SAFETY AND IMMUNOGENICITY OF MEASLES VACCINE, VARICELLA VACCINE AND HEPATITIS-A VACCINE IN HIV-EXPOSED AND HIV-UNEXPOSED SOUTH AFRICAN CHILDREN

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

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DECLARATION

I, Eleonora Alexandra Marie Louise Mutsaerts, declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Eleonora AML Mutsaerts

26th day of April 2020

DEDICATION

I dedicate this work to my beloved partner Michiel. Thank you - for your love, wisdom and encouragement throughout this adventure, for enabling me to follow my dreams. Above all, for supporting me to move to South Africa and for taking a leap of faith by joining me in Johannesburg.

To my wonderful daughter, Maud - despite your young age (6 months) you were there when I was completing my laboratory analyses (although still comfortably in utero) and whilst I was writing up this thesis (by my side in a baby sling).

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In memory of my father, Dries, for being an inspiration, an example in kindness and seeing endless opportunities.

PUBLICATIONS ARISING FROM THIS STUDY

This thesis is based on the following papers:

1. **Mutsaerts EAML**, Nunes MC, van Rijswijk MN, Klipstein-Grobusch K, Grobbee DE, Madhi SA. Safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected children: a systematic review and meta-analysis. EClinicalMedicine 2018; 1:28–42. (Appendix 1).
2. **Mutsaerts EAML**, Nunes MC, van Rijswijk MN, Klipstein-Grobusch K, Otwombe K, Cotton MF, Violari A, Madhi SA. Measles immunity at 4.5 years of age following vaccination at 9 and 15-18 months of age among HIV-infected, HIV-exposed-uninfected and HIV-unexposed children. Clin Infect Dis. 2019;69(4):687-96 (Appendix 2).
3. **Mutsaerts EAML**, Nunes MC, Bhikha S, Ikulinda BT, Boyce W, Jose L, Koen A, Moultrie A, Cutland CL, Grobbee DE, Klipstein-Grobusch K, Madhi SA. Immunogenicity and safety of an early measles vaccination schedule at 6 and 12 months of age in Human Immunodeficiency Virus (HIV)-unexposed and HIV-exposed, uninfected South African children. J Infect Dis. 2019;220(9):1529–38 (Appendix 3).
4. **Mutsaerts EAML**, Nunes MC, Bhikha S, Ikulinda BT, Jose L, Koen A, Moultrie A, Grobbee DE, Klipstein-Grobusch K, Weinberg A, Madhi SA. Short-term immunogenicity and safety of hepatitis-A and varicella vaccines in HIV-exposed uninfected and HIV-unexposed South African children. Vaccine. 2020 Apr 16. (Appendix 4).

PRESENTATIONS ARISING FROM THIS STUDY

Presentations during peer reviewed scientific meetings:

1. Safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected Children: a systematic review and meta-analysis. *37th Annual Meeting of the European Society for Paediatric Infectious Diseases in Ljubljana, Slovenia.* May 2019. (Poster presentation; ESPID19-0253).
2. Measles immunity at 4.5 years of age following vaccination at 9 and 15-18 months of age among HIV-infected, HIV-exposed-uninfected, and HIV-unexposed children. *37th Annual Meeting of the European Society for Paediatric Infectious Diseases in Ljubljana, Slovenia.* May 2019. (Oral presentation; ESPID19-0256).
3. Immunogenicity and safety of an early 2-dose measles vaccination schedule at 6 and 12 months of age in HIV-unexposed and HIV-exposed uninfected South African children. *37th Annual Meeting of the European Society for Paediatric Infectious Diseases in Ljubljana, Slovenia.* May 2019. (Oral presentation; ESPID19-0249).

Other presentations:

4. Measles immunity at 4.5 years of age following vaccination at 9 and 15-18 months of age among HIV-infected, HIV-exposed-uninfected and HIV-unexposed children. *Faculty of Health Sciences Research Day 2018.* September 2018. (Poster presentation)
5. Optimisation of Vaccination Schedules. *Summer School Child Health: A Global Perspective in Utrecht, the Netherlands.* August 2018. (Oral presentation).

6. Immunogenicity and safety of an early 2-dose measles vaccination schedule at 6 and 12 months of age. *Meeting of the National Advisory Group on Immunization of the Republic of South Africa.* February 2019. (Oral presentation).

ABSTRACT

Introduction: Children living with HIV and HIV-exposed uninfected (HEU) children have increased risk of infectious diseases that may be compounded by modified immune response to vaccination or faster waning of immunity. We evaluated persistence of measles antibodies in HEU children and children living with HIV. In addition, we assessed the immunogenicity and safety of early measles, varicella and hepatitis-A vaccination in HIV-unexposed and HEU children.

Methods: Durability of measles response at 4.5 years of age was measured following measles vaccination at 9 and 15-18 months of age in HIV-unexposed (n=95), HEU (n=84) and HIV-infected children previously randomized to start antiretroviral therapy (ART) when clinically/immunologically indicated (HIV/Def-ART; n=62) or to immediate ART, that was interrupted at 12 months (HIV/Immed-ART-12; n=70) or 24 months (HIV/Immed-ART-24; n=70) of age.

In a prospective cohort, HEU (n=71) and HIV-unexposed (n=212) children received measles vaccination at 6 and 12 months of age, and one dose of either varicella or hepatitis-A vaccine at 18 months of age. Vaccine-specific antibody concentrations were measured before and one month after each vaccine dose.

Results: At 4.5 year of age, in comparison with HIV-unexposed children, HIV/Immed-ART-12 and HIV/Immed-ART-24 groups had significantly lower proportions with measles seropositive antibody titers (≥330 mIU/mL), while these were similar in HEU and HIV/Def-ART children, who initiated ART at median 5.8 months of age.

In the prospective cohort study, after one dose of measles vaccine at 6 months of age, 42% of HIV-unexposed and 46% of HEU children had seropositive titers (≥330

mIU/mL) at 7 months of age, which increased to 99% in HIV-unexposed and 95% in HEU one month after receipt of the second dose at 12 months of age.

A single dose of hepatitis-A vaccine induced seropositive titers in 92% of HIV-unexposed and 83% of HEU children (p=0.144). Seroconversion (>50 mIU/mL) following a single dose of varicella vaccine was modest (44%) and the same in HIV-unexposed and HEU children.

No difference in immune response was observed between HIV-unexposed and HEU children following measles, varicella and hepatitis-A vaccination.

Conclusions: Children living with HIV who interrupted ART experienced faster waning of measles humoral immunity compared to HIV-unexposed children, emphasizing the need for continuous ART treatment. A two-dose measles vaccine schedule, with the first dose administered at 6 months of age, induced antibody responses in the majority of HIV-unexposed and HEU children.

Hepatitis-A vaccination resulted in most children achieving seropositive antibody levels. The modest seroconversion rates after varicella vaccination, which were lower than expected in both groups, warrant further investigation.

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ABBREVIATIONS

AE	adverse event
aOR	adjusted odds ratio
ART	antiretroviral therapy
CHBAH	Chris Hani Baragwanath Academic Hospital
CI	confidence interval
CMI	cell-mediated immunity
CMIA	chemiluminescent microparticle immunoassay
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Program on Immunization
FAMA	fluorescent-antibody-to-membrane-antigen assay
gp	glycoprotein
gpELISA	glycoprotein enzyme-linked immunosorbent assay
GMT	geometric mean titer
HAV	hepatitis-A virus
HIV	human immunodeficiency virus
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IQR	interquartile range
MMR	measles, mumps, rubella vaccine
MMRV	measles, mumps, rubella, varicella vaccine
MV	measles vaccine
OR	odds ratio
PBMC	peripheral blood mononuclear cell
PIP	public immunization program

PRN	plaque reduction neutralization
RCT	randomized controlled trial
RMPRU	Respiratory and Meningeal Pathogens Research Unit
PCR	polymerase chain reaction
PCV	pneumococcal conjugate vaccine
SAE	severe adverse event
SFU	spot forming unit
VZV	varicella zoster virus
WHO	World Health Organization

PREFACE

During my Master's degree (medicine with a special focus on clinical research at Utrecht University, the Netherlands), I spent seven months at the Respiratory and Meningeal Pathogens Research Unit (RMPRU) to conduct research on maternal influenza vaccination. It turned out I got infected with the 'research' bug. Upon completing medical school, I returned to RMPRU in order to pursue a PhD degree. The work particularly sparked my attention because of the great relevance and high impact of studies that were conducted. I am passionate about reducing vaccine-preventable child morbidity and mortality, particularly in low- and middle-income countries. Every child deserves to have an equal opportunity for a healthy life.

This thesis is structured according to the University of the Witwatersrand's recommended 'divided block' format. The thesis comprises of an Introduction and Methods chapter, followed by five individual chapters presenting the Results and Discussion of each objective; Figure 0.1 (overview of this thesis). Publications resulting from the conducted work have been used as the basis for the results chapters. All references are listed at the end of the thesis.

This thesis describes the immunogenicity and safety of measles vaccination (chapters 3-5), varicella vaccination (chapter 6) and hepatitis-A vaccination (chapter 7) in HIV-exposed and HIV-unexposed children.

The introduction describes the influence of HIV-exposure on vaccine responses and outlines the epidemiology and current literature on measles, varicella and hepatitis-A vaccines.

Chapter 2 describes materials and methods of the two studies undertaken: a retrospective analysis of archived serum samples and a prospective cohort study.

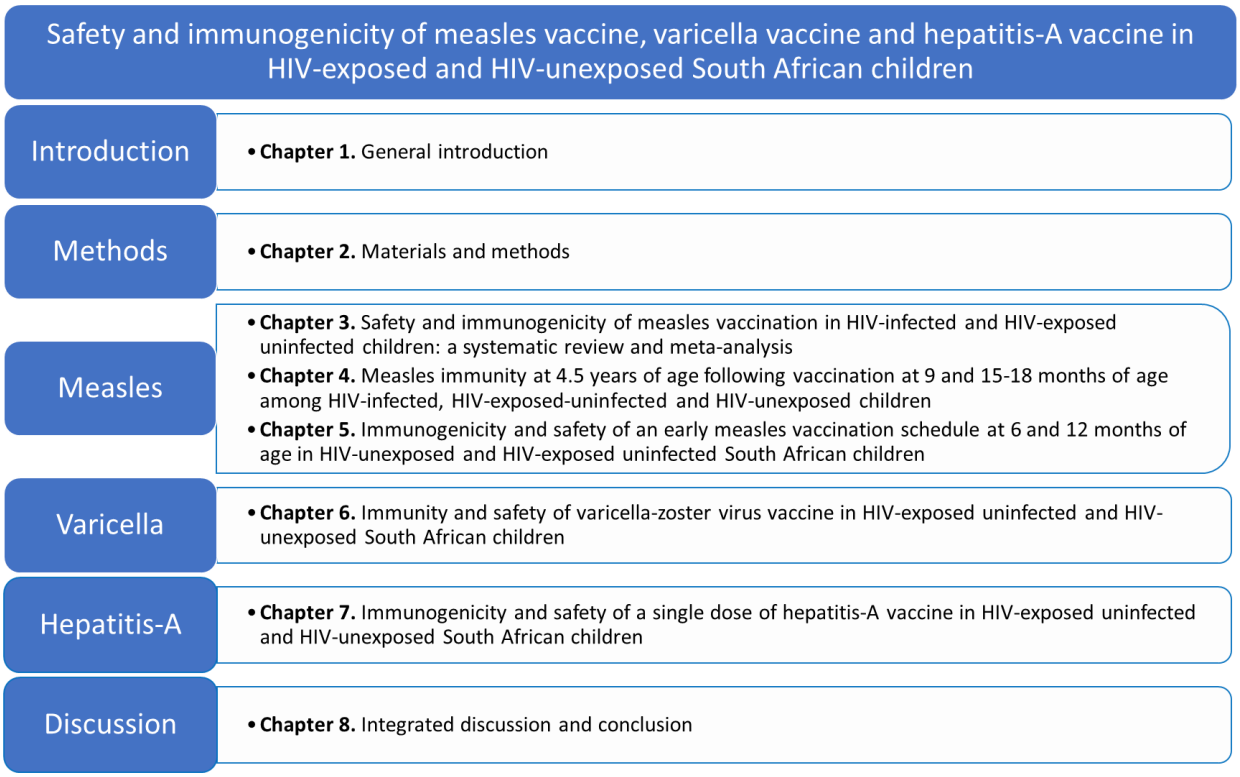
Chapter 3 is a systematic review and meta-analysis of literature on safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected (HEU) children.

In Chapter 4, the durability of measles antibodies is evaluated in children at 4.5 years of age among HIV-unexposed, HEU and children living with HIV randomized to differing timings of antiretroviral therapy initiation.

Chapters 5-7 evaluate the immunogenicity and safety of an early measles vaccination schedule (chapter 5), one dose of varicella-zoster vaccine (chapter 6) and one dose of hepatitis-A vaccine (chapter 7) in HEU and HIV-unexposed children.

This thesis concludes with a summation of the main findings in Chapter 8, which further discusses the validity of the results and directions for future research.

Figure 0.1 Overview of this thesis



STUDENT CONTRIBUTIONS

Protocol and ethics: The first draft of the study protocols was prepared by Eleonora Mutsaerts (EM). EM was responsible for obtaining ethical approval for all the studies.

Paper 1 is the systematic review and meta-analysis (chapter 3).

Project title: 'Safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected children: a systematic review and meta-analysis'

Role of the student: EM conducted the literature search, was responsible for screening of titles and abstracts and performed data extraction. Performed statistical analyses. Prepared, revised and submitted manuscript for publication.

Paper 2 is from the CIPRA 4 study – retrospective analysis of archived serum samples (chapter 4).

Project title: 'Evaluation of quantitative and qualitative antibody responses to Streptococcus pneumoniae and Haemophilus influenzae type B conjugate vaccines among HIV-1-exposed-infected children that are receiving vs. those not receiving antiretroviral therapy, as well as among HIV-1-exposed-uninfected children and HIV-1-unexposed-uninfected children' (CIPRA 4)

Role of the student: EM was responsible for locating of archived serum samples, data cleaning and matching of paper files with electronic database and physical cryogenic tubes. Performed measles ELISAs. Cleaned, analyzed and interpreted data. Prepared, revised and submitted manuscript for publication.

Papers 3 and 4 are from the MV/VV/Hep-AV study – prospective cohort study (chapters 5, 6 and 7)

Project title: 'Safety and immunogenicity of measles vaccine, varicella vaccine and hepatitis-A vaccine in HIV-exposed and HIV-unexposed South African children'

(MV/VV/Hep-AV)

Role of the student: Principal investigator. EM developed and revised case report forms. Trained staff. Oversaw patient recruitment, study staff reported directly to EM. Enrolment, consenting and follow-up visits were done by EM and study nurses. Oversaw data entry and performed database cleaning. Performed measles ELISA, varicella ELISA and varicella ELISPOT. Hepatitis-A chemiluminescent microparticle immunoassay was conducted at the National Health Laboratory Service. Conducted statistical analyses and interpreted data. Prepared, revised and submitted manuscripts for publication.

Chapter 1 Introduction

Immunization is one of the greatest public health successes of the 20th century (1). Forecasts predict that 17.7 million deaths in children under 5 could be prevented between 2011-2020 as a result of vaccination, of which approximately 52% will be averted in Africa (2). First-dose measles vaccine (at 9-12 months of age) is estimated to have the highest per person impact, with 16.5 deaths prevented per 1000 persons vaccinated (2).

Human Immunodeficiency Virus (HIV)-exposed children (i.e. children born to mothers living with HIV), who comprise almost 30% of South African children (3), have higher risk of morbidity and mortality from infectious causes than HIV-unexposed children (4–6). Recently, South Africa recommended a younger age for first measles vaccination, because unvaccinated young infants have increased risk of measles infection and complications (7,8). Varicella poses a high burden on healthcare systems and society (9,10). Because varicella vaccination has the potential to alleviate the burden of varicella disease, it is included in immunization programs of numerous countries, but not in South Africa. Hepatitis-A vaccination is recommended in settings detecting changes in endemicity from high to intermediate (11), which could be the case in South Africa. In light of the above, we evaluated immunogenicity and safety of measles, varicella and hepatitis-A vaccines in HIV-exposed and HIV-unexposed children.

1.1 HIV exposure

Globally, an estimated 1.1 million children were born to women living with HIV in 2018 (12). *In utero* HIV exposure in South Africa ranks amongst one of the highest in the world and has remained stable over the past decade. Thirty percent of South

African pregnant women were living with HIV between 2005-2015 (3). As a result of effective prevention of mother-to-child HIV transmission programs, vertical HIV transmission rates have dropped from 8% in 2008 to <1.5% in 2015 (13–15). An increasing population of children is therefore HIV-exposed uninfected (HEU) (16).

1.1.1 HIV-exposed uninfected children

HIV-exposure is associated with greater morbidity and mortality of infectious origin, and HEU children are more frequently hospitalized than HIV-unexposed children (5). A 2019 meta-analysis including 17 955 subjects found a higher risk of diarrhoea and pneumonia in HEU children compared with their HIV-unexposed counterparts (17). Underlying mechanisms explaining these differences in morbidity have not been fully elucidated.

HEU children have impaired T-cell maturation, in addition to hypo- and hyper-responsiveness to T-cell activation and lower absolute neutrophil counts (18). The aetiology of altered immune function in HEU children is multifactorial, including exposure to antiretroviral therapy (ART) and to maternal HIV infection (19). Any ART use during pregnancy and/or by the infant has been associated with lower haemoglobin concentrations and neutrophil, lymphocyte and CD4+ cell counts at 0-2 months of age compared with no ART, whereas combination ART has been associated with lower neonatal neutrophil, lymphocyte and CD8+ counts at 0-2 months of age compared with ART monotherapy (20). The maternal-fetal interface is also affected by HIV infection, which can lead to increased risk for chorioamnionitis and deciduitis (21). The neonatal immune system is thereby exposed *in utero* to antigens and an environment of cytokines and chemokines characterised by infection or inflammation. As a result, this pro-inflammatory milieu *in utero* may prime and change neonatal immunity (18).

For most vaccine-preventable diseases, HEU infants acquire lower levels of pathogen-specific antibodies at birth than HIV-unexposed infants (22–25). These low antibody concentrations have been attributed to reduced antibody concentrations in women living with HIV, as well as impaired transplacental transfer of antibody from the mother to the fetus (22–24,26–28). Kidzeru et al. showed that HIV exposure was associated with significant changes to CD4+ and CD8+ T-cell immune responses to vaccines and nonspecific antigens (29).

Studies comparing the immune responses following vaccination in HEU and HIV-unexposed children have not always found the same results. While some studies have reported reduced antibody responses in HEU children for hepatitis-B, BCG and oral polio vaccines in comparison with HIV-unexposed children (30–32), others found similar (BCG, hepatitis B, tetanus toxoid, diphtheria toxoid, *Haemophilus influenzae* type-b conjugate vaccine, oral polio) (22,25,30,33–38) or higher (pneumococcal conjugate vaccine, whole cell pertussis, measles) (22,24,34,39,40) antibody levels in HEU compared to HIV-unexposed children following vaccination. The greater immunogenicity to vaccines observed in HEU children could be explained by reduced interference of maternal antibody on development of the primary immune response or priming of the HEU immune system by the HIV-infected *in utero* milieu (18). After booster vaccination in the second year of life, HEU children had robust memory responses to diphtheria-toxoid, tetanus-toxoid, whole-cell-pertussis and hepatitis-B vaccines compared with HIV-unexposed children (41).

1.1.2 Children living with HIV

In 2018, there were 160 000 [uncertainty bounds 110 000–260 000] new HIV-infections in children globally (12). Since 2016, the World Health Organization (WHO) recommended to initiate all children living with HIV on ART, irrespective of

their clinical or immunological stage (42). Despite effective ART, children living with HIV have immune system aberrations, including significant dysfunction in the B-cell system (43). During the early stages of HIV infection, specific memory B-cell pools are depleted and cannot be re-established by the use of ART (44). Impairment of the immune system by HIV infection negatively impacts vaccine efficacy and immunogenicity, thereby potentially creating a vulnerable population of individuals at increased risk of disease (39,45,46). Children living with HIV who were initiated on ART later in life had lower immunogenicity and faster waning of antibodies following vaccination compared to HIV-uninfected children (37,39,40,47–50).

Immunological dysfunction primarily occurs during the early course of HIV-infection (51). Therefore, ART should be started immediately after birth in vertically infected children. Early initiation of ART is associated with normal development of the T-cell repertoire and preservation of high numbers of functional memory B-cells (44,52,53). ART initiation within the first year of life allowed for maintenance of the B-cell compartment in 70 Italian children living with HIV, whereas children treated later in life had lower number and functionality of B-cells despite attaining viral control (44). Improved primary immune responses to childhood vaccines were observed following early initiation of ART than when ART was delayed (25,40,44,50).

1.2 Measles

Measles is caused by infection with the measles virus, a single-stranded, enveloped RNA virus from the genus *Morbillivirus* of the *Paramyxoviridae* family (54). Humans are the only hosts of measles. The virus is transmitted via the respiratory route and presents clinically with a prodromal phase characterized by fever, coryza, cough and conjunctivitis (55). Two to four days after infection, a characteristic erythematous maculopapular rash appears. Measles is one of the most contagious human

diseases and remains a leading cause of death among children, despite availability of safe, efficacious and affordable vaccines (1,55).

1.2.1 Measles epidemiology

Before development and widespread use of measles vaccines (MV), measles caused approximately 5-8 million deaths annually (56). In 2018, global measles deaths amounted to 142 300 (95% confidence interval [CI] 93 600-387 900) (57). Measles mortality accounted for approximately 4.2% (95% CI 1.5-8.7%) of all under 5 deaths in 2017, of which >95% occurred in low-income countries (7,58).

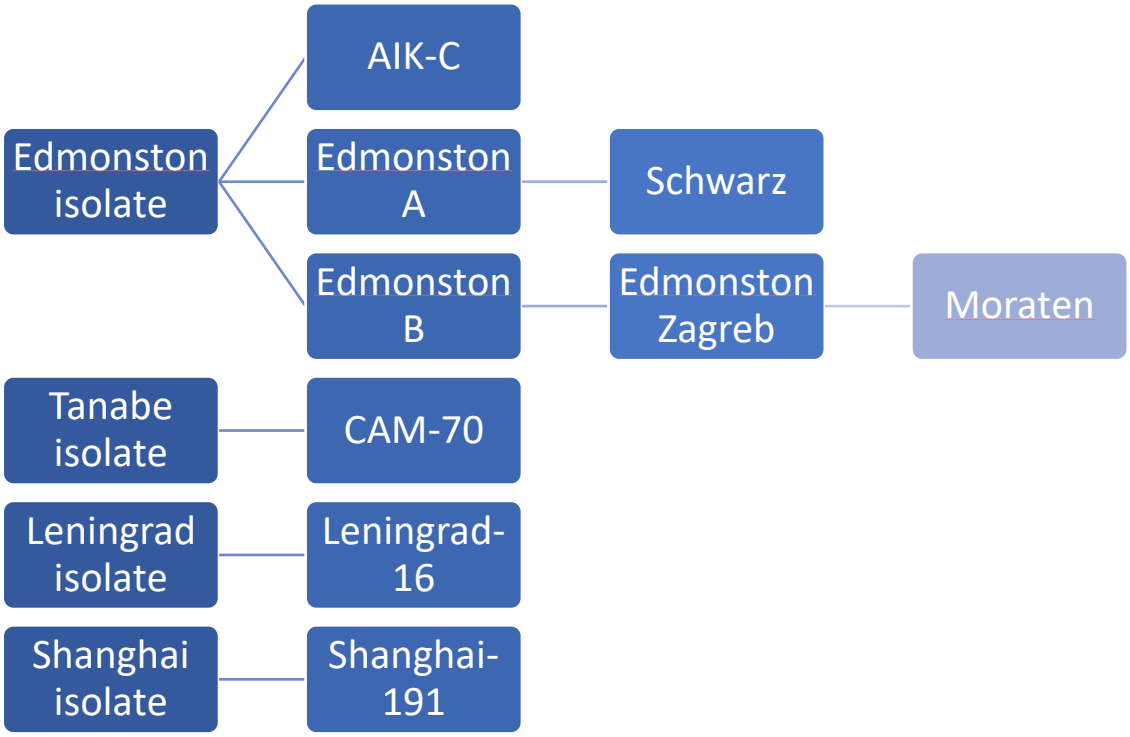
All six WHO regions have adopted goals for measles elimination by 2020. Substantial progress in measles elimination has been made between 2000 and 2018, with 66% reduction in reported measles incidence (from 145 to 49 cases per million population) and 73% decrease in measles deaths (from 535 600 to 142 300) (57). The only region that achieved measles elimination was the Americas. However, outbreaks still occur, due to low or suboptimal vaccination coverage (59). Between 2016 and 2018, measles incidence increased again from 19 to 49 cases per million, with increases observed in four of six WHO regions and reestablishment of endemic measles transmission in the Americas (57,60). Resurgence of measles has been attributed to be multiple factors, which vary between countries. Weak routine immunization programs were responsible for the majority of measles cases during 2017-2018 (57). Furthermore, gaps in immunity in older age groups, due to past deficiencies in routine immunization systems, contributed to ongoing transmission in countries with low measles incidence or prior elimination. Spread of measles was further facilitated by international travel by infected individuals (57).

In South Africa, the measles surveillance network recorded 210 confirmed measles cases in 2017, with an incidence of 9.9 cases per million population in children under 5 years of age (61). Infants, adults >20 years of age, pregnant women, malnourished children and immunocompromised individuals have increased risk of complications (62). Complications associated with measles infection include otitis media, pneumonia, diarrhoea, post-infectious encephalitis and subacute sclerosing panencephalitis.

1.2.2 Measles vaccination

The first MV was licensed in 1963 (54). At present, only live attenuated vaccines are used, either as monovalent vaccines or in combination with mumps, rubella, or varicella vaccines. Commonly used viral strains incorporated in the vaccines, including Edmonston-Zagreb, Schwarz, Moraten and AIK-C, are derived from a single clinical virus isolate called the Edmonston strain and all have a high degree of nucleotide sequence homology (63); Figure 1.1. Attenuation of the original wild-type strain occurred by passage in chick embryo fibroblasts. Independently developed measles vaccines have been derived from local wild-type strains, including CAM-70 (Japan), Leningrad-16 (Russia) and Shanghai-191 (China), and are genetically more diverse (55).

Figure 1.1 Origin of measles vaccine viral isolates



WHO recommends the first dose of measles vaccine (MV1), in countries with ongoing transmission, to be given at 9 months of age and the second dose (MV2) between 15-18 months of age (55). This recommendation is based on the assumption that maternally-derived antibody wanes at 7-8 months of age resulting in less impediment of the infant immune response and improved seroconversion rates (64). In settings with high HIV incidence and ongoing measles transmission, a 'zero dose' (MV0) is recommended at 6 months of age, followed by two additional doses according to the national public immunization program (PIP) (55).

MV is not only important for the control of measles, but also indirectly prevents diminishment of pre-existing antibodies to other pathogens by preventing measles infection, as evidenced by two recent studies (65,66). In 77 unvaccinated Dutch children, measles infection caused elimination of 11-73% of the antibody repertoire across individuals; these immune aberrations were not present in vaccinated

children (66). Petrova et al. reported that measles infection contributed to incomplete reconstitution of the naïve B-cell pool causing immunological immaturity. Additionally, previously expanded B memory clones were depleted, leading to compromised immune memory to previously encountered pathogens (65). Alterations in naïve and memory B-lymphocyte subsets following measles infection persist after clearance of clinical disease and thereby generate vulnerability to pathogens for which immunity may have been previously established through natural infection or vaccination.

1.2.3 Measles and HIV exposure

Infants born to women living with HIV acquire less maternal measles IgG than their HIV-unexposed peers, and maternally-derived antibody decays more rapidly in children living with HIV (67–70). Children living with HIV have an increased risk of severe measles infection and hospitalization compared to children without HIV (71,72). A Kenyan study found a 3.8 (95% CI 1.2-13.2) times higher risk of measles disease before 9 months of age in infants born to women living with HIV (10 of 109) than in control infants (5 of 194) (73).

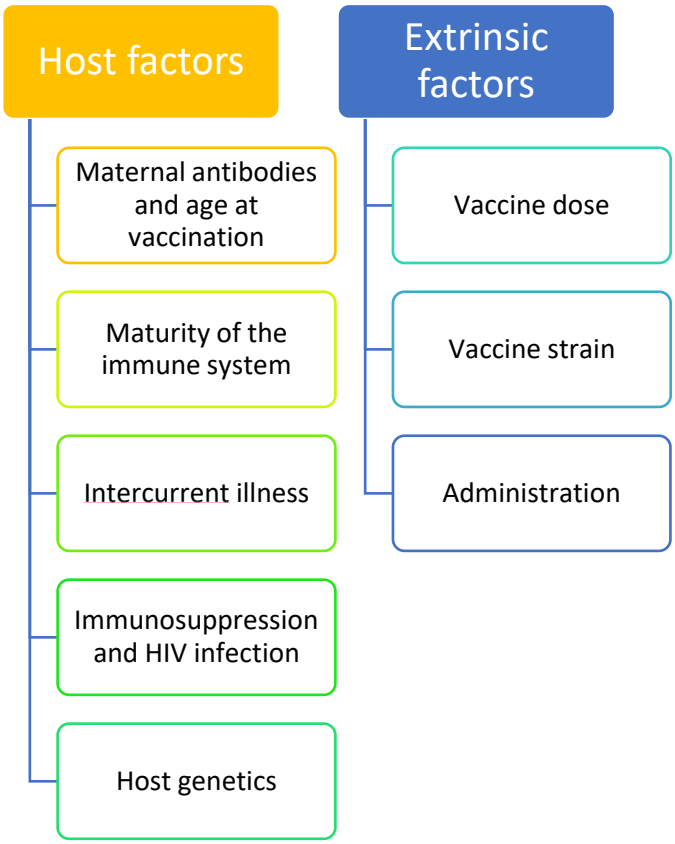
1.2.4 Determinants of measles vaccine immunogenicity

MV induces both neutralizing antibody and cellular immune responses of similar quality as wild-type measles virus infection, although levels of vaccine-induced antibodies are lower in magnitude and wane faster (74,75). The best correlate of protection against measles is based on antibodies targeting the haemagglutinin surface protein of the measles virus, with some contribution of antibodies against the fusion protein. The haemagglutinin protein mediates binding to host cellular receptors, whereas the fusion protein is responsible for viral uptake into the host cell (56). Antibodies are induced two weeks after vaccination and peak at 1-3 months

(54). The current gold standard for measuring measles immunity is based on quantification of neutralizing antibodies as measured by the plaque reduction neutralization (PRN) assay. Antibody titers of more than 120 milli-international units per milliliter (mIU/mL) by PRN are considered to be protective against disease (76). Most laboratories, however, use an enzyme-linked immunosorbent assay (ELISA) to assess measles immunity, which is less sensitive at lower antibody concentrations. A recent systematic review critically evaluated evidence supporting the 120 mIU/mL cutoff as a correlate of protection and concluded that this commonly used threshold is based on limited data (77). The role of cellular immunity in protection following vaccination is not fully understood, although vaccinated children with antibody titers <120 mIU/mL by PRN may be protected against measles, indicating involvement of cellular immunity (76,78).

The percentage of children achieving protective antibody titers following MV depends on both host and extrinsic factors; Figure 1.2. Host factors include: 1) the presence of inhibitory maternal antibodies and age at vaccination, 2) maturity of the recipient's immune system, 3) intercurrent illness, 4) immunosuppression and HIV infection, and 5) host genetics. Extrinsic factors include 1) vaccine dose, 2) vaccine strain and 3) route of administration. HIV-infection and age at vaccination as determinants of the immune response to MV are discussed in more detail in sections 1.2.5 and 1.2.7, respectively.

Figure 1.2 Determinants of immune response to measles vaccination



Maternal measles antibodies

During the first few months of life, passively-acquired antibodies protect young infants against measles. Immunoglobulin G (IgG) is transferred from the maternal circulation to the fetus via active transport through the placenta. This process starts during the first trimester and continues until birth (79). The protection conferred by these antibodies depends on the maternal antibody concentration, efficiency of transfer across the placenta and rate of antibody decay after birth (69). Maternally-acquired antibodies may interfere with the infant's primary immune response to MV by neutralizing vaccine virus before development of a full immune response (64,80,81). Maternal-fetal transport of IgG might be impaired by maternal infections such as malaria and HIV infection with high viral load during the third trimester

(68,82). The rate of antibody decay depends on the half-life of maternally-acquired antibody, which has been consistently reported to range from 40 to 61 days (56).

Maturity of the infant's immune system

Even in the absence of passively-acquired maternal antibodies, infants <6 months do not produce high concentrations of neutralizing antibodies following MV (56). The neonatal immune system is polarized against Th1 responses, causing some vaccines to be more effective later in life than in early infancy (83). In the first year of life, IgG and IgA responses to viruses and bacteria remain relatively weak (84). Vaccination to measles induces progressively higher IgG responses at older ages during the first 12 months. Limited infant IgG responses to T-cell dependent antigens may be attributed to reduced interaction between T-lymphocytes and antigen-presenting cells. This immaturity may result from unresponsiveness to lymphoid-mediated signals at the level of neonatal follicular dendritic cell precursors, followed by failure of follicular dendritic cell maturation and germinal center formation (85).

Intercurrent illness

There is controversy regarding the effect of interferon production during acute infection concurrent to vaccination, which may interfere with development of the vaccine-induced immune response (80). A number of small studies have reported lower seroconversion rates (80%) in children with rhinorrhea compared to well children (98%) (86). Thai children with an upper respiratory tract infection were found to have lower geometric mean antibody titers upon MV receipt at 9 months of age than those who were healthy (87). In contrast, most other studies have reported similar rates of seroconversion when ill and healthy children were vaccinated (88–94). As a result, mild illness is not considered as a reason to delay measles vaccination (94).

Host genetic factors

Host genetics play a role in immune responses to MV. Ovsyannikova et al. reported different human lymphocyte antigen haplotypes to be associated with increased or decreased humoral and cell-mediated immune responses to measles immunization (95). An association between human lymphocyte antigen homozygosity and low measles antibody concentrations after one dose of MMR vaccine have been reported (96), but this was no longer observed upon receipt of the second dose (97,98). Certain single nucleotide polymorphisms in cytokine and cytokine receptor genes have also been identified to be related to humoral and cellular measles immunity (99,100). Single nucleotide polymorphisms in signalling lymphocyte activation molecule and CD46 genes, which code for the two cellular receptors required for measles infection, have been associated with variations in antibody response to two doses of MV (101).

Vaccine dose

In the past, high-titer vaccines were developed in order to optimize humoral immune responses in 4-6 month old infants. By increasing the dose of the Edmonston-Zagreb vaccine from 10 000 to 40 000 plaque forming units, response rates increased from 73% to 100% in Gambian children (102). In 1989, the WHO recommended administration of high-titer vaccines to infants aged 6 months in areas where measles was a major health problem among young infants (103). Increased mortality rates were, however, observed among girls aged 1-2 years following receipt of high-titer vaccine (104–106) and consequently, the high-titer vaccine was withdrawn and is no longer in use.

Vaccine viral strain

Currently used live attenuated MVs are similarly immunogenic in infants at 9 months of age and older (80). However, a 1995 review of 30 studies reported that Edmonston Zagreb and AIK-C containing vaccines produced higher seroresponse rates than the Schwarz vaccine of equivalent titer in infants younger than 9 months of age (107,108). The reasons for these differences are unknown.

Combined MV with mumps and rubella antigens have consistently yielded the same high seroconversion rates as observed with each individual component (80).

Route of administration

No differences have been observed between intramuscular or subcutaneous administration of two doses of measles, mumps, rubella, varicella (MMRV) vaccine in healthy children (109). Aerosolized MVs were, however, inferior to subcutaneous vaccines in terms of seropositivity rates in a large open-label non-inferiority trial in Indian children (110). Similarly, two Mexican studies also reported lower proportions of infants developing cellular and humoral immunity to Edmonston-Zagreb MV when given by aerosol route compared with subcutaneous administration at 9 months (111) or 12 months of age (112).

1.2.5 Immunogenicity of measles vaccine in HIV-infected and HIV-exposed uninfected children

Children living with HIV can have an impaired humoral immune response to MV (56). Prospective studies conducted in the United States (USA) during the early 1990s reported approximately 25-37% of children living with HIV to be seropositive following a single dose of MV (113). A study from Malawi from 2000 through 2002 administered two doses of MV at 6 and 9 months of age and found 59% (95% CI 46-

71%) of infants living with HIV to be seropositive after MV1 at 6 months of age and 64% (95% CI 49-78%) after MV2 at 9 months of age, compared with 68% (95% CI 62-74%) and 94% (95% CI 89-97%) among HEU infants and 62% (95% CI 57-66%) and 92% (95% CI 89-95%) among HIV-unexposed infants using enzyme immunoassay (EIA) for antibody detection (114). In Zambia, during approximately the same time period, 88% (44 of 50) of children living with HIV developed protective antibody concentrations by PRN within six months of single dose measles vaccination compared with 94% (199 of 211) HEU children and 94% (92 of 98) HIV-uninfected children (115). However, 27 months after MV administration, only 50% (9 of 18) of HIV-infected children who survived and returned for follow-up maintained seroprotective titers, compared with 89% (63 of 71) of HIV-uninfected children (115).

It is important to note that these studies were conducted prior to the introduction of universal ART. In Zambia, 40% (32 of 81) of children living with HIV preserved their original measles IgG serostatus following initiation of ART after measles vaccination: 21 (26%) remained seropositive and 11 (14%) remained seronegative during 3 years of follow-up (116). The remaining 60% changed their serostatus: 38 (47%) were seronegative and seroconverted during follow-up and 11 seroreverted (14%). Upon revaccination, 95% (18 of 19) of seronegative children seroconverted by PRN (116). In a study from Latin America and the Caribbean following one dose of MV, more HIV-infected children with ART initiation before 12 months of age (87%) achieved measles titers ≥120 mIU/mL by ELISA measured at four years of age than when ART was initiated after 12 months of age (77%) or when no continuous ART was administered (73%) (48). In a recent South African study assessing immune responses after MV1 at 9 months of age, 96% (66 of 69) of infants living with HIV randomized to deferred ART (median age at ART initiation 5.8 months), 79% (69 of

87) of HIV-infected infants on early ART interrupted at 12 months of age, and 92% (82 of 89) of HIV-infected infants on early ART interrupted at 24 months of age achieved titers ≥330 mIU/mL (threshold for seroprotection) compared with 91% (102 of 112) of HIV-unexposed and 95% (110 of 116) HEU children (39). All children in the deferred arm were evaluated for ART initiation during an interim analysis. After MV2 at approximately 15-18 months of age, the deferred ART group had 96% (48 of 50) of participants with seroprotective titers, the early ART group interrupted at 12 and 24 months, 80% (49 of 61) and 86% (57 of 66) respectively, compared with 94% (100 of 106) of HIV-unexposed and 80% (82 of 103) of HEU measured 41 weeks after the booster dose (39). This indicates that following the booster dose, waning of measles immunity occurred among children living with HIV in whom ART was interrupted and also in HEU children.

Another South African study reported that antibody responses to MV were almost identical in HEU and HIV-unexposed children throughout the first two years of life (34). Responses to vaccination in HEU children beyond infancy have not been studied extensively.

1.2.6 Duration of protection

Duration of vaccine-induced immunity persists for decades, but is shorter and more variable than following wild-type measles infection (74,117,118). Secondary vaccine failure rates (defined as a vaccinated symptomatic individual showing a secondary immune response or clinical measles after vaccine-induced seroconversion) of 5-6% have been reported (119,120). Ten years post-vaccination, ≥94% of children remained seropositive for measles antibody (121). In the same study, geometric mean concentrations declined from 84 days after vaccination onwards (121). Despite waning antibody levels, protective immunity may still be present. After re-exposure to

measles virus, secondary immune responses develop, characterized by a rapid antibody rise and absence of clinical manifestation of disease (119). Individuals who become infected after vaccination are protected by developing an asymptomatic secondary immune response, but could be infectious to other members of the population and contribute to subclinical measles transmission (122). This is particularly a risk in elimination settings where natural boosting no longer occurs.

Children living with HIV maintain seroprotective titers for a shorter duration compared to those without HIV (114,115,123,124). A meta-analysis reported that 68% (95% CI 45-88%) of HIV-infected primary responders had seroprotective antibody titers 2 years after their last MV and 40% (95% CI 10-73%) after 5 years (46). The majority of studies were, however, conducted in the pre-ART era.

Two studies have reported measles immune responses in children living with HIV following early ART initiation, as currently recommended by the WHO. Pensieroso et al. reported that early ART-treated HIV-infected Italian children with mean age 6.8 years generated and preserved measles-specific memory B-cells comparable to age-matched healthy controls at mean 4.2 years after MV1 (44). Children starting ART after one year of age had lower measles antibody titers by ELISA compared with healthy controls and early-treated children (44). In contrast, Succi et al. observed no difference in measles seropositivity at 4 years of age between Latin American children on ART initiated at <12 months age, ≥12 months of age, or no ART after one measles-containing vaccine dose (48). Although early initiation of ART in HIV-infected infants is now recommended, lifelong continuous treatment may be problematic due to long-term toxicity, risk of ART resistance, waning adherence and resource constraints (125).

1.2.7 Early measles vaccination

Determining the optimal age for MV1 depends on various factors: the presence of transplacentally-acquired maternal antibodies, the average age of measles infection in the geographical area and maturity of the child's immune system (126).
A large group of children is susceptible to measles disease before reaching the eligible age of routine measles vaccination (127–130). Moreover, children <12 months of age have the highest measles-associated morbidity and case-fatality rates (131–134). Unvaccinated children also have increased risk of subacute sclerosing panencephalitis, a fatal complication of measles, particularly those infected during infancy (135).

During the 2009-2011 measles outbreak in South Africa, 24% of measles cases occurred among children younger than 9 months of age. Age-specific incidence of 302 (<6 months), 1083 (6-8 months), 724 (8-11 months) and 54 (≥5 years) per 100 000 population were reported (136). Another study in Zambia reported 23% of HIV-uninfected and 33% of HIV-infected children hospitalized with measles to be younger than 9 months (72). Despite the initial assumption that MV1 at 9 months would be enough to protect infants against infection, young infants disproportionally contribute to the burden of measles disease and mortality. In a re-analysis of two randomized controlled trials (RCT), mortality rates were lower (0.0 and 4.2 per 1000 person-years) when vaccinated with MV in the presence of maternal antibody than when vaccinated without maternal antibody (32.3 and 14.5 per 1000 person-years) (137).

The increase in measles incidence in young infants has partly been attributed to shorter duration of protection from passively-acquired antibodies. Infants born to immunized mothers acquire lower levels of maternal IgG which disappears at an earlier age than infants born to naturally measles infected mothers (138,139). Due to

reduced wild measles virus circulation after MV introduction and absence of natural boosting, mothers with naturally-acquired immunity also transferred low levels of antibody to their fetuses (140). Studies in South Africa and Guinea-Bissau reported <30% of infants with protective antibody titers by 4.5 months of age (39,67). As a result, although infants are more susceptible to measles infection, they are also responsive to vaccination at a younger age (64).

Reducing the age for MV1 to 6 months could prevent serious measles clinical complications and has been shown to reduce childhood mortality (137,141,142). This strategy has been recommended for countries with high incidence of measles and/or HIV (55). However, this is still considered as a supplemental dose because of concerns regarding lower seroconversion rates and shorter duration of protection after early MV (143). In order to achieve measles elimination, careful balancing between early infant protection and increased risk of reduced antibody response and secondary vaccine failure is required.

On 1st December 2015, the South African Department of Health recommended routine administration of MeasBio (Biovac), a live attenuated vaccine with the CAM-70 strain, at age 6 and 12 months, replacing Rouvax (Schwarz-strain) administered at 9 and 18 months of age (144). Schwarz strain was derived from the Edmonston isolate, whereas CAM-70 was developed from the Tanabe strain of measles virus adapted to the chorioallantoic membrane (CAM) of chick embryos (145–147). Reasons for lowering the vaccination age included the high measles incidence in infants <9 months of age during the 2009-2011 outbreak and restrictions regarding concomitant administration of Measbio with other vaccines (personal correspondence: South African National Advisory Group on Immunization, S.A. Madhi, February 27, 2019).

Immunogenicity of CAM-70 was established by clinical trials (147,148) and the vaccine was approved for single-dose administration to healthy children from 1 year of age in 1971 (produced by Biken Foundation; Osaka, Japan) (149). One Brazilian study from 1990 examined the serological response to a MV containing the CAM-70 strain administered at 6 and 11 months of age. Following MV2, seroconversion rates of 89% by immunofluorescence assay and 97% by ELISA were reported (150). Immunogenicity studies on early MV in Africa have shown variable results. Recent studies from Guinea-Bissau and Malawi on the standard-titer Edmonston-Zagreb strain concluded that an early two-dose MV schedule was associated with 77% and 97% protective antibodies at 9 and 24 months (after vaccination at 4.5 and 9 months) (151); and 62% and 92% seropositive antibodies at 9 and 12 months (after vaccination at 6 and 9 months) (114). However, a South African study from 1975 found suboptimal seroconversion rates in 16 of 38 (42%) infants vaccinated with a Moraten strain MV (Attenuvax) before 9 months (152).

A 2019 systematic review and meta-analysis reported that MV administration at <9 months of age is safe and immunogenic (153). Moreover, a second systematic review and meta-analysis concluded that early vaccination did not cause blunting of immune responses to subsequent doses of MV (154). The authors did, however, conclude that early MV is only appropriate in emergency or outbreak situations and should be followed by two scheduled doses. Infants vaccinated at <9 months of age had significantly lower antibody concentrations and seroconversion rates than those receiving MV1 at ≥9 months (153).

Measles vaccination at age 6 months resulted in similar levels of protection in American infants living HIV and HIV-unexposed infants (155).

1.2.8 Safety of measles vaccination

Following administration of MV, recipients may experience pain and tenderness at the injection site 24 hours after injection (156). Symptoms are generally mild and resolve within 2-3 days. Systemic reactions, including fever and rash, typically occur 7 to 12 days post-vaccination with a duration of 1-2 days. Temperature >39.4 ºC occurs in approximately 5-15% of vaccinees (156). Rash occurs in 2-5% of recipients 7-10 days after vaccination and commonly lasts for 2 days. Upon receipt of MV2, mild adverse events (AEs) occur less often than after MV1 (157).

Reported serious adverse events (SAEs) following MV include febrile seizures, thrombocytopenia and anaphylaxis. After MMR vaccination, 67% of admissions for convulsions were attributed to the measles component of the vaccine, with a risk of 1 in 3000 doses (158). Other studies reported the number of febrile seizures attributable to vaccination with MMR to be 1 in 3000 to 4000 doses (158,159) and 1 in 1150 doses (160). Rarely, measles containing vaccines induce thrombocytopenia. MMR-associated thrombocytopenia has been reported in 1 in 30 000 to 1 in 40 000 vaccinated children, which resolved within 6 months from diagnosis in 93% of children (161–163). There have been reports of hypersensitivity reactions to vaccine components, for example injection site urticaria. Anaphylaxis following MV is extremely rare, with a risk of 3.5 cases (95% CI 0.7-10.3) per million doses (164).

Risk of permanent neurological sequelae following MV has been widely studied. Published studies have not found evidence for increased risk of Guillain-Barré syndrome (165,166), inflammatory bowel disease (167–170) or autism (171–175) related to MV administration.

In 1973, Sangkawibha et al. evaluated reactions following MV with a CAM-70 strain in 200 Thai children aged 1 to 5 years old (148). Thirty-three percent of vaccinated children had a febrile reaction, with one child experiencing temperature >39 ºC and two children experiencing febrile convulsions. Similarly, of 456 healthy Japanese individuals aged 1.0-18.3 years receiving CAM-70 between 1982 and 1999, 29% experienced a temperature ≥37.5 ºC 5-14 days post-vaccination and 11% experienced a rash (149).

To our knowledge, no clinical trials have examined the safety of CAM-70 measles virus vaccine administered at 6 months of age. The recent systematic review and meta-analysis that evaluated the safety of MV administered at <9 months of age found no significant differences in the risk of fever, rash, diarrhoea, or local reactions between infants <9 months of age and those vaccinated at 9 months or older. Additionally in the 9 studies reporting on SAEs, none of 3848 infants analyzed had an event following immunization (153). Moreover, the WHO states that internationally available attenuated measles vaccines may be used interchangeably within immunization programs and considers them to be safe and effective (55).

Measles vaccine appears to be safe in children living with HIV and no increased risk of SAEs has been reported compared to HIV-unexposed children (71,155).

1.3 Varicella

Varicella and herpes zoster are both caused by the varicella-zoster virus (VZV) and are widespread vaccine preventable diseases in high-income countries (176). VZV is a double-stranded DNA virus belonging to the Herpes-viridae family (177). Transmission occurs via direct contact with skin lesions or through inhalation of aerosolized droplets. In general, VZV causes primary infection during childhood

(chicken pox), which presents as a benign, self-limiting disease in healthy immunocompetent children. Clinically, varicella is characterized by fever, malaise, loss of appetite, headache and a pruritic vesicular rash (178). However, complications may occur, including pneumonia, secondary bacterial infection of the skin, encephalitis and cerebellar ataxia, especially in neonates, pregnant women, immunocompromised and elderly individuals (9).

Following primary infection, VZV remains dormant in sensory nerve ganglia and can periodically reactivate in individuals with decreased cellular immunity, resulting in herpes zoster (shingles). Herpes zoster is characterized by a painful rash restricted to one dermatome. Complications of herpes zoster include post-herpetic neuralgia, which may persist for months or years (179).

1.3.1 Varicella epidemiology

VZV is prevalent worldwide and age at primary infection varies per geographic region. Annually, on average 4.2 million cases of varicella result in hospitalization and 4200 deaths worldwide (180). Estimating the global burden of VZV is difficult, due to most data coming from high-income countries and heterogeneity of surveillance systems. In high-income countries with temperate climates, prior to vaccine introduction, >90% of VZV infections occur during childhood and 5% of adults remain susceptible to primary infection (181). In tropical climates, infection occurs later in life (178). Infection during adolescence or adulthood is usually more severe than during childhood. Varicella seronegative prevalence in healthcare workers and college students ranged from <5% in the USA in the vaccine era to 14-19% in Saudi Arabia, 26% in India and 50% in Sri Lanka (178,182–185). Geographical differences in age of primary VZV acquisition may be attributed to climate, population density and probability of exposure to the virus (186). Varicella

has strong seasonality, peaking in winter and spring in temperate environments, and in the dry and cold months in tropical areas (187). Prior to varicella vaccine introduction, VZV epidemics occurred every 2-5 years (178).

Limited epidemiological data are available on the burden of VZV in Africa (188). In a systematic review from 2017, Hussey et al. reported African VZV prevalence ranging from 0-90% based on eight studies and incidence of VZV varied from 441 to 3420 cases per 100 000 population based on 3 studies, of which two were from Guinea Bissau (189). Mortality has mainly been reported in developed counties with case-fatality rates of approximately 2.6 per 100 000 cases in the USA between 1990-1994 prior to vaccine licensure (190). Fifty-fold higher case-fatality rates of 130 per 100 000 cases (2 deaths in 1539 varicella cases) have been reported in a smaller study from Guinea Bissau (191). A retrospective review of admissions to the only paediatric isolation facility in Durban, South Africa, reported that varicella (n=4376) accounted for 23% of admissions between 1985-1996, with 15% of varicella admissions (86 of 561) and 75% of varicella deaths (6 of 8) being associated with HIV-infection between 1994-1996 (192).

1.3.2 Varicella vaccine immunogenicity

VZV vaccines contain live attenuated virus from the OKA strain, developed in Osaka, Japan in 1974 (193). VZV-containing vaccines are available as a monovalent vaccine or a combined quadrivalent vaccine (measles, mumps, rubella, varicella [MMRV]), both licensed for children >12 months of age. The vaccine should be administered as a two-dose schedule, with a first dose at 12-15 months of age and a second at 4-6 years of age. Because VZV contributes to a high burden on the healthcare system and society, VZV vaccine has been introduced in the national vaccination programs of several countries (9,10). The WHO recommends varicella

immunization in settings where varicella poses an important public health burden, provided that at least 80% coverage is achieved (178). Varicella vaccine is rarely used in Africa and is not part of the South African public immunization program. Assays to measure VZV antibodies include fluorescent-antibody-to-membrane-antigen assay (FAMA), glycoprotein ELISA (gpELISA), complement-enhanced VZV neutralization assay and commercial ELISA, as detailed in section 2.3.8. Serological correlates of protection against varicella disease have been proposed as ≥1:64 antibody dilution by FAMA or ≥5 units/mL by gpELISA (76).

Vaccine effectiveness depends on the number of VZV vaccine doses administered. A recent meta-analysis estimated VZV vaccine effectiveness in preventing all varicella cases after single dose to be 81% (95% CI 78-84%) and after two doses to be 92% (95% CI 88-95%) (194). Humoral vaccine response may fail to develop (primary vaccine failure) and are normally lower than after natural VZV infection (195). Nonetheless, classical varicella disease is prevented in vaccine recipients.

Vaccine induced protection depends on both humoral and cell-mediated immunity (CMI), especially for disease control (196). One month after varicella vaccine administration, 85-89% of vaccinated healthy children developed protective antibody levels (by gpELISA cutoff ≥5 units/mL) after a single dose and more than 99% after two doses (186,197–204). It has been suggested that vaccine induced B- and T-cell responses collectively fight the infection. Cellular immune responses measured by lymphoproliferation assay are detected within 1-2 weeks after natural infection and 2 weeks after vaccination (196,205,206). Positive CMI responses, as measured by lymphocyte stimulation or skin testing, were found to be durable for many years in the USA and Japan in the presence of continued circulation of wild-type VZV (207–209).

A South African trial that examined immunogenicity of a single dose of VZV vaccine, co-administered with MMR in healthy children 15-18 months old, obtained seroprotection rates of 73-75%, which were slightly lower than expected (210).

In children living with HIV and with CD4+ T-cell levels ≥15%, including those receiving ART, varicella vaccine was found to be effective in preventing varicella (211–215). In these studies, children attaining varicella-specific protective antibody titers after vaccination ranged between 60% (6 weeks and 2 years post-booster) and 79% (2 months post-booster) in HIV-infected children (212,213,215). Table 1.1 summarizes studies evaluating humoral immune responses following VZV vaccination in HEU children. Literature search yielded only one study (216). Hundred percent of American HEU and HIV-infected children were seropositive after receiving varicella vaccine when tested <3 years from the last dose (216). Fewer HIV-infected children than HEU were, however seropositive when tested at 3-<7 years (73% vs 100%) and ≥7 years (77% vs 97%) following a single dose vaccination (216). In HIV-infected children, T-cell-mediated immune responses after vaccination are similar to those that follow natural infection (213). To our knowledge, there are no studies from the African continent that evaluated immunogenicity after varicella immunization in HEU children.

Table 1.1 Studies on VZV vaccination in HEU children

Author (year) country – study design	Enrolment years, eligible age children	Age in years at first VZV vaccine dose, median (IQR)	Age in years at last[a] VZV vaccine dose, median (IQR)	Time interval between last VZV vaccine dose and specimen collection	Proportion seropositive[b] after one dose of VZV vaccine, n/N (%)	Proportion seropositive[b] after two doses of VZV vaccine, n/N (%)
Purswani (2016) USA –	2007-2009, 7-16 yrs	1.5 (1.1-3.7)	9.1 (6.3-11.1)	Total 0 to <1 yr 1 to <3 yrs 3 to <7 yrs	55/56 (98) 1/1 (100) 4/4 (100) 13/13 (100)	121/121 (100) 22/22 (100) 41/41 (100) 54/54 (100)

cross-sectional				≥ 7 yrs	37/38 (97)	4/4 (100)

Abbreviations: yrs, years; IQR, interquartile range;
[a] Among subjects with at least two vaccine doses; [b] All samples were tested using whole-infected cell enzyme-linked immunosorbent assay, negative/equivocal samples were retested using glycoprotein enzyme-linked immunosorbent assay. Seropositivity was defined as a positive result from either assay (cutoffs not mentioned).

1.3.3 Safety of varicella vaccine

In all studies live attenuated VZV vaccine was found to be safe and well tolerated (217). Severe adverse vaccine-associated reactions have been rarely reported and were mostly related to administration in immunocompromised patients (218). Most frequently reported AEs following monovalent varicella vaccine included pain (28%), fever (27%), restlessness (20%) and cough (11%) (217). A systematic review reported 131/904 (14%) cases of fever and 30/911 (3%) cases of varicella-like rash after the first dose of varicella vaccine (219). The combined MMRV vaccine has also shown good safety profiles, except for higher incidence of fever and rash compared to single dose varicella in co-administration with MMR (220,221). In a South African trial, single dose of VZV vaccine, co-administered with MMR at 15-18 months in healthy children, was reported to be safe and well tolerated (210). In children living with HIV varicella vaccine was also found to be safe (211–214). Armenian et al. found low grade fever as the most commonly reported AE following varicella immunization in HIV-infected children (211).

1.4 Hepatitis-A

Hepatitis-A is an acute liver infection caused by the hepatitis-A virus (HAV). HAV is a single-stranded, linear RNA virus belonging to the *Picornaviridae* family. HAV is transmitted via the faecal-oral route through ingestion of contaminated food or water and direct person-to-person contact. The clinical spectrum of acute HAV infection ranges from asymptomatic infection revealed by serology to clinical infection

detected by abnormal liver tests, clinically apparent hepatitis, and, rarely, fulminant hepatitis, which is associated with high mortality (222). Clinical manifestations of acute hepatitis-A are similar to those caused by other forms of viral hepatitis (223). Most young children experience asymptomatic infections, whereas the majority of adults develop symptomatic disease (11). Clinical symptoms include jaundice, fatigue, malaise, anorexia, fever, myalgia, abdominal pain, nausea, vomiting and diarrhoea (223). Complications of hepatitis-A are rare and the disease resolves in >99% of the cases.

1.4.1 Hepatitis-A epidemiology

Hepatitis-A virus is the most common cause of acute viral hepatitis in South Africa and many other regions around the world (224). Major differences in endemicity between geographical regions exist, which are closely related to hygiene, sanitation and socioeconomic conditions (223). The global number of acute hepatitis-A cases has increased from 117 million in 1990 to 126 million in 2005, while deaths due to hepatitis-A have increased from 30 283 to 35 245 during the same period (225). The risk of developing symptomatic hepatitis-A virus infection is strongly related with age (225).

In regions of high hepatitis-A endemicity, including sub-Saharan Africa, nearly all children are exposed to the virus before 5 years of age, when asymptomatic infection predominates (226). There are few susceptible adults and therefore the burden of symptomatic disease is low. In areas of moderate endemicity, lower HAV transmission is associated with fewer HAV infections during childhood, but increased susceptibility in adolescents and young adults, who are more likely to have symptomatic disease (227). Countries with low HAV endemicity have few individuals infected during childhood and the majority of children and adults remain susceptible

to infection. Because of absence of viral circulation, the risk of acquiring HAV infection is low.

Studies suggest that a number of African countries experience an epidemiological transition from high to intermediate HAV endemicity (228–230). These countries are at greater risk for communitywide outbreaks and HAV-associated morbidity and mortality (225). A recent systematic review indicated that South Africa could be experiencing a transition from high to intermediate endemicity (231). This is corroborated by a seroprevalence study reporting intermediate HAV endemicity in South Africa between 2005 and 2015, with total antibody positivity of 53% at 1-4 years of age and >90% after 25 years of age (232).

1.4.2 Hepatitis-A vaccine immunogenicity

The WHO recommends inclusion of hepatitis-A vaccination in the national immunization schedules for children ≥1 year old in countries detecting changes in endemicity from high to intermediate (11). Two vaccine types are currently used: formaldehyde inactivated vaccines, which are used globally, and live attenuated vaccines, which are produced and used only in China (25).

Anti-HAV IgG levels of 10 to 33 mIU/mL using different assays have been proposed as thresholds for protection from HAV infection (76,223,233), although no absolute protective level has been defined (223). Available assays to measure HAV IgG include ELISA, modified radioimmunoassay and chemiluminescence immunoassay; section 2.3.8 elaborates on advantages and disadvantages of the different techniques. It has been proposed that any detectable amount of IgG may be denoting protection and, therefore, the lower limit of detection of the specific assay should also be considered protective (234).

Immunogenicity of all inactivated HAV vaccines is generally high, resulting in similar antibody responses, independently of manufacturer (235–237). The first dose of HAV vaccine administered at 12-23 months of age induced seroprotective antibody IgG titers (≥20 mIU/mL) in up to 95% of healthy children within one month post-vaccination (238). One year after receiving one dose of HAV vaccine at 11-23 months of age, 98.6% of Argentinian children maintained seropositive titers (≥10 mIU/mL by microparticle enzyme immunoassay) (239). In the following years seropositivity remained high at 100% (year 2) and 99.7% (year 3, 4 and 5) (239). Similarly, 95.3% of Chinese children vaccinated at 18-60 months of age were seropositive (≥20 mIU/mL by microparticle enzyme immunoassay) at 1 year following inactivated HAV vaccination, which decreased to 90.6% (year 2) and 85.9% (year 5) during follow-up (240).

Seroprotection rates in HIV-infected children ranged between 71% one month post-primary immunization (241,242) to 97-100% post-booster vaccination (241–244). Immunogenicity data following inactivated HAV vaccination in HEU children have been reported in only one Brazilian study (two publications) in which 72.0% (18/25) HEU children with a mean age 5.1 (range 1.6–9.4) years had antibody titers ≥20 mIU/mL 4-8 weeks after the first HAV vaccine and 100% after the second dose (243). Seven years after the primary immunization, 100% (10/10) HEU children that were available for follow-up maintained HAV antibodies ≥20 mIU/mL at median age 13.4 years (245).

Antibody levels achieved after HAV vaccination are 10-100 times lower than those occurring after natural infection (246). In addition, commercially available antibody tests may not be sensitive enough to measure vaccine-induced antibody at low

concentrations (247). Therefore, vaccinated children may be protected against disease, but have seronegative results by standard commercial ELISA.

1.4.3 Safety of Hepatitis-A vaccine

Inactivated hepatitis-A vaccines were reported to be safe in children older than 1 year, regardless of manufacturer or timing of immunization (248–250). Risk of vaccine-related systematic adverse events were comparable to placebo in a systematic review on the safety and efficacy of inactivated HAV vaccines in 41 690 participants (251). An integrated analysis from five clinical trials in the USA in children 12-23 months old reported that the most common local injection site reactions were pain/tenderness (26%) and erythema (14%) (252). The most common vaccine-related systemic AEs were fever (12%) and irritability (8%) (252).

Hepatitis-A virus vaccination is safe in children living with HIV (241). No vaccine-related grade 3 or 4 adverse events occurred in 233 HIV-infected children with evidence of immune reconstitution while on ART upon HAV vaccination (244).

1.5 Justification and Objectives

Despite measles being targeted for elimination, measles outbreaks continue to occur in both low middle-income and high-income countries. Contributing to this is under-immunization of children, as well as a shift in measles epidemiology towards infection of infants <9 months of age, who are generally not targeted for measles vaccination. Young infants may be at increased risk of infection due to changes in maternal immunity, which nowadays is predominantly derived from vaccination rather than natural infection, thereby reducing transplacental transfer of protective antibodies from mother to fetus resulting in decreased protection during early infancy. This might be further exacerbated in settings with a high prevalence of maternal HIV-infection, where there is waning of maternal immunity in women with HIV, that also results in lower concentrations of measles antibodies being transferred to their fetuses. Hence, HIV-exposed infants, including those who are HEU, are at increased susceptibility to measles infection during early infancy. This highlights the need for a review of the measles immunization strategy, particularly in settings with high prevalence of maternal HIV-infection, to inform future deliberations on alternate measles vaccine dosing strategies.

In the current era of universally recommended ART for children living with HIV and an increasing population of HEU children, it is important to evaluate the safety and immunogenicity of measles vaccine in these specific groups. Also, data on the persistence of antibody responses to measles vaccination in children living with HIV on early ART initiation and the consequences of interrupting ART are limited. Additionally, studying the safety and immunogenicity of measles vaccine (CAM-70 strain) administered at 6 months of age in South African children is relevant because

this vaccine formulation is part of the current PIP since 2015 and has mostly been investigated at older ages.

Varicella vaccination could alleviate the burden of VZV disease in sub-Saharan Africa, where the tropical climate impacts the viral epidemiology, leading to primary infection at older ages, increased susceptibility and severity of disease in adults (189). To anticipate the potential impact of varicella immunization it is required to evaluate the vaccine immunogenicity and safety in the local population, stratified by HIV status.

Alongside with improvements in sanitation conditions in South Africa, an epidemiological change in the burden of HAV is taking place with a decreasing incidence of asymptomatic hepatitis-A infection during childhood, but an increasing incidence of symptomatic hepatitis-A infection later in life, potentially adding to morbidity and socio-economic losses (224). In this setting, it is necessary to examine hepatitis-A prevalence and assess immunogenicity of a single dose of HAV vaccine.

The results from this thesis will be informative for decision making on vaccination schedules in Sub-Saharan Africa; for HEU, HIV-infected and HIV-unexposed children, in particular with regard to timing of MV and introduction of varicella and HAV vaccines in the routine vaccination schedule. This thesis further offers insight into differences in the level of vaccine-induced immunity between HIV-exposed and HIV-unexposed children.

The overall purpose of this thesis was to assess safety and immunogenicity of measles vaccine, varicella vaccine and hepatitis-A vaccine in HIV-exposed and HIV-unexposed South African children.

Objectives:

The objectives of this study were:

1. To systematically review the literature on the safety and immunogenicity of measles vaccine in HIV-infected and HEU children and compare immunogenicity outcomes in meta-analysis taking age at vaccination and number of doses received into consideration (chapter 3).

2. To evaluate persistence of measles antibody titers at 4.5 years of age in HIV-unexposed, HEU and HIV-infected children. HIV-infected children were previously randomized to initiate ART when clinically/immunologically indicated or within 6–12 weeks of age, followed by interruption at 12 or 24 months (chapter 4).

3. To evaluate and compare immunogenicity and safety of measles vaccine administered at 6 and 12 months of age in HEU and HIV-unexposed South African children (chapter 5).

4. To evaluate and compare antibody response, cell-mediated immunity and safety of one dose of varicella vaccine administered at 18 months of age in HEU and HIV-unexposed South African children (chapter 6).

5. To evaluate and compare immunogenicity and safety of one dose of hepatitis-A vaccine administered at 18 months of age in HEU and HIV-unexposed South African children (chapter 7).

Table 1.2 summarizes how these objectives were addressed.

Table 1.2 Summary of study design per objective

Chapter	Objective	Groups included	Study design
3	Systematic review of literature on safety and immunogenicity of **measles** vaccine in HIV-infected and HEU children	HIV-infected, HEU, HIV-unexposed	Systematic review of the literature and meta-analysis
4	Persistence of **measles** antibody titers at 4.5 years of age in HIV-unexposed, HEU, and HIV-infected children previously randomized to different timing of ART initiation and interruption	HIV-infected, HEU, HIV-unexposed	Retrospective analysis of samples collected during a cohort study including HIV-infected children enrolled in a randomized open-label trial on timing of ART initiation (Children with HIV Early Antiretroviral [CHER] study) and a parallel cohort of HEU and HIV-unexposed children
5	Immunogenicity and safety of **measles** vaccine administered at 6 and 12 months of age in HEU and HIV-unexposed children	HEU, HIV-unexposed	Prospective cohort study including HIV-unexposed children co-enrolled in a randomized open-label trial on alternate pneumococcal conjugate vaccine (PCV) dosing strategies (PCV1+1) and a parallel cohort of HEU children
6	Antibody response, cell-mediated immunity and safety of one dose of **varicella** vaccine in HEU and HIV-unexposed children	HEU, HIV-unexposed	
7	Immunogenicity and safety of one dose of **hepatitis-A** vaccine in HEU and HIV-unexposed children	HEU, HIV-unexposed	

Chapter 2 Materials and methods

This chapter describes the methods and techniques which are common to multiple objectives. Methods that are specific to one objective are discussed in the individual results chapters.

2.1 Study population

South Africa has an annual birth cohort of 1.17 million with an estimated 5.7 million children under 5 years of age and an under-5 mortality of 45 per 1000 in 2018 (253,254). National HIV prevalence among pregnant women 15-49 years of age attending antenatal clinics has remained stable between 2005 and 2015 at approximately 30% (3). The number of children 0-14 years of age living with HIV has decreased from 320 000 in 2010 to 260 000 in 2018 (255). In 2004, South Africa officially launched the prevention of mother-to-child HIV transmission (PMTCT) programs. Between 2011 and 2016 the estimated mother-to-child HIV transmission rates have fallen from 3.6% to 1.5% (256). In the same period, ART coverage among children 0-14 years of age living with HIV has increased from 32% to 63% (255). South Africa is classified as an upper middle-income country by the World Bank, yet it also has one of the highest income inequalities as measured by a Gini coefficient of 0.63 in 2015 (257).

Between 1995 and 2015, South Africa administered a Schwarz-strain MV (Rouvax) at 9 and 18 months of age (258). On the 1st of December 2015, the South African Department of Health updated the measles immunization schedule and advised routine injection of a live attenuated MV with the CAM-70 strain (Measbio, Biovac) at 6 and 12 months of age (144). Hepatitis-A and varicella vaccines are not part of the

South African PIP but are administered in the context of private practice at ages 12 and 18 months (hepatitis-A) or 12 months and 5-6 years (varicella).

2.2 Study design and methods: retrospective analysis of samples from a prospective cohort study

2.2.1 Study design and site

To investigate the long-term persistence of measles vaccine-induced immunogenicity we conducted a retrospective analysis of a prospective cohort of HEU, HIV-unexposed and HIV-positive children. The study was conducted on archived serum samples from children living with HIV enrolled in a randomized open-label trial on the timing of ART initiation (Children with HIV Early Antiretroviral [CHER] study) (259) and a parallel cohort of HEU and HIV-unexposed children (25,39,40). Children were enrolled at Chris Hani Baragwanath Academic Hospital (CHBAH), Johannesburg, and Tygerberg Hospital, Stellenbosch, South Africa, between April 2005 and June 2006. HIV-uninfected children were recruited from maternity wards of the study sites by health workers trained in HIV counselling. As part of PMTCT, HIV testing was done in all pregnant women. HEU children were recruited from those that were originally identified to participate in the CHER trial, but who tested HIV negative at 4 weeks of age by polymerase chain reaction (PCR). Children living with HIV were enrolled in the CHER study and were randomized to differing timings of ART initiation (2.2.3 Study procedures).

2.2.2 Inclusion and exclusion criteria

Inclusion criteria:

1. Age at recruitment ≥4 weeks and ≤10 weeks of age;
2. Birthweight ≥2000 grams;

3. Parent or legal guardian of the infant was able and willing to provide written informed consent and complied with all study requirements;
4. Parent or legal guardian of the infant indicated the intention to remain in the study area for the duration of the trial;
5. Documentation of mother's HIV status after 24 weeks of gestation of the child if the child is HIV uninfected.

Inclusion criteria for HIV-infected children

1. Co-enrolled in the CHER study (the CHER study enrolled HIV-infected infants between 6 to 12 weeks of age with CD4+ T-cell percentages ≥25%)
2. HIV infection must be identified by a positive DNA or RNA PCR at birth and confirmed by a second RNA PCR (plasma HIV RNA value >10 000 copies/mL) between 4 and 10 weeks of age;
3. ART naïve except for receipt of antiretroviral drugs used to prevent mother-to-infant HIV transmission.

