

**Hepatitis B virus infection and vaccine coverage among children living with HIV,
HIV-exposed uninfected, and HIV-unexposed uninfected children in the Western
Cape, South Africa**



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CLLCOU002

Submitted in partial fulfilment of the requirements for the degree

MASTER OF PUBLIC HEALTH

(Epidemiology and Biostatistics Specialisation)

At

UNIVERSITY OF CAPE TOWN

25 April 2025

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Acknowledgments

“I alone know the plans I have for you, plans to bring you prosperity and not disaster, plans to bring about the future you hope for.” - Jeremiah 29:11

This thesis would not have been completed without the guidance and support of my incredible supervisors, Dr. Edina Amponsah-Dacosta and Dr. Julie Copelyn. Thank you for your unwavering support, motivation, and expertise throughout this journey. Dr. Edina, in addition to your supervision, I am deeply grateful for your assistance with the administrative aspects of my research, abstract submissions, and my first research day.

A heartfelt thank you to the Vaccines for Africa Initiative (VACFA) team for providing continuous learning opportunities for students, fostering a welcoming environment, and supporting my academic growth.

I also extend my gratitude to Ms. Ntombi Blose for assisting me in compiling my dataset. Your help has been invaluable.

To my boyfriend, thank you for always being there for me throughout this journey and being my biggest supporter. I deeply appreciate everything you have done for me.

To my family and friends, thank you for always checking in, offering words of encouragement, and lending an ear when I needed to vent.

Lastly, I extend my gratitude to the University of Cape Town Financial Aid for funding my master’s degree and to the Division of Occupational Medicine for providing me with a part-time job.

List of Abbreviations

Antenatal Care	ANC
Case Report Forms	CRFs
Children living with HIV	CLWH
Essential Programme on Immunization	EPI
Global Health Sector Strategy	GHSS
Hepatitis B birth-dose	HepB-BD
Hepatitis B third dose	HepB3
Hepatitis B core antibody	Anti-HBc
Hepatitis B surface antibody	Anti-HBs
Hepatitis B surface antigen	HBsAg
Hepatitis B e-antigen	HBeAg
Hepatitis B virus	HBV
HIV-exposed uninfected	HEU
HIV-unexposed uninfected	HUU
Elimination of mother-to-child transmission	eMTCT
Road-to-Health card	RTHC
Socio-economic status	SES
Polymerase chain reaction	PCR

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Thesis organization

This thesis is organized in two sections: Part A and Part B.

The research protocol, which provides the context to why we embarked on this study, the research questions, aim and objectives, methods, ethical approval, and study timelines, is appended in Part B. Following the protocol are supplementary materials related to the study, including supporting data and analyses, the ethics clearance certificate, and the author submission guidelines for the targeted journal, *Vaccine*.

The study findings have been compiled into a journal-ready manuscript which is presented in Part A. This manuscript details the work done, the key findings from the research work, a detailed discussion appraising our findings against existing literature, an appropriate description of the study's limitations, and some concluding statements drawing key recommendations for policy, practice, and future research. Part A is formatted in line with the author guidelines of *Vaccine* (Appendix 6).

PART A: Journal ready manuscript

Hepatitis B virus infection and vaccine coverage among children living with HIV, HIV-exposed uninfected, and HIV-unexposed uninfected children in the Western Cape, South Africa

Targeted Journal: Vaccine¹

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¹ This manuscript will be submitted to the journal Vaccine. Author guidelines can be found in Appendix 6.

Abstract

Introduction: Limited evidence on the burden of hepatitis B virus (HBV) infection among children living with HIV (CLWH), HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) children hinders progress towards eliminating hepatitis B as a public health threat in South Africa.

Methods: This study used secondary data and archived sera (N= 671) obtained from children <13 years old presenting at health facilities in the Western Cape, South Africa. Hepatitis B vaccine coverage was assessed using vaccination records for doses 1 to 3 by 12 months of age. Timely uptake of the hepatitis B vaccine was calculated as the difference between the actual and expected date of vaccine receipt, defined as 4 days before and up to 28 days after the recommended age. The prevalence of HBV infection was determined based on the presence of HBsAg within each subgroup. Logistic regression analysis was performed to assess factors influencing incomplete and delayed vaccination.

Results: Coverage with all three doses by 12 months of age was 86.7% (13/15), 77.0% (57/74), and 80.9% (263/325) for CLWH, HEU, and HUU, respectively (p=0.645). Hepatitis B vaccine coverage decreased across all subgroups as the dosing schedule progressed from doses 1 to 3. Across the strata, the highest proportion of participants with delayed uptake for the third dose of hepatitis B vaccine was noted among CLWH at 23.1% (3/13), followed by 21.6% (58/269) among HUU and 15.3% (9/59) among HEU children (p= 0.540). The median delay in vaccination for dose 3 was highest among CLWH (11.3 weeks) compared to HUU (6.7 weeks) (p= 0.368). HBV infection was detected in 1.4% (1/74) of HEU and 0.3% (1/328) of HUU children, while no cases were observed among CLWH. Factors associated with completing the three-dose hepatitis B vaccine series included crèche attendance, low-to-middle socio-economic status (SES), timely uptake of dose 1, participant's age (in months), and HIV exposure status. For the third dose, crèche attendance reduced the likelihood of delayed uptake by 69%, while a monthly increase in the participant's age was associated with a 2% increase in delayed uptake in the multivariable model.

Conclusion: Our findings highlight disparities in timely hepatitis B vaccine uptake and coverage, emphasizing the need for targeted interventions ensuring timely vaccine uptake among HIV-exposed children.

Keywords: Hepatitis B virus, Vaccine coverage, Vaccine timeliness, Children living with HIV (CLWH), HIV-exposed uninfected (HEU), HIV-unexposed uninfected (HUU).

Highlights

- Vaccine coverage remains below global targets, with no significant differences among HIV-exposed versus unexposed children.
- Delays in vaccine uptake is higher among children living with HIV (CLWH), increasing their risk of infection.
- Despite high vaccine coverage rates, CLWH have the lowest levels of immune protection.
- Prevalence of hepatitis B is low in this cohort, although high core antibody positivity may indicate maternal antibody transfer and suggest a higher risk of exposure.

Introduction

Hepatitis B is a significant public health threat and a major cause of morbidity and mortality globally (1). Chronic hepatitis B virus (HBV) infection can lead to long-term complications such as cirrhosis and hepatocellular carcinoma (HCC) (2). Globally, approximately 254 million people are chronically infected with HBV, with an estimated 1.1 million HBV-associated deaths reported in 2022 (3).

Hepatitis B is transmitted either vertically (intrauterine, intrapartum, or postnatal) or horizontally through direct contact with infected blood or blood products (4). The natural history of chronic HBV infection progresses non-linearly through several phases, including HBeAg-positive infection, HBeAg-positive disease, HBeAg-negative infection, HBeAg-negative disease, the grey zone, and the occult hepatitis B phase (5). Markers of HBV infection and immunity detectable in the blood include hepatitis B surface antibody (Anti-HBs), indicating immunity; hepatitis B core antibody (Anti-HBc), which is a marker of past infection; and hepatitis B surface antigen (HBsAg), signifying active HBV infection (6). Diagnosis of active HBV infection depends on the serological detection of HBsAg and HBV DNA (6, 7). This infection can either be acute or chronic. Acute HBV infection can persist for up to six months and remains transmissible during this time, with possible symptoms such as fever, nausea, and jaundice (6). Recovery is confirmed by the absence of the virus in the blood (6). Chronic HBV infection occurs when HBsAg persists in the blood for more than six months (2, 6, 8). Chronic HBV infection may be symptomatic or asymptomatic, with symptoms including mild fever, jaundice or abdominal pain (2). Occult hepatitis B can also occur and is characterized by the absence of HBsAg in the blood with detectable HBV DNA in the liver, with or without detectable HBV DNA in the serum (8, 9). Its exact pathogenesis remains unclear (10).

Sub-Saharan Africa is among the regions with the highest HBV prevalence (4). Africa accounts for approximately 25% of the global infected population (11). Despite this high burden of chronic HBV infection, only 13% of cases had been diagnosed, and around 3% of individuals received treatment by the end of 2022 (3). The distribution of the disease burden across African countries highlights significant disparities (3). Nigeria has the highest number of HBV infections, with over 14 million cases recorded in 2022, followed by Ethiopia with approximately 7.6 million cases (3). In that same year, Ghana, Côte d'Ivoire, the Democratic Republic of Congo, and South Africa each reported between 2.1 and 2.9 million cases (3). In South Africa, a national cross-sectional study reported an HBsAg prevalence of 9.0% in the general population, with 6.4% classified as chronic HBV carriers (12). The high burden of HIV within the African region further compounds the disease epidemiology. The global prevalence of HBV/HIV co-infection has been estimated at 2.7 million (13), with the highest burden of 5.2% reported in the African region (14). However, the distribution of HBV/HIV co-infections within the African region is highly variable across geographies and population groups. A recent systematic review reported a co-infection rate of 3.3% among pregnant women living with HIV in the African region (15). Prevalence rates among this population varied by country, with Nigeria reporting a higher rate of 27.3%, compared to 4.7% in Congo and 3.2% in South Africa (16-18). In South Africa where both diseases are endemic, a previous study conducted in the Eastern Cape reported an HBV co-infection rate of 12.2% among

children living with HIV (CLWH) (1). The public health relevance of underlying HIV co-infection is the rapid acceleration in hepatitis B disease progression due to increased viral replication and reactivation in the absence of effective treatment (1).

Fortunately, HBV infection is vaccine preventable. In 2009, the World Health Organization (WHO) recommended the hepatitis B birth-dose (HepB-BD) to eliminate mother-to-child transmission (eMTCT) (11). This is an integral toolkit in the WHO's Global Health Sector Strategy (GHSS) for the elimination of viral hepatitis by 2030 which aims to achieve a 90% reduction in new infections and a 65% reduction in mortality (19). In line with this strategy, the WHO recommends administering HepB-BD to all newborns, followed by 2-3 additional doses of a hepatitis B containing vaccine during infancy (3). Globally, 115 countries have implemented a universal HepB-BD vaccination program, with coverage reaching 45% in 2022 (3). However, the African region lags with only 18% of newborns receiving the HepB-BD in 2022 (3). Coverage of the third dose of the hepatitis B vaccine (HepB3) administered during infancy, also varies globally, with the African region having the lowest coverage at 72% in 2022, while the Eastern Mediterranean (84%) and the Americas (83%) also fall short of the WHO 90% coverage targets (3). Despite these challenges, the introduction of the hepatitis B vaccine in infancy has led to a decline in the global HBV prevalence, with a 31.3 % reduction from 1990 to 2019 (20). The vaccine protects individuals and communities against the burden of HBV and its long-term complications (21). Given suboptimal HepB-BD and HepB3 vaccine coverage rates, the African region continues to experience a disproportionate burden, accounting for 63% of incident HBV cases and 25% of HBV-related deaths (3). This highlights the urgent need to scale up vaccination coverage to extend lifesaving benefits to populations most at need towards achieving global elimination targets.

It is important to note that vaccine coverage and timeliness are similar yet distinct concepts. Vaccine coverage provides an indication of the performance of a vaccination program (22). It is calculated as the proportion of children who received their vaccines by a certain age (typically <1 year or ≥ 1 year of age) (23, 24), or the total number of doses received, regardless of timing (23). Timeliness, however, refers to whether a child received a vaccine within specific timeframes, categorized as early, on-time, or delayed (24). Timely vaccination is critical to ensure children receive early protection against vaccine-preventable diseases (VPDs) (24). This is especially pertinent for the prevention of HBV infection, given that the risk of progressing to chronic disease and associated sequelae is directly proportional to the time at infection. Regions with the highest prevalence of chronic HBV infection typically report high rates of vertical transmission (13). Individuals who acquire chronic HBV infection during early childhood have a higher risk of developing serious complications during adulthood (25), whereas infections acquired during adulthood are usually symptomatic and rarely become chronic cases (26). Furthermore, early Hepatitis B e-antigen (HBeAg) seroconversion (before the age of 3), when accompanied by severe liver damage, significantly increases the risk of developing HCC (27). Delayed seroconversion (over the age of 40 years) is associated with an increased risk of cirrhosis and HCC (28). Additionally, those with HCC have a high risk of mortality if they do not receive appropriate treatment including liver transplantation in some instances (25). It is estimated that 15-40% of those chronically infected will develop liver cirrhosis, HCC or liver failure (29).

In South Africa, routine infant hepatitis B vaccination has been part of the Essential Programme on Immunization (EPI) since 1995 (30). The hepatitis B vaccine is administered as part of a hexavalent vaccine (DTaP-IPV-Hib-HepB) at 6, 10, and 14 weeks, with an additional dose available at 18 months of age (12). Until recently, South Africa has not had a HepB-BD vaccination program in place (31). A selective birth-dose vaccination program was rolled out in January 2024, targeting babies born to women with a laboratory confirmed chronic HBV infection (31). The reach of this birth-dose program in South Africa is yet to be understood.

Global elimination of HIV eMTCT programs have achieved significant success in reducing HIV infections in newborns. However, there has been limited success in HBV eMTCT due to gaps in universal screening of pregnant women for hepatitis B, linkage to care, and access to the HepB-BD. This has led to a growing and thriving population of HIV-exposed uninfected (HEU) children whose risk of HBV infection is not adequately prioritized. Among HIV-infected pregnant women, the burden of HBV infection remains high at 8.6% (32), and HEU children face an increased risk of infection (33). Additionally, HEU children have been reported to demonstrate reduced vaccine-induced immunity (34-36), leaving them at increased risk of infectious diseases, including HBV infection, compared to their HIV-uninfected counterparts. Despite this, comparable evidence on the burden of hepatitis B among CLWH, HEU, and HIV-unexposed uninfected (HUU) children in South Africa is scarce. This gap in knowledge limits our understanding of the need for targeted interventions, thereby hindering national progress toward eliminating hepatitis B as a public health threat. This study aims to describe the coverage and timely uptake of routine infant hepatitis B vaccination, and the burden of HBV infection among CLWH, HEU, and HUU children in South Africa.

Methods

Specific Objectives

1. To assess the coverage and timely uptake of the hepatitis B vaccine among CLWH, HEU, and HUU children in the Western Cape province of South Africa.
2. To determine the prevalence of HBV infection among CLWH, HEU, and HUU children in Western Cape, South Africa.
3. To explore factors associated with the completion and timely uptake of recommended hepatitis B vaccine doses among CLWH, HEU, and HUU children in Western Cape, South Africa.

Study design

We conducted a retrospective cross-sectional study, utilising a convenient sample of sera obtained from children participating in a parent study between 2012 and 2016 (37). The parent study, conducted in health facilities in the Western Cape, focused on investigating the incidence of pertussis in South African children who presented with mild to severe lower respiratory tract infections (37). From the original database containing 709 entries, 671 participants with available Case Report Forms (CRFs) were included in the current sub-study (Figure 1). Eligibility criteria² for analysis of vaccine coverage and timeliness included the availability of Road-to-Health Cards (RTHCs) containing participant's immunization history with a legible date and signature indicating the administration of the hepatitis B vaccine, as well as CRFs documenting the HIV status of both the mother and child. Socio-demographic and economic data were retrieved from the original study database. Socio-economic status (SES) was determined based on maternal asset ownership (including type of dwelling, access to basic amenities), employment status, and education level. HIV status was determined for both the mother and the child based on their confirmed HIV test results, while the child's exposure status was determined by the mother's HIV test result. Children categorized as CLWH, HEU, or HUU all had documented HIV test results. Briefly, HIV status was determined at the time of enrolment into the parent study by age-appropriate tests. This involved HIV polymerase chain reaction (PCR) for participants <18 months and two ELISA tests in older children. Participants under 18 months were categorised as HEU if they were ELISA positive but PCR negative. Older participants (>18 months) were categorised as HEU if their mother was HIV-positive during pregnancy, but the child tested negative for HIV (37). Where we could not confirm whether a child underwent HIV testing based on their clinical records, these participants were only classified as either HIV-exposed or HIV-unexposed. Where available, information on antiretroviral therapy (ART) use was collected for both mothers and children living with HIV. Data on duration of ART were available for CLWH. We did not have data on hepatitis B

²For Objective 2 of this study, the non-availability of RTHCs and ineligibility for the hepatitis B vaccine were not considered as exclusion criteria.

vaccination and infection status of mothers and as such, this could not be assessed in this study. Figure 1 presents a breakdown of study samples and assessments conducted in this study.

