI00407/18-SE	Anti-HBcII	8.81 S/CO		Reactive	No	No	Yes	No
CVI00407/18-SE	HBsAgQ2	0.22 S/CO		Nonreactive	No	No	Yes	No
CVI00434/18-SE	Anti-HBs 3	1.09 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00434/18-SE	Anti-HBcII	1.05 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00434/18-SE	HBsAgQ2	0.27 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI00467/18-SE	Anti-HBs 3	64.86 mIU/mL		Reactive	No	No	Yes	No
CVI00467/18-SE	Anti-HBcII	1.26 S/CO		Reactive	No	No	Yes	No
CVI00467/18-SE	HBsAgQ2	0.32 S/CO		Nonreactive	No	No	Yes	No
CVI00475/18-SE	Anti-HBs 3	192.64 mIU/mL		Reactive	No	No	Yes	No
CVI00475/18-SE	Anti-HBcII	1.11 S/CO		Reactive	No	No	Yes	No
CVI00475/18-SE	HBsAgQ2	0.74 S/CO		Nonreactive	No	No	Yes	No
CVI00576/18-SE	Anti-HBs 3	170.20 mIU/mL		Reactive	No	No	Yes	No
CVI00576/18-SE	Anti-HBcII	1.15 S/CO		Reactive	No	No	Yes	No
CVI00576/18-SE	HBsAgQ2	0.29 S/CO		Nonreactive	No	No	Yes	No
CVI00600/18-SE	Anti-HBs 3	266.23 mIU/mL		Reactive	No	No	Yes	No
CVI00600/18-SE	Anti-HBcII	2.74 S/CO		Reactive	No	No	Yes	No
CVI00600/18-SE	HBsAgQ2	0.13 S/CO		Nonreactive	No	No	Yes	No
CVI00695/18-SE	Anti-HBs 3	47.83 mIU/mL		Reactive	No	No	Yes	No
CVI00695/18-SE	Anti-HBcII	1.05 S/CO		Reactive	No	No	Yes	No
CVI00695/18-SE	HBsAgQ2	0.24 S/CO		Nonreactive	No	No	Yes	No
CVI00703/18-SE	Anti-HBs 3	66.68 mIU/mL		Reactive	No	No	Yes	No
CVI00703/18-SE	Anti-HBcII	1.28 S/CO		Reactive	No	No	Yes	No
CVI00703/18-SE	HBsAgQ2	0.23 S/CO		Nonreactive	No	No	Yes	No
CVI00762/18-SE	Anti-HBs 3	0.93 mIU/mL		Nonreactive	Yes	No	No	No
CVI00762/18-SE	Anti-HBcII	1.09 S/CO		Reactive	Yes	No	No	No
CVI00762/18-SE	HBsAgQ2	6072.46 S/CO	6741.14 S/CO	Reactive	Yes	No	No	No

CVI00828/18-SE	Anti-HBs 3	> 1000.00 mIU/mL		Reactive	No	No	Yes	No
CVI00828/18-SE	Anti-HBcII	1.12 S/CO		Reactive	No	No	Yes	No
CVI00828/18-SE	HBsAgQ2	0.22 S/CO		Nonreactive	No	No	Yes	No
CVI00829/18-SE	Anti-HBs 3	435.08 mIU/mL		Reactive	No	No	Yes	No
CVI00829/18-SE	Anti-HBcII	1.45 S/CO		Reactive	No	No	Yes	No
CVI00829/18-SE	HBsAgQ2	0.30 S/CO		Nonreactive	No	No	Yes	No
CVI00847/18-SE	Anti-HBs 3	> 1000.00 mIU/mL		Reactive	No	No	Yes	No
CVI00847/18-SE	Anti-HBcII	1.03 S/CO		Reactive	No	No	Yes	No
CVI00847/18-SE	HBsAgQ2	0.20 S/CO		Nonreactive	No	No	Yes	No
CVI00972/18-SE	Anti-HBs 3	0.77 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI00972/18-SE	Anti-HBcII	2.02 S/CO		Reactive	No	No	No	Only anti-HBc