Exclusion criteria:

1. Receipt of blood products prior to study entry;
2. HIV-uninfected: receipt of any vaccine, except for BCG and oral polio vaccine, prior to study entry; HIV-infected: prior vaccinations allowed to participate;
3. Receipt of immunosuppressant agents for >2 weeks, within one week of study entry;
4. Inability to tolerate oral medication;
5. Presence of any major, life threatening congenital abnormalities;
6. Infants with acute, intercurrent illness or fever of ≥38 ºC within 72 hours prior to immunization that requires hospitalization;

7. Presence of any Grade 2 allergic reactions or any ≥ Grade 3 clinical toxicity;
8. Presence of any Grade 4 or 4 clinical or laboratory toxicity;
9. Parent or guardian unable or unwilling to attend scheduled study visits;
10. Use of the following medications:
    a. All antiretroviral therapies other than the regimens described in the study;
    b. All investigational drugs;
    c. Systemic cytotoxic chemotherapy;
    d. Interleukin or other immune modulators.

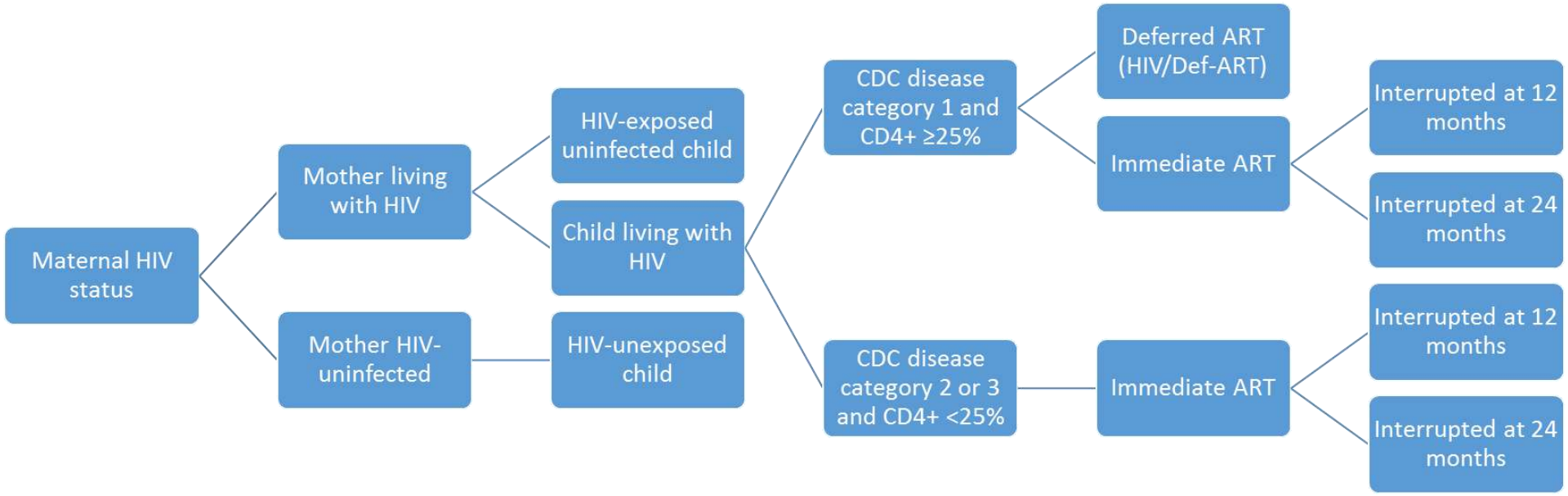
2.2.3 Study procedures

Children living with HIV with CD4+ T-cell percentages ≥25% were randomized to:

- Initiate immediate ART followed by interruption at 12 months (HIV/Immed-ART-12)
- Initiate immediate ART followed by interruption at 24 months (HIV/Immed-ART-24)
- Deferred ART until clinically or immunologically indicated (HIV/Def-ART), as per the WHO recommendations at the time of participant enrolment

A convenience sample of HIV-infected children with CD4+ T-cell percentage <25% (HIV+/CD4+<25%), who initiated ART at enrolment for 12 or 24 months followed by interruption, was included. ART was re-initiated if clinically or immunologically indicated. An interim analysis of the CHER trial showed greater risk of disease progression and death in the HIV/Def-ART group and thus all children in this group were evaluated for ART initiation and started treatment at median age 5.8 months (interquartile range [IQR] 4.4-10.3) (259).

Figure 2.1 Flow diagram of study groups



Antiretroviral therapy

At CHBAH, pregnant women living with HIV and their infants received single dose nevirapine (NVP) at the time of delivery. At Tygerberg hospital, HIV-infected pregnant women received zidovudine from 34 weeks gestation and single dose NVP at delivery, whereas the neonates received single dose NVP at birth plus zidovudine for seven days. The ART regimen during the study consisted of zidovudine, lamivudine and lopinavir-ritonavir. Second-line regimen consisted of didanosine, abacavir and nevirapine (259).

Study vaccines

The live attenuated measles vaccine (Schwarz strain, Rouvax; Aventis, France) was administered at 38-42 weeks (9 months) and 64-76 weeks (15-18 months) of age. The vaccine was injected intramuscularly into the right anterolateral thigh. Other childhood vaccines were administered per South African PIP (Table 2.1), except for administration of pneumococcal conjugate vaccine (PCV) primary series and randomization to PCV or *Haemophilus influenzae* type-b conjugate vaccine (HiBCV) booster dose at 64-76 weeks of age (40).

Table 2.1 Vaccination schedule for study participants

Age	**Vaccines as part of South African Public Immunization Program in 2005**	**Additional study vaccines**
Birth	Oral polio vaccine (0) Bacille Calmette-Guerrin	
6 weeks	Oral polio vaccine (1) Combined Diphtheria-tetanus-whole cell pertussis-*Haemophilus influenzae* b-vaccine (1) Hepatitis B vaccine (1)	Pneumococcal conjugate vaccine (1)
10 weeks	Oral polio vaccine (2) Combined Diphtheria-tetanus-whole cell pertussis-*Haemophilus influenzae* b-vaccine (2)	Pneumococcal conjugate vaccine (2)

	Hepatitis B vaccine (2)	
14 weeks	Oral polio vaccine (3) Combined Diphtheria-tetanus-whole cell pertussis-*Haemophilus influenzae* b-vaccine (3) Hepatitis B vaccine (3)	Pneumococcal conjugate vaccine (3)
9 months	Measles vaccine (1)	
18 months	Oral polio vaccine (4) Diphtheria-tetanus-whole cell pertussis vaccine (4) Measles vaccine (2)	Pneumococcal conjugate vaccine OR *Haemophilus influenzae* b- conjugate vaccine

Specimen collection

Clotted blood samples were collected at 4.5 years (232-236 weeks) of age at CHBAH and Tygerberg Hospital. After centrifugation, serum was aliquoted and stored at -20°C to -70°C at RMPRU, Johannesburg, South Africa.

2.2.4 Laboratory methods

Measles specific IgG antibodies were measured using an indirect ELISA (Enzygnost, Dade Behring, Marburg, Germany). The plaque reduction neutralization (PRN) assay is considered to be the gold standard for measuring measles-specific IgG due to its high sensitivity and specificity. Although PRN assays are able to detect very low levels of antibody, they are time-consuming, require extensive resources and are generally limited to specialized reference laboratories (80,260). In comparison, commercial ELISAs are widely available, cheaper and relatively easy to perform (260). Studies showed that seropositivity correlates with protection against clinical measles, however, children with seronegative ELISA results after vaccination are not necessarily susceptible (261,262). ELISAs have poor correlation with PRN at low levels of maternally derived antibody in infants less than 6 months of age (260,263,264). Because ELISA kits have differing sensitivity and specificity (265), a reference serum calibrated against the International Standard for anti-measles

serum, should be included in the ELISA assays in order to reduce inter-test and inter-laboratory variability. The manufacturers of the Enzygnost ELISA kit reported a sensitivity of 99.6% and specificity of 100% (Enzygnost anti-measles virus IgG kit insert). The assay included an internal reference for the quantitative assessment of measles IgG concentrations, calibrated against the first WHO International Reference Preparation (266,267).

The principle of ELISA is based on measles-specific IgG from the test sample binding to microtiter plate wells coated with measles antigen from permanent simian kidney cells infected with measles virus and antigen from non-infected cells (control antigen). Anti-human IgG conjugate is then added and binds to these specific antibodies. The conjugate has an enzyme portion which causes a color change in the solution. After the reaction is stopped, the color intensity can be measured. The difference in color intensity between wells coated with measles antigen and wells coated with control antigen yield a measure of the amount of measles-specific IgG antibodies in the sample. Test plates were read using a spectrophotometer (Labsystems Multiskan RC; USA) at 450 nm and a reference wavelength of 620 nm for background correction.

The difference in mean absorbance between the wells containing measles virus or control antigen was calculated and multiplied by a correction factor (the kit specific nominal value divided by the mean of the reference standards) to yield the corrected delta absorbance (∆A). Antibody titers were calculated using the following formula: Log_{10} mIU/mL= $\alpha \times \Delta A^{\beta}$, where α and β are lot-dependent constants. Measles seropositivity was defined as titers ≥330 mIU/mL (OD > 0.2). All negative (<150 mIU/mL; OD < 0.1) and equivocal (150–329 mIU/mL) samples were analyzed in duplicate. If a result of 150–329 mIU/mL was confirmed, the samples were classified

as equivocal, otherwise as positive or negative. Seronegative samples were assigned a titer half the value of the assay's detection limit (i.e. 75 mIU/mL).

2.2.5 Data analysis

Because this was an analysis on archived serum samples, a convenience sample of all sera available at 4.5 years of age was used. Measles antibody levels following vaccination may be influenced by sex (268,269), age and immune status in HIV-infected children. Therefore, we decided to adjust for sex, age at blood collection and CD4+ T-cell percentage at time of the 9-month measles dose. In order to account significant differences in baseline characteristics between groups, we also adjusted for race and study center in multivariable logistic regression models comparing the different proportions of children meeting serology cutoffs. To minimize the effect of other confounders, analysis was restricted to children who received two doses of MV and had an immunogenicity visit with serum collection at 4.5 ± 0.4 years of age.

Additional potential predictor variables for long-term measles antibody titers were assessed in logistic regression models including HIV status, ART initiation strategy, sex, race, age, time interval between vaccination and blood collection, and nutritional status at the primary measles dose. In HIV-infected children, we also evaluated the effect of ART (at time of primary and booster measles doses, immunogenicity visit) and CD4+ T-cell percentage (at enrolment and primary measles dose) on the proportion of participants with antibody titers ≥330 mIU/mL. Missing data were handled by listwise deletion. Variables with p-values ≤ 0.15 in univariate regression were included in multivariable regression models and HIV-unexposed children were used as the reference group.

2.2.6 Ethics

This study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M170391). The parent trials were approved by Ethics Committees of the University of the Witwatersrand and the Stellenbosch University, Medicine Control Council (South Africa), and the Division of AIDS of the National Institute of Health (USA). Written informed consent was obtained from the parent(s) of participants prior to study entry, including approval to analyse immune responses to other vaccines. The ClinicalTrials.gov registry numbers for the parent studies are NCT00099658 and NCT00102960.

2.2.7 Funding

The study was funded by the South African Research Chairs Initiative of the Department of Science and Technology, National Research Foundation in Vaccine Preventable Diseases, and the Medical Research Council. The parent study was funded by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH), through the Comprehensive International Program of Research on AIDS (CIPRA) network (grant U19 AI53217). Additional support for this work was provided with federal funds from the NIAID, NIH, Department of Health and Human Services under contract HHSN272200800014C.

2.3 Study design and methods: prospective cohort

2.3.1 Study design and site

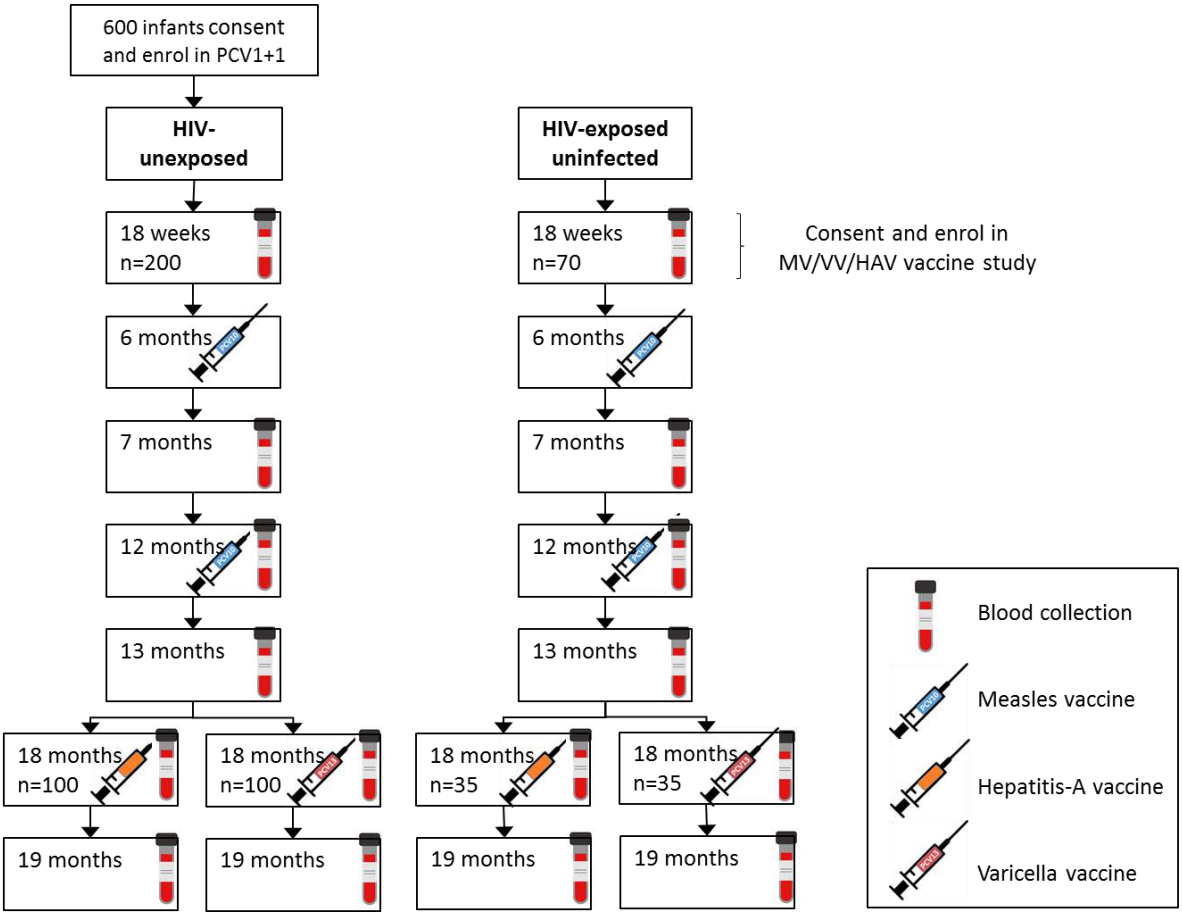
This was a prospective, observational cohort study enrolling HIV-unexposed and HEU infants 18 weeks of age at RMPRU between April 2017 and February 2019. HIV-unexposed children were co-enrolled in a randomized, open-label trial evaluating reduced dosing schedules of PCV (PCV1+1, NCT02943902).

The study participants were recruited from the population presenting CHBAH or one of the neighbouring primary health clinics, including midwife operated units and immunization clinics. CHBAH is the only public hospital in Soweto, a peri-urban black African suburb in Johannesburg. Data from the 2011 census indicated that 91.6% of Soweto households had access to flush toilets connected to sewerage, 93.1% to electricity for lighting and 55% had piped water inside dwellings (270). The total population of Soweto was estimated at 1.27 million with 84.2% living in formal dwellings (270), although no recent (sub)district level data are available. Community survey data from the greater Johannesburg region in 2016 indicated that 94.1% of households had access to safe drinking water and 90.2% to flush toilets (271).

The standard-of-care for pregnant women during the conduct of the study consisted of repeat HIV testing for HIV-uninfected women every 3 months during pregnancy and at labour/delivery. Upon positive HIV diagnosis, all women were initiated on immediate lifelong ART (fixed dose combination Tenofovir + Lamivudine (Emtricitabine) + Efavirenz). All HIV exposed neonates were tested by HIV PCR at birth and at 10 weeks of age. Testing was repeated at 6 weeks post cessation of breastfeeding (age appropriate HIV PCR or rapid HIV antibody test) and by rapid HIV antibody test at 18 months of age (272).

We aimed to enrol at least 200 HIV-unexposed and 70 HIV-exposed participants.

Figure 2.2 Study schedule of vaccination and evaluation of immune responses



2.3.2 Sample size calculation

The sample size was calculated at a 5% significance level (two-sided), 80% power and 1:3 ratio of HEU to HIV-unexposed participants. Immunogenicity of two doses standard titer MV, assuming seropositive rates post-booster of 99% in HIV-unexposed and a 10% reduction in seropositivity rate in HEU, resulted in a minimum sample size of 60 HEU and 178 HIV-unexposed children. The sample size was adjusted upward by 10% to account for loss to follow-up, resulting in a total minimal sample size of 270 participants: 70 HEU and 200 HIV-unexposed children.

With a sample size of 100 HIV-unexposed and 35 HIV-exposed children, this study was powered at 80% to detect a difference in seropositivity of 25% after varicella vaccine and 18% after HAV vaccine in HEU children compared to HIV-unexposed

children assuming seropositivity rates of 85% and 95% in HIV-unexposed children, respectively. The assumption of seropositivity after VZV vaccination was premised on 85%-89% of children having gpELISA titers ≥5 units/mL after a single dose VZV vaccine (178,186). Assumed seropositivity following HAV vaccination was based on 95% of healthy children having antibody IgG titers ≥20 mIU/mL within one month post-vaccination (238). A 3:1 ratio of HIV-unexposed to HIV-unexposed children was chosen because HIV-unexposed children were co-enrolled in the PCV1+1 study and study visits could be combined.

2.3.3 Inclusion and exclusion criteria

Research staff screened all children for eligibility when they were 18 weeks old at the study clinic in CHBAH.

Inclusion criteria:

- Age ≤18 weeks;
- Parent/guardian able to provide informed consent;
- Available for the duration of the study;
- Enrolled as a participant in the PVC1+1 trial AND born to HIV-uninfected woman OR born to HIV-infected mother AND infant CD4% ≥25% if HIV-infected;
- Birth weight >2499 g AND weight of >3.5 kg at time of proposed enrolment;
- Being a healthy child (except for HIV status in HIV-exposed cohort) based on medical history and physical examination by the study staff.

Exclusion criteria:

- Significant major congenital abnormalities;

- Receipt of measles vaccination, varicella vaccination or hepatitis-A vaccination since birth;
- Previous hospitalization for illness following discharge from hospital at birth;
- Known allergy to vaccine components;
- Febrile illness (axillary temperature ≥37.8°C) at time of screening;
- Known or suspected immunodeficiency condition other than HIV;
- Planning to relocate outside of the study area during the study period;
- Receipt of blood transfusion or any other blood products (including immunoglobulins) since birth, receipt of such products during the course of the study will require withdrawal of the child from the study;
- History of confirmed measles, varicella or hepatitis-A disease since birth.

2.3.4 Participant recruitment

Mothers of healthy infants were identified in postnatal wards of CHBAH or community health clinics in the Soweto area and informed about the study (PCV1+1 or HEU cohort). If interested, they were given specific dates on which to return to the RMPRU clinic for enrolment. In addition, maternity birth registers were used to identify mothers who were discharged with their baby after delivery and fulfilled eligibility criteria.

<u>HIV-unexposed cohort</u>

HIV-unexposed infants enrolled in the PCV1+1 study were invited to participate in the nested MV/VZV/HAV vaccine study. Children enrolled in the PCV1+1 study received all vaccines included in the South African PIP, except for the randomization to differing dosing schedules of PCV. Varicella vaccine or HAV vaccine was

assigned to the participants at 18 months of age as an additional benefit for participating in the PCV1+1 study.

HIV-exposed cohort

A separate cohort of HIV-exposed infants (not participating in the PCV1+1 study) was recruited. None of the HIV-exposed infants were HIV-infected at enrolment. These infants received measles vaccine, as well as VZV or HAV vaccine. Other vaccines included in the South African PIP were administered through routine visits at primary health immunization clinics, including three doses of PCV.

2.3.5 Outcome measures

Immunogenicity outcomes

Serum samples were obtained from all children prior to MV1 (4.5 months), one month post-MV1 (7 months), prior to MV2 (12 months), one month post-MV2 (13 months), prior to VZV/HAV vaccine (18 months), one month post-VZV/HAV vaccine (19 months). Geometric mean titers (GMT) and percentage of participants with vaccine specific IgG antibody titers above the putative threshold of positivity were calculated at the different time-points for each antigen.

Peripheral blood mononuclear cells (PBMCs) were collected prior to varicella vaccination (18 months) and one month post-vaccination (19 months). The outcome of interest was the mean number of spot-forming cells (SFCs)/10^6 PBMCS in VZV-stimulated wells, after subtracting the mean number of SFCs in control wells containing medium.

Safety outcome

Participants were observed for at least 30 minutes after each vaccination in order to observe and document any potential adverse reactions to the vaccine. Vaccine

safety was assessed using report cards. Parents were trained to record any solicited local (redness, swelling, tenderness, itching,) and systemic (fever, malaise, myalgia, nausea, headache, rash) reactions for 7 days following vaccination. In order to measure temperature and size of swelling/redness, they received a digital thermometer and ruler. Unsolicited adverse events (AEs) and serious adverse events (SAEs) were documented throughout the study.

2.3.6 Study vaccines

All study vaccines are licensed in South Africa and were stored at 2°C– 8°C in a temperature-monitored refrigerator until use. Measles vaccines were supplied as 10 dose multidose vials, containing lyophilized powder which were reconstituted with 5 mL diluent per vial. Participants received subcutaneous injection of 0.5 mL live attenuated MV (MeasBio®, Biovac, ≥1000 $CCID_{50}$ CAM-70 strain/0.5 mL) in the anterolateral external aspect of the upper thigh at 6 months (182 ± 14 days) and 12 months (365 ± 14 days) of age.

Participants were allocated consecutive study IDs. Based on having an even or odd study ID, children received either one dose of inactivated HAV vaccine (AVAXIM® - Pediatric, Sanofi Pasteur, Lyon, France, 80 U/0.5 mL) or one dose of live attenuated VZV vaccine (VARILRIX®, GlaxoSmithKline, Rixensart, Belgium, $\geq 10^{3.3}$ plaque-forming units of VZV/0.5 mL) at 18 months (547 days ± 14) of age. Qualified medical staff administered study vaccines. All children received other EPI vaccines as per South African public immunization guidelines, except for HIV-unexposed children being randomized to different dosing schedules of PCV (table 2.1).

Table 2.2 South African Public Immunization Program in 2017

Age	**Vaccines**
Birth	Oral polio vaccine Bacille Calmette-Guerrin

6 weeks	Oral polio vaccine Rotavirus vaccine Combined Diphtheria-tetanus-acellular pertussis-inactivated polio-*Haemophilus influenzae* b-Hepatitis B vaccine Pneumococcal conjugate vaccine
10 weeks	Combined Diphtheria-tetanus-acellular pertussis-inactivated polio-*Haemophilus influenzae* b-Hepatitis B vaccine
14 weeks	Rotavirus vaccine Combined Diphtheria-tetanus-acellular pertussis-inactivated polio-*Haemophilus influenzae* b-Hepatitis B vaccine Pneumococcal conjugate vaccine
6 months	Measles vaccine
9 months	Pneumococcal conjugate vaccine
12 months	Measles vaccine
18 months[a]	Combined Diphtheria-tetanus-acellular pertussis-inactivated polio-*Haemophilus influenzae* b-Hepatitis B vaccine

[a] Study participants received the booster dose of combined Diphtheria-tetanus-acellular pertussis-inactivated polio-Haemophilus influenzae b-Hepatitis B vaccine at 15 months of age

2.3.7 Study procedures

Study staff informed the parents/guardians of the children on the study procedures and presented a medical information sheet and informed consent form once eligibility was confirmed during visit 1. Participants eligible for enrolment were allocated a serial numerical study identifier. Demographic data and medical history were collected, and physical examination performed (including vital signs, measurement of weight and length, and targeted physical exam upon every subsequent visit, see Table 2.3).

Table 2.3 Data collection at enrolment

Maternal data	**Infant data**	**Infant physical examination**
Date of birth, race, contact details, HIV status, ART use during pregnancy	Date of birth, gender, race, gestational age at delivery, birth weight, mode of delivery, APGAR scores, pregnancy complications, history of immunizations, hospital admissions, infant illnesses, HIV exposure, ART use	Vital signs (temperature, blood pressure, heart rate, respiratory rate), weight, length and complete physical examination

HIV exposure status was determined based on maternal HIV-ELISA and infant HIV-PCR at the time of enrolment. The data was obtained from the participants' Road to Health booklet, or if not available, a blood sample from the infant was collected for HIV testing after parental informed consent was granted. Infants born to HIV-uninfected mothers were categorized as HIV-unexposed if maternal HIV-ELISA during pregnancy was negative, children born to mothers living with HIV were categorized as HEU if maternal HIV-ELISA was positive but infant HIV-PCR was negative, and as HIV-infected if infant HIV-PCR was positive. No participant was confirmed to be HIV-positive. Hereafter, participants will be stratified as HIV-unexposed and HEU.

All participants received a visit appointment card and the subsequent visits were scheduled at the end of each study visit. See tables 2.4 and 2.5 for an overview of study visits.

Table 2.4 Study schedule of HIV-unexposed cohort

Visit number	**1**	**2**	**3**	**4**	**5**	**5b**	**6**	**7**	**8**	**8b**	**9**	**10**	**10b**
	6 weeks	**10 weeks**	**14 weeks**	**18 weeks**	**6 months**	**7 months**	**9 months**	**10 months**	**12 months**	**13 months**	**15 months**	**18 months**	**19 months**
Age (days) or time since previous visit	Day 42-56	V1 +28-35	V2 +28-35	V3 +28-35	180 ±14	V5 +28-35	270 ±14	V6 +28-35	365 ±14	V8 +28-35	450 ±14	540 ±14	V10 +28-35
ICF signed for the MV/VZV/HAV study				X									
Inclusion/exclusion criteria				X									
Medical history				X									
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X
Measles vaccine					X				X				
Varicella or Hepatitis-A vaccine[1]												X	
Blood draw				X		X			X	X		X	X
PCV (depending on randomization arm)	X		X				X						
Hexaxim®	X	X	X								X		
Rotavirus vaccine	X		X										
Diary card hand out					X				X			X	

[1]Participants whose randomization number ends in an even number received hepatitis-A vaccine and those with an odd number the varicella vaccine. Grey marked study visits for PCV1+1 only.

Table 2.5 Study schedule of HIV-exposed cohort

Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	18 weeks	**6 months**	**7 months**	**12 months**	**13 months**	**18 months**	**19 months**
Age (days)	119 ±14	180 ±14	V2 +28-35	365 ±14	V4 +28-35	540 ±14	V6 +28-35
ICF signed	X						
Inclusion/exclusion criteria	X						
Medical history	X						
Physical examination	X	X	X	X	X	X	X
Measles vaccine		X		X			
Varicella or Hepatitis-A vaccine[1]						X	
Blood draw	X		X	X	X	X	X
Diary card hand out		X		X		X	

[1]Participants whose randomization number ends in an even number will receive hepatitis-A vaccine and those with an odd number the varicella vaccine.

2.3.8 Laboratory methods

Blood volumes of 3 mL were collected at each blood draw. At 18 and 19 months of age, an additional 5 mL of blood was collected from participants receiving varicella vaccine (n=135) for isolation of PBMCs. Samples were labelled using the unique study identifier code. Within 4 hours of collection, samples were delivered to the RMPRU laboratory, centrifuged and serum was stored at -70ºC until further processing. Only laboratory methods which are common across objectives are discussed in this section. Specific methods related to a single objective are discussed in the individual chapters.

Measles

Humoral responses to measles IgG were measured using commercially available ELISA (Enzygnost, Dade Behring, Marburg, Germany) in samples obtained at 4.5 months, 7 months, 12 months and 13 months of age. Section 2.2.4 describes the measles ELISA used in this cohort and the retrospective study in more detail.

Varicella

VZV antibodies in samples obtained at 18 and 19 months of age were measured by ELISA (SERION ELISA *classic* Varicella-Zoster IgG, Institut Virion\Serion GmbH, Würzburg, Germany), as further described in chapter 6. The following section elaborates on the reasons for choosing the particular assay to measure VZV antibodies.

Humoral immunity

The accepted gold standard assays for measurement of immunity and protection against VZV are FAMA and the complement-enhanced VZV neutralization assay. FAMA titers ≥1:4 following vaccination have been associated with protection against

varicella (273). Both FAMA and the VZV neutralization assay are technically complex, subjective and unsuitable for testing large numbers of samples (274). Upon VZV OKA-strain vaccine introduction in the USA, an assay with higher sensitivity was needed because VZV IgG titers were lower after vaccination compared with natural infection (275). In response, Merck developed a non-commercial gpELISA using concentrated, highly purified glycoproteins in the solid phase and tested it extensively in children (273). We initially planned on using Merck's gpELISA, however, this was not possible due to limited availability and the assay only being performed in a small number of specialist research laboratories. A gpELISA titer of ≥5 U/mL at 6 weeks after immunization has been reported as an approximate correlate of protection against break-through infection for individual vaccine recipients (276). This correlate is commonly used as the primary immunogenicity endpoint in VZV vaccine clinical trials. On the downside, gpELISA may be over-sensitive in detecting immunity to varicella (195).

For our study, we used a commercial ELISA (SERION ELISA *classic*) with purified VZV glycoprotein as antigen, which is methodologically different from the Merck enzyme immunoassay (EIA). The disadvantage of this technique is that there are limited data comparing the performance of commercially available EIAs to FAMA for measuring VZV IgG following vaccination (277). Moreover, commercial ELISAs are less sensitive than FAMA or gpELISA for detection of seroconversion after vaccination (273). The SERION ELISA *classic* VZV was reported to have 100% sensitivity for seropositivity in relation to FAMA when testing sera from latently infected persons. Sensitivity of 87% and specificity of 100% for seropositivity were achieved in post-vaccination samples (277). When using an optimized cutoff,

interpreting equivocal results as positive, sensitivity increased to 90% and specificity decreased to 98% in VZV vaccine recipients (277).

Cell-mediated immunity

Cell-mediated immunity to varicella vaccination was measured by double-color enzymatic interferon-gamma (IFN-γ) and interleukin-2 (IL-2) Enzyme-linked immunospot (ELISPOT) assay (Immunospot, Cellular Technology Limited, Bonn, Germany). More details are provided in chapter 6. The section below outlines different methods for measurement of CMI to VZV and the rationale for choosing ELISPOT.

CMI to VZV can be measured by stimulation of lymphocytes with VZV antigens *in vitro* (incubation of PBMCs in the presence of different VZV dilutions, the highest value expressed as a stimulation index) (278,279) and by lysis of histocompatible target cells by cytotoxic T-cells stimulated with VZV antigens (280). Another method to assess cellular immunity is by intradermal inoculation of VZV antigens (281,282). An individual's susceptibility to VZV is assessed by the erythematous change 24-48 hours after intradermal antigen injection. VZV skin testing is sensitive, specific and no laboratory equipment is needed, but may be difficult to perform in children (186). ELISPOT assays can measure the synthesis of cytokines, including IFN-γ and IL-2, by T-cells in response to antigenic VZV stimulation (283). This method for assessing CMI to VZV directly measures the number of cytokine secreting T-cells and is very sensitive and specific compared to other assays, such as the lymphoproliferative activity assay (284,285) or responder cell frequency method (286). ELISPOT is widely used for monitoring CMI in experimental and clinical settings and one of the most common methods for measuring antigen-specific T-cell responses.

ELISPOT requires the isolation of PBMCs, which are added in a known concentration to a capture antibody-coated plate, i.e. anti-IFN-γ and anti-IL-2. PBMCs are then stimulated with a known concentration of VZV-specific antigens, which leads to the secretion of cytokines by antigen-specific T-cells that are subsequently captured by the antibody on the coated plate. After incubation (16-24 hours) and sequential washing to remove the cells, cytokine-antibody complexes bound to the plate remain. The addition of an enzyme-conjugated reagent allows for cleavage of a chromogenic substrate and the generation of spots by creation of a stable and visible precipitate at the reaction site. Each individual cytokine-producing cell is represented by an individual spot. In doing so, ELISPOT measures the ability of individual T-cells *ex vivo* to produce a specific cytokine response to *in vitro* stimulation with a known antigen and gives an approximation of the frequency of responsive cells in the cell population (287,288). We chose a dual-color ELISPOT for simultaneous detection and enumeration of IL-2 and IFN-γ producing cells, because it allows for differentiation between different type of effector cells (total effector cells, effector memory cells, central memory cells and differentiated effector cells).

Hepatitis-A

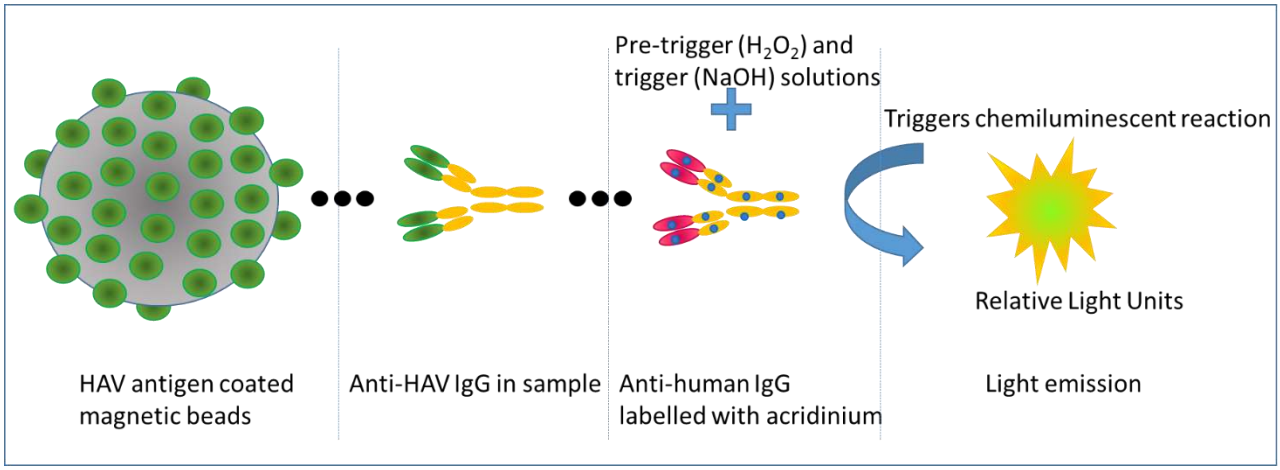
Antibodies to HAV were measured using a chemiluminescent microparticle immunoassay (CMIA; Abbott ARCHITECT® HAVAb- IgG, Wiesbaden, Germany), as described in detail in chapter 7.

Antibodies to HAV can be measured using ELISA, modified radioimmunoassay or chemiluminescence immunoassay. Neutralizing antibody can be detected by radioimmunofocus inhibition or HAV antigen reduction assay, but are very labour intensive, not widely available or well standardized (223). The advantage of CMIA is

the automated assay platform with short turnaround time and limited technical expertise. The disadvantage is that the assay was designed for the qualitative determination of HAV antibodies, hence quantitative antibody levels cannot be measured, thereby limiting direct comparison with other assays.

CMIA uses recombinant HAV antigen coated paramagnetic microparticles that bind to anti-HAV IgG in serum; Figure 2.3. After incubation and washing, an anti-human IgG acridinium labelled conjugate is added, which binds to anti-HAV IgG. After another incubation and wash step, pre-trigger solution, containing hydrogen peroxide, and trigger solution, containing sodium hydroxide, are added to the reaction mixture. The luminophore marker acridinium emits visible or near-visible (λ = 300-800 nm) radiation when an electron transitions from an excited state to ground state (289). The light emitted on antigen-antibody reaction is measured as relative light units.

Figure 2.3 Principles of chemiluminescent microparticle immunoassay



The amount of anti-HAV IgG is directly related to the relative light units detected. The chemiluminescent signal from the sample is divided by the cutoff signal from the ARCHITECT HAVAb-IgG calibrator (S/CO). S/CO values ≥1.00 are considered reactive for anti-HAV IgG and those with S/CO values <1.00 are considered non-

reactive (290). The manufacturer reports a sensitivity of ≥98% in samples from vaccinated individuals and patients recovering from acute HAV infection. The assay demonstrated ≥99.2% specificity in serum and plasma specimens from randomly selected blood donors and hospitalized patients (290).

2.3.9 Data analysis and statistical considerations

Data were entered onto paper case report forms (CRF) by study doctors and nurses during the study visits. Delivery information, gestational age at birth, birth weight, APGAR scores and HIV test results were collected from the Road to Health Card, which included antenatal HIV ELISA results of mothers and HIV PCR tests of infants. CRFs were reviewed for completeness, accuracy and consistency by the study investigator and study coordinator.

Paper CRFs were scanned and electronically sent to an external data capture company (CESAR, Johannesburg, South Africa), which performed double-data entry into a custom-designed database. Data cleaning was performed by checking for missing values and logic checks. Data queries were resolved by investigator and study coordinator.

Data were analyzed using STATA (version 13, Statacorp, College Station, Texas, USA) and R (version 3.5.1). Details of analyses are included in the methods sections of the following chapters.

2.3.10 Ethics

The study protocol was approved by the Human Research Ethics Committee (HREC) of the University of the Witwatersrand, South Africa (HREC reference number: M170276). Parents provided written informed consent before study entry. The ClinicalTrials.gov registry number is NCT03330171. Participants were

reimbursed for travel and incidental costs arising from study visits, if applicable. All data were anonymised and did not include any personal identifiers.

2.3.11 Funding

The measles sub-study was funded by The Biologicals and Vaccines Institute of Southern Africa (Biovac). Other analyses were supported by the Department of Science and Technology, Republic of South Africa / National Research Foundation. The parent PCV1+1 study was funded by the Bill & Melinda Gates Foundation (grant OPP1152352).

Chapter 3 Safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected children: a systematic review and meta-analysis

3.1 Abstract

Background: HIV-infected and HIV-exposed uninfected (HEU) children have an increased risk of measles that may be due to altered immune responses or suboptimal timing of measles vaccination. We aimed to evaluate the safety and immunogenicity of measles vaccination in HIV-infected and HEU children.

Methods: For this systematic review and meta-analysis, we searched PubMed, Embase, Cochrane Library, CINAHL, Global Health Library and IndMED on May 9, 2018. Studies were included if they reported on safety or seroresponse (either seroprotection/seropositivity/seroconversion) after measles vaccination in HIV-infected or HEU children. We calculated pooled estimates to compare immunogenicity outcomes between HIV-infected, HEU and HIV-unexposed children, using risk ratios [RRs] (with 95% CIs). PROSPERO registration number: CRD42017057411.

Findings: Seventy-one studies met the inclusion criteria (15,363 children). Twenty-eight studies reported on safety; vaccine-associated adverse events and deaths were uncommon. Sixty-two studies reported on immunogenicity, 27 were included in the meta-analysis. HIV-infected children had lower seroresponse rates after primary vaccination compared with HIV-unexposed (RR 0.74; 95% CI: 0.61-0.90, I^2=85.9%) and HEU children (0.78; 0.69-0.88, I^2=77.1%), which was mitigated by antiretroviral therapy and time interval between vaccination and serology. HEU and HIV-unexposed children had similar seroresponses. Vaccination at 6-months resulted in

similar proportions of HIV-infected children having seroresponse compared to HIV-unexposed (0.96; 0.77-1.19) and HEU children (1.00; 0.73-1.37, I^2=63.7%).

Interpretation: Primary measles vaccination at 6-months of age may provide protection against measles during early infancy in settings with high prevalence of maternal HIV-infection, however, further studies are needed to evaluate this strategy in HEU children and HIV-infected children receiving antiretroviral therapy.

3.2 Introduction

In 2015, an estimated 1.4 million births occurred in HIV-infected women, of which more than 95% lived in low- and middle-income countries (LMICs) (291). Increased implementation of Prevention of Mother-To-Child Transmission (PMTCT) programs has reduced vertical HIV transmission to around 1% in breastfeeding populations (292,293) and to less than 1% in non-breastfeeding populations in LMICs (294). As a result, a significant proportion of children born to HIV-infected mothers are HIV-exposed but uninfected (HEU). Recent studies showed that HEU children are at increased risk of morbidity and mortality compared to their HIV-unexposed peers (6,295–299), in particular from infectious diseases in the first 6-months of life (5,298,300–303). This increased susceptibility could be due to immune aberrations in HIV-exposed infants resulting from *in utero* exposure to HIV-virion particles or maternal antiretroviral treatment (18).

HIV-infected children have an increased risk of severe measles disease and complications compared to HIV-unexposed children (133,304,305). The increased susceptibility to developing measles during early infancy in HIV-exposed infants may be explained by lower levels of maternally acquired measles antibody than HIV-unexposed (129). Furthermore, HIV-infected, antiretroviral-naïve children have a

reduced serological response to primary measles vaccination and increased waning of immunity compared to HIV-uninfected and HEU children (114,123,124,306).

A previous systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected children undertaken by Scott et al. included studies up to February 2009 (71). Since then, the number of HEU children has increased globally and universal antiretroviral treatment for HIV-infected children is now recommended. Understanding the effects of HIV-infection and HIV-exposure on the immune response to measles vaccination is crucial for determining dosing schedules of immunization programs, especially in LMICs with a high burden of HIV.

This systematic review evaluated the safety and immunogenicity of measles vaccine in HIV-infected and HEU children and compared immunogenicity outcomes taking age at vaccination and number of doses received into consideration.

3.3 Methods

Search strategy and selection criteria

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (307).

We searched PubMed, Embase, Cochrane Library, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Global Health Library (including African Index Medicus, Latin American and Caribbean Health Sciences), and IndMED on 9 May 2018, for articles containing (“measles” and “vaccine”) and “HIV”. Additional studies were identified by searching reference lists of the articles included in full-text screening and ClinicalTrials.gov.

Studies were eligible for inclusion in the systematic review if they reported on immunogenicity or safety of any measles vaccination strategy in HIV-infected or HEU children aged 0-18 years. For inclusion in the immunogenicity meta-analysis, studies needed to report on primary or booster vaccination and had to include a comparator group of either HIV-uninfected children (HEU/HIV-unexposed) or HIV-infected children on a different antiretroviral therapy (ART) regimen. No restrictions regarding geographical region or year of publication were applied. Eligible study designs were interventional or observational. For assessment of safety, case reports were also included. Animal studies, systematic reviews, narrative reviews, reports of proceedings and publications not written in English, French, German, Spanish, Portuguese or Dutch were excluded.

The outcomes of interest were immunogenicity and safety. Immunogenicity: studies were included if data were reported as proportions of subjects with seroprotective (≥330mIU/mL or as indicated by authors), seropositive (as defined by authors), or seroconversion (4-fold rise in titer or change from seronegative to seropositive) measles antibody responses. A composite outcome named seroresponse was created using either i) seroprotection rates post-vaccination, and if not available, ii) seropositivity or iii) seroconversion rates. Safety: all reported safety outcomes post-vaccination were considered, including deaths, severe adverse events (SAEs) other than death and adverse events (AEs).

Two independent reviewers (EM, MvR) screened titles and abstracts of identified studies. Articles were retained if they met the inclusion criteria according to one or both of the reviewers. In case of duplicate publications of the same results, the most complete reference was included.

Data analysis

Data were extracted from manuscripts using a standardised data extraction form and authors were contacted in case of missing data. Data of interest included: study design, study population, vaccine type, age at vaccination, time-period between vaccination and measurement of the serological response, number of vaccine doses administered, use of ART, outcome measures, laboratory methods used to detect measles antibodies, serological cutoff values, proportions with seroresponse, and number and type of (S)AEs.

Standardised data extraction form:

Study characteristics:

	Study ID (author, year)	Date extraction	Name of researcher performing extraction	Year of publication	Study year (start)	Country	Study setting	Primary study	Publication type	Study design	HIV-exposed/infected	Comparison group	Study population	Groups examined
1														
2														
3														
4														

	Study ID (author, year)	Name of vaccine	Informed consent obtained	Ethical approval obtained	Potential references from ref list	Potential references from ref list	Potential references from ref list	Immunogenicity outcome measures; I0 not reported, I1 seropositivity after vaccination reported, I2 seroconversion (seronegative before vacc, seropos after vacc), I3 seroconversion (4-fold rise in titre), I4 might be either seropositivity, seroconversion or seroprotection, I5 summary measure GMT, I6 Seroprotection	Safety outcome measures; S0 no adverse event information reported, S1 explicit reporting on adverse events, S2 serious adverse events S3 information on deaths reported	Measles outcomes: M0 no info on occurrence of clinical measles or unclear when or which group, M1 reports explicitialy on clinical measles after vacccination and numbers given per group	Information on progression of HIV-related disease reported P1
1													
2													
3													
4													

Baseline characteristics:

	Study ID	Age at last vaccination	Age at MV1 (months)	Stratified age MV1	Age at MV2 (months)	Stratified age MV2	Period between vaccine and serology (months) HIV infected	Period between vaccine and serology (months) HIV-uninfected	Period between vaccine and safety assessment (months)	Number of doses of measles vaccine given prior to entry
1										
2										
3										
4										

	Study ID	Number of HIV-exposed uninfected (HEU)	number of HIV-infected (HI)	number of HIV-unexposed uninfected	Did HIV status change during study period?	If yes, were participants included in the final analysis?	Age (median in years) of participant	Gender (% female)	Ethnicity (%)	ART use	CD4 count performed?	CD4 count of HIV infected	Variance, IQR or range of CD4 count
1													
2													
3													
4													

Immunogenicity:

	Study ID	Laboratory assay used for measurement of immune response	Number of vaccinations	Blood draw for serology < 6 months after vaccination	HAART	Cut-off seropositivity	Unclear if seropositive prior to vaccination are exluded	Cut-off seroprotection	Cut-off seroconversion	Proportion seropositive HEU (n)	Total	Proportion seropositive HIV-infected (n)	Total	Proportion seropositive HUU (n)	Total
1															
2															
3															
4															

	Study ID	Proportion seroprotected HEU (n)	Total	Proportion seroprotected HIV-infected (n)	Total	Proportion seroprotected HUU (n)	Total	Proportion seroconverted HEU (n)	Total	Proportion seroconverted HIV-infected (n)	Total	Proportion seroconverted HUU (n)	Total
1													
2													
3													
4													

Safety:

	Study ID	Safety reported	Comments	Number of AEs total	Number of SAEs total	Time observed for SAEs	Number of grade 1 or more AEs HEU	Total HIV-exposed	Number of grade 1 or more AEs HIV-infected	Total HIV-infected	Number of grade 1 or more AEs HUU	Total HUU	Number of SAEs HEU	Total HIV-exposed	Number of SAEs HIV-infected	Total HIV-infected	Number of SAEs HUU	Total HUU
1																		
2																		
3																		
4																		

	Study ID	Vaccine related SAE in HEU	Vaccine related SAE in HIV-infected	Vaccine related SAE in HIV-uninfected	Type of SAEs HEU	Type of SAEs HIV-infected	Type of SAEs HUU	Duration mild AEs HEU	duration mild AEs HIV-infected	duration mild AEs HUU	Duration SAEs HEU	Duration SAEs HIV-infected	Duration SAEs HUU	Post-vaccination deaths in HIV-infected children	Vaccine related potentially life-threatening events or deaths	Time observed for deaths
1																
2																
3																
4																

The Cochrane Risk of Bias Tool was adapted to enable evaluation of observational studies (308). For five categories, risk of bias was assessed as low (=0), unclear (=1), or high (=2). Studies with a high summative risk of bias score (≥7) were excluded from meta-analysis.

When multiple time-points were reported for immune responses after the same vaccine dose, the time-point closest to vaccination was reported, except for two studies that had a smaller sample size at the earlier time-point (309,310). For the descriptive analyses, point estimates of the proportion of seroresponders for the individual studies under each group were calculated with 95% confidence intervals (CIs) assuming an exact binomial distribution.

Three different primary meta-analyses compared serological responses in HIV-infected vs. HIV-unexposed, HIV-infected vs. HEU and HEU vs. HIV-unexposed children using risk ratios (RRs) and 95% CIs stratified by vaccination dose and age at vaccination. In case of significant heterogeneity (I^2>50%), a random-effects model was applied. To explore statistical variation and heterogeneity between trials, pre-specified subgroup analyses were performed based on outcome (seroprotection), serological test, use of ART, study design, age at vaccination and time interval

between vaccination and measurement of the serological response. Meta-regression was used to explore between-study variance not explained by the covariates and risk of publication bias was assessed using normal and contour-enhanced funnel plots if ten or more articles were included in the meta-analysis. Small study effects were evaluated using Egger's-test for asymmetry.

We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for rating overall quality of evidence (311). All analyses were performed using Stata, version 13 (StataCorpLP, Texas, USA). The study was prospectively registered in PROSPERO (CRD42017057411) (312).

3.4 Results

We identified 897 unique articles (Figure 3.1). Seventy-one studies fulfilled the eligibility criteria (Table 3.1). Twenty-eight studies reported on safety and 62 reported on immunogenicity of which 27 were included in the primary meta-analyses (Table 3.2).

Included study designs were randomized controlled trial (RCT) (n=1) (155,313), cohort (n=35), cross-sectional (n=30), case reports (n=2) (314,315), retrospective audits (n=1) (316); two studies had an unclear study design (317,318). Studies were published from 1987 through 2018 and were conducted in Africa (n=28), USA (n=16), Europe (n=17), South America (n=5) and Asia (n=5).

Taking all studies together, 15 363 children vaccinated against measles were evaluated, of which 4867 were HIV-infected, 2733 were HEU, and 7763 were HIV-unexposed.

Figure 3.1 Flow chart of study selection

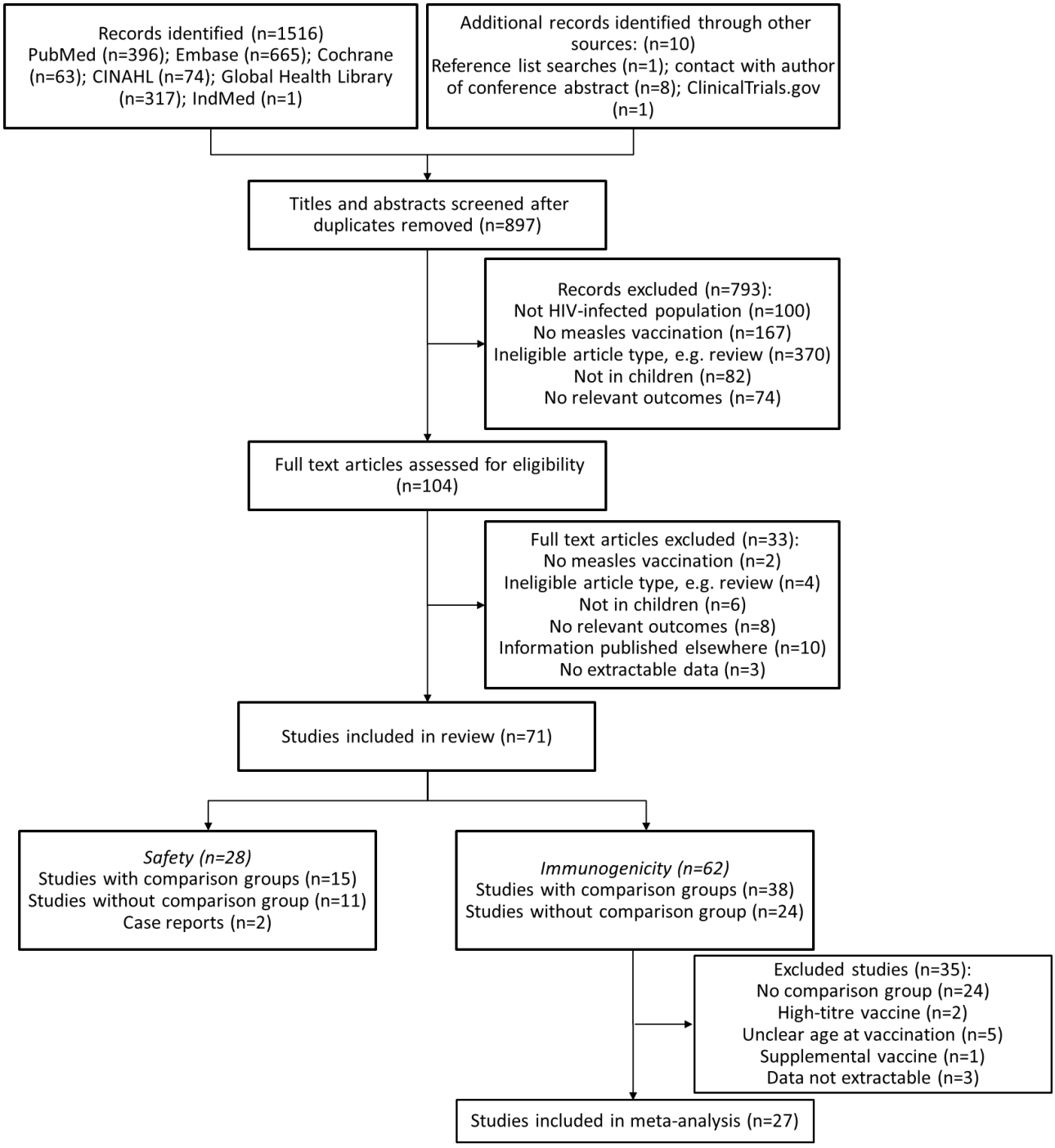


Table 3.1 Characteristics of included studies by study design

Randomized controlled trial and cohort studies												
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**
Abzug 2012 (319)	USA	Prospective cohort	2001	HIV-infected children 2 to <19 yr on HAART, HIV loads <30 000 copies/mL, CD4% ≥15, and ≥1 prior MMR	HI	Strain NR, MMR	2-19 yr (median 9.8 yr)	I1, I3, I5, I6, S2, S3	193	0	0	8 wks - 1.67 yr; revacc approx 7-28 days
Aurpibul 2007 (320)	Thailand	Prospective cohort	2005	Perinatally HIV-infected >5 yr, nadir CD4 <15%, immune recovery>15%, for at least 3 mo on HAART, measles seronegative	HI	Schwarz, MMR	Assume at age tested negative in previous study 9.9 yr ± 2.7	I5, I6, S1, S2	51	0	0	Approx 4-24 wks
Bekker 2006 (321)	Nether-lands	Prospective cohort	1997	HIV-1 infected, <18 yr, on HAART	HI	Strain NR, MMR	MV1 (n=3) at 14 mo, MV2 (n=15) at median 7.3 yr	I4, S0	59 (18 revacc)	0	0	median 48 wks (IQR 19-93 wks) (revacc)
Cagigi 2014 (322)	Italy	Prospective cohort	2007	Vertically HIV-infected on HAART at children's hospital	HI	Schwarz, MMR	NR	I6, S0	32	0	0	Approximately 2.5 yr
Chandwani 2011 (& Chandwani 1998) (155,313)	USA	Randomized controlled trial	1996	Children born to HIV-infected mothers	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx 12 mo	I5, I6, S1, S2, S3	15	95	0	0 - approx 2.5 yr
Cutts 1993 (323)	Zaire	Prospective cohort	1989	Infants born to HIV-infected mothers and non-HIV-infected mothers, perinatal transmission study	HI, HEU, HU	High-titer Edmonston-Zagreb strain, monovalent	Approx 6 mo (median 27 wks)	I3, S1, S2, S3	34	153	102	Approx 3 mo
Dunn 1998 (324)	Europe	Prospective cohort	1985	Children born to HIV-infected mothers, European Collaborative study in 10 paediatric centres	HI, HEU	Strain NR, MMR	NR	I0, S2	17	unclear	0	NR
Farquhar 2009 (325)	Kenya	Prospective cohort	2004	Previously vaccinated HIV-1 infected children before HAART initiation	HI	Strain NR, preparation NR	<1 yr	I1, S1	90	0	0	Approx 2 wks or 1 mo post-revaccination
Fernandez -Ibieta 2007 (326)	Spain	Retrospectiv e cohort	1997	HIV-infected children at Pediatric Infectious Disease and Immunodeficiency Unit, 1.5-19.0 yr	HI	Strain NR, MMR	Unclear	I0, S1, S2 based on adverse event statement	68 (55 vacc)	0	0	NA
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**

Fowlkes 2011 (& Helfand 2008) (114,327)	Malawi	Prospective cohort	2000	Children attending 14-week routine immunization visit at health centre	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx 9 mo	I1, I6, S1, S2, S3	85	334	1327	Approx 3-15 mo
Fowlkes 2016 (309)	Malawi	Prospective cohort	2000	Children enrolled in measles study who had received sMV >3 mo after their routine 9-month MV	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx 20 mo	I1, I5, I6, S2, S3	22	464 (HU)	401	<3 mo, 3-6 mo, 6-9 mo or >9 mo
Hilgartner 2001 (328)	USA	Prospective cohort	1989	Children and adolescents with haemophilia	HI, HU	Strain NR, preparation NR	Min 6 mo	I5, S0	52 (24 revacc)	0	23 (10 revacc)	Approx 3-9 mo
Jain 2017 (329)	India	Prospective cohort	2012	HIV-exposed infants at pediatric ART centre	HI, HEU	Edmonston-Zagreb, monovalent	6-7 mo	I1, I2, S1, S2	6	33	0	Approx 8-12 wks
Kizito 2013 (82)	Uganda	Prospective cohort	2003	Pregnant women and their offspring	HI, HEU, HU	Edmonston-Zagreb/Schwarz, monovalent	Approx 9 mo	I6, S0	12	62	637	Approx 3 mo
Lepage 1992 (330)	Rwanda	Prospective cohort	1988	Children born to HIV-seropositive and seronegative mothers	HI, HEU, HU	High-dose Edmonston Zagreb, monovalent	median 0.51 yr (0.48-0.84 yr)	I1, I2, I5, I6, S1, S2, S3	43	135	194	Approx 3 mo
Marczynska 2001 (substudy) (331)	Poland	Prospective cohort	NR	Children revaccinated if measles seronegative	HI	Schwarz, MMR	5-6 yr	I0, S1, S2	9	0	0	NA
McLaughlin 1988 (332)	USA	Retrospective cohort	1985	Children <12 yr, vaccinated before HIV diagnosis	HI	Strain NR, monovalent or MMR	Unclear	I0, S1, S2, S3	70	0	0	NA
Melvin 2003 (333)	USA	Retrospective cohort	NR	Perinatally infected children, primary immunization series before HAART initiation, revaccination on HAART	HI	Edmonston strain, MMR	median 7 yr (range 3-14 yr)	I4, S0	18	0	0	Approx 4 wks
Moss 2007 (115)	Zambia	Prospective cohort	2000	Children between 2-8 mo seeking routine childhood vaccinations	HI, HEU, HU	Edmonston-Zagreb, preparation NR	Approx 9 mo	I1, I3, I5, S1, S2, S2	66	258	117	Approx 1-6 mo
Nair 2009 (334)	Zambia	Prospective cohort	2000	Children in measles vaccine study and children hospitalized for measles	HI, HU	Edmonston-Zagreb, preparation NR	Approx 9 mo	I5, S0	15	29 (HU)	0	Approx 3 mo
Nduati 2016 (& Nduati 2012) (335,336)	Kenya	Prospective cohort	2009	Children at comprehensive care and research clinic	HEU, HU	Strain NR, preparation NR	Approx 9 mo	I5, I6, S0	0	55	48	Approx 9, 12 or 15 mo

Study (year)	Country	Type of study	Start study	Population	Groups	Vaccine used	Age at last vaccination	Outcomes reported	number of HI	number of HEU	number of HU	Interval between vaccination and serological testing
Newman 2017 (& Newman 2015) (337,338)	Kenya	Prospective cohort	2011	HIV-infected children 15 mo-12 yr	HI	Strain NR, preparation NR	Approx 9 mo	I1, S0	232	0	0	Approx 1, 12, 24 mo
Oldakowska 2001 (339)	Poland	Prospective cohort	NR	HIV-infected children at pediatric infectious disease clinic	HI	Strain NR, MMR	NR	I4, S1	13	0	0	Approx 3 mo
Oldakowska 2008 (340)	Poland	Prospective cohort	2001	Vertically HIV-infected children at pediatric infectious disease clinic	HI	Strain NR, preparation NR	NR	I4, S0	45	0	0	unclear
Omenda 2015 (341)	Kenya	Prospective cohort	NR	HEU, HU/malaria-exposed and HU/malaria-unexposed children 0-21 mo	HEU, HU	Strain NR, preparation NR	NR	I0, S0	0	13	43 (25 malaria-exp;18 malaria-unexp)	NR
Oxtoby 1989 (342)	Zaire	Prospective cohort	NR	Children born to HIV-infected and HIV-uninfected mothers	HI, HEU, HU	Strain NR, preparation NR	Approx 9 mo	I2, S1, S2, S3	37	157 (385 exposed vacc)	224 (569 vacc)	Approx 12 mo
Palumbo 1992 (& Hoyt 1992) (343,344)	USA	Prospective cohort and retrospective case finding	1990	Children approx 1-10 yr	HI	Edmonston strain, MMR	Unclear	I2, S1, S2, S3	127 (92 vacc)	0	0	Approx 4 wks
Rainwater-Lovett 2013 (116)	Zambia	Prospective cohort	2008	HIV-infected children 9-60 mo with documented history of MV and initiation of HAART	HI, HU (presumed)	Strain NR, preparation NR	Median 10 mo	I1, I2, S0	116	0	25	Median 11 mo
Reikie 2013 (34)	South Africa	Prospective cohort	2009	Children at academic hospital	HEU, HU	Strain NR, preparation NR	Approx 18 mo	I5, I6, S0	0	27	28	Approx 3, 9, 13 mo
Seth 2016 (345)	India	Prospective cohort	2011	Perinatally HIV-infected children 5-18 yr, ART>6 mo, CD4 count>15% at tertiary teaching hospital	HI	Edmonston-Zagreb, MMR	NR	I1, S1, S2	66	0	0	Approx 8-12 wks
Siberry 2015 (37)	USA	Prospective cohort	2007	Perinatally HIV-infected and HEU children aged 7-15 yr at 15 centres	HI, HEU	Edmonston-Zagreb, MMR	Median 4.32 yr (IQR 4.04-5.03 yr)	I6, S0	428	221	0	median 9.8 yr (IQR 6.9-12.1 yr)
Simani 2013 (39)	South Africa	Prospective cohort (archived serum samples)	2005	Children aged 6-12 wks	HI, HEU, HU	Schwarz, monovalent	Mean 67.8 wks ± 4.4	I1, I5, I6, S0	297	116	115	28 wks post MV1, 2 and 41 wks post MV2
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**

Succi 2018 (48)	Latin America	Prospective cohort	2002	Perinatally HIV-infected children and HEU children <4 yrs at 15 centres	HI, HEU	Strain NR, preparation NR	Approx 12 mo	I1, I5, S0	96	51	0	median 1037 days (2.8 yrs)
Sudfeld 2013 (346)	Tanzania	Prospective cohort	2005	Children enrolled in trial on multivitamin supplementation	HI, HEU	Edmonston-Zagreb, preparation NR	Approx 9 mo (8.5-12 mo)	I1, I5, S0	35	201	0	Approx 3-9.5 mo
Takano 2003 (347)	Brazil	Prospective cohort	NR	Children at paediatric HIV clinic	HI, HU	Strain NR, MMR	Min 1 yr	I1, I5, S0	70 (12 revacc)	0	69	Approx 1-3 mo
Thaithumyanon 2000 (123)	Thailand	Prospective cohort	NR	Children born to HIV-infected mothers	HI, HEU	Schwarz, monovalent	Approx 9 mo	I2, I5, S1, S2, S3	16	14	3	Approx 12 wks
Cross-sectional studies, prospective cohort studies/cross-sectional, retrospective cohort studies/cross-sectional												
Study	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported***	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serology**
al-Attar 1995 (348)	USA	Retrospective cohort / cross-sectional	1986	Children at HIV clinic, born to HIV-positive mothers or to mothers with high risk of being HIV-infected	HI, HEU	Strain NR, preparation NR	1.2-2.3 yr (median 1.3 yr)	I4, I5, S0	40	16 (HU)	0	1 mo - 6.7 yr (mean 1.6 yr)
Arpadi 1996 (& Arpadi 1992) (124,349)	USA	Cross-sectional	1991	Perinatally HIV-infected children 9-168 mo	HI	strain NR, monovalent or MMR	Unclear	I1, S0	81	0	0	1-155 mo (median 6 mo)
Aurpibul 2006 (350)	Thailand	Cross-sectional	2005	Perinatally HIV-infected children >5 yr, nadir CD4% <15%, immune recovery>15% for at least 3 mo on HAART	HI	Strain NR, preparation NR	Unclear	I5, I6, S0	93	0	0	79.6 ±38.6 mo
Berkelhamer 2001 (351)	USA	Retrospective cohort / cross-sectional	1999	Perinatally HIV-infected <12 yr, untreated, non-HAART, or HAART-regimen receiving medical care at children's hospital	HI	Strain NR, MMR	mean 6 yr (3.9-11 yr)	I6, S0	28	0	0	HAART mean 2.3 mo (range 1-4 mo), non-HAART mean 3.7 mo (range 1-9 mo)
Brena 1993 (352)	USA	Retrospective cohort / cross-sectional	NR	HIV-infected children and children born to HIV-infected mothers who seroreverted to negative	HI, HEU	Strain NR, MMR	median 1.3 yr (1.2-3 yr)	I1, I5, S0	20	13	0	median 2 mo (range 1-42 mo)
Cardemil 2016a (353)	Namibia	Cross-sectional	2008	Pregnant women aged 15-49 yr at first ANC visit, not referred from other health facility	HI, HU	Strain NR, preparation NR	NR	I4, S0	4 (aged 15-19 yr)	0	332 (aged 15-19 yr)	NR
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**

Cardemil 2016b (353)	Namibia	Cross-sectional	2010	Pregnant women aged 15-49 yr at first ANC visit, not referred from other health facility	HI, HU	Strain NR, preparation NR	NR	I4, S0	24 (aged 15-19 yr)	335 (aged 15-19 yr)	NR	NR
Echeverria Lecuona 1996 (354)	Spain	Retrospective cohort / cross-sectional	NR	Children born to HIV-infected mothers at study hospital	HI, HEU	Strain NR, MMR	Approx 12 mo	I1, S1, S2 based on adverse event statement	14 (10 vacc)	30	0	Approx 1-2 yr
Fitter 2013 (355)	Haiti	Cross-sectional	2012	Pregnant women aged 15-39 yr in national antenatal HIV sentinel serosurvey	HI	Strain NR, preparation NR	Unclear	I1, I6, S0	184 (aged 15-19 yr)	0	0	NR
Frenkel 1994 (& Frenkel 1992) (356,357)	USA	Prospective cohort / cross-sectional	NR	HIV-infected symptomatic children with documented history of MMR	HI	Strain NR, MMR	Unclear	I4, S1, S2	10	0	0	median 13 mo (range 1-130 mo)
Lindgren-Alves 2001 (358)	Brazil	Retrospective cohort / cross-sectional	1995	Perinatally infected children at teaching hospital HIV-centre	HI, HU	Strain NR, preparation NR	Unclear	I4, I5, S0	21	0	29	Mean 29.4 mo ±31.9 mo
Lowther 2009 (359)	Zambia	Cross-sectional	2006	Children 9 mo-5 yr from randomly selected households	HI, HU	Strain NR, preparation NR	Approx 9 mo	I4, S0	54	0	796	Unclear
Lyamuya 1999 (360)	Tanzania	Cross-sectional	1994	Children 18 mo-5 yr attending mother and child health clinics	HI, HIV-uninfected	Schwarz, preparation NR	Approx 9 mo	I5, I6, S0	9	0	663	Mean 26.1 mo
Marczynska 2001 (331)	Poland	Retrospective cohort / cross-sectional	NR	HIV-infected children on HAART; matched controls	HI, HIV-uninfected	Schwarz, monovalent or MMR	Unclear	I1, S0	19	0	19	Mean 3.1 yr (range 3 mo-13 yr)
Molyneaux 1993 (361)	UK	Retrospective cohort / cross-sectional	NR	Children in HIV perinatal transmission study aged >1 yr	HI, HEU	Strain NR, monovalent or MMR	Min 1 yr	I1, S1, S2	11 (9 vacc)	70 (61 vacc)	0	Approx 3-9 mo
Morris 2015 (362)	USA	Prospective cohort / cross-sectional	2011	Individuals with perinatally-acquired HIV aged 13-26 yr at HIV-clinic	HI	Strain NR, MMR	NR	I4, S0	34	0	0	non-immune median 13.5 mo (range 10.8-15 mo), immune: median 7.5 mo (range 6-9 mo)
Myers 2009 (363)	Switzerland	Prospective cohort / cross-sectional	NR	Children part of mother and child HIV cohort study	HI	Strain NR, MMR	Unclear	I4, S0	87	0	0	NR
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**

Ndikuyeze 1987 (364)	Rwanda	Cross-sectional	1985	Children 8-19 mo	HI	Strain NR, preparation NR	Approx 8-19 mo	I0, S1, S2	3	492 (HU)	0	NR
Newman 2014 (365)	Kenya	Cross-sectional	2011	HIV-infected children 15 mo-12 yr	HI	Strain NR, preparation NR	Approx 9 mo	I4, S0	232	0	0	NR
Oshitani 1996 (366)	Zambia	Cross-sectional	1993	Children 9-59 mo with measles in University teaching hospital	HI, HU	Biken-cam, preparation NR	Approx 9 mo	I0, S3	68 (37 vacc)	0	288 (111 vacc)	NR
Pensieroso 2009 (44)	Italy	Cross-sectional	2007	Vertically HIV-infected children at children's hospital; age matched healthy controls	HI, HU	Schwarz, MMR	NR	I2, I6, S0	70	0	50	Mean 4.7 yr
Polonsky 2015 (& Polonsky 2015) (367,368)	Malawi	Cross-sectional	2012	Individuals presenting for HIV testing at district hospital	HI, HU	Strain NR, preparation NR	NR	I4, I5, I6, S0	86 (aged 18 mo-17 yr)	0	55 (aged 18 mo - 17 yr)	NR
Rosso 2011 (369)	Italy	Retrospective cohort / cross-sectional	NR	Perinatally HIV-infected adolescents	HI	Strain NR, MMR	NR	I4, S0	39	0	0	NR
Rowson 2015 (370)	UK	Cross-sectional	NR	Previously vaccinated HIV-infected children	HI	Strain NR, preparation NR	NR	I4, S0	224	0	0	Approx 6-12 wks
Rudy 1994a (371)	USA	Unclear	1990	Children at immunology clinic, vaccinated < 12 mo	HI, HEU	Strain NR, monovalent	6-11 mo	I4, S1, S2	13	22	0	Approx 1-3 mo
Rudy 1994b (371)	USA	Unclear	1990	Children at immunology clinic, vaccinated ≥12 mo	HI, HEU	Strain NR, MMR	12-15 mo	I4, S1, S2	12	14	0	Approx 1.0-3.0 mo
Ruel 2008 (372)	Uganda	Cross-sectional	2005	HIV-infected children 1-10 yr at paediatric HIV clinic	HI	strain NR, preparation NR	NR	I0, S0, P1	300 (11 vacc and lab test)	0	0	NA
Singh 2013 (373)	UK	Retrospective cohort / cross-sectional	NR	HIV-infected children at paediatric HIV centre	HI	strain NR, MMR	NR	I4, S0	80	0	0	NR
Sticchi 2015 (374)	Italy	Cross-sectional	NR	Perinatally HIV-infected children and adults at regional reference hospital	HI	strain NR, MMR	NR	I4, S0	39	0	0	NR
Sutcliffe 2016 (375)	Zambia	Cross-sectional	2009	HIV-infected not on ART and HIV-infected youth receiving ART, HIV-uninfected youth (5-15 yr) in study of malaria and HIV	HI, HU	strain NR, preparation NR	Unclear	I4 (seroprevalence), S0	272	0	617	NR
Study (year)	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serological testing**

Tejiokem 2007 (45)	Cameroon, Central African Republic	Cross-sectional	2004	Children at 4 paediatric care centres between 18-36 mo	HI, HEU	strain NR, preparation NR	9 mo-1.3 yr	I1, I5, S0	51	78	0	Median 12.8 mo (90% range; 3.3-26.1 months)
Waibale 1999 (376)	Uganda	Retrospective cohort / cross-sectional	1995	Children at paediatric and HIV clinic	HI, HEU	Strain NR, monovalent	Median 9.4 mo (5.2 - 25.8 mo)	I1, I5, S0	50	193	0	Median 14.0 mo (2.7 - 30.8 mo)
Walter 1994 (310)	USA	Retrospective cohort / cross-sectional	1992	HIV-infected or born to HIV-infected mother at paediatric infectious disease clinic ≥15 mo	HI, HEU	Strain NR, MMR	Mean 20.4 month (±10.2 mo)	I4, I5, S0	35	49	0	mean 3.1, 13.3 and 43.6 mo
Other study designs												
Study	**Country**	**Type of study**	**Start study**	**Population**	**Groups**	**Vaccine used**	**Age at last vaccination**	**Outcomes reported**	**number of HI**	**number of HEU**	**number of HU**	**Interval between vaccination and serology**
Brunell 1995a (318)	USA	Unclear	1980	Perinatally HIV-infected infants; normal babies	HI, HU	Strain NR, MMR/MMRV	median 15 mo (range 8-26 mo)	I1, I5, S0	9	0	21	median 7 mo (range 2 - 29 mo)
Brunell 1995b (318)	USA	Unclear	1980	Children who received HIV-infected blood or blood products,1.5-9 yr; normal children	HI, HU	Strain NR, MMR	14-42 mo	I5, S0	17	0	49	median 29 mo (range 3 wks - 127 mo)
Dhesi 2012 (316)	UK	Retrospective audit	NR	HIV-positive children 4-16 yr at University hospital	HI	Strain NR, MMR	NR	I4, S0	19	0	0	NR
Embree 1989 (317)	Kenya	Unclear	NR	Perinatal HIV-transmission study	HI, HEU	Strain NR, preparation NR	Unclear	I4, S1, S2 based on adverse event statement	61	98	0	Unclear
Goon 2001 (314)	UK	Case report	NR	HIV-infected child	HI	Edmonston strain, monovalent	1.2 yr	I0, S1, S2, S3	1	0	0	NA
Ramon-Garcia 1995 (315)	Mexico	Case report	1989	HIV-infected children during measles outbreak	HI	Strain NR, preparation NR	Approx 12 mo	I0, S2, S3	2	0	0	NA

Abbreviations: ANC, antenatal care; exp, exposed; ART, antiretroviral therapy; HEU, HIV-exposed uninfected; HI, HIV-infected; HU, HIV-unexposed; HU, HIV-unexposed; mo, months of age; MV, measles vaccination; MMR, measles, mumps, rubella vaccine; MMRV, measles, mumps, rubella, varicella vaccine; NA, not applicable; NR, not reported; revacc, revaccinated; sMV, supplemental measles vaccination; unexp, unexposed; vacc, vaccinated; yr, years of age.