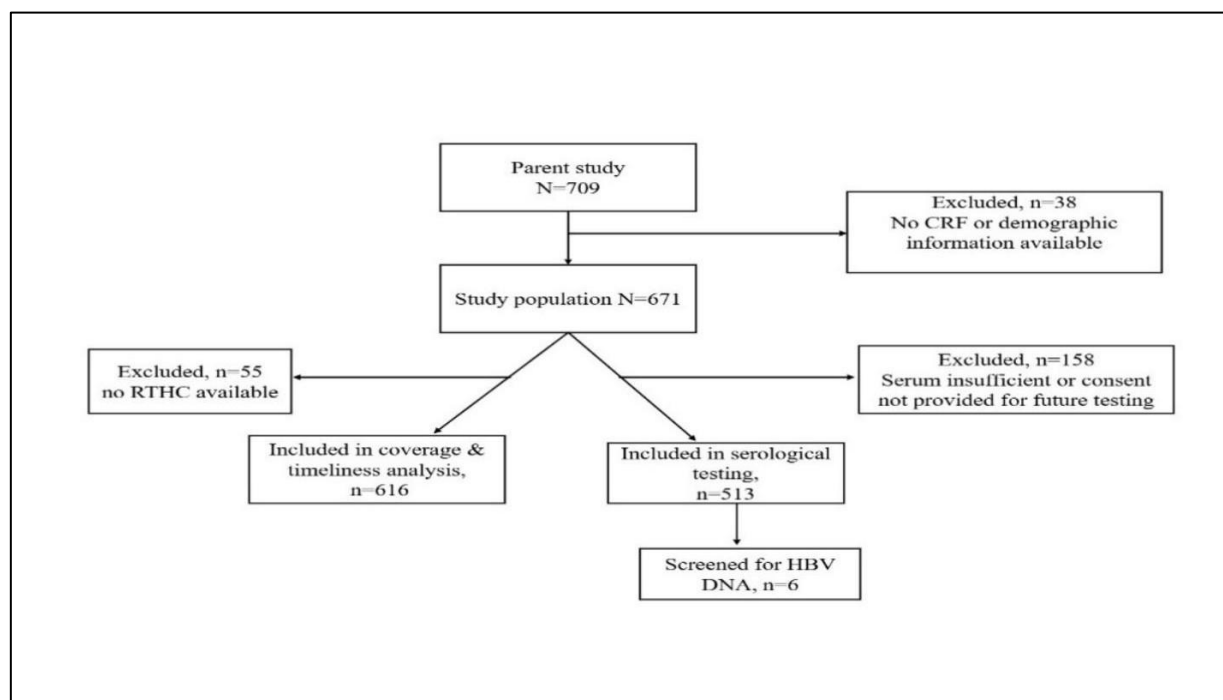


Figure 1: Study sample stratification and laboratory testing for serological markers

Note: RTHC = Road-to-Health Card, CRF = Case Report Form.

Assessment of hepatitis B vaccine coverage and timeliness

Hepatitis B vaccine coverage and timeliness were assessed for each dose, provided participants were age-eligible and had a RTHC available. Among the study population, 616 participants had RTHCs available. For vaccine coverage, 606 participants were age-eligible for dose 1, 566 for dose 2, and 530 for dose 3. For vaccine timeliness among eligible participants with appropriately documented RTHCs, 557 had received dose 1, 507 had received dose 2, and 440 had received dose 3.

Definitions

- i. Eligibility to receive the hepatitis B vaccine at 6, 10, and 14 weeks was defined as being not more than 4 days younger than the minimum age for which the vaccine is licensed, and not more than 4 days prior to the minimum dosing interval (38).
- ii. Coverage was defined as the proportion of age eligible children who had documented evidence (date and signature on RTHC) of receiving each scheduled dose of the hepatitis B vaccine by 12 months of age.
- iii. Complete coverage referred to receiving all three hepatitis B vaccine doses by 12 months of age.
- iv. Vaccine timeliness was defined as receiving a dose between 4 days before and up to 28 days after the national EPI recommended age, in accordance with previous studies (39-41).

- v. Early vaccine uptake included doses given more than 4 days before the recommended age or dosing interval (42),
- vi. Delayed vaccine uptake referred to doses administered more than 28 days after the recommended age (43).

Laboratory testing for HBV serological and molecular markers

Between 2023 and 2024, a total of 513 archived serum samples were tested for three key serological markers: HBsAg, anti-HBs, and anti-HBc, using Elecsys® test kits (Roche Diagnostics, Mannheim, Germany). Serological assays were performed on the Cobas® 6000 analyser series (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Following the manufacturers guidelines, samples were considered positive if the cut-off index for HBsAg was ≥ 1.0 COI, ≥ 10 IU/mL for anti-HBs, and ≤ 1.0 COI for anti-HBc. To determine the presence of HBV DNA among those testing positive for HBsAg and/or anti-HBc, we quantified viral loads using a real-time PCR (qPCR) assay performed on the CFX96 touch real-time PCR (Bio-Rad Laboratories, California, USA). For this purpose, HBV DNA was extracted from available sera using the High Pure Nucleic Acid Extraction Kit (Roche Diagnostics, Mannheim, Germany). The Luna® Universal qPCR Master Mix (New England Biolabs, Ipswich, MA, USA) which uses SYBR® Green 1 to measure DNA amplification in real-time, was used for the in-house qPCR reactions together with Taq1 and Taq2 primers targeting the viral surface gene as published previously [39]. A standard Accurun HBV positive control (SeraCare Life Sciences, Inc., USA) with a lower limit of detection of $ct=29$ was included in the assay. The positive cut-off was set at any amplification <29 ct value while anything amplified at >30 ct value was considered negative.

Data analysis

Numerical data were described using medians and interquartile ranges. Categorical variables were described using proportions as percentages. Associations between numerical data were analysed using Kruskal-Wallis tests and categorical variables were analysed using Chi-square or Fisher's exact tests where appropriate. To assess hepatitis B vaccine coverage rates, proportions of eligible participants who received age-specific hepatitis B vaccine doses by 12 months of age were described. Timeliness for each vaccine dose was calculated as the difference in the actual date and the expected date of receipt of the age-specific immunization. The median duration of delay in uptake was described for vaccine doses received 28 days past the scheduled date. The prevalence of HBV infection was determined based on the presence of HBsAg within each subgroup. Factors influencing the incomplete and delayed uptake of hepatitis B vaccine doses were assessed by logistic regression analyses using the binary outcomes incomplete/complete and delayed/timely administration, with complete coverage and timely administration used as the reference categories. As a priori, HIV exposure status, SES, maternal age, and crèche attendance were included in the multivariable model, along with variables that had a p-value <0.15 in the univariable model. All statistical analyses were conducted in R version 4.3.0 and R Studio version 2024.04.02+764 (45), with significance defined at a two-tailed p-value of <0.05 .

Ethical Considerations

We used secondary data and archived sera collected during a parent study approved by the University of Cape Town (UCT) Faculty of Health Sciences (FHS) Human Research Ethics Committee (HREC) [HREC Reference Number: 371/2011]. Ethical clearance to conduct this sub-study was granted by the UCT FHS HREC [HREC 303/2024]. Only samples from participants whose caregivers consented to future testing were included in this sub-study.

Results

Demographic characteristics of the study population

A total of 671 participants were included in this study and of these, 20 were categorised as CLWH, 93 HEU and 398 HUU, while 9 HIV-exposed and 151 HIV-unexposed had no records of their HIV infection status and thus could not be stratified into the three core study subgroups. Among CLWH, 40% (8/20) were on ART, with a median ART duration of 14 months. Overall, over half of the participants (53.7%, [360/671]) were male with a median age of 13.2 months [IQR 4.8, 40.6]. CLWH had a higher median age of 20.4 months [IQR 9.3, 65.1], compared to HEU (10.8 months, [IQR 4.8, 20.4]) and HUU children (11.1 months, [IQR 4.8, 28.8]). Half (50.0%, [10/20]) of CLWH had ever attended crèche compared to 26.1% of HEU (24/92) and 26.3% of HUU (104/395) children. Among the mothers in the study, 122 were HIV-positive, and only 41.8% (51/122) reported being on ART. Data on their ART regimen and duration of treatment were unavailable. The median maternal age was 29.1 years [IQR 24.5, 34.7]. Most mothers (90.4%, [596/659]) reported having completed secondary education. The majority could be categorised into the upper-middle socio-economic level (39.2%, [263/671]) and this was consistent across the HIV strata, with just over half of mothers of CLWH (57.9%, [11/19]) falling into this category. The demographic characteristics of the study population are presented in Table 1 with further details in Appendix 3.

Table 1: Baseline characteristics of study participants and caregivers

Characteristic	Total, n= 671 ¹	CLWH, n= 20	HEU, n=93	HUU, n=398
<i>n (%) or Median [IQR]</i>				
<i>Participant demographics</i>				
Sex				
Female	311(46.4)	9(45.0)	40(43.0)	187(47.0)
Male	360(53.7)	11(55.0)	53(57.0)	211(53.0)
Age (months)	13.2 [4.8,40.6]	20.4 [9.3,65.1]	10.8 [4.8,20.4]	11.1 [4.8,28.8]
Race				
Black	384(58.7)	18(90.0)	88(95.7)	223(57.6)
Coloured	268(41.0)	2(10.0)	4(4.4)	162(41.9)
White	1(0.1)	0	0	1(0.3)
Indian/Asian	1(0.1)	0	0	1(0.3)
Crèche²				
Yes	193(29.0)	10(50.0)	24(26.1)	104(26.3)
No	473(71.0)	10(50.0)	68(73.9)	291(73.7)
<i>Maternal demographics</i>				
Age (years)	29.1 [24.5,34.7]	28.9 [26.5,33.4]	31.8 [26.4,34.7]	28.3 [23.9,33.9]
Education				
Basic education	18(2.7)	0	2(2.1)	10(2.6)
Primary	30(4.5)	0	5(5.4)	18(4.6)
Secondary	596(90.4)	20(100.0)	85(91.4)	354(90.5)
Tertiary	15(2.3)	0	1(1.1)	9(2.3)
Socio-economic quartiles³				
High	76(11.3)	2(10.5)	11(13.8)	47(13.0)
Low	120(17.9)	4(21.0)	21(26.3)	72(20.0)
Lower-middle	49(7.3)	0	3(3.8)	35(9.7)
Upper-middle	263(39.2)	11(57.9)	38(47.5)	182(50.3)
Unknown	163(24.3)	2(10.5)	7(8.8)	26(7.2)

Note: Missing data shown in this table if greater than 5% of the category total.

¹This total includes the 160 children with known HIV exposure but unknown infection status (n=9) and those who were HIV unexposed with unknown infection status (n=151).

²Pre-school, Kindergarten, Nursery.

³Socio-economic status (SES) was categorized into quartiles based on a validated weighted composite score that included maternal asset ownership, employment, and education.

Coverage of the hepatitis B vaccine among HIV-exposed and unexposed children

The dose coverage for the hepatitis B vaccine within the study population is summarized in Table 2. While vaccine coverage rates were not statistically significantly different across the subgroups, HEU children had the highest decline in coverage from dose 1 to dose 3 (12.5%), followed by HUU children (11.1%) while CLWH had a minimal decline of 2.2%.

Table 2: Hepatitis B vaccine coverage rates among CLWH, HEU and HUU children

Hepatitis B vaccine coverage n (%)					
Hepatitis B dose	Total ¹	CLWH	HEU	HUU	p-value
	n=606	n=18	n=86	n=375	
Dose 1	556(91.7)	16(88.9)	77(89.5)	345(92.0)	0.627
	n=566	n=16	n=79	n=350	
Dose 2	505(89.2)	14(87.5)	69(87.3)	312(89.1)	0.777
	n=530	n=15	n=74	n=325	
Dose 3	426(80.4)	13 (86.7)	57 (77.0)	263(80.9)	0.645

¹This total includes participants whose mothers were confirmed as either HIV-infected or HIV-uninfected, but the child's HIV status was not tested.

Across the strata, vaccination coverage decreased as the dosing schedule progresses from dose 1 to dose 3 (Figure 2).

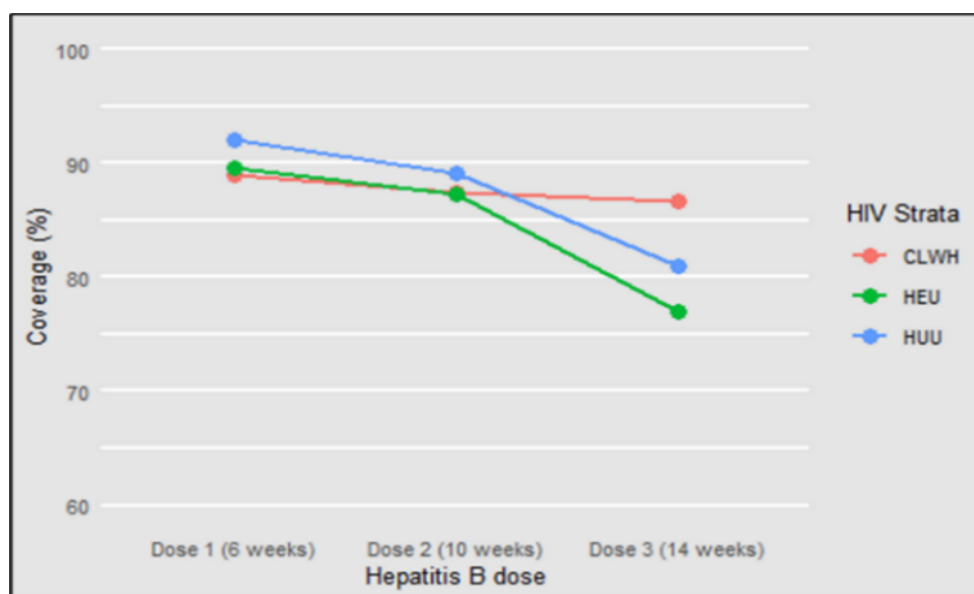


Figure 2: Hepatitis B vaccine coverage for doses 1, 2 and 3 among CLWH, HEU, and HUU

Timeliness and duration of delay of the hepatitis B vaccine

The majority of participants across all subgroups received their scheduled doses on time, as shown in Figure 3. Delayed vaccine uptake increased over the course of the schedule with the highest proportion of delayed uptake observed for HepB3 (Figure 3). Across the strata, the highest proportion (23.1%, [3/13]) of participants who experienced delayed uptake for HepB3 was noted among CLWH, followed by 21.6% (58/269) among HUU, and 15.3% (9/59) in the HEU subgroups ($p= 0.540$).

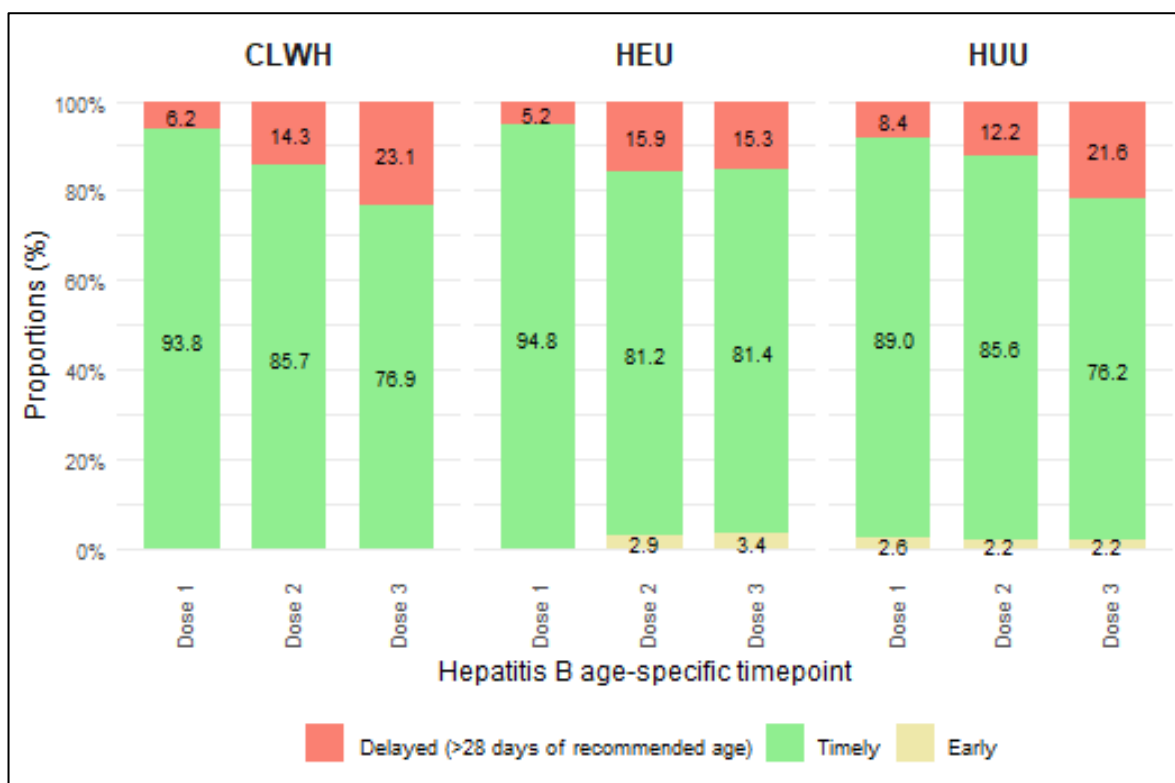


Figure 3: Timeliness of uptake of hepatitis B vaccine at 6, 10, and 14 weeks by HIV strata
Hepatitis B vaccination at 6 weeks (n = 557), 10 weeks (n= 507) and 14 weeks (n= 440).