CVI00972/18-SE	HBsAgQ2	0.29 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI01026/18-SE	Anti-HBs 3	16.21 mIU/mL		Reactive	No	No	Yes	No
CVI01026/18-SE	Anti-HBcII	1.21 S/CO		Reactive	No	No	Yes	No
CVI01026/18-SE	HBsAgQ2	0.22 S/CO		Nonreactive	No	No	Yes	No
CVI01061/18-SE	Anti-HBs 3	> 1000.00 mIU/mL		Reactive	No	No	Yes	No
CVI01061/18-SE	Anti-HBcII	4.32 S/CO		Reactive	No	No	Yes	No
CVI01061/18-SE	HBsAgQ2	0.22 S/CO		Nonreactive	No	No	Yes	No
CVI01150/18-SE	Anti-HBs 3	20.33 mIU/mL		Reactive	No	No	Yes	No
CVI01150/18-SE	Anti-HBcII	9.86 S/CO		Reactive	No	No	Yes	No
CVI01150/18-SE	HBsAgQ2	0.19 S/CO		Nonreactive	No	No	Yes	No
CVI01318/18-SE	Anti-HBs 3	657.95 mIU/mL		Reactive	No	No	Yes	No
CVI01318/18-SE	Anti-HBcII	1.44 S/CO		Reactive	No	No	Yes	No
CVI01318/18-SE	HBsAgQ2	0.26 S/CO		Nonreactive	No	No	Yes	No
CVI01492/18-SE	Anti-HBs 3	136.94 mIU/mL		Reactive	No	No	Yes	No
CVI01492/18-SE	Anti-HBcII	1.29 S/CO		Reactive	No	No	Yes	No

CVI01492/18-SE	HBsAgQ2	0.23 S/CO		Nonreactive	No	No	Yes	No
CVI01493/18-SE	Anti-HBs 3	37.50 mIU/mL		Reactive	No	No	Yes	No
CVI01493/18-SE	Anti-HBcII	2.14 S/CO		Reactive	No	No	Yes	No
CVI01493/18-SE	HBsAgQ2	0.29 S/CO		Nonreactive	No	No	Yes	No
CVI01639/18-SE	Anti-HBs 3	1.27 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI01639/18-SE	Anti-HBcII	1.17 S/CO		Reactive	No	No	No	Only anti-HBc
CVI01639/18-SE	HBsAgQ2	0.29 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI01645/18-SE	Anti-HBs 3	622.33 mIU/mL		Reactive	No	No	Yes	No
CVI01645/18-SE	Anti-HBcII	1.41 S/CO		Reactive	No	No	Yes	No
CVI01645/18-SE	HBsAgQ2	0.23 S/CO		Nonreactive	No	No	Yes	No
CVI01682/18-SE	Anti-HBs 3	> 1000.00 mIU/mL		Reactive	No	No	Yes	No
CVI01682/18-SE	Anti-HBcII	3.38 S/CO		Reactive	No	No	Yes	No
CVI01682/18-SE	HBsAgQ2	0.24 S/CO		Nonreactive	No	No	Yes	No
CVI01695/18-SE	Anti-HBs 3	> 1000.00 mIU/mL		Reactive	No	No	Yes	No
CVI01695/18-SE	Anti-HBcII	1.07 S/CO		Reactive	No	No	Yes	No
CVI01695/18-SE	HBsAgQ2	0.27 S/CO		Nonreactive	No	No	Yes	No
CVI01709/18-SE	Anti-HBs 3	409.34 mIU/mL		Reactive	No	No	Yes	No
CVI01709/18-SE	Anti-HBcII	8.33 S/CO		Reactive	No	No	Yes	No
CVI01709/18-SE	HBsAgQ2	0.19 S/CO		Nonreactive	No	No	Yes	No
CVI01712/18-SE	Anti-HBs 3	3.63 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI01712/18-SE	Anti-HBcII	1.27 S/CO		Reactive	No	No	No	Only anti-HBc