*I immunogenicity outcomes: I0, immunogenicity not reported; I1, seropositivity after vaccination reported; I2, seroconversion (seronegative before vaccination, seropositive after vaccination) reported; I3, seroconversion (4-fold rise in titer) reported; I4, measure which might be either seropositivity,

seroconversion or seroprotection after vaccination is reported; I5, summary immunological measure (e.g. geometric mean titer) reported; I6, seroprotection after vaccination reported;
*S Safety outcomes: S0, no adverse event information reported; S1, explicit reporting on adverse events; S2, explicit reporting on serious adverse events; S3, reporting on deaths.

Table 3.2 Characteristics and reported proportion seroprotected/seropositive/seroconverted in the studies that assessed immunogenicity after measles vaccination included in the primary meta-analyses

Author (year) Country	Study design (start year)	Groups	Vaccine used	Age at last vaccination	Outcomes reported*	Interval between vaccination and serology	Number and timing of MV	Serological assay and timing of serology	Serological cutoff	Events (n)/vaccinated HIV; proportion (95% CI)	Events (n)/vaccinated HEU; proportion (95% CI)	Events (n)/vaccinated HU; proportion (95% CI)
al-Attar (1995) USA (348)	Retrospective cohort / cross-sectional (1986)	HI, HEU (Vertically- and transfusion acquired)	Strain NR, preparation NR	1.2-2.3 yr (median 1.3 yr)	I4, I5, S0	1 mo - 6.7 yr (mean 1.6 yr)	Primary vaccine?	ELISA	Manufacturer definitions	25/40; 0.63 (0.46-0.77)	15/16; 0.94 (0.70-1.00)	
Brena (1993) USA (352)	Retrospective cohort / cross-sectional (NR)	HI, HEU	Strain NR, MMR	Median 1.3 yr (1.2-3.0 yr)	I1, I5, S0	Median 2 mo (range 1-42 mo)	Primary vaccine?	ELISA	≥20 EU/ml	11/20; 0.55 (0.32-0.77)	12/13; 0.92 (0.64-1.00)	
Brunell (1995a) USA (318)	Unclear (1980)	HI, HU	Strain NR, MMR/MMRV	Median 15 mo (range 8-26 mo)	I1, I5, S0	Median 7 mo (range 2 - 29 mo)	Primary vaccine	ELISA	OD>42	7/9; 0.78 (0.40-0.97)		21/21; 1.00 (0.84-1.00)
Chandwani (2011) USA (155)	Randomized controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx 12 mo	I4, I5, S1, S2, S3	0 - approx 2.5 yr	6 mo vaccination	PRNT, b	≥120 mIU/ml	7/7; 1.00 (0.59-1.00)	49/61; 0.80 (0.68-0.89)	
Chandwani (2011) USA (155)	Randomized controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx 12 mo	I4, I5, S1, S2, S3	0 - approx 2.5 yr	12 mo vaccination only	PRNT, b	≥120 mIU/ml	7/7; 1.00 (0.59-1.00)	22/22; 1.00 (0.85-1.00)	
Chandwani (2011) USA (155)	Randomized controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx 12 mo	I4, I5, S1, S2, S3	0 - approx 2.5 yr	6&12 mo vaccination	PRNT, b	≥120 mIU/ml	5/6; 0.83 (0.36-1.00)	55/56; 0.98 (0.90-1.00)	
Echeverria (1996) Spain (354)	Retrospective cohort / cross-sectional (NR)	HI, HEU	Strain NR, MMR	Approx 12 mo	I1, S1, S2 based on adverse event statement	Approx 1-2 yr	Primary vaccine	ELISA	>200 mIU/ml	5/8; 0.63 (0.24-0.91)	28/30; 0.93 (0.78-0.99)	
Embree (1989) Kenya (317)	Unclear (NR)	HI, HEU	Strain NR, preparation NR	Unclear	I4, S1, S2 based on adverse event statement	Unclear	Primary vaccine?	Unclear	Protective antibody	7/8; 0.88 (0.47-1.00)	10/15; 0.67 (0.38-0.88)	
Fowlkes (2011) Malawi (327)	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx 9 mo	I1, I6, S1, S2, S3	Approx 3-15 mo	6 mo first dose, 9 mo serology	ELISA, b	Package insert	36/61; 0.59 (0.46-0.71)	152/223; 0.68 (0.62-0.74)	288/467; 0.62 (0.57-0.66)

Author (year) Country	Study design (start year)	Groups	Vaccine used	Age at last vaccination	Outcomes reported*	Interval between vaccination and serology	Number and timing of MV	Serological assay and timing of serology	Serological cutoff	Events (n)/vaccinated HIV; proportion (95% CI)	Events (n)/vaccinated HEU; proportion (95% CI)	Events (n)/vaccinated HU; proportion (95% CI)
Fowlkes (2011) Malawi (327)	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx 9 mo	I1, I6, S1, S2, S3	Approx 3-15 mo	9 mo 2nd dose, 12 mo serology	ELISA, b	Package insert	29/45; 0.64 (0.49-0.78)	189/202; 0.94 (0.89-0.97)	385/417; 0.92 (0.89-0.95)
Jain (2017) India (329)	Prospective cohort (2012)	HI, HEU, a	Edmonston-Zagreb, monovalent	Approx 6 mo	I1, I2, S1, S2	Approx 2-3 mo	Primary vaccine	ELISA, b	Package insert	2/6; 0.33 (0.04-0.78)	13/33; 0.39 (0.23-0.58)	
Kizito (2013) Uganda (82)	Prospective cohort (2003)	HI, HEU, HU, a?	Edmonston-Zagreb/Schwarz, monovalent	Approx 9 mo	I6, S0	Approx 3 mo	Primary vaccine	ELISA, b	≥200 mIU/ml	4/12; 0.33 (0.10-0.65)	44/62; 0.71 (0.58-0.82)	482/637; 0.76 (0.72-0.79)
Lindgren-Alves (2001) Brazil (358)	Retrospective cohort / cross-sectional (1995)	HI, HU	Strain NR, preparation NR	Unclear	I4, I5, S0	Mean 29.4 mo ±31.9 mo	Revaccination	PRNT	>50 mIU/ml	12/21; 0.57 (0.34-0.78)		29/29; 1.00 (0.88-1.00)
Lyamuya (1999) Tanzania (360)	Cross-sectional (1994)	HI, HU, a?	Schwarz, preparation NR	Approx 9 mo	I5, I6, S0	Mean 26.1 mo	Primary vaccine	ELISA	≥200 mIU/ml	6/9; 0.67 (0.30-0.93)		617/663; 0.93 (0.91-0.95)
Molyneaux (1993) UK (361)	Retrospective cohort / cross-sectional (NR)	HI, HEU	Strain NR, monovalent or MMR	Min 1 yr	I1, S1, S2	Approx 3-9 mo	Primary vaccine?	ELISA	Any detectable antibody	9/9; 1.00 (0.66-1.00)	61/61; 1.00 (0.94-1.00)	
Moss (2007) Zambia (115)	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, preparation NR	Approx 9 mo	I1, I3, I5, S1, S2, S2	Approx 1-6 mo	Primary vaccine, 6 months post-vaccination, HIV+ at vaccination	PRNT, b	≥120 mIU/ml	44/50; 0.88 (0.76-0.95)	198/211; 0.94 (0.90-0.97)	92/98; 0.94 (0.87-0.98)
Moss (2007) Zambia (115)	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, preparation NR	Approx 10-27 mo	I1, I3, I5, S1, S2, S2	Approx 3-4 mo	Revaccination, 10-27 months	PRNT, b	≥120 mIU/ml	12/13; 0.92 (0.64-1.00)		111/115; 0.97 (0.91-0.99)
Nduati (2016) Kenya (335)	Prospective cohort (2009)	HEU, HU, a	Strain NR, preparation NR	Approx 9 mo	I5, I6, S0	Approx 9, 12 or 15 mo	Primary vaccine, 18 mo	ELISA	≥200 mIU/ml		39/47; 0.83 (0.69-0.92)	19/20; 0.95 (0.75-1.00)
Nduati (2016) Kenya (335)	Prospective cohort (2009)	HEU, HU, a	Strain NR, preparation NR	NR	I5, I6, S0	Approx 9, 12 or 15 mo	Primary vaccine?, >18 mo	ELISA	≥200 mIU/ml		8/8; 1.00 (0.63-1.00)	26/28; 0.93 (0.76-0.99)
Oxtoby (1989) Zaire (342)	Prospective cohort (NR)	HI, HEU, HU	Strain NR, preparation NR	Approx 9 mo	I2, S1, S2, S3	Approx 12 mo	Primary vaccine	Unclear	Seronegative to Seropositive	24/37; 0.65 (0.47-0.80)	140/157; 0.89 (0.83-0.94)	199/224; 0.89 (0.84-0.93)
Pensieroso (2009) Italy (44)	Cross-sectional (NR)	HI, HU, a	Schwarz, MMR	Approx 13-15 mo	I2, I6, S0	Mean 4.7 yr	Primary vaccine	ELISA	≥200 mIU/ml	33/70; 0.47 (0.35-0.59)		50/50; 1.00 (0.93-1.00)

Author (year) Country	Study design (start year)	Groups	Vaccine used	Age at last vaccination	Outcomes reported*	Interval between vaccination and serology	Number and timing of MV	Serological assay and timing of serology	Serological cutoff	Events (n)/vaccinated HIV; proportion (95% CI)	Events (n)/vaccinated HEU; proportion (95% CI)	Events (n)/vaccinated HU; proportion (95% CI)
Rainwater-Lovett (2013) Zambia (116)	Prospective cohort (2008)	HI, HU (presumed), a	Strain NR, preparation NR	Median 10 mo	I1, I2, S0	Median 11 mo	Primary vaccine	ELISA	>120 mIU/ml	46/116; 0.40 (0.31-0.49)		9/12; 0.75 (0.43-0.95)
Rainwater-Lovett (2013) Zambia (116)	Prospective cohort (2008)	HI, HU (presumed), a	Strain NR, preparation NR	Median 10 mo	I1, I2, S0	Median 11.0 mo	Revaccination	ELISA	>120 mIU/ml	18/19; 0.95 (0.74-1.00)		13/13; 1.00 (0.75-1.00)
Reikie (2013) South Africa (34)	Prospective cohort (2009)	HEU, HU	Strain NR, preparation NR	Approx 18 mo	I5, I6, S0	Approx 3, 9, 13 mo	Primary vaccine, 12 mo serology	ELISA, b	≥330 mIU/ml		22/27; 0.81 (0.62-0.94)	20/28; 0.71 (0.51-0.87)
Reikie (2013) South Africa (34)	Prospective cohort (2009)	HEU, HU	Strain NR, preparation NR	Approx 18 mo	I5, I6, S0	Approx 3, 9, 13 mo	Two doses, 24 mo serology	ELISA	≥330 mIU/ml		19/27; 0.70 (0.50-0.86)	13/27; 0.48 (0.29-0.68)
Rudy (1994a) USA (371)	Unclear (1990)	HI, HEU	Strain NR, monovalent	6-11 mo	I4, S1, S2	Approx 1-3 mo	Primary vaccine, monovalent <12 mo	ELISA, b	Unclear	9/13; 0.69 (0.39-0.91)	17/22; 0.77 (0.55-0.92)	
Rudy (1994b) USA (371)	Unclear (1990)	HI, HEU	Strain NR, MMR	12-15 mo	I4, S1, S2	Approx 1-3 mo	Primary vaccine MMR ≥12 mo	ELISA, b	Unclear	6/12; 0.50 (0.21-0.79)	13/14; 0.93 (0.66-1.00)	
Siberry (2015) USA (37)	Prospective cohort (2007)	HI, HEU, a	Edmonston-Zagreb, MMR	Median 4.32 yr (IQR 4.04-5.03 yr)	I6, S0	Median 9.8 yr (IQR 6.9-12.1 yr)	Revaccination (for 2% primary vaccine)	PRNT	≥120 mIU/ml	244/428; 0.57 (0.52-0.62)	219/221; 0.99 (0.97-1.00)	
Simani (2013) South Africa (39)	Prospective cohort (archived serum samples) (2005)	HI, HEU, HU	Schwarz, monovalent	Mean 67.8 wks ± 4.4	I1, I5, I6, S0	28 wks post MV1	Primary vaccine, 28 wks post-primary, HIV groups combined	ELISA	≥330 mIU/ml	225/253; 0.89 (0.84-0.93)	110/116; 0.95 (0.89-0.98)	102/112; 0.91 (0.84-0.96)
Simani (2013) South Africa (39)	Prospective cohort (archived serum samples) (2005)	HI, HEU, HU, a	Schwarz, monovalent	Mean 67.8 wks ± 4.4	I1, I5, I6, S0	28 wks post MV1, 2 and 41 wks post MV2	Two doses, 2 wks post-booster, def-ART	ELISA, b	≥330 mIU/ml	235/248; 0.95 (0.91-0.97)	104/114; 0.91 (0.84-0.96)	111/115; 0.97 (0.91-0.99)

Author (year) Country	Study design (start year)	Groups	Vaccine used	Age at last vaccination	Outcomes reported*	Interval between vaccination and serology	Number and timing of MV	Serological assay and timing of serology	Serological cutoff	Events (n)/vaccinated HIV; proportion (95% CI)	Events (n)/vaccinated HEU; proportion (95% CI)	Events (n)/vaccinated HU; proportion (95% CI)
Succi (2018) Latin America and the Caribbean (48)	Prospective cohort	HI, HEU, a	Strain NR, preparation NR	Approx 1 yr	I1, I5, S0	Approx 2.8 yrs	Primary vaccine	ELISA	≥120 mIU/ml	77/96; 0.80 (0.71-0.88)	51/51; 1.00 (0.93-1.00)	
Sudfeld (2013) Tanzania (346)	Prospective cohort (2005)	HI, HEU, a?	Edmonston-Zagreb, preparation NR	Approx 9 mo (9-12 mo)	I1, I5, S0	Approx 3-10 mo	Primary vaccine	ELISA, b	≥200 mIU/ml	16/35; 0.46 (0.29-0.63)	138/201; 0.69 (0.62-0.75)	
Tejiokem (2007) Cameroon, Central African Republic (45)	Cross-sectional (2004)	HI, HEU, a	Strain NR, preparation NR	9 mo-1.3 yr	I1, I5, S0	Median 12.8 mo (90% range; 3.3-26.1 months)	Primary vaccine, commercial ELISA kit	ELISA, b	≥335 mIU/ml	7/46; 0.15 (0.06-0.29)	45/72; 0.63 (0.50-0.74)	
Tejiokem (2007) Cameroon, Central African Republic (45)	Cross-sectional (2004)	HI, HEU, a	Strain NR, preparation NR	9 mo-1.3 yr	I1, I5, S0	Median 12.8 mo (90% range; 3.3-26.1 months)	Revaccination, commercial ELISA kit	ELISA, b	≥335 mIU/ml	1/4; 0.25 (0.01-0.81)	3/5; 0.60 (0.15-0.95)	
Thaithumyanon (2000) Thailand (123)	Prospective cohort (NR)	HI, HEU	Schwarz, monovalent	Approx 9 mo	I2, I5, S1, S2, S3	Approx 12 wks	Primary vaccine	ELISA, b	>150 mIU/ml	8/14; 0.57 (0.29-0.82)	14/14; 1.00 (0.77-1.00)	
Waibale (1999) Uganda (376)	Retrospective cohort / cross-sectional (1995)	HI, HEU	Strain NR, monovalent	Median 9.4 mo (5.2-25.8 mo)	I1, I5, S0	Median 14 mo (2.7-30.8 mo)	Primary vaccine (99%)	ELISA	≥15 EU/ml	24/50; 0.48 (0.34-0.63)	122/193; 0.63 (0.56-0.70)	
Walter (1994) USA (310)	Retrospective cohort / cross-sectional (1992)	HI, HEU	Strain NR, MMR	Mean 20.4 month (±10.2 mo)	I4, I5, S0	Mean 13.3 mo	Unclear, mean 13.3m post-vaccination	ELISA	≥0.065 OD	14/20; 0.70 (0.46-0.88)	11/11; 1.00 (0.72-1.00)	

Abbreviations: HEU, HIV-exposed uninfected; HI, HIV-infected; HU, HIV-unexposed; ELISA, enzyme-linked immunosorbent assay; EU/mL, ELISA units per milliliter; mIU/mL, milli international units per milliliter; mo, months of age; MV, measles vaccination; MMR, measles, mumps, rubella vaccine; MMRV, measles, mumps, rubella, varicella vaccine; NA, not applicable; NR, not reported; OD, optical density; PRNT, plaque reduction neutralization test; sMV, supplemental measles vaccination; yr, years of age.

*I Immunogenicity outcomes: I0, immunogenicity not reported; I1, Seropositivity after vaccination reported; I2, seroconversion (seronegative before vaccination, Seropositive after vaccination) reported; I3, seroconversion (4-fold rise in titer) reported; I4, measure, which might be either Seropositivity,

seroconversion or seroprotection after vaccination, is reported; I5, summary immunological measure (e.g. geometric mean titer) reported; I6, seroprotection after vaccination reported;
*S Safety outcomes: S0, no adverse event information reported; S1, explicit reporting on adverse events; S2, explicit reporting on serious adverse events; S3, reporting on deaths;
a: studies where children received antiretroviral therapy;
a?: studies where it is not clear if children received antiretroviral therapy;
b: studies where blood was drawn for measles serology less than six months after vaccination;
Primary vaccine?: studies where it is not clear if the children received primary vaccination.

Thirty-five studies with comparison groups reported post-vaccination seroresponses in HIV-infected children, of which twelve administered ART (Figure 3.2). HIV-infected children showed similar seroresponse rates after primary vaccination at 6-months (pooled estimate 71%; 95% CI 55-88; n=5) compared to later time points: 9-months (60%; 95% CI 43-77; n=12), 12-months (84%; 95% CI 48-120; n=2) and >12-months of age (64%; 95% CI 51-76; n=7). The pooled point estimate of HIV-infected children with seroresponse after booster vaccination was similar when administered at ≤24-months (77%; 95% CI 58-96; n=5) or >24-months (61%; 95% CI 39-83; n=4). Two studies assessed the effect of different ART-regimens on the response to primary vaccination (39,48) and four studies to booster vaccination (39,44,322,351). Children receiving ART or early-ART within the first year of life had greater seroresponses to booster vaccination compared to those who received late-ART or did not receive ART (44,322,351).

HEU children receiving primary vaccination at 12-months (pooled estimate 98%; 95% CI 91-104; n=2) or >12-months of age (99%; 95% CI 96-102; n=5) tended to have better seroresponse rates compared with HEU children vaccinated at 6 (70%; 95% CI 58-83; n=5) or 9-months (84%; 95% CI 76-91; n=13) of age (Figure 3.3).

Similar to HEU children, a trend towards improved seroresponse was observed in HIV-unexposed children receiving primary vaccination at >12-months (pooled estimate 100%; 95% CI 97-103; n=2) compared to 6-months (66%; 95% CI 50-82; n=3) or 9-months of age (88%; 95% CI 82-94; n=9) (Figure 3.4).

Figure 3.2 Descriptive analysis of studies reporting seroresponses after measles vaccination in HIV-infected children by dose and age at vaccination

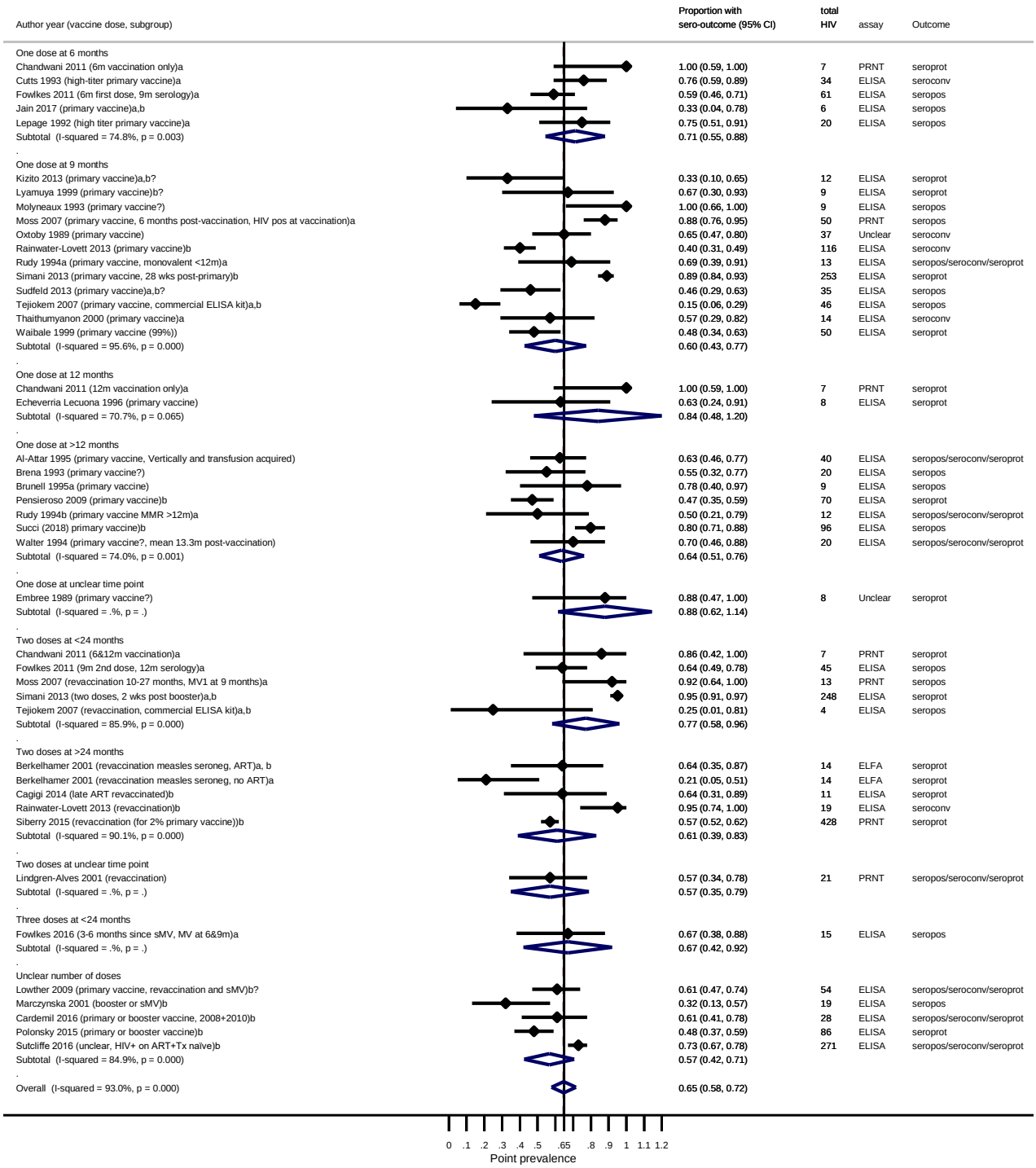


Figure 3.3 Descriptive analysis of studies reporting seroresponses after measles vaccination in HEU children by dose and age at vaccination

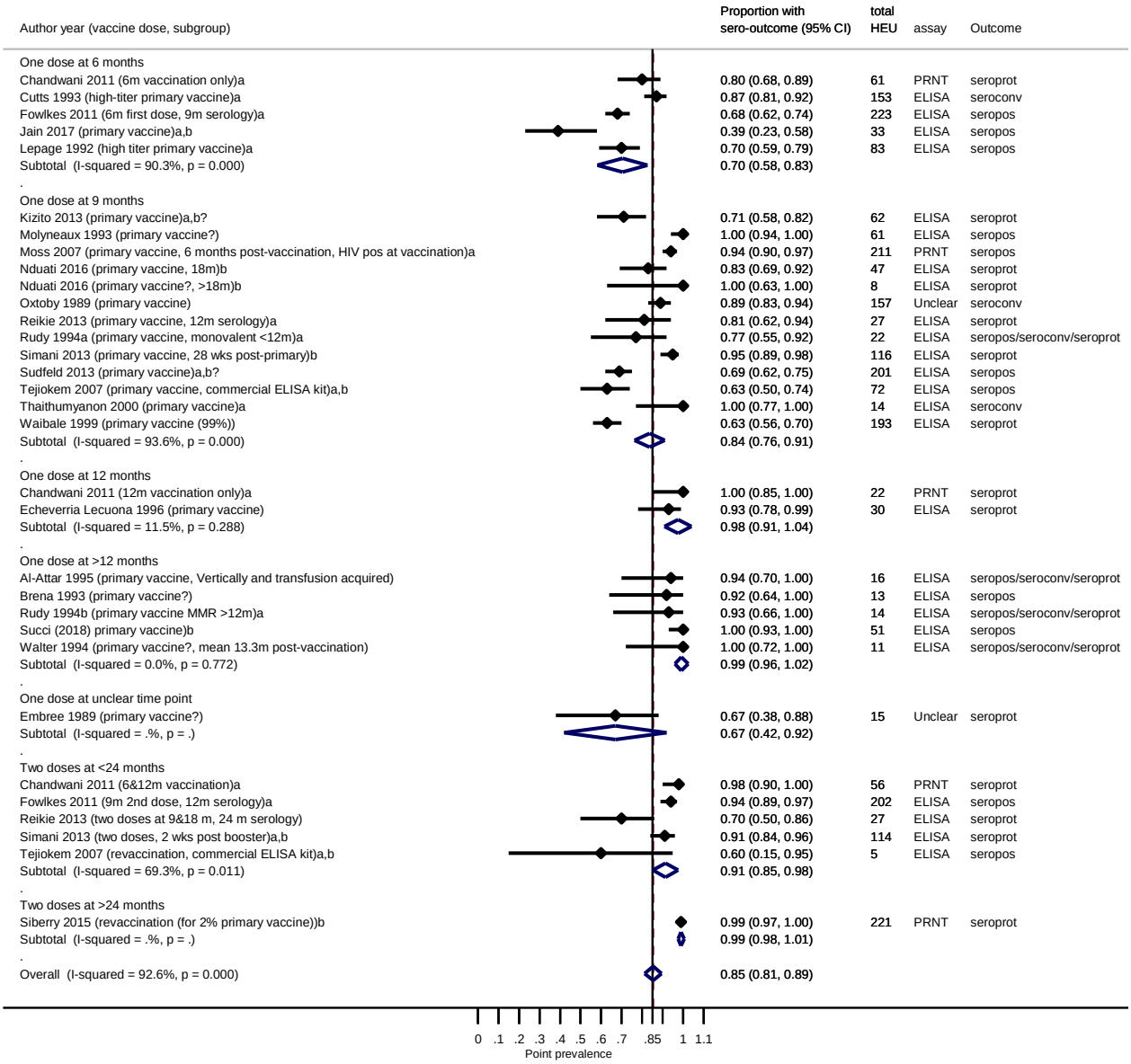
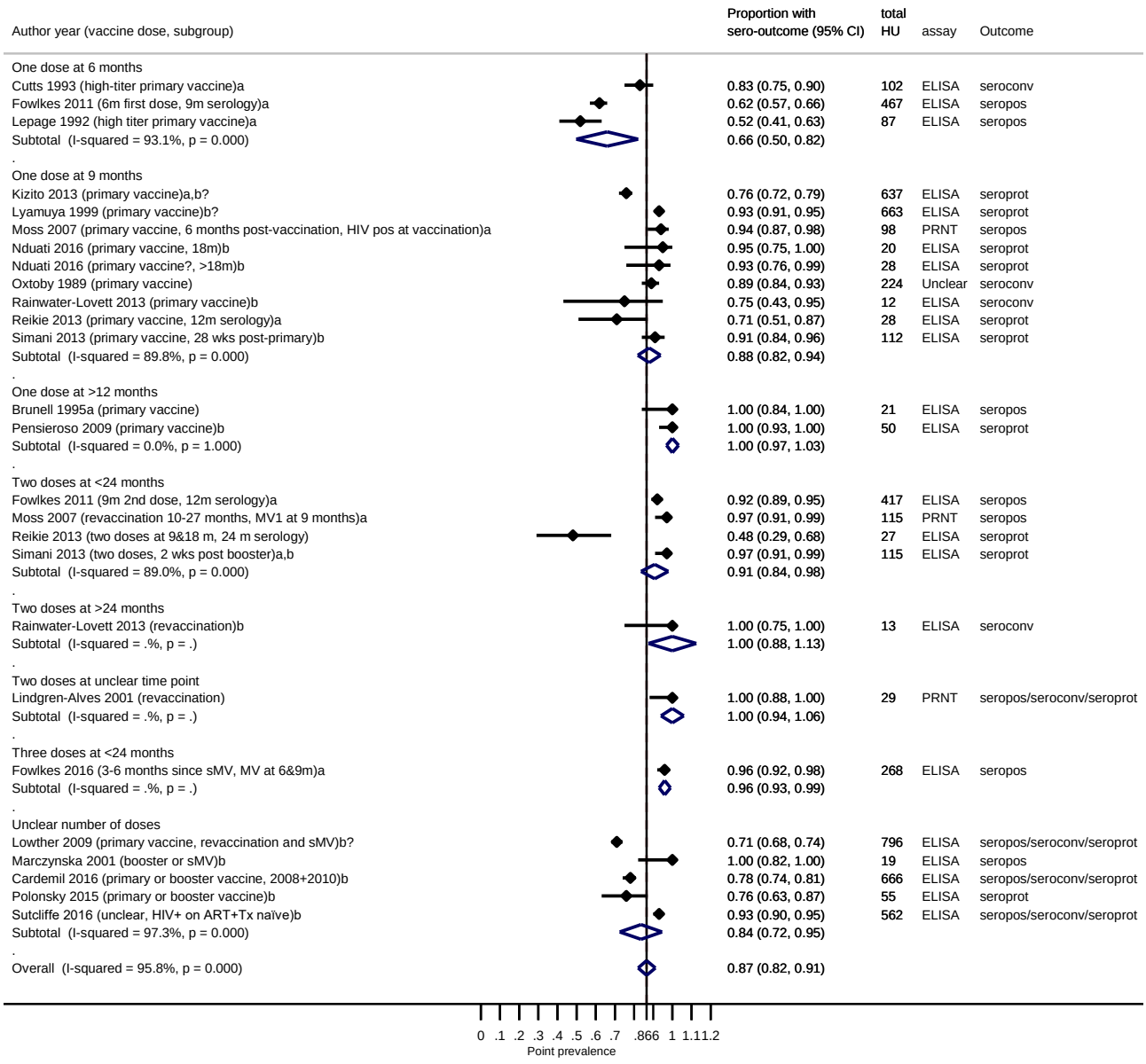


Figure 3.4 Descriptive analysis of studies reporting seroresponses after measles vaccination in HIV-unexposed children by dose and age at vaccination



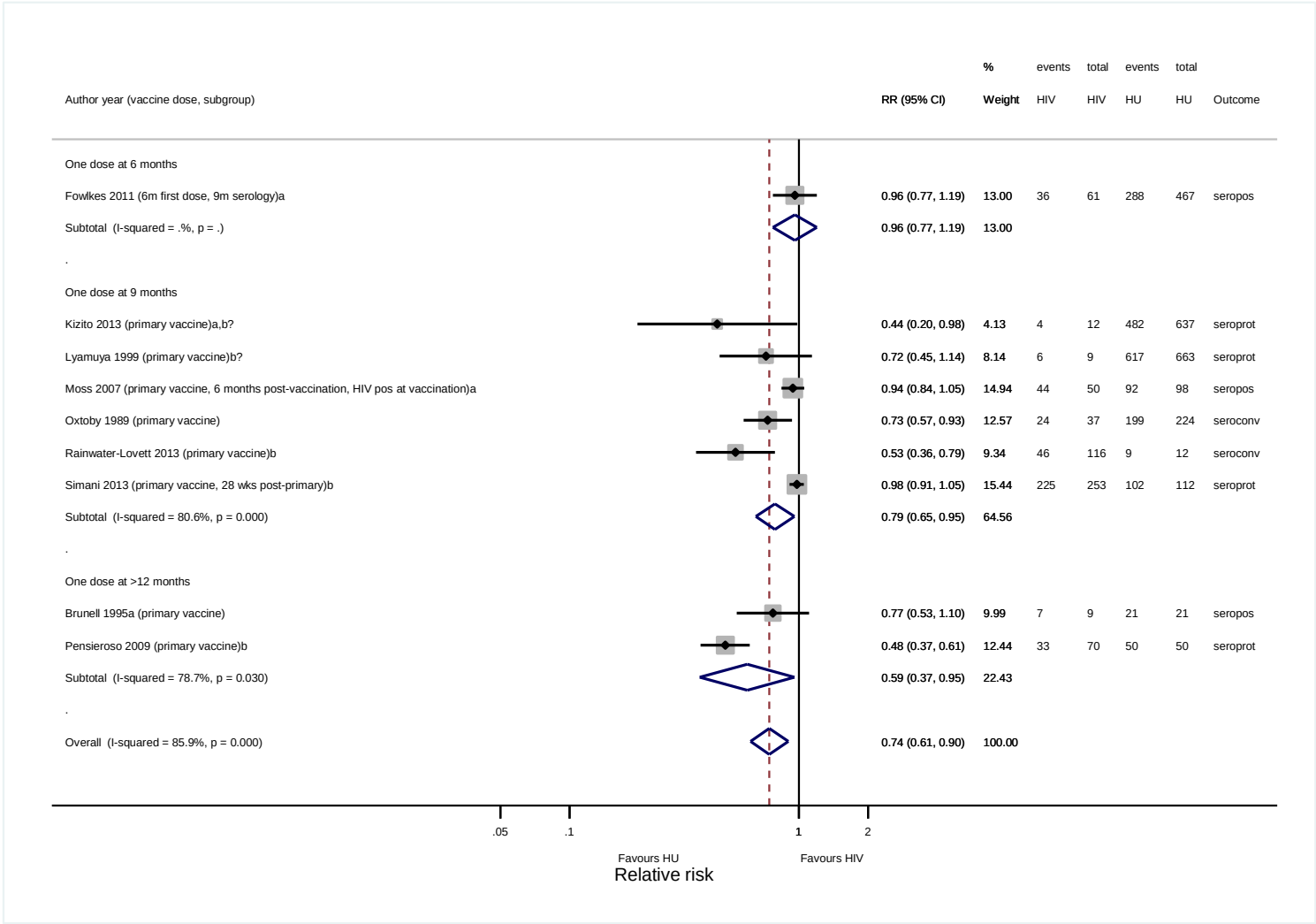
Abbreviations for Figures 3.2 – 3.4: ART, antiretroviral therapy; ELISA, enzyme-linked immunosorbent assay; HEU, HIV-exposed uninfected; HU, HIV-unexposed; MV, measles vaccination; MMR, measles, mumps, rubella vaccine; PRNT, plaque reduction neutralisation test; sMV, supplemental measles vaccination.
a: studies where blood was drawn for measles serology within six months after vaccination;
b: studies where children received antiretroviral therapy;
b?: studies where it is not clear if children received antiretroviral therapy.

Nine publications were included in the primary meta-analysis comparing immune responses after primary vaccination in HIV-infected and HIV-unexposed children (39,44,82,115,116,318,327,342,360). Relative risks for all studies were <1, although only significant in four studies (44,82,116,342). ART was administered in two of four studies with a significant RR (44,116), compared with one of five studies that did not find a significant difference (39). The pooled RR resulting from the random-effects model was 0.74 (95% CI 0.61-0.90; I^2=85.9%) (Figure 3.5a). Seroresponses after primary vaccination at 9-months (RR=0.79; 95% CI 0.65-0.95) and >12-months of age (RR=0.59; 95% CI 0.37-0.95) were significantly lower in HIV-infected compared with HIV-unexposed children, but not when vaccinated at 6-months (RR=0.96; 95% CI 0.77-1.19; n=1). Limiting analysis to studies that reported seroprotection (RR=0.64; 95% CI 0.36-1.14; n=4), administered ART (RR=0.63; 95% CI 0.34-1.19; n=3), or measured serology within 3 (RR=0.71; 95% CI 0.33-1.55; n=2) or 6-months post-vaccination (RR=0.90; 95% CI 0.73-1.11; n=3), resulted in non-significant combined RRs (Table 3.3).

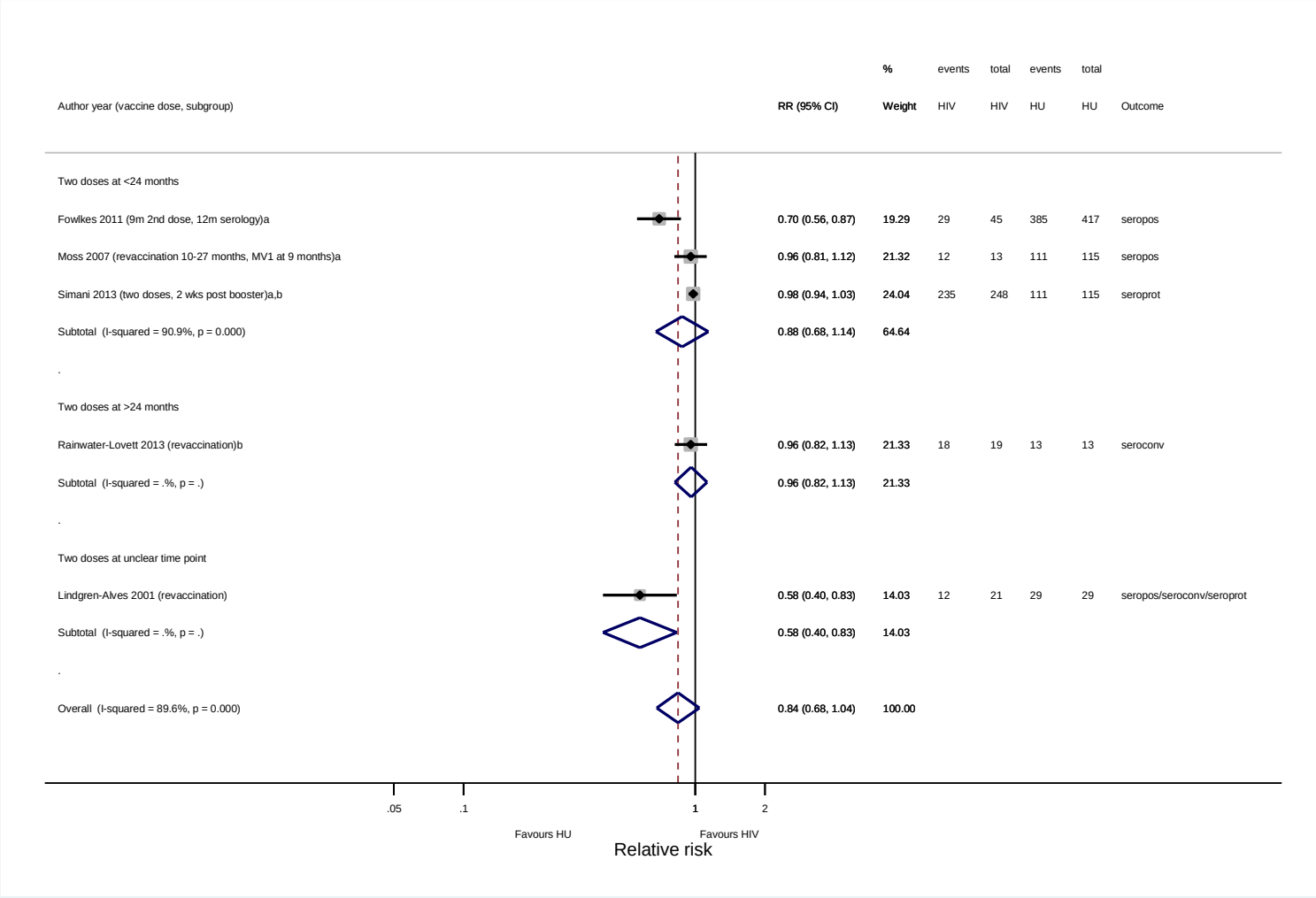
Meta-analysis in five studies comparing post-booster responses in HIV-infected and HIV-unexposed children found a pooled non-significant RR (0.84, 95% CI 0.68-1.04; I^2=89.6%) (Figure 3.5b), irrespective of subgroup analyses (Table 3.4) (39,115,116,327,358).

Figure 3.5 Forest plots for seroresponses comparing HIV-infected and HIV-unexposed children

(A) One dose of measles vaccine



(B) Two or more doses of measles vaccine



Abbreviations: ART, antiretroviral therapy; HU, HIV-unexposed; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seropos/seroconv/seroprot, might either be seropositivity, seroconversion or seroprotection; seroprot, seroprotection.
a: studies where blood was drawn for measles serology within six months after vaccination;
b: studies where children received antiretroviral therapy;
b?: studies where it is not clear if children received antiretroviral therapy.

Table 3.3 Subgroup analyses for immune response post-primary vaccination

Subgroup	HIV vs HIV-unexposed primary vaccination RR (95% CI)	HIV vs HEU primary vaccination RR (95% CI)	HEU vs HIV-unexposed primary vaccination RR (95% CI)
Outcome in main analysis	Overall: **0.74 (0.61-0.90)**; n=9 6 months: 0.96 (0.77-1.19) 9 months: **0.79 (0.65-0.95)** >12 months: **0.59 (0.37-0.95)**	Overall: **0.78 (0.69-0.88)**; n=21 6 months: 1.00 (0.73-1.37) 9 months: **0.73 (0.59-0.89)** **12 months: 0.72 (0.62-0.84)** >12 months: **0.66 (0.55-0.78)** Unclear age: 1.31 (0.84-2.05)	Overall: 1.03 (0.98-1.07); n=7 6 months: 1.11 (0.99-1.24) 9 months: 1.00 (0.96-1.04)
Limited to studies that reported on seroprotection	Overall: 0.64 (0.36-1.14); n=4 9 months: 0.74 (0.44-1.24) >12 month: **0.48 (0.37-0.61)**	Overall: 0.92 (0.74-1.15); n=7 6 months: 1.17 (0.94-1.46) 9 months: 0.78 (0.52-1.16) 12 months: 0.67 (0.39-1.16) Unclear age: 1.31 (0.84-2.05)	Overall: 0.99 (0.92-1.07); n=4 9 months: 0.99 (0.92-1.07)
Limited to studies that reported on serology within 3 months after primary vaccination	Overall: 0.71 (0.33-1.55); n=2 6 months: 0.96 (0.77-1.19) 9 months: **0.44 (0.20-0.98)**	Overall: 0.79 (0.60-1.04); n=8 6 months: 1.00 (0.73-1.37) 9 months: **0.68 (0.46-0.99)** >12 months: **0.54 (0.30-0.97)**	Overall: 1.06 (0.97-1.16); n=3 6 months: 1.11 (0.99-1.24) 9 months: 0.98 (0.84-1.13)
Limited to studies that reported on serology within 6 months after primary vaccination	Overall: 0.90 (0.73-1.11); n=3 6 months: 0.96 (0.77-1.19) 9 months: 0.68 (0.23-1.96)	Overall: **0.81 (0.67-0.99)**; n=10 6 months: 1.00 (0.73-1.37) 9 months: 0.74 (0.54-1.01) >12 months: **0.54 (0.30-0.97)**	Overall: 1.04 (0.97-1.11); n=4 6 months: 1.11 (0.99-1.24) 9 months: 0.99 (0.92-1.06)
Limited to studies administering primary vaccination ≤12 months of age	Overall: **0.83 (0.71-0.9)**; n=7 6 months: 0.96 (0.77-1.19) 9 months: **0.79 (0.65-0.95)**	Overall: **0.79 (0.67-0.92)**; n=15 6 months: 1.00 (0.73-1.37) 9 months: **0.73 (0.59-0.89)** 12 months: 0.67 (0.39-1.16)	No difference
Limited to studies administering ART	Overall: 0.63 (0.34-1.19); n=3 9 months: 0.74 (0.40-1.38) >12 months: **0.48 (0.37-0.61)**	Overall: 0.74 (0.54-1.00); n=4 6 months: 0.85 (0.25-2.83) 9 months: 0.49 (0.06-3.91) **>12 months: 0.81 (0.73-0.90)**	NA
Excluding studies with assumed primary vaccination dose*	No difference	Overall: **0.78 (0.68-0.88)**; n=17 6 months: 1.00 (0.73-1.37) 9 months: **0.73 (0.59-0.89)**	Overall: 1.03 (0.98-1.07); n=7 6 months: 1.11 (0.99-1.24) 9 months: 1.00 (0.96-1.04)

		12 months: 0.67 (0.39-1.16) >12 months: **0.72 (0.58-0.90)**	
Limited to studies with known age at vaccination	No difference	Overall: **0.76 (0.68-0.86)**; n=20 6 months: 1.00 (0.73-1.37) 9 months: **0.73 (0.59-0.89)** 12 months: 0.67 (0.39-1.16) >12 months: 0.72 (0.62-0.84)	No difference
Limited to studies that report the first time point after vaccination	No difference	Overall: **0.78 (0.69-0.89)**; n=20 6 months: 1.00 (0.73-1.37) 9 months: **0.73 (0.59-0.89)** 12 months: 0.67 (0.39-1.16) **>12 months: 0.70 (0.57-0.86)** Unclear age: 1.31 (0.84-2.05)	No difference

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, HIV-infected; HEU, HIV-exposed uninfected; NA, not applicable; No difference, if result of the sensitivity analysis is exactly the same as the main analysis; RR; risk ratio.
*Studies that did not explicitly mention that primary vaccine was administered, but where this was assumed based on the context of the paper.
Results with a significant p-value are marked in bold.

Table 3.4 Subgroup analyses for immune responses post-booster vaccination

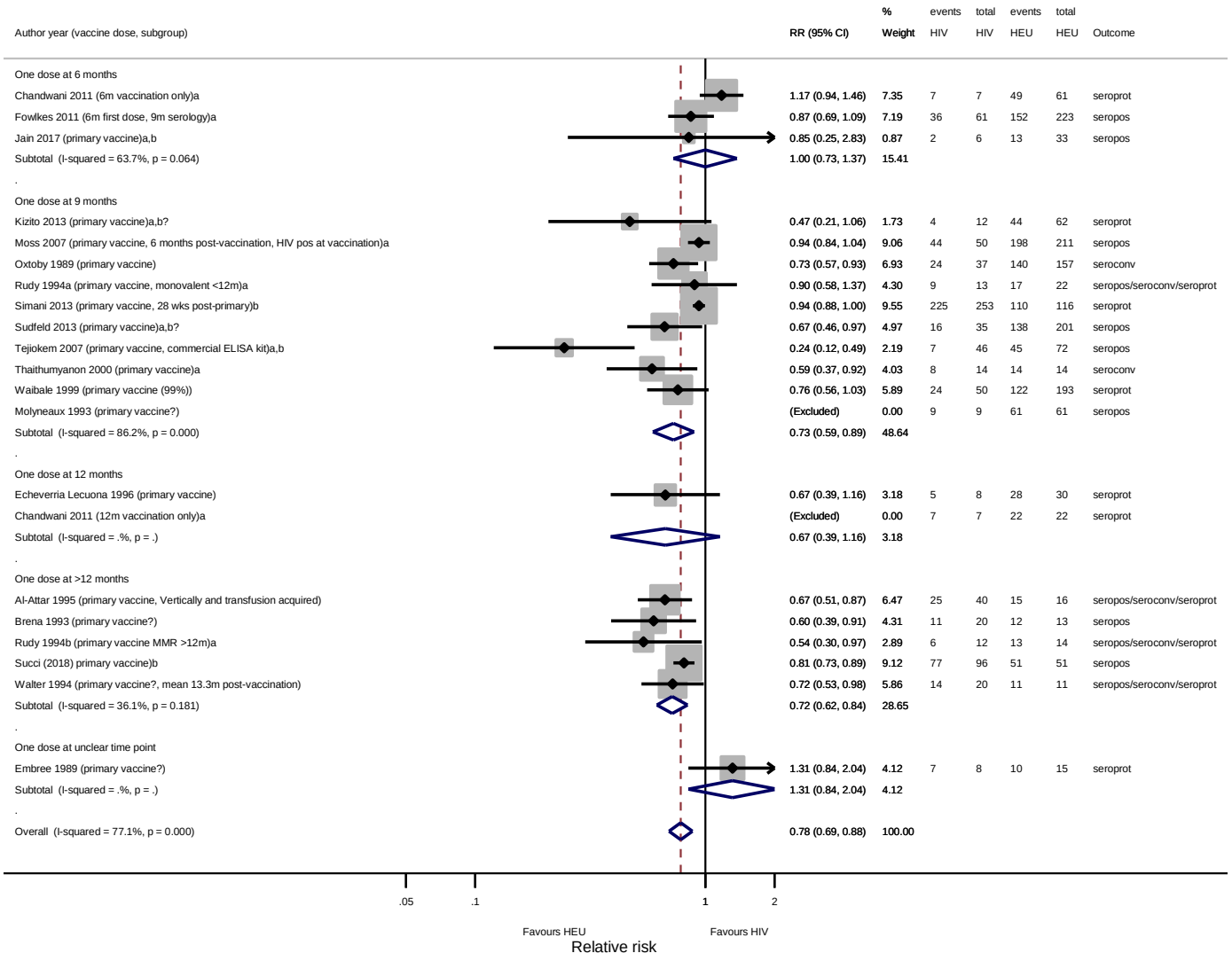
Subgroup	HIV vs HIV-unexposed booster vaccination RR (95% CI)	HIV vs HEU booster vaccination RR (95% CI)	HEU vs HIV-unexposed booster vaccination RR (95% CI)
Outcome in main analysis	Overall: 0.84 (0.68-1.04) ; n=5 ≤ 24 months: 0.88 (0.68-1.14) > 24 months: 0.96 (0.82-1.13) Unclear age: **0.58 (0.40-0.83)**	Overall: 0.75 (0.50-1.13); n=5 ≤ 24 months: 0.84 (0.59-1.19) > 24 months: **0.58 (0.53-0.63)**	Overall: 1.00 (0.91-1.09); n=3 ≤ 24 months: 1.00 (0.91-1.09)
Limited to studies that reported on seroprotection	Overall: 0.98 (0.94-1.03); n=1 ≤ 24 months: 0.98 (0.94-1.03)	Overall: 0.80 (0.48-1.33); n=3 ≤ 24 months: 1.02 (0.91-1.14) > 24 months: **0.58 (0.53-0.63)**	Overall: 1.13 (0.67-1.92); n=2 ≤ 24 months: 1.13 (0.67-1.92)
Limited to studies that reported on serology within 3 months after booster vaccination	Overall: 0.83 (0.49-1.43); n=2 ≤ 24 months: 0.83 (0.49-1.43)	Overall: 0.86 (0.61-1.22); n=3 ≤ 24 months: 0.86 (0.61-1.22)	Overall: 0.98 (0.92-1.05); n=2 ≤ 24 months: 0.98 (0.92-1.05)
Limited to studies that reported on serology within 6 months after booster vaccination	Overall: 0.88 (0.68-1.14); n=3 ≤ 24 months: 0.88 (0.68-1.14)	Overall: 0.86 (0.61-1.22); n=3 ≤ 24 months: 0.86 (0.61-1.22)	No difference
Limited to studies administering booster vaccination ≤24 months of age	Overall: 0.88 (0.68-1.14); n=3 ≤ 24 months: 0.88 (0.68-1.14)	Overall: 0.84 (0.59-1.19); n=4 ≤ 24 months: 0.84 (0.59-1.19)	No difference
Limited to studies administering ART	Overall: 0.98 (0.94-1.02); n=2 ≤ 24 months: 0.98 (0.94-1.03) >24 months: 0.96 (0.82-1.13)	Overall: 0.73 (0.40-1.35); n=3 ≤ 24 months: 1.02 (0.79-1.32) > 24 months: 0.84 (0.59-1.19)	NA
Limited to studies with known age at vaccination	Overall: 0.90 (0.76-1.08); n=4 ≤ 24 months: 0.88 (0.68-1.14) > 24 months: 0.96 (0.82-1.13)	No difference	No difference

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, HIV-infected; HEU, HIV-exposed uninfected; NA; not applicable; No difference, if result of the sensitivity analysis is exactly the same as the main analysis; RR; risk ratio.
Results with a significant p-value are marked in bold.

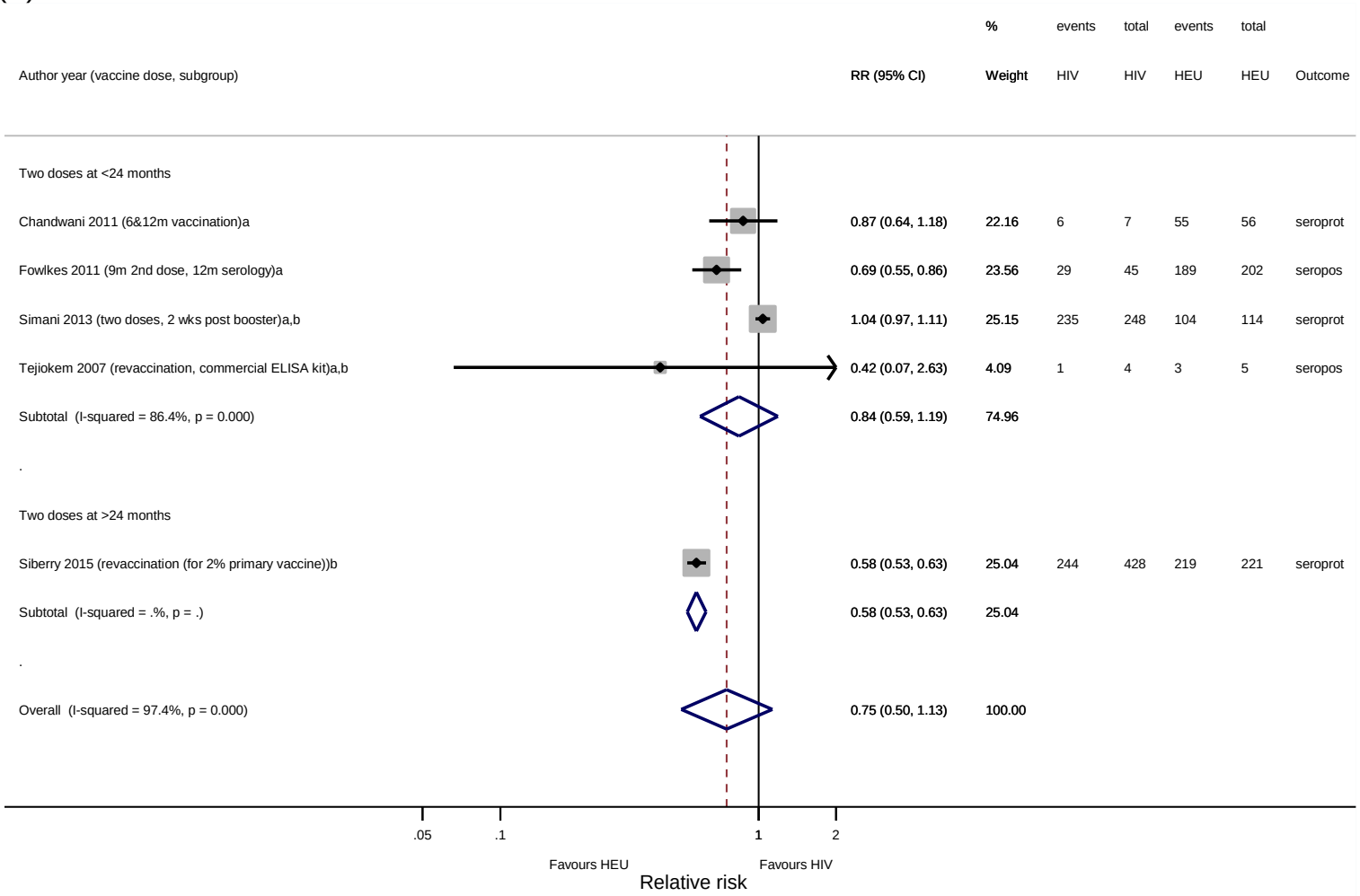
Twenty-one studies compared immunogenicity after primary measles vaccination between HIV-infected and HEU children. Nine studies reported significant RR estimates <1 (45,48,123,310,342,346,348,352,371), two included HIV-infected children on ART (45,48). The pooled RR comparing HIV-infected and HEU children after primary measles vaccination was 0.78 (95% CI 0.69-0.88; I^2=77.1%) (Figure 3.6a). The proportion of HIV-infected children with seroresponse after primary vaccination was lower compared to HEU when vaccinated at either 9-months (RR=0.73; 95% CI 0.59-0.89; n=10) or >12-months of age (RR=0.72; 95% CI 0.62-0.84; n=5), but not at 6-months (RR=1.00; 95% CI 0.73-1.37; n=3) of age. The combined RRs followed the same trend when limiting analysis to studies that administered ART (RR=0.74; 95% CI 0.54-1.00; n=4), analyzed serology within 3-months post-vaccination (RR=0.79; 95% CI 0.60-1.04; n=8), or reported seroprotection (RR=0.92; 95% CI 0.74-1.15; n=7), although non-significant (Table 3.3). Random effects meta-regression identified significant subgroup differences for studies with a different serological outcome measure (1.17; 95% CI 1.05-1.31), which could explain about 40% of between-study variance.

HIV-infected and HEU children showed similar immune responses after booster measles vaccination (RR=0.75; 95% CI 0.50-1.13; n=5) (Figure 3.6b) (39,45,155,327). When stratified by age at vaccination, HIV-infected children were less likely to show a seroresponse when vaccinated at >24-months (RR=0.58; 95% CI 0.53-0.63; n=1), but not at ≤ 24-months of age (RR=0.84; 95% CI 0.59-1.19; n=4). Pooled RRs in subgroup analyses yielded similar results (Table 3.4).

Figure 3.6 Forest plots for seroresponses comparing HIV-infected and HIV-exposed uninfected children
(A) One dose of measles vaccine



(B) Two or more doses of measles vaccine



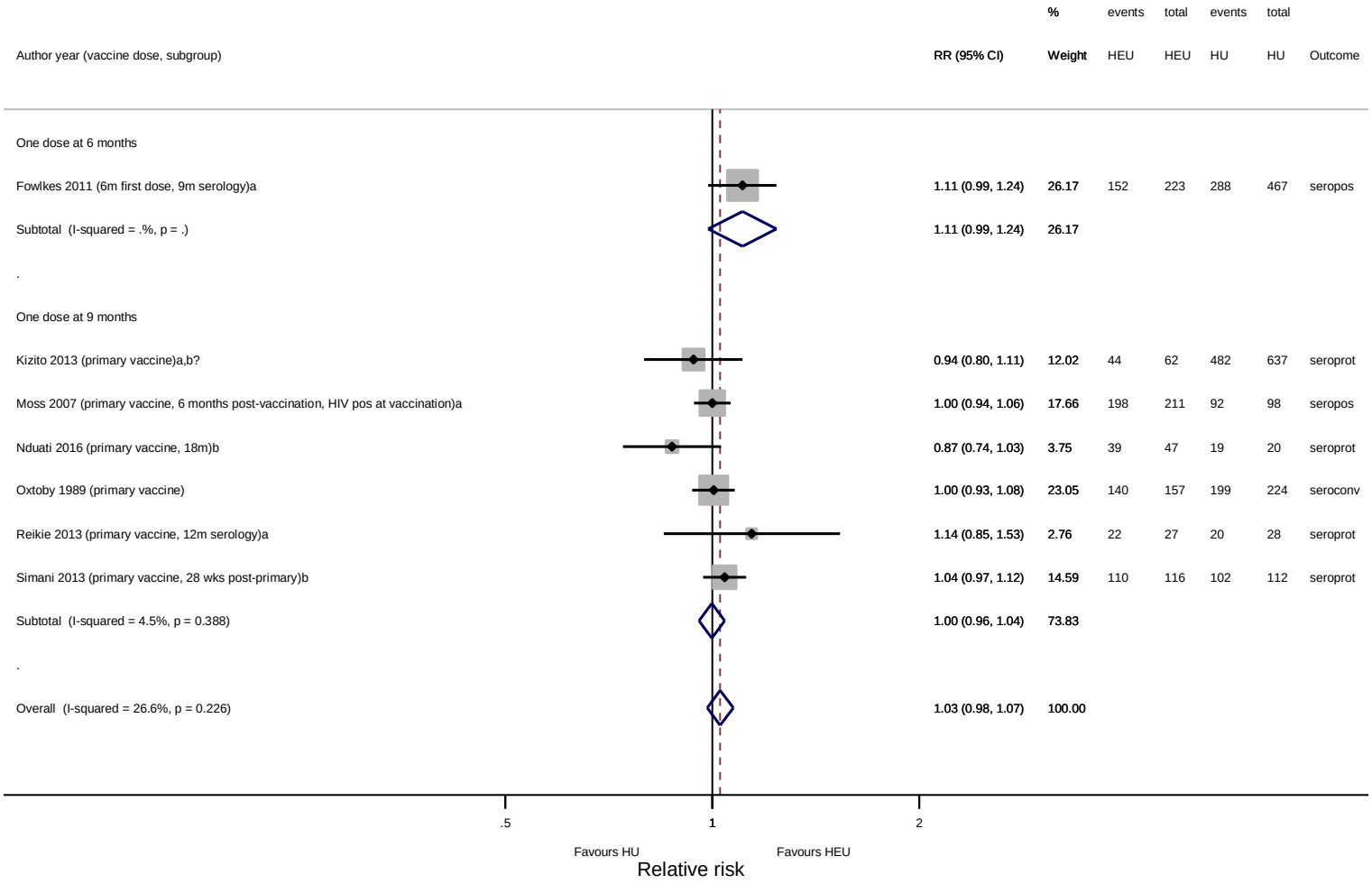
Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEU, HIV-exposed uninfected; MMR; Measles Mumps Rubella; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seropos/seroconv/seroprot, might either be seropositivity, seroconversion or seroprotection; seroprot, seroprotection. a: studies where blood was drawn for measles serology within six months after vaccination;
b: studies where children received antiretroviral therapy; b?: studies where it is not clear if children received antiretroviral therapy.

None of the seven studies reporting on immunogenicity outcomes after primary vaccination in HEU and HIV-unexposed children (34,39,82,115,327,335,342) found significant differences between the two groups. The pooled RR from a fixed-effects model showed similar seroresponses between HEU and HIV-unexposed children (RR=1.03; 95% CI 0.98-1.07; I^2=26.6%), irrespective of age or other covariates (Figure 3.7a, Table 3.3).

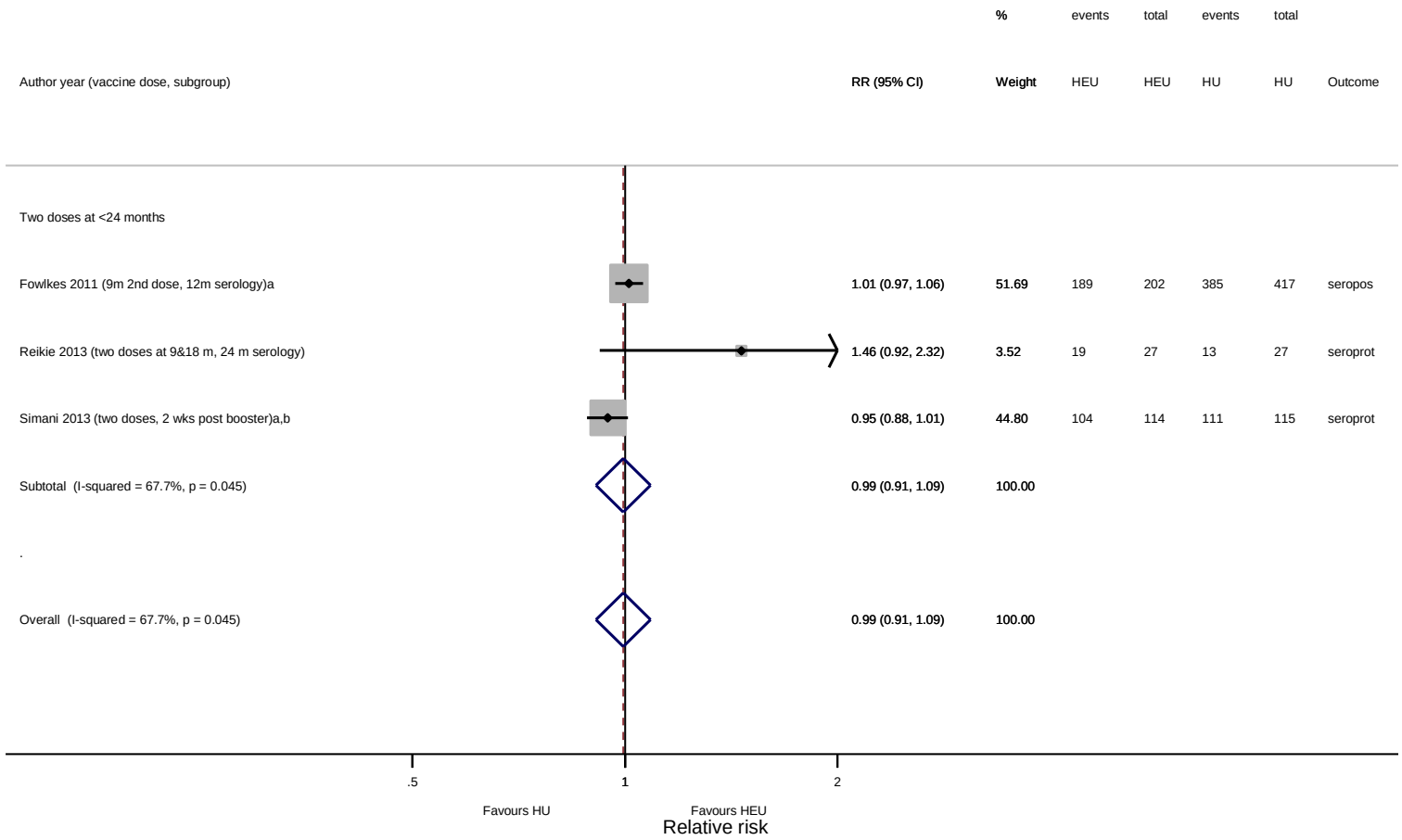
The meta-analysis comparing HEU to HIV-unexposed children after booster vaccination showed a similar likelihood of seroresponding among the two groups (R= 0.99; 95% CI 0.91-1.09; I^2=67.7%) (Figure 3.7b) (34,39,327).

Figure 3.7 Forest plots for seroresponses comparing HIV-exposed uninfected and HIV-unexposed children

(A) One dose of measles vaccine



(B) Two or more doses of measles vaccine



Abbreviations: HEU, HIV-exposed uninfected; HU, HIV-unexposed; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seroprot, seroprotection.
a: studies where blood was drawn for measles serology within six months after vaccination;
b: studies where children received antiretroviral therapy;
b?: studies where it is not clear if children received antiretroviral therapy.

Twenty-eight studies reported on safety (Table 3.5). In total, 102 HIV-infected and 21 HIV-uninfected children died after immunization. The median time between vaccine administration and end of study during which monitoring of deaths was performed was 38 weeks (range 4-144 weeks). For two deaths in HIV-infected children, the relation between vaccine administration and death could not be definitely ascertained, of which one occurred within a month post-vaccination (115,332). No relationship to vaccination was established for the remaining 121 deaths.

Twenty-three studies provided information on post-vaccination SAEs other than death in HIV-infected children (period of observation ranged 1-4 weeks post-vaccination). SAEs other than death were reported in 29 of 884 HIV-infected children (3.3%), 2 of 1337 HEU (0.1%), and 18 of 1898 HIV-unexposed children (0.9%). None of the verifiable SAEs were vaccine-related. One study reported a possible, but unverifiable vaccine-related SAE (332). HIV-uninfected children were more likely to experience AEs irrespective of association with measles vaccination (41%) compared to HIV-infected (33%) or HEU (25%) children ($p<0.001$).