Delayed hepatitis B vaccine uptake increased for each subsequent dose. From dose 1 to dose 2, delays increased by 8.0%, 10.8%, and 3.8% among CLWH, HEU, and HUU subgroups, respectively (Appendix 4). Delays further increased from dose 2 to dose 3 by 8.8% and 9.9% among CLWH and HUU. The median delay in weeks for HepB3 was highest among CLWH, at 11.3 weeks [IQR 9.6, 13.0], compared to 6.9 weeks [IQR 5.2,20.0] among HEU and 6.7 weeks [IQR 5.2, 10.0] among HUU children ($p= 0.368$). The median age for completing HepB3 was 15.9 weeks, 15.6 weeks and 15.1 weeks for CLWH, HEU and HUU, respectively ($p= 0.876$).

Prevalence of hepatitis B virus infection and immunity

The prevalence of serological markers of HBV infection and immunity across the study strata is presented in Table 3. Overall, 13.5% (69/513) of the study population were non-immune to HBV infection, with CLWH (52.6%, [10/19]) having significantly higher susceptibility rates than HEU (14.9%, [11/74]) and HUU participants (11.6%, [38/328]) ($p < 0.001$). Vaccine induced immunity, indicated by anti-HBs in the absence of other markers of infection, was prevalent in 69.0% (354/513) of the study population. Among the 354 participants with protective levels of immunity, 338 had valid RTHCs. Among these, 317 (93.8%) were confirmed to have received at least one dose of the hepatitis B vaccine.

When comparing HepB3 coverage rates and the proportion of age-eligible participants with vaccine-induced immunity across the strata, it was observed that although 86.7% (13/15) of CLWH completed their vaccination schedule, only 40% (6/15) had protective titres of anti-HBs (Figure 4). In contrast, 81.5% (216/265) of participants in the HUU subgroup were fully vaccinated, with 82.6% (219/265) having protective anti-HBs levels. Among the HEU subgroup, 80.7% (46/57) were fully vaccinated with all (80.7%, [46/57]) exhibiting a protective immune response (Figure 4).

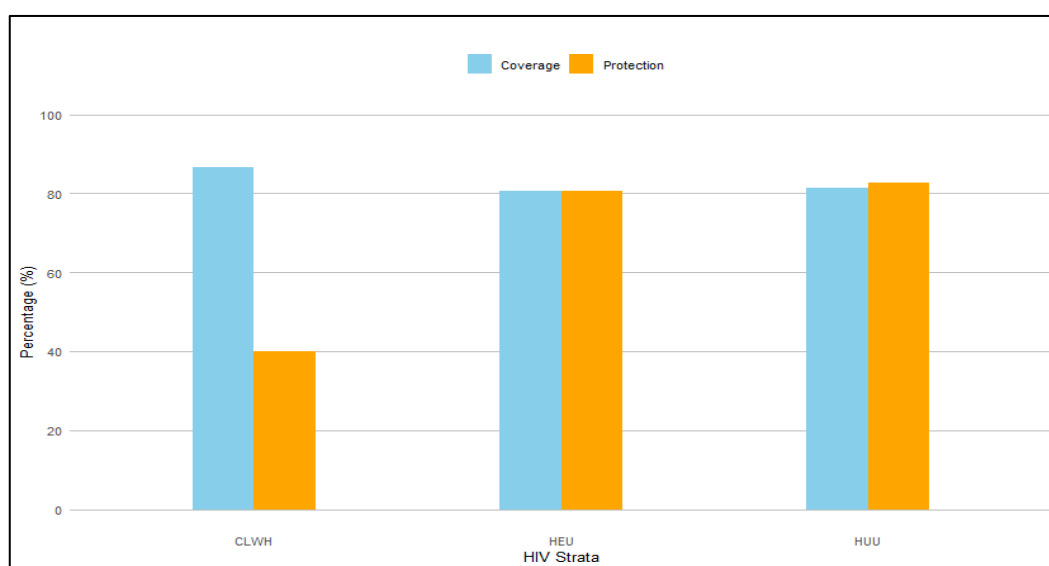


Figure 4: Vaccine coverage and proportion protected (anti-HBs positive) against hepatitis B among CLWH, HEU and HUU

In terms of vaccine non-response (participants who received all three hepatitis B vaccine doses but remained unprotected), we found a proportion of 8.3% (27/325) in the overall population. The non-response rate was highest among CLWH (53.8%, [7/13]), compared to HEU (6.8%, [3/44]) and HUU (5.6%, [12/213]) children.

Additionally, 0.8% (4/513) of the population tested positive for anti-HBc alone, indicating isolated total anti-HBc positivity or past infection (Table 3). Across the strata, only one HUU participant (0.3%, [1/328]) had markers of past infection and had received at least one vaccine dose. Another participant living with HIV was

both anti-HBc and anti-HBs positive and was not on ART. Using the qPCR assay, no HBV DNA was detected among children who tested positive for anti-HBc.

HBsAg was present in 2/513 participants (0.4%). One participant was HEU, aged 1.2 months, and had received their first eligible dose of the hepatitis B vaccine. The other participant was HUU and concurrently tested positive for anti-HBs despite not receiving any of their scheduled doses by 7 months of age. The simultaneous detection of HBsAg and anti-HBs in the absence of vaccination suggests this participant was in the process of seroconverting or transplacental transfer of anti-HBs. Among both HBsAg positive participants, HBV DNA was not detectable by qPCR.

Table 3: Prevalence of serological markers of hepatitis B virus infection and immunity among CLWH, HEU and HUU children

Interpretation	Serological markers	Total population n=513 ¹	CLWH, n=19	HEU, n=74	HUU, n=328	p-value
Not immune-Not protected <i>Has not been infected with HBV</i>	Anti-HBc ⁻ HBsAg ⁻ Anti-HBs ⁻	69 (13.5)	10 (52.6)	11 (14.9)	38 (11.6)	<0.001
Could be infected <i>Possible acute or past HBV infection</i>	Anti-HBc ⁺ HBsAg ⁻ Anti-HBs ⁻	4 (0.8)	0	0	1 (0.3)	1
Immune-Protected <i>Immunity from vaccination and not infected with HBV, or a past infection</i>	Anti-HBs ⁺ (≥10mIU/mL) Anti-HBc ⁻ HBsAg ⁻	354 (69.0)	7 (36.8)	46 (62.2)	233 (71.0)	<0.001
Immune Controlled-Protected <i>Surface antibodies resulting from natural infection, indicating recovery from a previous HBV infection.</i>	Anti-HBc ⁺ Anti-HBs ⁺ HBsAg ⁻	28 (5.5)	1 (5.3)	5 (6.8)	18 (5.5)	0.842
Infected <i>Positive HBsAg is a marker of active HBV infection.</i>	HBsAg ⁺	2 (0.4)	0	1 (1.4)	1 (0.3)	0.221

¹This total includes the 92 participants with known HIV-exposure but unknown infection status (n=8) and those who were HIV-unexposed with unknown infection status (n=84).

Factors associated with incomplete coverage and delayed uptake of hepatitis B vaccine

Factors associated with decreased odds of incomplete hepatitis B vaccine coverage in the univariable model included crèche attendance (OR:0.55, p= 0.021), low-middle SES (OR:0.17, p=0.024) and timely uptake of dose 1 (OR: 0.16, p<0.001). However, in the multivariable model, the only factors to retain statistical significance were timely uptake of dose 1 (OR: 0.08, p<0.001) and participants age in months (OR: 0.62, p<0.001), while HIV exposure status (OR:2.92, p=0.036) was only significant in the multivariable model. Findings from the logistic regression analysis are presented in Tables 4 and 5.

Table 4: Factors associated with incomplete coverage by 12 months of age

Variable		Univariable OR (95 % CI)	p-value	Multivariable OR (95% CI)	p-value
HIV exposure status	HIV-unexposed [Ref] ¹	1	—	1	—
	HIV-exposed	1.26(0.72 - 2.11)	0.402	2.92(1.06 - 8.03)	0.036
Sex	Male [Ref]	1	—		
	Female	0.80(0.52 - 1.23)	0.307		
Age (months)		0.77(0.67 - 0.87)	<0.001	0.62(0.49 - 0.76)	<0.001
Crèche attendance	No [Ref]	1	—	1	—
	Yes	0.55(0.32 - 0.90)	0.021	0.94(0.26 - 3.05)	0.922
Maternal age (years)		0.99(0.97 - 1.02)	0.684	0.99(0.93 - 1.06)	0.765
SES	High [Ref]	1	—	1	—
	Low	0.56(0.25 - 1.25)	0.154	0.88(0.22 - 3.49)	0.848
	Low-middle	0.17(0.03 - 0.65)	0.024	0.14(0.01 - 1.26)	0.121
	Upper-middle	0.97(0.51 - 1.92)	0.927	0.82(0.26 - 2.72)	0.742
Maternal age category	<18 [Ref]	1	—		
	>18	1.47(0.25 - 27.95)	0.722		
Maternal Education	Basic [Ref]	1	—		
	Primary	1.26(0.21 - 10.13)	0.804		
	Secondary	1.45(0.39 - 9.44)	0.629		
	Tertiary	1.20(0.13 - 11.54)	0.867		
Highest grade completed		1.04(0.90 - 1.22)	0.580		
Timely Dose 1	Delayed [Ref]	1	—	1	—

	Timely	0.16(0.08 - 0.31)	<0.001	0.08(0.02 - 0.25)	<0.001
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¹Reference category

Factors found to decrease the odds of delayed uptake of HepB3 in the univariable model included crèche attendance (OR: 0.44, p=0.004). In the multivariable model, crèche attendance remained a protective factor (OR: 0.31, p= 0.005), while participants age in months (OR:1.02, p=0.014) increased the odds of delayed vaccine uptake.

Table 5: Factors associated with delayed uptake of the 3rd dose of the hepatitis B vaccine

Variable		Univariable OR (95 % CI)	p-value	Multivariable OR (95% CI)	p-value
HIV exposure status	HIV-unexposed [<i>Ref</i>]	1	—	1	—
	HIV-exposed	0.65(0.32 - 1.23)	0.209	0.55(0.23 - 1.21)	0.162
Sex	Male [<i>Ref</i>]	1	—		
	Female	0.76(0.47 - 1.20)	0.243		
Age (months)		1.01(1.00 - 1.01)	0.085	1.02(1.00 - 1.03)	0.014
Crèche attendance	No [<i>Ref</i>]	1	—	1	—
	Yes	0.44(0.24 - 0.75)	0.004	0.31(0.13 - 0.67)	0.005
SES	High [<i>Ref</i>]	1	—	1	—
	Low	0.80(0.35 - 1.89)	0.609	0.69(0.26 - 1.84)	0.449
	Low-middle	0.58(0.18 - 1.66)	0.319	0.44(0.12 - 1.47)	0.198
	Upper-middle	0.79(0.38 - 1.72)	0.530	0.78(0.35 - 1.86)	0.564
Maternal age (years)		1.01(0.98 - 1.04)	0.634	1.01(0.97 - 1.05)	0.704
Maternal age category	<18 years [<i>Ref</i>]	1	—		
	>18 years	0.54(0.10 - 3.94)	0.482		
Maternal Education	Basic [<i>Ref</i>]	1	—		
	Primary	1.38(0.28 - 7.94)	0.695		
	Secondary	0.80(0.23 - 3.69)	0.749		
	Tertiary	0.75(0.08 - 5.69)	0.781		
Highest grade completed		0.90(0.78 - 1.04)	0.142	0.92(0.76 - 1.12)	0.421

Discussion

This study provides important insights into hepatitis B vaccine coverage, timeliness, and the burden of HBV infection among CLWH, HEU, and HUU children in the Western Cape province of South Africa. Previous studies conducted in South Africa have typically focused on the hepatitis B vaccine coverage or the disease burden in the general population and have not specifically looked at hepatitis B vaccine timeliness among HIV-exposed children (both infected and uninfected) compared to their unexposed counterparts (39, 46, 47). To the best of our knowledge, this study is the first of its kind to compare the hepatitis B vaccine coverage rates, timeliness, and burden of HBV infection among a population of CLWH, HEU, and HUU children in South Africa. Our findings highlight key disparities in vaccine uptake and immunity among these groups, emphasizing the need for targeted interventions to improve hepatitis B prevention efforts in high-risk populations like HIV-exposed children.

Low hepatitis B vaccine coverage and timeliness among HIV-exposed children

The overall hepatitis B vaccine coverage rate in this study was 80.4% and varied across subgroups: 86.7% among CLWH, 77.0% among HEU and 80.9% among HUU children. These coverage rates are comparable to the national coverage rate of 78.8% reported in 2023 (48). More specifically, the coverage rate in the years the participants were sampled (2012-2016) ranged from 86.1% in 2012 to 84.4% in 2016 (48). The overall coverage rate in our study aligns closely with both the national coverage rates during the sampling period and the current 2023 estimate. However, hepatitis B vaccine coverage was suboptimal across all groups, with a decline in coverage observed as the vaccine schedule progressed. The coverage rates for the third dose across all subgroups fall short of the 90% coverage target set by the WHO GHSS to eliminate viral hepatitis as a public health threat by 2030 (19).

Our study found lower hepatitis B vaccine coverage rates for the third dose compared to the first dose. This aligns with prior studies conducted in African countries and other low- and middle-income settings, demonstrating declining vaccine coverage rates for later doses in multi-dose vaccine series (49-51). An analysis across 47 African countries showed a decline in HepB3 coverage compared to the first dose, with a sharp decline observed in countries that administer the vaccine at 6, 10 and 14 weeks (49). For example, in Côte d'Ivoire, coverage dropped from 74% to 58%, while Liberia reported a decline from 80% to 53%, highlighting challenges in completing the vaccine schedule (49). Similarly, a study in Ethiopia (50) reported declining coverage rates for the pentavalent (DTP-HepB-Hib) doses, although the third-dose coverage was slightly higher (92.6%) compared to that reported in our study (80.4%). This higher coverage may be because they assessed coverage up to 23 months of age, allowing more time for children to catch-up on missed doses, thereby increasing overall coverage rates. In addition, a study conducted in Tanzania (51) reported a decline in coverage from the first to the third pentavalent dose, with coverage dropping from 79.4% for dose 1 to 72.8% for dose 3. This disparity may be due to a lack of access to immunization services, as the study included a significant proportion of children from rural areas who experienced longer delays in vaccine uptake.

The timeliness of vaccine administration also revealed critical gaps, particularly among CLWH, who experienced the highest median delay for HepB3 (11.3 weeks). This aligns with previous studies (52, 53) indicating that CLWH are more likely to experience delays in routine immunizations, potentially due to frequent healthcare visits for HIV care that disrupt adherence to standard immunization schedules. Delayed vaccination places children at prolonged risk of HBV infection, highlighting the need for interventions such as providing integrated healthcare services, promoting caregiver education on the importance of timely vaccination, and improved record keeping at crèches to ensure timely vaccine uptake, particularly in high-risk populations.

Factors influencing vaccine uptake and timeliness in this study included socio-economic status, timely uptake of dose 1, HIV-exposure status and crèche attendance. Children who attended crèche had higher odds of receiving timely vaccinations, possibly due to caregiver awareness and institutional immunization requirements. In South Africa, school entry regulations require parents to present proof of immunization when enrolling their children in public schools (54), which may contribute to higher third-dose coverage rates observed in our study. However, a child will not be excluded from school enrolment if they are not fully immunized. In the Western Cape, the education policy stipulates that in such cases, parents must write to the Head of the Education Department to secure admission (55). These measures tend to enhance parental adherence with the EPI schedule. This underscores the potential role of national health and educational policies, and the importance of early childhood education centres in improving vaccine uptake and timeliness. A systematic review assessing the impact of school vaccination policies on coverage rates reported that vaccination mandates for schoolchildren increased coverage, with a greater effect observed in regions where baseline vaccination rates were low (56). Strengthening the implementation of educational policies is essential to enhance adherence to the full immunization schedule particularly for HIV-exposed children. Furthermore, to improve vaccine uptake, interventions such as SMS reminders for caregivers has been associated with higher rates of complete coverage (57, 58). We also found that delays in the uptake of the first dose was associated with reduced dose completion rates by 12 months of age. This underscores the need to target at-risk populations that could benefit from adherence monitoring to ensure timely administration of subsequent doses. Strengthening health education during antenatal care (ANC) visits, promoting continuous use of healthcare services, and increasing community involvement, particularly among women living with HIV, could further improve vaccine timeliness and adherence.

Hepatitis B virus infection and immunity gaps

The prevalence of HBV infection in this study was low, with only two participants testing positive for HBsAg. This low prevalence is consistent with findings from other studies in sub-Saharan Africa (8, 59, 60), which have attributed the decline in childhood HBV prevalence to the widespread implementation of routine infant hepatitis B immunization programs. However, the presence of isolated anti-HBc positivity in some participants raises concerns about maternal exposure and risk of MTCT, occult HBV infection and the potential for underdiagnosis. Given the age of the participants and the absence of detectable HBV DNA, it is possible that the anti-HBc

detected among study participants was a result of transplacental transfer from an infected mother to child and would need further investigation (61).