CVI01712/18-SE	HBsAgQ2	0.20 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI02038/18-SE	Anti-HBs 3	54.49 mIU/mL		Reactive	No	No	Yes	No
CVI02038/18-SE	Anti-HBcII	1.76 S/CO		Reactive	No	No	Yes	No
CVI02038/18-SE	HBsAgQ2	0.30 S/CO		Nonreactive	No	No	Yes	No
CVI02055/18-SE	Anti-HBs 3	0.78 mIU/mL		Nonreactive	No	No	No	Only anti-HBc

CVI02055/18-SE	Anti-HBcII	1.17 S/CO		Reactive	No	No	No	Only anti-HBc
CVI02055/18-SE	HBsAgQ2	0.27 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI02366/18-SE	Anti-HBs 3	1.36 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI02366/18-SE	Anti-HBcII	1.08 S/CO		Reactive	No	No	No	Only anti-HBc
CVI02366/18-SE	HBsAgQ2	0.33 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI02454/18-SE	Anti-HBs 3	0.88 mIU/mL		Nonreactive	Yes	No	No	No
CVI02454/18-SE	Anti-HBcII	0.29 S/CO		Nonreactive	Yes	No	No	No
CVI02454/18-SE	HBsAgQ2	2.74 S/CO	2.05 S/CO	Reactive	Yes	No	No	No
CVI02524/18-SE	Anti-HBs 3	64.85 mIU/mL		Reactive	No	No	Yes	No
CVI02524/18-SE	Anti-HBcII	1.00 S/CO		Reactive	No	No	Yes	No
CVI02524/18-SE	HBsAgQ2	0.26 S/CO		Nonreactive	No	No	Yes	No
CVI02575/18-SE	Anti-HBs 3	300.39 mIU/mL		Reactive	No	No	Yes	No
CVI02575/18-SE	Anti-HBcII	2.40 S/CO		Reactive	No	No	Yes	No
CVI02575/18-SE	HBsAgQ2	0.40 S/CO		Nonreactive	No	No	Yes	No
CVI02842/18-SE	Anti-HBs 3	361.72 mIU/mL		Reactive	No	No	Yes	No
CVI02842/18-SE	Anti-HBcII	1.09 S/CO		Reactive	No	No	Yes	No
CVI02842/18-SE	HBsAgQ2	0.22 S/CO		Nonreactive	No	No	Yes	No
CVI02870/18-SE	Anti-HBs 3	0.38 mIU/mL		Nonreactive	No	No	No	Only anti-HBc
CVI02870/18-SE	Anti-HBcII	2.28 S/CO		Reactive	No	No	No	Only anti-HBc
CVI02870/18-SE	HBsAgQ2	0.26 S/CO		Nonreactive	No	No	No	Only anti-HBc
CVI03090/18-SE	Anti-HBs 3	15.59 mIU/mL		Reactive	No	No	No	No
CVI03090/18-SE	Anti-HBcII	0.41 S/CO		Nonreactive	No	No	No	No
CVI03090/18-SE	HBsAgQ2	0.17 S/CO		Nonreactive	No	No	No	No
CVI03521/18-SE	Anti-HBs 3	151.81 mIU/mL		Reactive	No	No	Yes	No
CVI03521/18-SE	Anti-HBcII	1.19 S/CO		Reactive	No	No	Yes	No
CVI03521/18-SE	HBsAgQ2	0.26 S/CO		Nonreactive	No	No	Yes	No

CVI03822/18-SE	Anti-HBs 3	381.80 mIU/mL		Reactive	No	No	Yes	No
CVI03822/18-SE	Anti-HBcII	1.05 S/CO		Reactive	No	No	Yes	No
CVI03822/18-SE	HBsAgQ2	0.18 S/CO		Nonreactive	No	No	Yes	No
CVI03856/18-SE	Anti-HBs 3	602.08 mIU/mL		Reactive	No	No	Yes	No
CVI03856/18-SE	Anti-HBcII	1.11 S/CO		Reactive	No	No	Yes	No
CVI03856/18-SE	HBsAgQ2	0.37 S/CO		Nonreactive	No	No	Yes	No