Table 3.5 Adverse events, serious adverse events and deaths in studies reporting on safety

Study	AEs in HIV-infected/ total HIV-infected	AEs in HEU/ total HEU	AEs in HIV-unexposed/ total HIV-unexposed	SAEs (other than death) in HIV-infected/ total HIV-infected	SAEs (other than death) in HEU/ total HEU	SAEs (other than death) in HIV-unexposed / total HIV-unexposed	Vaccine-related SAEs (other than death) in HIV-infected	Time observed for SAEs other than death	Post-vaccination deaths in HIV-infected/total post-vaccination deaths in all groups	Vaccine related potentially life-threatening events or deaths	Time observed for deaths
Abzug 2012 (319)	NR	-	-	4/193	-	-	NR	28 days	NR	NR	-
Aurpibul 2007 (320)	23/51	-	-	0/51	-	-	NA	28 days	NR	-	-
Chandwani 2011a (& Chandwani 1998) (155,313)	4/8	9/27	-	0/8	0/27	-	0	14 days	0/0	NA	NR
Chandwani 2011a (& Chandwani 1998) (155,313)	2/7	17/61	-	0/7	0/61	-	0	14 days	0/0	NA	NR
Cutts 1993 (323)	29/49[a]	18/376[b]		9/49	4/376		0	5-15 days	9/13	0	Median 1.7 years
Dunn 1998 (324)	NR	NR	-	0/56	1/616	-	0	NR	NR	-	-
Echeverria Lecuona 1996 (354)	10/14	NR	-	0/14	NR	-	NR	NR	0/NA	NA	NR
Embree 1989 (317)	NR	NR	-	0/unclear	0/unclear	-	NA	NR	NR	-	-
Farquhar 2009 (325)	NR	-	-	NR/18	-	-	-	NR	0/NA	NA	NR
Fernandez-Ibieta 2007 (326)	NR	-	-	NR/55	-	-	-	NR	0/NA	NA	NR
Fowlkes 2011 (& Helfand 2008)a (114,327)	31/83[c]	84/246[c]	186/512[c]	NER	NER	NER	0	28 days	34/NER	0	16.5 months
Fowlkes 2011 (& Helfand 2008)b (114,327)	25/59[d]	80/222[d]	152/453[d]								
Fowlkes 2016 (309)	NR	NR	NR	0/22	NR	0/865	NA	21 days	NER	0	36 months
Frenkel 1994 (Frenkel 1992) (356,357)	NR	-	-	0/10	-	-	NA	NR	NR	-	-
Goon 2001 (314)	NR	-	-	1/1	-	-	NR	10 days	0/NA	NA	1 year
Jain 2017 (329)	2/7	5/39	-	NR	NR	-	0	28 days	NER	0	1 month
Lepage 1992 (330)	20/36	71/121	68/166	0/36	1/121	0/166	0	8-14 days	15/17	0	18 months

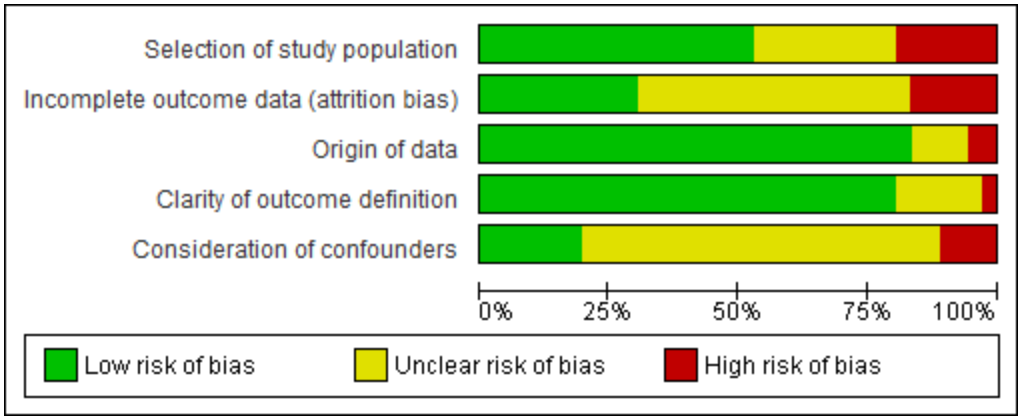
Study	AEs in HIV-infected/ total HIV-infected	AEs in HEU/ total HEU	AEs in HIV-unexposed/ total HIV-unexposed	SAEs (other than death) in HIV-infected/ total HIV-infected	SAEs (other than death) in HEU/ total HEU	SAEs (other than death) in HIV-unexposed / total HIV-unexposed	Vaccine-related SAEs (other than death) in HIV-infected	Time observed for SAEs other than death	Post-vaccination deaths in HIV-infected/total post-vaccination deaths in all groups	Vaccine related potentially life-threatening events or deaths	Time observed for deaths
Marczynska 2001 (substudy) (331)	NR	-	-	0/9	-	-	NA	28 days	0/0	NA	3 months
McLaughlin 1988 (332)	NR	-	-	1/70	-	-	Potentially 1, but relation to vaccination not verifiable	NR	Unclear, 41 of 221 HIV-infected patients (19%) died (vaccinated and unvaccinated)/NA	Potentially 1, but relation to vaccination not verifiable	NR
Molyneaux 1993 (361)	NR	NR	-	1/9[e]	0/61	-	NA	NR	NR	-	-
Moss 2007 (115)	41% of 66 with fever, 70% of 66 with cough	NR	41% of 375 with fever, 57% of 375 with cough	1/66	NR	2/375	NR	28 days	28/38	1 died with measles, but not known to be related to vaccination	27 months
Ndikuyeze 1987 (364)	NR	-	-	0/3	-	-	NA	NR	NR	NA	-
Oldakowska 2001 (339)	0/13	-	-	0/13	-	-	NA	28 days	NR	-	-
Oshitani 1996 (366)	NR	-	NR	11/37	-	5/111	NR	NR	11/16	NR	NR
Oxtoby 1989 (342)	NR	NR	NR	4/37[f]		11/381[f]	NER	NR	NER	-	NR
Palumbo 1992 (& Hoyt 1992) (343,344)	0/92[g]	-	-	4/94	-	-	NR	NR	2/NA	0	NR
Ramon-Garcia 1995 (315)	NR	-	-	2/2	-	-	NR	NR	2/NA	NR	NR
Rudy 1994a&b (371)	0/13 and 0/12	0/22 and 0/14	-	0/13 and 0/12	0/22 and 0/14	-	NA	NR	NR	-	-
Seth 2016 (345)	0/66	-	-	0/66	-	-	NA	28 days	NR	-	-
Thaithumyanon 2000 (123)	NR	NR	-	NR	NR	-	-	short term	1/NER	0	12 weeks

Studies were excluded from the safety table if they did not report on serious adverse events or deaths.
Abbreviations: AE, adverse event; HEU, HIV-exposed uninfected; HI, HIV-infected; HU, HIV-unexposed; HU, HIV-unexposed; NA, not applicable; NER, not explicitly reported; NR, not reported; SAE, serious adverse event.

[a] Incidence of symptoms with onset within 5-15 days after vaccination among HIV-infected infants: diarrhoea (n=22), cough (n=14), rhinorrhoea (n=12), fever (n=29), morbilliform rash (n=2), unscheduled consultation (n=6); highest number (n=29) used for calculations;
[b] Incidence of symptoms with onset within 5-15 days after vaccination among non-HIV-infected infants: diarrhoea (n=14), cough (n=15), rhinorrhoea (n=13), fever (n=18), conjunctivitis (n=3), unscheduled consultation (n=7); highest number (n=18) used for calculations;
[c] Parental reports of any symptoms during the first 21 days after measles vaccination at 6 months of age;
[d] Parental reports of any symptoms during the first 21 days after measles vaccination at 9-months of age;
[e] HIV-infected child who required hospital admission for severe measles, but unclear whether this was before or after vaccination;
[f] Only cases of clinical measles explicitly reported during follow-up at a mean of 9-months after vaccination;
[g] Unclear number of HIV-infected children vaccinated in case finding; number reported during outbreak.

Of the 71 studies, 59 (83%) had unclear or high-risk of confounding bias and 55 (77%) had unclear or high-risk of attrition bias due to incomplete outcome data. The origin of data and the clarity of outcome definition had low-risk of bias in 60 (85%) and 54 (76%) studies, respectively (Figure 3.8). No studies had a high summative risk of bias score (≥7). The GRADE quality of evidence was low or very low, except for the included RCT.

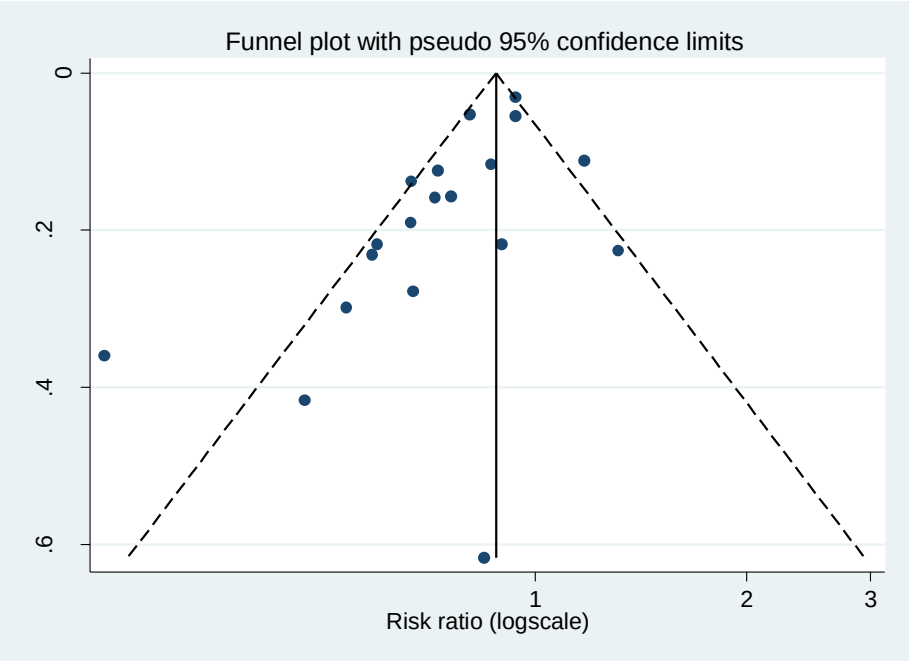
Figure 3.8 Summary of risk of bias evaluation



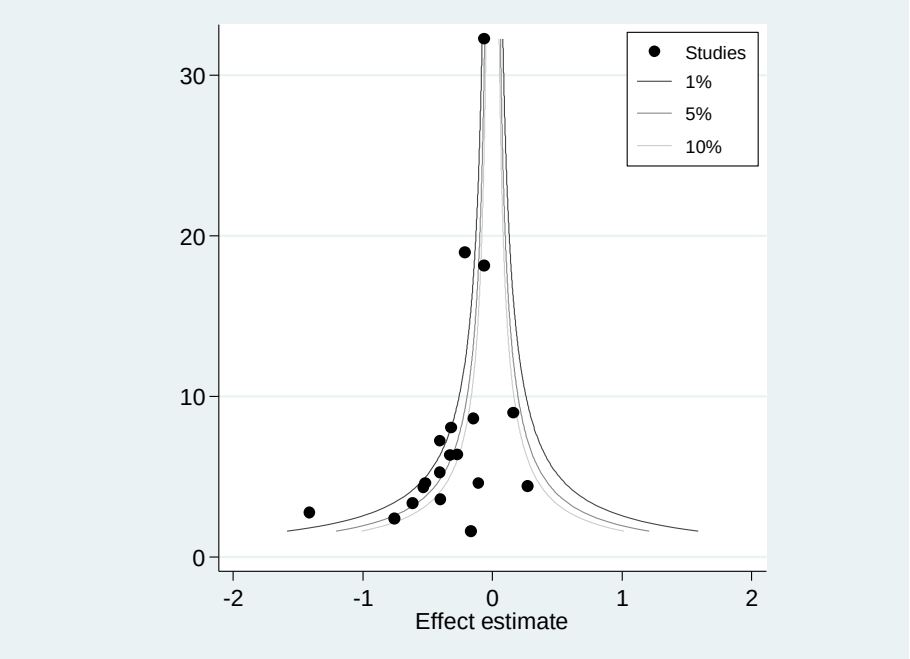
The funnel plot for comparisons containing ten or more studies (HIV-infected vs. HEU children after primary vaccination) had an asymmetrical appearance (Figure 3.9a). The contour-enhanced funnel plot showed that studies were missing in regions of both low and high statistical significance (Figure 3.9b), suggesting that the asymmetry cannot be explained by publication bias. Smaller studies were likely to have contributed to funnel plot asymmetry (Egger's test p=0.009).

Figure 3.9 Funnel plots

(A) Funnel plot HIV-infected vs HEU children after primary measles vaccination



(B) Contour-enhanced funnel plot HIV-infected vs HEU children after primary measles vaccination



3.5 Discussion

This review assessed the safety and immunogenicity of measles vaccination in 4867 HIV-infected, 2733 HEU and 7763 HIV-unexposed children. HIV-infected children had 26% (95% CI 10%-39%) lower seroresponse rate to primary measles vaccination compared to HIV-unexposed children, and 22% (95% CI 12%-31%) lower rate compared to HEU children. Differences between groups were no longer present after booster vaccination (123,349,377). This might be due to selection of HIV-infected children that survived to an older age, who were likely to be slow progressors and maintained their immunological status, or received ART. No association between death and measles vaccination was found in HIV-infected children. None of the verifiable SAEs were vaccine-related.

Primary measles vaccination with standard titer measles vaccine at 6-months of age resulted in similar seroresponse rates between groups of HIV-infected (327), HEU (155,327), and HIV-unexposed children. This finding is supported by studies using high-titer primary measles vaccination at 6-months (323,330).

Pooled RRs showed no difference between HIV-infected and HIV-unexposed or HEU children after primary vaccination when limiting the meta-analysis to studies that administered ART, reported on seroprotection, or measured serology within 3 or 6-months post-vaccination. Thus, reduced seroresponse to primary vaccination may particularly be evident in HIV-infected children when using a less stringent serological cutoff (seroconversion or seropositivity instead of seroprotection), in the absence of ART, or after a longer time-period between vaccination and serology.

Studies with different timing for ART initiation showed improved immune responses to booster vaccination in HIV-infected children after ART initiation (320,325,333,351)

or when started on early-ART (39,44,322), while late- or non-treated groups had reduced protective responses after revaccination.

HIV-exposed children showed a non-significant trend towards improved serological response when vaccinated at 6-months of age compared to HIV-unexposed children. This could be explained by reduced transplacental transfer of antibodies from HIV-infected women, resulting in lower levels of maternal antibodies in the infant and less interference with the B-cell response to vaccination (129). Maternal PMTCT regimens and breastfeeding recommendations for HIV-infected mothers varied substantially between 1987 and 2018 and may have contributed to differences between HEU and other groups. Fetal ART exposure has been associated with less hypergammaglobulinemia in HEU children (378) and higher transfer of transplacental pathogen-specific antibodies was reported in women on triple ART compared with women on short course zidovudine (379). In this meta-analysis, only two studies reported on maternal ART (329,346) and one on breastfeeding (346); no association with measles seroresponse was found.

HIV-infected children experienced slightly more SAEs other than death in the first 4-weeks post-vaccination compared with HEU or HIV-unexposed children. However, due to absence of direct comparisons between vaccinated and unvaccinated HIV-infected children and poor quality of reporting, limited conclusions can be drawn from this analysis. HIV-infected children may experience more SAEs due to their underlying illness, unrelated to vaccine administration.

A previous systematic review and meta-analysis of 39 studies analysing safety and immunogenicity of measles vaccination in HIV-infected children searched literature up to February 2009 (71). The analysis was not stratified according to primary or

booster vaccination. We included nine new studies on safety and 15 new studies on immunogenicity. In line with the previous review, we found a trend towards improved serological responses with increasing age at vaccination in HEU and HIV-unexposed children in the descriptive analysis.

Strengths of this review and meta-analysis are the comprehensive search in seven databases and the large number of studies identified. Also, this is the first meta-analysis on this topic to separately analyse primary and booster dose by age at vaccination.

Our systematic literature search was limited by restrictions in languages used. Our results need to be interpreted in the context of the risk of bias evaluation and low to very low quality of evidence. All studies included in this review were of observational nature, except for one RCT (155,313). Observational studies may be subject to selection and confounding bias. The majority of studies did not account for age, time since vaccination and CD4+ T-cell count, hence unadjusted outcome measures were used in the analysis. A large number of studies were cross-sectional, and single time-point data were used for assessment of immune responses, increasing the risk of selection bias.

In the different meta-analyses, substantial heterogeneity between studies was detected. Therefore, pooled results should be viewed as an average representing a wide distribution of seroresponses. Differences in the definition and cutoff points for serological outcomes partly explained the large heterogeneity. Due to inconsistent outcome reporting across studies, we used seroresponse, a composite of seroprotection, seropositivity or seroconversion. We encourage consistency in reporting to allow for comparison between studies.

The findings from this review support the 2017 recommendations by the World Health Organization to administer an initial dose of measles vaccination at 6-months of age in areas with high incidence of HIV-infection and measles, followed by two routine doses (55). To date, only three studies with comparison groups have evaluated immunogenicity after standard-titer measles vaccination at 6-months of age (155,327,329). Future studies should evaluate serological response to early measles vaccination in HIV-infected and HEU children. In addition, there are concerns regarding long-term immunogenicity of a 2-dose schedule given early in life, as antibody titers in HIV-infected children on ART wane over time (321,333). Therefore, we recommend future studies on long-term waning of immunogenicity after early vaccination in HIV-infected children treated with ART.

Chapter 4 Measles immunity at 4.5 years of age following vaccination at 9 and 15-18 months of age among HIV-infected, HIV-exposed-uninfected and HIV-unexposed children

4.1 Abstract

Background: HIV-infected and HIV-exposed-uninfected (HEU) children may be at increased risk of measles infection due to waning of immunity following vaccination. We evaluated persistence of antibodies to measles vaccination at 4.5 years of age in HIV-unexposed, HEU, and HIV-infected children with CD4+≥25% previously randomized to immediate antiretroviral therapy interrupted at 12 months (HIV/Immed-ART-12), 24 months (HIV/Immed-ART-24), or when clinically/immunologically indicated (HIV/Def-ART). The HIV/Def-ART group had ART initiated by median 5.8 (interquartile range 4.4-10.3) months of age.

Methods: This cohort study followed participants from 6-12 weeks through 4.5 years of age. HIV-unexposed (n=95), HEU (n=84), HIV/Immed-ART-12 (n=70), HIV/Immed-ART-24 (n=70), and HIV/Def-ART (n=62) children were scheduled to receive measles vaccination at 9 and 15-18 months of age. Anti-measles serum IgG titers were quantified using enzyme-linked immunosorbent assay at 4.5 years.

Results: Compared with HIV-unexposed children (2860 mIU/mL; 95% confidence interval [CI] 2373-3446), measles antibody geometric mean titers (GMTs) were significantly lower in both HIV/Immed-ART-12 (571; 95% CI 409-796; p<0.001) and HIV/Immed-ART-24 (1136; 95% CI 791-1633; p<0.001), but similar in the HIV/Def-ART (2777; 95% CI 2008-3841); p=0.675) and HEU (3242; 95% CI 2617-4014; p=0.525) groups. Furthermore, compared with HIV-unexposed, antibody titers ≥330 mIU/mL (i.e. presumed sero-correlate for protection; 99%) were also significantly

lower in HIV/Immed-ART-12 (70%; p<0.001) and HIV/Immed-ART-24 (83%; p<0.001); but similar in the HIV/Def-ART (90%) and HEU (98%) groups.

Conclusions: HIV-infected children in whom ART was interrupted at either 12 or 24 months of age had lower GMTs and lower proportions had seroprotective titers than HIV-unexposed children; indicating a potential impairment in immune responses with ART interruption.

4.2 Introduction

Measles infection contributed to 1.2% (134 200 deaths) of global under-5 mortality in 2015 (380). Despite safe and effective vaccines (55), sporadic outbreaks of measles still persist (381) due to low vaccine coverage, reduced maternal measles antibody transfer to infants born to vaccinated women compared with those with naturally-acquired immunity, and pockets of susceptible individuals with sub-optimal immune responses to vaccination (138,382).

Following measles vaccination, HIV-infected children maintain seroprotective titers for a shorter duration than HIV-uninfected children (46,114,115,123). A meta-analysis of five studies estimated that 68% (95% confidence interval [CI], 45%-88%) of HIV-infected primary responders had seroprotective antibody titers 2 years after their last measles vaccine and 40% (95% CI, 10%-73%) after 5 years, generally in the absence of combination antiretroviral therapy (ART) (46).

Increased ART coverage has improved survival of HIV-infected children, potentially creating a cohort of measles-susceptible children, due to quicker waning of antibodies over time (321). Two studies have been published on measles seroresponses in HIV-infected children receiving early ART initiation, as currently recommended by the World Health Organization (WHO) (383). Pensieroso et al.

reported that early ART treated HIV-infected children generated and preserved measles-specific memory B cells comparable to healthy controls (44). However, Succi et al. observed no difference in measles seropositivity at 4 years of age between children on ART initiated <12 months age, ≥12 months of age or no ART (48). Although early initiation of ART in HIV-infected infants is now recommended, lifelong continuous treatment may be problematic due to long-term toxicity, risk of ART resistance, waning adherence and resource constraints. Previous studies showed that early time-limited (interrupted) ART had improved clinical outcomes to deferred continuous ART (384).

Because of effective prevention of mother-to-child-transmission of HIV, a large proportion of children born to HIV-infected mothers is HIV-exposed but uninfected (HEU) (296). These children may have suboptimal immune response to vaccination due to intra-uterine exposure to HIV and/or maternal ART (22,30,385–387). HEU infants have reduced CD4+ and CD8+ cell-counts, impaired T-cell maturation, and both hypo- and hyper-responsiveness upon T-cell activation (18,29,388). We previously reported that the proportion with seroprotective measles antibody titers at approximately 9 months post-measles booster vaccination was lower in HEU (79.6%) than HIV-unexposed children (94.3%) (39). In contrast, other studies have reported similar measles antibody persistence between HEU and HIV-unexposed children up to two years of age (34,82,115,327,335,342).

A recent systematic-review highlighted the absence of published studies on long-term immunity to measles vaccination beyond two years of age in HEU and HIV-infected children, especially with early ART initiation (389).

This study aimed to evaluate the persistence of measles antibody titers at 4.5 years of age in HIV-unexposed, HEU, and HIV-infected children previously randomized to initiate ART when clinically/immunologically indicated or within 6-12 weeks of age, which was interrupted at 12 or 24 months.

4.3 Methods

Study population

This cohort study included HIV-infected children enrolled in a randomized open-label trial on timing of ART initiation (Children with HIV Early Antiretroviral [CHER] study) (259) and a parallel cohort of HEU and HIV-unexposed children (25,39,40). The CHER study enrolled HIV-infected infants between 6 to 12 weeks of age with CD4+ T-cell percentages ≥25% and were randomized to initiate immediate ART followed by interruption at 12 months (HIV/Immed-ART-12) or 24 months (HIV/Immed-ART-24), or deferred ART until clinically or immunologically indicated (HIV/Def-ART). A convenience sample of HIV-infected children with CD4+ T-cell percentage <25% was included (HIV+/CD4+<25%) who initiated ART at enrolment for 12 or 24 months followed by interruption. In parallel, children born to HIV-uninfected mothers (HIV-unexposed) and HIV-infected mothers who were themselves HIV-uninfected (HEU) were enrolled in this study.

Children included in the current study were enrolled between April 2005 and June 2006. The Schwarz measles vaccine (Rouvax; Aventis, France) was administered at 38-42 weeks (9 months) and 64-76 weeks (15-18 months) of age. Participants received other childhood vaccines according to the public immunization program (25,39). The ART regimen consisted of zidovudine, lamivudine and lopinavir-ritonavir. An interim analysis of the CHER trial showed greater risk of disease

progression and death in the HIV/Def-ART group and the data and safety monitoring board thus recommended they be evaluated for ART initiation if they were not receiving ART (259). Children in the HIV/Def-ART group began ART at median age 5.8 months (IQR4.4 – 10.3 months); 73% had been initiated on ART before receiving the first measles vaccine and 88% before receiving the booster measles dose. Those in HIV/Immed-ART-12 and HIV/Immed-ART-24 groups were initiated on ART at a median age of 7.4 (IQR 6.6 – 8.9) weeks.

Laboratory methods

Blood samples were collected at 4.5 years (232-236 weeks) of age. After centrifugation, serum was aliquoted and stored at -20°C to -70°C. Measles specific IgG antibodies were measured using an indirect enzyme-linked immunosorbent assay (Enzygnost, Dade Behring, Marburg, Germany), as described in chapter 2. The assay included an internal reference for the quantitative assessment of measles IgG concentrations, adjusted according to the first WHO International Reference Preparation (1964) (266). Antibody titers were calculated using the α-method according to the manufacturer's instructions. Measles seropositivity was classified as IgG titers ≥150 mIU/mL (optical density [OD] 0.1-0.2) and seroprotection as titers ≥330 mIU/mL (OD > 0.2) as per manufacturer's guidelines. All negative (<150 mIU/mL; OD < 0.1) and equivocal (150–329 mIU/mL) samples were analyzed in duplicate. Seronegative samples were assigned a titer half the value of the assay's detection limit (i.e. 75 mIU/mL).

Statistical analysis

Geometric mean titers (GMT) were calculated following log10 transformation of antibody titers and then compared between groups using multivariable linear

regression, considering age, sex, race, study centre and CD4+ T-cell percentage at the 9-month measles dose as covariates. Proportions of children meeting seropositivity and seroprotection cutoffs were compared between groups using multivariable logistic regression, adjusted for the aforementioned covariates.

Weight-for-height, weight-for-age, and height-for-age z-scores were calculated using WHO child growth references (390). Stunting was defined as height-for-age z-score ≤ 2 standard deviations (SD) from the WHO reference population mean, wasting as weight-for-height score of ≤ 2SD below the mean, and underweight as weight-for-age z-score ≤ 2SD below the mean.

Logistic regression was used to explore the association between long-term seroprotective antibody titers and HIV status, ART initiation strategy, sex, race, age, time interval between vaccination and blood collection, and nutritional status at the primary measles dose by reporting adjusted odds ratios (aOR) and 95% CI. In HIV-infected children, we further analyzed the effect of ART (at time of primary and booster measles doses, immunogenicity visit) and CD4+ T-cell percentage (at enrolment and primary measles dose) on the proportion of participants with seroprotective antibody titers. Variables with p-values ≤ 0.15 in univariate regression were included in multivariable regression models. HIV-unexposed children were used as the reference group. Participants were included in the analyses if they received two doses of measles vaccination and had an immunogenicity visit with serum collection around 4.5 years of age. Unadjusted p-values ≤ 0.05 and Bonferroni adjusted p-values ≤ 0.007, taking multiple comparisons into consideration, were considered statistically significant. All tests were two-sided. Data were analyzed using Stata version 13 (Stata Corporation, College Station, Texas, USA).

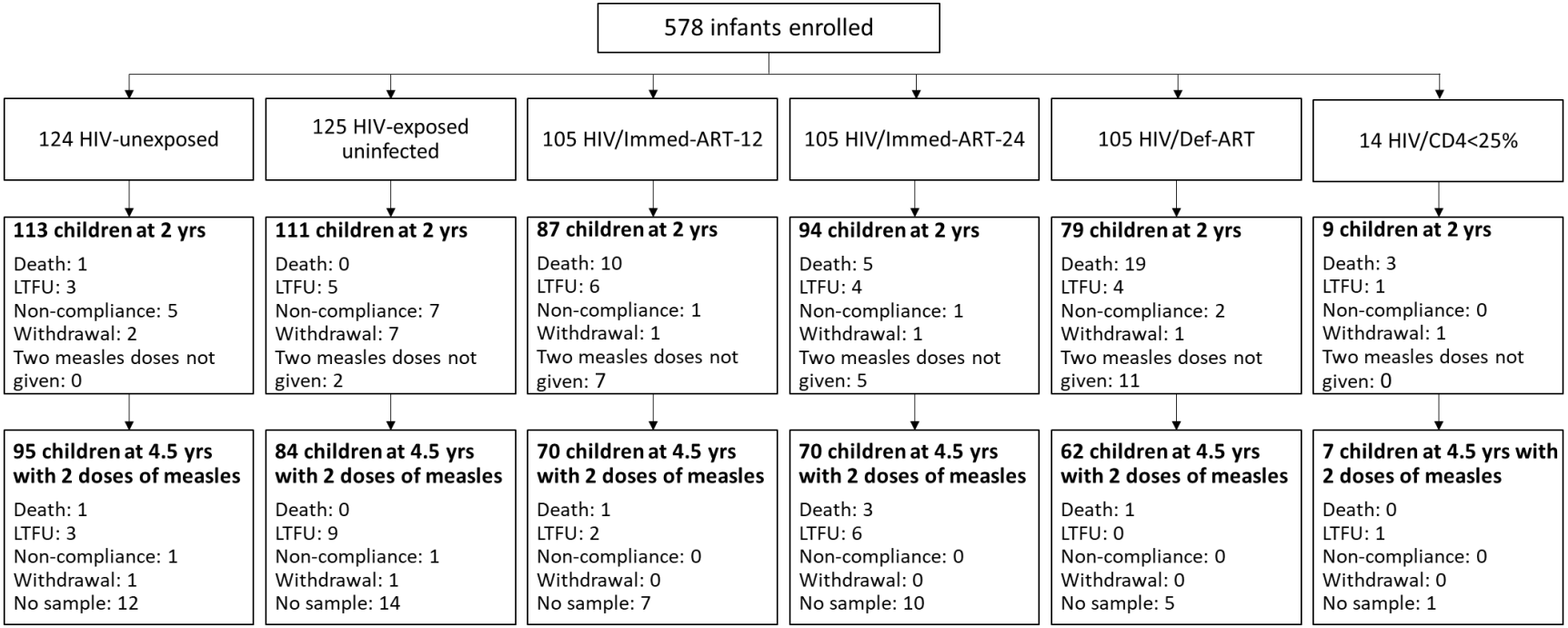
4.4 Results

Of 578 children enrolled, samples were unavailable for 141 (24%) participants at 4.5 years of age, as detailed in Figure 4.1, and included high infant mortality rates in HIV/Def-ART (19%; n=20), HIV/Immed-ART-12 (10%; n=11) and HIV-Immed-ART-24 (8%; n=8) groups (259). Of the 437 surviving children who received two doses of measles vaccine, 388 (89%) had serum samples for analysis at median age of 4.4 years: 95 HIV-unexposed, 84 HEU, 70 HIV/Immed-ART-12, 70 HIV/Immed-ART-24, 62 HIV/Def-ART, and 7 HIV-infected with CD4+<25% (Table 4.1). Baseline characteristics of children included in the current analyses were not significantly different from those who were excluded, except for deaths and those administered <2 doses of measles vaccine (Table 4.2).

Overall, the median age at time of the primary measles dose was 9.0 months and at booster dose 15.4 months with a median time interval of 37.7 months between primary vaccination and the immunogenicity visit (Table 4.1). Differences in age at vaccination between groups were unlikely to be of clinical relevance. More children were of Black-African descent in the HEU (95%), HIV/Immed-ART-12 (93%), HIV/Immed-ART-24 (96%), and HIV/Def-ART (98%) groups compared with HIV-unexposed children (80%). More HIV-infected children (HIV/Immed-ART-12: 31%; HIV/Def-ART: 45%) were stunted at the primary measles dose than HIV-unexposed (14%) and HEU (14%) children, however, nutritional status was similar by 4.5 years of age (Table 4.1).

Total number of HIV-infected children per group (re-)initiated on ART at the time of vaccinations and sample collection, duration of ART interruption is presented in Table 4.1 and Figure 4.2. Mean CD4+ percentage and median CD4+ count were significantly different between HIV groups (Table 4.1).

Figure 4.1 Study profile showing the study population from enrolment through the current analysis



Abbreviations: HIV/Immed-ART-12, HIV-infected children receiving immediate antiretroviral therapy until 12 months of age; HIV/Immed-ART-24, HIV-infected children receiving immediate antiretroviral therapy until 24 months of age; HIV/Def-ART, HIV-infected children on deferred antiretroviral therapy until clinically or immunologically indicated; HIV/CD4+<25%, convenience sample of HIV-infected children with CD4+<25% at enrolment and immediate initiation on ART; LTFU, lost to follow-up.

Table 4.1 Demographics and baseline characteristics of participants included in the immunogenicity analysis

	HIV-unexposed (n = 95)	**HEU (n = 84)**	**HIV/Immed-ART-12 (n = 70)**	**HIV/Immed-ART-24 (n = 70)**	**HIV/Def-ART (n = 62)**	**HIV/CD4+<25% (n = 7)**	**Total (n = 388)**
Male, n (%)	50 (53)	43 (51)	24 (34)	36 (51)	23 (37)	2 (29)	178 (46)
Black-African, n (%)	76 (80)[a, b, c, d]	80 (95)[a]	65 (93)[b]	67 (96)[c]	61 (98)[d]	6 (86)	355 (91)
Mixed ancestry, n (%)	19 (20)[a, b, c, d]	4 (5)[a]	5 (7)[b]	3 (4)[c]	1 (2)[d]	1 (14)	33 (9)
Age at primary measles dose, median months (IQR)	8.9 (8.8-9.0)[a, b, c d]	9.0 (8.9-9.1)[a]	9.0 (8.8-9.2)[b]	9.1 (8.9-9.4)[c]	9.2 (8.9-9.5)[d]	9.5 (8.9-9.7)	9.0 (8.8-9.2)
Age at booster measles dose, median months (IQR)	15.2 (15.2-15.4)[a, b, c, d]	15.4 (15.3-15.6)[a, g]	15.5 (15.3-15.9)[b]	15.5 (15.3-15.7)[c]	15.7 (15.3-16.5)[d, g]	15.7 (15.2-15.9)	15.4 (15.2-15.7)
Age at immunogenicity visit, median years (IQR)	4.4 (4.4-4.5)	4.4 (4.4-4.5)	4.4 (4.4-4.5)	4.4 (4.4-4.5)	4.4 (4.4-4.5)	4.5 (4.5-4.6)	4.4 (4.4-4.5)
Interval from primary measles dose to immunogenicity visit, median months (IQR)	37.7 (37.7-38.2)[b, d]	37.7 (37.7-38.1)[g]	37.6 (36.9-38.1)[b]	37.7 (37.5-38.0)	37.7 (36.7-38.1)[d, g]	38.5 (38.4-38.7)	37.7 (37.6-38.1)
Stunting[k] at primary measles dose, n (%)	13 (14)[b, d]	12 (14)[e, g]	22 (31)[b, e]	13 (19)[j]	27 (45)[d, g, j]	3 (43)	90 (24)
Wasting[l] at primary measles dose, n (%)	3 (3)	3 (4)	3 (4)	0 (0)	1 (2)	0 (0)	10 (3)
Underweight[m] at primary measles dose, n (%)	8 (8)	3 (4)	8 (11)	4 (6)	8 (13)	0 (0)	31 (8)
Stunting at immunogenicity visit, n (%)	10 (11)	18 (21)	18 (26)	16 (23)	11 (18)	3 (43)	76 (20)
Wasting at immunogenicity visit, n (%)	1 (1)	1 (1)	0 (0)	0 (0)	1 (2)	0 (0)	3 (1)
Underweight at immunogenicity visit, n (%)	5 (5)	1 (1)	3 (4)	1 (1)	3 (5)	0 (0)	13 (3)
Enrolment CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1980 (1695-2722)	2283 (1739-2924)	2612 (1771-3277)	1727 (963-2356)	2283 (1695-2982)
Enrolment CD4+ lymphocyte %, mean (±SD)	NA	NA	36.5 (8.3)	38.0 (8.9)	38.1 (7.8)	22.6 (4.3)	37.0 (8.7)
Primary measles dose CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	2104 (1579-2713)[i]	2149 (1604-2746)[j]	1518 (1207-2189)[i, j]	1495 (1209-2330)	1970 (1421-2583)
Primary measles dose CD4+ lymphocyte %, mean (±SD)	NA	NA	40.0 (9.3)[i]	39.1 (7.7)[j]	31.5 (6.8)[i, j]	32.0 (8.0)	36.9 (8.8)

Booster measles dose CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1286 (1016-1746)[h, i]	1860 (1306-2220)[h]	1734 (1357-2277)[i]	1612 (900-1976)	1614 (1181-2131)
Booster measles dose CD4+ lymphocyte %, mean (±SD)	NA	NA	26.3 (7.1)[h, i]	34.2 (7.9)[h]	34.2 (8.4)[i]	29.7 (10.2)	31.4 (8.6)
Immunogenicity visit CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1055 (723-1330)[i]	945 (715-1241)[j]	1202 (910-1524)[i, j]	No observations	1057 (779-1383)
Immunogenicity visit CD4+ lymphocyte %, mean (±SD)	NA	NA	31.7 (7.4)[i]	30.0 (7.4)[j]	36.6 (7.7)[i, j]	No observations	32.5 (7.9)
Total on ART at 9-month measles dose, n/N (%)	NA	NA	69/70 (99)[i]	70/70 (100)[j]	44/62 (71)[i, j]	7/7 (100)	190/209 (91)
Total on ART at 15-18-month measles dose, n/N (%)	NA	NA	21/70 (30)[h, i]	70/70 (100)[h, j]	53/62 (85)[i, j]	7/7 (100)	151/209 (72)
Total on ART at immunogenicity visit, n/N (%)	NA	NA	50/70 (71)[i]	44/70 (63)[j]	58/62 (94)[i, j]	7/7 (100)	159/209 (76)
Interval from ART initiation to primary measles dose, median months (IQR)	NA	NA	7.4 (7.4; 7.5)[i]	7.4 (7.4; 7.4)[j]	3.7 (-1.3; 5.5)[i, j]	7.4 (7.4; 7.5)	7.4 (5.6; 7.4)
Duration of ART interruption, median months (IQR)	NA	NA	7.3 (3.2; 45.3)[i]	16.5 (3.9; 34.1)[j]	0 (0; 0)[i, j]	0 (0; 0)	3.9 (0; 21.4)

Abbreviations: CD4+<25%, HIV-infected children with CD4+% <25% at enrolment who received immediate antiretroviral therapy; HEU; HIV-exposed-uninfected children; HIV/Immed-ART-12, HIV-infected children on immediate antiretroviral therapy interrupted at 12 months; HIV/Immed-ART-24, HIV-infected children on immediate antiretroviral therapy interrupted at 24 months; HIV/Def-ART, HIV-infected children on deferred antiretroviral therapy; IQR, interquartile range; NA, not applicable; SD, standard deviation.

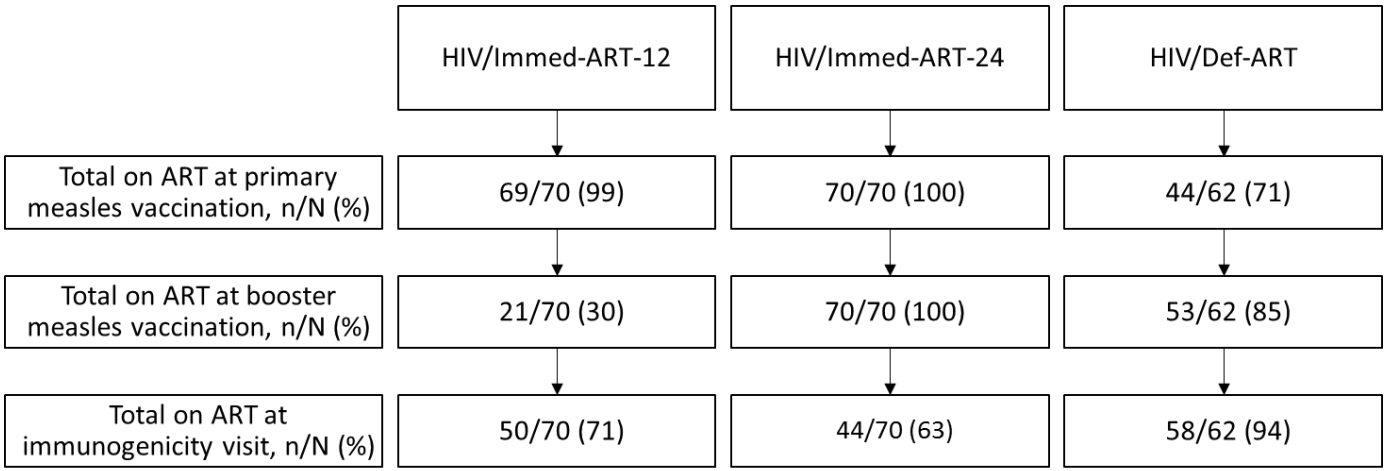
[a] significant difference between HIV-unexposed and HEU; [b] significant difference between HIV-unexposed and HIV/Immed-ART-12; [c] significant difference between HIV-unexposed and HIV/Immed-ART-24; [d] significant difference between HIV-unexposed and HIV/Def-ART; [e] significant difference between HEU and HIV/Immed-ART-12; [f] significant difference between HEU and HIV/Immed-ART-24; [g] significant difference between HEU and HIV/Def-ART; [h] significant difference between HIV/Immed-ART-12 and HIV/Immed-ART-24; [i] significant difference between HIV/Immed-ART-12 and HIV/Def-ART; [j] significant difference between HIV/Immed-ART-24 and HIV/Def-ART; [k] stunting: height-for-age z-score ≤2 SD; [l] wasting: weight-for-height score of ≤2SD; [m] underweight: weight-for-age z-score ≤ 2SD; p-values were calculated using a Kruskal-Wallis test and adjusted for multiple comparisons; Five participants had missing data on nutritional status primary measles dose vaccination; 12 (HIV/Def-ART group) and 25 (HIV/Immed-ART groups) participants had missing data on CD4+ T cell count/percentage at the immunogenicity visit.

Table 4.2 Comparison of included and excluded participants

	HIV-unexposed			HEU			HIV/Immed-ART			HIV/Def-ART		
Characteristic	Included	Excluded	p-value	Included	Excluded	p-value	Included	Excluded	p-value	Included	Excluded	p-value
Participants	95	30		84	41		140	70		62	43	
Categorical, n (%)												
Deaths	0 (0)	2 (7)	0.056	0 (0)	0 (0)	n/a	0 (0)	19 (27)	<0.001	1 (2)	19 (44)	<0.001
Received <2 doses of measles vaccine	0 (0)	0 (0)	n/a	0 (0)	2 (5)	0.106	0 (0)	12 (17)	<0.001	0 (0)	11 (26)	<0.001
Sex												
Female	45 (47)	10 (31)		41 (49)	16 (39)		80 (57)	40 (57)		39 (63)	25 (58)	
Male	50 (53)	20 (69)	0.120	43 (51)	25 (61)	0.302	60 (43)	30 (43)	1.000	23 (37)	18 (42)	0.623
Race												
Black race	76 (80)	19 (63)		80 (95)	37 (90)		132 (94)	69 (99)		61 (98)	41 (95)	
Mixed ancestry	19 (20)	10 (33)	0.107	4 (5)	4 (10)	0.437	8 (6)	1 (1)	0.277	1 (2)	2 (5)	0.566
Study centre												
Soweto	70 (74)	12 (41)		65 (77)	18 (44)		95 (68)	49 (70)		42 (68)	27 (63)	
Stellenbosch	25 (26)	17 (59)	0.001	19 (23)	23 (56)	<0.001	45 (32)	21 (30)	0.753	20 (32)	16 (37)	0.599
Continuous, mean (SD)												
Birthweight, g	3192 (448)	3046 (550)	0.258	3095 (479)	3049 (388)	0.406	2988 (448)	2869 (424)	0.045	2957 (466)	2927 (450)	0.595
Age at primary measles dose, months	8.92 (0.20)	8.98 (0.35)	0.886	9.09 (0.35)	9.04 (0.32)	0.163	9.16 (0.49)	9.06 (0.31)	0.222	9.29 (0.50)	9.27 (0.50)	0.975
Enrolment CD4+ lymphocyte %	NA	NA		NA	NA		37.26 (8.62)	38.15 (9.11)	0.457	38.11 (7.76)	38.00 (9.71)	0.602

Abbreviations: HEU, HIV-exposed-uninfected; HIV/Def-ART, HIV-infected children on deferred antiretroviral therapy; HIV/Immed-ART, HIV-infected children receiving immediate antiretroviral therapy.
Excluding HIV-infected children with CD4+<25% at enrolment.

Figure 4.2 Number of participants (re-)initiated on ART



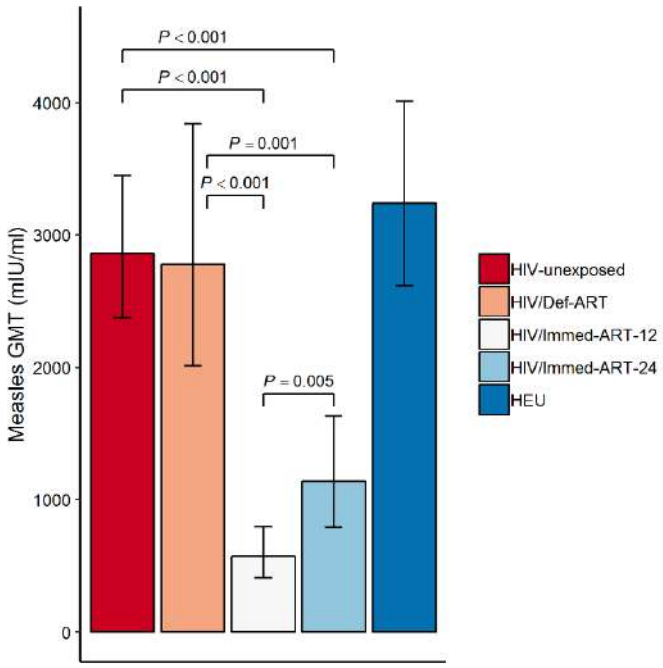
Abbreviations: ART antiretroviral therapy; HIV/Def-ART, HIV-infected children on deferred ART; HIV/Immed-ART-12; HIV-infected children with interruption of ART at 12 months of age; HIV/Immed-ART-24, HIV-infected children with interruption of ART at 24 months of age.

Persistence of measles antibodies at 4.5 years of age

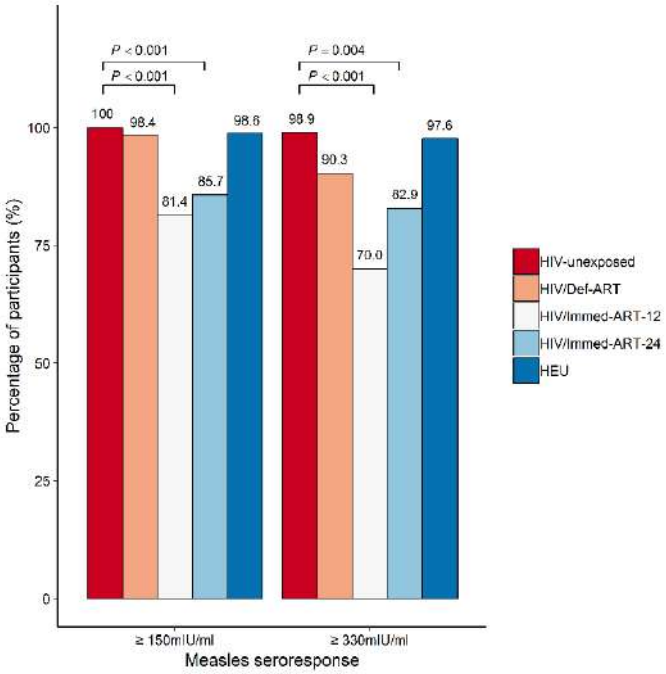
GMTs were lower in HIV/Immed-ART-12 (571 mIU/mL) and HIV/Immed-ART-24 (1136 mIU/mL) children than HIV-unexposed children (2860 mIU/mL; p<0.001 for both comparisons). Also, GMTs were lower in the HIV/Immed-ART-12 and HIV/Immed-ART-24 groups than the HIV/Def-ART children (2777 mIU/mL; p<0.001 and p=0.001, respectively) when adjusted for race, study centre, age and CD4+ percentage at time of the primary measles dose. Furthermore, GMTs were significantly lower in HIV/Immed-ART-12 children compared with HIV/Immed-ART-24 (p=0.005) (Figure 4.3a; Table 4.3).

Figure 4.3 Measles antibody geometric mean titers (Panel A) and proportion of children with seropositive and seroprotective antibody levels (Panel B) at 4.5 years of age

A



B



P value deemed significant at ≤.007 after Bonferroni correction. P values were either calculated by linear or logistic regression and adjusted for age at the immunogenicity visit, sex, race, study center, and CD4+ cell percentage at the primary measles dose in HIV-infected children or by Fisher exact test.

Table 4.3 Measles antibody geometric mean titers and proportion of children with seropositive and seroprotective antibody levels at 4.5 years of age

Measure	**HIV-unexposed**	**HEU**	**HIV/Def-ART**	**HIV/Immed-ART-12**	**HIV/Immed-ART-24**	**CD4+% <25%**	**Total**
Number of Participants evaluated	95	84	62	70	70	7	388
GMT (95% CI)	2860 (2373-3446)[a, b]	3242 (2617-4014)	2777 (2008-3841)[c, d]	571 (409-796)[a, c, e]	1136 (791-1633)[b, d, e]	2538 (561-11475)	1848 (1610-2120)
n (%) ≥ 150mIU/ml	95 (100.0) [a, b]	83 (98.8)	61 (98.4)	57 (81.4)[a]	60 (85.7)[b]	6 (85.7)	362 (93.3)
n (%) ≥ 330mIU/ml	94 (99.0) [a, b]	82 (97.6)	56 (90.3)	49 (70.0)[a]	58 (82.9)[b]	6 (85.7)	345 (88.9)

[a] significant difference between HIV-unexposed and HIV/Immed-ART-12, p<0.001 for all comparisons; [b] significant difference between HIV-unexposed and HIV/Immed-ART-24, p<0.001 for GMT and n ≥150mIU/mL titers, p=0.004 for n ≥330mIU/mL titers; [c] significant difference between HIV/Def-ART and HIV/Immed-ART-12, p<0.001; [d] significant difference between HIV/Def-ART and HIV/Immed-ART-24, p=0.001; [e] significant difference between HIV/Immed-ART-12 and HIV/Immed-ART-24, p=0.005;

P-value deemed significant at p ≤ 0.007 after Bonferroni correction; p-values were either calculated by linear or logistic regression and adjusted for age at the immunogenicity visit, sex, race, study centre, and CD4+ cell percentage at the primary measles dose in HIV-infected children; or by Fisher's exact test.

Measles seropositivity (≥150mIU/mL) was present in all HIV-unexposed children and 98.4% of the HIV/Def-ART groups (p=0.395). In contrast, fewer children in HIV/Immed-ART-12 (81.4%; p<0.001) and HIV/Immed-ART-24 (85.7%; p<0.001) groups were seropositive than HIV-unexposed children; Figure 4.3b, Table 4.3.

Similarly, the percentage of children with seroprotective titers (i.e. ≥330 mIU/mL) was significantly lower in the HIV/Immed-ART-12 (70.0%; p<0.001) and HIV/Immed-ART-24 (82.9%; p=0.004) groups compared with HIV-unexposed children (99.0%). In the HIV/Def-ART group, 90.3% had seroprotective titers. Of seven children with CD4+ T-cell<25% at enrolment, 85.7% were measles seropositive and 85.7% had seroprotective titers. There were no differences either in GMTs, seropositivity or percentage with seroprotective titers between the HEU and HIV-unexposed children; Figure 4.3b, Table 4.3.

Exclusion of participants in the early therapy groups, who did not interrupt ART for various reasons and proceeded on continuous ART (n=3 in HIV/Immed-ART-12, n=10 in HIV/Immed-ART-24 and n=6 in CD4+<25% groups), did not significantly alter results.

Determinants of long-term seroprotection

We examined the association between long-term measles seroprotection and HIV status, timing of ART initiation, sex, race, age at vaccination, age at immunogenicity visit, and nutritional status at the primary measles dose among all children (n=381) in univariate and multivariable logistic regression (Table 4.4). After controlling for timing

of ART initiation, sex, race, age at vaccination, age at immunogenicity visit, and nutritional status, HIV/Immed-ART-12 (aOR: 0.03; 95% CI 0.003-0.20), HIV/Immed-ART-24 (aOR: 0.05; 95% CI 0.006-0.41), and HIV/Def-ART (aOR: 0.11; 95% CI 0.01-0.90) had a lower odds for seroprotective titers relative to HIV-unexposed.

Among HIV-infected children (n=202), we assessed the association of measles seroprotection with the aforementioned covariates, in addition to receipt of ART at the time of the primary or booster dose, ART at 4.5 year of age, and CD4+ T-cell percentage at enrolment or primary dose. In multivariable logistic regression, HIV-infected children receiving ART at the time of the booster measles dose were 2.27 (95% CI 1.02-5.05) more likely to have antibody titers ≥330mIU/mL than those not receiving ART. Similarly, ART at time of measuring antibody persistence (aOR 3.07; 95% CI 1.39-6.84) was associated with higher likelihood of seroprotective titers ≥330mIU/mL; whereas this was not associated with CD4+ percentage at enrolment or at time of the primary vaccine dose; Table 4.4.

Table 4.4 Association of measles seroprotection at 4.5 years of age with HIV status, sex, age at vaccination, nutritional status at primary measles dose, ART regimen and immune status

Characteristic	**Non-seroprotected**	**Seroprotected**	**Univariate OR (95% CI) for seroprotection[c]**	**p-value**	**Adjusted OR (95% CI) for seroprotection[c]**	**p-value**
All children (n = 381[a, b])	n = 42	n = 339				
HIV status						
HIV-unexposed	1/42 (2.4)	94/339 (27.7)	Ref.		Ref.	
HIV-exposed uninfected	2/42 (4.8)	82/339 (24.2)	0.44 (0.04-4.90)	0.501	0.44 (0.04-4.93)	0.504
HIV/Immed-ART-12	21/42 (50.0)	49/339 (14.5)	0.02 (0.003-0.19)	<0.001	0.03 (0.003-0.20)	<0.001
HIV/Immed-ART-24	12/42 (28.6)	58/339 (17.1)	0.05 (0.007-0.41)	0.005	0.05 (0.006-0.41)	0.005
HIV/Def-ART	6/42 (14.3)	56/339 (16.5)	0.10 (0.01-0.85)	0.035	0.11 (0.01-0.90)	0.040
Sex						
Male	14/42 (33.3)	162/339 (47.8)	Ref.		Ref.	
Female	28/42 (66.7)	177/339 (52.2)	0.55 (0.28-1.07)	0.080	0.66 (0.32-1.36)	0.262
Race						
Black	41/42 (97.6)	308/339 (90.9)				
Mixed ancestry	1/42 (2.4)	31/339 (9.1)	NA	0.169	NA	
Age at primary measles dose (mo)	9.0 (8.8-9.1)	9.0 (8.8-9.2)	1.07 (0.49-2.36)	0.859	NA	
Age at booster measles dose (mo)	15.4 (15.2-15.8)	15.4 (15.2-15.7)	1.11 (0.76-1.63)	0.588	NA	
Age at immunogenicity visit (mo)	53.1 (52.9-53.5)	53.2 (52.9-53.7)	1.14 (0.72-1.82)	0.581	NA	
Interval from booster measles dose to immunogenicity visit (mo)	37.7 (37.5-37.9)	37.7 (37.5-38.1)	0.97 (0.78-1.21)	0.812	NA	
Stunting at primary measles dose						
No	32/41 (78.1)	257/335 (76.7)	Ref.			
Yes	9/41 (22.0)	78/335 (23.3)	1.08 (0.49-2.36)	0.849	NA	
Wasting at primary measles dose						
No	41/41 (100.0)	325/335 (97.0)				
Yes	0/41 (0.0)	10/335 (3.0)	NA	-	NA	
Underweight at primary measles dose						
No	38/41 (92.7)	307/335 (91.6)	Ref.			
Yes	3/41 (7.3)	28/335 (8.4)	1.16 (0.34-3.98)	0.819	NA	
HIV-infected children (n = 202)	**Non-seroprotected** (n=39)	**Seroprotected** (n=163)	**Univariate OR (95% CI) for seroprotection[c]**	**p-value**	**Adjusted OR (95% CI) for seroprotection[c]**	**p-value**
Sex						
Male	12/39 (30.8)	71/163 (43.6)	Ref.		Ref.	
Female	27/39 (69..3)	92/163 (56.4)	0.58 (0.27-1.22)	0.148	0.77 (0.34-1.72)	0.522
Race						
Black	38/39 (97.4)	155/163 (95.1)				
Mixed ancestry	1/39 (2.6)	8/163 (4.9)	NA	0.531	NA	
Age at primary measles dose (mo)	9.0 (8.8-9.1)	9.1 (8.9-9.4)	1.79 (0.73-4.36)	0.202	NA	
Age at booster measles dose (mo)	15.4 (15.2-15.8)	15.6 (15.3-15.9)	1.50 (0.81-2.76)	0.196	NA	
Age at immunogenicity visit (mo)	53.1 (52.9-53.5)	53.2 (52.7-53.8)	1.00 (0.60-1.68)	0.999	NA	
Interval from booster measles dose to immunogenicity visit (mo)	37.7 (37.5-37.9)	37.7 (36.8-38.1)	0.83 (0.59-1.16)	0.272	NA	
Stunting at primary measles dose[b]						
No	29/38 (76.3)	106/159 (66.7)	Ref.			

Yes	9/38 (23.7)	53/159 (33.3)	1.61 (0.71-3.65)	0.253	NA	
Wasting at primary measles dose[b]						
No	38/38 (0.0)	155/159 (97.5)	Ref.			
Yes	0/38 (0.0)	4/159 (2.5)	NA	-	NA	
Underweight at primary measles dose[b]						
No	35/38 (92.1)	142/159 (89.3)	Ref.			
Yes	3/38 (7.9)	17/159 (10.7)	1.40 (0.39-5.03)	0.609	NA	
ART at time of primary measles dose						
No	6/39 (15.4)	13/163 (7.98)	Ref.		Ref.	
Yes	33/39 (84.6)	150/163 (92.0)	2.10 (0.74-5.92)	0.162	2.16 (0.64-7.34)	0.216
ART at time of booster measles dose						
No	20/39 (51.3)	38/163 (23.3)	Ref.		Ref.	
Yes	19/39 (48.7)	125/163 (76.7)	3.46 (1.68-7.15)	0.001	2.27 (1.02-5.05)	0.044
ART at immunogenicity visit						
No	19/39 (48.7)	31/163 (19.0)	Ref.		Ref.	
Yes	20/39 (51.3)	132/163 (81.0)	4.05 (1.93-8.48)	<0.001	3.07 (1.39-6.84)	0.006
Enrolment CD4+ T lymphocyte %	37.2 (31.4-43.3)	36.4 (31.8-42.4)	1.00 (0.96-1.04)	0.942	NA	
Primary measles dose CD4+ T lymphocyte %	39.6 (32.6-47.0)	36.7 (30.5-42.2)	0.96 (0.93-1.00)	0.071	0.98 (0.93-1.03)	0.396

Data are proportion of subjects (%) or median (IQR);
Abbreviations: ART, antiretroviral therapy; NA, Not Applicable; OR, Odds Ratio; Ref., Reference group;
[a] Seven HIV-infected children with CD4+<25% at enrolment were excluded from analyses; [b] Five participants had missing information on nutritional status at the primary measles dose; [c] Seroprotection was defined as an IgG titer of ≥330 mIU/mL.

4.5 Discussion

The results from this study underscore a potential downside of ART interruption in HIV-infected infants who initiated ART during early-infancy, indicating greater waning of immunity against measles infection by 4.5 years of age than HIV-unexposed children. Furthermore, our study dispelled earlier concerns of HEU also being predisposed to greater waning of immunity, as suggested by the previous observation in the same cohort of children 9 months after the booster dose of measles vaccine (39). Notably, children in the HIV/Def-ART group, the majority (88%) of whom were on continuous ART by the time of their booster dose of measles vaccine, showed similar measles immunity to HIV-unexposed children.

The attenuated antibody response in HIV/Immed-ART compared with HIV/Def-ART may be explained by the lower number of children on ART at the time of the immunogenicity visit in HIV/Immed-ART groups and the lower number on ART at booster vaccination in HIV/Immed-ART-12 children. These results are in line with our previous findings at two years of age, showing greater waning of immunity in children with interrupted ART (39). Immune-cell activation during ART interruption may cause memory B cells to be drawn into effector pathways, resulting in depletion of memory B cells (391).

HIV/Immed-ART-12 children, compared with HIV/Immed-ART-24, had significantly lower GMTs. This may also be explained by fewer children in the HIV/Immed-ART-12 group on ART at the time of booster vaccination compared with HIV/Immed-ART-24.

The HIV/Def-ART group, who initiated continuous ART after the CHER interim analysis in June 2007, were less immunosuppressed and a significantly higher proportion was on ART at the time of booster vaccination and immunogenicity visit compared to the HIV/Immed-ART groups. The HIV/Def-ART group, however, might have been selectively biased by representing children with slower HIV progression within the group, as there was a higher mortality rate in these children (16%) compared to the HIV/Immed-ART children (4%) after a median follow-up of 40 weeks (259). Nevertheless, our study does represent the survivors initially randomized to this group and suggests that ART should be initiated prior to measles vaccine immunization.

Two other studies have evaluated long-term measles antibody persistence in a much smaller number of HIV-infected children initiated on ART within the first year of life;

and reported similar results to ours. These included a Latin American cohort study in which seropositive titers (≥120 mIU/mL) were present in 87% of children at 4 years of age (n=38) (48) and an Italian cross-sectional study in which 82% of children had seroprotective titers at 7 years (n=13) of age (44). The former, however, did not find a significant relation between the timing of ART initiation and measles serology (48). Of note, no studies in children evaluated the effect of systematically assigned early ART on long-term measles antibodies and the consequences of interrupting ART.

The CHER study hypothesised that if children are initiated on early-ART close to primary infection, disease progression could be prevented and children could be allowed a subsequent period off ART (384). In CHER, HIV-infected children on early-ART in whom ART was interrupted had a better clinical outcome than deferred ART, without increased risk for disease progression during the ART interruption period (384). Nevertheless, we showed that ART interruption is associated with long-term waning of measles protection, hence, suggesting sub-clinical consequences of ART interruption in these children, which could lend them to being susceptible to measles in the event of outbreaks. Of note, however, is that ART was reinitiated due to CD4+ T-cell depletion, with children being exposed to prolonged HIV viremia during interruption. Seventy percent of children in the main CHER trial had rebounded by two months off ART, with median viral load being log5 HIV RNA copies per mL (392). The need for further booster doses of measles vaccine in these children is currently being evaluated.

Prior studies reported that if ART initiation precedes immunization, vaccine responses are comparable to healthy children (25) and memory B cells are maintained over time (44). Likewise, our multivariable logistic regression analysis showed that long-term presence of seroprotective titers was associated with being

on ART at the time of booster vaccination, as well as at the immunogenicity visit in HIV-infected children, underlining the importance of early and continuous ART.

In contrast to our previous report at 2 years of age (measured in years 2005-2006), where HEU children had lower antibody levels than HIV-unexposed children (39), we did not observe such differences at 4.5 years of age. Possible reasons include that HEU children may have experienced natural boosting of measles antibody titers after exposure to wild-type measles infection (393), especially during the measles outbreak in 2009 in South Africa, which occurred prior to the sampling point for this study (133,136). Also, immune system aberrations of HEU children could resolve after the first two years of life (18). Other studies have also reported that HEU children produce robust anamnestic antibody responses to measles vaccination (34,82,115,327,335,342).

We selected for participants with CD4+ percentage ≥25%, which reduces the generalisability of our findings to HIV-infected children who are already severely immunocompromised by 4-8 weeks of age. Furthermore, we did not assess cellular immunity or avidity of the antibody response. The clinical implication of waning measles antibodies in HIV-infected children in whom ART has been interrupted remains uncertain. Strengths of this study include the early administration of ART in HIV-infected children as per current guidelines, the randomized nature of the ART initiation and interruption, the large sample size, and length of follow-up.

In order to achieve measles elimination by 2020, as targeted by the WHO, it is essential for HIV-infected children to receive timely and complete measles vaccination, in addition to early and continuous ART. This study showed that waning of immunity occurs in HIV-infected children, in particular if ART has been interrupted,

but not in HEU children. In a real-world situation, this may happen with poor adherence or missed visits. To prevent measles outbreaks and achieve sufficient levels of population immunity, HIV-infected children and adolescents may need supplemental immunization, especially if they were not on ART at the time of vaccination, or are not currently on ART.

Chapter 5 Immunogenicity and safety of an early measles vaccination schedule at 6 and 12 months of age in HIV-unexposed and HIV-exposed uninfected South African children

5.1 Abstract

Background: Measles morbidity and mortality are greatest in children <12 months old, with increased susceptibility in HIV-exposed children. We evaluated the immunogenicity and safety of an early 2-dose measles vaccine regimen administered at 6 and 12 months of age in South Africa.

Methods: HIV-unexposed (n=212) and HIV-exposed uninfected (HEU) (n=71) children received measles vaccination (CAM-70) at 6 and 12 months of age. Measles immunoglobulin G titers were measured by enzyme-linked immunosorbent assay before and one month after each vaccine dose.

Results: The majority of children (88.2% HIV-unexposed, 95.8% HEU; p=0.044) were seronegative (<150 mIU/mL) to measles at 4.2 months of age. This was particularly evident among infants of mothers born after 1992 (year of public nation-wide measles vaccine availability). One-month post-first measles vaccine, 42.3% HIV-unexposed and 46.4% HEU children were seropositive (≥330 mIU/mL). Post-second dose, the proportion seropositive increased to 99.0% in HIV-unexposed and 95.3% in HEU children. Safety profiles were similar between HIV-unexposed and HEU children.

Conclusions: Early 2-dose measles vaccination at 6 and 12 months of age was safe and induced antibody responses in HIV-unexposed and HEU children, which could partly offset the early loss of maternally derived antibodies in infants born to predominantly measles-vaccinated mothers.

5.2 Introduction

Measles virus infection remains an important cause of vaccine-preventable deaths. An estimated 110 000 deaths were attributed to measles globally in 2017, despite an 84% decline in measles mortality between 2000 and 2016 (394,395). Measles-associated morbidity and case-fatality rates are highest among children <12 months of age (131–134). The majority of children are susceptible to measles infection before reaching the age of routine measles immunization (127–130). In South Africa, during a measles outbreak in 2009-2011, 24% (4,284/17,530) of laboratory-confirmed cases were identified among children aged <9 months, with age-specific incidence of 302/100 000 in children <6 months, 1083/100 000 6 to 8 months, 724/100 000 8 to 11 months and 54/100 000 ≥5 years of age (136).

Measles vaccine (MV) was recommended for inclusion in public immunization programs (PIP) of low middle-income countries during the 1970s and 1980s by the World Health Organization (WHO)'s Expanded Program on Immunization (103). Vaccination coverage increased to 73% in the WHO-African Region since 2000 as per WHO/UNICEF Estimates of National Immunization Coverage (396). Consequently, current immunity against measles among women of child-bearing age is more likely to be derived from MV during childhood than from immunity acquired through previous natural infection. Children born to women who derived immunity mainly through vaccination have lower transplacental acquisition of measles antibodies from their mothers (138) and become susceptible to measles as early as 3.3 months of age (397). Furthermore, children born to HIV-infected women are at heightened risk of measles infection due to reduced transplacental transfer of measles-specific antibodies (22,26,27,129,398). The lower concentration of transplacentally-acquired antibody may, however, lend itself to earlier measles

vaccination in infancy, with immunogenicity less likely to be impeded by interference of maternally-derived antibody (64). Vaccinating at an earlier age could mitigate serious measles complications during early- and mid-infancy (137,141). Early vaccination at 6 months of age, however, induced lower antibody levels than vaccination at 9 or 12 months (399).

In settings with high incidence of measles and HIV-infection, WHO recommends a supplementary dose of MV from 6 months of age, followed by two doses at the recommended ages (usually at 9-12 and 15-18 months of age) (55). South Africa introduced MV in the PIP in 1983, but it only became widely available since 1992 (400). Until 2015, MV (Schwarz-strain) was administered at 9 and 18 months of age. As of December 2015, South Africa implemented an early 2-dose MV schedule of a CAM-70 strain (Measbio) administered at 6 and 12 months of age (144). While Schwarz was derived from the Edmonston strain, CAM-70 was developed from a Japanese wild-type isolate (401). The reasons for lowering the age of vaccination included the high incidence of measles in children aged <9 months during the outbreak in 2009-2011, and regulatory restrictions regarding co-administration of Measbio with other vaccines (personal correspondence: South African National Advisory Group on Immunization, S.A. Madhi).

HIV-seroprevalence rates in pregnant women in South Africa ranks amongst one of the highest in the world, with rates of approximately 30% between 2005 and 2015 (3). Because of effective prevention of mother-to-child transmission programs, an increasing proportion of South African children born to these mothers are HIV-exposed but uninfected (HEU) (16). In a recent systematic review, HEU children showed similar serological response when vaccinated at 6 months compared with

HIV-unexposed, with 68% and 94% being seropositive after the first and second dose, respectively (389).

The limited number of serological studies on early measles vaccination in HEU and HIV-unexposed children in low- and middle-income countries, highlight the need to provide evidence for the current South African recommendations. This study aimed to evaluate the immunogenicity and safety of 2-dose MV regimen administered at 6 and 12 months of age to HIV-unexposed and HEU children.

5.3 Methods

Study Design

This prospective observational cohort study included HIV-unexposed children co-enrolled in a randomized, open-label trial evaluating non-inferiority of two versus three doses of pneumococcal-conjugate vaccine (PCV) (NCT02943902) and a parallel cohort of HEU children (NCT03330171). Children were identified from hospital birth registers, postnatal wards and neighbouring primary health clinics and invited for screening at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), based at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa. Healthy children aged 6-18 weeks, ≥37 weeks' gestation at birth and birth weight >2499 grams were eligible for enrolment. Criteria for inclusion, exclusion and classification of HIV status are listed in chapter 2.3.3.

Participants were vaccinated under the current South African recommendations with subcutaneous injection of live attenuated MV (MeasBio, BioFarma, Bandung Indonesia) at 6 (182 ± 14 days) and 12 months (365 ± 14 days) of age. Participants received other childhood vaccines according to the PIP, except for the randomization of HIV-unexposed children to different PCV schedules in the parent protocol.

Assessment of Outcomes

Venous blood samples were collected from all participants approximately two months prior to the first measles dose (MV1) (4.2 months, 126 ± 14 days of age), one month post-MV1 (7 months, 28-35 days post-vaccination), prior to the second measles dose (MV2) (12 months; 365 ± 14 days) and one month post-MV2 (13 months, 28-35 days post-vaccination). Children were observed after each vaccine injection for 30 minutes. Safety evaluation for solicited adverse events was only included in the protocol as an amendment implemented in March 2017, resulting in 102/278 (37%) children with safety evaluation following MV1 and 260/262 (99%) children following MV2. Parents were provided with a vaccination report card to report local injection site (pain/tenderness, redness, swelling and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) symptoms on a daily basis for 7 days after each injection. Adverse events were graded on a 1-3 scale (and a 1-4 scale for fever) using symptom-specific definitions outlined in the vaccination report card (described below). Serious adverse events (SAEs) were documented throughout the study.

Laboratory methods

Blood samples were centrifuged and sera stored at -70ºC at the RMPRU laboratory, until testing. Measles immunoglobulin G (IgG) antibody levels were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Enzygnost, Dade Behring, Marburg, Germany) according to the manufacturer's instructions and detailed in chapter 2. Optical density values were converted to milli-International Units per milliliter (mIU/mL) using the α-method and calibration against the first measles antigen WHO International Reference Preparation (266,267). Measles

seronegativity was defined as IgG titers <150 mIU/mL (optical density [OD] <0.1), equivocal as titers 150-329 mIU/mL (OD 0.1-0.2), seropositivity as titers ≥330 mIU/mL (OD >0.2) and seroconversion as the change from seronegative pre-vaccination to seropositive post-vaccination. All equivocal samples were re-tested. If the result was confirmed, the samples were classified as equivocal, otherwise as positive or negative. Seronegative samples were assigned a titer half the value of the assay's detection limit (i.e. 75 mIU/mL).

Statistical analyses

The sample size was calculated based on a significance level of 5% (two-sided), 80% power, 1:3 ratio of HEU to HIV-unexposed children and a hypothesized 10% lower seropositivity rate between HEU and HIV-unexposed children after two doses of MV. The sample size was adjusted upward by 10% to account for loss to follow-up, resulting in a total minimal sample size of 270 participants.