Despite relatively high vaccine coverage rates, immunity levels differed significantly across HIV strata. Protective anti-HBs levels were significantly lower among CLWH (36.8%) compared to HEU (62.2%) and HUU (71.0%). This is consistent with previous studies (34-36), indicating that individuals living with HIV often exhibit suboptimal vaccine-induced immune responses due to immune dysregulation. Furthermore, the waning immunity observed among HIV-exposed children suggests a need for booster doses or targeted revaccination strategies to maintain long-term protection against HBV infection. Fortunately, the current South African EPI schedule includes a fourth dose of the hepatitis B vaccine at 18 months as part of the hexavalent vaccine. Given that hepatitis B immunity wanes over time and that CLWH in this study were relatively older, increasing uptake of the fourth dose at 18 months of age could enhance long-term protection in this vulnerable group (6, 35, 62). These findings highlight the need for comprehensive, targeted public health interventions and guidelines. This includes enhancing antenatal screening for HBV infection (59) particularly among pregnant women living with HIV and ensuring timely birth-dose vaccination for their exposed newborns, followed by completion of all four doses of the hepatitis B vaccine as per the South African EPI schedule. In addition, at-risk groups like CLWH should be screened for missed vaccinations during routine healthcare visits and caught-up with the schedule to enhance hepatitis B immunity.

Implications for public health policy and implementation

Our findings have significant implications for public health policy and implementation. First, the observed delays in hepatitis B vaccination among HIV-exposed children underscore the need for enhanced immunization tracking and reminder systems. Second, the lower levels of vaccine-induced immunity among CLWH and HEU children highlight the need for further research to enhance our understanding of the need for serological monitoring, or targeted vaccination strategies. Finally, the introduction of the HepB-BD in South Africa presents a critical opportunity to further reduce the burden of perinatal HBV transmission. While the selective birth-dose strategy targeting infants born to HBV-infected mothers is a step forward, a universal birth-dose policy may be more effective in closing immunity gaps and preventing early childhood HBV infection.

Strengths and limitations of this study

This study is the first of its kind to compare hepatitis B burden and vaccination history among CLWH, HEU and HUU participants in South Africa, providing critical evidence for further exploration in larger prospective studies. A key strength of this study is the availability of reliable vaccination records through participants' RTHCs, as few studies are able to confirm vaccination history using reliable clinical records and thus often depend on caregiver recall. The findings of this study should be considered in light of some limitations. The reliance on secondary data may have introduced selection bias, while the retrospective nature of the study and the absence of data on maternal HBV vaccination and infection status limits our ability to fully assess the risk of vertical transmission. Additionally, due to the possibility of maternal HIV seroconversion during pregnancy

or breastfeeding, some children classified as HUU may, in fact, have been HEU, particularly since maternal HIV testing was conducted during pregnancy. The relatively small sample size of CLWH, although a reflection of the success of HIV eMTCT programs in South Africa, may have reduced the statistical power to detect associations within this subgroup. Lastly, we did not assess the seroprevalence of acute HBV infection based on detection of anti-HBc IgM. Future research should explore longitudinal assessments of hepatitis B immunity in HIV-exposed children to better understand the durability of vaccine-induced protection and the potential need for targeted interventions, while also improving representativeness across rural and urban populations and including healthy, HIV-unexposed children.

Conclusions

This study highlights disparities in hepatitis B vaccine uptake, timeliness and immunity among HIV-exposed and unexposed children in South Africa. The findings underscore the need for targeted interventions to improve vaccine coverage and timeliness, particularly among CLWH. Given the lower levels of protective immunity observed in HIV-exposed children, enhanced vaccination strategies may be necessary to ensure long-term protection. Additionally, the introduction of the selective HepB-BD in South Africa should be carefully evaluated to determine its reach and impact on closing immunity gaps in high-risk populations. Strengthening immunization programs through improved caregiver education, health system integration, and innovative reminder systems will be crucial for achieving hepatitis B elimination targets in South Africa.

Data Availability

The dataset analysed in this study is available from the corresponding author upon reasonable request.

Author Contributions

EA-D conceptualized the study. CC managed data collection and analysis and prepared the first draft of the manuscript. EA-D and JC supervised the study, assisted in the interpretation of results, and contributed to reviewing and finalizing the manuscript.

Funding

This independent research was supported by the Gilead Sciences Research Scholars Program in Global Public Health.

Declaration of Competing Interest

All authors declare no known financial or personal conflicts of interest that could have influenced the work presented in this paper.

Acknowledgements

The authors would like to thank Ntombi Blose for her support with data retrieval and cleaning during this study.

References

1. Kaswa R, de Villiers M. Prevalence of hepatitis-B virus co-infection among people living with HIV in Mthatha region of South Africa. *Afr Health Sci.* 2023;23(1):149-56. <https://doi.org/10.4314/ahs.v23i1.17>.
2. Elgouhari HM, Abu-Rajab Tamimi TI, Carey WD. Hepatitis B virus infection: understanding its epidemiology, course, and diagnosis. *Cleve Clin J Med.* 2008;75(12):881-9. <https://doi.org/10.3949/ccjm.75a.07019>.
3. World Health Organization. Global hepatitis report 2024: action for access in low-and middle-income countries. World Health Organization; 2024 Apr 9.
4. McMahon BJ, editor *Epidemiology and natural history of hepatitis B*. Semin Liver Dis; 2005: Published in 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue. <https://doi.org/10.1055/s-2005-915644>.
5. World Health Organization. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection 2024.
6. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. *Can J Infect Dis Med Microbiol.* 2005;16(2):65. <https://doi.org/10.1155/2005/450574>.
7. Weber B. Recent developments in the diagnosis and monitoring of HBV infection and role of the genetic variability of the S gene. *Expert Rev Mol Diagn.* 2005;5(1):75-91. <https://doi.org/10.1586/14737159.5.1.75>.
8. Duri K, Munjoma PT, Mataramvura H, Mazhandu AJ, Chandiwana P, Marere T, et al. Antenatal hepatitis B virus seroprevalence, risk factors, pregnancy outcomes and vertical transmission rate within 24 months after birth in a high HIV prevalence setting. *BMC Infect Dis.* 2023;23(1):736. <https://doi.org/10.1186/s12879-023-08523-2>.
9. Im YR, Jagdish R, Leith D, Kim JU, Yoshida K, Majid A, et al. Prevalence of occult hepatitis B virus infection in adults: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2022;7(10):932-42. [https://doi.org/10.1016/s2468-1253\(22\)00201-1](https://doi.org/10.1016/s2468-1253(22)00201-1).
10. Mphahlele MJ, Lukhwari A, Burnett RJ, Moropeng LM, Ngobeni JM. High risk of occult hepatitis B virus infection in HIV-positive patients from South Africa. *J Clin Virol.* 2006;35(1):14-20. <https://doi.org/10.1016/j.jcv.2005.04.003>.
11. Sonderup MW, Spearman CW. Global disparities in hepatitis B elimination—a focus on Africa. *Viruses.* 2022;14(1):82. <https://doi.org/10.3390/v14010082>.
12. Moonsamy S, Pillay P, Prabdial-Sing N. Hepatitis B infection status among South Africans attending public health facilities over a five-year period: 2015 to 2019. *PLOS Glob Public Health.* 2023;3(9):e0000992. <https://doi.org/10.1371/journal.pgph.0000992>.
13. World Health Organization. Hepatitis B, <https://www.who.int/news-room/fact-sheets/detail/hepatitis-b>; 2024 [accessed 10 March 2025].
14. Platt L, French CE, McGowan CR, Sabin K, Gower E, Trickey A, et al. Prevalence and burden of HBV co-infection among people living with HIV: a global systematic review and meta-analysis. *J Viral Hepat.* 2020;27(3):294-315. <https://doi.org/10.1111/jvh.13217>.
15. Kafeero HM, Ndagire D, Ocamo P, Walusansa A, Sendagire H. Sero-prevalence of human immunodeficiency virus–hepatitis B virus (HIV–HBV) co-infection among pregnant women attending antenatal care (ANC) in sub-Saharan Africa (SSA) and the associated risk factors: a systematic review and meta-analysis. *Viol J.* 2020;17:1-19. <https://doi.org/10.1186/s12985-020-01443-6>.
16. Ya'aba Y, Mohammed S, Olatunji K, Abubakar A, Usoroh M, Daniel O, et al. Prevalence of hepatitis B and C among HIV infected pregnant women attending care and treatment at National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. *Microbiol Res J Int.* 2019:1-8. <https://doi.org/10.9734/mrji/2019/v28i430137>.
17. Mpody C, Thompson P, Tabala M, Ravelomanana NLR, Malongo F, Kawende B, et al. Hepatitis B infection among pregnant and post-partum women living with HIV and on antiretroviral therapy in Kinshasa, DR Congo: A cross-sectional study. *PloS One.* 2019;14(5):e0216293. <https://doi.org/10.1371/journal.pone.0216293>.
18. 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):307-9. <https://doi.org/10.7196/samj.7299>.

19. World Health Organization. Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis 2016.
20. Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol*. 2022;7(9):796-829. [https://doi.org/10.1016/s2468-1253\(22\)00124-8](https://doi.org/10.1016/s2468-1253(22)00124-8).
21. Al-Busafi SA, Alwassief A. Global perspectives on the hepatitis B vaccination: challenges, achievements, and the road to elimination by 2030. *Vaccines*. 2024;12(3):288. <https://doi.org/10.3390/vaccines12030288>.
22. Negash BT, Tediso Y, Yoseph A. Predictors of timeliness of vaccination among children of age 12–23 months in Boricha district, Sidama region Ethiopia, in 2019. *BMC Pediatr*. 2023;23(1):409. <https://doi.org/10.1186/s12887-023-04234-4>.
23. Luman ET, Barker LE, Shaw KM, McCauley MM, Buehler JW, Pickering LK. Timeliness of childhood vaccinations in the United States: days undervaccinated and number of vaccines delayed. *JAMA*. 2005;293(10):1204-11. <https://doi.org/10.1001/jama.293.10.1204>.
24. Dolan SB, Carnahan E, Shearer JC, Beylerian EN, Thompson J, Gilbert SS, et al. Redefining vaccination coverage and timeliness measures using electronic immunization registry data in low-and middle-income countries. *Vaccine*. 2019;37(13):1859-67. <https://doi.org/10.1016/j.vaccine.2019.02.017>.
25. Wright TL. Introduction to chronic hepatitis B infection. *AM J Gastroenterol*. 2006;101:S1-S6. <https://doi.org/10.1111/j.1572-0241.2006.00469.x>
26. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP, et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis*. 1985;151(4):599-603. <https://doi.org/10.1093/infdis/151.4.599>.
27. Chang M-H, Hsu H-Y, Hsu H-C, Ni Y-H, Chen J-S, Chen D-S. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. *Hepatology*. 1995;22(5):1387-92. [https://doi.org/10.1016/0270-9139\(95\)90141-8](https://doi.org/10.1016/0270-9139(95)90141-8).
28. Chen YC, Chu CM, Liaw YF. Age-specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. *Hepatology*. 2010;51(2):435-44. <https://doi.org/10.1002/hep.23348>.
29. Lok A, McMahon B. Chronic hepatitis. *N Engl J Med*. 2002;346(22):1682-3. <https://doi.org/10.1056/nejm200205303462202>.
30. 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):675-7. <https://doi.org/10.7196/samj.2016.v106i7.10956>.
31. Solomon-Rakiep T, Olivier J, Amponsah-Dacosta E. Weak adoption and performance of hepatitis B birth-dose vaccination programs in Africa: time to consider systems complexity? —A scoping review. *Trop Med Infect Dis*. 2023;8(10):474. <https://doi.org/10.3390/tropicalmed8100474>.
32. Millar J, Cromhout GZ, Mchunu N, Bengu N, Ndung'u T, Goulder PJ, et al., editors. Hepatitis B Virus Prevalence and Mother-to-Child Transmission Risk in an HIV Early Intervention Cohort in KwaZulu-Natal, South Africa. *Open Forum Infect Dis*; 2023: Oxford University Press US. <https://doi.org/10.1093/ofid/ofad366>.
33. Afran L, Garcia Knight M, Nduati E, Urban B, Heyderman RS, Rowland-Jones SL. HIV-exposed uninfected children: a growing population with a vulnerable immune system? *Clin Exp Immunol*. 2014;176(1):11-22. <https://doi.org/10.1111/cei.12251>.
34. Njom Nlend AE, Nguwoh PS, Ngounouh CT, Tchidjou HK, Pieme CA, Otélé JM, et al. HIV-infected or-exposed children exhibit lower immunogenicity to hepatitis B vaccine in Yaounde, Cameroon: an appeal for revised policies in Tropical settings? *PLoS One*. 2016;11(9):e0161714. <https://doi.org/10.1371/journal.pone.0161714>.
35. Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesseling AC. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. *JAMA*. 2011;305(6):576-84. <https://doi.org/10.1001/jama.2011.100>.
36. Rutstein RM, Rudy B, Codispoti C, Watson B. Response to hepatitis B immunization by infants exposed to HIV. *AIDS*. 1994;8(9):1281-4. <https://doi.org/10.1097/00002030-199409000-00010>.

37. Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. Incidence and diagnosis of pertussis in South African children hospitalized with lower respiratory tract infection. *Pediatr Infect Dis J*. 2016;35(6):611-6. <https://doi.org/10.1097/inf.0000000000001132>.
38. Miller E, Wodi PA, Centers for Disease Control and Prevention (CDC). General best practice guidance for immunization. *Aplicar*. 2020;1:2023-4.
39. Blose N, Amponsah-Dacosta E, Kagina BM, Muloiwa R. Descriptive analysis of routine childhood immunisation timeliness in the Western Cape, South Africa. *Vaccine: X*. 2022;10:100130. <https://doi.org/10.1016/j.jvacx.2021.100130>.
40. Mthiyane T, Walaza S, Cohen C, Norris S, Tempia S, Cohen A, et al. Factors associated with missed and delayed DTP3 vaccination in children aged 12-59 months in two communities in South Africa, 2012-2013. *S Afr Med J*. 2019;109(8):562-9. <https://doi.org/10.7196/SAMJ.2019.v109i8.13244>.
41. Laryea DO, Abbeyquaye Parbie E, Frimpong E. Timeliness of childhood vaccine uptake among children attending a tertiary health service facility-based immunisation clinic in Ghana. *BMC Public Health*. 2014;14:1-5. <https://doi.org/10.1186/1471-2458-14-90>.
42. Masters NB, Wagner AL, Boulton ML. Vaccination timeliness and delay in low-and middle-income countries: a systematic review of the literature, 2007-2017. *Hum Vaccin Immunother*. 2019. <https://doi.org/10.1080/21645515.2019.1616503>.
43. Sadoh AE, Eregie CO. Timeliness and completion rate of immunization among Nigerian children attending a clinic-based immunization service. *J Health Popul Nutr*. 2009;27(3):391. <https://doi.org/10.3329/jhpn.v27i3.3381>.
44. Daniel HDJ, Fletcher JG, Chandy GM, Abraham P. Quantitation of hepatitis B virus DNA in plasma using a sensitive cost-effective "in-house" real-time PCR assay. *Indian J Med Microbiol*. 2009;27(2):111-5. <https://doi.org/10.4103/0255-0857.45362>.
45. R Core Team. The R Project for Statistical Computing, <https://www.r-project.org/>; 2025 [accessed 11 February 2025].
46. Amponsah-Dacosta E, Lebelo RL, Rakgole JN, Burnett RJ, Selabe SG, Mphahlele MJ. 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*. 2014;86(6):918-24. <https://doi.org/10.1002/jmv.23910>.
47. Amponsah-Dacosta E, Lebelo RL, Rakgole JN, Selabe SG, Gededzha MP, Mayaphi SH, et al. Hepatitis B virus infection in post-vaccination South Africa: occult HBV infection and circulating surface gene variants. *J Clin Virol*. 2015;63:12-7. <https://doi.org/10.1016/j.jcv.2014.11.032>.
48. World Health Organization. Hepatitis B vaccination coverage, https://immunizationdata.who.int/global/wiise-detail-page/hepatitis-b-vaccination-coverage?CODE=AFR&ANTIGEN=HEPB_BD&YEAR=; 2025 [accessed 15 February 2025].
49. Schweitzer A, Akmatov MK, Krause G. Hepatitis B vaccination timing: results from demographic health surveys in 47 countries. *Bull World Health Organ*. 2017;95(3):199. <https://doi.org/10.2471/BLT.16.178822>.
50. Marefiaw TA, Yenesew MA, Mihirete KM. Age-appropriate vaccination coverage and its associated factors for pentavalent 1-3 and measles vaccine doses, in northeast Ethiopia: A community-based cross-sectional study. *PloS One*. 2019;14(8):e0218470. <https://doi.org/10.1371/journal.pone.0218470>.
51. Yelverton V, Hair NL, Ghosh SH, Mfinanga SG, Ngadaya E, Baumgartner JN, et al. Beyond coverage: Rural-urban disparities in the timeliness of childhood vaccinations in Tanzania. *Vaccine*. 2022;40(37):5483-93. <https://doi.org/10.1016/j.vaccine.2022.07.020>.
52. Bruzzese E, Pagano F, Diana A, Punzi L, Guarino A. Protection of vaccine preventable diseases in a population of HIV-infected children: a 3 years prospective study. *Vaccines*. 2021;9(11):1331. <https://doi.org/10.3390/vaccines9111331>.
53. Shen R, Wang A-L, Pan X-P, Qiao Y-P, Wang Q, Wang X-Y, et al. Levels of vaccination coverage among HIV-exposed children in China: a retrospective study. *Infect Dis Poverty*. 2021;10:1-12. <https://doi.org/10.1186/s40249-021-00797-5>.
54. Department of Basic Education. ADMISSION OF LEARNERS TO PUBLIC SCHOOLS, <https://www.education.gov.za/Informationfor/ParentsandGuardians/SchoolAdmissions.aspx>; 2025 [accessed 17 April 2025].