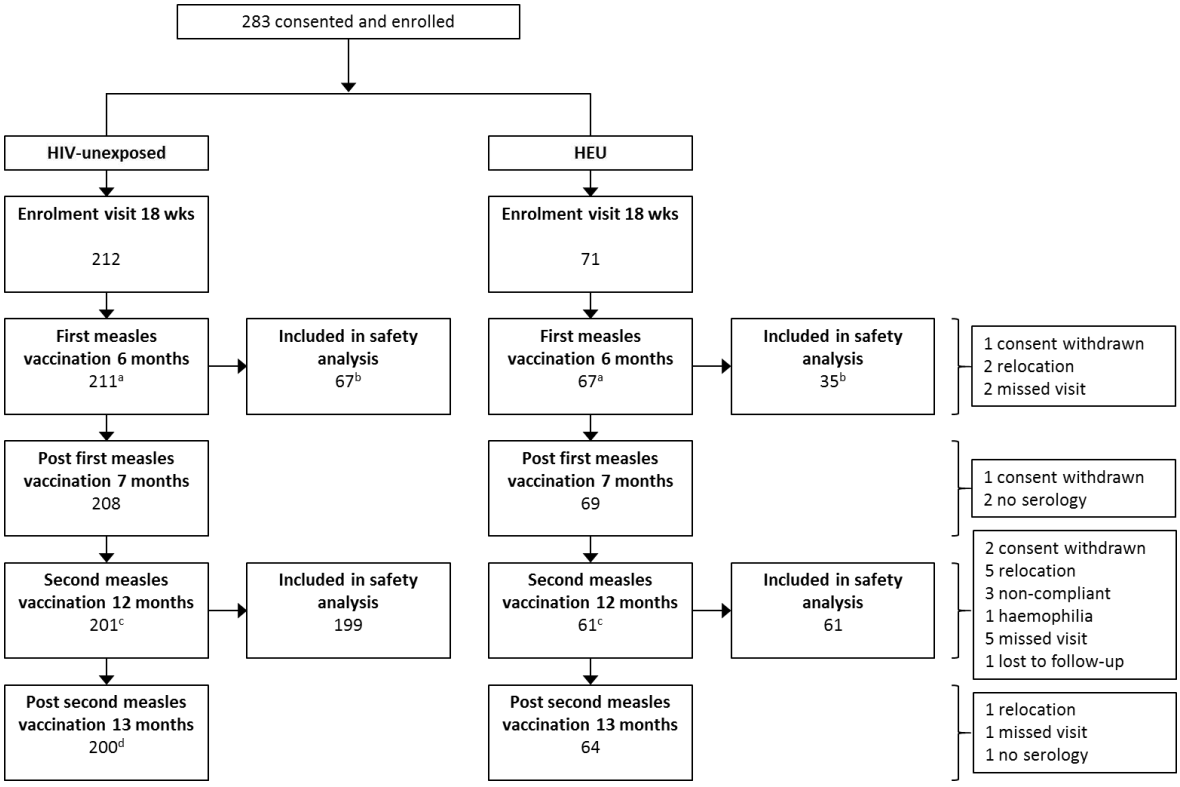
Geometric mean titers (GMT) of measles antibody concentrations and 95% confidence intervals (CI) were calculated following natural logarithmic transformation of titer values and were compared between the two study-groups by multivariable linear regression using the following covariates: sex, race, maternal age, antibody levels pre-MV1 and age at the serology visit. Comparison of the proportion of participants meeting the putative thresholds for seronegativity, seropositivity and seroconversion was done by multivariable logistic regression, adjusting for the abovementioned covariates. The association between maternal age in years or classified as maternal year of birth before 1992 or after 1992 (the year of wide public MV availability) and proportion of seronegative or seropositive children at pre-MV1 visit was explored using logistic regression, adjusting for HIV-exposure. Pre- and

post-MV GMTs were correlated using Spearman's correlation and the association between percentage seronegative pre- and post-MV evaluated using (exact) logistic regression. Safety analysis included the proportion of children with at least one event (including solicited local and systemic reactions, and serious adverse events) and the proportion with solicited grade 3 events. P-values of <0.05 were considered significant. Analysis was by modified intention-to-treat with all participants included if antibody results were available. A per-protocol analysis was performed including only those children who were vaccinated or had bloods collected within the protocol defined time-periods. Stata13 (StataCorp, LP, Texas, USA) and R (version 3.5.1) were used.

5.4 Results

From April to October 2017, 283 children were enrolled in the study, including 212 HIV-unexposed (75%) and 71 HEU (25%); Figure 5.1. Baseline characteristics at study initiation did not differ between HIV-unexposed and HEU children, except for mothers of HEU children being older (30.7 vs. 27.9 years); Table 5.1. Baseline characteristics of HIV-unexposed children who consented to the measles study were not significantly different from those who only enrolled in the main parent study (data not shown). Overall, the mean age at pre-MV1 serology visit was 4.2 (standard deviation ±0.2) months, at MV1 visit 6.0 (±0.1) months, at post-MV1 serology visit 7.0 (±0.1) months, at MV2 visit 12.0 (±0.2) months and at post-MV2 serology visit 13.0 (±0.2) months. HIV-unexposed children were slightly younger at the vaccination and serology visits compared to HEU (-0.1 month); Table 5.1.

Figure 5.1 Flow diagram of study participants



[a] 2 HEU children missed a visit at time of the first measles vaccination and were vaccinated at the local clinic; [b] Safety analysis was completed in a subset of participants at first measles vaccination, due to diary card introduction during the course of the study; [c] 4 HEU and 1 HIV-unexposed child missed a visit at time of the second measles vaccination and were vaccinated at the local clinic; [d] 1 HIV-unexposed child missed the visit at time of serology post second measles dose.

Table 5.1 Demographics and study participants' characteristics at enrolment

Characteristic	Total	HIV-unexposed	HIV-exposed uninfected
	n = 283	n = 212	n = 71
Male, n (%)	143 (51)	108 (51)	35 (49)
Black ethnicity, n (%)	281 (99)	210 (99)	71 (100)
Multiracial ethnicity, n (%)	2 (1)	2 (1)	0 (0)
Mean birthweight, grams (SD)	3227 (423)	3242 (442)	3181 (362)
Mean maternal age at delivery, years (SD)	28.6 (6.1)	27.9 (5.9)[a]	30.7 (6.4)[a]
Mean age, months (SD)	4.2 (0.2)	4.2 (0.2)	4.2 (0.2)
Primary measles dose	n = 278	n = 211	n = 67
Mean age, months (SD)	6.0 (0.1)	6.0 (0.1)[a]	6.1 (0.2)[a]
Serology post primary measles dose	n = 277	n = 208	n = 69
Mean age, months (SD)	7.0 (0.1)	6.9 (0.1)[a]	7.0 (0.2)[a]
Serology prior to booster measles dose	n = 262	n = 201	n = 61
Mean age, months (SD)	12.0 (0.2)	12.0 (0.2)[a]	12.1 (0.3)[a]
Booster measles dose	n = 267	n = 202	n = 65
Mean age, months (SD)	12.1 (0.2)	12.0 (0.2)[a]	12.1 (0.3)[a]
Serology post booster measles dose	n = 264	n = 200	n = 64
Mean age, months (SD)	13.0 (0.2)	13.0 (0.2)[a]	13.1 (0.3)[a]

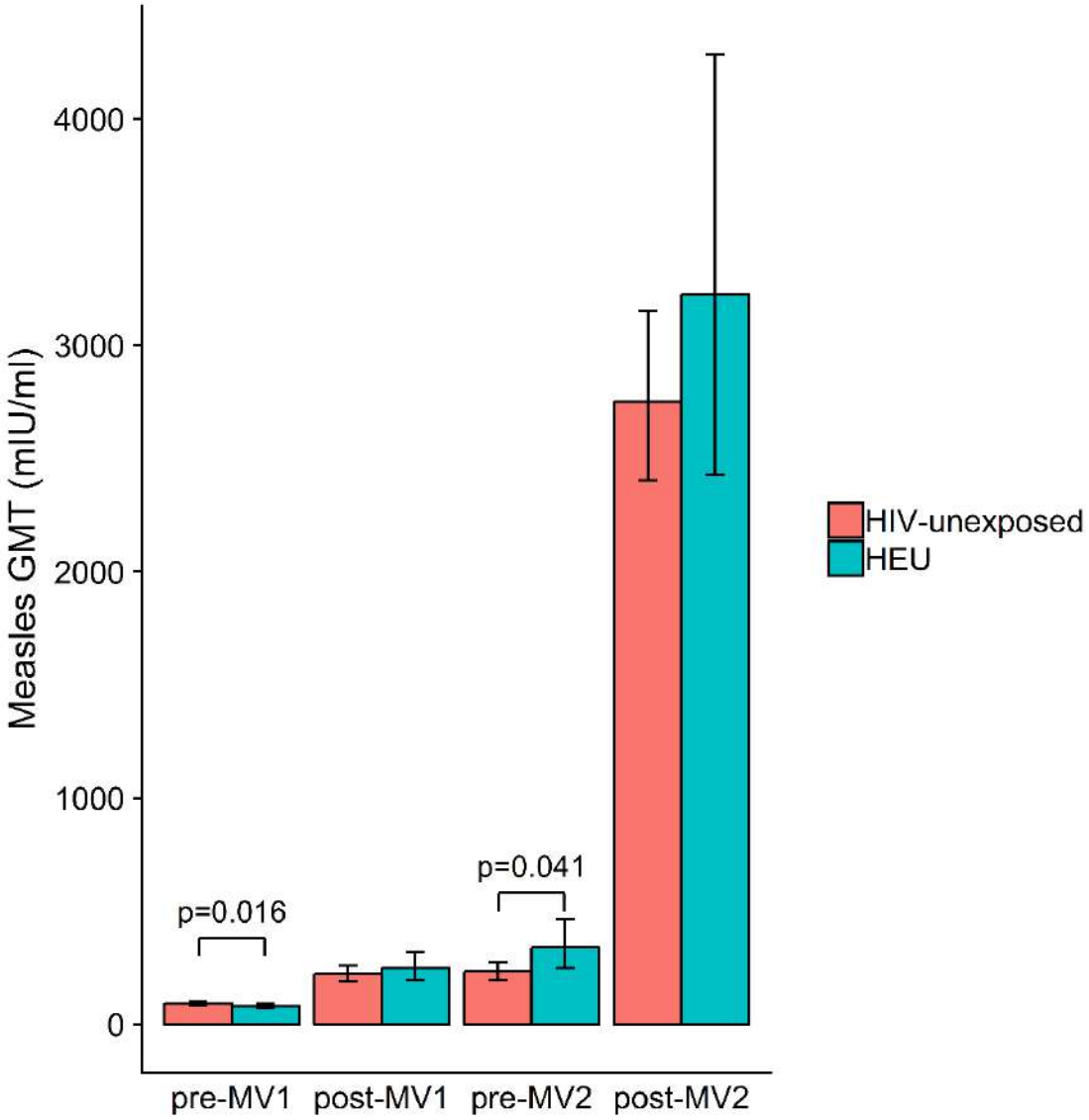
Abbreviation: SD, standard deviation; [a] difference $p<0.05$.

Measles antibodies titers

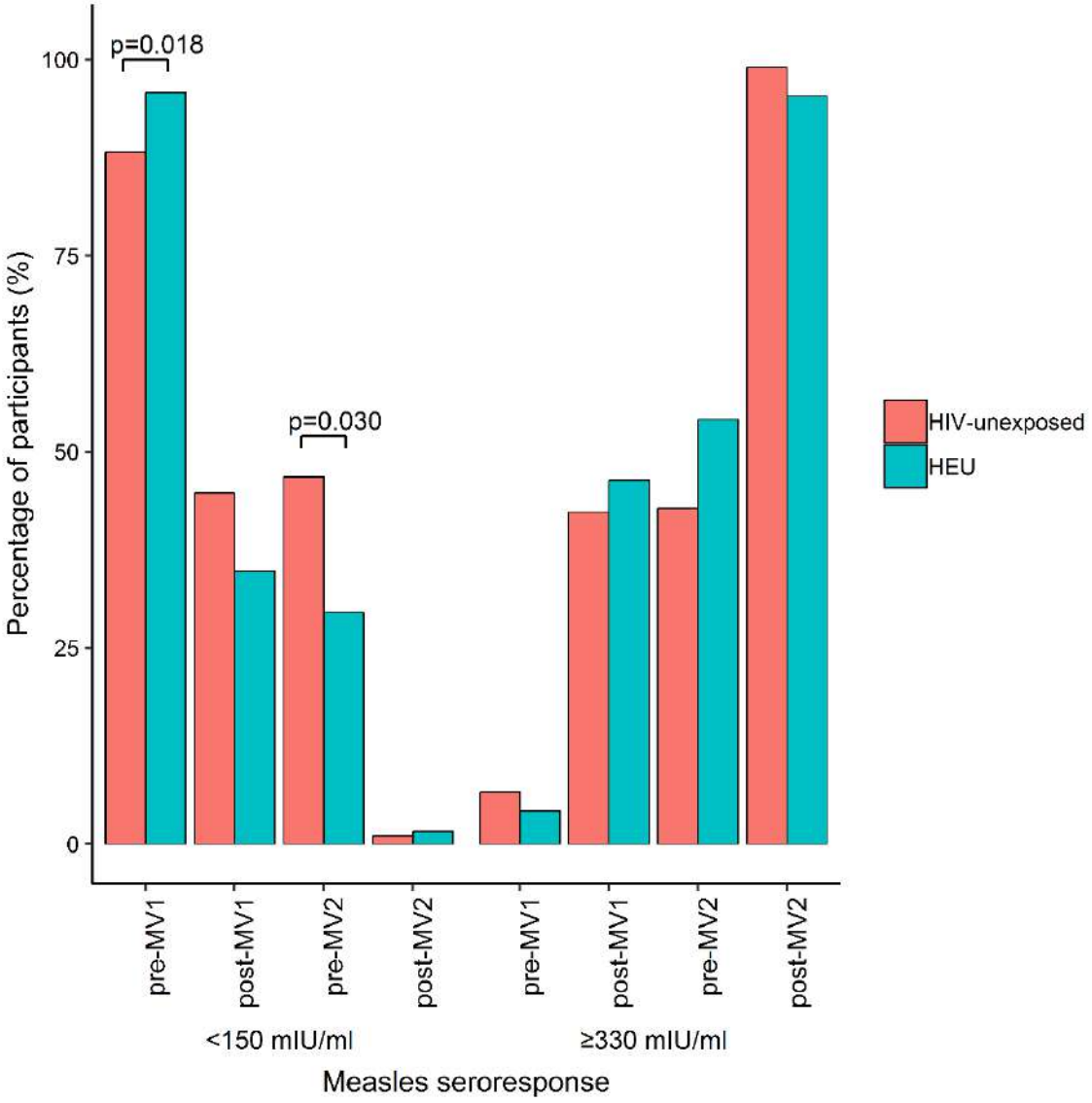
In analyses adjusted for sex, race, maternal age, antibody levels pre-MV1 (only for subsequent time points) and age at serology, HIV-unexposed children had higher GMTs than HEU children prior to MV1 (93 mIU/mL; 95%CI: 85-102 vs. 82 mIU/mL; 95%CI: 74-91; p=0.016). GMTs were similar between HIV-unexposed and HEU children post-MV1 (223 mIU/mL; 95%CI: 191-260 vs. 251 mIU/mL; 95%CI: 197-319) and post-MV2 (2751 mIU/mL; 95%CI: 2402-3152 vs. 3226 mIU/mL; 95%CI: 2429-4286). Prior to MV2, GMTs were, however, lower in HIV-unexposed children (233 mIU/mL; 95%CI: 196-277) than HEU (340 mIU/mL; 95%CI: 249-464; p=0.041); Figure 5.2a, Table 5.2.

Figure 5.2 Measles antibody responses before and after first and second measles vaccination

A) Measles antibody geometric mean titers



B) Proportion seronegative and seropositive children



P-values were either calculated by linear or logistic regression and adjusted for sex, race, maternal age, measles antibody levels prior to the first measles dose and age at serology.

Table 5.2 Measles antibody geometric mean titers and proportion of seropositive children pre- and post-first and second measles vaccination

Measure	**HIV-unexposed**	**HEU**	**Total**
Pre-measles dose 1	n = 212	n = 71	n = 283
GMT (95% CI)	93 (85-102)[a]	82 (74-91)[a]	90.0 (84-97)
n (%) <150 mIU/ml	187 (88.2)[b]	68 (95.8)[b]	255 (90.1)
n (%) ≥330 mIU/ml	14 (6.6)	3 (4.2)	17 (6.0)
Post-measles dose 1	n = 208	n = 69	n = 277
GMT (95% CI)	223 (191-260)	251 (197-319)	230 (202-261)
n (%) <150 mIU/ml	93 (44.7)	24 (34.8)	117 (42.2)
n (%) ≥330 mIU/ml	88 (42.3)	32 (46.4)	120 (43.3)
n (%) Seroconversion[c]	90 (48.1)	34 (50.0)	124 (48.6)
Pre-measles dose 2	n = 201	n = 61	n = 262
GMT (95% CI)	233 (196-277)[d]	340 (249-464)[d]	254 (218-296)
n (%) <150 mIU/ml	94 (46.8)[e]	18 (29.5)[e]	112 (42.8)
n (%) ≥330 mIU/ml	86 (42.8)	33 (54.1)	119 (45.4)
Post-measles dose 2	n = 200	n = 64	n = 264
GMT (95% CI)	2751 (2402-3152)	3226 (2429-4286)	2860 (2528-3235)
n (%) <150 mIU/ml	2 (1.0)	1 (1.6)	3 (1.1)
n (%) ≥330 mIU/ml	198 (99.0)	61 (95.3)	259 (98.1)
n (%) Seroconversion[f]	92 (97.9)[g]	15 (83.3)[g]	107 (95.5)

P-values were either calculated by linear or logistic regression and adjusted for sex, race, maternal age, measles antibody levels prior to the first measles dose and age at serology; Seronegativity was defined as an immunoglobulin G titer of <150 mIU/mL; Seropositivity was defined as an immunoglobulin G titer of ≥330 mIU/mL; Seroconversion was defined as a change from titers ≤150 mIU/mL pre-vaccination to ≥330 mIU/mL post-vaccination;

[a] p-value of 0.016; [b] p-value of 0.018; [c] A total of 255 children had titers <150 mIU/mL pre-MV1, of which 187 were HIV-unexposed and 68 HIV-exposed uninfected; [d] p-value of 0.041; [e] p-value of 0.030; [f] A total of 112 children had titers <150 mIU/mL pre-MV1, of which 94 were HIV-unexposed and 18 HIV-exposed uninfected; [g] p-value of 0.010.

At the pre-MV1 visit (mean age of 4.2 months), more HEU children were seronegative (titers <150 mIU/mL; 95.8%) compared with HIV-unexposed (88.2%; p=0.018); and only 4.2% and 6.6%, respectively, were seropositive (≥330 mIU/ml); Figure 5.2b, Table 5.2.

One month post-MV1, the percentages seropositive (42.3% HIV-unexposed and 46.4% HEU) and that seroconverted (48.1% HIV-unexposed and 50.0% HEU) were similar between HIV-unexposed and HEU groups.

Five months later, prior to MV2 at 12 months of age, 42.8% of HIV-unexposed and 54.1% (p=0.340) of HEU were seropositive; whereas 46.8% and 29.5%, respectively, were seronegative (p=0.030). The percentage of seropositive children increased to 99.0% in HIV-unexposed and 95.3% in HEU children one-month post-MV2; and only 1.0% and 1.6%, respectively, remained seronegative; Figure 5.2b, Table 5.2. Post-MV2, seroconversion rates from pre-MV2 were higher in HIV-unexposed (97.9%) than in HEU (83.3%; p=0.010).

Per-protocol analysis, excluding children who were vaccinated or had blood collected outside the protocol defined time-periods, resulted in similar results, except that differences between HIV-unexposed and HEU in pre-MV2 GMTs became marginally significant (p=0.055).

At 4.2 months of age (pre-MV1), maternal age was inversely associated with the percentage of seronegative children (adjusted odds ratio [aOR] 0.88; 95%CI: 0.82-0.94; p<0.001) and positively associated with percentage of seropositive children (aOR 1.17; 95%CI: 1.07-1.27; p<0.001); Table 5.3. Pre-vaccination, 0% (0/91) of infants of women born after 1992 were seropositive, compared to 8.9% (17/192) in infants of mothers born before 1992. Furthermore, a positive association was

observed between year of maternal birth category and percentage of seronegative children (aOR 5.01; 95%CI: 1.46-17.17; p=0.010), when adjusted for HIV-exposure; Table 5.3.

Table 5.3 Association of maternal age with percentage of seronegative and seropositive children prior to the first measles dose

Characteristic	Nonseronegative (n=28)	Seronegative[a] (n=255)	Adjusted OR (95% CI) for seronegativity[a]	p-value
Mean maternal age, months (SD)	32.3 (5.3)	28.2 (6.1)	0.88 (0.82-0.94)	<0.001
Maternal year of birth, n (%) <1992 ≥1992	 25 (89%) 3 (11%)	 167 (65%) 88 (35%)	 Ref. 5.01 (1.46-17.17)	 0.010
	Nonseropositive (n=266)	Seropositive[b] (n=17)	Adjusted OR (95% CI) for seroprotection[b]	p-value
Mean maternal age, months (SD)	28.3 (6.1)	33.6 (4.3)	1.17 (1.07-1.27)	<0.001
Maternal year of birth, n (%) <1992 ≥1992	 175 (66%) 91 (34%)	 17 (100%) 0 (0%)	 NA	 NA

Odds ratios were adjusted for HIV-exposure; Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio; Ref., reference group; [a] Seronegativity was defined as an immunoglobulin G titer of <150 mIU/mL; [b] Seropositivity was defined as an immunoglobulin G titer of ≥330 mIU/mL.

There was a negative correlation between pre- and post-MV1 GMTs (Spearman's correlation coefficient -0.27; p<0.001); Figure 5.3. Similarly, children with undetectable antibody levels pre-vaccination were more likely to have titers ≥150 mIU/mL (aOR 9.67; 95%CI: 3.25-28.84; p<0.001) or be seropositive post-MV1 (aOR 11.63; 95%CI: 2.70-50.20; p=0.001). A positive correlation was found between pre- and post-MV2 GMTs (Spearman's correlation coefficient 0.50; p<0.001); Figure 5.4.

Figure 5.3 Scatterplot of log transformed pre-MV1 (18 weeks) and post-MV1 (7 months) measles antibody titers

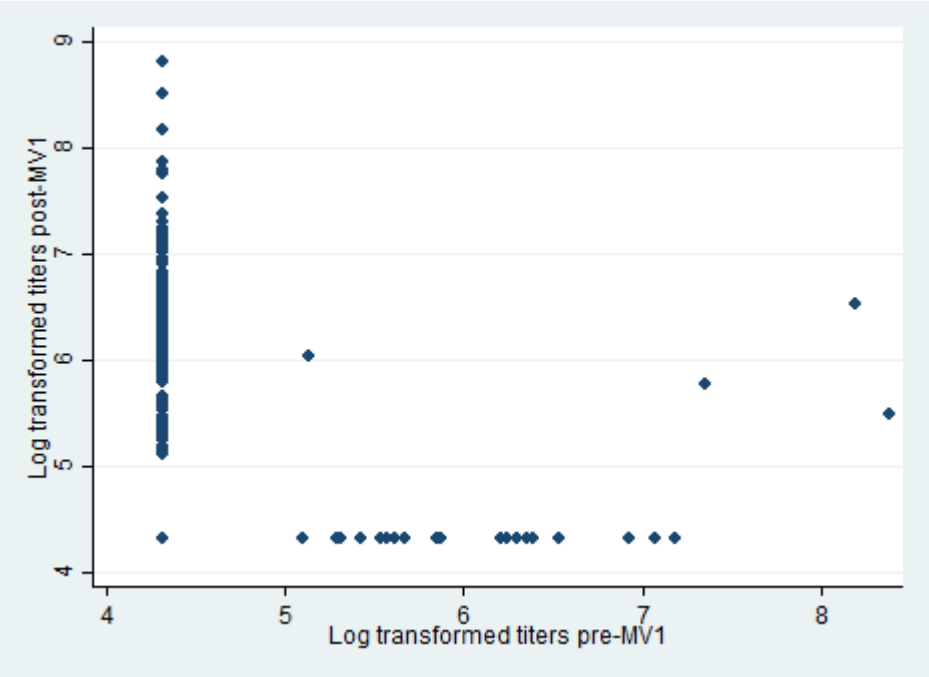
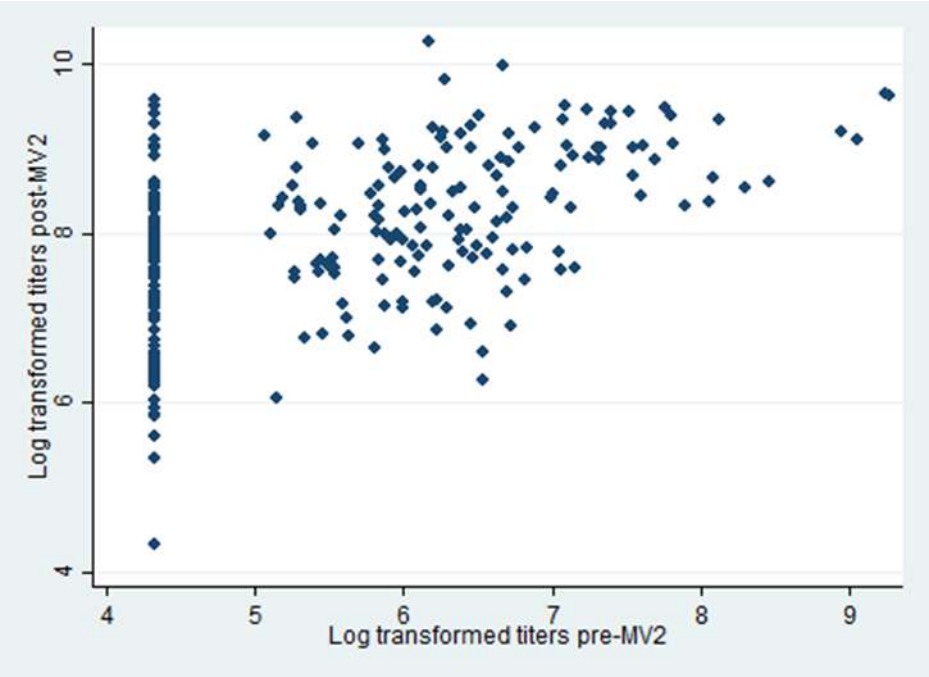


Figure 5.4 Scatterplot of log transformed pre-MV2 (12 months) and post-MV2 (13 months) measles antibody titers



Safety

The frequency and severity of solicited local and systemic reactions during the 7 days after each measles vaccination were similar between HIV-unexposed and HEU children; Table 5.4. Most children showed no solicited reactions. Post-MV1, no grade 3 local injection site reactions occurred, whereas 9% (6/67) HIV-unexposed and no HEU children experienced grade 3 systemic reactions. Post-MV2, grade 3 local reactions were recorded in 1% (2/199) HIV-unexposed and 5% (3/61) HEU children; and grade 3 systemic adverse events were recorded in 9% (17/199) HIV-unexposed and 10% (6/61) HEU children. The most common local reactions were pain/tenderness and redness. Common systemic adverse events were decreased appetite and irritability in HIV-unexposed, and decreased appetite and decreased activity in HEU children; Table 5.5. Pain/tenderness post-MV1 was higher in HEU (mild 23%, moderate 6%) compared to HIV-unexposed children (mild 16%; p=0.006).

Thirty SAEs occurred throughout the study, of which two occurred in HIV-unexposed children within 28 days after measles injection; Table 5.4. One child had bronchopneumonia and otitis media with onset 9 days post-MV1; another child had bronchiolitis and otitis media with onset 20 days post-MV2; Table 5.6. All SAEs had mild or moderate severity and none were classified as MV related. No deaths occurred. None of the HIV-exposed children became HIV-positive during the study period.

Table 5.4 Reported solicited local and systemic reactions and unsolicited serious adverse events following immunization with measles vaccine at 6 and 12 months of age by HIV-exposure

n/N (%)	HIV-unexposed	HIV-exposed uninfected
Solicited reactions during first 7 days after first measles dose		
Local reactions		
Any	17/67 (25)	12/35 (34)
Severe	0/67 (0)	0/35 (0)
Systemic reactions		
Any	36/67 (54)	15/35 (43)
Severe	6/67 (9)	0/35 (0)
Solicited reactions during first 7 days after second measles dose		
Local reactions		
Any	49/199 (25)	14/61 (23)
Severe	2/199 (1)	3/61 (5)
Systemic reactions		
Any	106/199 (53)	27/61 (44)
Severe	17/199 (9)	6/61 (10)
Unsolicited serious adverse event after measles vaccination[a]		
≤28 days after injection	2/211 (1)	0/67 (0)
Throughout the study period	24/211 (11)	6/67 (9)
Per study participant	20/211 (9)	4/67 (6)
Related to measles vaccination	0/211 (0)	0//67 (0)

Abbreviations: N, total number of participants with vaccination report card / serious adverse event assessment; n, number of participants having a reaction; [a] Serious adverse events could occur more than once per participant.

Table 5.5 Frequency of solicited adverse events (maximum severity per participant[a]) during the first seven days following first and second measles vaccination

	First measles dose		**Second measles dose**	
Adverse events, n (%)	**HIV-unexposed (n=67)**	**HIV-exposed uninfected (n=35)**	**HIV-unexposed (n=199)**	**HIV-exposed uninfected (n=61)**
Injection site				
Pain/tenderness[b]				
Grade 1 (mild)	11 (16%)[c]	8 (23%)[c]	31 (16%)	9 (15%)
Grade 2 (moderate)	0	2 (6%)	7 (4%)	2 (3%)
Grade 3 (severe)	0	0	1 (1%)	2 (3%)
Redness[d]				
Grade 1 (mild)	9 (13%)	3 (9%)	27 (14%)	3 (5%)
Grade 2 (moderate)	0	0	2 (1%)	1 (2%)
Grade 3 (severe)	0	0	0	1 (2%)
Swelling[e]				
Grade 1 (mild)	3 (4%)	4 (11%)	19 (10%)	4 (7%)
Grade 2 (moderate)	0	0	1 (1%)	1 (2%)
Grade 3 (severe)	0	0	0	1 (2%)
Itching[f]				
Grade 1 (mild)	3 (4%)	2 (6%)	13 (7%)	4 (7%)
Grade 2 (moderate)	0	0	3 (2%)	1 (2%)
Grade 3 (severe)	0	0	1 (1%)	2 (3%)
Systemic				
Fever[g]				
Grade 1 (mild)	1 (1%)	1 (3%)	9 (5%)	3 (5%)
Grade 2 (moderate)	0	0	8 (4%)	0
Grade 3 (severe)	0	0	0	1 (2%)
Grade 4 (potentially life threatening)	0	0	0	0
Vomiting[h]				
Grade 1 (mild)	13 (19%)	9 (26%)	31 (16%)	11 (18%)
Grade 2 (moderate)	4 (6%)	0	8 (4%)	0
Grade 3 (severe)	0	0	5 (3%)	1 (2%)
Decreased appetite[i]				
Grade 1 (mild)	16 (24%)	8 (23%)	63 (32%)	13 (21%)
Grade 2 (moderate)	8 (12%)	4 (11%)	30 (15%)	10 (16%)
Grade 3 (severe)	2 (3%)	0	9 (5%)	4 (7%)
Irritability[j]				
Grade 1 (mild)	23 (34%)	3 (9%)	45 (23%)	7 (11%)
Grade 2 (moderate)	3 (4%)	3 (9%)	19 (10%)	6 (10%)
Grade 3 (severe)	5 (7%)	0	6 (3%)	1 (2%)
Decreased activity[k]				
Grade 1 (mild)	18 (27%)	3 (9%)	37 (19%)	10 (16%)
Grade 2 (moderate)	4 (6%)	3 (9%)	20 (10%)	7 (11%)
Grade 3 (severe)	1 (1%)	0	4 (2%)	1 (2%)

[a] adverse events were solicited during 7 days following vaccination; an event could last several days, but is counted only once and is reported by its maximum severity per participant;

[b] mild: Injection spot is sore or painful when touched, but still able to move leg as usual or only slightly less than usual; moderate: Injection spot is sore or painful when touched, and moving leg a lot less than usual; Severe: Injection spot is sore or painful when touched and unable to move the leg OR needing hospitalization because of the pain;

[c] p-value of 0.006;

[d] mild: Redness <2,5 cm in diameter; moderate: Redness >2,5 cm in diameter, but not more than half the thigh surface; severe: More than half the thigh surface is red, or if it forms an abscess or sore at the injection spot;

[e] mild: Swelling <2,5 cm in diameter; moderate: Swelling >2,5 cm in diameter, but not more than half the thigh surface; severe: More than half the thigh surface is swollen;

[f] mild: Itching at the injection spot which goes away by itself or with medicine taken for less than 48 hours; moderate: Itching not only at the injection spot, but the rest of the leg as well but not the whole body OR itching at the injection spot that needs medicine for more than 48 hours; severe: Itching of the whole body, preventing normal activity;

[g] mild: 38 to <38.6ºC; moderate: 38.6 to <39.3ºC; severe: 39.3 to <40.0ºC; potentially life threatening: ≥40.0ºC;

[h] mild: 1-2 episodes/24 hr; moderate: 3-4 episodes/24 hr; severe: >4 episodes/24 hr;

[i] mild: Not interested in the food but still eats as much as usual; moderate: Not interested in food and eating less than usual; severe: Not interested in food, eating less than usual and losing weight;

[j] mild: Crying more than usual, but easily consoled; moderate: Crying more than usual and difficult to console, but eventually settles; severe: Inconsolable, continuous crying for 4 hours or more;

[k] mild: Not as alert as usual, but still responds and plays normally; moderate: Not as alert as usual and does not respond or want to play as usual; severe: Sleepy all the time. Does not want to play at all.

Table 5.6 Serious adverse events reported after measles vaccine administration until the end of the study period

Serious adverse event description	Onset of symptoms	Severity	Outcome
HIV-unexposed			
Bronchopneumonia, otitis media	9 days post-MV1	moderate	Resolved
Bronchiolitis, otitis media	20 post-MV2	mild	Resolved
Febrile seizures	>28 days post-MV2	moderate	Resolved
Right clavicle fracture	>28 days post-MV1	mild	Resolved
Bronchopneumonia, acute gastro-enteritis	1 day pre-MV1[a]	moderate	Resolved
Acute gastro-enteritis[b]	>28 days post-MV1	moderate	Resolved
Suspected septic arthritis[c]	>28 days post-MV1	moderate	Resolved
Lymphadenitis submandibular	>28 days post-MV1	mild	Resolved
Bronchiolitis	>28 days post-MV2	mild	Resolved
Pulmonary tuberculosis, severe acute malnutrition[d]	>28 days post-MV1	moderate	Resolved
Bronchiolitis	>28 days post-MV2	mild	Resolved
Acute gastro-enteritis[e]	>28 days post-MV1	moderate	Resolved
Bronchiolitis	>28 days post-MV2	mild	Resolved
Acute gastro-enteritis	>28 days post-MV1	moderate	Resolved
Pulmonary tuberculosis, Generalised desquamating dermatitis, severe acute malnutrition[d]	>28 days post-MV1	moderate	Continuing at protocol termination
Bronchopneumonia[c]	>28 days post-MV2	moderate	Resolved
Pulmonary tuberculosis	>28 days post-MV2	moderate	Continuing at protocol termination
Pneumonia[b]	>28 days post-MV1	moderate	Resolved
Febrile seizures, upper respiratory tract infection	>28 days post-MV1	moderate	Resolved
Febrile seizures, upper respiratory tract infection	>28 days post-MV2	moderate	Resolved
Dysentery	>28 days post-MV2	moderate	Resolved
Bronchiolitis	>28 days post-MV2	mild	Resolved
Pulmonary tuberculosis[e]	>28 days post-MV1	moderate	Resolved
Herpetic gingivostomatitis	>28 days post-MV2	mild	Resolved
HIV-exposed			
Laryngotracheobronchitis[f]	>28 days post-MV1	moderate	Resolved
Acute gastro-enteritis	>28 days post-MV1	moderate	Resolved
Acute gastro-enteritis[g]	>28 days post-MV1	moderate	Resolved
Pneumonia[g]	>28 days post-MV2	moderate	Resolved
Laryngotracheobronchitis, urinary tract infection[f]	>28 days post-MV2	moderate	Resolved
Pulmonary tuberculosis	>28 days post-MV2	moderate	Continuing at protocol termination

Abbreviations: MV1, first measles dose; MV2, second measles dose;

[a] Child was hospitalized four days after measles vaccination, but onset of symptoms was one day before measles vaccination; [b] Occurred in the same participant; [c] Occurred in the same participant; [d] Occurred in the same participant; [e] Occurred in the same participant; [f] Occurred in the same participant; [g] Occurred in the same participant.

5.5 Discussion

The results of this prospective cohort study showed that an early 2-dose measles vaccination schedule administered at 6 and 12 months of age is similarly safe and immunogenic in HIV-unexposed and HEU children. The vast majority (90.1%) of children aged 4.2 months in our study were seronegative for measles antibody. This was especially evident among infants of women born after 1992 (when measles vaccination became widely available in the South African PIP). These findings underscore the importance to reconsider measles vaccination dosing schedules in settings like ours.

Administration of two doses of MV at 6 and 12 months of age resulted in seropositivity rates of 99.0% and 95.3% in HIV-unexposed and HEU children, respectively, one-month post-MV2. Our results corroborate findings from other studies on early measles vaccination regimens in Africa (114,151,327,402,403). Moreover, a Brazilian study from 1990 that examined the humoral response to a CAM-70 strain containing MV administered at 6 and 11 months of age, reported 89% seroconversion rates by immunofluorescence assay and 97% by ELISA following the second dose (150). Yet, the rise in antibody levels induced by a second vaccination may be short-lived and could fall back to pre-boost levels (404). A recent study reported a decrease in long-term concentration and avidity of measles virus-specific neutralizing antibodies after early vaccination compared to vaccination at a later age (405). Hence, durability of the response to early vaccination needs to be established,

to rule out future vaccine failures and to prevent low maternal antibody transfer to future generations.

A high proportion of children had antibody levels below the assay detection limit at 4.2 months of age, similar to an earlier study from our setting (39). Even in areas that have eliminated measles, a number of children may be susceptible to infection before the age of first MV if given at 12 months of age (130,138,139). The increase in measles seronegativity among young children, has in part been attributed to lower levels of transplacental IgG transfer to fetus in women who derived antibody from measles vaccination rather than following natural viral infection (64). In our study, measles antibody seronegativity in infants prior to measles vaccination (4.2 months of age), was associated with younger maternal age and mothers being born after wide implementation of MV into the South African PIP. In addition lower concentrations of measles-specific antibody have been detected in children born to HIV-infected women compared to HIV-unexposed children (22,26,27,129,398), as corroborated by our findings of 95.8% seronegativity in HEU compared with 88.2% in HIV-unexposed prior to measles immunization. A previous study from our setting on cord blood samples collected in 2007 from mother-newborn dyads reported measles seronegativity prevalence of 5.6% in HIV-unexposed (6/107) and 8.7% (17/196) in HEU children (129). This indicates rapid waning of measles antibodies in the first 4 months after birth, thereby creating a group of infants susceptible to measles at a younger age.

Our results that after a single dose of MV at 6 months of age 55.3% HIV-unexposed and 65.2% HEU children had titers ≥150 mIU/mL are in line with a Malawian study on early measles vaccination reporting 62% of HIV-unexposed and 68% of HEU children with titers ≥120 mIU/mL (as measured by enzyme immunoassay) after one

dose of MV (114,327), but are lower than the 77% seroprotected (≥125 mIU/mL as measured by measles hemagglutination inhibition test) reported from Guinea-Bissau (151) and lower than the overall pooled estimate for seropositivity following MV at 6 months (75% [95%CI 68-82]) described in a meta-analysis that included studies until June 2015 (406). The seroconversion rate at age 6 months (48.6%), which may depend on the vaccine strain, was also lower than that reported in the meta-analysis evaluating MV at 6 months (76%, 95%CI: 71-82%) (406). Our findings after vaccination at 6 months are similarly lower when compared to findings from our setting when vaccination was done at 9 months of age with 91.1% of HIV-unexposed and 94.8% of HEU children having seropositive (≥330 mIU/mL as measured by the same ELISA kit) measles titers at 6.6 months post-MV1 (39). Our results suggest that a single early dose of MV is only partially effective in inducing humoral immune responses and administration of the second dose remains essential.

When evaluating the effect of maternal HIV-infection on infant vaccine-induced measles antibody responses, we observed that HEU children generated similar or higher post-MV1 GMTs and similar proportions had titers ≥330 mIU/mL compared with HIV-unexposed. Previous studies on responses to primary vaccination have reported similar findings (22,23,39). This may be explained by the association between pre-vaccination antibody levels in HEU children and a heightened humoral immune response to childhood vaccines, due to reduced interference of maternally acquired antibody (22). Similarly, our study reported pre-vaccination antibody concentrations to be lower in HEU than HIV-unexposed children. Additionally, we found children who were seronegative pre-vaccination to be more likely to have titers ≥150 mIU/mL or be seropositive post-MV1.

We observed an increase in antibody titers between post-MV1 and pre-MV2 study-visits. This could be explained by sub-clinical exposure to wild-type measles virus and avidity maturation. During 2017, a localised measles outbreak occurred in the Gauteng province in South Africa with a total of 96 laboratory-confirmed cases (61). No measles cases were, however, reported from Soweto, the area where most study participants resided. In response to the outbreak, a province-wide supplemental vaccination campaign was conducted from May to June 2017 (61). Only one study participant was reported to have received additional measles vaccination. No participant developed clinical measles infection during the study.

This study examined safety of CAM-70 measles virus vaccine given at 6 months of age. Prior studies have demonstrated the safety of other MV strains when administered before 9 months of age (155,407,408). The WHO states that internationally pre-qualified attenuated MV may be used interchangeably within immunization programs and considers them to be safe and effective (55), but strain-dependent differences in immunogenicity have been described (107). We report that an early 2-dose MV regimen is safe and well tolerated. Frequency of local and systemic adverse events were comparable to previous studies (55,155,389). Grade 3 solicited systemic reactions were reported more often than in a previous study that co-administered a fully liquid hexavalent vaccine and a measles/mumps/rubella and varicella vaccine at 15-18 months of age in healthy South African children (210). The children in our study were vaccinated at younger age, although meta-analysis did not identify increased risk of fever, rash, diarrhoea or local reactions between infants vaccinated before or after 9 months of age (153).

A limitation of our study was the late introduction of vaccination report cards, as a result only 37% of participants were included in the safety analysis post-MV1.

Furthermore, solicited adverse events were followed-up until day 7 post-vaccination, thereby excluding adverse events occurring during the second week post-MV. Also, we did not assess antibody titers further than one-month post-MV2. Long-term follow-up of study participants is currently ongoing. Another limitation is the use of an ELISA instead of the gold-standard plaque reduction neutralization test (PRNT), especially because ELISA has reduced sensitivity at low antibody levels and may therefore underestimate humoral immunity. As a result, currently undetectable antibodies by ELISA could be detected by PRNT and may interfere with vaccination response.

In conclusion, early 2-dose measles vaccination at 6 and 12 months of age with the CAM-70 strain is immunogenic and induces similar post-MV2 responses between HIV-unexposed and HEU children. A window of vulnerability exists prior to 6 months of age, as well as between 6-12 months of age. This is particularly important because an increased number of mothers will have vaccine-derived immunity instead of naturally-acquired immunity, which is associated with early loss of maternal antibodies. When combined with a reduction in measles vaccination coverage, outbreaks can occur, affecting those most susceptible (i.e. young infants). Earlier vaccination could narrow the vulnerability gap, suggesting the need for new MV that are more immunogenic in younger age-groups; possibly as early as 3-4 months of age in settings with high HIV incidence. In addition, future studies should optimize vaccination dosing schedules and evaluate the sustainability of protection with an accelerated measles vaccination schedule.

Chapter 6 Immunity and safety of varicella-zoster virus vaccine in HIV-exposed uninfected and HIV-unexposed South African children

6.1 Abstract

Background: Varicella vaccine is recommended in settings where varicella-zoster virus (VZV) poses a high public health burden, nonetheless it is seldom implemented in African countries. We aimed to study the immunogenicity and safety of a single dose of VZV vaccine in South African children.

Methods: 95 HIV-unexposed and 29 HIV-exposed uninfected (HEU) children received one dose of VZV vaccine at 18 months of age. Blood samples were tested for VZV immunoglobulin G and VZV-specific cell-mediated immunity (CMI) before and one month after vaccination. Geometric mean titer (GMT) and geometric mean fold-rise (GMFR) from baseline to one-month post-vaccination were measured by enzyme-linked immunosorbent assay. Levels of interferon-gamma (IFN-γ) and interleukin-2 (IL-2), markers of VZV-specific CMI, were measured by dual-color ELISPOT. Children were evaluated for solicited adverse events.

Results: GMTs increased from 8.1 mIU/mL (95% confidence interval 7.2-9.2) in HIV-unexposed and 9.0 (6.2-13.2) in HEU children pre-vaccination to 44.1 (35.9-54.1) and 44.4 (28.9-68.2) post-vaccination, respectively. VZV antibody GMFR was 5.6 (4.6-6.7) in HIV-unexposed and 5.1 (3.7-7.2) in HEU children (p=0.743); 44% of children in both groups seroconverted, defined as change from ≤50 mIU/mL pre-vaccination to >50 mIU/mL post-vaccination. A large proportion of ELISPOT results was considered negative (pre-vaccination 58% and 66%; post-vaccination 44% and 69% for IL-2 and IFN-γ) due to similar response in stimulated and background wells. Pre- and post-vaccination VZV-specific CMI was similar between HIV-unexposed

and HEU children. VZV vaccine was safe and AEs occurred with similar frequency and severity between groups.

Conclusions: Single dose VZV vaccine at 18 months of age was similarly safe and modestly immunogenic in HIV-unexposed and HEU children. Seroconversion rates after a single dose of VZV vaccine were lower than anticipated compared to reports from previous studies (85%-89%), which warrants further investigation.

6.2 Introduction

Varicella-zoster virus (VZV) is the causative agent of varicella (chickenpox), a common childhood infection (176). Following primary infection, VZV remains dormant in sensory nerve ganglia and periodically reactivates resulting in herpes zoster (shingles) in older adults and immunocompromised individuals. Varicella infection is usually self-limited, but may cause serious complications, including secondary bacterial infections, pneumonia or encephalitis (177). Populations at increased risk of severe disease and complications are the elderly and immunocompromised individuals (9), as well as pregnant women, newborns and infants (177). Differences in epidemiology between temperate and tropical climates have been described, with primary infection occurring at older age in tropical climates, thereby increasing the risk of developing complications (9).

Safe and effective vaccines against varicella and herpes zoster are available (178). The World Health Organization (WHO) recommends immunization with a live, attenuated varicella vaccine at 12-18 months of age in settings where varicella poses an important public health burden (178). Although varicella immunization has been introduced in the national vaccination programs of several high-income countries due to the high burden on healthcare systems and society (9,10), it is seldom used in

Africa (177). A theoretical modelling study claimed that if low and middle-income countries (LMIC), introduced a one dose VZV vaccine in children 12-18 months of age with coverage between 20% and 80%, there would be an increased risk of an epidemiological shift to older age at infection and increased mortality (409). Epidemiologic data from high-income countries have shown a decrease in varicella incidence in all age groups (410) or in persons under the age of 40 (411) after VZV vaccine introduction in the second year of life and, most importantly, of complications, hospitalizations and overall healthcare costs associated with varicella infections (412).

Prevention of mother-to-child HIV transmission programs have reduced vertical transmission of HIV to infants, however, there remains a growing population of children born to women with HIV but who are not infected by HIV (i.e. HIV-exposed uninfected; HEU) (16). HEU children have increased risk of infectious morbidity and mortality compared with HIV-unexposed children, particularly during early childhood (4,6). Nevertheless, HEU children have similar primary immune responses to BCG, diphtheria, tetanus and *Haemophilus influenza* type-b conjugate vaccine immunization (22,25,33–35) and increased responses to pertussis, pneumococcal capsular polysaccharide protein conjugate and measles vaccine immunization (22,24,34,39,40) compared with HIV-unexposed children. Unusual clinical manifestations, such as haemorrhagic varicella, have been reported in HEU children in South Africa after VZV infection (6). To our knowledge, there are no studies on the immunogenicity of VZV in HEU children from sub-Saharan Africa.

This study evaluated the immunogenicity and safety of a single dose of varicella vaccine administered at 18 months of age to HIV-unexposed and HEU South African children.

6.3 Methods

This prospective cohort study enrolled HIV-unexposed and HEU children aged 18 weeks of age who were evaluated for antibody response to measles vaccination (413) at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa from April to October 2017. HIV-unexposed children who were enrolled in a randomized, open-label trial evaluating reduced dosing schedules of pneumococcal conjugate vaccine (PCV) (NCT02943902) were invited to participate in consecutive order. Eligible participants were those born at ≥37 weeks gestation, birth weight >2499 grams, healthy based on medical history and physical examination, and absence of previous VZV vaccination or varicella disease since birth. Inclusion and exclusion criteria are detailed in section 2.3.3.

Procedures

One dose of live attenuated VZV vaccine (VARILRIX®, GlaxoSmithKline, Rixensart, Belgium) was administered by subcutaneous injection in the upper arm (deltoid region) at 18 months of age (547 days ± 14). Children were observed for 30 minutes after vaccination in order to observe anaphylactic reactions. All children received other childhood vaccines according to the South African public immunization program, except for HIV-unexposed children being randomized to different dosing schedules of PCV.

Venous heparinized blood specimens were obtained immediately before (age 18 months) and one-month after vaccination (age 19 months, 28-35 days post-vaccination).

Safety assessment

Vaccine safety was assessed using report cards. Parents were trained to record any solicited local (pain/tenderness at injection site, redness, swelling and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) reactions for 7 days following vaccination. Monitoring for serious adverse events (SAEs) was done during the whole follow-up period, i.e. one-month post-vaccination, through passive surveillance.

Immunologic endpoints

VZV antibody measurements

Blood samples were centrifuged, and sera were stored at -70ºC. VZV antibodies were measured by enzyme-linked immunosorbent assay (ELISA) (SERION ELISA *classic* Varicella-Zoster IgG, Institut Virion\Serion GmbH, Würzburg, Germany). The kit was referenced against the First International Standard for Varicella Zoster Immunoglobulin provided by the WHO (NIBSC Code W1044). The assay's lower limit of quantification (LLOQ) was 15 mIU/mL and samples below this value were assigned a titer half of the LLOQ (7.5 mIU/mL). For assay validation, each ELISA plate had to meet the following criteria of validity as per manufacturer's instructions:

- The substrate blank must be <0.25 optical density (OD);
- The negative control must be negative;
- The mean OD value (after subtraction of the substrate blank) of the standard serum must be within the validity range, which is given on the lot specific quality control certificate;

- The variation of OD values of the standard serum must not be higher than 20% within a plate.

If these criteria were not met, the test was not valid and samples were re-tested.

A high and low internal control were used for further assessment of inter-assay variability. The following criteria had to be met:

- The high and low control must be within 2 standard deviations of the mean;
- The high control must be positive;
- The low control must be negative (≤50 mIU/ml).

Antibody levels were calculated by the Microsoft Excel-based software tool named SERION *Activity*, as provided by the manufacturer. Following recommendations by Sauerbrei and colleagues for measuring post-vaccination VZV responses using this ELISA kit, positive results were defined as >50 mIU/mL (277). Seroconversion was defined as a change from seronegative (≤50 mIU/mL) pre-vaccination, to seropositive (>50 mIU/mL) post-vaccination. Correlates of protection against varicella disease have been proposed as ≥1:64 dilution by fluorescent-antibody-to-membrane-antigen assay (FAMA) or ≥5 units/mL by glycoprotein ELISA (gpELISA) (76). Although FAMA is the gold standard for measuring VZV antibodies, it is rarely available and technically complex (186,274), whilst gpELISA is not commercially available (186,414). The SERION ELISA *classic* has 90% sensitivity and 98% specificity compared to modified standard FAMA (277).

IFN- γ and IL-2 ELISPOT assay

Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood using Histopaque-1077 (Sigma-Aldrich) density gradient centrifugation and cryopreserved as described below.

Blood samples were centrifuged at 400 g for 10 minutes within 4-6 hours of collection. Plasma was aspirated from the top layer using a sterile pipette without disturbing the red layer and stored at -80ºC. The remainder of cells were diluted 1:1 with phosphate-buffered saline (PBS) and resuspended in a conical tube. After inverting, blood diluted in PBS was pipetted on top of Ficoll-Histopaque (equilibrated at room temperature). After centrifugation at 900 g for 15 minutes (break turned off to preserve the formed layers), the PBMC layer was transferred from the overlay to a new sterile conical tube. PBMCs were then washed twice with PBS and centrifuged at 400 g for 10 minutes. Subsequently, supernatant was aspirated and the pellet of cells was resuspended in 1 mL of PBS. For cell counting, 10 µL of suspension was transferred to a U-bottom well plate and mixed with 10 µL 0.4% Trypan Blue. The cell-Trypan Blue suspension was placed on a counting slide (Bio-Rad) and viability and cell concentration measured (TC20™ Automated Cell Counter; Bio-Rad). After counting, cells were mixed with PBS and centrifuged at 400 g for 10 minutes. Cells were resuspended in freezing medium (10% DMSO, HI-FBS) to obtain a cell concentration of $1x10^7$ cells/mL. Cell suspensions were aliquoted in cryogenic freezing tubes and placed in a controlled-rate freezing container (Mr. Frosty) and placed in the freezer at -80 ºC overnight. On the next day, cryogenic tubes were transferred on liquid nitrogen to a -150 ºC freezer until further use.

Thawing of peripheral blood mononuclear cells

On day 1 PBMCs were thawed slowly, as per the IMPAACT/ACTG/HPTN/HVTN cross-network standard operating procedure (http://www.hanc.info/labs/Pages/SOPs.aspx). Cells were rested overnight in a 37 ºC, 5% CO_2 humidified incubator.

IFN-γ and IL-2 ELISPOT assay

Human interferon-gamma (IFN-γ) and interleukin-2 (IL-2) double-color enzymatic ELISPOT assays (Immunospot, Cellular Technology Limited [CTL], Bonn, Germany) were performed at the RMPRU laboratory per manufacturer's instructions. Test and control wells were analyzed in duplicate and both visits (pre- and post-vaccination) from the same participant and were included in the same plate; Figure 6.1. An assay control of donor PBMCs, who was experiencing zoster at the time of specimen collection, was included in each run.

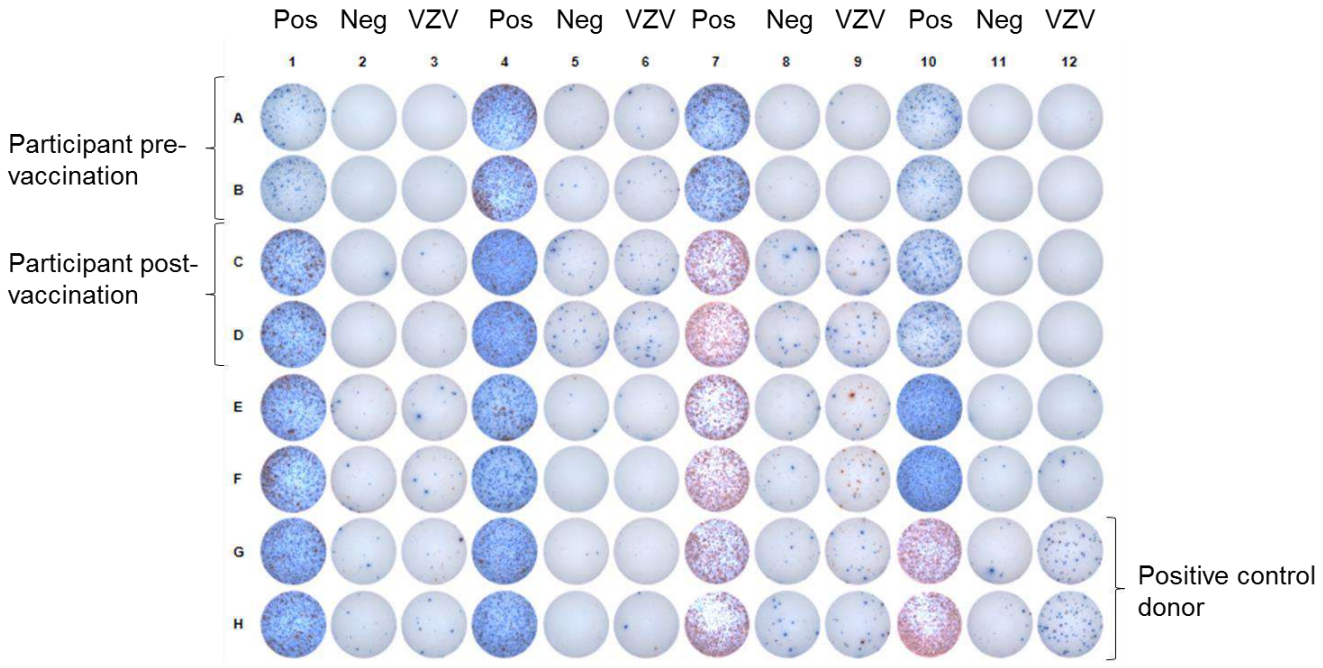
On day 1, plate membranes were activated using 70% ethanol. After washing with PBS, membranes were coated with human IFN-γ and IL-2 capture solution and incubated overnight at 4ºC.

On day 2, plates were washed and plated with 100 µl/well UV-inactivated lysate of VZV-infected cells (University of Colorado–Denver Anschutz Medical Campus, Aurora, USA), mock-infected (negative) control (University of Colorado–Denver Anschutz Medical Campus, Aurora, USA) or 5 µg/mL phytohemagglutinin A (PHA; Sigma-Aldrich, positive control). To ensure optimal pH and temperature, the antigen-containing plate was placed in a 37ºC incubator at 5% CO_2 while processing the PBMCs. PBMCs were centrifuged at 400 g for 10 minutes and the pellet diluted into 1 mL of testing medium for cell counting. After adjustment of PBMC concentrations,

cells with viability ≥70% were plated at 2-3 x10^5 cells/well in duplicate (100 µl/well) and placed for 24 hours in a 37 ºC, humidified 5% CO_2 incubator. Plates were not disturbed in order to avoid 'smudging' of cells.

On day 3, plates were washed and spots were developed using anti-IFN-γ (FITC) and anti-IL2 (Biotin) detection solutions (2 hours incubation at room temperature). Spots were revealed using FITC-HRP (IFN-γ) and Strep-AP (IL-2) (1 hour incubation at room temperature), followed by addition of colorimetric developer solutions (blue: IL-2, red: IFN-γ). Membranes were rinsed using tap water and air-dried on a paper towel for 24 hours.

Figure 6.1 ELISPOT plate layout



PBMCs analyzed in duplicate stimulated with a positive control (PHA), negative control (mock-infected cell lysate) and VZV; Blue spots represent IL-2 and red spots IFN-γ.

Reading of ELISPOT plates

Spots were counted using CTL Immunospot® analyzers and ImmunSpot® Software (version 7.0.22.1) based at CTL Europe, Bonn, Germany. Parameters for optimal

spot counting were as follows: counted area percentage: 100; normalize counts of mask: off; background balance level: 40; base color blue RGB: Channel 1: 141.16, Channel 2: 160.44, Channel 3: 206.58; base color red RGB: Channel 1: 212.71, Channel 2: 164.03, Channel 3: 142.8. Settings blue spots: blue double-color amplification: 58; sensitivity: 120; edge effect compensation: 1.0; diffuse processing level: large; spot separation: 0; maximum spot size: 0.0723 mm^2; minimum spot size: 0.0038 mm^2. Settings red spots: red double-color amplification: 50; sensitivity: 119; edge effect compensation: off; diffuse processing level: large; spot separation: 1; maximum spot size: 0.0740 mm^2; minimum spot size: 0.0022 mm^2. Settings were applied uniformly to all plates. Quality control was performed using software enabling review of aberrant counts. Absolute number of counts were exported using Excel. Results were expressed as mean number of spot-forming cells (SFCs)/10^6 PBMCs after subtracting mock-infected control wells from the VZV-stimulated wells.

Based on production of IFN-γ and IL-2, SFCs were classified as: total effector cells (secreting IFN-γ, with or without IL-2), effector memory cells (secreting IFN-γ and IL-2), total memory cells (secreting IL-2, with or without IFN-γ), central memory cells (secreting IL-2, but no IFN-γ), and differentiated effector cells (secreting IFN-γ, but no IL-2) (415).

Statistical analyses

This study was powered at 80% to detect a difference in seropositivity of at least 25% after VZV vaccination in HEU children compared to HIV-unexposed children, assuming a seropositivity rate of 85% in HIV-unexposed children (premised on 85%-89% of children to have gpELISA titers ≥5 units/mL after single dose VZV vaccine (178,186)), requiring a sample size of 95 HIV-unexposed and 29 HEU children.

GMTs were calculated following log_{10} transformation of ELISA titer values and were compared between groups using multiple linear regression adjusting for maternal age at delivery and baseline antibody levels (post-vaccination only). Geometric mean fold-rise (GMFR) of VZV antibody was calculated as the geometric mean of the ratio of post-vaccination to pre-vaccination titers and compared using multiple linear regression with maternal HIV status, maternal age and pre-vaccination titers as covariates. Percentage of children meeting serological cutoffs was presented with exact binomial 95% confidence intervals (CI) and compared between groups using multivariable logistic regression adjusting for the abovementioned covariates. VZV IgG antibody increase was also assessed as ≥2, ≥3 and ≥4 fold-rise from baseline.

Geometric mean counts (GMCs) from IFN-γ, IL-2 and IFN-γ /IL-2 ELISPOT assays with ≥70% viable cells were presented in descriptive analysis. Safety analyses evaluated the proportion of children with at least one adverse event and severe adverse events. Adverse events were summarized by HIV-exposure group. Analyses were performed using Stata13 (StataCorp, LP, Texas, USA).

6.4 Results

Demographic characteristics

A total of 95 HIV-unexposed and 29 HEU children received one dose of VZV vaccine. Four participants (3 HIV-unexposed and 1 HEU) were not available for follow-up serology (withdrawals n=2 [also not available for safety analysis], missing blood samples n=2). Table 6.1 describes the demographic characteristics of the participants, who were 18.0 months of age (interquartile range [IQR] 18.0-18.1) at vaccination and 19.0 months of age (IQR 19.0-19.1) at the serology visit following vaccination. Characteristics were similar between groups, expect for HEU children having older mothers; Table 6.1. Baseline characteristics of the HIV-unexposed participants who were included in this VZV study were similar to those enrolled in the main PCV parent study.

Table 6.1 Demographic characteristics of participants

Characteristic	**Total**	**HIV-unexposed**	**HIV-exposed uninfected**
Number of participants	124	95	29
Male, n (%)	54 (44)	43 (45)	11 (38)
Race Black-African, n (%) Mixed ancestry, n (%)	 122 (98) 2 (2)	 93 (98) 2 (2)	 29 (100) 0 (0)
Mean birthweight, grams (SD)	3256 (380)	3245 (393)	3291 (339)
Median maternal age at delivery, years (IQR)	27.9 (25.1-33.4)	27.0 (24.6-31.4)[a]	33.4 (27.1-39.0)[a]
Median age at varicella vaccination and serology pre-vaccination, months (IQR)	18.0 (18.0-18.1)	18.0 (18.0-18.1)	18.0 (18.0-18.1)
Median age at serology following vaccination, months (IQR)[b]	19.0 (19.0-19.1)	19.0 (19.0-19.1)	19.0 (19.0-19.2)

Abbreviations: IQR, interquartile range; SD, standard deviation; [a] p-value = 0.002; [b] total of 122 children available at post-vaccination visit: 93 HIV-unexposed and 29 HIV-exposed uninfected.

Antibody responses

Prior to vaccination in analyses adjusted for maternal age, GMTs were similar between HIV-unexposed (8.1 mIU/mL; 95% CI: 7.2-9.2) and HEU (9.0 mIU/mL; 95% CI: 6.2-13.2) children (p=0.373). Three children (2 HIV-unexposed and 1 HEU) had VZV antibody titers >50 mIU/mL and were excluded from post-vaccination analyses; Table 6.2.

One-month after VZV vaccination, GMTs increased to 41.6 mIU/mL (95% CI: 34.4-50.3) in HIV-unexposed and to 38.6 mIU/mL (95% CI: 27.8-53.7) in HEU children; Table 6.2. GMFR was similar in HIV-unexposed (5.6; 95% CI: 4.6-6.7) and HEU (5.1; 95% CI: 3.7-7.2) children (p=0.743) in analyses adjusted for maternal age and pre-vaccination titers. The proportion of seropositive children increased from 2.4% (95% CI: 0.5-6.9) to 44.4% (95% CI: 35.3-53.9) in both groups combined and less than half seroconverted (HIV-unexposed: 44.4%, 95% CI: 34.0-55.3 and HEU: 44.4%, 95% CI: 25.5-64.7). Most children (HIV-unexposed: 87.8% , 95% CI: 79.2-93.7 and HEU: 88.9%, 95% CI: 70.8-97.6) had at least a 2-fold rise in VZV antibody titer post-vaccination; Table 6.2.

Table 6.2 Varicella zoster virus IgG antibody response and proportion of children achieving seropositive titers and seroconversion

Measure	**Total**	**HIV-unexposed**	**HIV-exposed uninfected**	**p-value**
Pre-vaccination	n = 124	n = 95	n = 29	
GMT (95% CI)	8.3 (7.3-9.5)	8.1 (7.2-9.2)	9.0 (6.2-13.2)	0.373
Seronegative (≤50 mIU/mL), n % (95% CI)	121 97.6 (93.1-99.5)	93 97.9 (92.6-99.7)	28 96.6 (82.2-99.9)	0.524
Seropositive (>50 mIU/mL)[a], n % (95% CI)	3 2.4 (0.5-6.9)	2 2.1 (0.2-7.4)	1 3.4 (0.1-17.8)	0.524

Post-vaccination[a]	n = 117	n = 90	n = 27	
GMT (95% CI)	40.9 (34.8-48.1)	41.6 (34.4-50.3)	38.6 (27.8-53.7)	0.743
Seronegative (≤50 mIU/mL), n % (95% CI)	65 55.5 (46.1-64.7)	50 55.6 (44.7-66.0)	15 55.6 (35.3-74.5)	0.536
Seropositive (>50 mIU/mL), n % (95% CI)	52 44.4 (35.3-53.9)	40 44.4 (34.0-55.3)	12 44.4 (25.5-64.7)	0.536
GMFR[b] (95% CI)	5.5 (4.6-6.4)	5.6 (4.6-6.7)	5.1 (3.7-7.2)	0.743
Seroconversion[c], n % (95% CI)	52 44.4 (35.3-53.9)	40 44.4 (34.0-55.3)	12 44.4 (25.5-64.7)	0.536
≥2 fold-rise from baseline, n % (95% CI)	103 88.0 (80.7-93.3)	79 87.8 (79.2-93.7)	24 88.9 (70.8-97.6)	0.817
≥3 fold-rise from baseline, n % (95% CI)	94 80.3 (72.0-87.1)	73 81.1 (71.5-88.6)	21 77.8 (57.7-91.4)	0.590
≥4 fold-rise from baseline, n % (95% CI)	80 68.4 (59.1-76.7)	63 70.0 (59.4-79.2)	17 63.0 (42.4-80.6)	0.824

Abbreviations: CI, confidence interval; GMFR, geometric mean fold-rise; GMT, geometric mean titer; P-values were either calculated by linear or logistic regression and adjusted for maternal age at delivery and baseline antibody levels (post-vaccination comparison only); [a] Three participants (2 HIV-unexposed and 1 HEU) with seropositive titers pre-varicella vaccination are excluded from post-vaccination analyses; [b] Geometric mean of the ratio of post-vaccination titer to the pre-vaccination titer; [c] Change from ≤50 mIU/mL pre-vaccination to >50 mIU/mL post-vaccination.

ELISPOT responses

Cell-mediated immunity to VZV vaccine was assessed by measuring the number of cells secreting IFN-γ and IL-2, which are both T-helper type 1 (Th1) cytokines; Table 6.3. Typically, IFN-γ is produced by effector T cells with a peak at 7 days following vaccination, whereas IL-2 is produced by memory T cells and peaks at 30 to 40 days post-vaccination (415). Only a subset of participants had PBMCs with viability ≥70%,

below which ELISPOT results have reduced interpretability (416,417). Pre-vaccination, 33 participants (21 HIV-unexposed and 12 HEU) had samples included in the analysis and post-vaccination 36 (20 HIV-unexposed and 16 HEU). Of these, only 16 paired samples were available: 6 HIV-unexposed and 10 HEU.

Overall, few spots developed, in particular for IFN-γ. Pre-vaccination, 58% participants (IL-2) and 67% participants (IFN-γ) had counts that were assigned a value of zero because VZV stimulated wells did not yield a response above background. Post-vaccination, 44% participants (IL-2) and 56% participants (IFN-γ) were assigned a value of zero.

One month after vaccination, no differences were observed between HIV-unexposed and HEU children for all cell populations. Counts of VZV-specific total memory cells increased from 4.2 (95% CI: 2.8-6.4) to 7.6 (95% CI: 4.8-12.1) SFCs/10^6 PBMCs and central memory cells from 3.8 (95% CI: 2.6-5.6) to 6.7 (95% CI: 4.0-11.3) SFCs/10^6 PBMCs; Table 6.3. Counts of other VZV-specific T-cell subsets were similar before and one month after vaccination, although no statistical tests were performed.

Table 6.3 Cell-mediated immunity determined by the number of varicella zoster virus-specific cells

End point	**No.**	**Total**	**No.**	**HIV-unexposed**	**No.**	**HIV-exposed uninfected**
VZV-specific total effector cells						
Pre-vaccination GMC, SFCs no. (95% CI)	33	5.4 (2.3-12.7)	21	3.4 (1.4-8.5)	12	9.5 (1.5-62.3)
Post-vaccination GMC, SFCs no. (95% CI)	36	6.2 (3.2-12.3)	20	3.8 (1.5-9.4)	16	7.8 (3.0-20.6)
GMFR, SFCs no. (95% CI)	7	0.5 (0.2-1.3)	2	0	5	0.5 (0.2-1.3)
VZV-specific effector memory cells						
Pre-vaccination GMC, SFCs no. (95% CI)	33	2.4 (1.3-4.5)	21	1.7 (1.6-1.7)	12	4.1 (0.3-53.5)
Post-vaccination GMC, SFCs no. (95% CI)	36	2.7 (1.6-4.7)	20	1.7 (1.7-1.7)	16	3.1 (1.5-6.4)
GMFR, SFCs no. (95% CI)	3	0.4 (0.0-5.3)	1	0	2	0.4 (0.0-5.3)
VZV-specific total memory cells						
Pre-vaccination GMC, SFCs no. (95% CI)	33	4.2 (2.8-6.4)	21	4.4 (2.2-8.7)	12	4.1 (2.1-8.0)
Post-vaccination GMC, SFCs no. (95% CI)	36	7.6 (4.8-12.1)	20	5.5 (3.3-9.1)	16	10.6 (4.7-24.0)
GMFR, SFCs no. (95% CI)	8	2.0 (0.3-11.1)	1	0	7	2.0 (0.3-11.1)
VZV-specific central memory cells						
Pre-vaccination GMC, SFCs no. (95% CI)	33	3.8 (2.6-5.6)	21	3.8 (2.1-6.9)	12	3.8 (2.0-7.2)
Post-vaccination GMC, SFCs no. (95% CI)	36	6.7 (4.0-11.3)	20	5.3 (3.2-8.7)	16	8.8 (3.0-25.6)
GMFR, SFCs no. (95% CI)	8	1.3 (0.04-41.4)	1	0	7	1.3 (0.04-41.4)
VZV-specific differentiated effector cells						
Pre-vaccination GMC, SFCs no. (95% CI)	33	6.1 (2.2-16.8)	21	4.9 (1.2-19.5)	12	7.2 (0.9-59.0)
Post-vaccination GMC, SFCs no. (95% CI)	36	6.3 (2.9-13.5)	20	3.3 (1.2-9.1)	16	9.0 (2.9-27.5)

GMFR, SFCs no. (95% CI)	6	0.4 (0.1-1.8)	1	0	5	0.4 (0.1-1.8)

VZV-specific total effector cells are defined as cells secreting interferon γ, with or without secretion of interleukin 2. VZV-specific effector memory cells are defined as cells secreting interferon γ and interleukin 2. VZV-specific total memory cells are defined as cells secreting interleukin 2, with or without interferon γ. VZV-specific central memory cells are defined as cells secreting interleukin 2 only, without interferon γ. VZV-specific differentiated effector cells are defined as cells secreting interferon γ only, without interleukin 2.
Data are mean number of SFCs/10^6 peripheral blood mononuclear cells in VZV-stimulated wells after subtracting the mean number of SFCs in control wells containing mock-infected control. Abbreviations: CI, confidence interval; GMC, geometric mean count; GMFR, geometric mean fold-rise from pre-vaccination; SFC, spot-forming cell.

Safety

In general, VZV vaccine was well tolerated; Table 6.4. Seven days following vaccination, 27% HIV-unexposed and 18% HEU children (p-value=0.346) experienced ≥1 local adverse reaction, whereas 57% HIV-unexposed and 29% HEU children (p-value=0.007) experienced ≥1 systemic adverse reaction. Overall, the frequency and severity of the individual solicited adverse events were similar between groups, except for decreased appetite, which was 22% in HIV-unexposed and 14% in HEU (p-value=0.026); Table 6.5. No vaccine-related adverse events and no SAEs were reported during the 28 days following vaccination.

Table 6.4 Adverse events following single dose varicella vaccination, by HIV-exposure

n/N (%)	**HIV-unexposed**	**HIV-exposed uninfected**
Solicited reactions during first 7 days after varicella vaccination		
Local reactions		
≥1	25/94 (27)	5/28 (18)
Severe	2/94 (2)	1/28 (4)
Systemic reactions		
≥1	54/94 (57)[a]	8/28 (29)[a]
Severe	6/94 (6)	4/28 (14)
Unsolicited serious AE after varicella vaccination		
Serious AE ≤28 days after injection	0/94 (0)	0/28 (0)
Vaccine-related serious AE	0/94 (0)	0/28 (0)

Abbreviations: AE, adverse events; N, total number of participants with vaccination report card / serious adverse event assessment; n, number of participants having a reaction; [a] p-value=0.007.

Table 6.5 Frequency of solicited adverse events (maximum severity per participant[a]) during the first seven days following varicella vaccination

Adverse events, n (%)	**HIV-unexposed (n=94)**	**HIV-exposed uninfected (n=28)**
Injection site		
Pain/tenderness		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	13 (14%) 4 (4%) 2 (2%)	2 (7%) 0 0
Redness		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	11 (12%) 0 2 (2%)	2 (7%) 0 1 (4%)
Swelling		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	5 (5%) 1 (1%) 1 (1%)	2 (7%) 1 (4%) 0
Itching		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	9 (10%) 1 (1%) 0	2 (7%) 0 0
Systemic		
Fever		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe) Grade 4 (potentially life threatening)	4 (4%) 1 (1%) 0 0	2 (7%) 0 1 (4%) 0
Vomiting		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	16 (17%) 2 (2%) 3 (3%)	3 (11%) 1 (4%) 1 (4%)
Decreased appetite		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	21 (22%)[b] 16 (17%) 3 (3%)	4 (14%)[b] 0 3 (11%)
Irritability		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	22 (23%) 11 (12%) 4 (4%)	1 (4%) 0 2 (7%)
Decreased activity		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	17 (18%) 3 (3%) 3 (3%)	2 (7%) 1 (4%) 2 (7%)

[a] adverse events were solicited during 7 days following vaccination; an event could last several days, but is counted only once and is reported by its maximum severity per participant; [b] p-value = 0.026; Please see footnotes below table 5.5 for definition of local and systemic events.

6.5 Discussion

This study demonstrated that a single dose of live attenuated VZV vaccine administered at 18 months of age was similarly safe and modestly immunogenic in both HIV-unexposed and HEU South African children. This is in line with previous studies reporting no differences between HIV-unexposed and HEU children after vaccination with other childhood vaccines (22,25,33–35), although seroconversion rates after VZV vaccination were lower than expected in both groups in our study. Our study fills a data gap in providing evidence on VZV immunization in HEU children.

HIV-unexposed and HEU children had comparable antibody responses to VZV vaccine. A previous study from the USA reported that 98% (55/56) of HEU children achieved seropositive titers following one dose of VZV vaccine at median 1.5 (IQR 1.1-3.7) years of age as measured by whole-infected cell ELISA and confirmed by gpELISA if results were equivocal or negative (cutoffs not mentioned) (216).

In our study seropositivity rates following single dose VZV vaccination were strikingly lower than previously reported, where 85%-89% of vaccinated children in the USA and Canada had antibody levels ≥5 units/mL (based on gpELISA) after a single dose of VZV vaccine (186,197–204). Our findings were also lower than a South African trial on the immunogenicity of a single dose of VZV vaccine, co-administered with measles/mumps/rubella (MMR) vaccine at 15-18 months in healthy children; which showed a varicella seroresponse of 73% to 75% (measured by FAMA ≥4 [1/dil]) (210). We followed Sauerbrei and colleagues' recommendation to use an optimized cutoff value of 50 mIU/mL for assessment of post-vaccine immunity (277). Despite using this cutoff, seropositivity and seroconversion rates remained less than 50%, which is lower than reported after one dose of the same vaccine alone (195,418,419)

or when combined with MMR (420,421). A literature search did not identify any published study evaluating VZV antibodies following immunization using the same Virion\Serion ELISA kit. An Indian study, using a different commercial ELISA kit, found a similar percentage of children experiencing a 3- or 4-fold increase in VZV-specific IgG titer from baseline (73% and 62%, respectively) in children aged 12 months to 12 years after one dose of the same VZV vaccine (422). Commercial ELISA may not be sensitive enough to detect seroconversion after vaccination, since it is calibrated for diagnosis of natural infection (423) and may therefore underestimate the immunogenicity of vaccines. The choice for commercial ELISA was due to limited availability of the gpELISA assay.

Pre-vaccination VZV seroprevalence was low (2%), which is corroborated by a recent systematic review showing seropositivity to VZV antibodies in African children aged 1-12 years of 23% (95% CI 17%-30%) (189). The authors of the review suggested that primary VZV infection occurs at a later age in Africa compared to other regions and noted a positive association between age and VZV seropositivity (189).

CMI plays an important role in the prevention of symptomatic VZV infection (424,425) and T cell-mediated immunity is normally induced after vaccination (196). Our findings of increased VZV-specific total memory and central memory cells post-vaccination may suggest induction of immune memory, although no statistical tests were performed due to the small sample size. Our study was limited by only a small number of participants having PBMCs available. We observed very low IL-2 and IFN-γ responses, which may be attributed to under-stimulation by VZV antigen, since the assay was optimized using blood from a donor experiencing zoster at the time of specimen collection. Another possible explanation for the low ELISPOT counts is a

limited number of viable cells during stimulation. Overnight resting of PBMCs after thawing has been suggested to improve antigen-induced spot counts in ELISPOT assays, however, resting also leads to losing about half of the PBMCs available for testing (426). T-cell functionality and viability may have also been affected by temperature fluctuations that occurred due to an inadvertent temporary transfer of samples from a -150 ºC freezer to a -70 ºC freezer (427). Moreover the observed low IFN-γ response may be explained by an inhibitory effect resulting from IL-2 absorption on the plate-membrane, which is thereafter no longer available for the cellular pathway of IFN-γ production and release (428). This could be overcome by addition of anti-CD28 antibody, although this effect was not observed during optimization of the assay.

VZV vaccine was found to be safe and well tolerated. The percentage of children with any local or systemic solicited reaction (14%-51%) was similar to that reported in a South African study co-administering varicella vaccine with MMR (10%-68%) (210). The percentage of children with severe systemic reactions was, however, higher in our study (2%-14%) compared to the co-administration study (0%-4%). No serious adverse reactions occurred during the 28 days following vaccination.

This study was limited by the sample size assumption of 85% seropositivity following VZV vaccination in HIV-unexposed children not being met. With the present sample size and 44% seropositivity in HIV-unexposed children, we were only adequately powered (at 80% power) to detect a difference of at least 27% between HEU and HIV-unexposed children after varicella immunization. Another limitation was the short duration of follow-up because persistence of antibodies and long-term safety could not be assessed; long-term follow-up is currently ongoing. Moreover, assessment of humoral immunity was done using a commercially available ELISA, which may be

less sensitive in post-vaccination samples and could therefore underestimate the antibody response.

In many LMIC, other vaccine-preventable diseases, with greater public health burden or severity, are prioritized over VZV. As a result, routine VZV immunization is rarely considered in this context. Despite the low mortality of VZV, the burden of varicella and herpes zoster on healthcare systems and society, in absence of preventive measures, can be considerable (9). A retrospective review of admissions between 1994-1996 to a paediatric isolation facility in Durban, South Africa, demonstrated that varicella accounted for 23% of admissions between 1986-1996, with 15% of varicella admission (n=86) and 75% of varicella deaths (n=6) being associated with HIV-infection (192). Before routine VZV vaccination can be implemented, countries first need to consider the burden of varicella disease and predict achievable vaccination coverage (409).

In conclusion, we have shown that a single dose of live attenuated VZV at 18 months of age was safe but resulted in less than half of children with seropositive rates as measured by a commercially available ELISA. A second dose of VZV is expected to improve seropositivity rates. Future studies should evaluate long-term humoral and cellular response to VZV vaccine in the African context, in both HIV-unexposed and HEU children.

Chapter 7 Immunogenicity and safety of a single dose of hepatitis-A vaccine in HIV-exposed uninfected and HIV-unexposed South African children

7.1 Abstract

Background: In countries experiencing an epidemiological transition from high to intermediate hepatitis-A virus (HAV) endemicity, universal single-dose HAV immunization may prevent a rise in symptomatic HAV infections. We evaluated the immunogenicity and safety of single dose inactivated HAV vaccine in South African children.

Methods: 100 HIV-unexposed and 35 HIV-exposed uninfected (HEU) South African children received one dose of HAV vaccine at 18 months of age. Blood samples were tested for hepatitis-A antibodies before and one month after vaccination by chemiluminescent microparticle immunoassay. Solicited adverse events (AEs) were monitored and compared between groups.

Results: 6.0% HIV-unexposed and 2.9% HEU children had seropositive (sample signal/cutoff calibration signal ≥1.00) antibodies before HAV vaccination. One-month post-vaccination, 91.8% HIV-unexposed and 82.9% HEU children were seropositive for hepatitis-A (p=0.144). Both HIV-unexposed and HEU children experienced similar frequency and severity of AEs.

Conclusion: Single dose HAV vaccine was safe and immunogenic in HIV-unexposed and HEU children. We did not identify differences in humoral immunity between HIV-unexposed and HEU children after administration of an inactivated vaccine.

7.2 Introduction

Hepatitis-A virus (HAV) is a common cause of viral hepatitis, particularly in low- and middle-income countries (225). HAV is transmitted via the faecal-oral route through ingestion of contaminated food or water and direct person-to-person contact. The global number of acute hepatitis-A cases has increased from 117 million in 1990 to 126 million in 2005, while deaths due to hepatitis-A have increased from 30 283 per year to 35 245 per year in the same period (429). The risk of developing symptomatic HAV infection is strongly related with age and viral prevalence at the population level. In regions with high HAV prevalence, including sub-Saharan Africa, most children are exposed to the virus before 5 years of age when hepatitis-A infection commonly has an asymptomatic course of disease (11). Recent studies suggest that a number of African countries are experiencing an epidemiological transition to lower HAV endemicity (228–230,232), which is associated with absence of HAV infection during childhood but increased susceptibility to symptomatic infection later in life (11). A recent serological study found intermediate HAV endemicity in South Africa between 2005 and 2015, with total antibody positivity of 53% at 1-4 years of age and >90% after 25 years of age (232). The World Health Organization (WHO) recommends inclusion of HAV vaccination in the national immunization schedules for children ≥1 year old in countries experiencing an epidemiological transition from high to intermediate HAV endemicity, which could be the case in South Africa (11,232).

Traditionally, two doses of hepatitis A vaccine are recommended, however, comparable immune responses have been achieved after one or two doses during childhood (239,430). Single dose vaccination may be a preferred option in low- and middle-income countries. After one dose of HAV vaccine, 86% and 71% of children

living with HIV in Switzerland (431) and the Netherlands (241), respectively, seroconverted (≥10 mIU/mL by ELISA and ≥ 20 mIU/mL by enzyme-linked fluorescent assay, respectively). Despite the growing population of HIV-exposed uninfected (HEU) children, few studies explored the humoral immune responses to HAV vaccine in HEU children. A Brazilian study on single dose inactivated HAV vaccine reported seroconversion (antibody titer >20 mIU/mL as measured by enzyme-linked immunosorbent assay) in 72% HEU and 87% children living with HIV (243).

This study evaluated the immunogenicity and safety of a single dose of HAV vaccine administered to HIV-unexposed and HEU South African children at 18 months of age.

7.3 Methods

Study design

This prospective observational cohort study was conducted at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa between April 2017 and February 2019. The study included HIV-unexposed children enrolled in a randomized, open-label trial evaluating alternate dosing schedules of pneumococcal conjugate vaccine (PCV) (NCT02943902) and a parallel cohort of HEU children (NCT03330171). To be enrolled in the study, children had to be 6-18 weeks of age, healthy based on medical history and physical examination, ≥37 weeks' gestation at birth and birth weight >2499 grams. See section 2.3.3 for detailed inclusion and exclusion criteria.

Study participants received one dose of inactivated HAV vaccine (AVAXIM® - Pediatric, 80 U/0.5 mL, Sanofi Pasteur, Lyon, France) intramuscularly at 18 months

of age (547 days ± 14) together with the other childhood vaccines according to the public national immunization program, except for the randomization to different dosing schedules of PCV in the parent protocol. Blood samples were collected just before (18 months; 547 days ±14) and one-month after HAV vaccine administration (19 months, 28-35 days post-vaccination). To assess safety, parents recorded local (injection site pain/tenderness, redness, swelling and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) adverse events (AEs) daily for 7 days following vaccination using a vaccination report card. Serious adverse events (SAEs) were recorded for one month following vaccination.

Laboratory methods

Venous blood samples were centrifuged and sera stored at -70ºC until testing. HAV antibodies were measured using a chemiluminescent microparticle immunoassay (CMIA; Abbott ARCHITECT® HAVAb- IgG) using the ARCHITECT HAVAb 6C29 kit and the ARCHITECT iSystem (Abbott, Wiesbaden, Germany). This automated two-step immunoassay determines the presence of IgG anti-HAV using CMIA technology (290). Following the manufacturer's guidelines, thawed samples were vortexed at low speed until specimens were visibly homogenous. Samples were centrifuged for 10 minutes at ≥10 000 Relative Centrifugal Force to remove particulate matter and ensure consistency. The microparticle bottle was inverted 30 times to resuspend all microparticles and the reagent kit loaded on the ARCHITECT iSystem. After loading HABAb-IgG Calibrator 1, Controls and samples, the system was run. During the automated procedure, sample, assay diluent and HAV coated paramagnetic microparticles were combined, allowing for binding of anti-HAV IgG to the HAV coated microparticles. Following washing, the reaction mixture was created by adding anti-human IgG acridinium-labeled conjugate. After another wash, pre-trigger

(containing 1.32% hydrogen peroxide) and trigger solutions (containing 0.35 N sodium hydroxide) were added. The resulting chemiluminescent reaction was measured. For assay validation, the control values must be within the acceptable range specified in the control package insert.

IgG results were calculated by dividing relative light units of the sample by relative light units of the calibrator cutoff signal (S/CO). Seropositivity was defined as S/CO ≥1.00 and seroconversion as a change from nonreactive (S/CO <1.00) pre-vaccination, to reactive (S/CO ≥1.00) post-vaccination, as per manufacturer's instructions. Anti-HAV IgG levels of 10 to 33 mIU/mL using different assays have been proposed as thresholds for protection from HAV infection (76,223,233), although no absolute protective level has been defined (223). It has been proposed that any detectable concentration of IgG anti-HAV may even suggest protection (223,234). The Architect HAVAb-IgG assay has a sensitivity of ≥98% and a specificity of ≥99.2 % (290).

Statistical analyses

With a sample size of 100 HIV-unexposed and 35 HIV-exposed children, this study was powered at 80% to detect a difference in seropositivity of 18% after HAV vaccination in HEU children compared to HIV-unexposed children, assuming a seropositivity rate of 95% in HIV-unexposed children. Assumed seropositivity following HAV vaccination was based on 95% of healthy children having antibody IgG titers ≥20 mIU/mL within one month post-vaccination (238).

Multiple linear regression was conducted to compare HAV IgG antibody responses measured as S/CO between the two study groups and multivariable logistic regression to compare the percentage of participants meeting the criteria for

seropositivity and seroconversion after controlling for baseline antibody levels (post-vaccination only). Safety analyses evaluated the proportion of children with at least one AE, severe AEs and SAEs. Categorical variables were compared between groups using Chi-square test and Fisher's exact test and continuous variables using the Student's t-test or Mann-Whitney U test. Analyses were performed using Stata13 (StataCorp, LP, Texas, USA).

7.4 Results

100 HIV-unexposed and 35 HEU children received one dose of HAV vaccine at a median age of 18.0 (interquartile range [IQR] 18.0-18.1) months. Two HIV-unexposed participants missed post-vaccination blood collection due to withdrawal (n=1) and loss to follow-up (n=1). The post-vaccination blood collection occurred at the median age of 19.0 (IQR 19.0-19.2) months; Table 7.1. Baseline characteristics were similar between groups. Demographics of HIV-unexposed participants who were included in this HAV vaccine study were not significantly different from those who enrolled in the main PCV parent study.

Table 7.1 Study participants' characteristics

Characteristic	**Total**	**HIV-unexposed**	**HIV-exposed uninfected**
	n = 135	n = 100	n = 35
Male, n (%)	75 (56)	56 (56)	19 (54)
Race-Black African descent, n (%)	135 (100)	100 (100)	35 (100)
Median birthweight, grams (IQR)	3090 (2810-3420)	3120 (2840-3428)	3070 (2790-3410)
Median maternal age at delivery, years (IQR)	28.5 (23.4-32.9)	28.0 (22.3-32.6)	29.3 (25.8-34.9)
Mean height, cm (SD)	80.7 (3.9)	81.0 (3.9)	79.7 (4.0)
Mean weight, cm (SD)	10.9 (2.0)	11.0 (2.2)	10.6 (1.4)
Median age at hepatitis-A vaccination and serology pre-vaccination, months (IQR)	18.0 (18.0-18.1)	18.0 (17.9-18.1)[a]	18.0 (18.0-18.1)[a]
Median age at serology following vaccination, months (IQR)[b]	19.0 (19.0-19.2)	19.0 (19.0-19.2)	19.0 (19.0-19.2)

Abbreviations: IQR, interquartile range; SD, standard deviation; [a] p-value = 0.037; [b] total of 133 children available at post-vaccination visit: 98 HIV-unexposed and 35 HIV-exposed uninfected.

Immunogenicity

Overall 5.2% (95% CI: 2.1-10.4) children were seropositive for HAV pre-vaccination; including 6.0% (95% CI: 2.4-12.6) and 2.9% (95% CI: 0.1-14.9; p=0.974) of HIV-unexposed and HEU children, respectively; Table 7.2. Prior to HAV vaccination, the median HAV antibody S/CO was 0.21 (IQR 0.16-0.32) in HIV-unexposed and 0.20 (IQR 0.15-0.30) in HEU children.

One month after administration of HAV vaccine, overall 89.5% (95% CI: 83.0-94.1) of children had seropositive titers, with 91.8% (95% CI: 84.5-96.4) of HIV-unexposed and 82.9% of HEU children (95% CI: 66.4-93.4; p=0.144); Table 7.2. Of the 126 children seronegative before vaccination, the majority (88.9%; 95% CI: 82.1-93.8) seroconverted after HAV vaccination (91.3% HIV-unexposed and 82.4% HEU;

p=0.196). Post-vaccination, the median S/CO was 3.17 (IQR 2.41-4.38) in HIV-unexposed and 2.98 (IQR 1.66-4.17) in HEU children.

Table 7.2 Hepatitis-A IgG antibody response and proportion of children achieving seropositive titers and seroconversion

Measure	**Total**	**HIV-unexposed**	**HIV-exposed uninfected**	**p-value**
Pre-vaccination				
Seropositive[a], n/N % (95% CI)	7/135 5.2 (2.1-10.4)	6/100 6.0 (2.4-12.6)	1/35 2.9 (0.1-14.9)	0.974
S/CO, median (IQR)	0.21 (0.16-0.30)	0.21 (0.16-0.32)	0.20 (0.15-0.30)	0.860
Post-vaccination				
Seropositive[a], n/N % (95% CI)	119/133 89.5 (83.0-94.1)	90/98 91.8 (84.5-96.4)	29/35 82.9 (66.4-93.4)	0.144
Seroconversion[b], n/N in HAV seronegative % (95% CI)	112/126 88.9 (82.1-93.8)	84/92 91.3 (83.6-96.2)	28/34 82.4 (65.5-93.2)	0.196
S/CO, median (IQR)	3.06 (2.30-4.34)	3.17 (2.41-4.38)	2.98 (1.66-4.17)	0.160

Abbreviations: CI, confidence interval; HAV, hepatitis-A virus; IQR, interquartile range; S/CO, sample signal to cutoff ratio; n, number of participants; N, total number of participants tested; P-values were calculated by Mann-Whitney or Fisher's exact test (pre-vaccination) and linear or logistic regression adjusting for baseline antibody values (post-vaccination);
[a] Seropositivity was defined as sample signal to cutoff ratio (S/CO) ≥1.00 per manufacturer's specification; [b] Seroconversion was defined as a change from S/CO <1.00 to S/CO ≥1.00.

Safety

Solicited local and systemic reactions occurred with similar frequency and severity in HIV-unexposed and HEU children; Table 7.3 and Table 7.4. There were no vaccine-related adverse events. One SAE (hospitalization for herpetic gingivostomatitis) occurring 24 days following HAV vaccination in an HIV-unexposed child was reported.

Table 7.3 Reported adverse events following single dose hepatitis-A vaccination by HIV-exposure

n/N (%)	HIV-unexposed	HIV-exposed uninfected
Solicited reactions 0-7 days after hepatitis-A vaccination		
Local reactions		
Any	25/100 (25)	10/35 (29)
Severe	0/100 (0)	2/35 (6)
Systemic reactions		
Any	47/100 (47)	15/35 (43)
Severe	3/100 (3)	4/35 (11)
Unsolicited serious adverse event after hepatitis-A vaccination		
≤28 days after injection	1/100 (1)[a]	0/35 (0)
Related to hepatitis-A vaccination	0/100 (0)	0/35 (0)

Abbreviations: n, number of participants having a reaction; N, total number of participants with vaccination report card / serious adverse event assessment; See footnotes below table 5.5 for definition of local and systemic events.

[a] One child was hospitalized with herpetic gingivostomatitis, symptom started 24 days following hepatitis-A vaccination, resolved completely.

Table 7.4 Frequency of solicited adverse events (maximum severity per participant[a]) during the first seven days following hepatitis-A vaccination

Adverse events, n (%)	**HIV-unexposed (n=100)**	**HIV-exposed uninfected (n=35)**
Injection site		
Pain/tenderness		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	18 (18%) 2 (2%) 0	7 (20%) 2 (6%) 1 (3%)
Redness		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	9 (9%) 2 (2%) 0	8 (23%) 1 (3%) 0
Swelling		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	7 (7%) 2 (2%) 0	5 (14%) 2 (6%) 0
Itching		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	9 (9%) 1 (1%) 0	6 (17%) 1 (3%) 1 (3%)
Systemic		
Fever		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe) Grade 4 (potentially life threatening)	2 (2%) 1 (1%) 0 0	1 (3%) 0 0 0
Vomiting		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	9 (9%) 5 (5%) 0	3 (9%) 1 (3%) 1 (3%)
Decreased appetite		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	27 (27%) 15 (15%) 1 (1%)	9 (26%) 7 (20%) 1 (3%)
Irritability		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	21 (21%) 6 (6%) 2 (2%)	4 (11%) 2 (6%) 2 (6%)
Decreased activity		
Grade 1 (mild) Grade 2 (moderate) Grade 3 (severe)	18 (18%) 7 (7%) 2 (2%)	6 (17%) 3 (9%) 1 (3%)

[a] adverse events were solicited during 7 days following vaccination; an event could last several days, but is counted only once and is reported by its maximum severity per participant; See footnotes below table 5.5 for definition of local and systemic events.

7.5 Discussion

This study demonstrated that a single dose of inactivated HAV vaccine administered at 18 months of age was safe and immunogenic (89.5% of children had seropositive titers post-vaccination) in HIV-unexposed and HEU South African children. Our study addresses a gap in the literature on HAV vaccination in HEU children.

We found no statistical difference in the percentage of HEU to be HAV seropositive post-vaccination compared with HIV-unexposed children. To our knowledge, immunogenicity data following a single dose of inactivated HAV vaccine regimen in HEU children are limited to one Brazilian study evaluating humoral immune responses to HAV vaccine (Havrix; Glaxo SmithKline Beecham) in 25 older HEU children vaccinated at the mean age of 5.1 (range 1.6–9.4) years (243). Our findings of 82.9% seropositivity and 82.4% seroconversion in HEU were slightly higher than in that study, in which 72.0% (18/25) HEU children seroconverted (titer >20 mIU/mL measured by a competitive ELISA kit; Adaltis) 4-8 weeks after one dose of HAV vaccine (243). Long-term persistence of antibodies following a single dose HAV regimen in HEU children requires further investigation.

In the present study, a single dose of HAV vaccine generated seropositive antibody responses in 91.8% of HIV-unexposed children. In comparison, 98.6% of Argentinian children had seropositive titers (≥10 mIU/mL as measured by microparticle enzyme immunoassay) one year after receiving one dose of the same HAV vaccine at 11-23 months of age (239). Similarly, 95.3% of Chinese children vaccinated at 18-60 months of age had seropositive responses by microparticle enzyme immunoassay at one year following inactivated HAV vaccination (240). Our results are slightly lower than seropositivity rates reported in literature, which may partially be explained by the small sample size and the difference in the laboratory assay and cutoffs used.

Despite 10.5% of children in our study not showing a humoral immune response to HAV vaccination, previous studies showed that children with low or undetectable antibody levels after one HAV dose were able to elicit a robust humoral response after booster challenge, indicative of an anamnestic response (432). This memory recall response may reflect residual B-cell response capacity. It has also been shown that a single HAV vaccine dose induces HAV-specific T-cell immunity that persists independently of circulating antibody levels and produces a HAV-specific memory response similar to that induced by natural infection (433). The T-cell immunity may contribute to protection against hepatitis-A viral infection in children without seroconversion.

Prior to vaccine administration, HAV seroprevalence at 18 months of age was low (5.2%). In a previous South African study in 2015, 22% of children 1-2 years of age presenting for routine or emergency medical treatment in the Western Cape tested positive for HAV antibodies (434). The differences in these findings may be explained by the time period when the studies were performed, geographical location and study population; including improved water supply and sanitation coverage, as well as the younger age at which antibodies were tested in our study. Our study was conducted in the urban setting of Soweto, Johannesburg. Community survey data from 2016 indicated that 94.1% of households had access to safe drinking water and 90.2% to flush toilets in the Johannesburg region (271).

Limitations of this study include that our sample size provided 80% power to detect at least a 20% difference following HAV vaccination between HEU and HIV-unexposed, based on 92% seropositivity in HIV-unexposed children as identified in our study. Consequently, we may have missed detecting differences of lesser magnitude in HAV antibody responses between the two groups. Our study was

limited by the short duration of follow-up and use of a chemiluminescent microparticle immunoassay to measure HAV IgG response. This assay did not allow for calculation of exact titer values in mIU/mL. Due to differences between individual assays and vaccines used, it is difficult to make direct comparisons with previous studies. Even more so because there is no universally accepted anti-HAV threshold that confers protection against infection (248). During the study period, no symptomatic cases of HAV infection were detected, but we were unable to ascertain if asymptomatic infections occurred. The likelihood of natural infection between vaccination and post-vaccination visit was considered to be small, as supported by the low HAV seroprevalence at 18 months of age.

Based on the WHO recommendation to integrate universal vaccination against HAV in the national immunization schedules in countries with declining endemicity from high to intermediate, several countries have introduced the HAV vaccine during childhood, which led to a considerable decrease in HAV incidence in both vaccinated and non-vaccinate age groups (435). Before universal HAV vaccination can be considered, seroprevalence across age groups and regions needs to be established, in combination with country-specific cost-effectiveness assessment. Follow-up studies are needed on long-term persistence after administration of a single HAV vaccine dose, both in HIV-unexposed and HEU children.

In conclusion, we have shown that a single dose of inactivated HAV vaccine at 18 months of age was safe and resulted in most children achieving HAV seropositive rates.

Chapter 8 Integrated discussion and conclusion

The studies undertaken as part of this thesis resulted in several important findings. Our systematic review suggested that primary measles vaccination at 6-months of age may provide protection against measles during early infancy in settings with high prevalence of measles and maternal HIV-infection, while indicating that more studies on early measles vaccination should be undertaken to address persistence of antibodies. This thesis highlighted the importance of early and continuous ART in children living with HIV in order to sustain measles antibodies over time to 4.5 years of age. The prospective study showed that early 2-dose measles vaccination at 6 and 12 months of age was safe and induced antibody responses in HIV-unexposed and HEU children, although less than half were seropositive after one dose of measles vaccine and persistence of antibodies needs to be established. In general, HEU and HIV-unexposed children had similar humoral immune responses to measles, varicella and hepatitis-A vaccines.

We conducted a systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected and HEU children. The meta-analysis showed that HIV-infected children were less likely to serorespond after primary measles vaccination compared to HIV-unexposed or HEU children, while HEU and HIV-unexposed children had generally similar antibody levels. When vaccinated at 6-months of age, similar proportions of HIV-infected and HEU children had a seroresponse compared to HIV-unexposed children. We found that vaccine-associated adverse events and deaths were uncommon. One previous systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected children included studies up to February 2009. Since then, ART has become widely available in many countries and the number of HEU children has

increased globally due to effective prevention of mother-to-child HIV transmission programs. Our study builds on the previous systematic review by incorporating additional evidence published since 2009 (nine new studies on safety and 15 new studies with comparison group for immunogenicity outcomes). To our knowledge, this is the first meta-analysis on this topic which compares measles immunogenicity outcomes considering both age at vaccination and number of doses received. We further extended the results by detailed subgroup analyses to explore heterogeneity in seroresponse estimates and improved the robustness of the evidence by using GRADE to assess the quality of evidence. In order to sufficiently protect children born to HIV-infected mothers, primary vaccination at 6 months of age is recommended. However, we only identified three studies evaluating measles vaccination at 6 months of age in HIV-infected and HEU children, underlining the need for further investigation before widely adopting an early vaccination strategy.

This thesis provides novel data on the persistence of antibody responses to measles vaccination at 4.5 years of age, approximately 3 years after booster vaccination, in HIV-infected children managed either with ART initiated early in life, or subsequently as clinically/immunologically indicated per WHO ART management guidelines at the time that vaccination occurred. This is the first study to analyse the effect of early ART initiation and the consequences of interrupting ART on long-term measles antibodies. HIV-infected children in whom ART was interrupted at either 12 or 24 months of age had lower geometric mean titers and smaller proportions with seroprotective titers at 4.5 years of age than HIV-unexposed children. The results underscore a potential downside of ART interruption in HIV-infected infants who initiated ART during early-infancy. In a real-world situation, this may happen with poor adherence to treatment or missed visits. We also found that measles immunity

in HEU children was similar to that in HIV-unexposed children, despite earlier concerns of waning immunity in this group.

An early-two dose measles vaccination schedule administered at 6 and 12 months of age was similarly safe and immunogenic in HIV-unexposed and HEU children. We found that the vast majority of young infants aged 4.2 months was seronegative for measles antibody, in particular infants from mothers born after introduction of measles vaccination in the South African public immunization program. High (90%) prevalence of measles IgG antibody seronegativity by 4.2 months of age may indicate that maternally-derived antibody now plays a lesser role in attenuating immune responses to measles vaccine at 6 months of age. Our results underscore the importance to reconsider measles dosing schedules in settings with and without high prevalence of maternal HIV infection and where measles epidemics are ongoing.

This thesis fills a data gap in providing evidence on varicella and hepatitis-A immunization in HEU children. We observed no differences in humoral immunity after administration of either a live attenuated (VZV) or an inactivated (HAV) vaccine in HEU and HIV-unexposed children, although the study was underpowered to detect small differences. One dose of live attenuated varicella vaccine resulted in less than half of children achieving seropositive rates for both HIV-unexposed and HEU children by commercial ELISA, which was lower than expected. Most children, however, became seropositive for hepatitis-A antibody after vaccination. Hepatitis-A vaccination could be useful in countries experiencing an epidemiological transition from high to intermediate endemicity, in order to prevent an increase in symptomatic infections.

8.1 Considerations when assessing validity of results

Individual study limitations have been described in the respective chapters. Below, a summary of limitations and validity of the conclusions are discussed.

Systematic review and meta-analysis

Our systematic review was limited to published studies and ongoing trials. Grey literature or unpublished trial results may have been missed by our search. In particular, post-marketing safety surveillance and data from vaccine manufacturers could give valuable information on vaccine safety.

Studies included in the review were mostly observational, except for one randomized controlled trial. The overall level of evidence was rated as low to very low. Furthermore, most studies were conducted in the pre-ART era, limiting the generalizability of findings to children currently living with HIV, who are receiving early and continuous ART.

Measles immunogenicity

Ideally, measles immunity should be assessed by challenging vaccine recipients and unvaccinated controls with wild-type measles virus and observe clinical manifestations of disease (80). However, this is unethical in humans. Previous animal studies showed reduced clinical symptoms and viral replication in vaccinated animals compared to unvaccinated upon wild-type measles virus challenge (436). In humans, outbreak settings provide an opportunity to evaluate vaccine effectiveness, but outbreaks cannot be planned. Protection may also be assessed by measuring the immune response of MV recipients who undergo challenge with live virus through revaccination. This generally results in an anamnestic immune response (80,437).

Measles antibody concentrations are most often used as evidence of protection. Although the presence of measles antibody detected by ELISA correlates with immunity, neutralizing antibodies measured by PRN provide the best correlate of protection against measles infection (80,438). Recently, it was highlighted that the threshold of measles protection is based on a limited number of studies and needs further characterization (77). The choice for ELISA to measure measles antibody responses was made because this assay is widely used. However, samples with low measles antibody concentration by PRN often yield negative results by ELISA, and thus ELISA may underestimate measles immunity (439).

We quantified presence of measles-specific IgG but did not assess antibody function. Higher avidity of measles antibodies allows for binding at lower concentrations and improved protection (440). A previous study found reduced antibody quality and quantity following measles vaccination in HIV-infected children compared with HIV-uninfected children in absence of ART (334). Antibody quality requires further investigation in HIV-infected children on immediate continuous ART and HEU children after receiving early measles vaccination.

Retrospective analysis of prospective cohort

Evaluation of measles antibodies had not been planned *a priori* in the parent study (CIPRA 4) (40). Consequently, this limited the collection of important variables for the current analysis, such as maternal measles vaccination status. Instead we used maternal year of birth as a proxy for maternal vaccination status.

Our study may have been biased by selective loss-to follow-up. In particular, the HIV/Def-ART group experienced higher mortality in the first year of life than HIV/Immed-ART children and may therefore represent a group of survivors with

slower HIV progression. Also, only a convenience sample of HIV-infected children with CD4+ T-lymphocyte <25% was included, limiting generalizability.

Prospective cohort – measles vaccine

Because early measles vaccination was already part of the South African national immunization program at the time of our project initiation, it was not possible to randomize children to receiving the first measles vaccine dose at different ages. Also, no direct comparison was possible between the new 6 and 12-month schedule and the previous 9 and 15-18-month schedule after implementation.

There is controversy on the optimal age of the first dose of measles vaccination. It has been assumed that primary immunization should be delayed until maternal measles antibodies are cleared, because they interfere with the infant's ability to mount an adequate immune response. However, a study in Canadian infants published in 2019 suggested that quality, rather than quantity, of maternal measles IgG is important (441). The authors showed that pre-existing low-avidity neutralizing maternally-derived measles antibodies did not interfere with production of high levels of high-avidity measles antibodies in children following vaccination at 12 months of age (441). In our study, levels of maternal antibodies were already low in children at 4 months of age, but avidity was not assessed. Data on pre-vaccination avidity of maternal measles antibody in our study population would have added important information when correlated with post-vaccine immunogenicity.

Our study did not evaluate long-term antibody levels following early measles vaccination. Information on persistence of immunity following MV at 6 and 12 months of age is critical to inform deliberations on whether early vaccination with two doses of MV should be considered in other countries. For this reason, we are currently

following-up the cohort until 5 years of age. This is particularly important because the current South African guidelines on measles vaccination are not in line with the WHO recommendations, which recommend that an early MV dose should be considered as a “zero-dose” and should be followed by two doses according to the national schedule (55).

The sample size of our cohort study was insufficient to detect rare adverse events following MV and the short follow-up by active surveillance limited our ability to identify vaccine-related AEs >7 days follow vaccination.

Prospective cohort – varicella and hepatitis-A vaccines

Although no differences between HEU and HIV-unexposed children in immunogenicity were reported after live attenuated varicella vaccine (44% seroconversion in both groups), we found a possible trend towards reduced seropositivity following inactivated hepatitis-A vaccination in HEU (83%) compared with HIV-unexposed (92%) children. The clinical relevance of this difference remains uncertain. Due to assumptions of sample size calculations not being met, we were underpowered to detect statistically significant differences between HIV-unexposed and HEU children after varicella or hepatitis-A vaccination. A larger sample size would be required based on the seropositivity rates reported in our study.

Varicella antibodies were measured by commercial ELISA rather than gpELISA or FAMA. A limitation of commercial ELISA is the lower sensitivity in detecting post-vaccine antibodies. Our results may therefore be an underestimation of the humoral response. Nevertheless, the SERION ELISA classic VZV IgG was found to have a specificity of 98% and a sensitivity of 90% in vaccinees when compared to FAMA when using the cutoff of 50 mIU/mL (277).

There were several issues with the ELISPOT assay measuring CMI after varicella vaccination. In general, a limited number of samples had sufficient viability, which resulted in a low number of evaluable results and VZV stimulated wells had low responses overall. This may possibly be attributed to understimulation by VZV, suboptimal storage conditions of the PBMCs or an inhibitory effect resulting from IL-2 absorption. As a result, no final conclusions were drawn based on these findings.

8.2 Future research

This thesis has identified several areas for further research. Future studies should evaluate long-term immune responses to early measles vaccination in HIV-unexposed, HEU and children living with HIV. This is particularly important to prevent future vaccine failures and ensure sufficient maternal antibody transfer in future generations. In addition, studies should measure the functionality of measles antibodies and explore these results in relation to primary vaccine responses. A high-throughput assay to measure measles neutralizing antibodies may be useful in future measles vaccine studies. This high-throughput assay was found to be sensitive (95%) and specific (100%) when compared to PRN (373). Future work should also explore the development of new measles vaccines that are more immunogenic in younger age-groups, particularly in settings with high HIV incidence.

We underscore the need for studies on (persistence of) immunity following hepatitis-A and varicella immunization in HEU children conducted in settings similar to ours. A further recommendation is for studies to include larger sample sizes to detect potential differences in antibody response following varicella vaccination between HIV-unexposed and HEU children. Finally, hepatitis-A seroprevalence in African countries across age groups requires further evaluation.

8.3 Implications

- To prevent measles outbreaks and achieve sufficient levels of population immunity, children and adolescents living with HIV may need supplemental vaccination, especially if they were not on ART at the time of earlier immunization, or are currently not on ART.
- Early measles vaccination offers an opportunity to reduce the susceptibility gap for the age-group at highest risk for severe measles disease. With the present early measles immunization schedule in South Africa, a window of vulnerability exists prior to 6 months of age, as well as between 6-12 months of age. Durability of the response to early measles vaccination needs to established.
- Reassuringly, our studies did not identify differences in vaccine responses between HEU and HIV-unexposed children.

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APPENDICES

Appendix 1: Mutsaerts EAML, Nunes MC, van Rijswijk MN, Klipstein-Grobusch K, Grobbee DE, Madhi SA. Safety and immunogenicity of measles vaccination in HIV-infected and HIV-exposed uninfected children: a systematic review and meta-analysis. EClinicalMedicine 2018; 1:28–42.

Appendix 2: Mutsaerts EAML, Nunes MC, van Rijswijk MN, Klipstein-Grobusch K, Otwombe K, Cotton MF, Violari A, Madhi SA. Measles immunity at 4.5 years of age following vaccination at 9 and 15-18 months of age among HIV-infected, HIV-exposed-uninfected and HIV-unexposed children. Clin Infect Dis. 2019;69(4):687-96.

Appendix 3: Mutsaerts EAML, Nunes MC, Bhikha S, Ikulinda BT, Boyce W, Jose L, Koen A, Moultrie A, Cutland CL, Grobbee DE, Klipstein-Grobusch K, Madhi SA. Immunogenicity and safety of an early measles vaccination schedule at 6 and 12 months of age in Human Immunodeficiency Virus (HIV)-unexposed and HIV-exposed, uninfected South African children. J Infect Dis. 2019;220(9):1529–38.

Appendix 4: Immunogenicity and safety of hepatitis-A and varicella vaccines in HIV-exposed uninfected and HIV-unexposed South African children

Appendix 5: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee on the 15th March 2017 (HREC number: M170391)

Appendix 6: Certificate of approval granted by the University of Witwatersrand Human Research Ethics Committee on the 24th February 2017 (HREC number: M170276)

Appendix 1

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Safety and Immunogenicity of Measles Vaccination in HIV-Infected and HIV-Exposed Uninfected Children: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: HIV-infected and HIV-exposed uninfected (HEU) children have an increased risk of measles that may be due to altered immune responses or suboptimal timing of measles vaccination. We aimed to evaluate the safety and immunogenicity of measles vaccination in HIV-infected and HEU children.

Methods: For this systematic review and meta-analysis, we searched PubMed, Embase, Cochrane Library, CINAHL, Global Health Library and IndMED on May 9, 2018. Studies were included if they reported on safety or seroresponse (either seroprotection/seropositivity/seroconversion) after measles vaccination in HIV-infected or HEU children. We calculated pooled estimates to compare immunogenicity outcomes between HIV-infected, HEU and HIV-unexposed children, using risk ratios [RRs] (with 95%CIs). PROSPERO registration number: CRD42017057411.

Findings: Seventy-one studies met the inclusion criteria (15,363 children). Twenty-eight studies reported on safety; vaccine-associated adverse events and deaths were uncommon. Sixty-two studies reported on immunogenicity, 27 were included in the meta-analysis. HIV-infected children had lower seroresponse rates after primary vaccination compared with HIV-unexposed (RR 0.74; 95%CI: 0.61–0.90, $I^2 = 85.9\%$) and HEU children (0.78; 0.69–0.88, $I^2 = 77.1\%$), which was mitigated by antiretroviral therapy and time interval between vaccination and serology. HEU and HIV-unexposed children had similar seroresponses. Vaccination at 6-months resulted in similar proportions of HIV-infected children having seroresponse compared with HIV-unexposed (0.96; 0.77–1.19) and HEU children (1.00; 0.73–1.37, $I^2 = 63.7\%$).

Interpretation: Primary measles vaccination at 6-months of age may provide protection against measles during early infancy in settings with high prevalence of maternal HIV-infection, however, further studies are needed to evaluate this strategy in HEU children and HIV-infected children receiving antiretroviral therapy.

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1. Introduction

In 2015, an estimated 1.4 million births occurred in HIV-infected women, of which more than 95% lived in low- and middle-income countries (LMICs) [1]. Increased implementation of Prevention of Mother-To-Child Transmission (PMTCT) programs has reduced vertical HIV transmission to around 1% in breastfeeding populations [2, 3] and to less than 1% in non-breastfeeding populations in LMICs [4]. As a result, a significant proportion of children born to HIV-infected mothers is HIV-exposed but uninfected (HEU). Recent studies showed that HEU children are at increased risk of morbidity and mortality compared with their HIV-unexposed peers [5–11], in particular from infectious diseases in the first 6-months of life [9, 12–16]. This increased susceptibility could be due to immune aberrations in HIV-exposed infants resulting from in utero exposure to HIV-virion particles or maternal antiretroviral treatment [17].

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Research in context

Evidence before this study

Despite measles being targeted for elimination, outbreaks of measles continue to occur in low-middle income and high income countries. Contributing to this is under-immunization of children, as well as a shift in measles epidemiology towards infection of infants <9 months of age, who are not generally targeted for measles vaccination. Young infants may be at increased risk of infection due to changes in maternal immunity, which nowadays is predominantly derived from vaccination rather than natural infection, thereby reducing transplacental transfer of protective antibodies from mother to fetus and lowering protection during early infancy. This might be further exacerbated in settings with a high prevalence of maternal HIV-infection, where there is waning of maternal immunity in HIV-infected women, that also results in lower concentrations of measles antibodies being transferred to their fetuses. Hence, HIV-exposed infants, including those who are HIV-exposed uninfected (HEU), are at increased susceptibility to measles infection during early infancy. This calls for a review of measles immunization strategy, particularly in settings with high prevalence of maternal HIV-infection, to inform future deliberations on alternate measles vaccine dosing schedule strategies.

One previous systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected children included studies up to February 2009. Since then, antiretroviral treatment (ART) has become widely available in many countries and the number of HEU children has increased globally due to effective Prevention of Mother-to-Child Transmission programs.

We did a systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected and HEU children. We searched seven databases (PubMed, Embase, Cochrane Library, CINAHL, Global Health Library, including African Index Medicus, Latin American and Caribbean Health Sciences, and IndMED) for articles in English, French, German, Spanish, Portuguese, or Dutch published before 9 May 2018, using the key words ("measles" and "vaccine") and "HIV". Reference lists of the articles that were included in full-text screening were searched manually to identify additional studies. The online database ClinicalTrials.gov was accessed for ongoing and unpublished trials. The inclusion criteria were limited to observational or interventional studies in HIV-infected or HEU children that measured safety or antibody seroresponses after measles vaccination. For inclusion in the meta-analysis a comparison group was required. Case reports were included for assessment of safety.

Added value of this study

The meta-analysis showed that HIV-infected children were less likely to serorespond after primary measles vaccination compared to HIV-unexposed or HEU children, while HEU and HIV-unexposed children had similar immune responses. When vaccinated at 6-months of age, similar proportions of HIV-infected and HEU children had a seroresponse compared to HIV-unexposed children. We found that vaccine-associated adverse events and deaths were uncommon.

Our study builds on the previous systematic review by incorporating additional evidence published since 2009 (nine new studies on safety and 15 new studies with comparison group on immunogenicity). To our knowledge, this is the first meta-analysis on this topic which compares measles immunogenicity outcomes considering both age at vaccination and number of doses received. We further extended previous work through detailed subgroup analyses to explore heterogeneity in seroresponse estimate and improve the robustness of the evidence by using GRADE to assess quality of evidence. HIV-infected children had a reduced immune response to primary vaccination in absence of ART, when measuring immunogenicity as seroprotection and if serology was assessed more than 3 or 6-months post-immunisation.

Implications of all the available evidence

In order to sufficiently protect children born to HIV-infected mothers, primary vaccination at 6-months of age is recommended. Our findings are in line with World Health Organization recommendations to administer the primary dose of measles vaccine at 6-months of age in areas with high incidence of HIV-infection and measles, followed by two routine doses according to the national immunization schedules. However, we only identified three studies evaluating measles vaccination at 6-months of age in HIV-infected and HEU children, underlining the need for further investigation before widely adopting an early vaccination strategy. Future studies should evaluate immune responses to early measles vaccination and long-term waning of immunity in HEU children and HIV-infected children treated with ART in settings with high incidence of measles and HIV.

HIV-infected children have an increased risk of severe measles disease and complications compared with HIV-unexposed children [18–20]. The increased susceptibility to developing measles during early infancy in HIV-exposed infants may be explained by lower levels of maternally acquired measles antibody than HIV-unexposed [21]. Furthermore, HIV-infected, antiretroviral-naïve children have a reduced serological response to primary measles vaccination and increased waning of immunity compared with HIV-uninfected and HEU children [22–25].

A previous systematic review and meta-analysis on the safety and immunogenicity of measles vaccination in HIV-infected children undertaken by Scott et al. included studies up to February 2009 [26]. Since then, the number of HEU children has increased globally and universal antiretroviral treatment for HIV-infected children is now recommended. Understanding the effects of HIV-infection and HIV-exposure on the immune response to measles vaccination is crucial for determining dosing schedules of immunisation programs, especially in LMICs with a high burden of HIV.

This systematic review evaluated the safety and immunogenicity of measles vaccine in HIV-infected and HEU children, and compared immunogenicity outcomes taking age at vaccination and number of doses received into consideration.

2. Methods

2.1. Search Strategy and Selection Criteria

This systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [27].

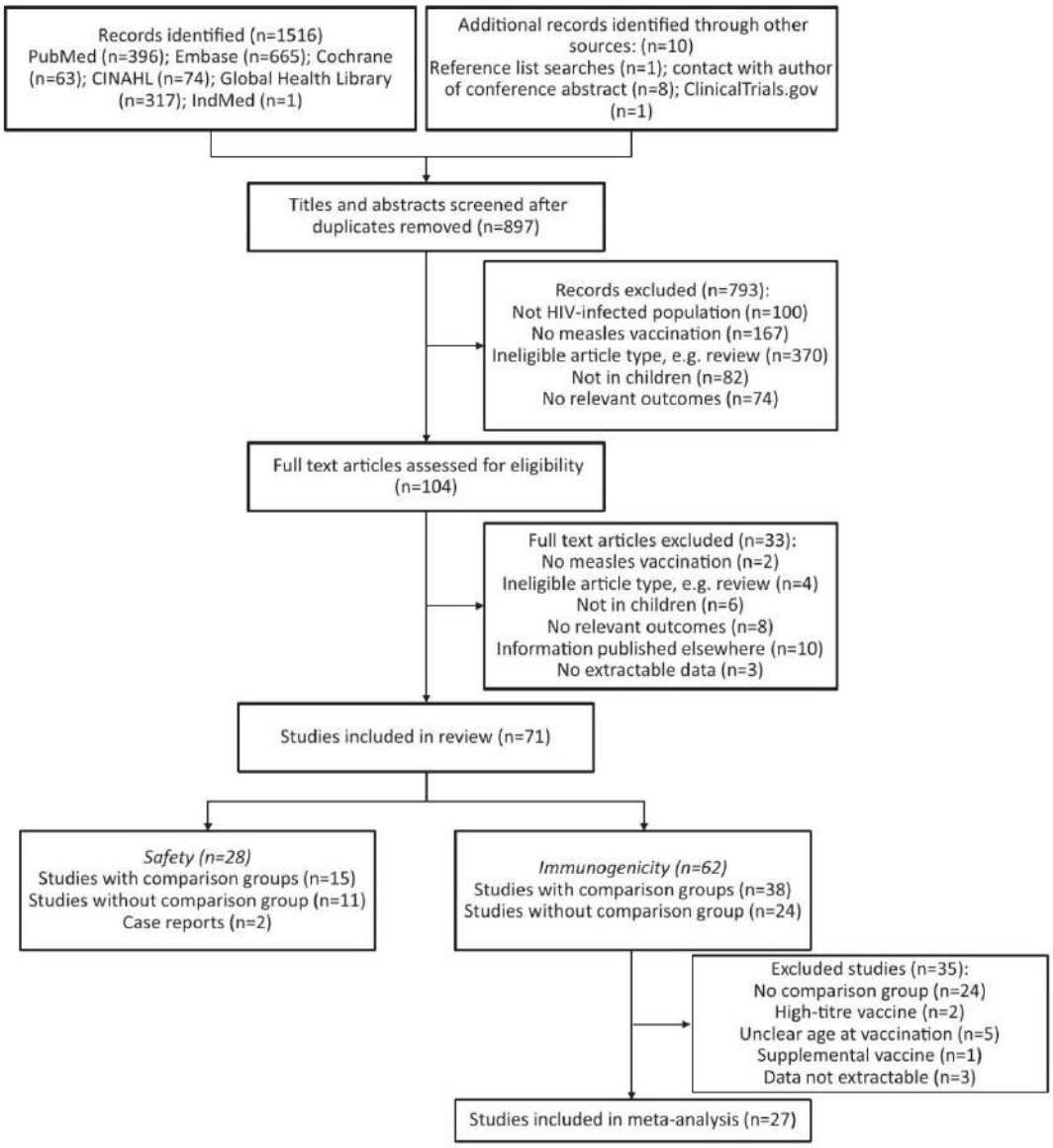


Fig. 1. Flow chart of study selection.

We searched PubMed, Embase, Cochrane Library, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Global Health Library (including African Index Medicus, Latin American and Caribbean Health Sciences), and IndMED on 9 May 2018, for articles containing ("measles" and "vaccine") and "HIV" (Supplementary data 1). Additional studies were identified by searching reference lists of the articles included in full-text screening and ClinicalTrials.gov.

Studies were eligible for inclusion in the systematic review if they reported on immunogenicity or safety of any measles vaccination strategy in HIV-infected or HEU children aged 0–18 years. For inclusion in the immunogenicity meta-analysis, studies needed to report on primary or booster vaccination and had to include a comparator group of either HIV-uninfected children (HEU/HIV-unexposed) or HIV-infected children on a different antiretroviral therapy (ART) regimen. No restrictions regarding geographical region or year of publication were applied. Eligible study designs were interventional or observational. For assessment of safety, case reports were also included. Animal studies, systematic reviews, narrative reviews, reports of proceedings and publications not written in English, French, German, Spanish, Portuguese or Dutch were excluded.

The outcomes of interest were immunogenicity and safety. Immunogenicity: studies were included if data were reported as proportions of subjects with seroprotective (≥330 mIU/mL or as indicated by authors), seropositive, or seroconversion (4-fold rise in titre or change from seronegative to seropositive) measles antibody responses. A composite outcome for seroresponse was created using seroprotection rates post-vaccination, and if not available, seropositivity or seroconversion rates were considered. Safety: all reported safety outcomes post-vaccination were considered, including deaths, severe adverse events (SAEs) other than death and adverse events (AEs).

Two independent reviewers (EM, MvR) screened titles and abstracts of identified studies. Articles were retained if they met the inclusion criteria according to one or both of the reviewers. In case of duplicate publications of the same results, the most complete reference was included.

2.2. Data Analysis

Data were extracted from manuscripts using a standardised data extraction form (Supplementary data 2) and authors were contacted in case of missing data. Data of interest included: study design, study population, vaccine type, age at vaccination, time-period between vaccination and measurement of the serological response, number of vaccine doses administered, use of ART, outcome measures, laboratory methods used to detect measles antibodies, serological cut-off values, proportions with seroresponse, and number and type of (S)AEs.

The Cochrane Risk of Bias Tool was adapted to enable evaluation of observational studies (Supplementary data 3) [28]. For five categories, risk of bias was assessed as low (=0), unclear (=1), or high (=2). Studies with a high summative risk of bias score (≥7) were excluded from meta-analysis.

When multiple time-points were reported for immune responses after the same vaccine dose, the time-point closest to vaccination was reported, except for two studies that had a smaller sample size at the earlier time-point [29, 30]. For the descriptive analyses, point estimates of the proportion of seroresponders for the individual studies under each group were calculated with 95% confidence intervals (CIs) assuming an exact binomial distribution.

Three different primary meta-analyses compared serological responses in HIV-infected vs. HIV-unexposed, HIV-infected vs. HEU and HEU vs. HIV-unexposed children using risk ratios (RRs) and 95%CIs stratified by vaccination dose and age at vaccination. In case of significant heterogeneity ($I^2 > 50\%$), a random-effects model was applied. To explore statistical variation and heterogeneity between trials, prespecified subgroup analyses were performed based on outcome (seroprotection), serological test, use of ART, study design, age at vaccination and time interval between vaccination and measurement of the serological response. Meta-regression was used to explore between-study variance not explained by the covariates and risk of publication bias was assessed using normal and contour-enhanced funnel plots if ten or more articles were included in the meta-analysis. Small study effects were evaluated using Egger's-test for asymmetry.

We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for rating overall quality of evidence [31]. All analyses were performed using Stata, version 13 (StataCorpLP, Texas, USA). The study was prospectively registered in PROSPERO (CRD42017057411) [32].

2.3. Role of the Funding Source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and all authors had final responsibility for the decision to submit for publication.

3. Results

We identified 897 unique articles (Fig. 1). Seventy-one studies fulfilled the eligibility criteria (Supplementary data 4). Twenty-eight studies reported on safety [24, 25, 29, 33–61] and 62 reported on immunogenicity [23–25, 29, 30, 33–37, 39–41, 43–45, 47, 48, 50, 51, 53, 55–57, 59–105], of which 27 were included in the primary meta-analyses (Table 1).

Included study designs were randomised controlled trial (RCT) (n = 1) [35, 36], cohort (n = 35), cross-sectional (n = 30), case reports (n = 2) [46, 58], retrospective audits (n = 1) [72]; two studies had an unclear study design [40, 68]. Studies were published from 1987 through 2018 and were conducted in Africa (n = 28), the United States (n = 16), Europe (n = 17), South America (n = 5) and Asia (n = 5).

Taking all studies together, 15,363 children vaccinated against measles were evaluated, of which 4867 were HIV-infected, 2733 were HEU, and 7763 were HIV-unexposed.

Thirty-five studies with comparison groups reported post-vaccination seroresponses in HIV-infected children, of which twelve administered ART (Supplementary data 5.1). HIV-infected children showed similar seroresponse rates after primary vaccination at 6-months (pooled estimate 71%; 95%CI 55–88; n = 5) compared with later time points: 9-months (60%; 95%CI 43–77; n = 12), 12-months (84%; 95%CI 48–120; n = 2) and >12-months of age (64%; 95%CI 51–76; n = 7). The pooled point estimate of HIV-infected children with seroresponse after booster vaccination was similar when administered at ≤24 months (77%; 95%CI 58–96; n = 5) or >24 months (61%; 95%CI 39–83; n = 4). Two studies assessed the effect of different ART-regimens on the response to primary vaccination [97, 105] and four studies to booster vaccination [66, 70, 89, 97]. Children receiving ART or early-ART within the first year of life showed improved seroresponses to booster vaccination compared with those who received late-ART or did not receive ART [66, 70, 89].

HEU children receiving primary vaccination at 12-months (pooled estimate 98%; 95%CI 91–104; n = 2) or >12-months of age (99%; 95% CI 96–102; n = 5) tended to have better seroresponse compared with HEU children vaccinated at 6 (70%; 95%CI 58–83; n = 5) or 9-months (84%; 95%CI 76–91; n = 13) of age (Supplementary data 5.2).

Similar to HEU children, a trend towards improved seroresponse was observed in HIV-unexposed children receiving primary vaccination at >12-months (pooled estimate 100%; 95%CI 97–103; n = 2) compared with 6-months (66%; 95%CI 50–82; n = 3) or 9-months of age (88%; 95%CI 82–94; n = 9) (Supplementary data 5.3).

Nine publications were included in the primary meta-analysis comparing immune responses after primary vaccination in HIV-infected and HIV-unexposed children [43, 51, 55, 68, 75, 78, 89, 92, 97]. Relative risks for all studies were <1, although only significant in four studies [55, 75, 89, 92]. ART was administered in two of four studies with a significant RR [89, 92], compared with one of five studies that did not find a significant difference [97]. The pooled RR resulting from the random-effects model was 0.74 (95%CI 0.61–0.90; $I^2 = 85.9\%$) (Fig. 2A). Seroresponses after primary vaccination at 9-months (RR = 0.79; 95%CI 0.65–0.95) and >12-months of age (RR = 0.59; 95%CI 0.37–0.95) were significantly lower in HIV-infected compared with HIV-unexposed children, but not when vaccinated at 6-months (RR = 0.96; 95%CI 0.77–1.19; n = 1). Limiting analysis to studies that reported seroprotection (RR = 0.64; 95%CI 0.36–1.14; n = 4), administered ART (RR = 0.63; 95%CI 0.34–1.19; n = 3), or measured serology within 3 (RR = 0.71; 95%CI 0.33–1.55; n = 2) or 6-months post-vaccination (RR = 0.90; 95%CI 0.73–1.11; n = 3), resulted in non-significant combined RRs (Supplementary data 6 and 8.1).

Meta-analysis in five studies comparing post-booster responses in HIV-infected and HIV-unexposed children found a pooled non-significant RR (0.84, 95%CI 0.68–1.04; $I^2 = 89.6\%$) (Fig. 2B), irrespective of subgroup analyses (Supplementary data 7 and 8.2) [43, 51, 76, 92, 97].

Twenty-one studies compared immunogenicity after primary measles vaccination between HIV-infected and HEU children. Nine studies reported significant RR estimates <1 [25, 30, 55, 59, 62, 67, 100, 103, 105], two included HIV-infected children on ART [103, 105]. The pooled RR comparing HIV-infected and HEU children after primary measles vaccination was 0.78 (95%CI 0.69–0.88; $I^2 = 77.1\%$) (Fig. 3A). The proportion of HIV-infected children with seroresponse after primary vaccination was lower compared with HEU when vaccinated at either 9-months (RR = 0.73; 95%CI 0.59–0.89; n = 10) or >12-months of age (RR = 0.72; 95%CI 0.62–0.84; n = 5), but not at 6-months (RR = 1.00; 95%CI 0.73–1.37; n = 3) of age. The combined RRs followed the same trend when limiting analysis to studies that administered ART (RR = 0.74; 95%CI 0.54–1.00; n = 4), analysed serology within 3-months post-vaccination (RR = 0.79; 95%CI 0.60–1.04; n = 8), or reported

Table 1
Characteristics and reported proportion seroprotected/seropositive/seroconverted in the studies that assessed immunogenicity after measles vaccination included in the primary meta-analyses.

Author (year) country	Study design (start year)	Groups	Vaccine used	Age at last vaccination	Outcomes reported*	Interval between vaccination and serology	Number and timing of MV	Serological assay and timing of serology	Serological cut-off	Events (n)/vaccinated HIV; proportion (95%CI)	Events (n)/vaccinated HEU; proportion (95%CI)	Events (n)/vaccinated HU; proportion (95%CI)
al-Attar [62] (1995) USA	Retrospective cohort/cross-sectional (1986)	HI, HEU	Strain NR, preparation NR	1.2–2.3 yr (median 1.3 yr)	I4, I5, S0	1 mo–6.7 yr (mean 1.6 yr)	Primary vaccine? Vertically- and transfusion acquired	ELISA	Manufacturer definitions	25/40; 0.63 (0.46–0.77)	15/16; 0.94 (0.70–1.00)	
Brena [67] (1993) USA	Retrospective cohort/cross-sectional (NR)	HI, HEU	Strain NR, MMR	Median 1.3 yr (1.2–3.0 yr)	I1, I5, S0	Median 2 mo (range 1–42 mo)	Primary vaccine?	ELISA	≥20 EU/ml	11/20; 0.55 (0.32–0.77)	12/13; 0.92 (0.64–1.00)	
Brunell [68] (1995a) USA	Unclear (1980)	HI, HU	Strain NR, MMR/MMRV	Median 15 mo (range 8–26 mo)	I1, I5, S0	Median 7 mo (range 2–29 mo)	Primary vaccine	ELISA	OD > 42	7/9; 0.78 (0.40–0.97)		21/21; 1.00 (0.84–1.00)
Chandwani [35] (2011) USA	Randomised controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx. 12 mo	I4, I5, S1, S2, S3	0 – approx. 2.5 yr	6 mo vaccination	PRNT, b	≥120 mIU/ml	7/7; 1.00 (0.59–1.00)	49/61; 0.80 (0.68–0.89)	
Chandwani [35] (2011) USA	Randomised controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx. 12 mo	I4, I5, S1, S2, S3	0–approx. 2.5 yr	12 mo vaccination only	PRNT, b	≥120 mIU/ml	7/7; 1.00 (0.59–1.00)	22/22; 1.00 (0.85–1.00)	
Chandwani [35] (2011) USA	Randomised controlled trial (1996)	HI, HEU	Enders' attenuated Edmonston strain, MMR	Approx. 12 mo	I4, I5, S1, S2, S3	0–approx. 2.5 yr	6&12 mo vaccination	PRNT, b	≥120 mIU/ml	5/6; 0.83 (0.36–1.00)	55/56; 0.98 (0.90–1.00)	
Echeverria [39] (1996) Spain	Retrospective cohort/cross-sectional (NR)	HI, HEU	Strain NR, MMR	Approx. 12 mo	I1, S1, S2 based on adverse event statement	Approx. 1–2 yr	Primary vaccine	ELISA	>200 mIU/ml	5/8; 0.63 (0.24–0.91)	28/30; 0.93 (0.78–0.99)	
Embree [40] (1989) Kenya	Unclear (NR)	HI, HEU	Strain NR, preparation NR	Unclear	I4, S1, S2 based on adverse event statement	Unclear	Primary vaccine?	Unclear	Protective antibody	7/8; 0.88 (0.47–1.00)	10/15; 0.67 (0.38–0.88)	
Fowlkes [43] (2011) Malawi	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx. 9 mo	I1, I6, S1, S2, S3	Approx. 3–15 mo	6 mo first dose, 9 mo serology	ELISA, b	Package insert	36/61; 0.59 (0.46–0.71)	152/223; 0.68 (0.62–0.74)	288/467; 0.62 (0.57–0.66)
Fowlkes [43] (2011) Malawi	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, monovalent	Approx. 9 mo	I1, I6, S1, S2, S3	Approx. 3–15 mo	9 mo 2nd dose, 12 mo serology	ELISA, b	Package insert	29/45; 0.64 (0.49–0.78)	189/202; 0.94 (0.89–0.97)	385/417; 0.92 (0.89–0.95)
Jain [61] (2017) India	Prospective cohort (2012)	HI, HEU, a	Edmonston-Zagreb, monovalent	Approx. 6 mo	I1, I2, S1, S2	Approx. 2–3 mo	Primary vaccine	ELISA, b	Package insert	2/6; 0.33 (0.04–0.78)	13/33; 0.39 (0.23–0.58)	
Kizito [75] (2013) Uganda	Prospective cohort (2003)	HI, HEU, HU, a?	Edmonston-Zagreb/Schwarz, monovalent	Approx. 9 mo	I6, S0	Approx. 3 mo	Primary vaccine	ELISA, b	≥200 mIU/ml	4/12; 0.33 (0.10–0.65)	44/62; 0.71 (0.58–0.82)	482/637; 0.76 (0.72–0.79)
Lindgren-Alves [76] (2001) Brazil	Retrospective cohort/cross-sectional (1995)	HI, HU	Strain NR, preparation NR	Unclear	I4, I5, S0	Mean 29.4 mo ± 31.9 mo	Revaccination	PRNT	>50 mIU/ml	12/21; 0.57 (0.34–0.78)		29/29; 1.00 (0.88–1.00)
Lyamuya [78] (1999) Tanzania	Cross-sectional (1994)	HI, HU, a?	Schwarz, preparation NR	Approx. 9 mo	I5, I6, S0	Mean 26.1 mo	Primary vaccine	ELISA	≥200 mIU/ml	6/9; 0.67 (0.30–0.93)		617/663; 0.93 (0.91–0.95)
Molyneaux [50] (1993) UK	Retrospective cohort/cross-sectional (NR)	HI, HEU	Strain NR, monovalent or MMR	Min 1 yr	I1, S1, S2	Approx. 3–9 mo	Primary vaccine?	ELISA	Any detectable antibody	9/9; 1.00 (0.66–1.00)	61/61; 1.00 (0.94–1.00)	
Moss [51] (2007) Zambia	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, preparation NR	Approx. 9 mo	I1, I3, I5, S1, S2, S2	Approx. 1–6 mo	Primary vaccine, 6 months post-vaccination, HIV + at vaccination	PRNT, b	≥120 mIU/ml	44/50; 0.88 (0.76–0.95)	198/211; 0.94 (0.90–0.97)	92/98; 0.94 (0.87–0.98)
Moss [51] (2007) Zambia	Prospective cohort (2000)	HI, HEU, HU	Edmonston-Zagreb, preparation NR	Approx. 10–27 mo	I1, I3, I5, S1, S2, S2	Approx. 3–4 mo	Revaccination, 10–27 months	PRNT, b	≥120 mIU/ml	12/13; 0.92 (0.64–1.00)		111/115; 0.97 (0.91–0.99)
Nduati [84] (2016) Kenya	Prospective cohort (2009)	HEU, HU, a	Strain NR, preparation NR	Approx. 9 mo	I5, I6, S0	Approx. 9, 12 or 15 mo	Primary vaccine, 18 mo	ELISA	≥200 mIU/ml		39/47; 0.83 (0.69–0.92)	19/20; 0.95 (0.75–1.00)
Nduati [84] (2016) Kenya	Prospective cohort (2009)	HEU, HU, a	Strain NR, preparation NR	NR	I5, I6, S0	Approx. 9, 12 or 15 mo	Primary vaccine?, >18 mo	ELISA	≥200 mIU/ml		8/8; 1.00 (0.63–1.00)	26/28; 0.93 (0.76–0.99)
Oxtoby [55] (1989) Zaire	Prospective cohort (NR)	HI, HEU, HU	Strain NR, preparation NR	Approx. 9 mo	I2, S1, S2, S3	Approx. 12 mo	Primary vaccine	Unclear	Seronegative to Seropositive	24/37; 0.65 (0.47–0.80)	140/157; 0.89 (0.83–0.94)	199/224; 0.89 (0.84–0.93)

Pensieroso [89] (2009) Italy	Cross-sectional (NR)	HI, HU, a	Schwarz, MMR	Approx. 13–15 mo	I2, I6, S0	Mean 4.7 yr	Primary vaccine	ELISA	≥200 mIU/ml	33/70; 0.47 (0.35–0.59)		50/50; 1.00 (0.93–1.00)
Rainwater-Lovett [92] (2013) Zambia	Prospective cohort (2008)	HI, HU (presumed), a	Strain NR, preparation NR	Median 10 mo	I1, I2, S0	Median 11 mo	Primary vaccine	ELISA	>120 mIU/ml	46/116; 0.40 (0.31–0.49)		9/12; 0.75 (0.43–0.95)
Rainwater-Lovett [92] (2013) Zambia	Prospective cohort (2008)	HI, HU (presumed), a	Strain NR, preparation NR	Median 10 mo	I1, I2, S0	Median 11.0 mo	Revaccination	ELISA	>120 mIU/ml	18/19; 0.95 (0.74–1.00)		13/13; 1.00 (0.75–1.00)
Reikie [93] (2013) South Africa	Prospective cohort (2009)	HEU, HU	Strain NR, preparation NR	Approx. 18 mo	I5, I6, S0	Approx. 3, 9, 13 mo	Primary vaccine, 12 mo serology	ELISA, b	≥330 mIU/ml		22/27; 0.81 (0.62–0.94)	20/28; 0.71 (0.51–0.87)
Reikie [93] (2013) South Africa	Prospective cohort (2009)	HEU, HU	Strain NR, preparation NR	Approx. 18 mo	I5, I6, S0	Approx. 3, 9, 13 mo	Two doses, 24 mo serology	ELISA	≥330 mIU/ml		19/27; 0.70 (0.50–0.86)	13/27; 0.48 (0.29–0.68)
Rudy [59] (1994a) USA	Unclear (1990)	HI, HEU	Strain NR, monovalent	6–11 mo	I4, S1, S2	Approx. 1–3 mo	Primary vaccine, monovalent <12 mo	ELISA, b	Unclear	9/13; 0.69 (0.39–0.91)	17/22; 0.77 (0.55–0.92)	
Rudy [59] (1994b) USA	Unclear (1990)	HI, HEU	Strain NR, MMR	12–15 mo	I4, S1, S2	Approx. 1–3 mo	Primary vaccine MMR ≥12 mo	ELISA, b	Unclear	6/12; 0.50 (0.21–0.79)	13/14; 0.93 (0.66–1.00)	
Siberry [96] (2015) USA	Prospective cohort (2007)	HI, HEU, a	Edmonston-Zagreb, MMR	Median 4.32 yr (IQR 4.04–5.03 yr)	I6, S0	Median 9.8 yr (IQR 6.9–12.1 yr)	Revaccination (for 2% primary vaccine)	PRNT	≥120 mIU/ml	244/428; 0.57 (0.52–0.62)	219/221; 0.99 (0.97–1.00)	
Simani [97] (2013) South Africa	Prospective cohort (archived serum samples) (2005)	HI, HEU, HU	Schwarz, monovalent	Mean 67.8 wks ± 4.4	I1, I5, I6, S0	28 wks post MV1	Primary vaccine, 28 wks post-primary, HIV groups combined	ELISA	≥330 mIU/ml	225/253; 0.89 (0.84–0.93)	110/116; 0.95 (0.89–0.98)	102/112; 0.91 (0.84–0.96)
Simani [97] (2013) South Africa	Prospective cohort (archived serum samples) (2005)	HI, HEU, HU, a	Schwarz, monovalent	Mean 67.8 wks ± 4.4	I1, I5, I6, S0	28 wks post MV1, 2 and 41 wks post MV2	Two doses, 2 wks post-booster, def-ART	ELISA, b	≥330 mIU/ml	235/248; 0.95 (0.91–0.97)	104/114; 0.91 (0.84–0.96)	111/115; 0.97 (0.91–0.99)
Succi [105] (2018) Latin America and the Caribbean	Prospective cohort	HI, HEU, a	Strain NR, preparation NR	Approx. 1 yr	I1, I5, S0	Approx. 2.8 yrs	Primary vaccine	ELISA	≥120 mIU/ml	77/96; 0.80 (0.71–0.88)	51/51; 1.00 (0.93–1.00)	
Sudfeld [100] (2013) Tanzania	Prospective cohort (2005)	HI, HEU, a?	Edmonston-Zagreb, preparation NR	Approx. 9 mo (9–12 mo)	I1, I5, S0	Approx. 3–10 mo	Primary vaccine	ELISA, b	≥200 mIU/ml	16/35; 0.46 (0.29–0.63)	138/201; 0.69 (0.62–0.75)	
Tejiokem [103] (2007) Cameroon, Central African Republic	Cross-sectional (2004)	HI, HEU, a	Strain NR, preparation NR	9 mo–1.3 yr	I1, I5, S0	Median 12.8 mo (90% range; 3.3–26.1 months)	Primary vaccine, commercial ELISA kit	ELISA, b	≥335 mIU/ml	7/46; 0.15 (0.06–0.29)	45/72; 0.63 (0.50–0.74)	
Tejiokem [103] (2007) Cameroon, Central African Republic	Cross-sectional (2004)	HI, HEU, a	Strain NR, preparation NR	9 mo–1.3 yr	I1, I5, S0	Median 12.8 mo (90% range; 3.3–26.1 months)	Revaccination, commercial ELISA kit	ELISA, b	≥335 mIU/ml	1/4; 0.25 (0.01–0.81)	3/5; 0.60 (0.15–0.95)	
Thaithumyanon [25] (2000) Thailand	Prospective cohort (NR)	HI, HEU	Schwarz, monovalent	Approx. 9 mo	I2, I5, S1, S2, S3	Approx. 12 wks	Primary vaccine	ELISA, b	>150 mIU/ml	8/14; 0.57 (0.29–0.82)	14/14; 1.00 (0.77–1.00)	
Waibale [104] (1999) Uganda	Retrospective cohort/cross-sectional (1995)	HI, HEU	Strain NR, monovalent	Median 9.4 mo (5.2–25.8 mo)	I1, I5, S0	Median 14 mo (2.7–30.8 mo)	Primary vaccine (99%)	ELISA	≥15 EU/ml	24/50; 0.48 (0.34–0.63)	122/193; 0.63 (0.56–0.70)	
Walter [30] (1994) USA	Retrospective cohort/cross-sectional (1992)	HI, HEU	Strain NR, MMR	Mean 20.4 month (± 10.2 mo)	I4, I5, S0	Mean 13.3 mo	Unclear, mean 13.3 m post-vaccination	ELISA	≥0.065 OD	14/20; 0.70 (0.46–0.88)	11/11; 1.00 (0.72–1.00)	

HEU, HIV-exposed uninfected; HI, HIV-infected; HU, HIV-unexposed; ELISA, enzyme-linked immunosorbent assay; EU/ml, ELISA units per milliliter; mIU/ml, milli international units per milliliter; mo, months of age; MV, measles vaccination; MMR, measles, mumps, rubella vaccine; MMRV, measles, mumps, rubella, varicella vaccine; NA, not applicable; NR, not reported; OD, optical density; PRNT, plaque reduction neutralization test; sMV, supplemental measles vaccination; yr, years of age.
a: studies where children received antiretroviral therapy.
a?: studies where it is not clear if children received antiretroviral therapy.
b: studies where blood was drawn for measles serology less than six months after vaccination.
* I Immunogenicity outcomes: I0, immunogenicity not reported; I1, Seropositivity after vaccination reported; I2, seroconversion (seronegative before vaccination, Seropositive after vaccination) reported; I3, seroconversion (4-fold rise in titre) reported; I4, measure, which might be either Seropositivity, seroconversion or seroprotection after vaccination, is reported; I5, summary immunological measure (e.g. geometric mean titre) reported; I6, seroprotection after vaccination reported; S Safety outcomes: S0, no adverse event information reported; S1, explicit reporting on adverse events; S2, explicit reporting on serious adverse events; S3, reporting on deaths.

seroprotection (RR = 0.92; 95%CI 0.74–1.15; n = 7), although non-significant (Supplementary data 6 and 8.3). Random effects meta-regression identified significant subgroup differences for studies with a different serological outcome measure (1.17; 95%CI 1.05–1.31), which could explain about 40% of between-study variance.