55. Government of South Africa. Western Cape Education on admissions process for the 2022 school year, <https://www.gov.za/news/media-statements/western-cape-education-admissions-process-2022-school-year-28-feb-2021>; 2021 [accessed 17 April 2025].
56. Greyson D, Vriesema-Magnuson C, Bettinger JA. Impact of school vaccination mandates on pediatric vaccination coverage: a systematic review. *CMAJ*. 2019;7(3):E524-E36. <https://doi.org/10.9778/cmajo.20180191>.
57. Ndwandwe D, Ndlovu M, Mayeye A, Lumphondo N, Muvhulawa N, Ntamo Y, et al. Trends in Vaccine Completeness in Children Aged 0–23 Months in Cape Town, South Africa. *Vaccines*. 2023;11(12):1782. <https://doi.org/10.3390/vaccines11121782>
58. Kazi AM, Ali M, Zubair K, Kalimuddin H, Kazi AN, Iqbal SP, et al. Effect of mobile phone text message reminders on routine immunization uptake in Pakistan: randomized controlled trial. *JMIR Public Health Surveill*. 2018;4(1):e7026. <https://doi.org/10.2196/publichealth.7026>.
59. Chotun N, Nel E, Cotton MF, Preiser W, Andersson MI. Hepatitis B virus infection in HIV-exposed infants in the Western Cape, South Africa. *Vaccine*. 2015;33(36):4618-22. <https://doi.org/10.1016/j.vaccine.2015.06.076>.
60. Baruti K, Lentz K, Anderson M, Ajibola G, Phinius BB, Choga WT, et al. Hepatitis B virus prevalence and vaccine antibody titers in children HIV exposed but uninfected in Botswana. *PloS One*. 2020;15(8):e0237252. <https://doi.org/10.1371/journal.pone.0237252>.
61. Wang J-S, Chen H, Zhu Q-R. Transformation of hepatitis B serologic markers in babies born to hepatitis B surface antigen positive mothers. *World J Gastroenterol*. 2005;11(23):3582. <https://doi.org/10.3748/wjg.v11.i23.3582>.
62. Moss WJ, Clements CJ, Halsey NA. Immunization of children at risk of infection with human immunodeficiency virus. *Bull World Health Organ*. 2003;81:61-70.

PART B: Appendices

Appendix 1: Research Protocol

Hepatitis B virus infection and vaccine coverage among children living with HIV, HIV-exposed uninfected, and HIV-unexposed uninfected children in the Western Cape, South Africa

Introduction

Globally, hepatitis B prevalence rates vary (Figure 1) with an estimated 245 million individuals living with HBV infection and 1.1 million HBV-associated deaths reported in 2022 (1). Chronic HBV infection can lead to hepatitis, cirrhosis, or hepatocellular carcinoma (2). The age at which HBV infection occurs plays a crucial role in disease outcomes, with infections occurring during infancy or childhood carrying a higher risk of chronic complications compared to those acquired during adulthood (3). Around one in four people with chronic hepatitis B will develop cirrhosis or liver cancer (4). The mortality rates of HBV-related liver cirrhosis exhibit geographical variation (5). In 2019, an estimated 331,000 deaths were attributed to HBV-related liver cirrhosis and chronic liver disease (6). Additionally, an estimated 192,000 deaths due to HBV-related liver cancer were recorded in the same year (6).

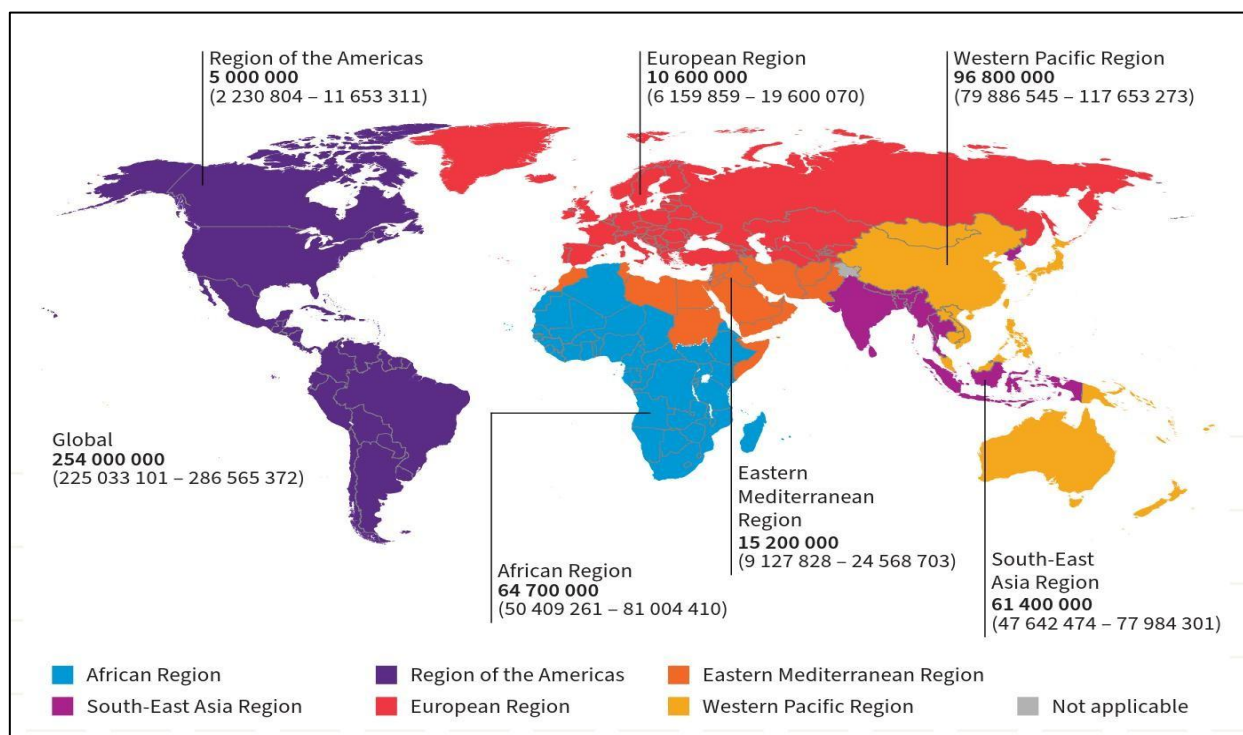


Figure 1: Global distribution of chronic hepatitis B prevalence in 2022

In 2019, the global prevalence of HBV surface antigen (HBsAg), a marker of acute or chronic HBV infection, among infants and children under 5 years of age was 1% (5). The highest prevalence among children under 5 was observed in the African region at 2.7%, while the lowest prevalence was in the Americas at 0.1% (5). A study conducted in South Africa, comparing pre- and post-HBV vaccine introduction in children and adults aged 1 to 25 years, demonstrated a decline of 2.8% in HBsAg prevalence and a 44% increase in HBV immunity (7). Despite the decreasing prevalence of HBV infection in children, vertical transmission of HBV remains a concern (8).

Globally, 7.6% of people living with HIV are co-infected with HBV (9). In South Africa, approximately 7.4% of people are co-infected with HIV and HBV (9). This co-infection significantly elevates the risk of perinatal HBV transmission and contributes to a more severe progression of chronic hepatitis B (10). Despite these significant implications, there remains a gap in understanding the prevalence of viral hepatitis among people living with HIV.

The national HIV Vertical Transmission Prevention program recommends routine HIV testing for all pregnant women whose HIV status is unknown or negative, along with rapid initiation of lifelong antiretroviral therapy in all women newly diagnosed with HIV (8). This initiative has resulted in a growing population of HIV-exposed uninfected (HEU) children. In 2018, it was reported that 21.6% of children under 15 years old were categorized as HEU (11, 12). It has been demonstrated that children exposed to HIV show a lower antibody response to HBV vaccination compared to children unexposed to HIV (13). This increases the susceptibility of HIV-exposed children to HBV transmission, especially in the presence of elevated viral DNA levels in HBV/HIV co-infected mothers not on treatment (14). However, there is limited understanding of the disease burden in HEU children compared to children living with HIV (CLWH).

The Global Health Sector Strategy (GHSS) on viral hepatitis, introduced in 2016 by the World Health Assembly, aims to eliminate viral hepatitis as a public health threat by 2030 (15). This includes reducing hepatitis-related deaths by 65%, new chronic HBV and HCV infections by 90%, and achieving a hepatitis B third dose vaccine coverage of 90% (15). To meet the targets of eliminating viral hepatitis, an expansion of interventions aimed at preventing transmission, coupled with increased testing and treatment efforts to mitigate the consequences of infections, are necessary (15, 16).

Hepatitis B immunization is crucial for eliminating the disease as a public health threat (17). In 2015, global hepatitis B vaccine coverage among infants reached 84% (18), which was close to the 2022 and 2030 targets (90% coverage), outlined in the GHSS. However, there are geographical disparities in coverage across World Health Organization (WHO) regions (17). The Western Pacific (90%), Americas (89%), and Southeast Asia (87%) regions reported the highest coverage in 2015, approaching the GHSS target (17). Conversely, the European (81%), Eastern Mediterranean (80%), and African (75%) regions had the lowest coverage rates (17).

Low coverage of hepatitis B vaccine birth-dose is particularly concerning, as individuals with HBV infections acquired at birth represent the majority of chronic liver disease cases later in life (18). Timely administration of

the birth-dose (within 24 hours of birth) of the hepatitis B vaccine is crucial for preventing vertical transmission of HBV (19). In the WHO African region, the limited availability of hepatitis B birth-doses is concerning, with only 15 out of 47 countries having introduced routine hepatitis B birth-dose vaccination programs (20). In 2022, hepatitis B birth-dose coverage in the WHO African region stood at a mere 18% (21), indicating a significant gap in protecting newborns against this vaccine preventable disease. With GAVI support, it is anticipated that more countries within the region will introduce the birth-dose although scaling up coverage remains a challenge. This issue is particularly concerning in South Africa, where infants born to mothers with HBV are at risk of vertical transmission in the absence of a universal hepatitis B birth-dose vaccination program. Addressing the low coverage of hepatitis B birth-dose vaccination is critical to advancing progress toward the targets set by the GHSS.

To prevent long-term complications of chronic hepatitis B and mitigate further transmission, improving HBV diagnosis and enhancing access to care are necessary (22). Simultaneously, ongoing efforts should focus on enhancing vaccination coverage and timeliness which are crucial to eliminating HBV infection as a public health threat. In South Africa, the hepatitis B vaccine was incorporated into the national Essential Program on Immunization (EPI) schedule in 1995, administered at weeks 6, 10, and 14 (23). Until recently, the country has not had a hepatitis B birth-dose vaccination program in place. In January 2024, the National Department of Health rolled out a selective hepatitis B birth-dose vaccination program, targeting exposed newborns. The high burden of HBV/HIV co-infections in South Africa presents additional challenges to national viral hepatitis elimination efforts. To the best of our knowledge, data on HBV prevalence among the growing population of HEU compared to HIV-unexposed uninfected (HUU) children in South Africa also remains limited. Therefore, we aim to investigate vaccine coverage and HBV infection among CLWH, HEU, and HUU children to better inform current policy and practice in South Africa.

Problem Statement

The dearth of research on the burden of HBV infections among CLWH and HEU children in South Africa hinders the capacity to implement effective and tailored interventions. This knowledge gap directly impacts the goal of eliminating viral hepatitis as a public health threat, as outlined by the GHSS. To effectively reach the targets set by the GHSS, it is essential to understand the vaccine coverage rates and burden of hepatitis B among these high-risk populations. By addressing these knowledge gaps, public health strategies can be designed to meet the specific needs of CLWH and HEU populations, ultimately advancing progress toward the elimination of viral hepatitis as a significant public health concern.

Research Questions

This study seeks to address the following research questions:

1. How do hepatitis B vaccine coverage rates and timely uptake among CLWH and HEU differ from that among HUU children?
2. How does the burden of HBV infection among CLWH and HEU differ from that among HUU children?
3. What are the determinants of hepatitis B vaccine coverage and timely uptake among CLWH, HEU, and HUU children?

Aim

To describe the coverage and timely uptake of routine infant hepatitis B vaccination and the burden of HBV infection among CLWH, HEU, and HUU children in South Africa.

Objectives

1. To assess the coverage and timely uptake of the hepatitis B vaccine among CLWH, HEU, and HUU children in Western Cape, South Africa.
2. To determine the prevalence of HBV infection among CLWH, HEU, and HUU children in Western Cape, South Africa.
3. To explore factors associated with the completion and timely uptake of recommended hepatitis B vaccine doses among CLWH, HEU, and HUU children in Western Cape, South Africa.

Methods

a. Study design

We will adopt a retrospective cross-sectional study design, utilising a convenient sample of sera obtained from children during a parent study conducted between 2012 and 2016 (24). This parent study, conducted in health facilities in the Western Cape, focused on investigating the incidence of pertussis in South African children who presented with mild to severe lower respiratory tract infections (24).

For the purpose of the current study, clinical, demographic, and socio-economic records from 671 participants, and 535 archival sera (serum samples) which were collected from children aged 27 days to 12 years will be assessed.

b. Sample size

There are 671 participant data available for this secondary data analysis, with an estimated minimum of 330 children's records expected to meet the inclusion criteria.

The sample size of 330 was calculated using the following formula:

$$n = \frac{p(1-p)(1.96)^2}{d^2} \quad \text{where } p \text{ is the expected proportion and } d \text{ is the precision.}$$

HBV:

p= 85.3 %, The 2022 national immunization coverage for the third dose of the hepatitis B vaccine in South Africa

d= 3.8%, The prevalence of hepatitis B infection in children under 5 years of age in 2019 (25).

c. Inclusion criteria:

- Secondary data (laboratory, clinical, demographic, and socio-economic records) from children under the age of 13 who were admitted to Red Cross War Memorial Children's Hospital for lower respiratory tract infections between 07 September 2012 to 06 September 2013 will be included in this study.
- Only participants whose caregivers consented to having their samples undergo additional testing will be included to address Objective 2.

Exclusion criteria³:

- Non-availability of Road-to-Health Cards (RTHCs)
- Ineligible for hepatitis B vaccine
- Non-Availability of Case Report Forms (CRFs)⁴

d. Data collection

Variables such as hepatitis B vaccination history, including the number of doses received and age at vaccination, will be retrieved from RTHCs. Results from hepatitis B serological testing will be collected, encompassing three key markers: HBsAg, anti-HBs, and anti-HBc. Briefly, serum samples were tested for these three markers using Elecsys® test kits, and serological assays were performed on the Cobas® 6000 analyser series (Roche Diagnostics, Germany). HBsAg serves as an indicator for acute and chronic HBV infection, anti-HBs signifies immunity from vaccination or previous acute infection that resolved, and anti-HBc is indicative of exposure to HBV. Additionally, socio-demographic and economic data, such as participant and maternal age, maternal education, and socio-economic status (SES), will be extracted from the parent study research database (Table 1).

³ For Objective 2 of the study, the non-availability of RTHCs and ineligibility for the hepatitis B vaccine will not apply.

⁴ A template of the CRF used in data collection is provided in Appendix 2.

Table 1: Participant and caregiver variables extracted from the parent study

	Type	Scale	Categories/units
<i>Participant Demographics</i>			
Age	Numerical	Continuous	Months
Sex	Categorical	Binary	Male, Female
Race	Categorical	Polytomous	Black, Coloured, Asian/Indian or White
HIV exposure status	Categorical	Binary	Exposed, Unexposed
HIV status	Categorical	Polytomous	Positive, negative, unknown
Number of hepatitis B doses	Numerical	Discrete	
HBsAg titre	Numerical	Continuous	mIU/mL
Anti-HBs titre	Numerical	Continuous	mIU/mL
Anti-HBC titre	Numerical	Continuous	mIU/mL
<i>Caregiver Demographics</i>			
Age	Numerical	Continuous	Years
Highest grade Education¹	Numerical	Discrete	Grade
Education¹	Categorical	Binary	Primary, secondary, tertiary
Socio-economic status²	Categorical	Polytomous	High, low, upper-middle, lower-middle, unknown

¹Education was defined according to the South African school system as follows: primary (grades 1-7), secondary (grades 8-12) and tertiary (higher education). Basic education is a combination of primary and secondary school (grades 1-12).