HIV-infected and HEU children showed similar immune responses after booster measles vaccination (RR = 0.75; 95%CI 0.50–1.13; n = 5) (Fig. 3B) [35, 43, 96, 97, 103]. When stratified by age at vaccination, HIV-infected children were less likely to show a seroresponse when vaccinated at >24 months (RR = 0.58; 95%CI 0.53–0.63; n = 1), but not at ≤24 months of age (RR = 0.84; 95%CI 0.59–1.19; n = 4). Pooled RRs in subgroup analyses yielded similar results (Supplementary data 7 and 8.4).

None of the seven studies reporting on immunogenicity outcomes after primary vaccination in HEU and HIV-unexposed children [43, 51, 55, 75, 84, 93, 97] found significant differences between the two groups. The pooled RR from a fixed-effects model showed similar seroresponses between HEU and HIV-unexposed children (RR = 1.03; 95%CI 0.98–1.07; $I^2 = 26.6\%$), irrespective of age or other covariates (Fig. 4A, Supplementary data 6 and 8.5).

The meta-analysis comparing HEU to HIV-unexposed children after booster vaccination showed a similar likelihood of seroresponding among the two groups (R = 0.99; 95%CI 0.91–1.09; $I^2 = 67.7\%$) (Fig. 4B) [43, 93, 97].

Twenty-eight studies reported on safety (Table 2). In total, 102 HIV-infected and 21 HIV-uninfected children died after immunisation. For two deaths in HIV-infected children, the relation between vaccine administration and death could not be definitely ascertained, of which one occurred within a month post-vaccination [49, 51]. The median time between vaccine administration and end of study during which monitoring of deaths was performed was 38 weeks (range 4–144 weeks).

Twenty-three studies provided information on post-vaccination SAEs other than death in HIV-infected children (period of observation ranged 1–4 weeks post-vaccination). SAEs other than death were reported in 29 of 884 HIV-infected children (3.3%), 2 of 1337 HEU (0.1%), and 18 of 1898 HIV-unexposed children (0.9%). None of the verifiable SAEs were vaccine-related. One study reported a possible, but unverifiable vaccine-related SAE [49]. HIV-uninfected children were more likely to experience AEs (41%) compared with HIV-infected (33%) or HEU (25%) children ($p < 0.001$) (Supplementary data 9).

Of the 71 studies, 59 (83%) had unclear or high-risk of confounding bias and 55 (77%) had unclear or high-risk of attrition bias due to incomplete outcome data. The origin of data and the clarity of outcome definition had low-risk of bias in 60 (85%) and 54 (76%) studies, respectively (Fig. 5, Supplementary data 10). No studies had a high summative risk of bias score (≥7).The GRADE quality of evidence was low or very low, except for the included RCT (Supplementary data 11–14).

The funnel plot for comparisons containing ten or more studies (HIV-infected vs. HEU children after primary vaccination) had an asymmetrical appearance (Supplementary data 15a). The contour-enhanced funnel plot showed that studies were missing in regions of both low and high statistical significance (Supplementary data 15b), suggesting that the asymmetry cannot be explained by publication bias. Smaller studies were likely to have contributed to funnel plot asymmetry (Egger's test $p = 0.009$).

4. Discussion

This review assessed the safety and immunogenicity of measles vaccination in 4867 HIV-infected, 2733 HEU and 7763 HIV-unexposed children. HIV-infected children had 26% (95%CI 10%–39%) lower seroresponse rate to primary measles vaccination compared with HIV-unexposed children, and 22% (95%CI 12%–31%) lower rate compared with HEU children. Differences between groups were no longer present after booster vaccination [25, 63, 106]. This might be due to selection of HIV-infected children that survived to an older age, who were likely to be slow progressors and maintained their immunological status, or received ART. No association between death and measles vaccination was found in HIV-infected children. None of the verifiable SAEs were vaccine-related.

Primary measles vaccination with standard titre measles vaccine at 6-months of age resulted in similar seroresponse rates between groups of HIV-infected [43], HEU [35, 43], and HIV-unexposed children. This finding is supported by studies using high-titre primary measles vaccination at 6-months [37, 47].

Pooled RRs showed no difference between HIV-infected and HIV-unexposed or HEU children after primary vaccination when limiting the meta-analysis to studies that administered ART, reported on seroprotection, or measured serology within 3 or 6-months post-vaccination. Thus, reduced seroresponse to primary vaccination may particularly be evident in HIV-infected children when using a less stringent serological cut-off (seroconversion or seropositivity instead of seroprotection), in the absence of ART, or after a longer time-period between vaccination and serology.

Studies with different timing for ART initiation showed improved immune responses to booster vaccination in HIV-infected children after ART initiation [34, 41, 66, 79] or when started on early-ART [70, 89, 97], while late- or non-treated groups had reduced protective responses after revaccination.

HIV-exposed children showed a non-significant trend towards improved serological response when vaccinated at 6-months of age compared with HIV-unexposed children. This could be explained by reduced transplacental transfer of antibodies from HIV-infected women, resulting in lower levels of maternal antibodies in the infant and less interference with the B-cell response to vaccination [21]. Maternal PMTCT regimens and breastfeeding recommendations for HIV-infected mothers varied substantially between 1987 and 2018, and may have contributed to differences between HEU and other groups. Fetal ART exposure has been associated with less hypergammaglobulinemia in HEU children [107] and higher transfer of transplacental pathogen-specific antibodies was reported in women on triple ART compared with women on short course zidovudine [108]. In this meta-analysis, only two studies reported on maternal ART [61, 100] and one on breastfeeding [100]; no association with measles seroresponse was found.

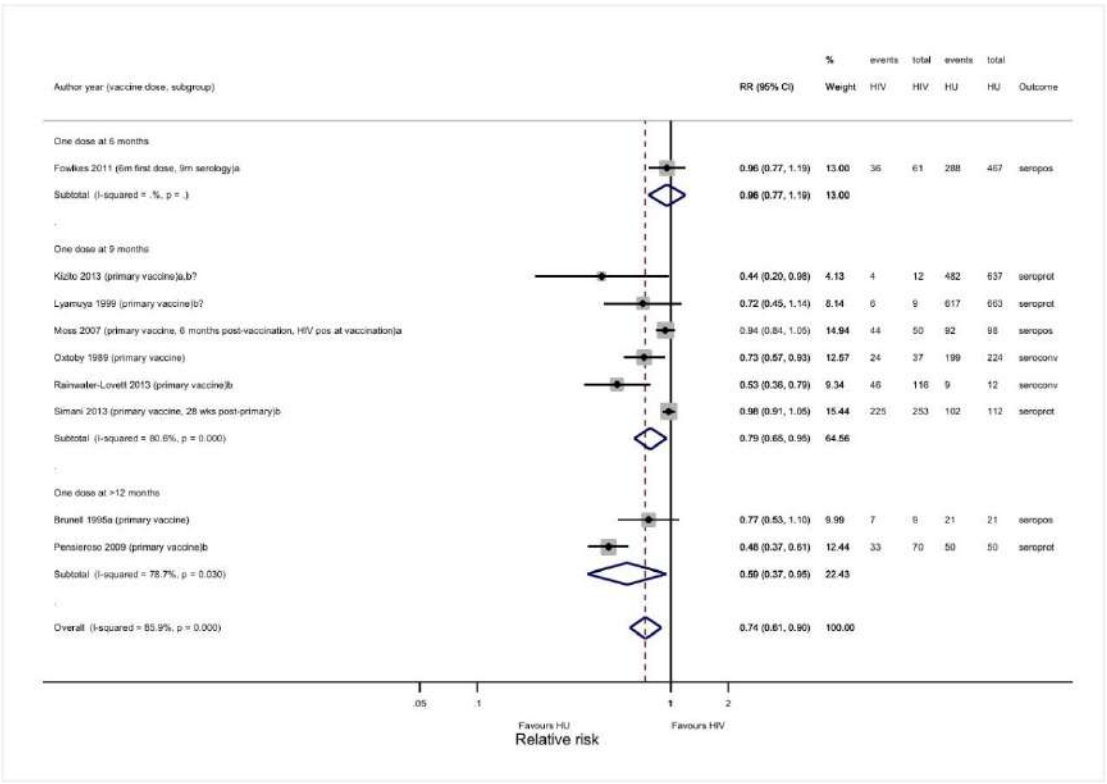
HIV-infected children experienced slightly more SAEs other than death in the first 4-weeks post-vaccination compared with HEU or HIV-unexposed children. However, due to absence of direct comparisons between vaccinated and unvaccinated HIV-infected children and poor quality of reporting, limited conclusions can be drawn from this analysis. HIV-infected children may experience more SAEs due to their underlying illness, unrelated to vaccine administration.

A previous systematic review and meta-analysis of 39 studies analysing safety and immunogenicity of measles vaccination in HIV-infected children searched literature up to February 2009 [26]. The analysis was not stratified according to primary or booster vaccination. We included nine new studies on safety and 15 new studies on immunogenicity. In line with the previous review, we found a trend towards improved serological responses with increasing age at vaccination in HEU and HIV-unexposed children in the descriptive analysis.

Strengths of this review and meta-analysis are the comprehensive search in seven databases and the large number of studies identified. Also, this is the first meta-analysis on this topic to separately analyse primary and booster dose by age at vaccination.

Our results need to be interpreted in the context of the risk of bias evaluation and low to very low quality of evidence. All studies included in this review were of observational nature, except for one RCT [35, 36]. Observational studies may be subject to selection and confounding bias. The majority of studies did not account for age, time since vaccination and CD4+ T-cell count, hence unadjusted outcome measures were used in the analysis. A large number of studies were cross-sectional,

(A)



(B)

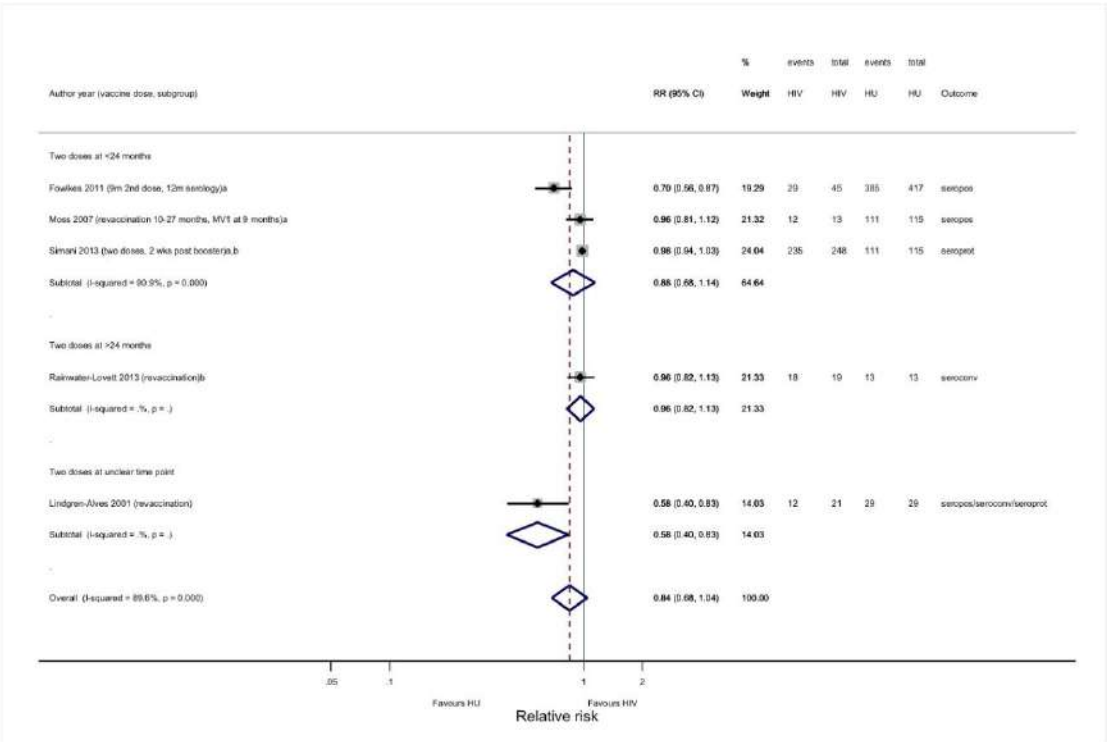


Fig. 2. Forest plots for seroresponses comparing HIV-infected and HIV-unexposed children. (A) One dose of measles vaccine; (B) Two or more doses of measles vaccine. ART, antiretroviral therapy; HU, HIV-unexposed; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seropos/seroconv/seroprot, might either be seropositivity, seroconversion or seroprotection; seroprot, seroprotection; a: studies where blood was drawn for measles serology within six months after vaccination; b: studies where children received antiretroviral therapy; b?: studies where it is not clear if children received antiretroviral therapy.

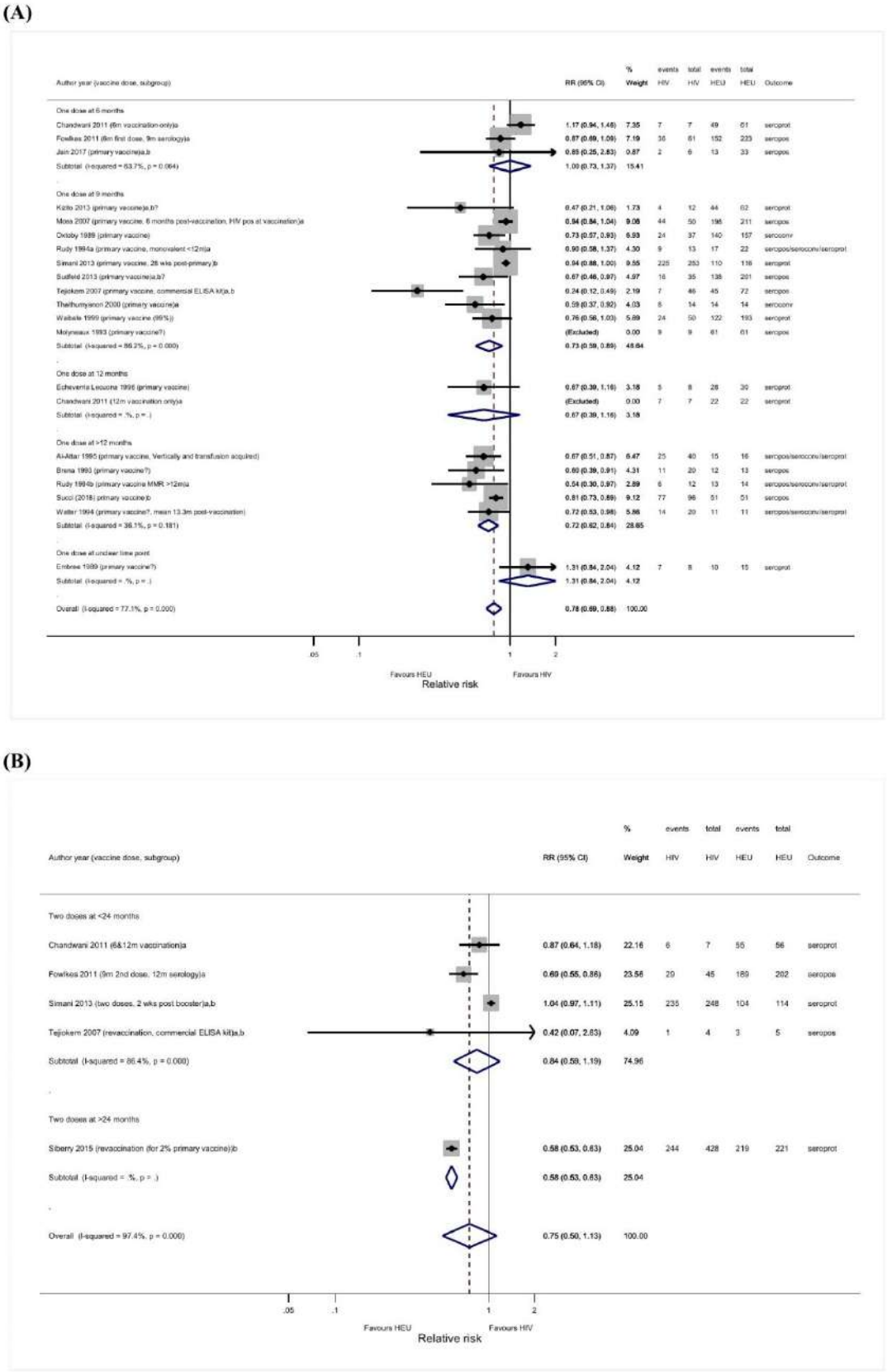
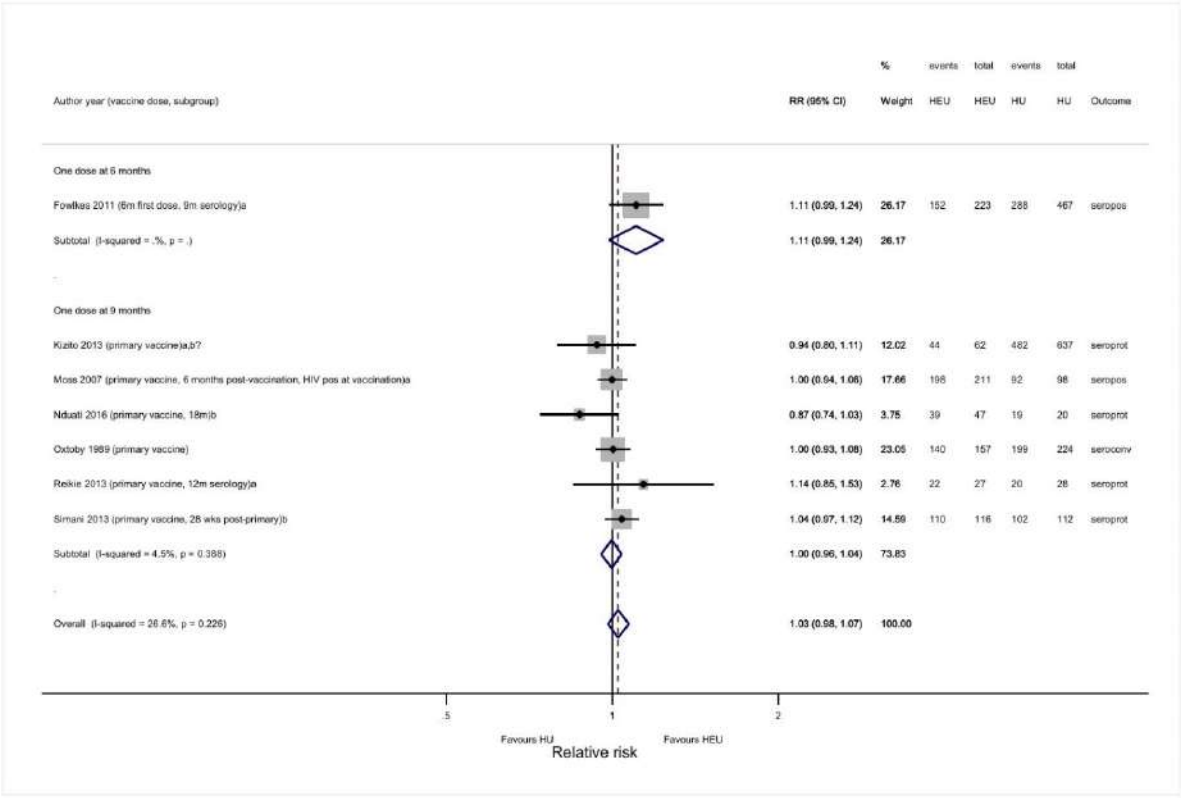


Fig. 3. Forest plots for seroresponses comparing HIV-infected and HIV-exposed uninfected children. (A) One dose of measles vaccine; (B) Two or more doses of measles vaccine. ELISA, enzyme-linked immunosorbent assay; HEU, HIV-exposed uninfected; MMR; Measles Mumps Rubella; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seropos/seroconv/seroprot, might either be seropositivity, seroconversion or seroprotection; seroprot, seroprotection; a: studies where blood was drawn for measles serology within six months after vaccination; b: studies where children received antiretroviral therapy; b?: studies where it is not clear if children received antiretroviral therapy.

(A)



(B)

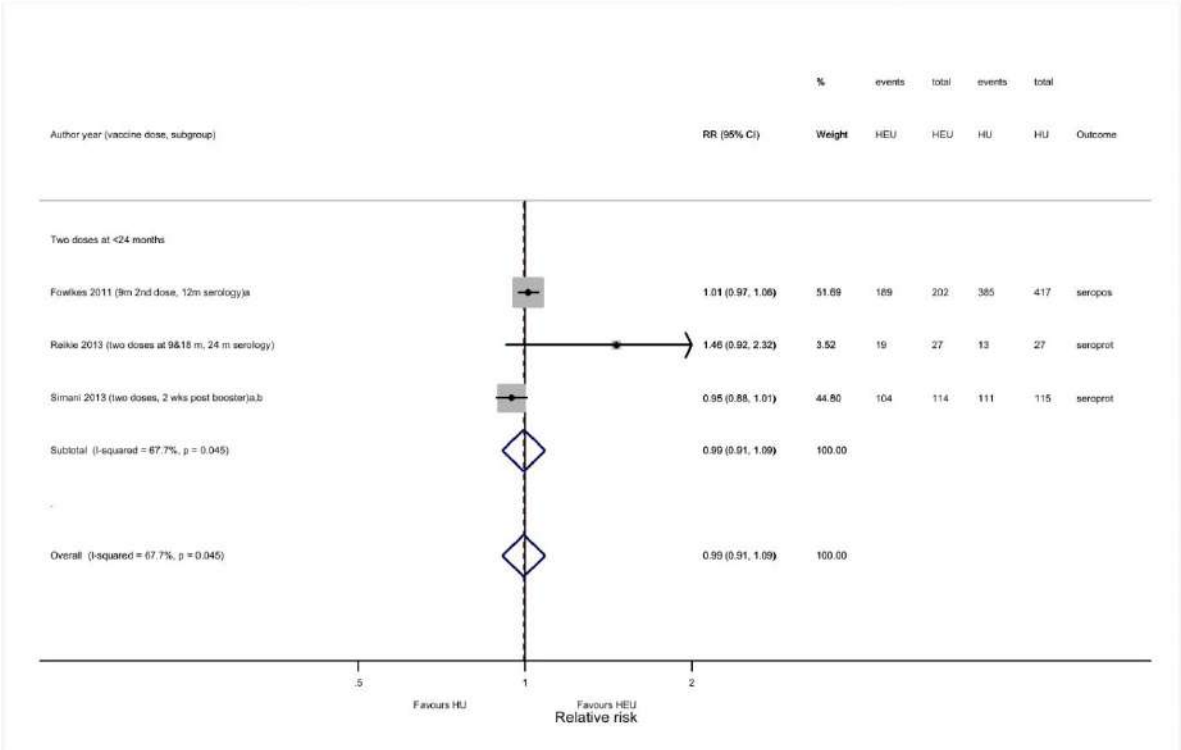


Fig. 4. Forest plots for seroresponses comparing HIV-exposed uninfected and HIV-unexposed children. (A) One dose of measles vaccine; (B) Two or more doses of measles vaccine. HEU, HIV-exposed uninfected; HU, HIV-unexposed; RR, Risk Ratio; seroconv, seroconversion; seropos, seropositivity; seroprot, seroprotection; a: studies where blood was drawn for measles serology within six months after vaccination; b: studies where children received antiretroviral therapy; b?: studies where it is not clear if children received antiretroviral therapy.

Table 2
Adverse events, serious adverse events and deaths in studies reporting on safety.

Study	AEs in HIV-infected/total HIV-infected	AEs in HEU/total HEU	AEs in HIV-unexposed/total HIV-unexposed	SAEs (other than death) in HIV-infected/total HIV-infected	SAEs (other than death) in HEU/total HEU	SAEs (other than death) in HIV-unexposed/total HIV-unexposed	Vaccine-related SAEs (other than death) in HIV-infected	Time observed for SAEs other than death	Post-vaccination deaths in HIV-infected/total post-vaccination deaths in all groups	Vaccine related potentially life-threatening events or deaths	Time observed for deaths
Abzug [33] 2012	NR	–	–	4/193	–	–	NR	28 days	NR	NR	–
Aurpibul [34] 2007	23/51	–	–	0/51	–	–	NA	28 days	NR	–	–
Chandwani [35] 2011a (& Chandwani [36] 1998)	4/8	9/27	–	0/8	0/27	–	0	14 days	0/0	NA	NR
Chandwani [35] 2011b (& Chandwani [36] 1998)	2/7	17/61	–	0/7	0/61	–	0	14 days	0/0	NA	NR
Cutts [37] 1993	29/49[a]	18/376[b]		9/49	4/376		0	5–15 days	9/13	0	Median 1.7 years
Dunn [38] 1998	NR	NR	–	0/56	1/616	–	0	NR	NR	–	–
Echeverria Lecuona [39] 1996	10/14	NR	–	0/14	NR	–	NR	NR	0/NA	NA	NR
Embree [40] 1989	NR	NR	–	0/unclear	0/unclear	–	NA	NR	NR	–	–
Farquhar [41] 2009	NR	–	–	NR/18	–	–	–	NR	0/NA	NA	NR
Fernandez-Ibieta [42] 2007	NR	–	–	NR/55	–	–	–	NR	0/NA	NA	NR
Fowlkes [43] 2011 (& Helfand [24] 2008)a	31/83[c]	84/246[c]	186/512[c]	NER	NER	NER	0	28 days	34/NER	0	16.5 months
Fowlkes [43] 2011 (& Helfand [24] 2008)b	25/59[d]	80/222[d]	152/453[d]								
Fowlkes [29] 2016	NR	NR	NR	0/22	NR	0/865	NA	21 days	NER	0	36 months
Frenkel [44] 1994 (Frenkel [45] 1992)	NR	–	–	0/10	–	–	NA	NR	NR	–	–

Goon [46] 2001	NR	–	–	1/1	–	–	NR	10 days	0/NA	NA	1 year
Jain [61] 2017	2/7	5/39	–	NR	NR	–	0	28 days	NER	0	1 month
Lepage [46] 1992	20/36	71/121	68/166	0/36	1/121	0/166	0	8–14 days	15/17	0	18 months
Marczynska [48] 2001 (substudy)	NR	–	–	0/9	–	–	NA	28 days	0/0	NA	3 months
McLaughlin [49] 1988	NR	–	–	1/70	–	–	Potentially 1, but relation to vaccination not verifiable	NR	Unclear, 41 of 221 HIV-infected patients (19%) died (vaccinated and unvaccinated)/NA	Potentially 1, but relation to vaccination not verifiable	NR
Molyneaux [50] 1993	NR	NR	–	1/9[e]	0/61	–	NA	NR	NR	–	–
Moss [51] 2007	41% of 66 with fever, 70% of 66 with cough	NR	41% of 375 with fever, 57% of 375 with cough	1/66	NR	2/375	NR	28 days	28/38	1 died with measles, but not known to be related to vaccination	27 months
Ndikuyeze [52] 1987	NR	–	–	0/3	–	–	NA	NR	NR	NA	–
Oldakowska [53] 2001	0/13	–	–	0/13	–	–	NA	28 days	NR	–	–
Oshitani [54] 1996	NR	–	NR	11/37	–	5/111	NR	NR	11/16	NR	NR
Oxtoby [54] 1989	NR	NR	NR	4/37[f]		11/381[f]	NER	NR	NER	–	NR
Palumbo [56] 1992 (& Hoyt [57] 1992)	0/92[g]	–	–	4/94	–	–	NR	NR	2/NA	0	NR
Ramon-Garcia [58] 1995	NR	–	–	2/2	–	–	NR	NR	2/NA	NR	NR
Rudy [59] 1994a&b	0/13 and 0/12	0/22 and 0/14	–	0/13 and 0/12	0/22 and 0/14	–	NA	NR	NR	–	–
Seth [60] 2016	0/66	–	–	0/66	–	–	NA	28 days	NR	–	–
Thaithumyanon [25] 2000	NR	NR	–	NR	NR	–	–	short term	1/NER	0	12 weeks

Studies were excluded from the safety table if they did not report on serious adverse events or deaths.

AE, adverse event; HEU, HIV-exposed uninfected; HI, HIV-infected; HU, HIV-unexposed; HU, HIV-unexposed; NA, not applicable; NER, not explicitly reported; NR, not reported; SAE, serious adverse event.

[a] Incidence of symptoms with onset within 5–15 days after vaccination among HIV-infected infants: diarrhoea (n = 22), cough (n = 14), rhinorrhoea (n = 12), fever (n = 29), morbilliform rash (n = 2), unscheduled consultation (n = 6); highest number (n = 29) used for calculations.

[b] Incidence of symptoms with onset within 5–15 days after vaccination among non-HIV-infected infants: diarrhoea (n = 14), cough (n = 15), rhinorrhoea (n = 13), fever (n = 18), conjunctivitis (n = 3), unscheduled consultation (n = 7); highest number (n = 18) used for calculations.

[c] Parental reports of any symptoms during the first 21 days after measles vaccination at 6 months of age.

[d] Parental reports of any symptoms during the first 21 days after measles vaccination at 9 months of age.

[e] HIV-infected child who required hospital admission for severe measles, but unclear whether this was before or after vaccination.

[f] Only cases of clinical measles explicitly reported during follow-up at a mean of 9 months after vaccination.

[g] Unclear number of HIV-infected children vaccinated in case finding; number reported during outbreak.

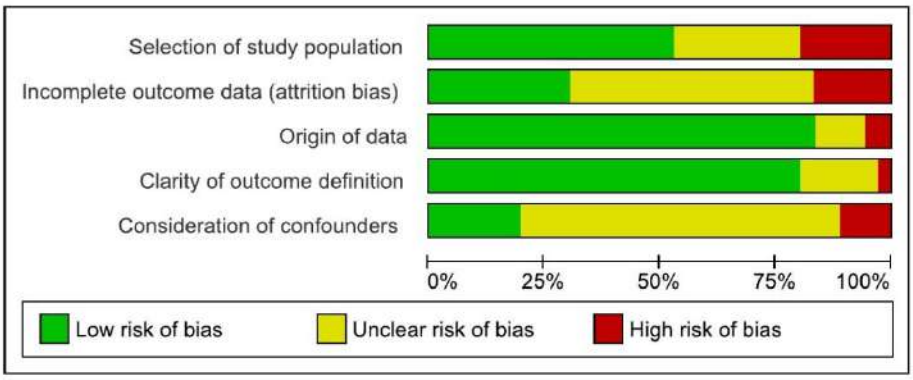


Fig. 5. Summary of risk of bias evaluation using adapted Cochrane framework.

and single time-point data were used for assessment of immune responses, increasing the risk of selection bias.

In the different meta-analyses, substantial heterogeneity between studies was detected. Therefore, pooled results should be viewed as an average representing a wide distribution of seroresponses. Differences in the definition and cut-off points for serological outcomes partly explained the large heterogeneity. Due to inconsistent outcome reporting across studies, we used seroresponse, a composite of seroprotection, seropositivity or seroconversion. We encourage consistency in reporting to allow for comparison between studies.

The findings from this review support the 2017 recommendations by the World Health Organization to administer an initial dose of measles vaccination at 6-months of age in areas with high incidence of HIV-infection and measles, followed by two routine doses [109]. To date, only three studies with comparison groups have evaluated immunogenicity after standard-titre measles vaccination at 6-months of age [35, 43, 61]. Future studies should evaluate serological response to early measles vaccination in HIV-infected and HEU children. In addition, there are concerns regarding long-term immunogenicity of a 2-dose schedule given early in life, as antibody titres in HIV-infected children on ART wane over time [65, 79]. Therefore, we recommend future studies on long-term waning of immunogenicity after early vaccination in HIV-infected children treated with ART.

Contributors

EM, MCN, KKG participated in the conception, design and implementation of the study. EM and MvR performed screening and data extraction. EM did the statistical analysis. EM wrote the first draft of the report with input from MCN, MvR, KKG, DEG and SAM. All authors have approved the final manuscript.

Declaration of Interests

MCN reports personal fees from Pfizer and non-financial support from Sanofi outside the submitted work. SAM reports grants from Medical Research Council South Africa, grants from Department Science and Technology/National Research Foundation during the conduct of the study; grants and personal fees from the Bill and Melinda Gates Foundation, grants from GSK, grants and personal fees from Sanofi, grants from Pfizer outside the submitted work. All other authors declare no competing interests.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eclinm.2018.06.002.

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Appendix 2

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MAJOR ARTICLE

Measles Immunity at 4.5 Years of Age Following Vaccination at 9 and 15–18 Months of Age Among Human Immunodeficiency Virus (HIV)–infected, HIV-exposed–uninfected, and HIV-unexposed Children

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Background. Human immunodeficiency virus (HIV)–infected and HIV-exposed–uninfected (HEU) children may be at increased risk of measles infection due to waning of immunity following vaccination. We evaluated persistence of antibodies to measles vaccination at 4.5 years of age in HIV-unexposed, HEU, and HIV-infected children with CD4+ ≥25% previously randomized to immediate antiretroviral therapy (ART) interrupted at 12 months (HIV/Immed-ART-12), 24 months (HIV/Immed-ART-24), or when clinically/immunologically indicated (HIV/Def-ART). The HIV/Def-ART group initiated ART by median 5.8 (interquartile range, 4.4–10.3) months of age.

Methods. In this study, HIV-unexposed (n = 95), HEU (n = 84), HIV/Immed-ART-12 (n = 70), HIV/Immed-ART-24 (n = 70), and HIV/Def-ART (n = 62) children were scheduled to receive measles vaccination at age 9 and 15–18 months. Antimeasles serum immunoglobulin G titers were quantified using enzyme-linked immunosorbent assay at 4.5 years.

Results. Compared with HIV-unexposed children (2860 mIU/mL), measles antibody geometric mean titers (GMTs) were significantly lower in both HIV/Immed-ART-12 (571; $P < .001$) and HIV/Immed-ART-24 (1136; $P < .001$) but similar in the HIV/Def-ART (2777) and HEU (3242) groups. Furthermore, compared with HIV-unexposed, antibody titers ≥330 mIU/mL (ie, presumed serocorrelate for protection; 99%) were also significantly lower in HIV/Immed-ART-12 (70%; $P < .001$) and HIV/Immed-ART-24 (83%; $P < .001$) but similar in the HIV/Def-ART (90%) and HEU (98%) groups.

Conclusions. HIV-infected children in whom ART was interrupted at either 12 or 24 months had lower GMTs and lower proportions with seroprotective titers than HIV-unexposed children, indicating a potential downside of ART treatment interruption.

Clinical Trials Registration. NCT00099658 and NCT00102960.

Keywords. antibody response; measles vaccine; HIV; HIV exposure; persistence.

In 2015, measles infection contributed to 1.2% (134 200 deaths) of global mortality in children aged <5 years [1]. Despite safe and effective vaccines [2], sporadic outbreaks of measles still persist [3] due to low vaccine coverage, reduced maternal measles antibody transfer to infants born to vaccinated women compared with those with naturally acquired immunity, and pockets of susceptible individuals with suboptimal immune responses to vaccination [4, 5].

Following measles vaccination, human immunodeficiency virus (HIV)–infected children maintain seroprotective titers for a shorter duration than HIV-uninfected children [6–9]. A meta-analysis [7] of 5 studies estimated that 68% (95% confidence interval [CI], 45%–88%) of HIV-infected primary responders had seroprotective antibody titers 2 years after their last measles vaccine and 40% (95% CI, 10%–73%) after 5 years, generally in the absence of combination antiretroviral therapy (ART) [10].

Increased ART coverage has improved survival of HIV-infected children, potentially creating a cohort of measles-susceptible children due to quicker waning of antibodies over time [11]. Two studies have been published on measles seroresponses in HIV-infected children receiving early ART initiation, as currently recommended by the World Health Organization (WHO) [12]. Pensieroso et al reported that early ART-treated HIV-infected children generated and preserved measles-specific memory B cells comparable to healthy controls

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[13]. However, Succi et al observed no difference in measles seropositivity at 4 years of age between children on ART initiated <12 months age, ≥12 months of age, or no ART [14]. Although early initiation of ART in HIV-infected infants is now recommended, lifelong continuous treatment may be problematic due to long-term toxicity, risk of ART resistance, waning adherence, and resource constraints. Previous studies showed that early time-limited (interrupted) ART had improved clinical outcomes compared with deferred continuous ART [15].

Because of effective prevention of mother-to-child-transmission, a large proportion of children born to HIV-infected mothers are HIV exposed but uninfected (HEU) [16]. These children may have suboptimal immune response to vaccination due to intrauterine exposure to HIV and/or maternal ART [17–21]. HEU infants have reduced CD4+ and CD8+ cell counts, impaired T-cell maturation, and both hypo- and hyperresponsiveness upon T-cell activation [22–24]. We previously reported that the proportion with seroprotective measles antibody titers at approximately 9 months post-measles booster vaccination was lower in HEU (79.6%) than HIV-unexposed children (94.3%) [25]. In contrast, other studies have reported similar measles antibody persistence between HEU and HIV-unexposed children up to 2 years of age [8, 26–30].

A recent systematic review highlighted the absence of published studies on long-term immunity to measles vaccination beyond 2 years of age in HEU and HIV-infected children, especially with early ART initiation [31].

In this study, we aimed to evaluate persistence of measles antibody titers at 4.5 years of age in HIV-unexposed, HEU, and HIV-infected children previously randomized to initiate ART when clinically/immunologically indicated or within 6–12 weeks of age, which was interrupted at 12 or 24 months.

METHODS

Study Population

This cohort study included HIV-infected children enrolled in a randomized open-label trial on timing of ART initiation (Children with HIV Early Antiretroviral [CHER] study) [32] and a parallel cohort of HEU and HIV-unexposed children [25, 33, 34]. The CHER study enrolled HIV-infected infants aged 6 to 12 weeks with CD4+ T-cell percentages ≥25% and randomized to initiate immediate ART followed by interruption at 12 months (HIV/Immed-ART-12) or 24 months (HIV/Immed-ART-24), or deferred ART until clinically or immunologically indicated (HIV/Def-ART). A convenience sample of HIV-infected children with CD4+ T-cell percentage <25% was included (HIV+/CD4+ <25%) who initiated ART at enrollment for 12 or 24 months followed by interruption. In parallel, children born to HIV-uninfected mothers (HIV-unexposed) and HIV-infected mothers who were themselves HIV-uninfected (HEU) were enrolled in this study.

Children included in this study were enrolled between April 2005 and June 2006. The Schwarz measles vaccine (Rouvax, Aventis, France) was administered at 38–42 weeks (9 months) and 64–76 weeks (15–18 months) of age. Participants received other childhood vaccines according to the public immunization program [25, 34]. The ART regimen consisted of zidovudine, lamivudine, and lopinavir-ritonavir. An interim analysis of the CHER trial showed greater risk of disease progression and death in the HIV/Def-ART group and thus recommended they be evaluated for ART initiation [32]. Children in the HIV/Def-ART group began ART at median age 5.8 months (interquartile range [IQR], 4.4–10.3 months); 73% had been initiated on ART before receiving the first measles vaccine and 88% before receiving the booster measles dose. Those in HIV/Immed-ART-12 and HIV/Immed-ART-24 groups were initiated on ART at a median age of 7.4 (IQR, 6.6–8.9) weeks.

Laboratory Methods

Blood samples were collected at 4.5 years (232–236 weeks) of age. After centrifugation, serum was aliquoted and stored at −20°C to −70°C. Measles-specific immunoglobulin G (IgG) antibodies were measured using an indirect enzyme-linked immunosorbent assay (Enzygnost, Dade Behring, Marburg, Germany). The assay included an internal reference for the quantitative assessment of measles IgG concentrations, adjusted according to the WHO International Reference Preparation (1964) [35]. Antibody titers were calculated using the α-method according to the manufacturer's instructions. Measles seropositivity was classified as IgG titers ≥150 mIU/mL (optical density [OD], 0.1–0.2) and seroprotection as titers ≥330 mIU/mL (OD >0.2) as per manufacturer's guidelines, which has been supported by other studies [36, 37]. All negative (<150 mIU/mL; OD <0.1) and equivocal (150–329 mIU/mL) samples were analyzed in duplicate. Seronegative samples were assigned a titer half the value of the assay's detection limit (ie, 75 mIU/mL).

Statistical Analyses

Geometric mean titers (GMTs) were calculated following $\log_{10}$ transformation of antibody titers and then compared between groups using multivariable linear regression, considering age, sex, race, study center, and CD4+ T-cell percentage at the 9-month measles dose as covariates. Proportions of children who met seropositivity and seroprotection cutoffs were compared between groups using multivariable logistic regression, adjusted for the aforementioned covariates.

Weight-for-height, weight-for-age, and height-for-age z scores were calculated using WHO child growth references [38]. Stunting was defined as height-for-age z score ≤2 standard deviations (SD) from the WHO reference population mean, wasting as weight-for-height score of ≤2 SD below the mean, and underweight as weight-for-age z score ≤2 SD below the mean.

Logistic regression was used to explore the association between long-term seroprotective antibody titers and HIV status, ART initiation strategy, sex, race, age, time interval between vaccination and blood collection, and nutritional status at the primary measles dose by reporting adjusted odds ratios (aORs) and 95% CIs. In HIV-infected children, we further analyzed the effect of ART (at time of primary and booster measles doses, immunogenicity visit) and CD4+ T-cell percentage (at enrollment and primary measles dose) on the proportion of participants with seroprotective antibody titers. Variables with P values ≤ .15 in univariate regression were included in multivariable regression models. HIV-unexposed children were used as the reference group. Participants were included in the analyses if they received 2 doses of measles vaccination and had an immunogenicity visit with serum collection around 4.5 years of age. Unadjusted P values ≤ .05 and Bonferroni adjusted P values ≤ .007, taking multiple comparisons into consideration, were considered statistically significant. All tests were 2 sided. Data were analyzed using Stata version 13 (Stata Corporation, College Station, TX).

Ethics

The Human Research Ethics Committee of the University of the Witwatersrand approved this substudy (M170391). The parent trials were approved by ethics committees of the University of the Witwatersrand and the Stellenbosch University, Medicine Control Council (South Africa), and the Division of AIDS of the National Institutes of Health. Written informed consent was obtained from the parent(s) of participants prior to study entry, including approval to analyze immune responses to other vaccines.

RESULTS

Of 578 children enrolled, samples were unavailable for 141 (24%) participants at 4.5 years of age, as detailed in Figure 1, and included high infant mortality rates in HIV/Def-ART (19%; n = 20), HIV/Immed-ART-12 (10%; n = 11), and HIV-Immed-ART-24 (8%; n = 8) groups [32]. Of the 437 children who received 2 doses of measles vaccine, 388 (89%) had serum samples for analysis at median age of 4.4 years: 95 HIV-unexposed, 84 HEU, 70 HIV/Immed-ART-12, 70 HIV/Immed-ART-24, 62 HIV/Def-ART, and 7 HIV-infected with CD4+ <25% (Table 1). Baseline characteristics of children included in the current analyses were not significantly different from those who were excluded, except for deaths and those administered <2 doses of measles vaccine (Supplementary Table 1).

Overall, the median age at time of the primary measles dose was 9.0 months and at booster dose 15.4 months with a time interval of median 37.7 months between primary vaccination and the immunogenicity visit (Table 1). Differences in age at vaccination between groups were unlikely to be of clinical relevance. More children were of black-African descent in the HEU (95%), HIV/Immed-ART-12 (93%), HIV/Immed-ART-24 (96%), and HIV/Def-ART (98%) groups compared with HIV-unexposed children (80%). More HIV-infected children (HIV/Immed-ART-12, 31%; HIV/Def-ART, 45%) were stunted at the primary measles dose than HIV-unexposed (14%) and HEU (14%) children; however, nutritional status was similar by 4.5 years of age (Table 1).

Total number of HIV-infected children per group (re-)initiated on ART at the time of vaccinations and sample collection and duration of ART interruption are presented in Table 1 and Supplementary Figure 1. Mean CD4+ percentage and median CD4+ count were significantly different between HIV groups (Table 1).

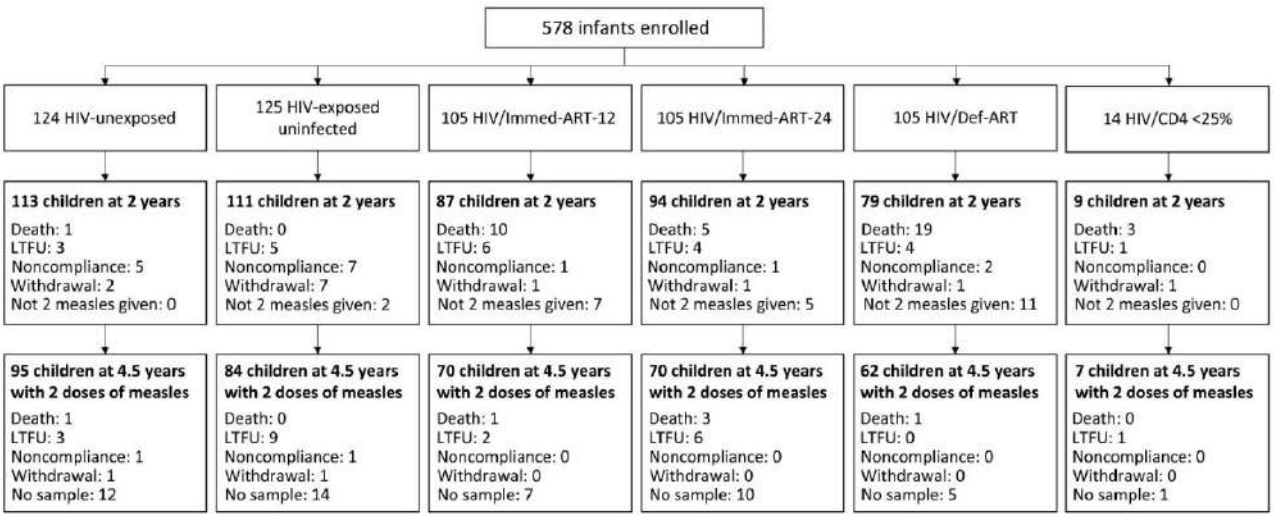

Figure 1. Study profile showing the study population from enrollment through the current analysis. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; HIV/Immed-ART-12, HIV-infected children receiving immediate ART until 12 months of age; HIV/Immed-ART-24, HIV-infected children receiving immediate ART until 24 months of age; HV/Def-ART, HIV-infected children on deferred ART until clinically or immunologically indicated; HIV/CD4+ <25%, convenience sample of HIV-infected children with CD4+ <25% at enrollment and immediate initiation on ART; LTFU, loss to follow-up.

Table 1. Demographics and Baseline Characteristics of Participants Included in the Immunogenicity Analysis

Characteristic	HIV-Unexposed (n = 95)	HEU (n = 84)	HIV/Immed-ART-12 (n = 70)	HIV/Immed-ART-24 (n = 70)	HIV/Def-ART (n = 62)	HIV/CD4+ <25% (n = 7)	Total (n = 388)
Male, n (%)	50 (53)	43 (51)	24 (34)	36 (51)	23 (37)	2 (29)	178 (46)
Black-African, n (%)	76 (80)[a,b,c,d]	80 (95)[a]	65 (93)[b]	67 (96)[c]	61 (98)[d]	6 (86)	355 (91)
Mixed ancestry, n (%)	19 (20)[a,b,c,d]	4 (5)[a]	5 (7)[b]	3 (4)[c]	1 (2)[d]	1 (14)	33 (9)
Age at primary measles dose, median months (IQR)	8.9 (8.8–9.0)[a,b,c,d]	9.0 (8.9–9.1)[a]	9.0 (8.8–9.2)[b]	9.1 (8.9–9.4)[c]	9.2 (8.9–9.5)[d]	9.5 (8.9–9.7)	9.0 (8.8–9.2)
Age at booster measles dose, median months (IQR)	15.2 (15.2–15.4)[a,b,c,d]	15.4 (15.3–15.6)[a,e]	15.5 (15.3–15.9)[b]	15.5 (15.3–15.7)[c]	15.7 (15.3–16.5)[d,e]	15.7 (15.2–15.9)	15.4 (15.2–15.7)
Age at immunogenicity visit, median years (IQR)	4.4 (4.4–4.5)	4.4 (4.4–4.5)	4.4 (4.4–4.5)	4.4 (4.4–4.5)	4.4 (4.4–4.5)	4.5 (4.5–4.6)	4.4 (4.4–4.5)
Interval from primary measles dose to immunogenicity visit, median months (IQR)	37.7 (37.7–38.2)[b,d]	37.7 (37.7–38.1)[e]	37.6 (36.9–38.1)[b]	37.7 (37.5–38.0)	37.7 (36.7–38.1)[d,e]	38.5 (38.4–38.7)	37.7 (37.6–38.1)
Stunting[f] at primary measles dose, n (%)	13 (14)[b,d]	12 (14)[e]	22 (31)[b]	13 (19)[h]	27 (45)[d,e,h]	3 (43)	90 (24)
Wasting[i] at primary measles dose, n (%)	3 (3)	3 (4)	3 (4)	0 (0)	1 (2)	0 (0)	10 (3)
Underweight[j] at primary measles dose, n (%)	8 (8)	3 (4)	8 (11)	4 (6)	8 (13)	0 (0)	31 (8)
Stunting at immunogenicity visit, n (%)	10 (11)	18 (21)	18 (26)	16 (23)	11 (18)	3 (43)	76 (20)
Wasting at immunogenicity visit, n (%)	1 (1)	1 (1)	0 (0)	0 (0)	1 (2)	0 (0)	3 (1)
Underweight at immunogenicity visit, n (%)	5 (5)	1 (1)	3 (4)	1 (1)	3 (5)	0 (0)	13 (3)
Enrollment CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1980 (1695–2722)	2283 (1739–2924)	2612 (1771–3277)	1727 (963–2356)	2283 (1695–2982)
Enrollment CD4+ lymphocyte %, mean (±SD)	NA	NA	36.5 (8.3)	38.0 (8.9)	38.1 (7.8)	22.6 (4.3)	37.0 (8.7)
Primary measles dose CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	2104 (1579–2713)[k]	2149 (1604–2746)[h]	1518 (1207–2189)[h,k]	1495 (1209–2330)	1970 (1421–2583)
Primary measles dose CD4+ lymphocyte %, mean (±SD)	NA	NA	40.0 (9.3)[k]	39.1 (7.7)[h]	31.5 (6.8)[h,k]	32.0 (8.0)	36.9 (8.8)
Booster measles dose CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1286 (1016–1746)[k,l]	1860 (1306–2220)[l]	1734 (1357–2277)[k]	1612 (900–1976)	1614 (1181–2131)
Booster measles dose CD4+ lymphocyte %, mean (±SD)	NA	NA	26.3 (7.1)[k,l]	34.2 (7.9)[l]	34.2 (8.4)[k]	29.7 (10.2)	31.4 (8.6)
Immunogenicity visit CD4+ lymphocyte count, median cells/mL (IQR)	NA	NA	1055 (723–1330)[k]	945 (715–1241)[h]	1202 (910–1524)[h,k]	No observations	1057 (779–1383)
Immunogenicity visit CD4+ lymphocyte %, mean (±SD)	NA	NA	31.7 (7.4)[k]	30.0 (7.4)[h]	36.6 (7.7)[h,k]	No observations	32.5 (7.9)
Total on ART at 9-month measles dose, n/N (%)	NA	NA	69/70 (99)[k]	70/70 (100)[h]	44/62 (71)[h,k]	7/7 (100)	190/209 (91)
Total on ART at 15–18-month measles dose, n/N (%)	NA	NA	21/70 (30)[k,l]	70/70 (100)[h,l]	53/62 (85)[h,k]	7/7 (100)	151/209 (72)

Table 1. Continued

Characteristic	HIV-Unexposed (n = 95)	HEU (n = 84)	HIV/Immed-ART-12 (n = 70)	HIV/Immed-ART-24 (n = 70)	HIV/Def-ART (n = 62)	HIV/CD4+ <25% (n = 7)	Total (n = 388)
Total on ART at immunogenicity visit, n/N (%)	NA	NA	50/70 (71)[k]	44/70 (63)[h]	58/62 (94)[h,k]	7/7 (100)	159/209 (76)
Interval from ART initiation to primary measles dose, median months (IQR)	NA	NA	7.4 (7.4; 7.5)[k]	7.4 (7.4; 7.4)[h]	3.7 (−1.3; 5.5)[h,k]	7.4 (7.4; 7.5)	7.4 (5.6; 7.4)
Duration of ART interruption, median months (IQR)	NA	NA	7.3 (3.2; 45.3)[k]	16.5 (3.9; 34.1)[h]	0 (0; 0)[h,k]	0 (0; 0)	3.9 (0; 21.4)

Five participants had missing data on nutritional status primary measles dose vaccination; 12 (HIV/Def-ART group) and 25 (HIV/Immed-ART groups) participants had missing data on CD4+ T cell count/percentage at the immunogenicity visit. *P* values were calculated using a Kruskal–Wallis test and adjusted for multiple comparisons.

Abbreviations: ART, antiretroviral therapy; CD4+ <25%, HIV-infected children with CD4+% <25% at enrollment who received immediate ART; HEU; HIV-exposed–uninfected children; HIV, human immunodeficiency virus; HIV/Immed-ART-12, HIV-infected children on immediate ART interrupted at 12 months; HIV/Immed-ART-24, HIV-infected children on immediate ART interrupted at 24 months; HIV/Def-ART, HIV-infected children on deferred ART; IQR, interquartile range; NA, not applicable; SD, standard deviation.

[a]Significant difference between HIV-unexposed and HEU.

[b]Significant difference between HIV-unexposed and HIV/Immed-ART-12.

[c]Significant difference between HIV-unexposed and HIV/Immed-ART-24.

[d]Significant difference between HIV-unexposed and HIV/Def-ART.

[e]Significant difference between HEU and HIV/Def-ART.

[f]Stunting: height-for-age *z* score ≤2 SD.

[g]Significant difference between HEU and HIV/Immed-ART-12.

[h]Significant difference between HIV/Immed-ART-24 and HIV/Def-ART.

[i]Wasting: weight-for-height score ≤2 SD.

[j]Underweight: weight-for-age *z* score ≤2 SD.

[k]Significant difference between HIV/Immed-ART-12 and HIV/Def-ART.

[l]Significant difference between HIV/Immed-ART-12 and HIV/Immed-ART-24.

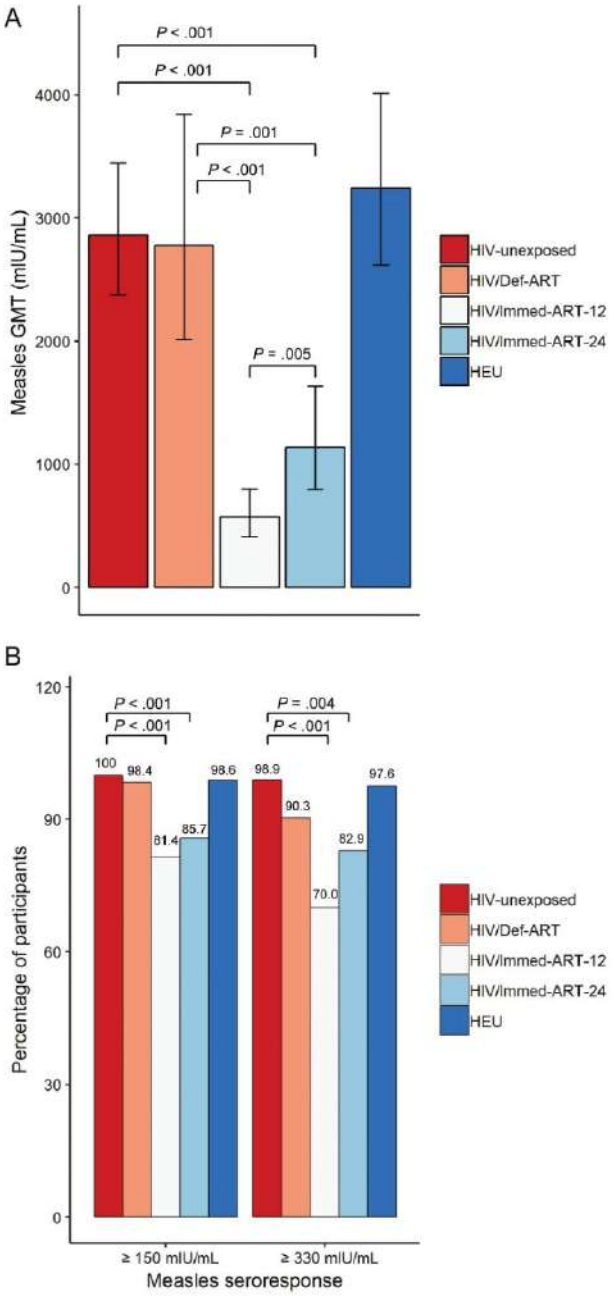

Figure 2. Measles antibody geometric mean titers and proportion of children with seropositive and seroprotective antibody levels at 4.5 years of age. *P* value deemed significant at ≤.007 after Bonferroni correction. *P* values were either calculated by linear or logistic regression and adjusted for age at the immunogenicity visit, sex, race, study center, and CD4+ cell percentage at the primary measles dose in HIV-infected children or by Fisher exact test. Abbreviations: ART, antiretroviral therapy; GMT, geometric mean titer; HEU, HIV-exposed uninfected; HIV, human immunodeficiency virus; HIV/Def-ART, HIV-infected children on deferred ART until clinically or immunologically indicated; HIV/Immed-ART-12, HIV-infected children receiving immediate ART until 12 months of age; HIV/Immed-ART-24, HIV-infected children receiving immediate ART until 24 months of age.

Persistence of Measles Antibodies at 4.5 Years of Age

GMTs were lower in HIV/Immed-ART-12 (571 mIU/mL) and HIV/Immed-ART-24 (1136 mIU/mL) children than in HIV-unexposed children (2860 mIU/mL; $P < .001$ for both comparisons). Also, GMTs were lower in the HIV/Immed-ART-12 and HIV/Immed-ART-24 groups than in the HIV/Def-ART children (2777 mIU/mL; $P < .001$ and $P = .001$, respectively) when adjusted for race, study center, age and CD4+ percentage at time of the primary measles dose. Furthermore, GMTs were significantly lower in HIV/Immed-ART-12 children compared with HIV/Immed-ART-24 children ($P = .005$; Figure 2A; Supplementary Table 2).

Measles seropositivity (≥150 mIU/mL) was present in all HIV-unexposed children and 98.4% of the HIV/Def-ART groups ($P = .395$). In contrast, fewer children in the HIV/Immed-ART-12 (81.4%; $P < .001$) and HIV/Immed-ART-24 (85.7%; $P < .001$) groups were seropositive than HIV-unexposed children (Figure 2B, Supplementary Table 2).

Similarly, the percentage of children with seroprotective titers (ie, ≥330 mIU/mL) was significantly lower in the HIV/Immed-ART-12 (70.0%; $P < .001$) and HIV/Immed-ART-24 (82.9%; $P = .004$) groups compared with HIV-unexposed children (99.0%). In the HIV/Def-ART group, 90.3% had seroprotective titers. Of 7 children with CD4+ T-cell <25% at enrollment, 85.7% were measles seropositive and 85.7% had seroprotective titers. There were no differences in GMTs, seropositivity, or percentage with seroprotective titers between the HEU and HIV-unexposed children (Figure 2B, Supplementary Table 2).

Exclusion of participants in the early therapy groups who did not interrupt ART for various reasons and proceeded on continuous ART (n = 3 in HIV/Immed-ART-12, n = 10 in HIV/Immed-ART-24 and n = 6 in CD4+<25% groups) did not significantly alter results (Supplementary Table 3).

Determinants of Long-term Seroprotection

We examined the association between long-term measles seroprotection and HIV status, timing of ART initiation, sex, race, age at vaccination, age at immunogenicity visit, and nutritional status at the primary measles dose among all children (n = 381) in univariate and multivariable logistic regression (Table 2). After controlling for timing of ART initiation, sex, race, age at vaccination, age at immunogenicity visit, and nutritional status, HIV/Immed-ART-12 (aOR, 0.03; 95% CI, 0.003–0.20), HIV/Immed-ART-24 (aOR, 0.05; 95% CI, 0.006–0.41), and HIV/Def-ART (aOR, 0.11; 95% CI, 0.01–0.90) children had a lower odds for seroprotective titers relative to HIV-unexposed children.

Among HIV-infected children (n = 202), we assessed the association of measles seroprotection with the aforementioned covariates, in addition to receipt of ART at the time of the primary or booster dose, ART at 4.5 year of age, and CD4+ T-cell percentage at enrollment or primary dose. In multivariable logistic regression, HIV-infected children who received ART at the time of the booster measles dose were 2.27 (95% CI, 1.02–5.05) more likely to have antibody titers ≥330 mIU/mL than those who did not receive ART. Similarly, ART at time of measuring antibody persistence (aOR, 3.07; 95% CI, 1.39–6.84)

Table 2. Association of Measles Seroprotection at 4.5 Years of Age With Human Immunodeficiency Virus Status, Sex, Age at Vaccination, Nutritional Status at Primary Measles Dose, Antiretroviral Therapy Regimen, and Immune Status

Characteristic	Nonseroprotected	Seroprotected	Univariate OR (95% CI) for Seroprotection[a]	*P* Value	Adjusted OR (95% CI) for Seroprotection[a]	*P* Value
All children (n = 381[b,c])	n = 42	n = 339	...	...	...	...
HIV status						
HIV unexposed	1/42 (2.4)	94/339 (27.7)	Ref.	...	Ref.	...
HIV exposed, uninfected	2/42 (4.8)	82/339 (24.2)	0.44 (0.04–4.90)	.501	0.44 (0.04–4.93)	.504
HIV/Immed-ART-12	21/42 (50.0)	49/339 (14.5)	0.02 (0.003–0.19)	<.001	0.03 (0.003–0.20)	<.001
HIV/Immed-ART-24	12/42 (28.6)	58/339 (17.1)	0.05 (0.007–0.41)	.005	0.05 (0.006–0.41)	.005
HIV/Def-ART	6/42 (14.3)	56/339 (16.5)	0.10 (0.01–0.85)	.035	0.11 (0.01–0.90)	.040
Sex						
Male	14/42 (33.3)	162/339 (47.8)	Ref.	...	Ref.	...
Female	28/42 (66.7)	177/339 (52.2)	0.55 (0.28–1.07)	.080	0.66 (0.32–1.36)	.262
Race						
Black	41/42 (97.6)	308/339 (90.9)	...	...	...	...
Mixed ancestry	1/42 (2.4)	31/339 (9.1)	NA	.169	NA	...
Age at primary measles dose (mo)	9.0 (8.8–9.1)	9.0 (8.8–9.2)	1.07 (0.49–2.36)	.859	NA	...
Age at booster measles dose (mo)	15.4 (15.2–15.8)	15.4 (15.2–15.7)	1.11 (0.76–1.63)	.588	NA	...
Age at immunogenicity visit (mo)	53.1 (52.9–53.5)	53.2 (52.9–53.7)	1.14 (0.72–1.82)	.581	NA	...
Interval from booster measles dose to immunogenicity visit (mo)	37.7 (37.5–37.9)	37.7 (37.5–38.1)	0.97 (0.78–1.21)	.812	NA	...
Stunting at primary measles dose						
No	32/41 (78.1)	257/335 (76.7)	Ref.	...	...	...
Yes	9/41 (22.0)	78/335 (23.3)	1.08 (0.49–2.36)	.849	NA	...
Wasting at primary measles dose						
No	41/41 (100.0)	325/335 (97.0)	...	...	...	...
Yes	0/41 (0.0)	10/335 (3.0)	NA	...	NA	...
Underweight at primary measles dose						
No	38/41 (92.7)	307/335 (91.6)	Ref.	...	...	...
Yes	3/41 (7.3)	28/335 (8.4)	1.16 (0.34–3.98)	.819	NA	...
HIV-infected children (n = 202)	n = 39	n = 163				
Sex						
Male	12/39 (30.8)	71/163 (43.6)	Ref.	...	Ref.	...
Female	27/39 (69.3)	92/163 (56.4)	0.58 (0.27–1.22)	.148	0.77 (0.34–1.72)	.522
Race						
Black	38/39 (97.4)	155/163 (95.1)	...	...	...	...
Mixed ancestry	1/39 (2.6)	8/163 (4.9)	NA	.531	NA	...
Age at primary measles dose (mo)	9.0 (8.8–9.1)	9.1 (8.9–9.4)	1.79 (0.73–4.36)	.202	NA	...
Age at booster measles dose (mo)	15.4 (15.2–15.8)	15.6 (15.3–15.9)	1.50 (0.81–2.76)	.196	NA	...
Age at immunogenicity visit (mo)	53.1 (52.9–53.5)	53.2 (52.7–53.8)	1.00 (0.60–1.68)	.999	NA	...
Interval from booster measles dose to immunogenicity visit (mo)	37.7 (37.5–37.9)	37.7 (36.8–38.1)	0.83 (0.59–1.16)	.272	NA	...
Stunting at primary measles dose[c]						
No	29/38 (76.3)	106/159 (66.7)	Ref.	...	...	...
Yes	9/38 (23.7)	53/159 (33.3)	1.61 (0.71–3.65)	.253	NA	...
Wasting at primary measles dose[c]						
No	38/38 (0.0)	155/159 (97.5)	Ref.	...	...	...
Yes	0/38 (0.0)	4/159 (2.5)	NA	...	NA	...
Underweight at primary measles dose[c]						
No	35/38 (92.1)	142/159 (89.3)	Ref.	...	...	...
Yes	3/38 (7.9)	17/159 (10.7)	1.40 (0.39–5.03)	.609	NA	...

Table 2. Continued

Characteristic	Nonseroprotected	Seroprotected	Univariate OR (95% CI) for Seroprotection[a]	*P* Value	Adjusted OR (95% CI) for Seroprotection[a]	*P* Value
ART at time of primary measles dose						
No	6/39 (15.4)	13/163 (7.98)	Ref.	…	Ref.	…
Yes	33/39 (84.6)	150/163 (92.0)	2.10 (0.74–5.92)	.162	2.16 (0.64–7.34)	.216
ART at time of booster measles dose						
No	20/39 (51.3)	38/163 (23.3)	Ref.	…	Ref.	…
Yes	19/39 (48.7)	125/163 (76.7)	3.46 (1.68–7.15)	.001	2.27 (1.02–5.05)	.044
ART at immunogenicity visit						
No	19/39 (48.7)	31/163 (19.0)	Ref.	…	Ref.	…
Yes	20/39 (51.3)	132/163 (81.0)	4.05 (1.93–8.48)	<.001	3.07 (1.39–6.84)	.006
Enrollment CD4+ T lymphocyte %	37.2 (31.4–43.3)	36.4 (31.8–42.4)	1.00 (0.96–1.04)	.942	NA	…
Primary measles dose CD4+ T lymphocyte %	39.6 (32.6–47.0)	36.7 (30.5–42.2)	0.96 (0.93–1.00)	.071	0.98 (0.93–1.03)	.396

Data are proportion of patients (%) or median (IQR).