²Socio-economic status was categorized into quartiles based on a validated weighted composite score that included maternal asset ownership, employment, and education.

e. Outcomes of interests

This study aims to establish a baseline for further research on hepatitis B vaccine coverage, timely uptake, and the burden of hepatitis B among CLWH and HEU children, comparing them to their HUU counterparts in South Africa.

The evidence will play a crucial role in highlighting gaps in our current understanding of vaccine coverage, timeliness, and HBV infection among high-risk populations, particularly CLWH and HEU. Our goal is to contribute robust evidence needed to inform the prioritization of viral hepatitis elimination efforts among CLWH and HEU children in the South African context.

f. Data analysis

Descriptive data including participant demographics will be presented in a table. Numerical data will be analysed using either the mean (standard deviation) or median (interquartile range) depending on the distribution. Categorical variables will be described using proportions as percentages. Statistical tests, including Kruskal-Wallis tests, will be used to analyse associations between numerical data. For categorical variables, associations will be assessed using Chi-square or Fisher's exact tests, as appropriate. To assess hepatitis B vaccine coverage

rates among CLWH, HEU and HUU children, the study will examine the proportions of participants who received age-specific hepatitis B vaccine doses by 12 months of age. Timely uptake of the hepatitis B vaccine will be calculated as the difference in the actual date and expected date of receipt of the age-specific immunization. The coverage and timeliness will be assessed for participants who are age eligible for the hepatitis B vaccine at weeks 6, 10 and 14. The prevalence of HBV infection will be determined based on the presence of HBsAg within each subgroup. Factors influencing the (in)complete⁵ and (un)timely⁶ administration of HBV vaccination will be assessed using logistic regression analyses, with binary outcomes incomplete/complete and delayed/timely administration. All statistical analyses will be conducted using R version 4.3.0 and R studio version 2024.04.02+764 (30). Where hypothesis testing has been done, significance level will be set at a two-tailed $P < 0.05$.

g. Risks and benefits

This retrospective cross-sectional study poses no harm or threat to any participants, given that there will be no direct human participation. The advancement of knowledge has the potential to benefit both the Western Cape Department of Health and the national health sector at large. The findings of this study can inform policies aimed at enhancing vaccine coverage and addressing the burden of viral hepatitis in South Africa, thereby contributing to the overall improvement of public health initiatives.

h. Study limitations

This study relies on data collected from health facilities in Cape town, Western Cape province, South Africa, limiting generalisability of the study findings. Using a hospital-based population may introduce biases due to differing risk profiles and health-seeking behaviours compared to the general population. Methodological limitations also need to be acknowledged, including the modest sample size and the retrospective design of the study, which relies on samples collected between 2012 and 2016. This time frame may not accurately reflect the current South African context in terms of the burden of disease in the paediatric population or advancements in the prevention of mother-to-child transmission of HIV and how this has impacted vertical transmission rates.

Consequently, caution is advised when interpreting the study findings. It is important to note that inaccuracies may be introduced due to missing or incomplete data from the research database of the parent study.

⁵Dose completion (completeness) will be defined as receiving all three scheduled doses of hepatitis B vaccine at 14 weeks (+4 weeks) of age 26. Chiabi A, Nguetack FD, Njapndounke F, Kobela M, Kenfack K, Nguetack S, et al. Vaccination of infants aged 0 to 11 months at the Yaounde Gynaeco-obstetric and pediatric hospital in Cameroon: how complete and how timely? *BMC pediatrics*. 2017;17:1-7.

⁶Timeliness will be defined as receiving the vaccine less than 5 days before and 28 days after the recommended age administration, as reported in previous studies 27. Blose N, Amponsah-Dacosta E, Kagina BM, Muloiwa R. Descriptive analysis of routine childhood immunisation timeliness in the Western Cape, South Africa. *Vaccine*: X. 2022;10:100130, 28. Mthiyane T, Walaza S, Cohen C, Norris S, Tempia S, Cohen A, et al. Factors associated with missed and delayed DTP3 vaccination in children aged 12-59 months in two communities in South Africa, 2012-2013. *South African medical journal*. 2019;109(8):562-9, 29. Laryea DO, Abbeyquaye Parbie E, Frimpong E. Timeliness of childhood vaccine uptake among children attending a tertiary health service facility-based immunisation clinic in Ghana. *BMC public health*. 2014;14:1-5. Any administration beyond this time period will be classified as delayed.

i. Privacy and confidentiality

To maintain confidentiality, the study will refrain from collecting any personal identifiable data, such as names. Instead, each participant will be assigned a unique identification number. All participant data will be securely stored and backed up in a database accessible only to the primary researcher and the supervisors overseeing this study.

j. Dissemination

The anticipated study outcomes will be disseminated via publication in an internationally accredited journal *Vaccine*. Additionally, the findings will be disseminated at various workshops and conferences, including the Annual African Vaccinology Course hosted by the Vaccines for Africa Initiative. These dissemination efforts are designed to actively contribute to the expansion and reinforcement of strategies aimed at eliminating viral hepatitis in South Africa.

Expected significance of the study

The purpose of the study is to assess hepatitis B vaccine coverage rates, timely uptake, and the burden of hepatitis B, along with describing the socio-economic and demographic determinants associated with the coverage and timely uptake of the hepatitis B vaccine among CLWH and HEU children compared to their HUU counterparts. This evidence can be used to inform and shape policies and interventions with a particular focus on improving vaccine coverage rates and effectively addressing and mitigating the impact of hepatitis B within these specific populations. This is in line with global efforts to eliminate viral hepatitis as a global public health threat by 2030.

Ethical approval

Ethics approval for the parent study has been secured from the University of Cape Town Human Research Ethics Committee (HREC) [HREC Reference Number: 371/2011]. Ethics clearance to utilise secondary data and archived sera collected during the parent study as part of the primary researcher's MPH degree will be sought from the School of Public Health Departmental Research Committee (DRC), and HREC.

Study timeline

Table 2: Timeline for proposed study

Research activities	2023	2024				2025	
	Q1	Q1	Q2	Q3	Q4	Q1	Q2
Protocol development							
DRC and HREC submission							
Data extraction and entry							
Data analysis							
Manuscript Write-up phase							
Mini-dissertation submission							

References

1. World Health Organization. Global hepatitis report 2024: action for access in low-and middle-income countries. World Health Organization; 2024 Apr 9.
2. Liaw Y-F, Chu C-M. Hepatitis B virus infection. *Lancet*. 2009;373(9663):582-92. [https://doi.org/10.1016/s0140-6736\(09\)60207-5](https://doi.org/10.1016/s0140-6736(09)60207-5).
3. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet*. 2014;384(9959):2053-63. [https://doi.org/10.1016/s0140-6736\(14\)60220-8](https://doi.org/10.1016/s0140-6736(14)60220-8).
4. Maddrey WC. Hepatitis B: an important public health issue. *J Med Virol*. 2000;61(3):362-6. [https://doi.org/10.1002/10969071\(200007\)61:3%3C362::aid-jmv14%3E3.0.co;2-i](https://doi.org/10.1002/10969071(200007)61:3%3C362::aid-jmv14%3E3.0.co;2-i)
5. Sheena BS, Hiebert L, Han H, Ippolito H, Abbasi-Kangevari M, Abbasi-Kangevari Z, et al. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Gastroenterol Hepatol*. 2022;7(9):796-829. [https://doi.org/10.1016/s2468-1253\(22\)00124-8](https://doi.org/10.1016/s2468-1253(22)00124-8).
6. Hsu Y-C, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. *Nat Rev Gastroenterol Hepatol*. 2023;20(8):524-37. <https://doi.org/10.1038/s41575-023-00760-9>.
7. Amponsah-Dacosta E, Lebelo RL, Rakgole JN, Burnett RJ, Selabe SG, Mphahlele MJ. 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*. 2014;86(6):918-24. <https://doi.org/10.1002/jmv.23910>.
8. Tamandjou Tchuem C, Cotton MF, Nel E, Tedder R, Preiser W, Violari A, et al. Viral hepatitis B and C in HIV-exposed South African infants. *BMC Pediatr*. 2020;20:1-7. <https://doi.org/10.1186/s12887-020-02479-x>.
9. Platt L, French CE, McGowan CR, Sabin K, Gower E, Trickey A, et al. Prevalence and burden of HBV co-infection among people living with HIV: a global systematic review and meta-analysis. *J Viral Hepat*. 2020;27(3):294-315. <https://doi.org/10.1111/jvh.13217>.
10. Kourtis AP, Bulterys M, Hu DJ, Jamieson DJ. HIV–HBV coinfection—A global challenge. *N Engl J M*. 2012;366(19):1749-52. <https://doi.org/10.1056/nejmp1201796>.
11. Slogrove AL, Powis KM, Johnson LF, Stover J, Mahy M. Estimates of the global population of children who are HIV-exposed and uninfected, 2000–18: a modelling study. *Lancet Glob Health*. 2020;8(1): e67-e75. [https://doi.org/10.1016/s2214-109x\(19\)30448-6](https://doi.org/10.1016/s2214-109x(19)30448-6).
12. De Beer ST, Slogrove AL, Eley B, Ingle SM, Jones HE, Phelanyane F, et al. Change in HIV-related characteristics of children hospitalised with infectious diseases in Western Cape, South Africa, 2008–2021: a time trend analysis. *J Int AIDS Soc*. 2023;26: e26151. <https://doi.org/10.1002/jia2.26151>.

13. Baruti K, Lentz K, Anderson M, Ajibola G, Phinius BB, Choga WT, et al. Hepatitis B virus prevalence and vaccine antibody titers in children HIV exposed but uninfected in Botswana. *PloS One*. 2020;15(8): e0237252. <https://doi.org/10.1371/journal.pone.0237252>.
14. Mancinelli S, Pirillo MF, Liotta G, Andreotti M, Mphwere R, Amici R, et al. Antibody response to hepatitis B vaccine in HIV-exposed infants in Malawi and correlation with HBV infection acquisition. *J Med Virol*. 2018;90(6):1172-6. <https://doi.org/10.1002/jmv.25049>.
15. World Health Organization. Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis 2016.
16. Nayagam S, Thursz M, Sicuri E, Conteh L, Wiktor S, Low-Beer D, et al. Requirements for global elimination of hepatitis B: a modelling study. *Lancet Infect Dis*. 2016;16(12):1399-408. [https://doi.org/10.1016/s1473-3099\(16\)30204-3](https://doi.org/10.1016/s1473-3099(16)30204-3).
17. Hutin YJF, Bulterys M, Hirschall GO. How far are we from viral hepatitis elimination service coverage targets? *J Int AIDS Soc*. 2018;21:e25050. <https://doi.org/10.1002/jia2.25050>.
18. World Health Organization. Global hepatitis report 2017.
19. Shimakawa Y, Lemoine M, Njai HF, Bottomley C, Ndow G, Goldin RD, et al. Natural history of chronic HBV infection in West Africa: a longitudinal population-based study from The Gambia. *Gut*. 2016;65(12):2007-16. <https://doi.org/10.1136/gutjnl-2015-309892>.
20. U.S. Centers for Disease Control and Prevention. Partnering to Increase Hepatitis B Birth Dose Vaccination in Africa, <https://www.cdc.gov/global-immunization/stories/hepatitis-b-birth-dose-vaccination-in-africa.html>; 2024 [accessed 16 February 2025].
21. World Health Organization. Hepatitis B vaccination coverage, https://immunizationdata.who.int/global/wiise-detail-page/hepatitis-b-vaccination-coverage?CODE=AFR&ANTIGEN=HEPB_BD&YEAR=; 2022 [accessed 15 February 2025].
22. Spearman CW, Afihene M, Ally R, Apica B, Awuku Y, Cunha L, et al. Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. *Lancet Gastroenterol Hepatol*. 2017;2(12):900-9. [https://doi.org/10.1016/s2468-1253\(17\)30295-9](https://doi.org/10.1016/s2468-1253(17)30295-9).
23. 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):3919-26. [https://doi.org/10.1016/s0264-410x\(01\)00120-7](https://doi.org/10.1016/s0264-410x(01)00120-7).
24. Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. Incidence and diagnosis of pertussis in South African children hospitalized with lower respiratory tract infection. *Pediatr Infect Dis J*. 2016;35(6):611-6. <https://doi.org/10.1097/inf.0000000000001132>.
25. Moonsamy S, Suchard M, Pillay P, Prabdial-Sing N. Prevalence and incidence rates of laboratory-confirmed hepatitis B infection in South Africa, 2015 to 2019. *BMC Public Health*. 2022;22(1):29. <https://doi.org/10.1186/s12889-021-12391-3>.
26. Chiabi A, Nguetack FD, Njapndounke F, Kobela M, Kenfack K, Nguetack S, et al. Vaccination of infants aged 0 to 11 months at the Yaounde Gynaeco-obstetric and pediatric hospital in Cameroon: how complete and how timely? *BMC Pediatr*. 2017;17:1-7. <https://doi.org/10.1186/s12887-017-0954-1>.
27. Blose N, Amponsah-Dacosta E, Kagina BM, Muloiwa R. Descriptive analysis of routine childhood immunisation timeliness in the Western Cape, South Africa. *Vaccine: X*. 2022;10:100130. <https://doi.org/10.1016/j.jvacx.2021.100130>.
28. Mthiyane T, Walaza S, Cohen C, Norris S, Tempia S, Cohen A, et al. Factors associated with missed and delayed DTP3 vaccination in children aged 12-59 months in two communities in South Africa, 2012-2013. *S Afr Med J*. 2019;109(8):562-9. <https://doi.org/10.7196/SAMJ.2019.v109i8.13244>.
29. Laryea DO, Abbeyquaye Parbie E, Frimpong E. Timeliness of childhood vaccine uptake among children attending a tertiary health service facility-based immunisation clinic in Ghana. *BMC Public Health*. 2014;14:1-5. <https://doi.org/10.1186/1471-2458-14-90>.
30. R Core Team. The R Project for Statistical Computing, <https://www.r-project.org/>; 2025 [accessed 11 February 2025].

Appendix 2: Template of Case Report Form

CASE REPORT FORM

STUDY TITLE	
Pertussis in children hospitalised with Lower Respiratory Tract infection (LRTI)	
Study reference number:	371/2011

CLINICAL TRIAL SITE/UNIT:	Red Cross War Memorial Children's Hospital Department of Paediatrics University of Cape Town Klipfontein Road Rondebosch, 7700
PRINCIPAL INVESTIGATOR:	Rudzani Muloiwa

Patient's Name Hospital sticker

Subject Study Number:	<table border="1"> <tr> <td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td> </tr> </table>								

<i>I am confident that the information supplied in this case record form is complete and accurate data. I confirm that the study was conducted in accordance with the protocol and any protocol amendments and that written informed consent was obtained prior to the study.</i>																	
Investigator's Signature:	_____																
Date of signature:	<table border="1"> <tr> <td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td><td> </td> </tr> <tr> <td>d</td><td>d</td><td>m</td><td>m</td><td>y</td><td>y</td><td>y</td><td>y</td> </tr> </table>									d	d	m	m	y	y	y	y
d	d	m	m	y	y	y	y										

Parent contact details& Inclusion criteria

Care giver's First and Middle Names: _____

Care giver's Last Name: _____

Home Address *(If different from child's address)*

Home Telephone Number: _____

Work Telephone Number: _____

Cellular Telephone Number: _____

Alternate contact details

1. Name _____ Relationship _____

Home Telephone Number: _____

Work Telephone Number: _____

Cellular Telephone Number: _____

Inclusion Criteria		Yes	No*
1	Is the subject a child aged 13 years or younger?	<input type="checkbox"/>	<input type="checkbox"/>
2	Has the subject's parent/legal guardian willingly given written informed consent?	<input type="checkbox"/>	<input type="checkbox"/>
3	Has the subject been admitted for LRTI or apnoea?	<input type="checkbox"/>	<input type="checkbox"/>
<i>*If any inclusion criteria are ticked 'No' then the patient is not eligible for the study.</i>			
Exclusion Criteria		Yes*	No
1	Is child too ill to be enrolled?	<input type="checkbox"/>	<input type="checkbox"/>
2	Has the child been admitted for more than 72 hours?	<input type="checkbox"/>	<input type="checkbox"/>
<i>*If any exclusion criteria are ticked 'Yes' then the patient is not eligible for the study.</i>			

I. Parent Interview Form

Pertussis study number: _____ : _____

A. GENERAL: Complete all questions

1.	Are you the primary caregiver for this child?	<input type="checkbox"/> Yes	<input type="checkbox"/> No
2.	What is your relationship to him or her?	<input type="checkbox"/> Mother <input type="checkbox"/> Grandparent <input type="checkbox"/> Caregiver	<input type="checkbox"/> Father <input type="checkbox"/> Other relative <input type="checkbox"/> Other: _____
3.	During the first 4 months of life, how was your child fed?	<input type="checkbox"/> Breast milk <input type="checkbox"/> Formula (never breastfed)	<input type="checkbox"/> Breast milk and formula <input type="checkbox"/> Unknown
4.	If older than 4 months: how is your child being fed at present? (In addition to solids)	<input type="checkbox"/> Breast milk <input type="checkbox"/> Formula <input type="checkbox"/> Unknown	<input type="checkbox"/> Breast milk and formula <input type="checkbox"/> Other: _____ <input type="checkbox"/> Not applicable
5.	What is your house made of?	<input type="checkbox"/> Bricks <input type="checkbox"/> Tin/iron sheeting <input type="checkbox"/> Unknown	<input type="checkbox"/> Mud/traditional <input type="checkbox"/> Other: _____
6.	How many people sleep in the same room as the child (not counting the child)?	_____ people	<input type="checkbox"/> Unknown
7.	Do you use any of the following for heating and/or cooking in the household? (check all that apply)	<input type="checkbox"/> Electricity <input type="checkbox"/> Coal <input type="checkbox"/> Wood <input type="checkbox"/> Unknown	<input type="checkbox"/> Gas <input type="checkbox"/> Paraffin <input type="checkbox"/> Other: _____
8.	What is the main source of water in the household?	<input type="checkbox"/> In-door tap water <input type="checkbox"/> River water <input type="checkbox"/> Other: _____	<input type="checkbox"/> Borehole <input type="checkbox"/> Outdoor/communal tap <input type="checkbox"/> Unknown
9.	What type of toilet do you have at the house?	<input type="checkbox"/> Flush toilet <input type="checkbox"/> Bucket system <input type="checkbox"/> Other: _____	<input type="checkbox"/> Pit latrine <input type="checkbox"/> None/ Outdoors <input type="checkbox"/> Unknown
10.	Does the person who usually cares for the child smoke inside the house?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
11.	Does anyone else smoke inside the house?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
12.	Does your child attend a nursery or crèche? (at least 2 other children for at least 4 hours per day, 3 days per week)	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown
13.	What is the highest level of education the mother has completed?	<input type="checkbox"/> No School <input type="checkbox"/> Higher education <input type="checkbox"/> Unknown	<input type="checkbox"/> Schooling: highest grade completed _____

B. MEDICAL HISTORY

14.	Did your child have any of the following symptoms during the current illness?		
14.1	Fever	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 14.2 if No or Unknown)
14.1.1	If yes: Duration	_____ days <input type="checkbox"/> Unknown	
14.2	Cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 14.3 if No or Unknown)
14.2.1	If yes: Duration	_____ days <input type="checkbox"/> Unknown	
14.2.2	Night time cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.3	Paroxysms of cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.4	Whooping cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.5	Vomiting after cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.6	Facial cyanosis	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.7	Barking cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.2.8	Normal state between cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.3	Apnoea (stopping breathing)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.4	Loss of weight	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.5	Fits (seizures)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
14.6	Presence of any of these symptoms	<input type="checkbox"/> Runny nose <input type="checkbox"/> Wheeze <input type="checkbox"/> Fast breathing <input type="checkbox"/> Sneezing <input type="checkbox"/> None <input type="checkbox"/> Unknown	
15.	Did you seek health care prior to this hospitalization?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 16 if No or Unknown)

Staff initials: _____

Date completed: ____ / ____ / 20 ____

I. Parent Interview Form – (continue)

15.1	If Yes, did you go to:	<input type="checkbox"/> Clinic <input type="checkbox"/> General practitioner	<input type="checkbox"/> Traditional healer <input type="checkbox"/> Other:
16.	Did the child receive an antibiotic before coming to the hospital?	<input type="checkbox"/> Yes, oral antibiotic <input type="checkbox"/> Yes, injection	<input type="checkbox"/> Unknown (skip to 17 if No or Unknown) <input type="checkbox"/> No <input type="checkbox"/> Unknown
16.1	If Yes, name of antibiotic If Yes, number of days on antibiotics?	_____ days <input type="checkbox"/> Unknown	
17.	Does your child take cotrimoxazole (bactrim)?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 18 if No or Unknown)
17.1	If Yes, for how long?	_____ months or _____ weeks	<input type="checkbox"/> Unknown
18.	Has any one at home been coughing for more than two weeks? If yes, how many people? Relationship with coughing person/s	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown _____	
19.	Has your child been in contact with an adult known to have TB in the past 6 months?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 19 if No or Unknown)
19.1	If Yes, is the person with TB currently on treatment?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
20.	Is your child currently receiving INH prophylaxis	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 19 if No or Unknown)
20.1	If Yes, about how long has your child been taking it?	_____ months or _____ weeks <input type="checkbox"/> Unknown	
21.	Has the child ever been admitted to hospital? (Before this admission)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 20 if No or Unknown)
21.1	If Yes,		
21.2	How long ago was the last admission?	_____ months _____ weeks _____ days <input type="checkbox"/> Unknown	
21.3	Number of previous hospitalizations (not including current illness):	<input type="checkbox"/> 1-2 <input type="checkbox"/> 3-5 <input type="checkbox"/> >5 <input type="checkbox"/> Unknown	
21.4	Were any of these previous admissions for apnoea?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
21.5	Were any of these previous admissions for a chest infection?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	
22.	Has your child ever been diagnosed with one of the following chronic medical conditions? (before this admission)	<input type="checkbox"/> TB Disease: If yes: <input type="checkbox"/> On treatment <input type="checkbox"/> Asthma <input type="checkbox"/> Completed treatment <input type="checkbox"/> Heart problem <input type="checkbox"/> Defaulted <input type="checkbox"/> Pertussis/Whooping cough <input type="checkbox"/> Other lung problems Specify _____ <input type="checkbox"/> Cerebral palsy <input type="checkbox"/> Malnutrition <input type="checkbox"/> Kidney problem Specify _____ <input type="checkbox"/> Other Specify _____ <input type="checkbox"/> None known	
23.	Did the child's mother have an HIV test during pregnancy?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to 22 if No or Unknown)
23.1	If Yes, what was the latest result?	<input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Unknown	
24.	Has the child ever been tested for HIV?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to end if No / Unknown)
24.1	If Yes, What was the result?	<input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Unknown	(skip to end if Neg / Unknown)
25.	Is the child currently receiving Antiretroviral Therapy (ART)?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown	(skip to end if No / Unknown)
25.1	For how long has the child taken ART?	_____ months <input type="checkbox"/> Unknown	

2. RTHC / Vaccination History

Pertussis study number: _____

1.	Does the child have a road to health card available?	<input type="checkbox"/> Yes (<i>Skip to 2</i>)	<input type="checkbox"/> No
1.1	If No, Has the child ever received any vaccines other than the vaccines he or she received at birth?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown

Copy the following information from the card, if no RTHC available, ask person completing interview

2.	Date of Birth (dd/mm/yy)	___ / ___ / 20__	<input type="checkbox"/> Unknown
2.1	If DOB unknown, enter age in months:	_____	
3.	Is the child failing to thrive/crossing centiles	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unsure
4.	Birth weight	___ . ___ kg	<input type="checkbox"/> Unknown
5.	Gestational age	___ weeks	<input type="checkbox"/> Unknown
5.1	If gestational age unknown	<input type="checkbox"/> Term	<input type="checkbox"/> Unknown
		<input type="checkbox"/> Premature (<37 weeks)	

If the RTHC is available, please complete

 No RTHC available

6.	Vaccine	Date given (dd/mm/yyyy)	
6.1	BCG	___ / ___ / 20__	<input type="checkbox"/> Not given
6.2	OPV	0 ___ / ___ / 20__	<input type="checkbox"/> Not given
		1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.3	PENTAXIM LOCATION GIVEN -	(i.e. Health facility where the child received vaccine)	
6.3		1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
OR DTP/HIB		1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
		4 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.4	PCV <input type="checkbox"/> 7 <input type="checkbox"/> 13 <input type="checkbox"/> Unknown	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
		3 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.5	ROTAVIRUS	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
6.6	MEASLES	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
7.	INFLUENZA	1 ___ / ___ / 20__	<input type="checkbox"/> Not given
		2 ___ / ___ / 20__	<input type="checkbox"/> Not given
8.	OTHER: _____	___ / ___ / 20__	
9.	Did the child have any of the following within 6 weeks before or after any of the vaccines in 6.3?	<input type="checkbox"/> Steroid use	<input type="checkbox"/> Measles
		<input type="checkbox"/> Immunoglobulin therapy	<input type="checkbox"/> None
		<input type="checkbox"/> Other: _____	<input type="checkbox"/> Unknown
	If yes, which vaccines? Enter 1- 4	_____	
10.	Has the child received Vitamin A in the previous six months?	<input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Unknown

Staff initials: _____

Date completed: ___ / ___ / 20__

3. Medical Records

The following questions relate to the current admission. Information should be obtained from clinical records or measured (e.g. weight and temperature) if not available in records. Temperature, heart rate and respiratory rate should be the maximum recorded within 24 hours of admission

Pertussis study number: _____ : _____

1.	Sex	<input type="checkbox"/> Male <input type="checkbox"/> Female
2.	Date of admission	____ / ____ / 20 ____ (dd/mm/yyyy)
3.	Time of admission	____ : ____ <input type="checkbox"/> Am <input type="checkbox"/> Pm <input type="checkbox"/> Unknown
4.	Race	<input type="checkbox"/> Asian/Indian <input type="checkbox"/> Black <input type="checkbox"/> White <input type="checkbox"/> Coloured
5.	Admission height/length	____ , ____ cm
6.	Admission weight	____ . ____ kg
7.	Mid upper arm circumference (MUAC)	____ , ____ cm
8.	Head Circumference	____ , ____ cm
9.	Temperature	____ . ____ °C
10.	Heart rate	____ beats / min <input type="checkbox"/> Not recorded
11.	Respiratory rate	____ breaths / min <input type="checkbox"/> Not recorded
12.	Oxygen saturation	____ % <input type="checkbox"/> Room air <input type="checkbox"/> On oxygen <input type="checkbox"/> Not recorded
13.	Presence of oedema	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
14.	Did the child receive any of: Bronchodilators Antibiotics If Yes, name of antibiotic	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No _____
15.	HIV Stage of the child	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> Not Applicable
16.	Lower chest wall indrawing:	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
17.	Crackles/crepitations	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
18.	Wheezing	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
19.	Clubbing	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded

Outcome Summary – to be obtained on all participants when discharged.

20.	Did child receive supplemental oxygen	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
21.	Admission to a High Care unit or ICU	<input type="checkbox"/> ICU <input type="checkbox"/> High Care <input type="checkbox"/> None <input type="checkbox"/> Unknown
	If admitted to above, number of days	ICU days: ____ High Care days: ____ <input type="checkbox"/> Unknown
22.	Assisted ventilation	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not recorded
	If Yes, Duration	____ days <input type="checkbox"/> Not recorded
	Type of support	<input type="checkbox"/> CPAP <input type="checkbox"/> IPPV
	Number of days	____ days <input type="checkbox"/> Not recorded
23.	Outcome of child	<input type="checkbox"/> Discharged <input type="checkbox"/> Refused Hospital Treatment (RHT) <input type="checkbox"/> Died <input type="checkbox"/> Transferred to another hospital
24.	Date of discharge/ death/ transfer/RHT	____ / ____ / 20 ____ (dd/mm/yyyy)
25.	If discharged, on what treatment was child discharged? (mark all that apply)	<input type="checkbox"/> Antibiotics, specify: _____ <input type="checkbox"/> Bronchodilators <input type="checkbox"/> Inhaled steroids <input type="checkbox"/> Anti TB treatment <input type="checkbox"/> Other, specify _____
26.	Discharge diagnosis (mark all that apply)	<input type="checkbox"/> Bronchopneumonia <input type="checkbox"/> Bronchiolitis <input type="checkbox"/> Lobar Pneumonia <input type="checkbox"/> Tuberculosis <input type="checkbox"/> Pertussis <input type="checkbox"/> Pneumocystis pneumonia <input type="checkbox"/> Chronic lung disease <input type="checkbox"/> Sepsis <input type="checkbox"/> Underweight/Kwashiorkor/Marasmus <input type="checkbox"/> Not recorded <input type="checkbox"/> Other, specify _____

Staff initials: _____

Date completed: ____ / ____ / 20 ____

4. Care-Giver Information

Pertussis study number: _____: _____

1.	Sex	<input type="checkbox"/> Male <input type="checkbox"/> Female
2.	Date of birth	____ / ____ / 19 ____ (dd/mm/yyyy)
3.	What is your relationship to child	<input type="checkbox"/> Mother <input type="checkbox"/> Father <input type="checkbox"/> Grandparent <input type="checkbox"/> Other relative <input type="checkbox"/> Caregiver <input type="checkbox"/> Other: _____
4.	Race	<input type="checkbox"/> Asian/Indian <input type="checkbox"/> Black <input type="checkbox"/> White <input type="checkbox"/> Coloured
5.	Do you sleep in the same room as child?	<input type="checkbox"/> Yes <input type="checkbox"/> No
6.	Do you smoke? If Yes, do you smoke inside the house?	<input type="checkbox"/> Yes <input type="checkbox"/> No (skip to next question if No) <input type="checkbox"/> Yes <input type="checkbox"/> No
Did you experience any of these symptoms before or during the course of the child's illness?		
7.	Fever?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
8.	Wheezing?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
9.	Runny or congested nose?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.	Cough?	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown (skip to 11 if No or Unknown)
	If Yes, duration?	_____ days _____ weeks _____ months <input type="checkbox"/> Unknown
10.1	Night time cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.2	Paroxysms of cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.3	Whooping cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.4	Vomiting after cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.5	Barking cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.6	Normal state between cough	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Unknown
10.7	Did you receive any of the following: Bronchodilators Antibiotics If Yes, Name of antibiotic	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Yes <input type="checkbox"/> No _____
11.	Have you ever been diagnosed with one of the following chronic medical conditions?	<input type="checkbox"/> TB Disease: If yes: <input type="checkbox"/> On treatment <input type="checkbox"/> Completed treatment <input type="checkbox"/> Defaulted <input type="checkbox"/> Asthma <input type="checkbox"/> Other lung problems <input type="checkbox"/> Pertussis/Whooping cough <input type="checkbox"/> Heart problems <input type="checkbox"/> None <input type="checkbox"/> Other, Specify _____
12.	Have you ever had an HIV test?	<input type="checkbox"/> Yes <input type="checkbox"/> No
13.	If Yes, what was the result of the latest test?	<input type="checkbox"/> Positive <input type="checkbox"/> Negative
14.	Are you currently receiving Antiretroviral Therapy (ART)?	<input type="checkbox"/> Yes <input type="checkbox"/> No

5. Investigations & Results Form

Pertussis study number: _____

Questions 1 and 2 to be completed for all participants

1.	HIV testing <input type="checkbox"/> Not done (skip to 2)		
	Test	Date of test	Result
1.1	<input type="checkbox"/> ELISA - child	___/___/20__	<input type="checkbox"/> Reactive <input type="checkbox"/> Non reactive
1.2	<input type="checkbox"/> ELISA - mother	___/___/20__	<input type="checkbox"/> Reactive <input type="checkbox"/> Non reactive
1.3	<input type="checkbox"/> PCR - child	___/___/20__	<input type="checkbox"/> Positive <input type="checkbox"/> Negative
1.4	<input type="checkbox"/> Most recent CD4 count - child	___/___/20__	_____ Absolute CD4 _____ % of lymphocytes
1.5	<input type="checkbox"/> Most recent viral load - child:	___/___/20__	_____ copies/ml <input type="checkbox"/> >750 000
2.	Micro/Virology results - Obtained in the first 48 hours of admission <input type="checkbox"/> Not done (skip to 3)		
	Lab number	Date of test	Site
2.1		___/___/20__	
2.2		___/___/20__	
2.3		___/___/20__	
2.4		___/___/20__	
3.	CRP (C-reactive protein) (<72 hrs of admission) <input type="checkbox"/> Not done (skip to 4)		
	Date ___/___/20__	Time ___:___ <input type="checkbox"/> Am <input type="checkbox"/> Pm	Result _____ mg/l
	<input type="checkbox"/> Not recorded		
3.1	Haematology	Result	
3.1	Haemoglobin	_____	
3.2	Platelets	_____	
3.3	White cell count	_____ (Total)	
4.	Differential count	Lymphocytes	Absolute _____ Percentage _____
4.1		Neutrophils	Absolute _____ Percentage _____
4.2		Monocytes	Absolute _____ Percentage _____
4.3		Bands	Absolute _____ Percentage _____
4.4		Other	Absolute _____ Percentage _____
5.	STUDY SPECIMENS TAKEN	Date ___/___/20__	
		<input type="checkbox"/> NP Swab for culture	<input type="checkbox"/> NP Swab for PCR (Child)
		<input type="checkbox"/> Induced sputum	<input type="checkbox"/> NP Swab for PCR (Care-Giver)
6.	TB Results <input type="checkbox"/> Not done (skip to 6)		
	Date ___/___/20__	Specimen	Result -AFB smear Culture requested
6.1		<input type="checkbox"/> Induced sputum	<input type="checkbox"/> Positive <input type="checkbox"/> Yes
		<input type="checkbox"/> Gastric Washings	<input type="checkbox"/> Negative <input type="checkbox"/> No
	<input type="checkbox"/> Not recorded		
7.	Other (skip to 8 if not done)		
	Test	Date of test	Result
7.1	<input type="checkbox"/> Albumin	___/___/20__	_____ g/dL
7.2	<input type="checkbox"/> Total protein	___/___/20__	_____ g/dL
7.3	<input type="checkbox"/> Chemistry	___/___/20__	Na _____ K _____ Urea _____ Creatine _____
8.	Radiology		
8.1	Date of chest x-ray ___/___/20__		
8.2	X-ray findings	<input type="checkbox"/> Normal	<input type="checkbox"/> Interstitial infiltrate
		<input type="checkbox"/> Pleural effusion	<input type="checkbox"/> Consolidation or air bronchograms
		<input type="checkbox"/> Cavitations	<input type="checkbox"/> Lobar consolidation
		<input type="checkbox"/> Sub-optimal quality	<input type="checkbox"/> Other

Staff initials: _____

Date completed: ___/___/20__

Appendix 3: Baseline Characteristics

Table 6: Participant and caregiver baseline characteristics for entire cohort (N = 671)

Characteristic	Total, n= 671	CLWH, n= 20	HEU, n=93	HUU, n=398	HIV-E, n=9	HIV-U, n= 151
<i>n(%) or Median</i>						
<i>Participant demographics</i>						
Sex						
Female	311 (46.3)	9 (45.0)	40 (43.0)	187 (47.0)	3 (33.3)	72 (47.7)
Male	360 (53.7)	11 (55.0)	53 (57.0)	211 (53.0)	6 (66.7)	79 (52.3)
Age (months)	13.2 (4.8, 40.6)	20.4 (9.3, 65.1)	10.8 (4.8, 20.4)	11.1 (4.8, 28.8)	7.2 (3.6, 15.6)	31.1 (9.3, 70.4)
Race						
Black	384 (57.2)	18 (90.0)	88 (94.6)	223 (56.0)	6 (66.7)	49 (32.5)
Coloured	268 (39.9)	2 (10.0)	4 (4.3)	162 (40.7)	1 (11.1)	99 (65.6)
White	1(0.1)	0	0	1(0.3)	0	0
Indian/Asian	1(0.1)	0	0	1(0.3)	0	0
Unknown	17 (2.5)	0	1 (1.1)	11 (2.8)	2 (22.2)	3 (2.0)
Crèche¹						
Yes	193 (28.8)	10 (50.0)	24 (25.8)	104 (26.1)	4 (44.4)	51 (33.8)
No	473 (70.5)	10 (50.0)	68 (73.1)	291 (73.1)	5 (55.6)	99 (65.6)
Unknown	5 (0.7)	0	1 (1.1)	3 (0.8)	0	1 (0.7)

<i>Caregiver demographics</i>						
Age (years)	29.1 [24.5, 34.7]	28.9 [26.5, 33.4]	31.8 [26.4, 34.7]	28.3 [23.9, 33.9]	33.4 [28.6, 37.3]	29.6 [24.6, 37.6]
Education						
Basic education	18 (2.7)	0	2 (2.2)	10 (2.5)	2 (22.2)	4 (2.6)
Primary	30 (4.5)	0	5 (5.4)	18 (4.5)	1 (11.1)	6 (4.0)
Secondary	596 (88.8)	20 (100.0)	85 (91.4)	354 (88.9)	6 (66.7)	131 (86.8)
Tertiary	15 (2.2)	0	1 (1.1)	9 (2.3)	0	5 (3.3)
Unknown	12 (1.8)	0	0	7 (1.8)	0	5 (3.3)
Socio-economic IQR						
High	76 (11.3)	2 (10.0)	11 (11.8)	47 (11.8)	0	16 (10.6)
Low	120 (17.9)	4 (20.0)	21 (22.6)	72 (18.1)	2 (22.2)	21 (13.9)
Lower-middle	49 (7.3)	0	3 (3.2)	35 (8.8)	1 (11.1)	10 (6.6)
Upper-middle	263 (39.2)	11 (55.0)	38 (40.9)	182 (45.7)	3 (33.3)	29 (19.2)
Unknown	163 (24.3)	2 (10.0)	7 (7.5)	26 (6.5)	3 (33.3)	75 (49.7)

¹Preschool, Kindergarten, Nursery

Appendix 4: Age-appropriate vaccine timeliness and delay by HIV strata at 6, 10, and 14 weeks

Age	Total ¹		Timeliness of vaccination n (%)															p- value ²
			CLWH					HEU					HUU					
			n	Early	Timely	Delayed	Delay in weeks Median [IQR]	n	Early	Timely	Delayed	Delay in weeks Median [IQR]	n	Early	Timely	Delayed	Delay in weeks Median [IQR]	
Dose 1	557	16	0	15 (93.8)	1 (6.3)	4.3 [4.3, 4.3]	77	0	73 (94.8)	4 (5.2)	11.1 [10.0, 11.3]	345	9 (2.6)	307 (89.0)	29 (8.4)	6.4 [5.3, 9.9]	0.618	
Dose 2	507	14	0	12 (85.7)	2 (14.3)	9.8 [7.5, 12.0]	69	2 (2.9)	56 (81.2)	11 (15.9)	11.6 [6.7, 13.2]	312	7 (2.2)	267 (85.6)	38 (12.2)	7.9 [5.3, 10.1]	0.813	
Dose 3	440	13	0	10 (76.9)	3 (23.1)	11.3 [9.6, 13.0]	59	2 (3.4)	48 (81.4)	9 (15.3)	6.9 [5.2, 20.0]	269	6 (2.2)	205 (76.2)	58 (21.6)	6.7 [5.2, 10.0]	0.727	

¹This total includes participants with known HIV exposure but unknown infection status and who are HIV unexposed with unknown infection status.

²The p-value represents the association between early, timely, and delayed vaccination across the exposure groups.

Appendix 5: Ethics Approval Letter



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



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

03 May 2024

HREC REF: 303/2024

Dr E Amponsah-Dacosta
 Vaccines for Africa Initiative
 School of Public Health-FHS
 Email: edina.amponsah-dacosta@uct.ac.za
 Student: CLLCOU002@myuct.ac.za

Dear Dr Amponsah-Dacosta

PROJECT TITLE: HEPATITIS B VIRUS INFECTION AND VACCINE COVERAGE AMONG CHILDREN LIVING WITH HIV, HIV-EXPOSED UNINFECTED, AND HIV-UNEXPOSED UNINFECTED CHILDREN IN THE WESTERN CAPE, SOUTH AFRICA--SUB-STUDY LINKED TO 371/2011 (MASTER OF PUBLIC HEALTH-MISS COURTNEY COLLINS)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

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

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

Please submit a progress form, using the standardised Annual Report Form (FHS016) or FHS017 if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Miss Courtney Collins will also be involved in this study.

Please quote HREC REF 303/2024 in all your correspondence.

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

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

Yours sincerely

PROFESSOR M BLOCKS
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

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

HREC/ref 303.2024

Appendix 6: Vaccine journal author guidelines

Introduction

Vaccine publishes high quality science across all disciplines relevant to the field of vaccinology - all original article submissions across basic and clinical research, vaccine manufacturing, history, public policy, behavioral science and ethics, social sciences, safety, and many other related areas are welcomed.

Types of paper

Vaccine publishes primary research papers, review articles, short communications, conference reports and letters on the following topics:

- Basic Science Review
- Clinical Science Review
- Commentary/Editorial
- History of Vaccinology
- Human Fungal/Parasite/Other Vaccines
- Human Non-Infectious Disease Vaccines (cancer, allergy, other)
- Human Viral Vaccines: Basic Research
- Letter to the Editor
- Novel Pathogen Vaccines (Biodefense/High Consequence Pathogens/Emerging Diseases)
- Vaccine Acceptance/Hesitancy
- Vaccine Basic Science (Immunology/Animal Models)
- Vaccine Ethics
- Vaccine Manufacturing and Bioprocessing
- Vaccine Operational Research (Evaluation/Epidemiology/Informatics/Models /Big Data and Analytics)
- Vaccine Policy Legislation/Economics/Digital Health)
- Vaccine Regulatory Science (Implementation/Guidelines/Public Health)
- Vaccine Safety Science
- Vaccine Technology (Vectors/Adjuvants/Delivery Systems/Nanotechnology)
- Veterinary Bacterial Vaccines
- Veterinary Fungal/Parasite/Other Vaccines
- Veterinary Viral Vaccines
- Visual Vaccinology

For more specific guidelines for each article type please go to: [Article Type - Guidelines](#)

Vaccine also welcomes **Review articles** thoughtful **Opinion pieces** and **Commentaries** on topics of interest to the readership of the journal. Authors should contact the Editor-in-Chief Dr. Gregory Poland via jvac@elsevier.com, before preparing **Opinion pieces** or **Commentaries** in order to solicit approval to submit.

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Please note that ALL articles must now carry a single sentence before the article's bibliography stating: "All authors attest they meet the ICMJE criteria for authorship" and all authors must submit written confirmation in their cover letter that "All authors attest they meet the ICMJE criteria for authorship".

Authors must ensure that any documentation submitted to *Vaccine* for review purposes may be published should their article be accepted. Therefore, confidential and/or proprietary information contained in documentation submitted for review should be redacted or removed prior to submission.

Contact details for submission

Papers should be submitted using the *Vaccine* online submission system at: <https://www.editorialmanager.com/jvac>

Essentials to ensure fast handling

- Manuscript is in accordance with [ARTICLE TYPE - GUIDELINES](#)
- Manuscript-text is saved as a Word-file, line-numbers are added and text is double spaced
- Clinical trial registry is mentioned at the end of the abstract if applicable
- Conflict of interest statement is included at the end of the manuscript
- Figures and tables are prepared as separate files and are clearly labeled
- Cover letter is prepared, introducing your article and explaining the novelty of the research
- Keywords are prepared
- Contact details of 8 suggested reviewers (Name, affiliation and email address) are prepared
- Highlights are prepared (a birds' eye view of your article in 3-5 points, 85 characters each)
- The work presented in the article has been carried in line with all relevant ethical guidelines
- Please note that even though Vaccine is a transformative journal, certain article types are not supported by Open Access. This concerns article types such as Correspondence, Discussion, Conference Reports. If you are in doubt whether your article type can be published as Open Access in the journal please contact the Journal Manager at: jvac@elsevier.com

For any further information please consult this Guide For Authors or visit our [Support Center](#).

Submission checklist**Submission checklist**

Ensure that the following items are taken care of during submission:

One author has been designated as the corresponding author with contact details:

- E-mail address
- Full postal address

All necessary files have been uploaded:

Manuscript:

- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)

Supplemental files (where applicable)

Further considerations

- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A Conflict of Interest statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed and adhered to
- Suggestions for 8 reviewers and their contact details have been provided

Before you begin**Ethics in publishing**

Please see our information on [Ethics in publishing](#).

Studies in humans and animals

If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with [The Code of Ethics of the World Medical Association](#) (Declaration of Helsinki) for experiments involving humans. The manuscript

should be in line with the [Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals](#) and aim for the inclusion of representative human populations (sex, age and ethnicity) as per those recommendations. The terms [sex and gender](#) should be used correctly.

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1. that autonomous consent free from coercion was obtained from the donor(s) or their next of kin; and
2. that organs/tissues were not sourced from executed prisoners or prisoners of conscience.

All animal experiments should comply with the [ARRIVE guidelines](#) and should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, [EU Directive 2010/63/EU for animal experiments](#), or the National Research Council's [Guide for the Care and Use of Laboratory Animals](#) and the authors should clearly indicate in the manuscript that such guidelines have been followed. The sex of animals must be indicated, and where appropriate, the influence (or association) of sex on the results of the study.

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Informed consent

Studies on human subjects require ethics committee approval and informed consent, which should be documented in the paper. The statement must indicate that informed consent was obtained after the nature and possible consequences of the study had been fully explained to the subjects.

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Authors using experimental animals must state that their care was in accordance with institutional guidelines. For animals subjected to invasive procedures, the anesthetic, analgesic and tranquilizing agents used, as well as the amounts and frequency of administration, must be stated.

Availability of Materials

Publication of an article in *Vaccine* is taken to imply that the authors are prepared to freely distribute materials used in the published experiments (e.g. antibodies, cell lines) to academic researchers for their own use.

Declaration of competing interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors should complete the declaration of competing interest statement using Elsevier's [Declaration of Interests](#) form and upload to the submission system at the Attach/Upload Files step. **Note: Please do not convert the .docx template to another file type. Author signatures are not required.** If there are no interests to declare, you will still need to complete the form and confirm. For further guidance on using the declaration tool, please view this [short video](#).

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Reporting sex- and gender-based analyses

Reporting guidance

For research involving or pertaining to humans, animals or eukaryotic cells, investigators should integrate sex and gender-based analyses (SGBA) into their research design according to funder/sponsor requirements and best practices within a field. Authors should address the sex and/or gender dimensions of their research in their article. In cases where they cannot, they should discuss this as a limitation to their research's generalizability. Importantly, authors should explicitly state what definitions of sex and/or gender they are applying to enhance the precision, rigor and reproducibility of their research and to avoid ambiguity or conflation of terms and the constructs to which they refer (see Definitions section below). Authors can refer to the [Sex and Gender Equity in Research \(SAGER\) guidelines](#) and the [SAGER guidelines checklist](#). These offer systematic approaches to the use and editorial review of sex and gender information in study design, data analysis, outcome reporting and research interpretation - however, please note there is no single, universally agreed-upon set of guidelines for defining sex and gender.

Definitions

Sex generally refers to a set of biological attributes that are associated with physical and physiological features (e.g., chromosomal genotype, hormonal levels, internal and external anatomy). A binary sex categorization (male/female) is usually designated at birth ("sex assigned at birth"), most often based solely on the visible external anatomy of a newborn. Gender generally refers to socially constructed roles, behaviors, and identities of women, men and gender-diverse people that occur in a historical and cultural context and may vary across societies and over time. Gender influences how people view themselves and each other, how they behave and interact and how power is distributed in society. Sex and gender are often incorrectly portrayed as binary (female/male or woman/man) and unchanging whereas these constructs actually exist along a spectrum and include additional sex categorizations and gender identities such as people who are intersex/have differences of sex development (DSD) or identify as non-binary. Moreover, the terms "sex" and "gender" can be ambiguous--thus it is important for authors to define the manner in which they are used. In addition to this definition guidance and the SAGER guidelines, the [resources on this page](#) offer further insight around sex and gender in research studies.

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For transparency, we require corresponding authors to provide co-author contributions to the manuscript using the relevant CRediT roles. The [CRediT taxonomy](#) includes 14 different roles describing each contributor's specific contribution to the scholarly output. The roles are: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; and Writing - review & editing. Note that not all roles may apply to every manuscript, and authors may have contributed through multiple roles. [More details and an example](#).

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All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship

Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

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All scientific communications describing immunogenicity, effectiveness, or efficacy of a human or veterinary vaccine must include the following details: Vaccine characteristics: Vaccine lot number, manufacturer, dosing interval and number of doses, vaccine route of administration, if an injection - the anatomic site of injection, technique for vaccine administration (if by injection, specify needle length), concomitant vaccines administered, cold chain or storage effects if relevant, and a specification of what vaccine antigens and adjuvants were administered. Subject characteristics: Age, race, ethnicity, body mass index or body weight, smoking status, gender, medical/immunologic status, and concomitant drug use.

Statistical and analytical reporting

For further information on Vaccine guidelines for statistical and analytical reporting, please visit:

[AUTHOR GUIDELINES](#)

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[STATISTICAL AND ANALYTICAL GUIDELINES CHECKLIST](#)

Reporting clinical trials

Randomized controlled trials should be presented according to the [CONSORT guidelines](#). At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization, withdrawal and completion, and a detailed description of the randomization procedure.

All scientific communications describing immunogenicity, effectiveness, or efficacy of a human or veterinary vaccine must include the following details: Vaccine characteristics: Vaccine lot number, manufacturer, dosing interval and number of doses, vaccine route of administration, if an injection - the anatomic site of injection, technique for vaccine administration (if by injection, specify needle length), concomitant vaccines administered, cold chain or storage effects if relevant, and a specification of what vaccine antigens and adjuvants were administered. Subject characteristics: Age, race, ethnicity, body mass index or body weight, smoking status, gender, medical/immunologic status, and concomitant drug use.

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Registration in a public trials registry is a condition for publication of clinical trials in this journal in accordance with [International Committee of Medical Journal Editors](#) recommendations. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. A clinical trial is defined as any research study that

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