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; HIV, human immunodeficiency virus; HIV/Immed-ART-12, HIV-infected children on immediate ART interrupted at 12 months; HIV/Immed-ART-24, HIV-infected children on immediate ART interrupted at 24 months; HIV/Def-ART, HIV-infected children on deferred ART; NA, not applicable; OR, odds ratio; Ref., reference group.

[a]Seroprotection was defined as an immunoglobulin G titer of ≥330 mIU/mL.

[b]Seven HIV-infected children with CD4+ <25% at enrollment were excluded from analyses.

[c]Five participants had missing information on nutritional status at the primary measles dose.

was associated with higher likelihood of seroprotective titers ≥330 mIU/mL, whereas this was not associated with CD4+ percentage at enrollment or at time of the primary vaccine dose (Table 2).

DISCUSSION

The results from this study underscore a potential downside of ART interruption in HIV-infected infants who initiated ART during early infancy, indicating greater waning of immunity against measles infection by 4.5 years of age than in HIV-unexposed children. Furthermore, our study dispelled earlier concerns of HEU also being predisposed to greater waning of immunity, as suggested by the previous observation in the same cohort of children 9 months after the booster dose of measles vaccine [25]. Notably, children in the HIV/Def-ART group, the majority (88%) of whom were on continuous ART by the time of their booster dose of measles vaccine, had measles immunity that was similar to that of HIV-unexposed children.

The attenuated antibody response in HIV/Immed-ART children compared with that in HIV/Def-ART children may be explained by the lower number of children on ART at the time of the immunogenicity visit in HIV/Immed-ART groups and the lower number on ART at booster vaccination in HIV/Immed-ART-12 children. These results are in line with our previous findings at 2 years of age, showing greater waning of immunity in children with interrupted ART [25]. Immune-cell activation during ART interruption may cause memory B cells to be drawn into effector pathways, resulting in depletion of memory B cells [39]. HIV/Immed-ART-12 children compared with HIV/Immed-ART-24 children had significantly lower GMTs. This may also be explained by fewer children in the HIV/Immed-ART-12 group on ART at the time of booster vaccination compared with HIV/Immed-ART-24.

The HIV/Def-ART group, who initiated continuous ART after the CHER interim analysis in June 2007, were less immunosuppressed, and a significantly higher proportion were on ART at the time of booster vaccination and immunogenicity visit compared with the HIV/Immed-ART group. The HIV/Def-ART group, however, might have been selectively biased by representing children with slower HIV progression within the group, as there was a higher mortality rate in these children (16%) compared with the HIV/Immed-ART children (4%) after a median follow-up of 40 weeks [32]. Nevertheless, our study does represent the survivors initially randomized to this group and suggests that ART should be initiated prior to measles vaccine immunization.

Two other studies have evaluated long-term measles antibody persistence in a much smaller number of HIV-infected children initiated on ART within the first year of life and reported results similar to ours. These included a Latin American cohort study in which seropositive titers (≥120 mIU/mL) were present in 87% of children at 4 years of age (n = 38) [14] and an Italian cross-sectional study in which 82% of children had seroprotective titers at 7 years (n = 13) of age [13]. The former, however, did not find a significant relation between the timing of ART initiation and measles serology [14]. Of note, no studies in children evaluated the effect of systematically assigned early ART on long-term measles antibodies and the consequences of interrupting ART.

The CHER study hypothesized that if children are initiated on early ART close to primary infection, disease progression

could be prevented and children could be allowed a subsequent period off ART [15]. In CHER, HIV-infected children on early ART in whom ART was interrupted had a better clinical outcome than those on deferred ART, without increased risk for disease progression during the ART interruption period [15]. Nevertheless, we showed that ART interruption is associated with long-term waning of measles protection, hence, suggesting subclinical consequences of ART interruption in these children, which could make them susceptible to measles in the event of outbreaks. Of note, however, is that ART was reinitiated due to CD4+ T-cell depletion, with children being exposed to prolonged HIV viremia during interruption. Seventy percent of children in the main CHER trial had rebounded by 2 months off ART, with median viral load being $\log_5$ HIV RNA copies/mL [40]. The need for further booster doses of measles vaccine in these children is currently being evaluated.

Prior studies reported that if ART initiation precedes immunization, vaccine responses are comparable to those in healthy children [34] and memory B cells are maintained over time [13]. Likewise, our multivariable logistic regression analysis showed that long-term presence of seroprotective titers was associated with being on ART at the time of booster vaccination, as well as at the immunogenicity visit in HIV-infected children, underlining the importance of early and continuous ART.

In contrast to our previous report at 2 years of age (measured in years 2005–2006), where HEU children had lower antibody levels than HIV-unexposed children [25], we did not observe such differences at 4.5 years of age. Possible reasons include that HEU children may have experienced natural boosting of measles antibody titers after exposure to wild-type measles infection [41], especially during the measles outbreak in 2009 in South Africa, which occurred prior to the sampling point for this study [42, 43]. Also, immune system aberrations of HEU children could resolve after the first 2 years of life [22]. Other studies have also reported that HEU children produce robust anamnestic antibody responses to measles vaccination [8, 26–30].

The participants we selected had CD4+ percentage ≥25%, which reduces the generalizability of our findings to HIV-infected children who are already severely immunocompromised by 4–8 weeks of age. Furthermore, we did not assess cellular immunity or avidity of the antibody response. The clinical implication of waning measles antibodies in HIV-infected children in whom ART has been interrupted remains uncertain. Strengths of this study include the early administration of ART in HIV-infected children as per current guidelines, the randomized nature of the ART initiation and interruption, the large sample size, and length of follow-up.

In order to achieve measles elimination by 2020, as targeted by the WHO, it is essential for HIV-infected children to receive timely and complete measles vaccination, in addition to early and continuous ART. This study showed that waning of immunity occurs in HIV-infected children, in particular, if ART has been interrupted, but not in HEU children. In a real-world situation, this may happen with poor adherence or missed visits. To prevent measles outbreaks and achieve sufficient levels of population immunity, HIV-infected children and adolescents may need supplemental immunization, especially if they were not on ART at the time of vaccination or are not currently on ART.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Appendix 3

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MAJOR ARTICLE

Immunogenicity and Safety of an Early Measles Vaccination Schedule at 6 and 12 Months of Age in Human Immunodeficiency Virus (HIV)–Unexposed and HIV-Exposed, Uninfected South African Children

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Background: Measles morbidity and mortality rates are greatest in children <12 months old, with increased susceptibility in human immunodeficiency virus (HIV)–exposed children. We evaluated the immunogenicity and safety of an early 2-dose measles vaccine regimen administered at 6 and 12 months of age in South Africa.

Methods: HIV-unexposed (HU) (n = 212) and HIV-exposed, uninfected (HEU) (n = 71) children received measles vaccination (CAM-70) at 6 and 12 months of age. Measles immunoglobulin G titers were measured by means of enzyme-linked immunosorbent assay before and 1 month after each vaccine dose.

Results: The majority of children (88.2% HU and 95.8% HEU; P = .04) were seronegative (<150 mIU/mL) to measles at 4.2 months of age. This was particularly evident among infants of mothers born from 1992 onwards (year of public nationwide measles vaccine availability). One month after the first measles vaccine, 42.3% of HU and 46.4% of HEU children were seropositive (≥330 mIU/mL). After the second dose, the proportion seropositive increased to 99.0% in HU and 95.3% in HEU children. Safety profiles were similar between HU and HEU children.

Conclusions: Early 2-dose measles vaccination at 6 and 12 months of age was safe and induced antibody responses in HU and HEU children, which could partly offset the early loss of maternally derived antibodies in infants born to predominantly measles-vaccinated mothers.

Clinical Trials Registration: NCT03330171

Keywords. measles vaccine; early dose; safety; immunity; HIV exposure.

Measles virus infection remains an important cause of vaccine-preventable deaths. An estimated 110 000 deaths were attributed to measles globally in 2017, despite an 84% decline in measles mortality between 2000 and 2016 [1, 2]. Measles-associated morbidity and case-fatality rates are highest among children <12 months of age [3–6]. The majority of children are susceptible to measles infection before reaching the age of routine measles immunization [7–10]. In South Africa, during a measles outbreak in 2009–2011, 24% of laboratory-confirmed cases (4284 of 17 530) were identified among children aged <9 months, with age-specific incidences of 302, 1083, 724, and 54 per 100 000 population, respectively, in children aged <6 months, 6–8 months, 8–11 months, and ≥5 years [11].

Measles vaccine (MV) was recommended for inclusion in public immunization programs (PIPs) of low- and middle-income countries during the 1970s and 1980s by the Expanded Programme on Immunization of the World Health Organization (WHO) [12]. Vaccination coverage increased to 73% in the WHO African Region since 2000, according to WHO/UNICEF estimates of national immunization coverage [13]. Consequently, current immunity against measles among women of childbearing age is more likely to be derived by MV during childhood than by immunity acquired through previous natural infection.

Children born to women who derived immunity mainly through vaccination have lower transplacental acquisition of measles antibodies from their mothers [14] and become susceptible to measles as early as 3.3 months of age [15]. Furthermore,

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children born to human immunodeficiency virus (HIV)–infected women are at heightened risk of measles infection owing to reduced transplacental transfer of measles-specific antibodies [8, 16–19]. The lower concentration of transplacentally acquired antibody, however, may lend itself to earlier measles vaccination in infancy, with immunogenicity less likely to be impeded by interference of maternal-derived antibody [20]. Vaccinating at an earlier age could mitigate serious measles complications during early and middle infancy [21, 22]. Early vaccination at 6 months of age, however, induced lower antibody levels than vaccination at 9 or 12 months [23].

In settings with high incidence of measles and HIV-infection, WHO recommends a supplementary dose of MV from 6 months of age, followed by 2 doses at the recommended ages (usually at 9–12 and 15–18 months) [24]. South Africa introduced MV in the PIP in 1983, but it has only been widely available since 1992 [25]. Until recently MV (Schwarz strain) was administered at 9 and 18 months of age. As of December 2015, South Africa implemented an early 2-dose MV schedule of a CAM-70 strain (Measbio) administered at 6 and 12 months of age [26]. Whereas Schwarz was derived from the Edmonston strain, CAM-70 was developed from a Japanese wild-type isolate [27]. The reasons for lowering the age at vaccination included the high incidence of measles in children aged <9 months during the outbreak in 2009–2011 and regulatory restrictions regarding coadministration of Measbio with other vaccines (personal correspondence, S.A. Madhi, South African National Advisory Group on Immunization, 27 February 2019).

HIV infection in pregnant women in South Africa ranks among the highest in the world, with rates of approximately 30% between 2005 and 2015 [28]. Because of effective prevention of mother-to-child transmission programs, an increasing proportion of South African children born to these mothers are HIV-exposed but uninfected (HEU) [29]. In a recent systematic review, HEU children showed similar serological response when vaccinated at 6 months compared with HIV-unexposed (HU) children, with 68% and 94% being seropositive after the first and second doses, respectively [30].

The limited number of serological studies on early measles vaccination in HEU and HU children in low- and middle-income countries highlights the need to provide evidence for the current South African recommendations. The current study aimed to evaluate the immunogenicity and safety of 2-dose MV regimen administered to HU and HEU children at 6 and 12 months of age.

METHODS

Study Design

This prospective observational cohort study included HU children coenrolled in a randomized, open-label trial evaluating the noninferiority of 2 versus 3 doses of pneumococcal conjugate vaccine (NCT02943902) and a parallel cohort of HEU children (NCT03330171). Children were identified from hospital birth registers, postnatal wards and neighboring primary health clinics and invited for screening at the Respiratory and Meningeal Pathogens Research Unit, based at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa. Healthy children aged 6–18 weeks, ≥37 weeks' gestation at birth and birth weight >2499 g, were eligible for enrollment. Criteria for inclusion, exclusion and classification of HIV status are listed in the Supplementary Data.

Participants were vaccinated under the current South African recommendations, with subcutaneous injection of live attenuated MV (MeasBio; BioFarma) at 6 months (182 ± 14 days) and 12 months (365 ± 14 days) of age. Participants received other childhood vaccines according to the PIP, except for the randomization to different pneumococcal conjugate vaccine schedules in the parent protocol.

Assessment of Outcomes

Venous blood samples were collected from all participants approximately 2 months before the first MV dose (MV1) (age, 4.2 months; mean [standard deviation], 126 [14] days), 1 month after MV1 (age 7 months; 28–35 days after vaccination), before the second MV dose (MV2) (age 12 months; 365 ± 14 days), and 1 month after MV2 (age 13 months; 28–35 days after vaccination). Children were observed after each vaccine injection for 30 minutes. Safety evaluation for solicited adverse events was only included in the protocol as an amendment, resulting in 102 of 278 children (37%) with safety evaluation after MV1 and 260 of 262 (99%) after MV2. Parents were provided with a vaccination report card to report local injection site (pain/tenderness, redness, swelling, and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) symptoms on a daily basis for 7 days after each injection. Adverse events were graded on a 1–3 scale, using symptom-specific definitions outlined in the vaccination report card. Serious adverse events were documented throughout the study.

Laboratory Methods

Blood samples were centrifuged and serum samples stored at −70°C at the Respiratory and Meningeal Pathogens Research Unit laboratory, until testing. Measles immunoglobulin G (IgG) antibody levels were analyzed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Enzygnost; Dade Behring), according to the manufacturer's instructions. Optical density (OD) values were converted to milli-international units per milliliter using the alpha method with calibration against the first measles antigen WHO international reference preparation [31, 32]. Measles seronegativity was defined as IgG titers <150 mIU/mL (OD, <0.1), equivocal as titers 150–329 mIU/mL (OD, 0.1–0.2), seropositivity as titers ≥330 mIU/mL (OD, >0.2) and seroconversion as the change from seronegative before to seropositive after vaccination. All equivocal samples were

retested. If the result was confirmed, the samples were classified as equivocal, otherwise as positive or negative. Seronegative samples were assigned a titer half the value of the assay's detection limit (ie, 75 mIU/mL).

Statistical Analyses

The sample size was calculated based on a significance level of 5% (2 sided), 80% power, 1:3 ratio of HEU to HU children, and a hypothesized 10% lower seropositivity rate between HEU and HU children after 2 MV doses. The sample size was adjusted upward by 10% to account for loss to follow-up, resulting in a total minimal sample size of 270 participants.

Geometric mean titers (GMTs) of measles antibody concentrations and 95% confidence intervals (CIs) were calculated following natural logarithmic transformation of titer values and were compared between the 2 study groups by means of multivariable linear regression, using the following covariates: sex, race, maternal age, antibody levels before MV1. and age at the serology visit. The proportions of participants meeting the putative thresholds for seronegativity, seropositivity, and seroconversion were compared by means of multivariable logistic regression, adjusting for the above-mentioned covariates. The association between maternal age in years or classified as maternal year of birth before 1992 (the year of wide public MV availability) or 1992 and beyond, and the proportions of seronegative and seropositive children at the pre-MV1 visit were explored using logistic regression, adjusting for HIV exposure.

Pre- and post-MV GMTs were correlated using Spearman correlation, and the association between proportions seronegative before and after MV were evaluated using (exact) logistic regression. Safety analysis included the proportion of children with ≥1 event (including solicited local and systemic reactions and serious adverse events) and the proportion with solicited grade 3 events. Differences were considered significant at $P < .05$. Analysis was by modified intention to treat, with all participants included if antibody results were available. A per-protocol analysis was performed including only those children who were vaccinated or had blood samples collected within the protocol-defined time periods. Stata13 (StataCorp) and R (version 3.5.1) software were used.

Ethics

The study protocol was approved by the Human Research Ethics Committee of the University of the Witwatersrand, South Africa (the Human Research Ethics Committee reference number: M170276). Parents provided written informed consent before study entry.

RESULTS

From April to October 2017, a total of 283 children were enrolled in the study, including 212 HU (75%) and 71 HEU (25%) (Figure 1). Baseline characteristics at study initiation did not differ between HU and HEU children, except that mothers of HEU children were older (30.7 vs 27.9 years for HU children) (Supplementary Table 1). The baseline characteristics of HU children who consented to the measles study did not differ significantly from those of children who were enrolled only in the main parent study (data not shown). Overall, the mean (standard deviation) age at was 4.2 (0.2) months at the pre-MV1 serology visit, 6.0 (0.1) months at the MV1 visit, 7.0 (0.1) months at the post-MV1 serology visit, 12.0 (0.2) months at the MV2 visit, and 13.0 (0.2) months at the post-MV2 serology visit. HU children were slightly younger than HEU children at the vaccination and serology visits (difference, −0.1 month) (Supplementary Table 1).

Measles Antibodies Titers

In analyses adjusted for sex, race, maternal age, antibody levels before MV1 (only for subsequent time points), and age at serology, HU children had higher GMTs than HEU children before MV1 (93 [95% CI, 85–102] vs 82 [74–91] mIU/mL, respectively; $P = .02$). GMTs were similar between HU and HEU children after MV1 (223 [95% CI, 191–260] vs 251 [197–319] mIU/mL, respectively) and after MV2 (2751 [2402–3152] vs 3226 [2429–4286] mIU/mL). Before MV2, however, GMTs were lower in HU than in HEU children (233 [95% CI, 196–277] vs 340 [249–464] mIU/mL; $P = .04$) (Figure 2*A* and Table 1).

At the pre-MV1 visit (mean age, 4.2 months), more HEU than HU children were seronegative (titers <150 mIU/mL, 95.8% vs 88.2%, respectively; $P = .02$), and only 4.2% and 6.6%, respectively, were seropositive (≥330 mIU/mL) (Figure 2*B* and Table 1). One month after MV1, the percentage of seronegative children was nonsignificantly higher in HU (44.7%) than in HEU (34.8%) children, but the percentages who were seropositive (42.3% and 46.4%, respectively) or had seroconverted (48.1% and 50.0%) were similar between groups.

Five months later, before MV2 at age 12 months, 42.8% of HU and 54.1% of HEU children were seropositive ($P = .34$), whereas 46.8% and 29.5%, respectively, were seronegative ($P = .03$). The percentage of seropositive children increased to 99.0% in HU and 95.3% in HEU children 1 month after MV2; and only 1.0% and 1.6%, respectively, remained seronegative (Figure 2*B* and Table 1). After MV2, seroconversion rates from before MV2 were higher in HU (97.9%) than in HEU (83.3%) children ($P = .01$).

Per-protocol analysis, excluding children who were vaccinated or had blood samples collected outside the protocol-defined time periods, yielded similar results, except that differences between HU and HEU in pre-MV2 GMTs became marginally significant ($P = .055$) (Supplementary Table 2).

At 4.2 months of age (before MV1), maternal age was inversely associated with the percentage of seronegative children (adjusted odds ratio [aOR], 0.88; 95% CI, .82–.94; $P < .001$) and positively associated with percentage of seropositive children

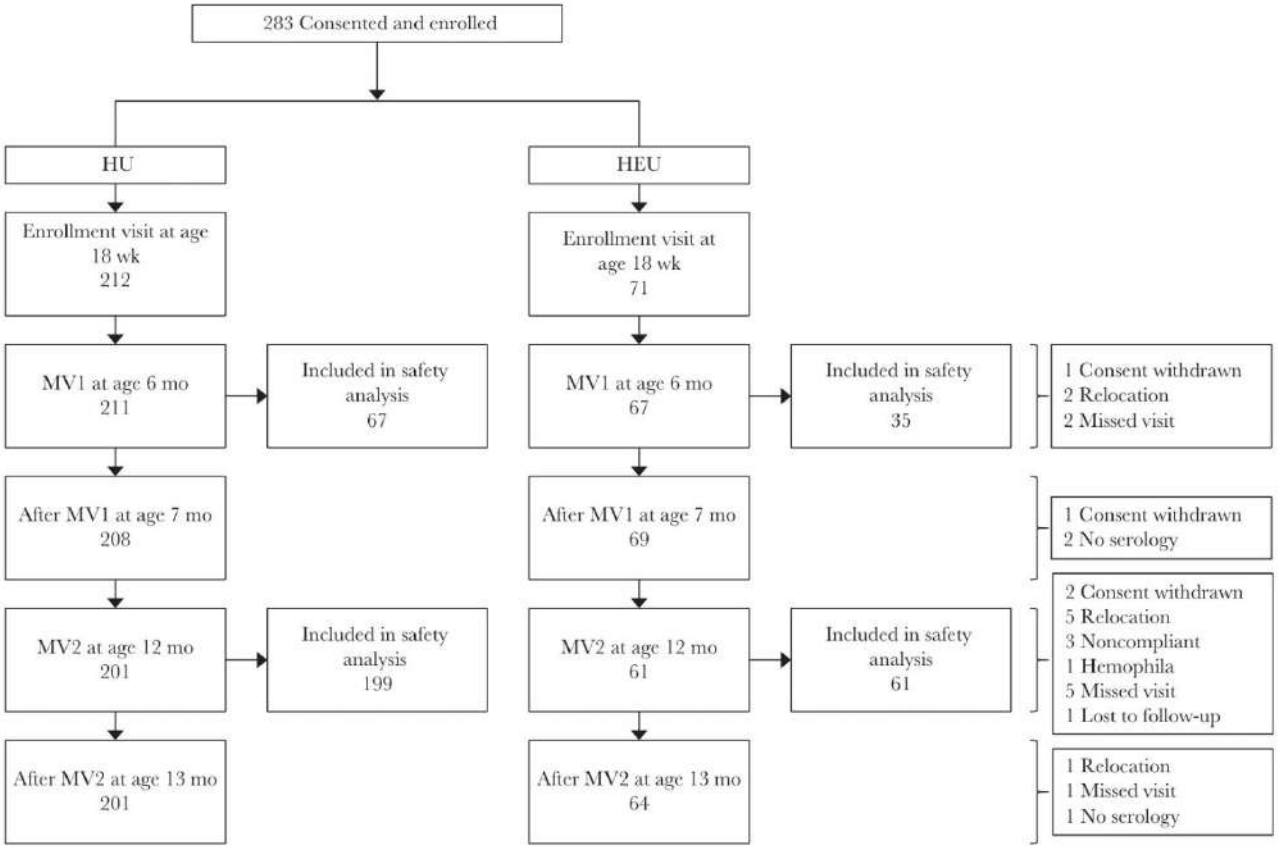

Figure 1. Flow diagram of study participants. One human immunodeficiency virus (HIV)–exposed, uninfected (HEU) and 1 HIV-unexposed (HU) child missed a visit at time of the first measles vaccine dose (MV1) and were vaccinated at the local clinic. Safety analysis was completed in a subset of participants at MV1, owing to diary card introduction during the course of the study. Four HEU and 1 HU child missed a visit at time of the second measles vaccination (MV2) and were vaccinated at the local clinic, and 1 HU child missed the serology visit after MV2.

(1.17; 1.07–1.27; $P < .001$) (Table 2). Before vaccination, 0% of infants (0 of 91) with mothers born since 1992 were seropositive, compared with 8.9% (17 of 192) with mothers born before 1992. Furthermore, a positive association was observed between year of maternal birth category and the percentage of seronegative children (aOR, 5.01; 95% CI, 1.46–17.17; $P = .01$), with adjustment for HIV exposure (Table 2).

There was a negative correlation between pre- and post-MV1 GMTs (Spearman correlation coefficient, −0.27; $P < .001$) (Supplementary Figure 1*A*). Similarly, children with undetectable antibody levels before vaccination were more likely to have titers ≥150 mIU/mL (aOR, 9.67; 95% CI, 3.25–28.84; $P < .001$) or to be seropositive after MV1 (11.63; 2.70–50.20; $P = .001$). A positive correlation was found between pre- and post-MV2 GMTs (Spearman correlation coefficient, 0.50; $P < .001$) (Supplementary Figure 1*B*).

Safety

The frequency and severity of solicited local and systemic reactions during the 7 days after each measles vaccination were similar in HU and HEU children (Table 3). Most children showed no solicited reactions. After MV1, no grade 3 local injection site reactions occurred; 9% (6 of 67) HU and no HEU children experienced grade 3 systemic reactions. After MV2, grade 3 local reactions were recorded in 1% (2 of 199) HU and 5% (3 of 61) HEU children; and grade 3 systemic adverse events in 9% (17 of 199) HU and 10% (6 of 61) HEU children. The most common local reactions were pain or tenderness and redness. Common systemic adverse events were decreased appetite and irritability in HU and decreased appetite and decreased activity in HEU children (Supplementary Table 3). The proportion with pain or tenderness after MV1 was higher in HEU children (mild pain or tenderness in 23%, moderate in 6%) than in HU children (mild in 16%) ($P = .006$).

Thirty serious adverse events occurred throughout the study, 2 in HU children within 28 days after measles injection (Table 3). One child had bronchopneumonia and otitis media with onset 9 days after MV1, and another had bronchiolitis and otitis media with onset 20 days after MV2 (Supplementary Table 4). All serious adverse events had mild or moderate severity, and none were classified as MV related. No deaths occurred. None of the HIV-exposed children became HIV positive during the study period.

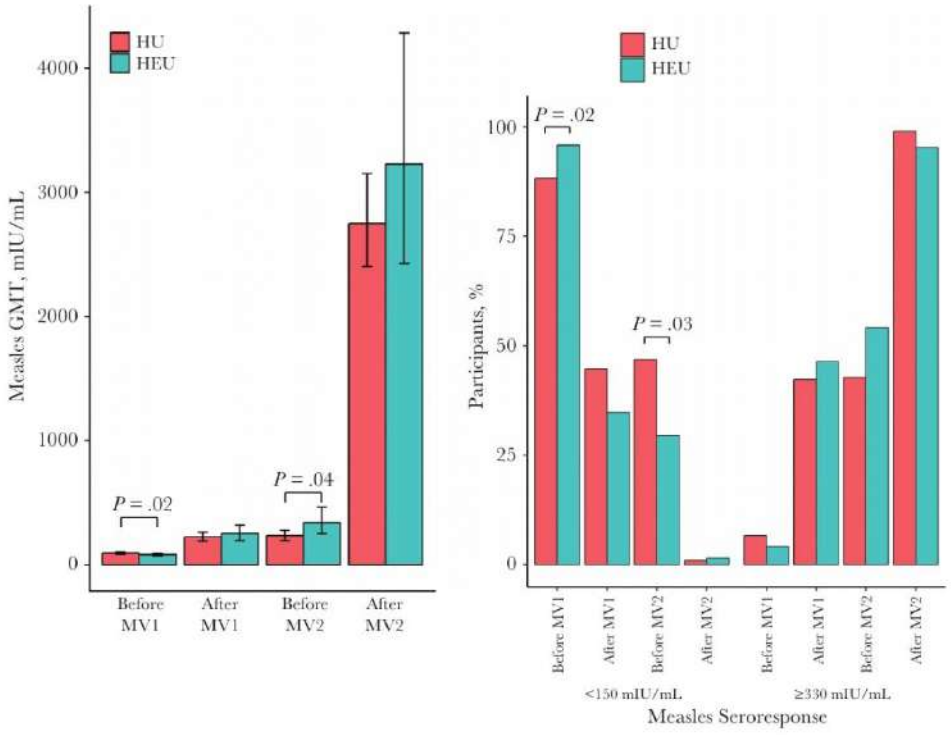

Figure 2. *A*, Measles antibody geometric mean titers (GMTs) in human immunodeficiency virus (HIV)–exposed, uninfected and HIV-unexposed (HU) children, before and after first and second measles vaccine dose (MV1 and MV2) *B*, Proportions of seronegative and seropositive children before and after MV1 and MV2. *P* values were calculated by means of either linear or logistic regression, with adjustment for sex, race, maternal age, measles antibody levels before MV1, and age at serology.

DISCUSSION

The results of this prospective cohort study showed that an early 2-dose measles vaccination schedule administered at 6 and 12 months of age is similarly safe and immunogenic in HU and HEU children. The vast majority (90.1%) of children aged 4.2 months in our study were seronegative for measles antibody. This was especially evident among infants of women born since 1992 (when measles vaccination became widely available in the South African PIP). These findings underscore the importance of reconsidering measles dosing schedules in settings similar to ours.

Administration of 2 doses of MV at 6 and 12 months of age resulted in seropositivity rates of 99.0% and 95.3% in HU and HEU children, respectively, 1 month after MV2. Our results corroborate findings from other studies on early measles vaccination regimens in Africa [33–37] and a Brazilian study from 1990 that examined the humoral response to a CAM-70 strain containing MV administered at 6 and 11 months of age, reporting 89% seroconversion rates by immunofluorescence assay and 97% by ELISA after the second dose [38]. However, the rise in antibody levels induced by a second vaccination may be short-lived, and titers could fall back to preboost levels [39]. A recent study reported a decrease in long-term concentration and avidity of measles virus-specific neutralizing antibodies after early vaccination compared with vaccination at a later age [40]. Hence, durability of the response to early vaccination needs to established, to rule out future vaccine failures and to prevent reductions in maternal antibody transfer in future generations.

A high proportion of children had antibody levels below the assay detection limit at 4.2 months of age, similar to findings of an earlier study from our setting [41]. Even in areas that have eliminated measles, a number of children may be susceptible to infection before receiving MV1 if given at age 12 months [10, 14, 42]. The increase in measles seronegativity among young children has been attributed in part to lower levels of transplacental IgG transfer to the fetus in women who derived antibody from measles vaccination rather than after natural viral infection [20]. In our study, measles antibody seronegativity in infants before measles vaccination (4.2 months of age), was associated with younger maternal age and women born after wide implementation of MV into the South African PIP. Even lower concentrations of measles-specific antibody have been detected in children born to HIV-infected women compared with HU children [8, 16–19], as corroborated by our findings of 95.8% seronegativity in HEU compared with 88.2% in HU children before measles immunization. A previous study from our setting on cord blood samples collected in 2007 from mother-newborn dyads reported measles seronegativity prevalence of 5.6% in HU (6 of 107) and 8.7% of HEU (17 of

Table 1. Measles Antibody Geometric Mean Titers and Proportions of Seropositive and Seronegative Children Before and After Both Measles Vaccines

Serological Status Before and After MV1 and MV2	Children, No. (%)[a]		
	HU	HEU	Total
Before MV1	n = 212	n = 71	n = 283
Measles antibody, GMT (95% CI)	93 (85–102)[b]	82 (74–91)[b]	90.0 (84–97)
Seronegative (IgG titer <150 mIU/mL)	187 (88.2)[b]	68 (95.8)[b]	255 (90.1)
Seropositive (IgG titer ≥330 mIU/mL)	14 (6.6)	3 (4.2)	17 (6.0)
After MV1	n = 208	n = 69	n = 277
GMT (95% CI)	223 (191–260)	251 (197–319)	230 (202–261)
Seronegative	93 (44.7)	24 (34.8)	117 (42.2)
Seropositive	88 (42.3)	32 (46.4)	120 (43.3)
Seroconversion[c]	90 (48.1)	34 (50.0)	124 (48.6)
Before MV2	n = 201	n = 61	n = 262
GMT (95% CI)	233 (196–277)[d]	340 (249–464)[d]	254 (218–296)
Seronegative	94 (46.8)[e]	18 (29.5)[e]	112 (42.8)
Seropositive	86 (42.8)	33 (54.1)	119 (45.4)
After MV2	n = 200	n = 64	n = 264
GMT (95% CI)	2751 (2402–3152)	3226 (2429–4286)	2860 (2528–3235)
Seronegative	2 (1.0)	1 (1.6)	3 (1.1)
Seropositive	198 (99.0)	61 (95.3)	259 (98.1)
Seroconversion[f]	92 (97.9)[g]	15 (83.3)[g]	107 (95.5)

Abbreviations: CI, confidence interval; GMT, geometric mean titer; HEU, human immunodeficiency virus (HIV)–exposed, uninfected; HU, HIV-unexposed; IgG, immunoglobulin G; MV1, first measles vaccine dose; MV2, second measles vaccine dose.

[a]Data represent no. (%) of children unless otherwise specified.

[b]$P = .02$. (P values were calculated by means of either linear or logistic regression and adjusted for sex, race, maternal age, measles antibody levels before MV1, and age at serology.)

[c]A total of 255 children had titers <150 mIU/mL before MV1 (187 HU and 68 HEU children). Seroconversion was defined as a change from titers ≤150 mIU/mL before to ≥330 mIU/mL after vaccination.

[d]$P = .04$.

[e]$P = .03$.

[f]A total of 112 children had titers <150 mIU/mL before MV2 (94 HU and 18 HEU children).

[g]$P = .01$.

196) children [8]. This indicates rapid waning of measles antibodies in the first 4 months after birth, thereby creating a group of infants susceptible to measles at a younger age.

We found that 55.3% of HU and 65.2% of HEU children had titers ≥150 mIU/mL after a single MV dose at age 6 months, findings in line with those of a Malawian study on early measles vaccination in which 62% of HU and 68% of HEU children had titers ≥120 mIU/mL (as measured by enzyme immunoassay) after 1 dose of MV [33, 34]. Our rates, however, are lower than the 77% seroprotected (≥125 mIU/mL, as measured by measles hemagglutination inhibition test) reported from Guinea-Bissau [35] and lower than the overall pooled estimate for seropositivity after MV at 6 months (75%; 95% CI, 68%–82%) [43]. The seroconversion rate at age 6 months (48.6%), which may depend on the vaccine strain, was also lower than that reported in a meta-analysis evaluating MV at 6 months (76%; 95% CI, 71%–82%) [43]. Our seropositivity rates after vaccination at age 6 months are similarly lower compared with findings from our setting when vaccination was done at age 9 months, with 91.1% of HU and 94.8% of HEU children seropositive (≥330 mIU/mL, as measured by ELISA) 6.6 months after MV1 [41]. Our results suggest that a single early dose of MV is only partially effective

Table 2. Association of Maternal Age With Percentage of Seronegative and Seropositive Children Before the First Measles Dose

Characteristic	Nonseronegative (n = 28)	Seronegative[a] (n = 255)	Adjusted OR (95% CI) for Seronegativity[b]	*P* Value	Nonseropositive (n = 266)	Seropositive[c] (n = 17)	Adjusted OR (95% CI) for Seroprotection[b]	*P* Value
Maternal age, mean (SD), y	32.3 (5.3)	28.2 (6.1)	.88 (.82–.94)	<.001	28.3 (6.1)	33.6 (4.3)	1.17 (1.07–1.27)	<.001
Maternal year of birth, no. (%)								
Before 1992	25 (89)	167 (65)	Reference	.01	175 (66)	17 (100)	…	…
1992 or later	3 (11)	88 (35)	5.01 (1.46–17.17)		91 (34)	0 (0)	NA	NA

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio.

[a]Seronegativity was defined as an immunoglobulin G titer <150 mIU/mL.

[b]ORs were adjusted for human immunodeficiency virus exposure.

[c]Seropositivity was defined as an immunoglobulin G titer ≥330 mIU/mL.

Table 3. Reported Adverse Events After Immunization With Measles Vaccine at 6 and 12 Months of Age, by Human Immunodeficiency Virus Exposure

Adverse Events	Children With Reaction/Total,[a] No. (%)	
	HIV Unexposed	HIV Exposed, Uninfected
Solicited reactions during 1st 7 d after MV1		
Local reactions		
Any	17/67 (25)	12/35 (34)
Severe	0/67 (0)	0/35 (0)
Systemic reactions		
Any	36/67 (54)	15/35 (43)
Severe	6/67 (9)	0/35 (0)
Solicited reactions during 1st 7 d after MV2		
Local reactions		
Any	49/199 (25)	14/61 (23)
Severe	2/199 (1)	3/61 (5)
Systemic reactions		
Any	106/199 (53)	27/61 (44)
Severe	17/199 (9)	6/61 (10)
Unsolicited serious adverse event after measles vaccination[b]		
≤28 d after injection	2/211 (1)	0/67 (0)
Throughout the study period	24/211 (11)	6/67 (9)
Per study participant	20/211 (9)	4/67 (6)
Related to measles vaccination	0/211 (0)	0//67 (0)

Abbreviations: HIV, human immunodeficiency virus; MV1, first measles vaccine dose; MV2, second measles vaccine dose.

[a]Total number with vaccination report card/serious adverse event assessment.

[b]Some participants had >1 serious adverse event. Serious adverse events are reported in Results and in Supplementary Table 5.

in inducing humoral immune responses and that administration of the second dose remains essential.

When evaluating the effect of maternal HIV infection on infant vaccine-induced measles antibody responses, we observed that HEU children, compared with HU children, had similar or higher post-MV1 GMTs, and similar proportions had titers ≥330 mIU/mL. Previous studies on responses to primary vaccination have reported similar findings [19, 41, 44]. This may be explained by the association between prevaccination antibody levels in HEU children and a heightened humoral immune response to childhood vaccines, owing to reduced interference of maternally acquired antibody [19]. Similarly, our study found prevaccination antibody concentrations to be lower in HEU than in HU children. In addition, we found that children who were seronegative before vaccination were more likely to have titers ≥150 mIU/mL or to be seropositive after MV1.

We observed an increase in antibody titers between post-MV1 and pre-MV2 study visits. This could be explained by subclinical exposure to wild-type measles virus and avidity maturation. During 2017, a localized measles outbreak occurred in the Gauteng province in South Africa, with a total of 96 laboratory-confirmed cases [45]. The measles cases were not detected in Soweto, the area where most study participants resided. In response to the outbreak, a province-wide supplemental vaccination campaign was conducted from May to June 2017 [45]. However, only 1 study participant was reported to have received additional measles vaccination. No participant had clinical measles infection during the study.

The current study examined the safety of CAM-70 measles virus vaccine given at 6 months of age. Prior studies have demonstrated the safety of other MV strains when administered before 9 months of age [46–48]. The WHO states that internationally qualified attenuated MV may be used interchangeably within immunization programs and considers them to be safe and effective [24], but strain-dependent differences in immunogenicity have been described [49]. We report that an early 2-dose MV regimen is safe and well tolerated. The frequencies of local and systemic adverse events were comparable to those in previous studies [24, 30, 46]. Grade 3 solicited systemic reactions were reported more often than in a previous study that coadministered a fully liquid hexavalent vaccine and a measles-mumps-rubella and varicella vaccine at age 15–18 months in healthy South African children [50].

A limitation of our study was the late introduction of vaccination report cards; as a result, only 37% of participants were included in the safety analysis after MV1. Furthermore, solicited adverse events were followed up until day 7 after vaccination, thereby excluding adverse events occurring during the second week after MV. Furthermore, we did not assess antibody titers for more than 1 month after MV2. Long-term follow-up of study participants is currently ongoing. Another limitation is the use of an ELISA instead of the reference-standard

plaque reduction neutralization test, especially because ELISA has reduced sensitivity at low antibody levels and may therefore underestimate humoral immunity. As a result, currently undetectable maternal antibodies by ELISA could be detected by plaque reduction neutralization test and may interfere with vaccination response.

In conclusion, early 2-dose measles vaccination at age 6 and 12 months with the CAM-70 strain is immunogenic and induces similar post-MV2 responses in HU and HEU children. A window of vulnerability exists before 6 months of age, as well as between 6 and 12 months of age. This is particularly important, because an increased number of mothers will have vaccine-derived instead of naturally acquired immunity, which is associated with early loss of maternal antibodies. When combined with a reduction in measles vaccination coverage, outbreaks can occur, affecting those most susceptible (ie, young infants). Earlier vaccination could narrow the vulnerability gap, suggesting the need for new MVs that are more immunogenic in younger age groups, possibly as young as 3–4 months in settings with high HIV incidence. In addition, future studies should optimize vaccination dosing schedules and evaluate the sustainability of protection with an accelerated measles vaccination schedule.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Appendix 4

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Short-term immunogenicity and safety of hepatitis-A and varicella vaccines in HIV-exposed uninfected and HIV-unexposed South African children

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ABSTRACT

Background: HIV-exposed uninfected (HEU) children have increased risk of infectious morbidity during early childhood. We evaluated the short-term immunogenicity and safety of single dose inactivated hepatitis-A virus (HAV) vaccine and live attenuated varicella zoster virus (VZV) vaccine in South African children.

Methods: 195 HIV-unexposed and 64 HEU children received either one dose of HAV or VZV vaccine at 18 months of age. Blood samples were tested for hepatitis-A or VZV antibodies before and one month after vaccination by chemiluminescent microparticle immunoassay and enzyme-linked immunosorbent assay, respectively. All children were evaluated for solicited adverse events (AEs).

Results: One-month post-vaccination, a similar percentage of HIV-unexposed (91.8%) and HEU (82.9%) children were seropositive for hepatitis-A ($p = 0.144$). VZV antibody geometric mean fold-rise was also similar in HIV-unexposed (5.6; 95%CI: 4.6–6.7) and HEU children (5.1; 95%CI: 3.7–7.2); however, only 44% HIV-unexposed and HEU seroconverted (titers > 50 mIU/ml). AEs occurred with similar frequency and severity between groups, except for more systemic AEs after VZV vaccination in HEU than HIV-unexposed children.

Conclusions: Single dose HAV and VZV vaccine was similarly immunogenic in HIV-unexposed and HEU children. We did not identify differences in short-term humoral immunity after administration of either a live attenuated or inactivated vaccine. Seroconversion rates after a single dose of VZV vaccine were, however, lower compared to reports from previous studies (85–89%).

Clinical trials registration: NCT03330171.

1. Introduction

Prevention of mother-to-child HIV transmission programs have reduced vertical transmission of HIV to newborns, however, there remains a growing population of children born to women with HIV but who are not infected by HIV (i.e. HIV-exposed uninfected; HEU) [1]. HEU children have increased risk of infectious morbidity and mortality compared with HIV-unexposed children, particularly in early childhood [2,3].

Hepatitis-A virus (HAV) is a common cause of viral hepatitis, particularly in low- and middle-income countries [4]. The global incidence of acute hepatitis-A has increased from 150 (141–159) million in 1990 to 170 (161–180) million in 2017 [5]. Most children are exposed to HAV before 5 years of age when hepatitis-A infection commonly has an asymptomatic/mild clinical course [6]. African countries may, however, be experiencing an epidemiological transition to lower HAV endemicity [7–10], which

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is associated with absence of HAV infection during early childhood and predisposition to more severe illness when acquired later in life [6]. In South Africa, sero-epidemiological studies indicate intermediate HAV endemicity between 2005 and 2015, with total antibody positivity of 53% at 1–4 years of age and > 90% after 25 years of age [10]. This indicates a need to consider the World Health Organization (WHO) recommendation for inclusion of HAV vaccination in South Africa's national immunization program for children ≥ 1 year of age [6,10]. Given the sizeable number of HEU children, it is important to study HAV vaccination in this group.

Although two doses of Hepatitis A vaccine are traditionally recommended, similar immune responses have been achieved after a single compared to two doses [11,12] and single dose vaccination is programmatically more realistic and feasible. To our knowledge, immunogenicity data following a single dose of inactivated HAV vaccine regimen in HEU children are limited to one Brazilian study [13]. Seroconversion (antibody titer > 20 mIU/ml as measured by enzyme-linked immunosorbent assay) was reported in 72% (18/25) of HEU children following HAV vaccination at mean 5.1 years of age [13].

Varicella infection is usually self-limited, but may cause serious complications, including secondary bacterial infections, pneumonia or encephalitis [14]. Differences in epidemiology between temperate and tropical climates have been described, with primary infection occurring at older age in tropical climates, thereby increasing the risk of developing complications [15]. Safe and effective vaccines against varicella and herpes zoster are available [16]. The WHO recommends immunization with a live attenuated varicella zoster virus (VZV) vaccine at 12–18 months of age in settings where varicella is of public health importance [16]. Although VZV immunization has been introduced in the national vaccination programmes of several countries [15,17,18], it is seldom used in Africa [14] and may be of use in South Africa. Unusual clinical manifestations, such as haemorrhagic varicella, have been reported in HEU children in South Africa [3]. To our knowledge, there are no VZV vaccine studies in HEU children from sub-Saharan Africa.

This study evaluated the short-term immunogenicity and safety of a single dose of HAV and VZV vaccines administered to HIV-unexposed and HEU South African children at 18 months of age.

2. Methods

2.1. Study design and setting

This prospective cohort study enrolled HIV-unexposed and HEU children aged 18 weeks of age at the Respiratory and Meningeal Pathogens Research Unit (RMPRU), Chris Hani Baragwanath Academic Hospital (CHBAH), Soweto, South Africa between April 2017 and February 2019. All children participating in the present study were also evaluated for measles vaccination antibody response and these results have been published separately [19]. Here we report on the immune response to varicella and hepatitis-A vaccination (NCT03330171). HIV-unexposed children who were additionally enrolled in a randomized, open-label trial evaluating reduced dosing schedules of pneumococcal conjugate vaccine (PCV) (NCT02943902) were invited to participate in consecutive order. All participants were recruited from the same population during the same time period. Eligible participants were those born at ≥ 37 weeks gestation, birth weight > 2499 g, healthy based on medical history and physical examination, and absence of previous varicella or hepatitis-A disease or vaccination since birth. Inclusion and exclusion criteria are detailed in Supplementary data 1.

2.2. Procedures

Participants in the measles study were allocated consecutive study identifiers. Study staff and participants were not blinded. For the current study, half of the children participating in the measles study received either one dose of inactivated HAV vaccine (AVAXIM® - Pediatric, 80 U/0.5 mL, Sanofi Pasteur, Lyon, France) intramuscularly or live attenuated VZV vaccine (VARILRIX®, GlaxoSmithKline, Rixensart, Belgium) subcutaneously in the deltoid region at 18 months of age (547 days ± 14) based on having an even or odd numeric study identifier. The vaccines were stored at 2–8 °C prior to use. Parents were asked about compatible illness prior to vaccine administration and received hepatitis-A vaccine if chicken pox was reported. All children received other childhood vaccines according to the South African public immunization programme, except for HIV-unexposed children being randomized to different dosing schedules of PCV during the first year of life. Children were observed for 30 min after vaccination to monitor for anaphylactic reactions. Venous blood specimens were obtained immediately before (18 months) and one-month after vaccination (19 months, 28–35 days post-vaccination).

2.3. Safety assessment

Vaccine safety was assessed using report cards. Parents were trained to record any solicited local (pain/tenderness at injection site, redness, swelling and itching) and systemic (fever, vomiting, poor appetite, irritability and decreased activity) reactions for 7 days following vaccination. Monitoring for serious adverse events (SAEs) was done during one-month post-vaccination, through passive surveillance.

2.4. Laboratory methods

Blood samples were centrifuged and sera were stored at −70 °C until testing. HAV antibodies were measured using a chemiluminescent microparticle immunoassay (Abbott ARCHITECT® HAVAb- IgG). IgG results were calculated by dividing relative light units of the sample by relative light units of the cut-off (S/CO). Seropositivity was defined as S/CO ≥ 1.00 and seroconversion as a change from nonreactive (S/CO < 1.00) pre-vaccination to reactive (S/CO ≥ 1.00) post-vaccination, as per manufacturer's instructions [20]. Further details are provided in Supplementary data 2.

VZV antibodies were measured by commercial enzyme-linked immunosorbent assay (ELISA) (SERION ELISA *classic* Varicella-Zoster IgG, Institut Virion\Serion GmbH, Würzburg, Germany) according to manufacturer's instructions. Following recommendations by Sauerbrei and colleagues for measuring post-vaccination VZV responses using this ELISA kit, positive results were defined as > 50 mIU/ml [21]. Seroconversion was defined as a change from seronegative (≤50 mIU/ml) pre-vaccination to seropositive (>50 mIU/ml) post-vaccination.

2.5. Statistical analyses

With a sample size of 100 HIV-unexposed and 35 HEU children that was calculated for the measles study, this study had 80% power to detect an 18% lower seropositivity after HAV vaccination in HEU children compared to HIV-unexposed children, assuming post-vaccination seropositivity rate of 95% in HIV-unexposed children. This study was also adequately powered at 80% to detect a reduction in seropositivity of at least 25% after VZV vaccination in HEU children compared to HIV-unexposed children, assuming a seropositivity rate of 85% in HIV-unexposed children (premised on 85–89% of children to have gpELISA titers ≥ 5 units/ml after single dose VZV vaccine [16,22]).

HAV IgG antibody responses were compared between groups after controlling for pre-vaccination antibody levels (post-vaccination analysis only). Geometric mean titers (GMT) following VZV vaccination were calculated following $\log_{10}$ transformation of ELISA titer values and were compared between groups using multiple linear regression adjusting for maternal age at delivery and pre-vaccination antibody levels (post-vaccination analysis only). Geometric mean fold-rise (GMFR) of VZV antibody was calculated as the geometric mean of the ratio of post-vaccination to pre-vaccination titers, and compared using multiple linear regression with maternal HIV status, maternal age and pre-vaccination titers as covariates. VZV IgG antibody increase was also assessed as ≥ 2, ≥ 3 and ≥ 4 fold-rise from baseline. Percentage of children meeting serological cut-offs was presented with exact binomial 95% confidence intervals (CI) and compared between groups using multivariable logistic regression adjusting for the above mentioned covariates.

Safety analyses evaluated the proportion of children with at least one adverse event, severe adverse events and SAEs. Categorical variables were compared between groups using Chi-square test and Fisher's exact test and continuous variables using the Student's *t*-test or Mann-Whitney *U* test. Analyses were performed using Stata13 (StataCorp, LP, Texas, USA).

2.6. Ethics

The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M170276), South Africa. Written informed consent was obtained from all parents or guardians prior to enrolment.

3. Results

3.1. Demographic characteristics

100 HIV-unexposed and 35 HEU children received one dose of HAV vaccine. Two HIV-unexposed participants missed post-HAV vaccine blood collection (withdrawal n = 1, loss to follow-up n = 1). 95 HIV-unexposed and 29 HEU children received one dose of VZV vaccine. Four participants (3 HIV-unexposed and 1 HEU) were unavailable for follow-up serology after VZV immunization (withdrawals n = 2, missing blood samples n = 2). Table 1 describes the demographic characteristics of the participants, who were 18.0 months of age (interquartile range [IQR] 18.0–18.1) at vaccination and 19.0 months of age (IQR 19.0–19.1) at the post-vaccination serology visit. Characteristics were similar between groups, except for HEU children who received VZV vaccine having older mothers; Table 1. Demographics of HIV-unexposed children who received either hepatitis-A vaccine or VZV vaccine were not significantly different from those who enrolled in the main PCV parent study (Supplementary Tables 2 and 3).

3.2. Hepatitis-A vaccine immunogenicity

Overall 5.2% (95% CI: 2.1–10.4) of children were seropositive for HAV pre-vaccination, including 6.0% (95% CI: 2.4–12.6) and 2.9% (95% CI: 0.1–14.9) in HIV-unexposed and HEU children, respectively; Table 2. Prior to HAV vaccination, the median HAV antibody S/CO was 0.21 (IQR 0.16–0.32) in HIV-unexposed and 0.20 (IQR 0.15–0.30) in HEU children.

One month after administration of HAV vaccine, overall 89.5% (95% CI: 83.0–94.1) of children had seropositive titers, including 91.8% (95% CI: 84.5–96.4) of HIV-unexposed and 82.9% of HEU children (95% CI: 66.4–93.4; p = 0.144); Table 2. Of the 126 children seronegative before vaccination, the majority (88.9%; 95% CI: 82.1–93.8) seroconverted after HAV vaccination (91.3% HIV-unexposed and 82.4% HEU; p = 0.196). Post-vaccination, the median S/CO was 3.17 (IQR 2.41–4.38) in HIV- unexposed and 2.98 (IQR 1.66–4.17) in HEU children.

3.3. Varicella vaccine immunogenicity

Prior to vaccination in analyses adjusted for maternal age, GMTs were similar between HIV-unexposed (8.1 mIU/ml; 95% CI: 7.2–9.2) and HEU (9.0 mIU/ml; 95% CI: 6.2–13.2) children (p = 0.373). Three children (2 HIV-unexposed and 1 HEU) had VZV antibody titers > 50 mIU/ml and were excluded from post-vaccination analyses; Table 3.

One-month after VZV vaccination, GMTs increased to 41.6 mIU/ml (95% CI: 34.4–50.3) in HIV-unexposed and to 38.6 mIU/ml (95% CI: 27.8–53.7) in HEU children; Table 3. GMFR was similar in HIV-unexposed (5.6; 95% CI: 4.6–6.7) and HEU (5.1; 95% CI: 3.7–7.2) children in analyses adjusted for maternal age and pre-vaccination titers. The proportion of seropositive children

Table 1
Demographics and study participants' characteristics.

Characteristic	Hepatitis-A vaccine recipients			Varicella vaccine recipients		
	Total n = 135	HIV-unexposed n = 100	HIV-exposed uninfected n = 35	Total n = 124	HIV-unexposed n = 95	HIV-exposed uninfected n = 29
Male, n (%)	75 (56)	56 (56)	19 (54)	54 (44)	43 (45)	11 (38)
Race	135	100 (1 0 0)	35	122	93	29
Black African, n (%)	(1 0 0)	0	(1 0 0)	(98)2	(98)2	(1 0 0)0
Mixed ancestry, n (%)	0		0	(2)	(2)	(0)
Median birthweight, grams (IQR)	3090 (2810–3420)	3120 (2840–3428)	3070 (2790–3410)	3288 (2985–3463)	3280 (2930–3475)	3305 (3095–3425)
Median weight at vaccination, kilograms (IQR)	10.6 (9.6–11.6)	10.6 (9.6–11.7)	10.6 (10.0–11.5)	10.5 (10.0–11.8)	10.8 (10.0–11.9)	10.3 (10.0–11.4)
Median maternal age at delivery, years (IQR)	28.5 (23.4–32.9)	28.0 (22.3–32.6)	29.3 (25.8–34.9)	27.9 (25.1–33.4)	27.0 (24.6–31.4)[a]	33.4 (27.1–39.0)[a]
Median age at vaccination and serology pre-vaccination, months (IQR)	18.0 (18.0–18.1)	18.0 (17.9–18.1)[b]	18.0 (18.0–18.1)[b]	18.0 (18.0–18.1)	18.0 (18.0–18.1)	18.0 (18.0–18.1)
Median age at serology following vaccination, months (IQR)[c]	19.0 (19.0–19.2)	19.0 (19.0–19.2)	19.0 (19.0–19.2)	19.0 (19.0–19.1)	19.0 (19.0–19.1)	19.0 (19.0–19.2)

Abbreviations: IQR, interquartile range;
[a] p-value = 0.002;
[b] p-value = 0.037;
[c] total of 133 children available at post hepatitis-A vaccination visit: 98 HIV-unexposed and 35 HIV-exposed uninfected; total of 122 children available at post varicella vaccination visit: 93 HIV-unexposed and 29 HIV-exposed uninfected;

Table 2
Hepatitis-A IgG antibody response and proportion of children achieving seropositive titers and seroconversion.

Measure	Total	HIV-unexposed	HIV-exposed uninfected	p-value
Pre-vaccination				
Seropositive[a], n/N	7/135	6/100	1/35	0.974
% (95% CI)	5.2 (2.1–10.4)	6.0 (2.4–12.6)	2.9 (0.1–14.9)	
S/CO, median (IQR)	0.21 (0.16–0.30)	0.21 (0.16–0.32)	0.20 (0.15–0.30)	0.860
Post-vaccination				
Seropositive[a], n/N	119/133	90/98	29/35	0.144
% (95% CI)	89.5 (83.0–94.1)	91.8 (84.5–96.4)	82.9 (66.4–93.4)	
Seroconversion[b], n/N in HAV seronegative	112/126	84/92	28/34	0.196
% (95% CI)	88.9 (82.1–93.8)	91.3 (83.6–96.2)	82.4 (65.5–93.2)	
S/CO, median (IQR)	3.06 (2.30–4.34)	3.17 (2.41–4.38)	2.98 (1.66–4.17)	0.160

Abbreviations: CI, confidence interval; HAV, hepatitis-A virus; IQR, interquartile range; S/CO, sample signal to cut-off ratio;
P-values were calculated by Mann-Whitney or Fisher's exact test (pre-vaccination) and linear or logistic regression adjusting for baseline antibody values (post-vaccination);
[a] Seropositivity was defined as sample signal to cut-off ratio (S/CO) $\geq$ 1.00 per manufacturer's specification;
[b] Seroconversion was defined as a change from S/CO < 1.00 to S/CO $\geq$ 1.00.

Table 3
Varicella zoster virus IgG antibody response and proportion of children achieving seropositive titers and seroconversion.

Measure	Total	HIV-unexposed	HIV-exposed uninfected	p-value
Pre-vaccination	n = 124	n = 95	n = 29	
GMT (95% CI)	8.3 (7.3–9.5)	8.1 (7.2–9.2)	9.0 (6.2–13.2)	0.373
Seronegative (≤50 mIU/ml), n	121	93	28	0.524
% (95% CI)	97.6 (93.1–99.5)	97.9 (92.6–99.7)	96.6 (82.2–99.9)	
Seropositive (>50 mIU/ml)[a], n	3	2	1	0.524
% (95% CI)	2.4 (0.5–6.9)	2.1 (0.2–7.4)	3.4 (0.1–17.8)	
Post-vaccination[a]	n = 117	n = 90	n = 27	
GMT (95% CI)	40.9 (34.8–48.1)	41.6 (34.4–50.3)	38.6 (27.8–53.7)	0.743
Seronegative (≤50 mIU/ml), n	65	50	15	0.536
% (95% CI)	55.5 (46.1–64.7)	55.6 (44.7–66.0)	55.6 (35.3–74.5)	
Seropositive (>50 mIU/ml), n	52	40	12	0.536
% (95% CI)	44.4 (35.3–53.9)	44.4 (34.0–55.3)	44.4 (25.5–64.7)	
Change from pre- to post-vaccination				
GMFR[b] (95% CI)	5.5 (4.6–6.4)	5.6 (4.6–6.7)	5.1 (3.7–7.2)	0.743
Seroconversion[c], n	52	40	12	0.536
% (95% CI)	44.4 (35.3–53.9)	44.4 (34.0–55.3)	44.4 (25.5–64.7)	
≥2 fold-rise from baseline, n	103	79	24	0.817
% (95% CI)	88.0 (80.7–93.3)	87.8 (79.2–93.7)	88.9 (70.8–97.6)	
≥3 fold-rise from baseline, n	94	73	21	0.590
% (95% CI)	80.3 (72.0–87.1)	81.1 (71.5–88.6)	77.8 (57.7–91.4)	
≥4 fold-rise from baseline, n	80	63	17	0.824
% (95% CI)	68.4 (59.1–76.7)	70.0 (59.4–79.2)	63.0 (42.4–80.6)	

Abbreviations: CI, confidence interval; GMFR, geometric mean fold-rise; GMT, geometric mean titer;
P-values were either calculated by linear or logistic regression and adjusted for maternal age at delivery and baseline antibody levels (post-vaccination comparison only);
[a] Three participants (2 HIV-unexposed and 1 HEU) with seropositive titers pre-varicella vaccination are excluded from post-vaccination analyses;
[b] Geometric mean of the ratio of post-vaccination titer to the pre-vaccination titer;
[c] Change from ≤ 50 mIU/ml pre-vaccination to > 50 mIU/ml post-vaccination.

increased to 44.4% (95% CI: 35.3–53.9) in both groups and less than half seroconverted (44.4% HIV-exposed and 44.4% HEU). Most children (87.8% HIV-unexposed and 88.9% HEU) had at least a 2-fold rise in VZV antibody titer post-vaccination; Table 3.

3.4. Hepatitis-A vaccine safety

Solicited local and systemic reactions occurred with similar frequency and severity in HIV-unexposed and HEU children; Table 4 and Supplementary table 4. There were no vaccine-related adverse events. One SAE (hospitalization for herpetic gingivostomatitis) occurring 24 days following HAV vaccination in an HIV-unexposed child was reported, which completely resolved.

3.5. Varicella vaccine safety

During the seven days following vaccination, 27% HIV-unexposed and 18% HEU experienced ≥ 1 local adverse reaction, whereas 57% HIV-unexposed and 29% HEU (p-value = 0.007) experienced ≥ 1 systemic adverse reaction; Table 4. Overall, the frequency and severity of solicited adverse events were similar between groups, except for decreased appetite, which was 22% in HIV-unexposed and 14% in HEU (p-value = 0.026); Supplementary table 4. No vaccine-related adverse events and no SAEs were reported during the 28 days following vaccination.

4. Discussion

This study demonstrated that a single dose of inactivated HAV vaccine or live attenuated VZV vaccine administered at 18 months of age was similarly immunogenic in both HIV-unexposed and HEU South African children, albeit seroconversion rates after VZV vaccination were lower than expected. Our study fills a data gap in providing evidence on HAV and VZV immunization in HEU children.

We found no statistical difference in the percentage of HEU children to be HAV seropositive post-vaccination compared with HIV-unexposed children. Our findings of 82.9% seropositivity and 82.4% seroconversion in HEU were slightly higher than the previous study on single dose HAV vaccine in HEU children, in which

Table 4
Reported adverse events following single dose hepatitis-A or varicella vaccination by HIV-exposure.

	Hepatitis-A vaccine recipients		Varicella vaccine recipients	
n/N (%)	HIV-unexposed	HIV-exposed uninfected	HIV-unexposed	HIV-exposed uninfected
Solicited reactions 0–7 days after vaccination				
Local reactions				
≥1	25/100 (25)	10/35 (29)	25/94 (27)	5/28 (18)
Severe	0/100 (0)	2/35 (6)	2/94 (2)	1/28 (4)
Systemic reactions				
≥1	47/100 (47)	15/35 (43)	54/94 (57)[a]	8/28 (29)[a]
Severe	3/100 (3)	4/35 (11)	6/94 (6)	4/28 (14)
Unsolicited serious adverse event after vaccination				
Serious AE ≤ 28 days after injection	1/100 (1)[b]	0/35 (0)	0/94 (0)	0/28 (0)
Vaccine-related serious AE	0/100 (0)	0/35 (0)	0/94 (0)	0/28 (0)

Abbreviations: AE, adverse events; N, total number of participants with vaccination report card / serious adverse event assessment; n, number of participants having a reaction;
[a] p = 0.007;
[b] Participant hospitalized with herpetic gingivostomatitis.

72.0% HEU children seroconverted 4–8 weeks after vaccination [13].

In the present study, a single dose of HAV vaccine generated seropositive antibody responses in 91.8% (95% CI: 84.5–96.4) of HIV-unexposed children. In comparison, 98.6% of Argentinian children had seropositive titers (≥10 mIU/mL as measured by microparticle enzyme immunoassay) one year after a single dose of the same HAV vaccine administered at 11–23 months of age [12]. Similarly, 95.3% of Chinese children vaccinated at 18–60 months of age had seropositive responses by microparticle enzyme immunoassay one year following inactivated HAV vaccination [23].

Despite 10.5% of children in our study not showing a humoral immune response to HAV vaccination, previous studies showed that children with low or undetectable antibody levels after one HAV dose, measured prior to booster administration, were able to elicit a strong humoral response after booster challenge, indicative of a robust anamnestic response [24]. This memory recall response may reflect residual B-cell response capacity. It has also been shown that a single HAV vaccine dose induces HAV-specific T-cell immunity that persists independently of circulating antibody levels and produces a HAV-specific memory response similar to that induced by natural infection [25]. The T-cell immunity may contribute to protection against hepatitis-A viral infection in children without seroconversion.

HIV-unexposed and HEU children had comparable antibody responses to VZV vaccine. A cross-sectional study from the United States reported 98% (55/56) of HEU children with seropositive titers following one dose of VZV vaccine at median 1.5 (IQR 1.1–3.7) years of age as measured either by whole-infected cell ELISA or gpLISA (cutoffs not mentioned), although relation to vaccination could not definitely be ascertained [26].

Seropositivity rates in our study following a single dose VZV vaccination were strikingly lower than previously reported after a single dose of VZV vaccine in different settings; 85–89% had antibody levels ≥ 5 units/mL (based on gpELISA) and an estimated vaccine efficacy of 94.4% during the 10-years following vaccination [22,27–29]. Our findings were also lower than a South African trial on the immunogenicity of a single dose of VZV vaccine, co-administered with measles/mumps/rubella (MMR) vaccine at 15–18 months in healthy children, which showed a varicella response of 73% to 75% (measured by FAMA ≥ 4 [1/dil]) [30]. We followed Sauerbrei and colleagues' recommendation to use an optimized cutoff value of 50 mIU/ml for assessment of post-vaccine immunity [21]. Despite using this cutoff, seropositivity and seroconversion rates remained<50%, which is lower than reported after one dose of the same vaccine [31–33] or when combined with MMR [34,35]. A literature search did not identify any published study evaluating VZV antibodies following immunization using the same Virion\Serion ELISA kit. An Indian study, using a different commercial ELISA kit, found a similar percentage of children experiencing a 3- or 4-fold increase in VZV-specific IgG titer from baseline (73% and 62%, respectively) in children aged 12 months to 12 years after one dose of the same VZV vaccine [36]. Commercial ELISA may not be sensitive enough to detect seroconversion after vaccination, since it is calibrated for diagnosis of natural infection [37] and may therefore underestimate the immunogenicity of vaccines. The choice for commercial ELISA was investigator-driven and due to limited availability of gpELISA.

Pre-vaccination VZV seroprevalence was low (2%), which is corroborated by a recent systematic review showing seropositivity to VZV antibodies in African children aged 1–12 years of 23% (95% CI 17–30%) [38]. The authors of the review suggested that primary VZV infection occurs at a later age in Africa compared to other regions and noted a positive association between age and VZV seropositivity [38].

Both HAV and VZV vaccines were found to be safe and well tolerated. Following VZV vaccination, more HIV-unexposed children experienced one or more systemic reaction than HEU children. The underlying reason for this difference remains unclear. The percentage of children with any local or systemic solicited reaction was similar to that reported in a South African study co-administering VZV vaccine with MMR (10–68%) [30]. The percentage of children with severe systemic reactions was, however, higher in our study (2–14%) compared to the co-administration study (0–4%), particularly in HEU children. No serious adverse events occurred during the 28 days following vaccination.

Limitations of this study include that our sample size only provided 80% power to detect at least a 20% difference following HAV vaccination between HEU and HIV-unexposed (based on our result of 92% seropositivity in HIV-unexposed). Consequently, we may have missed detecting differences of lesser magnitude in HAV antibody responses between the two groups. Also, due to lower than anticipated response rate to VZV vaccination, the study lacked power for this comparison. With the present sample size and 44% seropositivity in HIV-unexposed children, we were only adequately powered (at 80% power) to detect a difference of at least 27% between HEU and HIV-unexposed children after varicella immunization. Nevertheless, our study still yields important information that needs to be pursued. In addition, our study is limited by the absence of an HIV-positive cohort and short duration of follow-up. Long-term follow-up is currently ongoing. Solicited adverse events were followed-up until day 7 post-vaccination, thereby excluding adverse events with a later onset if not reported

by the parent during the next study visit. Assessment of humoral immunity following VZV immunization was done using a commercially available ELISA kit, which may be less sensitive in post-vaccination samples and could therefore underestimate the antibody response.

Based on the WHO recommendation to integrate universal vaccination against HAV in the national immunization schedules in countries with declining endemicity from high to intermediate, several countries have introduced the HAV vaccine during childhood, which led to a considerable decrease in HAV incidence in both vaccinated and non-vaccinatedgroups [39]. Before universal HAV vaccination can be considered, seroprevalence across different age groups and regions needs to be established, in combination with country-specific cost-effectiveness assessment.

In many low- and middle-income countries (LMIC), other vaccine-preventable diseases with greater public health burden or severity, are prioritized over VZV vaccine. Despite the low morbidity and mortality of VZV, the burden of varicella and herpes zoster on healthcare systems and society, in absence of preventive measures, can be considerable [15]. A retrospective review of admissions to a paediatric isolation facility in Durban, South Africa, demonstrated that varicella accounted for 23% of admissions between 1986 and 1996, with 15% of varicella admission (n = 86) and 75% of varicella deaths (n = 6) being associated with HIV-infection between 1994 and 1996 [40].

A theoretical modelling study claimed that if LMIC introduced a one dose VZV vaccine in children 12–18 months of age with coverage between 20% and 80%, there would be an increased risk of an epidemiological shift to older age at infection and increased mortality [41]. In contrast, epidemiologic data from high-income countries have shown a decrease in varicella incidence in all age groups [42] or under the age of 40 [43] after VZV vaccine introduction in the second year of life and, most importantly, of complications, hospitalizations and overall healthcare costs associated with varicella infections [44]. Before routine VZV vaccination can be implemented, countries first need to consider the burden of varicella disease and predict achievable vaccination coverage [41].

In conclusion, we have shown that a single dose of inactivated HAV vaccine at 18 months of age was safe and resulted in most children becoming HAV seropositive in the short-term. Durability of a single dose HAV vaccine needs to be established. Single dose of live attenuated VZV vaccination was safe, but resulted in seropositivity in less than half of children as measured by a commercially available ELISA. A second dose of VZV is expected to improve rate of seroconversion [22]. Future studies should evaluate long-term humoral and cellular response to HAV and VZV vaccines in the African context, in both HIV-unexposed and HEU children.

Author contributions

EAMLM, MCN and SAM contributed to the conception and design of the study; EAMLM, MCN and SAM oversaw the clinical trial, clinical data collection and clinical data management; SB, BTI, LJ and AK were responsible for clinical aspects of the study; AM was responsible for laboratory management; EAMLM conducted laboratory analyses and wrote the first draft of the paper. All authors contributed to subsequent drafts, read and approved the final version of the report.

CRediT authorship contribution statement

Eleonora A.M.L. Mutsaerts: Conceptualization, Formal analysis, Investigation, Writing - original draft, Project administration. **Marta C. Nunes:** Conceptualization, Writing - review & editing, Supervision. **Sutika Bhikha:** Investigation. **Benit T. Ikulinda:** Investigation. **Lisa Jose:** Investigation. **Anthonet Koen:** Investigation. **Andrew Moultrie:** Resources, Project administration. **Diederick E. Grobbee:** Writing - review & editing. **Kerstin Klipstein-Grobusch:** Writing - review & editing, Supervision. **Adriana Weinberg:** Resources, Writing - review & editing, Supervision. **Shabir A. Madhi:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Weinberg receives research grants from Merck and GSK. Moneys go to the University of Colorado. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.03.045.

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Appendix 5

R14/49 Dr Eleonora Mutsaerts

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170391

NAME: (Principal Investigator)	Dr Eleonora Mutsaerts
DEPARTMENT:	Clinical Microbiology and Infectious Diseases DST/NRF Vaccine Preventable Diseases RMPRU
PROJECT TITLE:	Evaluation of quantitatve and qualitative antibody responses to measles, diphtheria, tetanus, pertussis, Haemophilus influenzae type b and hepatitis B vaccines amongst HIV-1 exposed-infected children that are receiving vs. those not receiving antiretroviral therapy,...
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Substudy under primary study WHC 040704 (Prof Shabir Madhi) Specimens under WHC 040704 will be used.
SUPERVISOR:	Prof Shabir Madhi and MC Nunes
APPROVED BY:	Prof P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/03/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in March and will therefore be due in the month of March each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

28/03/2017
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 6

R14/49 Dr Eleonora A.M.L. Mutsaerts et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170276

NAME: (Principal Investigator)	Dr Eleonora A.M.L. Mutsaerts et al
DEPARTMENT:	Respiratory and Meningeal Pathogens Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Safety and Immunogenicity of Measeles Vaccine, Varicella Vaccine and Hepatitis-A Vaccine in HIV-Exposed and HIV-Unexposed South African Children
DATE CONSIDERED:	24/02/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S.A. Madhi and Dr M.C. Nunes
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/04/2017

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 10-04-2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES